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Research Article

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Effect of DL–Ethionine on the Intestinal Absorption and Transport of Palmitic Acid–1–^{14}C and Tripalmitin–^{14}C. Role of Intramucosal Factors in the Uptake of Luminal Lipids

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ABSTRACT The effect of DL-ethionine on the uptake and transport of lipid by the rat small intestine was investigated. A cottonseed oil emulsion containing ^{14}C-labeled tripalmitin or palmitic acid was administered intragastrically to rats pretreated with DL-ethionine, DL-ethionine plus methionine, or saline, and the rats were sacrificed 2, 4, and 6 hr later. Lipids from the plasma, the stomach, the colon, the luminal contents of the small intestine, and the wall of the small intestine were extracted, fractionated, and their radioactivity assayed.

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The degree of inhibition of mucosal reesterification and the degree of inhibition of transport of wall lipids were directly related to the degree of inhibition of uptake of luminal radioactivity. This relationship suggests that the rate of reesterification, the level of mucosal FFA, and the rate of transport of intramucosal TG may be of importance in determining the extent of uptake of intraluminal lipid by the mucosal cells.

Since a significant fraction of the wall radioactivity was in the form of TG, the decreased transport of wall lipids was attributed to an impairment of chylomicron completion due to inhibition of either the synthesis of chylomicron apoprotein or the association of preformed TG with the protein moiety of chylomicrons. Experiments with labeled amino acids support the first possibility.

INTRODUCTION

Recent evidence indicates that the absorption and transport of dietary lipids is closely related to the capacity of the gastrointestinal tract for synthesis of a number of enzymatic, carrier, and structural proteins (1, 2). Enzymes are essential for the intraluminal lipolysis of ingested fat (3, 4) and for the intramucosal reesterification of the products of digestion, i.e., fatty acids (FA) and partial glycerides (3, 5, 6). It has also been shown that the chylomicron apoprotein may be synthesized by the epithelium of the small intestine (7, 8). Although the protein moiety of chylomicrons has been measured as low as 0.2–0.5% (9), its presence appears to be essential for the transport of intramucosal triglyceride (TG) to the lacteals (1, 10). In addition, the very active turnover rate of the epithelial lining of the intestine (11) imposes a continuous need for replenishment of structural protein. Recently we have shown a very close

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correlation between the level of amino acid-activating enzymes (AAAE) in homogenates of rat intestinal epithelium and prolonged fasting (12), a condition known to be associated with impaired intestinal absorption of fat (13, 14). Since AAAE initiate the sequence of reactions involved in the synthesis of protein (15), the above observation suggested the possibility that substances having an inhibitory effect on the synthesis of protein may similarly impair the intestinal absorption of lipids. Indeed, administration of a number of protein synthesis inhibitors (puromycin and cycloheximide) (1) and, very recently, of DL-ethionine (2, 16) resulted in a marked impairment of the intestinal absorption of long-chain FA and TG.

In this communication we report on the effects of DL-ethionine on a number of reactions involved in the intestinal absorption of dietary lipids. The results confirm and extend the recently reported results of Hyams, Sabesin, Greenberger, and Isselbacher (2) and of Karvinen and Miettinen (16) and provide further understanding of the factors related to the uptake, intramucosal conversion, and transport of long-chain FA and TG by the small intestine.

METHODS

Female rats of the Sprague-Dawley strain (Quebec Breeding Laboratories, LaPrairie, Quebec) were maintained on a regular laboratory chow and were fasted the night before and throughout the experiment. Free access to water was allowed until sacrifice. The different groups of animals were matched so that the difference in body weight did not exceed 15–17%.

Tripalmitin carboxyl-^{14}C (18.4 μc/μmole) and palmitic acid-1-^{14}C (5.0 μc/μmole)[1] were purified further by dissolving in benzene and fractionation into esterified FA and free fatty acids (FFA) by the method of Borgström (17). The purity of each fraction was checked by thin-layer chromatography (TLC) and over 98% of the radioactivity was recovered in the TG or FFA spots, respectively. Gas chromatography (GLC) of the methyl esters of each fraction showed a single peak corresponding to palmitic acid. The purified fractions were added dropwise to a warmed (38°–40°C) emulsion of cottonseed oil[2] through which nitrogen was bubbled until the odor of benzene had disappeared completely. The final concentration and radioactivity of the two emulsions was 1.0 μc/ml with a specific activity of 128 μmoles palmitic acid/μc. The latter was calculated from the GLC composition of Lipomul[2] and the amount of the radioactivity of the added tripalmitin-^{14}C or palmitate-1-^{14}C. DL-ethionine and methionine[3] were used without further purification. All organic solvents were redistilled before use.

[1] New England Nuclear Corp., Boston, Mass.

[2] Lipomul, The Upjohn Co., Kalamazoo, Mich.; contains 15% cotonseed oil (with a FA composition of C14, 0.4%; C16, 21.9%; C16:1, 2.0%; C18, 2.1%; C18:1, 33.4%; C18:2, 39.7%; and C20, 0.6%), 4% dextrose, 1.2% lecithin, and 0.3% oxyethyleneoxypropylene polymer.

[3] Nutritional Biochemicals Corp., Cleveland, Ohio.

The ethionine-treated rats received intraperitoneally a total dose of 3 ml/100 g body weight (18) of a 2% solution of ethionine divided into equal hourly injections of 1 ml or less. Control animals were treated in an identical manner, except that equivalent volumes of isotonic saline or of a solution of 2% ethionine plus 1.83% methionine (18) were injected intraperitoneally. 24 hr after the first injection, each rat received through a gastric tube 2 ml of ^{14}C-labeled Lipomul, followed by 1 ml of saline. Preliminary experiments showed that the radioactivity retained in the syringe and gastric tube varied but never exceeded 0.3% of the original activity.

In a number of experiments Lipomul containing tripalmitin-^{14}C was administered intragastrically to control and ethionine-treated rats in which a jejunal fistula was constructed 5 hr previously by insertion of a polyethylene tube (O.D. 0.5 cm) 1 cm distal to the ligament of Treitz. The distal end of the tube was brought out through the skin, and the rats were placed in plastic restrainers to prevent biting of the tube. The intraluminal contents were collected in iced tubes for 2 hr and the collections (between 25 and 53% of the administered radioactivity) from 10 rats, respectively, were pooled and homogenized. Aliquots of pooled intraluminal contents from control animals were administered intragastrically to ethionine-treated rats and vice versa, and the experiments continued as described. The animals were sacrificed under light ether anesthesia 2, 4, and 6 hr after the intragastric administration of labeled fat. The abdominal and chest cavities were exposed, blood was withdrawn from the heart and collected into chilled syringes and tubes with heparin as an anticoagulant, and the plasma separated after centrifugation at 4°C.

The entire length of the gastrointestinal tract was removed after double ligatures were placed at the level of the gastroduodenal and ileocecal junctions. Mesenteric fat was meticulously separated from the walls and each portion (esophagus plus stomach, small and large intestine) was sectioned between the ligatures. The intraluminal contents of the small intestine were thoroughly flushed with 150 ml of ice-cold saline containing 0.2 M NaF. The intestine was then everted over a glass rod, and adsorbed radioactivity on the mucosal surface was removed by three consecutive washings in 50 ml of 0.2 M NaF in saline. The second and third washings had virtually no radioactivity. All the washings were combined, centrifuged, and homogenized in a Waring Blendor. The wall of the small intestine plus the pellets obtained after centrifugation of the washings were homogenized in saline with a Potter-Elvehjem homogenizer. The stomach and the colon were homogenized along with their contents. Feces passed after the administration of the test meal were homogenized together with the colon and its contents.

Lipids from plasma and homogenates were extracted according to the method of Folch, Lees, and Sloane-Stanley (19). The recovery of radioactive lipid added to homogenates from animals given nonradioactive Lipomul was better than 90%. The chloroform extracts were dried over sodium sulfate, filtered, and evaporated to dryness at 40°C in a vacuum oven. The lipids were then redissolved in a known volume of chloroform and aliquots taken for further analyses.

For radioactivity determinations, aliquots of lipid extract were transferred to counting vials, the solvent evaporated, and 12 ml of toluene containing 0.01% *p*-bis[2-(5-phenyloxazolyl)]benzene and 0.3% 2,5-diphenyloxazole was added. The samples were counted in a liquid scintillation spectrometer with a counting efficiency of close to 75% for ^{14}C. The

TABLE I

Effect on Gastric Emptying, Transport, and Distribution of Tripalmitin-^{14}C

Treatment	Time	Small intestine			Colon*	Transported‡
		Stomach	Lumen	Wall		
	hr	*% of administered radioactivity*				
Saline	2	40.9 ±4.7	8.4 ±1.5	1.2 ±0.3	0.4 ±0.3	49.2 ±5.9
(9)§	4	26.0 ±6.5	4.1 ±1.9	0.6 ±0.3	0.5 ±0.8	68.8 ±8.8
	6	11.8 ±4.0	3.9 ±2.6	0.6 ±0.4	0.4 ±0.8	83.4 ±4.7
Ethionine	2	42.9 ±5.2	19.1 ±2.0	12.8 ±1.2	3.7 ±1.3	21.5 ±6.1
(9)	4	28.8 ±4.8	11.5 ±1.7	16.8 ±3.0	4.1 ±1.2	34.6 ±7.2
	6	14.5 ±5.3	9.9 ±0.8	23.0 ±2.3	3.4 ±1.6	49.2 ±4.5
Ethionine + methionine	2	39.1 ±5.4	8.4 ±1.7	1.3 ±0.4	0.8 ±0.3	50.4 ±6.8
(9)	4	25.6 ±5.7	3.9 ±1.9	0.6 ±0.3	0.5 ±0.3	69.3 ±3.3
	6	11.3 ±4.4	3.9 ±2.5	0.7 ±0.4	0.4 ±0.3	83.2 ±3.1

* Radioactivity in lumen and wall.

‡ Transported = 100 − (A + B + C + D). A = per cent of administered radioactivity recovered in the stomach; B,C,D = per cent of administered radioactivity recovered in the lumen of the small intestine (B); in the wall of the small intestine (C); and in the colon (D).

§ No. of animals in each group.

samples were corrected for quenching by the channels ratio method (20).

Results were expressed as per cent of the administered radioactivity or as per cent of the radioactivity delivered from the stomach to the small intestine. Gastric emptying was calculated from the difference between the administered radioactivity and the radioactivity recovered in the stomach. Uptake of luminal lipid by the small intestine was calculated by subtracting from the administered radioactivity the radioactivity recovered in the stomach, in the lumen of the small intestine, and in the colon. Transport or net absorption was calculated by subtracting the radioactivity in the wall of the small intestine from the value for uptake of lipid radioactivity by the small intestine.

Preliminary results obtained with lipid extracts from small intestinal wall homogenates or from homogenates of mucosal scraping consistently showed that over 90% of the wall radioactivity was localized in the mucosal scrapings.

Lipid classes were separated by thin-layer chromatography on Silica Gel G using a solvent system of *n*-hexane–diethyl ether–acetic acid–methanol 90:20:2:3 v/v (21). Compounds were visualized with iodine and identified by comparison with standards run simultaneously (Applied Science Laboratories Inc., State College, Pa., and The Hormel Institute, Austin, Minn.). After the iodine had sublimed, the areas containing lipids were scraped into vials containing 12 ml of the counting solution with 2.5% of Cab-O-Sil (Packard Instrument Co., Inc., Downers Grove, Ill.). Radioactivity in the different lipid fractions was expressed as per cent of the radioactivity in the total lipid extract applied on the TLC plates. Recoveries ranged from 91 to 104% with a mean (±SD) of 98.5 ±9.6 (n = 42).

Fatty acid composition was determined by gas chromatography. Methyl esters were prepared according to the method of Nelson and Freeman (22) and separated on a Gas Chromatograph, Model 600, (Research Specialties Co., Richmond, Calif.) equipped with an argon detector (10 mc ^{90}Sr foil). U-shaped stainless steel columns (1.8 m × 0.65 cm I.D.) were packed with Gas-chrom P, 80-100 mesh,[4] coated with 17% (w/w) diethylene glycol succinate polyester and operated at 185°C. Quantitative results with standards of methyl esters of FA[4] agreed with the stated composition with a relative error of less than 8% for major components and less than 15% for minor components.

RESULTS

Effect on body weight. It has been shown previously that administration of ethionine results in a significant reduction of body weight (23, 24). This has been attributed mainly to a reduction in food intake. Our experiments indicate, however, that irrespective of the treatment, the extent of weight loss by the three groups of animals was approximately equal (ethionine, 19.2 ±3.6 g or 7.9 ±1.0%; saline, 19.7 ±4.2 g or 8.0 ±1.2%; and ethionine + methionine, 19.9 ±9.2 or 7.7 ±2.5%).

Since in these experiments the animals were fasted for approximately equal periods of time, and thus the effect of differences in food intake eliminated, the comparable degrees of weight loss can be attributed solely to the effect of fasting. The possibility that differences in the handling of the administered large volumes of fluids could have accounted for the absence of greater weight loss in the ethionine-treated rats cannot be completely excluded by our results. However, the absence of any gross differences in organ weights and hematocrits of the blood samples would seem to exclude this possibility as a major factor.

[4] Mixtures KA, KB, KD, and KF, Applied Science Laboratories Inc.

TABLE II

Effect on Uptake and Transport of Tripalmitin-^{14}C by the small Intestine.

Treatment	Time	Recovered in lumen	Recovered in wall	Uptake*	Transported‡
	hr		*% of radioactivity delivered from stomach*		
Saline	2	14.3 ±3.4	2.1 ±0.6	85.1 ±4.0	83.0 ±4.2
(9)	4	5.5 ±2.9	0.8 ±0.5	93.7 ±3.5	93.0 ±4.3
	6	4.5 ±3.3	0.8 ±0.5	95.1 ±4.5	94.3 ±4.2
Ethionine	2	33.4 ±4.6	22.5 ±3.4	60.1 ±4.6	37.6 ±8.7
(9)	4	16.1 ±2.9	23.4 ±4.2	78.0 ±3.0	54.6 ±3.9
	6	11.6 ±1.2	26.9 ±2.3	84.4 ±2.7	57.5 ±2.0
Ethionine + methionine	2	14.2 ±3.7	2.0 ±0.5	84.5 ±4.1	82.5 ±4.1
	4	5.2 ±2.2	0.8 ±0.3	94.2 ±2.6	93.4 ±3.1
(9)	6	4.4 ±3.0	1.1 ±1.1	95.0 ±3.7	93.9 ±4.9

* Uptake = (100 − A) − (B + C)/100 − A × 100. A, B, and C = per cent of administered radioactivity in the stomach (A), in the lumen of the small intestine (B), and in the colon (C).

‡ Transport = uptake − fraction in the wall.

Effect on gastric emptying, transport, and distribution of TG radioactivity. The stomachs of the ethionine-treated rats were dilated and contained a large amount of swallowed hair. However, the radioactivity recovered in the gastric lumen of the ethionine-treated rats was not statistically different from that of the control animals (Table I). A considerable degree of gastric retention of a glucose solution was observed by Lupu and Farber (25) in rats 6 hr after the administration of either ethionine or methionine. In our experiments emulsified fat (Lipomul) was administered 24 hr after the first intraperitoneal injection, and it is possible that at this time any effect of ethionine or methionine on gastric motility could have subsided. In addition, it is well known that fat inhibits gastric emptying, and it is possible that the effect of the administered fat could have exceeded the inhibition induced by ethionine or methionine alone.

The effect of ethionine on the distribution and extent of transport of lipid radioactivity in the intestinal tract is of interest. Ethionine markedly inhibited the transport of TG radioactivity through the intestinal wall, and in Table I it can be seen that this can be accounted for mainly by the radioactivity recovered in the lumen and walls of the small intestine. Also, a significantly greater fraction of lipid radioactivity was recovered in the colon and feces of the ethionine-treated rats. Administration of ethionine together with methionine prevented the effect of ethionine alone. In the group of rats treated with ethionine and methionine the extent of transport and the distribution of the radioactivity in the intestine was comparable to that of the saline-treated animals.

Effect on uptake and transport of TG radioactivity by the wall of the small intestine. In Table II the radioactivity recovered in the lumen and wall of the small intestine is expressed as a fraction of the radioactivity delivered from the stomach. This allowed calculation of the amount of luminal radioactivity which was taken up

TABLE III

Effect on Gastric Emptying, Transport, and Distribution of Palmitic Acid-1-^{14}C

Treatment	Time	Small intestine			Colon*	Transported‡
		Stomach*	Lumen	Wall		
	hr		*% of administered dose*			
Saline	2	45.0 ±7.1	9.5 ±1.7	2.3 ±0.9	0.5 ±0.4	42.7 ±6.4
(6)	6	12.1 ±3.0	3.9 ±1.5	1.0 ±0.6	0.9 ±0.6	82.0 ±2.2
Ethionine	2	45.6 ±5.2	14.8 ±2.9	11.6 ±2.1	3.3 ±1.0	24.8 ±2.7
(5)	6	11.4 ±1.8	8.7 ±0.5.	25.3 ±2.9	2.9 ±0.6	51.7 ±3.5
Ethionine + methionine	2	44.5 ±5.2	9.4 ±1.6	1.5 ±0.7	1.3 ±0.5	43.5 ±5.6
(6)	6	13.5 ±3.0	3.6 ±1.7	1.9 ±0.8	0.7 ±0.3	80.8 ±3.6

* Radioactivity in lumen and wall.

‡ Calculated as in Table I.

by the wall and the amount which was transported (net absorption) from the wall into the body.

It is seen that in the ethionine-treated rats the uptake by the intestinal wall was significantly impaired, particularly during the 1st 2 hr. Subsequently the uptake by the small intestine improved but remained consistently below that of the control animals. It is further seen that in the ethionine-treated rats the transport of the wall radioactivity was markedly inhibited and that this inhibition remained consistently above 40% throughout the experiment.

The observed impairment of transport of luminal and wall lipids to the lacteals and blood stream was also reflected in the plasma levels of triglycerides and lipid radioactivity of the ethionine-treated rats, (Fig. 1) which remained consistently below that of the control animals. The lower levels of plasma TG and lipid radioactivity could also be attributed to differences in recirculation of the absorbed lipid at 4 and 6 hr and to decreased hepatic release of lipoprotein (26) in the ethionine-treated rats. Even though the possibility that absorbed lipid might have been diverted to the liver cannot be excluded by our results, the persistently low levels of plasma radioactivity and the well established fact that absorbed long-chain TG enters the venous circulation before reaching the liver (3) indicate that transport of luminal and wall lipids was markedly impaired in the ethionine-treated rats.

Effect on intraluminal lipolysis. Fig. 2 shows the effect of the respective treatments on the intraluminal

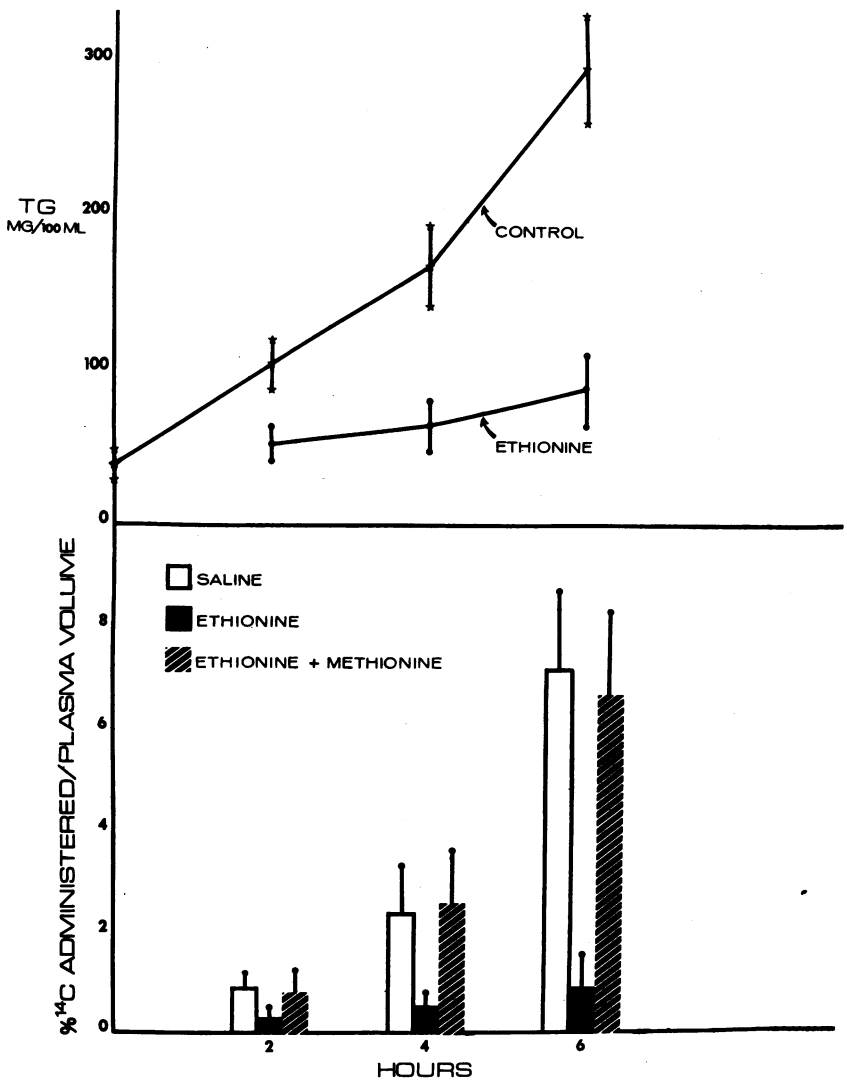

FIGURE 1 Effect on plasma TG and lipid radioactivity. Plasma volume taken as 4% of body wt at time of sacrifice (27).

lipolysis in the small intestine. It can be seen that at 2 hr the lipolysis was markedly inhibited in the ethionine-treated rats. Since at this time the uptake of radioactivity by the wall of the small intestine was also significantly inhibited (Table II), it would appear that the two were closely related. However, at 4 and 6 hr the intraluminal lipolysis in the ethionine-treated rats approached and even exceeded the lipolysis in the control animals at 2 hr (Fig. 2). Even though there was some improvement in uptake of radioactivity by the wall of the small intestine at 4 and 6 hr, the uptake remained consistently below that of the control groups (Table II).

To determine whether the degree of lipolysis was the rate-limiting factor, palmitic acid-1-^{14}C was administered instead of labeled tripalnitin. The results in Tables III and IV indicate that the effect of ethionine on gastric emptying, uptake, and transport of palmitic acid-1-^{14}C was analagous to that on tripalmitin. Table IV shows that although the radioactivity was administered in the form of a fatty acid, and thus a possible relationship to degree of lipolysis was eliminated, the uptake by the wall of the small intestine remained below that of the control animals. These results indicate that the uptake of luminal lipids by the intestinal wall was not dependent solely upon the degree of lipolysis.

To investigate the effect on biliary and pancreatic secretion, and thus eliminate any possible effects on emulsification, activation of pancreatic lipase, and micelle formation, cottonseed oil emulsion containing radioactive tripalmitin was administered to saline-treated rats and the products of digestion were collected for 2 hr through a tube placed approximately 1 cm distal to the ligament of Treitz. The distribution of the radioactivity in pooled predigested fat from 10 animals with duodenal

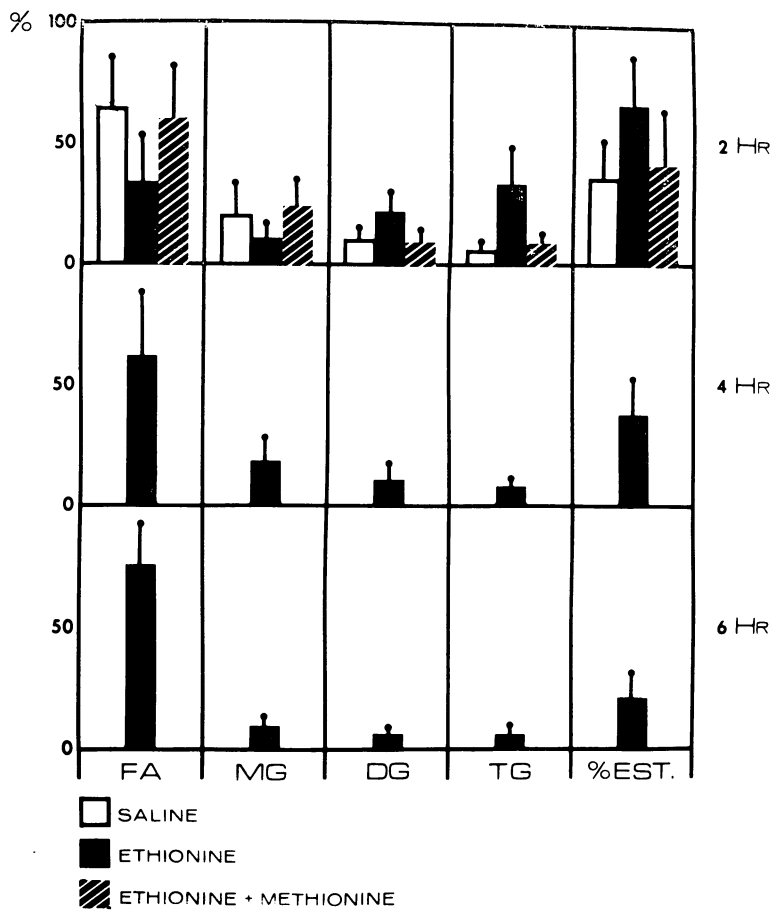

FIGURE 2 Effect on intraluminal lipolysis in the small intestine. Lipid fractions from extracts of intraluminal contents were separated by TLC. Radioactivity in each fraction is expresssed as per cent of the radioactivity of the total lipid extract. At 4 and 6 hr the radioactivity of the lipid fractions of the control animals was too low for assay. FA = fatty acids; MG = monoglyceride; DG = diglyceride; TG = triglyceride; % est. (% esterified) = % radioactivity in MG, DG, and TG.

TABLE IV

Effect on Uptake and Transport of Palmitic Acid-1-^{14}C by the Small Intestine

Treatment	Time	Recovered in lumen	Recovered in wall	Uptake*	Transported*
	hr		*% of radioactivity delivered from stomach*		
Saline	2	17.1 ±3.5	4.1 ±1.5	81.6 ±4.6	77.5 ±4.8
(6)	6	4.4 ±1.6	1.2 ±0.7	94.5 ±4.3	93.3 ±5.8
Ethionine	2	27.0 ±3.2	21.3 ±3.2	66.8 ±4.8	45.5 ±8.7
(5)	6	9.8 ±0.9	28.4 ±3.1	86.7 ±6.3	58.3 ±6.0
Ethionine + methionine	2	16.8 ±3.8	2.8 ±1.2	80.6 ±3.6	77.8 ±3.9
(6)	6	4.2 ±1.9	2.3 ±1.2	95.7 ±4.0	93.4 ±6.5

* Calculated as in Table II.

fistulas was similar to that of the luminal washings from the control animals of the previous experiments (Fig. 2). Aliquots of pooled predigested fat were administered intragastrically to six ethionine-treated rats and the experiment continued as described in Methods. The results were analagous to those obtained in the previous experiments with ethionine-treated animals (Tables I–IV). The possibility that substances inhibiting uptake and transport of luminal radioactivity by the wall of the small intestine could have been present in duodenal or biliary secretions of the ethionine-treated rats was excluded by administering predigested fat obtained from ethionine-treated rats with duodenal fistulas to control animals. These observations indicate that alterations in biliary and pancreatic secretions could not have been of major importance in inducing the observed impairment of fat uptake and transport in the ethionine-treated rats.

Effect on distribution of tripalmitin and palmitic acid radioactivity in small intestinal wall lipids. In Table V it is seen that ethionine altered significantly the distribution of the radioactivity in the lipid classes of the intestinal wall. The effect of ethionine was most pronounced on the incorporation of the label into TG of the wall of the small intestine. Reesterification of FA was significantly inhibited throughout the experiment, but it was most pronounced during the 1st 2 hr.

Fig. 3 shows a direct linear relationship between the degree of impairment of reesterification and the degree of inhibition of transport of wall radioactivity. This is in agreement with the present evidence that reesterification of intramucosal long-chain FA precedes their incorporation into chylomicrons and transport to the lacteals.

In Table V it is seen, however, that in the ethionine-treated rats a considerable fraction of the wall radioactivity was found in the form of TG. This would indi-

TABLE V

Effect on Distribution of Tripalmitin and Palmitic Acid Radioactivity in the Wall of the Small Intestine

Treatment	Time	MG + PL	DG	FA	TG	CE	Per cent esterified
	hr			*% of wall radioactivity*			
Saline	2*	4.0 ±2.4	14.4 ±6.2	4.2 ±1.8	75.6 ±10.4	1.8 ±0.9	95.8
(15)							
Ethionine + methionine	2*	4.0 ±3.1	14.1 ±5.7	3.8 ±2.1	76.4 ±11.6	1.7 ±0.9	96.2
(15)							
Ethionine	2	2.5 ±1.8	5.1 ±2.4	32.3 ±9.4	58.6 ±10.2	1.5 ±0.7	67.7
(9)	4	3.6 ±2.2	8.4 ±3.9	17.5 ±6.3	69.6 ±9.8	0.9 ±0.4	82.5
	6	4.2 ±2.8	9.1 ±2.9	15.4 ±6.1	70.4 ±10.1	0.9 ±0.4	84.6
Ethionine‡	2	4.1 ±3.3	8.3 ±2.7	20.4 ±7.2	65.3 ±9.9	1.9 ±0.9	79.6
(5)	4	4.0 ±1.9	10.2 ±1.6	12.3 ±3.8	72.5 ±10.3	1.0 ±0.6	87.7

Aliquots of extracts from the intestinal wall were applied on TLC plates. Radioactivity in each fraction is expressed as per cent of the radioactivity of the total lipid extract.

* Radioactivity at 4 and 6 hr was too low for assay.

‡ Palmitic acid-1-^{14}C was administered to these animals. PL = phospholipid; CE = cholesterol ester; rest of abbreviations as in Fig. 2.

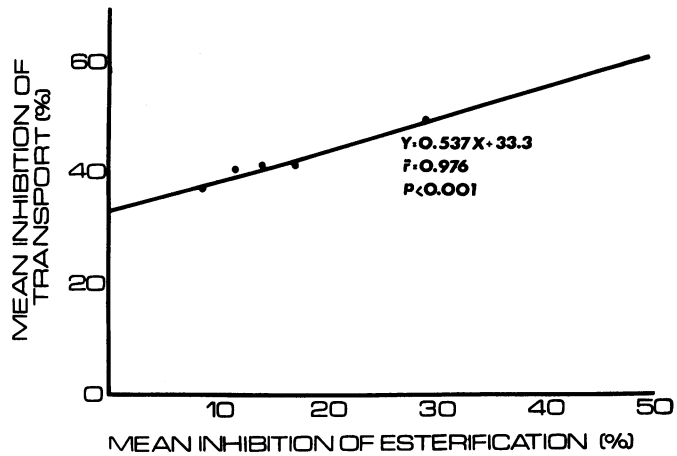

FIGURE 3 Relationship between inhibition of transport of wall radioactivity and inhibition of esterification in ethionine-treated rats.

cate that inhibition of reesterification could not have been the only factor accounting for the impaired transport of wall radioactivity in these animals.

Factors affecting mucosal uptake of intraluminal lipid radioactivity. Since ethionine affected a number of intramuscosal reactions related to the intestinal absorption of fat, attempts were made to determine the effect of these factors on the uptake of intraluminal lipids by the wall of the small intestine.

Fig. 4 *A* shows a direct linear relationship between the degree of inhibition of esterification and the impairment in uptake of radioactivity by the wall of the small intestine. A similar rlationship existed between the degree of inhibition of uptake of luminal radioactivity and the fraction of the radioactivity in the FFA (Fig. 4 *B*) of the intestinal wall. These findings would indicate that the rate of FA esterification and the level of FFA in the intestinal wall are factors which may determine the extent of uptake of luminal lipids by the mucosal cells.

Fig. 4 *C* shows that the inhibition of uptake was also related in a direct linear fashion to the degree of inhibition of transport of wall lipids. This would suggest that the uptake of FA and partial glyceride by the luminal side of the cell and the rate of exit of intramucosal lipid do not proceed independently of each other but are closely related, and alteration of one can affect the other.

DISCUSSION

Administration of a number of protein synthesis inhibitors (1), including ethionine (2), has been shown to inhibit significantly the synthesis of lipoprotein and protein by the intestinal mucosa. This effect was associated with an impaired absorption of intragastrically administered lipids and a marked accumulation of long-chain TG in the intestinal mucosa. The above observations are consistent with the concept that transport of intramucosal TG is dependent upon the intestinal capacity for synthesis of chylomicron apoprotein (1, 3). However, the absorption and transport of dietary lipid is preceded or accompanied by a number of intraluminal and intramucosal reactions (3–6) catalyzed by enzymatic proteins synthesized by the pancreas and by the intestinal epithelium. The inhibitors of protein synthesis can conceivably also inhibit the synthesis of enzymatic proteins, and furthermore, they can also interfere with fat absorption by mechanisms not necessarily related to their effect on protein synthesis. The results reported here indicate that ethionine indeed inhibits a number of the reactions essential for the absorption and transport of dietary lipid. Although a significant inhibition of intraluminal lipolysis was observed 2 hr after the administration of emulsified TG (Fig. 2) to the ethionine-treated rats, the subsequent improvement of the lipolysis at 4 and 6 hr, and the results with palmitic acid (Tables III and IV) and predigested TG excluded intraluminal factors as a major cause of the impaired absorption.

The effect on the mucosal phase of fat absorption appears to be of greater importance. Ethionine inhibited markedly the reesterification of FFA radioactivity to TG (Table V). From our results it is impossible to conclude whether the effect of ethionine on reesterification could have been due to decreased activity or rate of synthesis of the esterifying enzymes, or to substrate inhibition by the high levels of intramucosal TG, resulting presumably from impaired synthesis of chylomicron apoprotein (1, 2). However, in experiments with intestinal rings and mucosal homogenates from ethionine-treated rats,[5] we have found a very close correlation between the impaired capacity for reesterifica-

[5] Kessler, J. I., and J. Stein. Unpublished results.

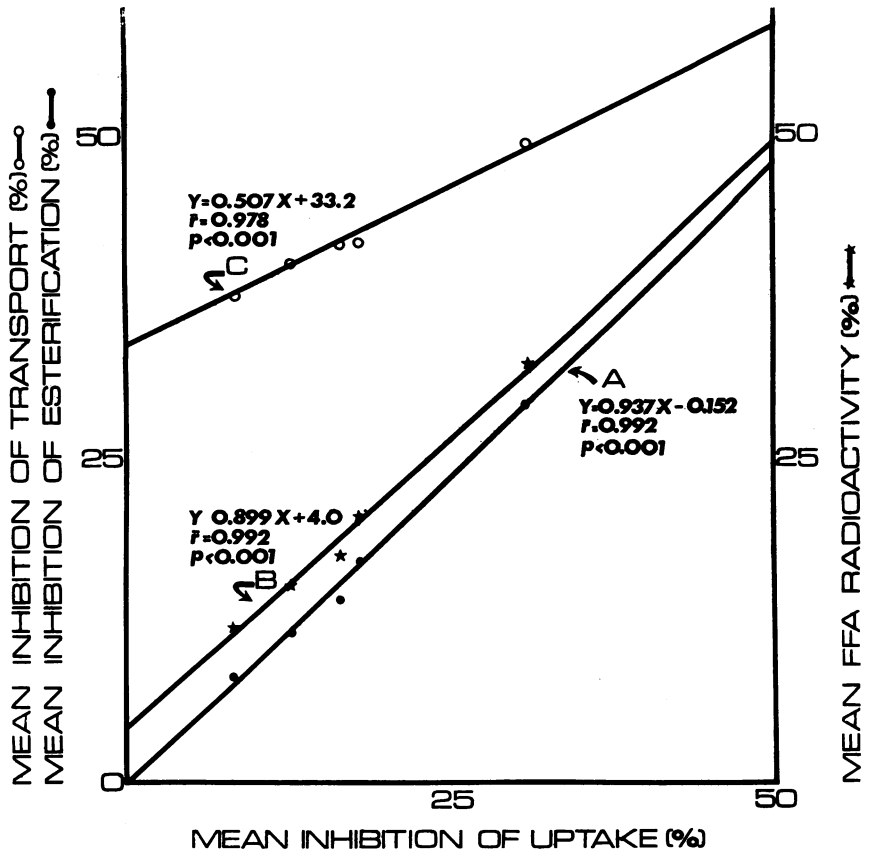

FIGURE 4 Relationship between inhibition of uptake of radioactivity by the intestinal wall and inhibition of esterification (*A*), wall FFA radioactivity (*B*), and inhibition of transport (*C*) in ethionine-treated rats.

tion and the activity of the enzymes essential for TG synthesis, i.e., FA : CoA ligase and mono- and diglyceride acylase. It has been shown (28) that ethionine produces an acute hepatic deficiency of ATP. Since ATP is essential for the acylation reactions of TG synthesis (3–6), the impaired capacity for reesterification could therefore be attributed to a similar effect on the ATP content of intestinal mucosa. However, in a recent study by Hyams et al. (2) no reduction of intestinal ATP levels was demonstrated in ethionine-treated rats. According to available evidence TG synthesis precedes chylomicron formation (1–3), and the effect of ethionine on reesterification could have in part accounted for the observed impairment of transport of intestinal wall lipid. However, in Table V it can be seen that a significant fraction of the radioactivity in the intestinal wall of the ethionine-treated rats was in the form of TG. This would indicate that the high levels of lipid radioactivity in the intestinal wall could be attributed mostly to a block in the transport of newly formed TG. Since transport of intramucosal TG can be accomplished only in the form of chylomicrons (1–3, 10), a major effect of ethionine may therefore be related to an impairment of chylomicron completion, either by inhibition of the synthesis of chylomicron apoprotein or of the association between preformed TG and apoprotein. Recently Hyams et al. (2) have demonstrated a decreased incorporation of L-leucine-^{14}C into protein by jejunal slices of ethionine-treated rats. In similar experiments we have observed a significant inhibition of incorporation of ^{14}C-labeled amino acids into intestinal and medium very low density lipoproteins.[6] Although these results indicate that ethionine may inhibit the synthesis of chylomicron apoprotein, the possibility of impaired association between the lipid and protein moieties or of secretion of chylomicrons cannot be completely excluded.

Ethionine also inhibited the uptake of intraluminal radioactivity by the wall of the small intestine (Tables II and IV). This effect was independent of alterations in the intraluminal factors such as degree of lipolysis, or

[6] Kessler, J. I., J. Stein, and P. Narcessian. Unpublished results.

presence of substance(s) with inhibitory effect on uptake of intraluminal radioactivity. Ethionine inhibited the uptake by the intestinal wall regardless of whether the radioactivity was administered in the form of TG, FA, or predigested TG obtained through a jejunal fistula from saline-treated rats. On the other hand, a significant correlation was found between the degree of inhibition of uptake and the degree of inhibition of mucosal esterification (Fig. 4). This relationship would indicate that the mucosal capacity for TG synthesis and the level of mucosal FFA may be important factors in determining the extent of uptake of intraluminal lipid.

This study does not allow conclusions regarding distribution of labeled FA, partial glycerides, and TG within different compartments of the epithelial cells. It is conceivable that alterations in the equilibrium among FFA, partial glycerides, and TG within different compartments can influence the rate of uptake of luminal lipids. In this respect, the recent observations of Sjostrand and Borgström (29) on the distribution of FA and TG radioactivity within the contents and the smooth-surfaced membranes of the apical vesicles of the mucosal cells are of interest. During earlier stages of fat absorption, a greater fraction of FFA radioactivity was found in smooth-surfaced membranes and in partially filled vesicles than during later stages when over 70% of the radioactivity in the fat-filled apical vesicles was in the form of TG. The authors' conclusion that the enzymes of TG synthesis are located in the smooth-surfaced membranes of the apical vesicles lends support to the possibility that the distribution and the equilibrium between FA and TG in different compartments might depend upon the activity of the esterifying enzymes. Normally, reesterification of long-chain FA is a very active process (5), leading to very low concentrations of FFA in the mucosal cells. This would presumably provide an acceptor "sink" for continuous uptake of luminal FA. The possibility that impaired mucosal reesterification might abolish the effectiveness of such an acceptor "sink" therefore merits further investigation.

In experiments with hamster intestinal rings, Johnston and Borgström (30) have shown, however, a dissociation between the uptake of oleic acid from a micellar solution and the synthesis of TG. Oleic acid was taken up by the intestinal rings even in the presence of effective inhibition of TG synthesis by temperature changes or by a number of metabolic inhibitors. It is difficult to reconcile our findings with those of Johnston and Borgström (30), although differences in the experimental conditions may have accounted for this discrepancy. The possibility that the permeability of the intestinal slices may have been altered by the metabolic inhibitors and by the effects of low (0°C) or high (100°C) temperatures should also be considered. We did not investigate the possibility that ethionine could have affected uptake and penetration of FA and partial glyceride by altering the properties of the epithelial plasma membrane. However, Hyams et al. (2) did not observe any significant electron microscopic changes of the microvilli, mitochondria, or endoplasmic reticulum 9 hr after the administration of ethionine.

In Fig. 4 it is seen that the transport of intramucosal radioactivity was lineraly related to the uptake of luminal radioactivity by the intestinal wall. This would indicate that the rate of exit of intramucosal lipid, i.e., formation and secretion of chylomicrons, and the rate of uptake of FA and partial glyceride by the luminal side of the mucosal cells are interrelated and can affect each other. Our data do not allow conclusions on which of the two phenomena is of primary importance. However, the observation that the mucosal cells of the ethionine-treated rats contained an excess of lipid and the impaired capacity of ethionine-treated rats for incorporation of amino acids into intestinal proteins (2) and very low density lipoproteins[6] would seem to support the possibility that alterations in the rate of exit of intramucosal lipid may be the primary factor in affecting the rate of uptake of luminal FA and partial glyceride. This assumption is supported further by the steatorrhea observed in patients with a-β-lipoproteinemia (10), a condition attributed to a hereditary deficit of chylomicron apoprotein, resulting also in a block in the transport of intramucosal TG to the lacteals and accumulation of TG within the epithelial cells.

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