This finding is interpreted as implying that induced lipemia may contribute to, or result in, an *in vivo* hypercoagulable state.

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Received April 10, 1959. P.S.E.B.M., 1959, v101.

Labeling of Intestinal and Lymph Cholesterol After Administration of Tracer Doses of Cholesterol-4-C^{14}.* (25002)

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In earlier studies(1,2) a tentative mechanism of cholesterol absorption was proposed in which it was postulated that transfer of cholesterol from lumen of intestine to lymph involves complete mixing with a pool of free cholesterol in the intestinal wall. Free cholesterol may be drawn from this pool for synthesis of lymph cholesterol esters and as a structural component of chylomicron. The data in those studies were obtained by feeding 40-44 mg cholesterol-4-C^{14} and determining changes taking place in both chemical and radioactive cholesterol fractions in the lumen, intestinal wall, and lymph. Under such conditions there was a marked increase in total lymph cholesterol in comparison with a fasting animal over 24 hour period, and of total cholesterol, 80-90% was esterified. Administration of 1-3 mg cholesterol-4-C^{14} dissolved in oil does not produce a chemical increase in total lymph cholesterol or change in percent esterified cholesterol over feeding of fat alone (3-5). Comparison of data obtained with administration of 40-44 mg cholesterol-4-C^{14} with that obtained by other workers(3-5) using 1-3 mg suggested that the latter amounts should be considered as *tracer* doses which labeled the endogenous cholesterol in the lumen and later the free cholesterol pool of intestinal wall. Thus, appearance of cholesterol-4-C^{14} in lymph appears to be a measure of reabsorption of endogenous cholesterol when fat alone is fed rather than a measure of exogenous cholesterol absorption. In this context, large doses of cholesterol-4-C^{14} employed in our earlier experiments(1,2) would be considered as *absorptive* doses since they increased pool size and turnover rate of free cholesterol in the mucosa and produced increases in levels of lymph cholesterol fractions. To test above suppositions and supply additional data on free cholesterol pool, the effect of feeding a small (tracer) dose of cholesterol-4-C^{14} on size and labeling of free and ester cholesterol pool of intestinal wall and of cholesterol fractions of lymph have been determined.

Methods and materials. Rats with thoracic lymph fistula were prepared and given saline to drink(1). Twenty-four hours after operation each animal received, by gastric intubation without anesthesia, 3 ml of aqueous emulsion containing 50 mg albumin, 150 mg glucose, and 3.27 mg cholesterol-4-C^{14} (0.5 μc) or one containing these constituents plus 292 mg oleic acid and 279 mg sodium taurocholate. Lymph was collected from 0-6 hours following administration of test meal and animals were sacrificed. The intestine was removed, washed, sectioned, and lipid extracts of intestine and lymph were prepared as de-

* This study supported by grants from Am. Heart Assn. and the PHS.

TABLE I. Cholesterol and Cholesterol-4-C^{14} of Lymph and Intestine after Feeding Small Amount of Cholesterol-4-C^{14} to Lymph Fistula Rats.

Tissue†	Cholesterol, mg						Admin. cholesterol-4-C^{14} recovered, %
	Free	Free-C^{14}	Ester	Ester-C^{14}	Total	Total-C^{14}	
			Fasting‡				
Lymph	.79 ± .20*		1.42 ± .35		2.21 ± .19		
			Cholesterol-4-C^{14}				
Lymph	.74 ± .14	.02 ± .00	1.55 ± .30	.08 ± .01	2.29 ± .17	.10 ± .01	3.1 ± 1.0
Small intestine	12.55 ± 2.06	.34 ± .06	1.24 ± .28	.04 ± .00	13.79 ± 2.34	.38 ± .07	11.7 ± 2.8
			Cholesterol-4-C^{14} + oleic acid + sodium taurocholate				
Lymph	1.50 ± .42	.09 ± .02	3.00 ± .70	.30 ± .06	4.50 ± 1.12	.39 ± .08	11.9 ± 2.4
Small intestine	13.05 ± 2.26	.58 ± .14	1.24 ± .23	.09 ± .02	14.29 ± 2.49	.67 ± .16	20.5 ± 3.1

* Stand. error of mean.

† Values are avg of 5 lymph fistula rats killed at 6 hr after test meal. Cholesterol-4-C^{14} administered per rat was 3.27 mg (1×10^5 cpm/mg).

‡ Represents 6 hr fasting lymph sample from 4 rats.

scribed earlier(1,2). Free and total cholesterol of each extract were determined colorimetrically by the method of Sperry and Webb (6). C^{14}-activity of free and esterified cholesterol fractions of all lipid extracts were determined as previously described(1,2).

Results. (Table I). Administration of 3.27 mg cholesterol-4-C^{14} alone did not increase levels of lymph cholesterol fractions above those of fasting control. However, during the 6-hour period, 0.1 mg of labeled material was transferred to lymph and 80% of it was esterified. Addition of sodium taurocholate and oleic acid to cholesterol-4-C^{14} test meal produced a greater transfer of cholesterol-4-C^{14} to lymph (0.39 mg) and a significant increase in lymph cholesterol level. Levels of lymph free and esterified cholesterol fractions were approximately double those of the group fed cholesterol-4-C^{14} alone. The increase in total cholesterol of 2.21 mg (4.50-2.29 mg) was not accounted for by presence of fed cholesterol-4-C^{14} (0.29 mg). Thus, addition of oleic acid and sodium taurocholate to the test meal led to increase of approximately 2 mg of non-radioactive cholesterol in the lymph. Comparison of levels of free and esterified cholesterol in the intestinal wall in the 3 groups indicates that there were no significant increases in either free or esterified fractions upon administration of 3.27 mg cholesterol-4-C^{14} with or without oleic acid and sodium taurocholate. However, in the group which received cholesterol-4-C^{14} alone, 0.38 mg or 11.7% of amount fed, was present in the intestinal wall at end of 6 hours. Approximately 90% of this was present as free cholesterol-4-C^{14}. When sodium taurocholate and oleic acid were added to test meal there was a much greater uptake of cholesterol-4-C^{14} by the intestinal wall (0.67 mg *vs.* 0.38 mg).

Confirmatory information on free cholesterol pool of intestine, the relative endogenous dilution of both small and large doses of fed cholesterol-4-C^{14} were determined (Table II). When a small dose (3.27 mg) of cholesterol-4-C^{14} was fed, it was diluted to considerably greater extent in the intestine and lymph than a large dose (40 mg). Degree of this dilution was related to size of dosage fed. Since the small dose was one-tenth the large dose, it was diluted approximately 10 times in its transfer from lumen to lymph. It is thus suggestive that the relative effects obtained when large and small doses are fed

TABLE II. Endogenous Dilution of Small and Large Doses of Fed Cholesterol-4-C^{14}.

Cholesterol-4-C^{14} admin., mg*	Total cholesterol, cpm/mg		Endogenous dilutions lumen cholesterol-4-C^{14}, No.
	Lumen	Lymph	
3.27	45200	8667	5.20
40	4348	2350	.54

* Values are avg of 5 lymph fistula rats killed at 6 hr after test meal. Test meals also contained 292 mg oleic acid and 279 mg sodium taurocholate.

are related to dosage fed, pool size, and turnover of pool.

Discussion. Our results indicate that feeding a small dose (3.27 mg) of cholesterol-4-C^{14} without sodium taurocholate and oleic acid did not influence size or turnover rate of cholesterol pool of intestinal wall or produce an increase in lymph cholesterol level. Addition of sodium taurocholate and oleic acid to test meal produced an increase in transfer of fed cholesterol-4-C^{14} to the lymph. However, the amount transferred did not account for the observed increase in lymph cholesterol level. This increased amount of cholesterol in lymph may have been derived from increased synthesis of cholesterol in the intestine. It was previously postulated(2) that during fat absorption an increase in cholesterol synthesis in the intestine would occur to support chylomicron and lipoprotein formation.

The data on cholesterol levels and specific activity of the cholesterol in lymph and intestinal wall after feeding small doses of cholesterol-4-C^{14} and the relative dilution of large and small doses are in agreement with the concept that cholesterol entering from the lumen is mixed with a pool of free cholesterol in the mucosa prior to incorporation into the chylomicron.

Summary. Small doses of fed cholesterol-4-C^{14} lead to labeling of cholesterol fractions of mucosa and lymph without increase in level or turnover rate of free cholesterol pool of the mucosa or in amount of cholesterol in lymph. Feeding tracer dose with sodium taurocholate and oleic acid increases the turnover rate of pool which leads to an increased amount of labeled and unlabeled cholesterol in lymph.

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Received April 13, 1959. P.S.E.B.M., 1959, v101.

Propagation of Duck Hepatitis Virus in Tissue Culture.* (25003)

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Virus-induced hepatitis has been described in man(1,2), duck(3-5), dog(6), and mouse (7-9), and agents of the latter 2 have been propagated in tissue culture(10-14). Much interest in comparative viral hepatitis stems from failure to propagate the virus(es) of human hepatitis in the laboratory(15). Since identification of this agent now depends on hazardous exploitation of man, alternate procedures with other host-specific hepatitis agents have been employed as possible prototypes. In this respect, some biological properties of duck hepatitis virus (DHV) have been studied.

Materials and methods. Assay and identification of DHV.† DHV-infected chick embryos were emulsified (10% w/v) in Hank's balanced salt solution (BSS). The preparation was partially clarified by centrifugation at 3,000 rpm for 20 minutes, ampulated, and stored at dry-ice temperature. Assays for DHV were performed by inoculation of 0.1 ml amounts of 10-fold dilutions of test fluids in phosphate buffered saline (pH 7.2) into

* Supported in part by Zelda Zinn Casper Fund and James W. McLaughlin Fellowship Fund.

† Kindly supplied by Dr. L. E. Hanson, Dept. of Veterinary Science, University of Illinois, Urbana.