PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 147, 826-829 (1974)

Sources of Cholesterol in the Intestinal Lymph in Rats Fed a Cholesterol-Free Diet[1] (38448)

KANG-JEY HO AND C. BRUCE TAYLOR

Department of Pathology, University of Alabama in Birmingham, Medical Center, Birmingham, Alabama 35294; and Veterans Administration Hospital, Albany, New York 12208

The importance of the intestine in regulation of body cholesterol metabolism cannot be overemphasized. The intestine is the portal of entry of the exogenous cholesterol into the body, but is also the major route of excretion of body cholesterol. Furthermore, active cholesterol synthesis has been shown to take place in the intestinal mucosa (1, 2). Chevallier and Lutton (3) believed that the intestine is the major site of cholesterol synthesis in the rat.

Newly synthesized cholesterol in the intestinal mucosa can be either excreted into the lumen or transported to the plasma through the lymphatics. Chevallier (4) and our group (5) have shown in rats that approximately two-thirds of the fecal neutral sterols was newly synthesized in the intestine and possibly actively secreted from the mucosa to the lumen (4). Lindsey and Wilson (6) gave direct evidence for a contribution by the intestinal wall to the plasma cholesterol of the rat; however, the quantitative significance of this source of serum cholesterol has not been established. The present study was designed to identify the sources of cholesterol in the intestinal lymph and to evaluate their quantitative significance in a group of rats fed a cholesterol-free diet.

Materials and Methods. Animals. Eighteen adult male albino rats of the Sprague–Dawley strain were used in the experiment. The animals weighed an average of 290 g at the beginning of the study and 350–400 g at the time of sacrifice. Two rats were kept together in one cage in a well-ventilated animal facility.

Diet. The cholesterol-free diet was prepared by General Biochemicals, Laboratory Park, Chagrin Falls, OH, and had the following composition: vitamin-free casein 17.8%, sucrose 49.4%, alphacel cellulose 13.6%, salt mixture (7) 3.4%, vitamin mix AOAC (General Biochemicals) 0.8%, linoleic acid 10.9%, oleic acid 0.3%, and saturated fatty acids 1.1%. The diet and water were given to the animals *ad libitum*.

Experimental design. The animals were fed the cholesterol-free diet throughout the experiment. One month after the commencement of such a dietary regimen, each rat received an intracardiac injection of a single dose of cholesterol-4-^{14}C (4 μCi per rat) which was prepared by mixing the cholesterol-4-^{14}C in a small amount of acetone with the plasma from a donor rat, shaken at 37° for 2 hr.

A state of complete equilibrium of isotope-labeled cholesterol between the plasma and the intestinal mucosa could be attained in 10 days to 2 weeks after the initial administration (5). In order to make sure that the isotope-labeled cholesterol in the plasma was also in equilibrium with that in other organs, the collection of the intestinal lymph was carried out 40–60 days after the injection.

Cannulation of the intestinal lymphatics was done by the method described by Bollman *et al.* (8). Animals were kept in a restraining cage described by Bollman (9). Lymph from the cannula was continuously collected over a period of 6 hr. Eight rats were excluded from the experiment because of the failure of operation or the obstruction of lymph flow soon after the cannulation. Lymph was successfully collected for a 6-hr period in the remaining ten rats.

At the termination of the lymph collection, the animal was killed by exsanguination through the abdominal aorta under ether anesthesia. The liver and the entire length of the gastrointestinal tract were removed from the animal. The small

[1] Supported by U.S. Public Health Service Grant No. HL-13612-05 from the National Heart and Lung Institute and by research funds from Veterans Administration Hospital, Albany, NY.

intestine was divided into three segments of equal length (proximal, middle, and distal). The gastrointestinal tract was opened longitudinally and washed thoroughly with normal saline to eliminate all the luminal contents. The mucosa of the stomach, three segments of the small intestine, cecum, and colon were then separated from the tunica muscularis by scraping with a sharp scalpel.

Chemical analysis. The total cholesterol contents of the plasma, liver, and mucosa of various segments of the gastrointestinal tract were determined by the method of Sperry and Webb (10). Their radioactivity was measured in a liquid-scintillation spectrometer (Packard).

Results. Relative specific activity of cholesterol. The specific activity (SA) of the serum cholesterol varied from animal to animal since they were killed at different periods of time after the injection of a single dose of cholesterol-4-^{14}C. The SA declined with the increase of the time period. In order to make the comparison among the 10 animals possible, the SA of tissue cholesterol relative to that of the plasma, instead of their absolute values, was used for statistical analyses. Such relative SA of cholesterol in the liver, lymph, and mucosa of various segments of the gastrointestinal tract are shown in Table I.

The SA of cholesterol in the liver was identical to that of the plasma cholesterol indicating that hepatic cholesterol was totally exchangeable and in complete equilibrium with plasma cholesterol. The cholesterol in the mucosa of various segments of gastrointestinal tract, however, had a SA less than two-thirds of that of plasma cholesterol (Table I). The average SA of the cholesterol in the lymph was 81.5% that of the plasma cholesterol (Table I).

TABLE I. Relative Specific Activity of Total Cholesterol in Liver, Lymph, and Mucosa of Various Segments of Gastrointestinal Tract as Compared with the Specific Activity of Plasma Total Cholesterol in 10 Rats Under the Study.

	100(%)
Plasma	
Liver	99.3 ± 4.4[a]
Mucosa	
Stomach	62.4 ± 8.6
Small Intestine: proximal third	56.0 ± 9.0
middle third	57.0 ± 9.8
distal third	58.8 ± 8.8
Average	57.2 ± 9.0
Cecum	62.7 ± 6.5
Colon	63.5 ± 6.9
Lymph[b]	81.5 ± 4.3

[a] Mean of 10 rats ± standard deviation.

[b] Lymph collected over a period of six hr before sacrifice.

Sources of cholesterol in the intestinal lymph. The amount of cholesterol in the lymph was 0.49 ± 0.070 μM/hr (mean ± SD) (Table II). Since these rats were fed a cholesterol-free diet, this amount of cholesterol in the lymph was entirely of endogenous origin. The SA of lymph cholesterol was always lower than that of plasma cholesterol (81.5%). The lower SA of lymph cholesterol would appear to be due to the dilution of radioactive cholesterol by the newly synthesized nonradioactive cholesterol from the mucosa. The relative SA of lymph cholesterol indicates that 81.5% of lymph cholesterol was derived from a pool in the intestine totally exchangeable and completely equilibrated with plasma cholesterol and the remaining 18.5% came from cholesterol synthesized *de novo* in the intestinal mucosa.

Similar results could also be obtained by application of simple mathematical proportions when we took the mucosal preparation into consideration. The portion of cholesterol in the intestinal lymph contributed by the cholesterol from the intestine other than the mucosal preparation (X%) could be calculated by the equation: $100X + \text{RSA mucosa}(100 - X) = \text{RSA lymph} \times 100$, where RSA mucosa and RSA lymph are the relative SA of mucosal and lymph cholesterol, respectively. The portion of lymph cholesterol contributed by mucosal cholesterol would then be $(100 - X)$%, which could be further divided into two portions: cholesterol synthesized *de novo* in the intestinal mucosa, and mucosal cholesterol exchangeable with plasma cholesterol. The latter might be considered as in the same pool as plasma cholesterol. The portion of lymph cholesterol synthesized *de novo* in the intestinal mucosa (Y%) could also be calculated by the equation:

$$Y\% = (100 - X)\left(\frac{100 - \text{RSA mucosa}}{100}\right),$$

where X is the percent lymph cholesterol contributed by the exchangeable cholesterol in the intestine other than the mucosal preparation.

Since the cannulated intestinal lymphatics drained only the lymph from the small intestine (8), the mean relative SA of the cholesterol in the small intestine only was used for the above mathematical manipulation. The relative and ab-

TABLE II. Sources of Cholesterol in the Intestinal Lymph.

Sources of cholesterol	μM/6 hr	μM/hr	% of total	
Total cholesterol in the intestinal lymph	2.918 ± 0.419[a]	0.491 ± 0.070	100	
Cholesterol from small intestine other than the mucosal preparation, in equilibrium with plasma cholesterol[b]	1.736 ± 0.541	0.279 ± 0.070	56.6 ± 7.6	
Cholesterol from the mucosal preparation of small intestine				81.5%[c]
Mucosal cholesterol in equilibrium with plasma[b] cholesterol	0.704 ± 0.119	0.119 ± 0.026	24.9 ± 6.2	
Cholesterol synthesized *de novo* in mucosal preparation	0.538 ± 0.085	0.088 ± 0.015	18.5 ± 4.3	
Total cholesterol from mucosal preparation	1.244 ± 0.109	0.207 ± 0.018	43.4 ± 7.6	

[a] Mean of 10 rats ± standard deviation.

[b] The portion of cholesterol freely exchangeable with plasma cholesterol and assumed to have same SA as that of plasma cholesterol.

[c] 81.5% of lymph cholesterol or 0.398 μM/hr which was derived from exchangeable cholesterol in the small intestine to be considered as in the same pool as the plasma cholesterol.

solute quantities of the intestinal lymph cholesterol of various sources are displayed in Table II.

Of the cholesterol in the intestinal lymph 56.6% or 0.279 μM/hr came from the small intestine other than the mucosa which was exchangeable with plasma cholesterol, 18.5% or 0.088 μM/hr synthesized *de novo* in the intestinal mucosa, and 24.9% or 0.119 μM/hr derived from the mucosal cholesterol exchangeable with the plasma cholesterol (Table II).

Discussion. The intestine has been shown to be very active in synthesizing cholesterol and lipoproteins including chylomicrons, very low density, and high density lipoproteins (1, 2, 11–13). The fact that SA of mucosal cholesterol was consistently lower than that of plasma cholesterol was apparently due to the *de novo* synthesized, nonradioactive cholesterol in the mucosa which diluted the radioactive–isotope-labeled cholesterol derived from plasma. Of the total cholesterol in the intestinal mucosa preparation, 42.8% was synthesized *de novo* in the mucosa and 57.2% was in complete equilibrium with plasma cholesterol (Table I).

The newly synthesized cholesterol in the intestinal mucosa was transported in one direction to the lumen and in another direction to the lymph and plasma. Our previous study showed that 77% of the fecal cholesterol in rats fed a cholesterol-free diet was derived from the cholesterol synthesized *de novo* in the intestinal mucosa and that the transport of cholesterol from mucosa to lumen was not merely by desquamation of epithelium but primarily by active secretion (5).

The present study, on the other hand, provided not only evidence for but also quantitative information on the contribution of *de novo* synthesized cholesterol in the intestinal mucosa to the lymph and eventually to the plasma—81.5% or 0.398 μM/hr of lymph cholesterol was derived from the intestinal cholesterol in the same pool as the plasma cholesterol, whereas 18.5% or 0.088 μM/hr was the cholesterol synthesized *de novo* in the intestinal mucosa. If the lymph flow were constant over a period of 24 hr the cholesterol synthesized *de novo* in the small intestinal mucosa would contribute daily 2.11 μM or 0.82 mg to the lymph or plasma. We have found in previous studies that the average daily fecal excretion of neutral sterols in rats was 16.5 mg (14), and 0.4 mg came from bile, 12.7

mg synthesized *de novo* in mucosa and 3.4 mg originated in plasma (5). If this were also true in this group of rats, the amount of plasma cholesterol contributed by mucosal *de novo* synthesis (0.32 mg/day) would account for 22% of its daily turnover (3.4 + 0.4 mg/day). However, the actual contribution of the cholesterol synthesized in the entire gastrointestinal tract to the plasma cholesterol and its daily turnover could be higher than the above values (0.82 mg or 22% of daily turnover) because in this study we only measured the lymph from the small intestine and there are also evidences for the transport of high-density lipoproteins synthesized in the intestine but transported through the portal vein which could not be measured in the lymph (13).

Summary. The intestinal lymphatics were successfully cannulated in 10 adult male Sprague–Dawley rats fed a cholesterol-free diet; 40–60 days prior to cannulation, these animals received an intracardiac injection of a single dose of cholesterol-4-^{14}C. The intestinal lymph was collected over a period of 6 hr. The animals were then sacrificed. The cholesterol and its specific activity in plasma, liver, and mucosa of various segments of gastrointestinal tract were measured and the sources of cholesterol in the intestinal lymph were determined by comparison of their relative specific activities. Of the total cholesterol in the intestinal lymph, 0.491 μM/hr, 81.5% or 0.398 μM/hr was derived from exchangeable cholesterol in the intestine considered to be the same pool as the plasma cholesterol, and 18.5% or 0.088 μM/hr was synthesized *de novo* in the intestinal mucosa. The latter accounted for 22% of the daily turnover of the plasma cholesterol.

The authors express their appreciation to Miss Belma Mikkelson and Miss Janice Drummond for their technical assistance.

1. Srere, P. A., Chaikoff, I. L., Treitman, S. S., and Burstein, L. S., J. Biol. Chem. **182,** 629 (1950).

2. Siperstein, M. D., and Guest, M. J., J. Clin. Invest. **39,** 642 (1960).

3. Chevallier, F., and Lutton, C., Nature **242,** 61 (1973).

4. Chevallier, F., Bull. Soc. Chim. Biol. **42,** 633 (1960).

5. Pang, S. K., Ho, K. J., and Taylor, C. B., Exp. Mol. Pathol. **21,** 138 (1974).

6. Lindsey, C. A., and Wilson, J. D., J. Lipid Res. **6,** 173 (1965).

7. Draper, H. H., Bergan, J. G., Chiu, M., Casallany, A. S., and Board, A. V., J. Nutr. **84,** 395 (1964).

8. Bollman, J. L., Cain, J. C., and Gridlay, J. H., J. Lab. Clin. Med. **33,** 1349 (1948).

9. Bollman, J. L., J. Lab. Clin. Med. **33,** 1348 (1948).

10. Sperry, W. M., and Webb, M., J. Biol. Chem. **187,** 97 (1950).

11. Rodbell, M., Fredrickson, D. S., and Ono, K., J. Biol. Chem. **234,** 567 (1959).

12. Roheim, P. S., Gidez, L. I., and Eder, H. A., J. Clin. Invest. **45,** 297 (1966).

13. Windmueller, H. G., and Sapeth, A. E., J. Lipid Res. **13,** 92 (1972).

14. Pang, S. K., Ho, K. J., Mikkelson, B., and Taylor, C. B., Atherosclerosis **18,** 197 (1973).

Received June 7, 1974. P.S.E.B.M., 1974, Vol. 147.