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Lipide Changes in Chylomicra and Subnatant Fractions of Rat Lymph During Cholesterol Absorption.* (24473)

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The effects of sodium taurocholate, oleic acid, and albumin, singly and in combinations, on absorption of exogenous and endogenous cholesterol in the rat were reported by Vahouny *et al.*(1,2,3). It was shown that lipide composition of lymph during absorption was related to composition of the test emulsion administered. Laurell(4) has reported the percentage distribution of lipides in chylomicra and lipoproteins of the lymph of rats which were fed one type of emulsion. Byers and Friedman(5) reported that dietary cholesterol travelled almost completely in the chylomicron fraction in the lymph of rats which were fed cholesterol in olive oil. Jobst and Schetter(6) isolated chylomicrons from chylic ascites and chylic pleural discharge of a patient suffering from Whipple's disease and found no differences in these types of chylomicra. In the experiment described below the lipide changes in the chylomicron and subnatant fractions of the lymph of rats which were administered various test emulsions were determined in order to evaluate more clearly the roles played by chylomicra and lipoproteins in lymph lipide transport.

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Methods. Adult male rats of the Carworth Farms strain, weighing between 175-250 g were employed. Collection of lymph, preparation and intragastric administration of the test emulsions were as described by Vahouny *et al.*(1). The basic emulsion contained 3 ml of isotonic saline solution and 50 mg of albumin. The test emulsions contained, in addition to the basic emulsion, amounts of cholesterol, oleic acid, triolein and sodium taurocholate shown in Table I. Ten groups of 3 or 4 rats each were used and they are numbered in the tables corresponding to the number of the emulsion shown in Table I which they received. Lymph was collected

TABLE I. Composition of Test Emulsions.

	Emulsion No.									
	1	2	3	4	5	6	7	8	9*	10
Albumin, 50 mg	+	+	+	+	+	+	+	+	+	+
Sodium taurocholate, 287.4 mg					+	+	+	+	+	+
Oleic acid, 290.9 mg			+	+			+	+	+	
Triolein, 303.9 mg										+
Cholesterol, 50 mg		+		+		+		+	+	+
.9% saline to 3 ml	+	+	+	+	+	+	+	+	+	+

* Oleic acid was increased to 878.7 mg.

TABLE II. Changes in Neutral Fat and Phospholipide in Lymph Fractions during Absorption.

Group and emulsion	2		3		4		7		8		9		10	
	C*	S	C	S	C	S	C	S	C	S	C	S	C	S
Neutral fat	6	30	37	64	47	54	58	46	80	46	117	74	108	103
Stand. error	±2	±4	±10	±14	±3	±7	±11	±6	±15	±9	±34	±6	±6	±5
"Extra" neut. fat			31	34	41	24	52	16	74	16	111	44	102	73
Phospholipide	1.6	3.2	2.6	8.6	2.3	8.6	3.6	6.6	7.5	7.8	12.2	6.4	5.3	7.7
Stand. error	±.3	±.6	±.8	±2.4	±.8	±.8	±.5	±1.7	±1.7	±.6	±.2	±1.4	±.9	±.9
"Extra" phospholipide			1.0	5.4	.7	5.4	2.	3.4	5.9	4.6	10.6	3.2	3.7	4.5

* C = Chylomicron; S = Subnatant.

All values are in mg/12 hr lymph collection.

Stand. errors of means were calculated using the formula: $S.E. = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

for 12 hrs after administration of the emulsion. From a portion of each 12 hr lymph sample the chylomicron and subnatant fractions were separated at 10,300 g for 1 hour, as described by Lindgren *et al.*(7). The samples of chylomicrons were washed twice with saline of density 1.006. Alcohol-acetone (1-1) extracts of each sample of whole lymph, and of each chylomicron, and subnatant fraction were prepared(1) and analyzed for total lipide(8), free and total cholesterol(9) and phospholipide(1). Neutral fat was calculated by difference(1). The average values for all fractions are given in the tables as mg per 12 hr lymph sample. "Extra" cholesterol, neutral fat, and phospholipide refer to the increase in these fractions over the appropriate control levels. Group 2 was used as control for neutral fat and phospholipide shown in Table II. Group 3 was used as control for Group 4, Group 5 for Group 6, and Group 7 for Groups 8, 9, and 10 in evaluation of changes in cholesterol content (Table III). In all groups the sum of the lipide fractions in the chylomicron and subnatant fractions agreed closely with the levels in the parent 12 hr lymph samples. These lymph data are not included in the tables but they indicated satisfactory recovery of the lipides in the fractions. Emulsion 1 gave the same levels and distribution of lipides in the chylomicron and subnatant fractions as Emulsion 2 and it has been omitted from the tables.

Results. The data on effects of test emulsions on neutral fat and phospholipide of the lymph fractions are shown in Table II. Inclusion of oleic acid alone (Group 3) produced comparable increases in neutral fat in both chylomicron and subnatant fractions. When cholesterol or taurocholate was also included (Groups 4 and 7) with oleic acid the magnitude of the increases in neutral fat was the same as with oleic acid alone but there was a shift in distribution so that the major part of the increase was in the chylomicron fraction. Administration of both cholesterol and taurocholate with oleic acid produced a further increase in lymph neutral fat which was entirely in the chylomicron fraction. Tripling the oleic acid gave additional increments in neutral fat of comparable magnitude in both fractions. Triolein gave twice as much "extra" neutral fat as a comparable amount of oleic acid (Groups 8 and 10).

Relative increases and distribution of phospholipide between the fractions did not follow the pattern for neutral fat. There was a significant and relatively constant increase (3-5 mg) in the subnatant fraction in all groups. There were small insignificant increases in the chylomicron fraction of Groups 3 and 4, those in which highly significant increases occurred in neutral fat. Relative proportions of phospholipide in the 2 fractions were different from neutral fat in Groups 7, 8, 9, and 10.

Changes in cholesterol content of the lymph fractions are shown in Table III. Comparison of Groups 3 and 4 shows that addition of cholesterol to the oleic acid emulsion gave increases in both fractions with 76% of the total increase occurring in the subnatant frac-

TABLE III. Changes in Cholesterol in Lymph Fractions during Absorption.

Group and emulsion	3		4		5		6		7		8		9		10	
	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
Cholesterol	1.4*	3.9	3.1	9.4	.4	5.9	.9	7.6	1.4	3.2	3.9	7.2	8.6	8.6	1.5	5.3
Stand. error	±.2†	±1.3	±.9	±1.9	±.2	±1.0	±.2	±.9	±.3	±.7	±.4	±.9	±.7	±.6	±.9	±2.5
"Extra" cholesterol			1.7	5.5			.5	1.7			2.5	4.0	7.2	5.4	.1	2.1

* All values are in mg/12 hr lymph collection.

† Stand. errors of the means were calculated using the formula: $\text{S.E.} = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

tion. Administration of cholesterol (Group 6) with taurocholate gave a smaller increase than with oleic acid but 77% of the increase was in the subnatant fraction. Likewise in Groups 8 and 10 the major part of the increase was in the subnatant fraction. Only in Group 9 was the increase greater in the chylomicron fraction; here the oleic acid was 3 times that in Group 8. The differences between Group 8 and 10 indicate that triolein gave only one-third the increase in cholesterol given by an equivalent quantity of oleic acid.

The percentage composition of the total lipides of the chylomicron and subnatant fractions is shown in Table IV. It is quite evident that the lipide mixture in the 2 fractions differed for each emulsion and that the chylomicron fraction was different for each emulsion except for Groups 3 and 7. Also there was considerable variation in composition of the subnatant fractions but Groups 2 and 9, 3 and 7, and 4 and 8 were similar. In all chylomicron fractions more than half of the lipide mixture was neutral fat. This was also true for the subnatant fractions except Group 6.

Discussion. The present experiment clearly demonstrates that the increase in lymph lipides which occurs during fat absorption is due to increases in both chylomicron lipides and those of the lipoproteins of the subnatant fractions. Thus the chylomicrons are not the sole means for lymphatic transport of either absorbed fatty acid or cholesterol. In most of the groups the chylomicrons carried the major part of the increase in neutral fat. However, in Group 10, which received a triglyceride, there was 92% more neutral fat in the lymph than in Group 8 which received an equivalent amount of fatty acid but 67% of the increase was carried in the subnatant fraction. Absorbed cholesterol was carried principally in the subnatant fraction except when a relatively large amount of free fatty acid (Group 9) was administered. In agreement with earlier findings the data show that free fatty acid may be a limiting factor in cholesterol absorption and that the fatty acids of triglycerides are not quantitatively equivalent to free fatty acids in promoting cholesterol absorption.

TABLE IV. Lipid Composition of Chylomicron and Subnatant Fractions.

Group and emulsion	2	3	4	5	6	7	8	9	10
	%								
Chylomicron fractions									
Neutral fat	74.4	89.1	86.9	57.9	80.9	91.1	85.2	82.6	93.0
Phospholipide	19.5	6.3	4.2	Trace	Trace	5.6	8.2	8.6	4.6
Free cholesterol	2.5	1.2	1.3	10.5	2.8	.8	1.0	.9	1.5
Cholesterol esters	3.6	3.4	7.6	31.6	16.7	2.5	5.6	7.9	.9
Subnatant fractions									
Neutral fat	75.5	81.8	70.2	61.7	32.5	80.4	70.6	78.9	86.6
Phospholipide	8.8	11.0	11.2	16.5	24.9	11.4	12.1	6.8	6.6
Free cholesterol	3.9	1.5	3.0	4.2	5.6	1.5	2.3	1.7	1.5
Cholesterol esters	11.8	5.7	15.6	17.6	37.0	6.7	15.0	12.6	5.3

Summary. Changes in distribution of neutral fat, phospholipide, and cholesterol between chylomicron and subnatant fractions of rat lymph have been produced by feeding emulsions containing cholesterol, fatty acid, triglyceride, and taurocholate in various combinations. Increases in neutral fat were carried largely in the chylomicron fraction while increases in cholesterol were carried largely in the subnatant fraction. The emulsions also produced changes in percentage composition of the lipide mixture in each fraction.

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Immunological and Enzymological Specificity of Antibodies Produced by Injection of Purified Acetylcholinesterase. (24474)

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This paper is concerned with results of experiments in which an attempt was made to produce specific inhibitory antibodies against a particular type of cholinesterase enzyme. Reports have been published in which specific inhibitory antibodies have been obtained against certain enzymes. Mansour *et al.*(1) immunized roosters with rabbit muscle lactic dehydrogenase and obtained sera which markedly inhibited the activity of this enzyme but had no effect on lactic dehydrogenase of *Schistosoma mansoni* or *Schistosoma japonicum*. Conversely, it was shown(2) that antisera to the lactic dehydrogenase of *Schistosoma mansoni* markedly inhibited the activity of this enzyme and that from *Schistosoma japonicum* and *haematobium* but not the lactic dehydrogenase from rabbit muscle. The antigenicity of a cholinesterase enzyme had been suggested by Holland(3). He reported that sera from rabbits immunized with purified ox erythrocyte cholinesterase inhibited

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