Composition of Lymph Cholesterol Ester Fatty Acids after Feeding of Cholesterol and Oleic Acid.* (25707)

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It has been shown(1) that administration of several different fatty acids will increase degree of absorption of administered cholesterol. However, very little data are available on extent to which administered fatty acid appears in cholesterol ester fatty acids of lymph. Clement and Mead(2) reported that following feeding of cholesterol and C^{14}-oleic acid, administered oleic acid in lymph sterol esters was diluted with endogenous oleic, linoleic and stearic acids. It has been shown(3,4) that free cholesterol, after entering mucosa from the lumen, is mixed with free cholesterol pool prior to its esterification and transfer to lymph. Considerable dilution of fed cholesterol-4-C^{14} with endogenous sterol occurred in its transfer from lumen to lymph. If there occurs an endogenous dilution of administered cholesterol during absorption, it also appears likely that the accompanying administered fatty acid would also be diluted with endogenous fatty acid in the mucosa prior to esterification and transfer to the lymph. The present study was undertaken to determine effect of feeding cholesterol and oleic acid on composition of cholesterol ester fatty acid(s) (CEFA) of lymph. For comparative purposes CEFA composition of fasting lymph was also determined.

Methods and materials. Rats with thoracic lymph fistulas were prepared and given saline to drink(3). Twenty-four hours after operation each animal of one group (6 rats) received, by gastric intubation without anesthesia, 3 ml of aqueous emulsion containing 40 mg cholesterol, 292 mg oleic acid, 279 mg sodium taurocholate, 50 mg blood albumin and 150 mg glucose. Lymph was collected from 0-24 hours following administration of test meal. Lymph was also collected from 0-24 hours in a comparable fasting control group (6 animals). Lipid extracts of individual lymph samples were prepared as described earlier(3,4). Free and total cholesterol of each extract were determined by the method of Sperry and Webb(5). Cholesterol esters were separated from other lipid components by chromatography on silicic acid. The isolated cholesterol esters were interesterified in HCl-methanol and the methyl esters were sublimed according to procedure of Stoffel *et al.*(6). Gas-liquid chromatography was carried out as previously described(7) using a succinate polyester of diethylene glycol as the stationary phase(8).

Results. The data on lymph CEFA of both groups of animals are given in Table I. A wide spectrum of CEFA was found in fasting lymph. The major fatty acids present were palmitic, linoleic and oleic acids; those acids comprised 77.1% of the total CEFA. The presence of arachidonic acid (7.2%) is also of interest.

Following feeding of test meal containing cholesterol and oleic acid considerable changes in the other CEFA besides oleic acid occurred in lymph. The oleic acid fraction increased from 16.1 to 42.3%, but in terms of total amount of fatty acid the increase was 6-fold. There were decreases in percentages of palmitic, linoleic and linolenic acids, but on a weight basis there was a slight increase in palmitic acid, while linoleic and linolenic acids remained the same. There were also increases in absolute amounts of the other fatty acids of the CEFA fraction of lymph, the most notable being a 2-fold increase in arachidonic acid level.

Discussion. These results confirmed and extended earlier observations by Clement and Mead(2). Fasting rat lymph contains a wide spectrum of CEFA and it is of interest that a considerable portion (36%) of total CEFA is present as polyunsaturated fatty acids. Comparison of the CEFA composition of fasting

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TABLE I. Cholesterol Ester Fatty Acid Composition of Lymph of Fasted and Cholesterol-Oleic Acid Fed Rats.*

Fatty acid†		% total fatty acids		mg fatty acid/24 hr lymph sample‡	
Chain length carbons	No. double bonds	Fasting	Cholesterol-oleic acid	Fasting	Cholesterol-oleic acid
6 to 12		.5 ± .2§	1.1 ± .4	.03	.12
14	0	.5 ± .2	1.1 ± .3	.03	.12
14	1	.7 ± .2	.6 ± .3	.04	.07
16	0	34.4 ± 3.1	22.3 ± 8.9	1.79	2.52
16	1	2.7 ± .4	7.5 ± 1.8	.14	.85
18	0	9.0 ± 2.4	7.0 ± 1.3	.47	.79
18	1	16.1 ± 2.8	42.3 ± 5.4	.84	4.77
18	2	26.6 ± 2.6	11.6 ± 3.2	1.38	1.31
18	3	2.1 ± 1.7	.7 ± .7	.11	.08
20	4	7.2 ± 2.6	5.6 ± 2.3	.37	.63
20	5	.2 ± .2	tr	.01	tr
Total fatty acid				5.21	11.26

* Values represent avg of 6 animals; lymph collection period 24 hr.

† Represents major fatty acids found; very small amounts of others were also detected.

‡ The mg of individual CEFA in lymph were calculated from total CEFA content of lymph (derived from lymph cholesterol ester levels) and percentage of individual fatty acids.

§ Stand. dev.

lymph with that of fasting rat serum† indicates that they are markedly different. Rat serum CEFA consist predominantly of polyunsaturated fatty acids (70%) with arachidonic acid comprising 50% of the total.

The data clearly show that, when oleic acid was fed, other CEFA than oleic acid changed in amount and in percentage of total acids. If the fed cholesterol had been simply esterified with the dietary oleic acid then amount of cholesterol oleate appearing in lymph should have been substantially greater than that observed. The data are in agreement with the concept that both exogenous cholesterol and fatty acids are mixed with mucosal pools of these substances prior to esterification and transfer to lymph(9). Under these circumstances the composition of lymph CEFA would be a reflection of the composition of the mucosal fatty acid pool after entrance and mixing of the fed oleic acid in the mucosa.

Summary. Fasting lymph and lymph following feeding of a mixture containing cholesterol and oleic acid were analyzed for their CEFA composition by gas-liquid chromatography. The major fatty acids of fasting lymph were palmitic, linoleic and oleic acids, with polyunsaturated fatty acids comprising 36% of total CEFA. After feeding oleic acid only 42.3% of total CEFA was present as oleic acid. Our results support the concept that CEFA composition is not determined solely by dietary fatty acid, but by the composition of the fatty acid pool in the mucosa from which fatty acids are drawn for esterification of cholesterol.

† Unpublished observations.

1. Vahouny, G. V., Treadwell, C. R., *Am. J. Physiol.,* 1959, v196, 881.

2. Clement, G. E., Mead, J. F., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 285.

3. Swell, L., Trout, E. C., Jr., Hopper, J. R., Field, H., Jr., Treadwell, C. R., *J. Biol. Chem.,* 1958, v232, 1.

4. ———, *ibid.,* 1958, v233, 49.

5. Sperry, W. M., Webb, M., *ibid.,* 1950, v187, 97.

6. Stoffel, W., Chu, F., Ahrens, E. H., Jr., *Anal. Chem.,* 1959, v31, 307.

7. Swell, L., Field, H., Jr., Schools, P. E., Jr., Treadwell, C. R., PROC. SOC. EXP. BIOL. AND MED., in press.

8. Lipsky, S. R., Landowne, R. A., Godet, M. R., *Biochim. Biophys. Acta,* 1959, v31, 336.

9. Swell, L., Trout, E. C., Jr., Hopper, J. R., Field, H., Jr., Treadwell, C. R., *Ann. N. Y. Acad. Sc.,* 1959, v72, 813.

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