Inhibition of Protein Synthesis and Absorption of Lipid into Thoracic Duct Lymph of Rats (33653)

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(Introduced by D. B. Zilversmit)
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In mammals long chain fatty acids in the diet are absorbed by the intestinal mucosal cells, resynthesized to triglyceride, and then appear in thoracic duct lymph as chylomicrons. These small fat particles contain about 0.5% protein situated at their surface (1). Labeled amino acids have been shown to be incorporated into chylomicrons during fat absorption (2, 3) and it was assumed that mucosal synthesis of the protein coat is essential to chylomicron formation (4).

The aim of the present experiments was to investigate the effect of actinomycin D and cycloheximide both inhibitors of protein synthesis, on fat absorption into the lymph. By infusing micellar lipid intraduodenally, complicating effects of variable gastric emptying or of defective digestion were avoided. Measuring the amounts of fat in the lymph under steady state conditions rather than in plasma also avoided possible complications of changes in plasma clearance of chylomicron esterified fatty acids or in preexisting fasting plasma lipid levels.

Materials. Actinomycin D was a gift of Merck, Sharp and Dohme, Rahway, New Jersey. A stock solution of 1 mg/ml in 95% ethanol was diluted with 0.9% saline before intravenous injection.

Cycloheximide (Actidione) Calbiochem was dissolved in 0.9% saline, 1 mg/ml, and administered through a chronic jugular venous cannula. Uniformly-labeled (^{14}C) protein hydrolysate, specific activity 640 μCi/mg was obtained from the Radiochemical Centre, Amersham, Bucks, England.

Lymphatic absorption. Male albino rats of a Wistar strain interbred locally for 10 years, weighing 200–220 g were used throughout. Rats were prepared with a thoracic duct fistula, and with intraduodenal and chronic jugular venous cannulas as described previously (5). Postoperatively food was withheld and 150 m*M* NaCl, 4 m*M* KCl was infused steadily by the duodenal cannula at 1.5 ml/hr. Animals were allowed to drink 100 m*M* NaCl, 5 m*M* KCl solution *ad libitum* until the night preceding the absorptive test.

On the morning of the second postoperative day saline infusion was continued for 1 hr while a basal lymph sample was collected, and then infusion of a micellar solution of lipid was begun at 3 ml/hr and continued for 10 hr. After the fifth hour animals were injected via the jugular cannula with the drug solutions. Control rats were injected with dilute ethanol or saline alone. Lymph was collected hourly into graduated heparinized centrifuge tubes. Cells were removed by centrifugation and samples were stored at 4° until analysis. Esterified fatty acid concentration in the lymph was measured by the method of Stern and Shapiro (6).

The micellar solutions contained pure 20 m*M* sodium taurocholate and oleic acid and monoolein in a molar ratio of 3:1 to a final lipid concentration of about 15 m*M*. Their preparation has been described previously (7).

Mucosal protein synthesis. Rats were prepared with intraduodenal and jugular venous cannulae. The next day they were injected with 50 μg of actinomycin D intravenously. Control rats were given dilute alcohol only. After 4 hr 1 ml of a 15% olive oil emulsion was infused over a period of 5 min via the intraduodenal tube. One hr later the upper small intestine was removed under ether anesthesia, washed through three times with ice-cold saline and the mucosa was then extruded with a spatula onto a cold glass plate. The mucosal tissue was divided

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TABLE I. Effect of Actinomycin D and Cycloheximide on Lymph Flow and Lipid Absorption.[a]

Group	Hours:	Lymph flow (ml/hr) 4 and 5	Lymph flow (ml/hr) 9 and 10	Fat output in lymph (μeq of esterified fatty acid/hr) 4 and 5	Fat output in lymph (μeq of esterified fatty acid/hr) 9 and 10
Controls (10)		2.8 ± 0.10	2.0 ± 0.17	54.5 ± 1.88	55.4 ± 2.24
Actinomycin D 50 μg (12)		2.7 ± 0.13	1.3 ± 0.09[b]	50.7 ± 2.10	46.1 ± 3.94
Actinomycin D 200 μg (10)		2.8 ± 0.10	0.2 ± 0.08[b]	59.2 ± 2.37	12.2 ± 1.61[b]
Cycloheximide 300 μg (8)		2.2 ± 0.09	0.4 ± 0.07[b]	59.5 ± 3.22	14.0 ± 3.67[b]
(Saline Infusion) actinomycin D 200 μg (8)		2.7 ± 0.11	1.3 ± 0.20[b,c]		

[a] Results are means ± SE; (n) = number of observations.

[b] Difference between means statistically significant ($p < .001$), compared with hours 4 and 5 in same group; comparison by Students t test.

[c] Compared with control group (line 1) hours 9 and 10 infused with lipid, difference is significant ($p < .05$).

into three portions which were added to 5 ml of Krebs–Ringer bicarbonate solution, pH 7.4, containing glucose (1 mg/ml) in glass stoppered tubes. After addition of 0.2 μCi of (^{14}C) labeled protein hydrolysate to two of the tubes the mucosa was incubated under 95% O_2, 5% CO_2 at 37° for 1 hr. At the end of the incubation period 0.6 mg of an unlabeled amino acid mixture was added, and label was added to the third tube which served as a zero-time control. The tissue was homogenized with a plastic mortar and pestle and aliquots were taken for determination of protein (8). Protein was precipitated from 2 ml of the homogenate with 10 ml of 10% trichloroacetic acid overnight at 4°. The precipitate was washed twice with cold 10% trichloroacetic acid, dried under a stream of nitrogen at 40°, and dissolved in Nuclear Chicago solubilizer. Then 10 ml of toluene scintillant solution containing 4 g/liter of 2,-5-diphenyloxazole and 0.05 g/liter of 1,-4-bis(5-phenyloxazolyl-2)-benzene was added and activity was counted in a Nuclear Chicago Mark I liquid scintillation system. Quench correction was determined by the channels ratio method (9).

Results. In Table I lymph flow and fat output before and after the drugs have been compared. A dose of 50 μg of actinomycin D produced no change in lymph fat output despite a 50% reduction in lymph flow. The pattern of these changes is apparent in Fig. 1. The second hour after injection shows a steep decline in flow compared with the rate before injection of the drug. This is compensated by a progessive rise in fat concentration in the lymph and so total fat output is maintained at about preinjection levels. The same dose of actinomycin D inhibited incorporation of labeled amino acids into mucosal protein by about 75% (Table II).

Fat output in the lymph decreased markedly after administration of 300 μg of cycloheximide or a larger dose of actinomycin D, 200 μg (Table I, Figs. 2 and 3). As shown in Fig. 2 and 3, a fairly steady output of ester fat in the lymph was obtained in hours 4 and 5; and in Table I, the output in these hours is compared with the output in hours 9 and 10, i.e., 4 and 5 hr after adminis-

TABLE II. Incorporation of (^{14}C)-labeled Amino Acids into Mucosal Protein.

	Net incorporation[a] (dpm/mg of mucosal protein)
Actinomycin 50 μg	86.1 ± 18.8
Control	371 ± 80.9
	$p < .01$

[a] Net incorporation is radioactivity recovered in trichloroacetic-precipitable protein after incubation of mucosal tissue with (^{14}C)-labeled chlorella hydrolysate corrected for activity in zero-time control incubations. Results are means ± SE; there were 6 observations of 3 rats in each group.

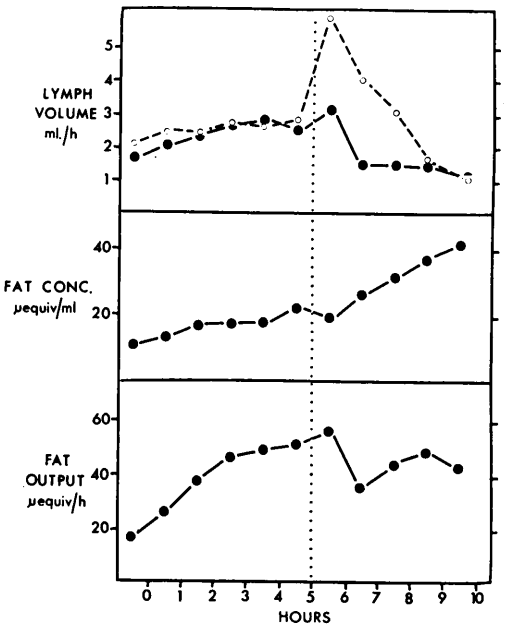

FIG. 1. Lipid absorption and lymph flow after actinomycin D, 50 μg; and lymph flow after actinomycin D, 200 μg: (●) actinomycin D, 50 μg, given at end of hour 5 during infusion of micellar lipid; (○) actinomycin D, 200 μg, given at end of hour 5 during infusion of saline only. The smaller dose of actinomycin D did not impair fat recovery in the lymph, although lymph flow declined, and concentration in the lymph increased. The changes during absorption were much less marked than after 200 μg (Fig. 3). Actinomycin D, 200 μg, given during infusion of saline rather than micellar lipid produced an initial sharp increase in flow, and then flow decreased but not as markedly as during infusion of micelles (Fig. 3).

tration of the drugs. Each rat was its own control.

The reduction in lymph flow seen after 50 μg of actinomycin was more obvious after a larger dose. However, now the increase in concentration of fat in the lymph was not sufficient to compensate the reduction in flow so hourly output decreased. Actinomycin D, in the absence of fat absorption, produced a similar effect on lymph flow (Table I, line 5). After cycloheximide the fat concentration in the lymph remained constant and fat output paralleled the reduction in flow. Transient increases in flow following injection appeared to be due to ethanol in the vehicle. Similar changes were seen in control rats receiving ethanol but no actinomycin D (Fig. 3).

Discussion. Many factors can cause malabsorption of long chain triglyceride. These include alterations in gastric emptying and intestinal motility, in bile salt and pancreatic lipase secretion, in mucosal function and in chylomicron formation and transport into the lymphatics. Also alterations in circulatory hemodynamics might influence fat absorption by affecting the microcirculation of the mucosa and intestinal lymph production.

Drugs which inhibit protein synthesis produce alterations in many of these factors. Gastric emptying is known to be impaired by ethionine (10) and in the present experiments gastric dilatation was apparent after actinomycin D. Pancreatic damage and defective lipolysis has been reported after inhibition of protein synthesis with ethionine (11–13). Impairment of incorporation of labeled amino acids into chylomicrons and oth-

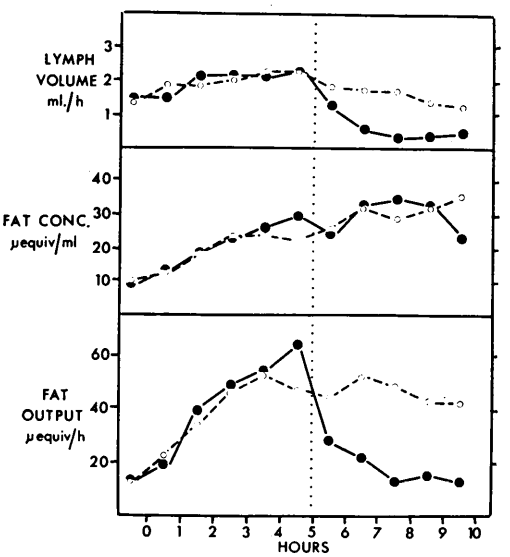

FIG. 2. Lipid absorption and lymph flow after cycloheximide, 300 μg. Infusion of micellar lipid commenced at the end of hour 0: cycloheximide, 300 μg (●); or saline (○); was injected at the end of hour 5. Cycloheximide produced a rapid decline in lymph flow and fat recovery in the lymph, although the concentration of fat in the lymph remained fairly constant, approximating values in the control rats.

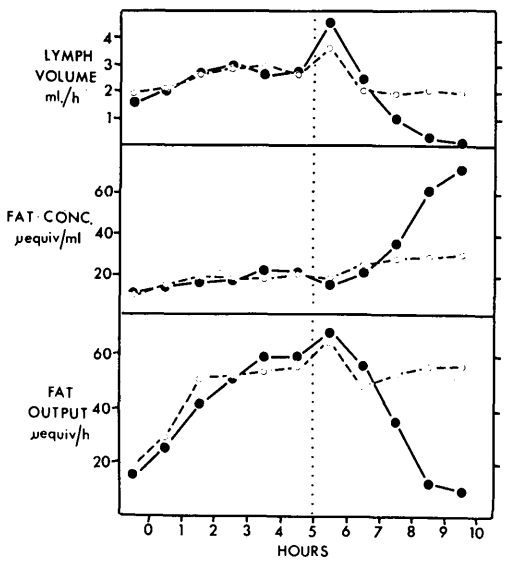

FIG. 3. Lipid absorption and lymph flow after actinomycin D, 200 μg. Infusion of micellar lipid commenced at the end of hour 0: actinomycin D, 200 μg (●); or saline–ethanol only (○); was injected at the end of hour 5. Actinomycin D produced a marked depression of fat output in the lymph. This was associated with an increased concentration of fat in the lymph. Lymph flow decreased markedly after an initial increase. An increase in flow was also seen after the control injections of saline–ethanol.

er lipoproteins *in vitro* has been demonstrated by several investigators (14, 15). Several agents known to inhibit protein synthesis have been shown to lower blood pressure in rats (16, 17). As well, the levels of plasma lipoprotein and plasma lipids are reduced, and chylomicron clearance is affected by these drugs (13).

Experiments in which triglyceride is introduced into the stomach (4, 18) or into isolated loops of intestine (19, 20) are subject to complicating factors as outlined above, and interpretation of absorptive defects is correspondingly difficult. The present studies were designed to avoid complications of variable gastric emptying, defective lipolysis, and changes in plasma clearance of lipids. Also, by studying changes in absorption into the lymph from steadily infused micellar solutions the recovery of fat in the lymph could be correlated with changes in lymph flow. Actinomycin D in a dosage of 50 μg caused suppression of protein synthesis by the mucosa, but this dose did not change fat output in the lymph. A larger dose of actinomycin D, 200 μg, produced a marked decline in lymph flow and associated impairment of fat absorption. However, fat concentration in the lymph was rising which indicated that chylomicron formation and transport were not impaired to the same extent as was lymph formation.

Cycloheximide, 300 μg/rat, produced a marked decline in lymph flow. Again this was accompanied by malabsorption of the infused lipid whereas the concentration of esterified fat in the lymph remained fairly constant, suggesting similarly that the malabsorption was related more directly to reduced lymph flow.

Previous workers suggested that the malabsorption of fat produced by puromycin or acetoxycycloheximide (4) and ethionine (19) was related to inhibition of protein synthesis. In particular, synthesis of β-lipoproteins by mucosal cells was proposed to be involved in chylomicron formation, and parallels were drawn with the absorptive defect present in patients with congenital absence of β-lipoproteins (21). Although β-lipoprotein metabolism was not studied in the present experiments, a dose of actinomycin D which suppressed mucosal protein synthesis did not impair lipid absorption. Only doses large enough to produce marked reduction in lymph flow were associated with malabsorption. Further experiments are needed to investigate the origin and significance of the protein associated with chylomicrons, but the results reported here suggest that this protein might not be essential for chylomicron formation or transport.

Summary. Absorption of micellar lipid into thoracic duct lymph of unanesthetized rats was studied after administration of actinomycin D or cycloheximide, agents known to inhibit protein synthesis. Micellar lipid was infused steadily into the upper small intestine to avoid complications by defective gastric emptying or defective lipolysis. Actinomycin D, 50 μg/rat, inhibited mucosal protein syn-

thesis, but absorption of fat into the lymph was unimpaired after this dosage. Malabsorption produced by larger doses of actinomycin D or cycloheximide was accompanied by a marked decline in lymph flow. The change in lymph flow was closely related to decreased recoveries of lipid in the lymph, and reduced flow appeared to be more important than suppression of mucosal protein synthesis. The results indicate that chylomicron formation is not dependent on normal mucosal protein synthesis.

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