Cholesterol Esterase Activity of Rat Lymph.* (24327)

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Our studies have shown that absorbed cholesterol in thoracic duct lymph of rats is approximately 90% esterified regardless of conditions which alter rate or extent of cholesterol absorption from the intestinal lumen (1-4). Conditions in the intestinal lumen are unfavorable for esterification of cholesterol, and produce a rapid and extensive splitting of dietary cholesterol esters(5,6). The intestinal mucosa has been suggested as the primary site of esterification during cholesterol absorption, since it has been demonstrated that extracts of intestinal mucosa are enzymatically active in the conversion of free cholesterol to the esterified form(7-9). However, based on studies which failed to demonstrate the presence of esterifying activity in the intestinal mucosa, Metzger and Favarger (10) suggested that lymph itself might be active in esterification of dietary cholesterol, after its passage into the intestinal lacteals. If lymph can synthesize or hydrolyze esters of cholesterol, either enzymatically or otherwise, it would be necessary to reevaluate the role of the esterification mechanism in cholesterol absorption. If, however, lymph does not participate in these processes, esterification of cholesterol during intestinal absorption may be attributed directly to the intestinal mucosa. Apparently a critical study of cholesterol esterase activity of lymph has not been reported. It was therefore of interest to investigate the influence of rat thoracic duct lymph on cholesterol partition of lymph itself, and the ability of this medium to catalyze esterification of added cholesterol and oleic acid, or hydrolysis of cholesterol oleate.

Methods. Adult male rats of Carworth Farms strain were employed. Cannulation of the thoracic duct was carried out according to the procedure of Bollman *et al.*(11), with minor modifications, and the animals were subsequently confined to restraining cages with 0.9% saline but no food for duration of lymph collection period. Following fasting from 18-24 hours, the rats were given 3 ml of a cholesterol-containing emulsion(1) by stomach tube and lymph was collected and treated as described below. In the lymph incubation experiments incubation temperature was 37°C, and samples were withdrawn at 0 hours and 24 hours for analysis of free and total cholesterol by the method of Sperry and Webb(12).

Results. In the preliminary study, lymph was collected for 3, 6, or 24 hours in vessels mounted in dry ice, so that the fluid was immediately frozen. The samples were then thawed and divided into 3 fractions. One was immediately extracted with 25 volumes of boiling acetone-alcohol (1:1); a second fraction was refrozen for 24 hours, and the third was incubated for 24 hours in a Dubnoff shaker. These were allowed to reach room temperature prior to extraction and analysis for total and free cholesterol. This experiment failed to show any changes in the lymph cholesterol partition, regardless of volume of the lymph sample collected, or the conditions employed. However, it appeared necessary to extend this study due to the possibility that free and esterified fractions of total lymph cholesterol had attained equilibrium prior to time of collection.

The second study was designed to determine the effect of lymph on esterification of added cholesterol and oleic acid, and on hydrolysis of added cholesterol oleate. Three ml of a saline-albumin emulsion containing 291 mg of oleic acid, 288 mg of sodium taurocholate, and 50 mg of cholesterol were administered to fasted, lymph-fistula rats, and lymph collected for 24 hours. The emulsions prepared for addition to lymph are described in Tables I and II. One ml of the appropriate test emulsion was added to 5 ml of lymph and the digest was thoroughly mixed. Where

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no additions to lymph were made, 1 ml of isotonic saline was substituted for the emulsion. In some digests, the pH was adjusted to 6.2 for the esterification study, and to 6.6 for hydrolysis, by addition of 0.154 molar sodium dihydrogen phosphate. The mixture was then made to 10 ml volume with 0.154 molar phosphate buffer at pH 6.2, or pH 6.6.

The data on esterifying activity of rat lymph on added cholesterol, oleic acid, and sodium taurocholate, are summarized in Table I. Incubation of lymph alone did not significantly alter the ratio of free and esterified cholesterol (Digest 1), nor did rat lymph cause esterification of added cholesterol when included alone, with oleic acid, or with oleic acid and sodium taurocholate (Digests 3-5). To check the effect of pH, lymph alone and lymph containing added cholesterol, oleic acid. and taurocholate were adjusted to pH 6.2. However, this alteration also did not result in cholesterol esterase activity in lymph.

A comparable study on hydrolytic activity of rat lymph is described in Table II. As in the previous study, no significant changes occurred after incubation of lymph alone (Digest 1), with added cholesterol oleate (Digest 2), or with cholesterol oleate and taurocholate (Digest 3). Adjustment of the pH to 6.6 also failed to result in any change of cholesterol partition in lymph (Digest 4).

Under our conditions, it is evident that rat thoracic duct lymph is ineffective in promoting synthesis or hydrolysis of esterified cholesterol, either of endogenous origin, or of that included in the incubation mixtures. These results therefore do not support the suggestion that lymph is responsible for cholesterol esterification following absorption from the intestinal tract(10). In addition, since the conditions in the intestinal lumen do not favor esterification of dietary cholesterol, it appears that the intestinal mucosa is the active site for esterification of absorbed cholesterol.

TABLE I. Synthetic Cholesterol Esterase Activity of Lymph.

Digest No.	Additions to lymph*: Cholesterol, mg	Oleic acid, mg	Sodium taurocholate, mg	Cholesterol esterified per 24 hr, %
1				−3.2
2†				.0
3	5			1.3
4	"	10.8		1.1
5	"	"	14.4	1.6
6†	"	"	"	1.1
7‡	"	"	"	3.9
8§	"	"	"	.0

* Additions were emulsified in 1 ml of isotonic saline containing 5 mg of albumin.
† pH adjusted to 6.2.
‡ 5 ml of isotonic saline substituted for lymph.
§ Lymph was heated to 80°C for 10 min. prior to use.

TABLE II. Hydrolytic Cholesterol Esterase Activity of Lymph.

Digest No.	Additions to lymph*: Cholesterol oleate, mg	Sodium taurocholate, mg	Oleate hydrolysis/24 hr, %
1			−2.0
2	8.4		−2.9
3	"	14.4	.0
4†	"	"	.0
5‡	"	"	.0
6§	"	"	−3.2

* Additions were emulsified in 1 ml of isotonic saline containing 5 mg of albumin.
† pH adjusted to 6.6.
‡ 5 ml of isotonic saline substituted for lymph.
§ Lymph was heated to 80°C for 10 min. prior to use.

Summary. Incubation of rat thoracic duct lymph at 37°C did not affect lymph cholesterol partition. Lymph also failed to cause esterification of added cholesterol when included alone, or with oleic acid and sodium taurocholate. Likewise, there was a lack of hydrolysis of added cholesterol oleate, with or without sodium taurocholate inclusion. Adjustment of the pH of incubation digests containing cholesterol or cholesterol oleate did not result in changes in the ratio of free and esterified cholesterol. From the present data it is apparent that rat thoracic duct lymph does not possess synthetic or hydrolytic cholesterol esterase activity.

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Comparison of Effect of Reserpine and Syrosingopine on Gastric Acid Secretion. (24328)

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In the search for Rauwolfia derivatives preserving the hypotensive action but free of the undesirable side effects of reserpine, a new alkaloid, carbethoxysyringoyl methylreserpate, (Syrosingopine, Ciba) has been prepared.* The gastric acid-stimulating action of this compound was compared with that of reserpine.

Methods. The effects of oral and parenteral administration of the drugs in humans were tested by measuring volume and acidity of continuously-aspirated gastric contents at hourly intervals. The subjects were known or suspected patients with peptic ulcer, fully ambulated and with no complaints at time of study. Output of acid in meq was calculated by multiplying volume of secretion by concentration of free acid. The control period before administration of drug was one or 2 hours; when a 2 hour control period was obtained, the higher of the 2 hours was taken as the basal secretion. Since average basal secretion varied considerably from one group of patients to another, post-drug output was recorded as increments over basal, as well as absolute values.

Results. Ordinary therapeutic doses of reserpine orally, as used in the management of hypertension (0.25 to 0.5 mg), stimulate secretion of HCl to a mild extent; higher doses, both oral and parenteral, induce a very definite hypersecretion of acid. The excellent absorption on oral administration is indicated by the similarity in acid secretory response after oral and parenteral administration.

Syrosingopine in oral doses of 0.5 to 1 mg stimulates less acid than does reserpine. Intramuscularly, reserpine stimulates more acid secretion than does an equal dose of Syrosingopine by the same route.

TABLE I. HCl Output in Gastric Juice after Reserpine and Syrosingopine.

			HCl, meq/hr				
				Hr after			
	Dose, mg	No. tests	Hr before	1	2	3	4
Oral							
Reserpine	.5	9	1.8	2.9	2.8	2.4	3.1
"	1.0	7	1.2	2.9	4.2	6.1	5.3
Syrosingopine	.5	6	1.4	2.0	1.8	1.0	3.1
"	1.0	10	2.5	4.0	3.2	3.4	3.5
Intramusc.							
Reserpine	1.0	6*	.8	1.8	3.4	7.6	8.2
Syrosingopine	1.0	6*	.8	1.3	1.6	2.5	3.7

* Same subjects.

* Supplied by Ciba Pharmaceutical Co.