Intestinal Lymphatic Transport of Absorbed Cholesterol.* (19167)

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One of the many facets of cholesterol metabolism which needs clarification is more detailed information concerning the absorption and transport of ingested cholesterol. A large body of evidence has accumulated to demonstrate that oral cholesterol is partially absorbed. The quantitative aspects of this absorption, however, seem to depend upon such factors as: (1) the experimental animal used; (2) the presence or absence of fat in the diet; (3) the flow of bile; (4) the physical state of the cholesterol administered; and (5) the method of assaying the absorbed cholesterol(1-4).

Bloom and co-workers(5) have demonstrated the importance of the lymph circulation in the transport of absorbed fatty acids. Since fat and cholesterol absorption appear to be linked in some as yet undefined way, it is of importance to know whether or not the transport of absorbed cholesterol occurs via the lymphatics and thoracic duct as well. The investigation to be described herein was designed to determine whether the thoracic duct lymph is an important vehicle for the transport of absorbed cholesterol to the systemic circulation.

Experimental. The intestinal lymph was collected in normal male adult rats (Long Evans) according to the technic described by Bloom *et al.*(5). Briefly, this consisted of the introduction, under ether anesthesia, of a plastic cannula (O.D.: 0.06″) into the abdominal segment of the thoracic duct. A second cannula was fixed in the stomach by a purse string suture. This stomach tube allowed the introduction of the experimental mixtures of fat and tritium-cholesterol. The animals were maintained in restraining cages. In this way, a continuous collection of lymph could be obtained from unanesthetized animals for a period of 48 hours. The rats were allowed to drink 0.4% NaCl solution *ad lib.*

Six rats were selected for the experiment. Rats No. 1 and No. 2, following cannulation of the thoracic duct, were each given 15 mg of tritium labeled cholesterol(6) (SA = 0.61 μc/mg) dissolved in 3 cc of oleic acid through the gastric cannula. The lymph flow from these 2 animals was collected over a 12 hour period. At the end of this time the animals were sacrificed following removal of a blood sample of approximately 8 cc. The lymph collected was subdivided into samples corresponding to time increments of 0 to 2 hours, 2 to 6 hours, and 6 to 12 hours. Rat No. 3 was used as a control animal. A sham operation was performed but the thoracic duct was not intubated. Fifteen mg of cholesterol (SA = 0.61 μc/mg) in 3 cc of oleic acid were administered as above. This animal was killed after 12 hours; a sample of whole blood was obtained. The samples of lymph and whole blood were processed for tritium-cholesterol specific activity as previously described(4,7). Rats No. 4 and No. 5 received 20 mg of tritium-cholesterol (SA = 0.61 μc/mg) in 4 cc of olive oil after cannulation of the thoracic duct. The lymph was collected for 48 hours in two time increments, 0 to 24 hours and 24 to 48 hours. At the end of the 48 hour experimental period the animals were sacrificed. A sample of whole blood and the livers were retained for exami-

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TABLE I.

Rat No.	Wt, g	Sample designation	Cholesterol content	Cholesterol specific activity (μc/mg) $\times 10^{-2}$	μc in total sample cholesterol	% of administered activity in total sample cholesterol
1 2	367 307	Combined (0-2 hr) lymph	.83 mg	2.84	2.36×10^{-2}	.26
1 2		Combined (2-6 hr) lymph	2.48 ''	5.38	.133	.73
1 2		Combined (6-12 hr) lymph	3.25 ''	6.09	.198	1.09
1		Whole blood	73 mg %	N.D.†		
2		'' ''	67 ''	N.D.		
3‡	301	'' ''	81 ''	.50	approx. .113*	approx. 1.24
4	265	(0-24 hr) lymph	9.5 mg	5.98	.569	4.66
		(24-48 hr) ''	5.3 ''	3.46	.184	1.51
		Whole blood	80 mg %	.067	.013*	.11
		Liver	17.5 mg	.076	.013	.11
5	289	(0-24 hr) lymph	5.3 ''	4.61	.246	2.02
		(24-48 hr) ''	3.7 ''	3.29	.121	.99
		Whole blood	73 mg %	N.D.		
		Liver	14.2 mg	.018	.003	.03
6‡	283	Whole blood	66 mg %	1.52	.322*	2.64
		Liver	19.1 mg	1.40	.267	2.19

* Estimated using a total blood volume of 7.5 cc/100 g rat.
† Non-detectable. Measured electrometer drift was less than two times background. Samples contained less than 2×10^{-4} μc/mg.
‡ Control.

nation. Rat No. 6 received only the sham operation. It was given 20 mg of tritium-cholesterol as above, and was killed at the end of 48 hours. The liver and a whole blood sample were preserved for cholesterol specific activity determination.

Results. A summary of the collected data is given in Table I. A total of 2.08% of the administered dose of tritium-cholesterol was recovered in the cholesterol of the intestinal lymph (collected for 12 hours) of rats No. 1 and No. 2; however, no tritium-cholesterol could be demonstrated in the blood of either animal. On the other hand, the blood cholesterol of the control rat (No. 3) at the end of 12 hours had a specific activity of 0.50 x 10^{-2} μc/mg. This activity would account for approximately 1.24% of the administered dose in the circulating blood.

The lymph cholesterol collected during the first 24 hours as well as that collected during the second 24 hours in rats No. 4 and No. 5 showed a high specific activity. The combined lymph collected during the 48 hour period contained 6.17% of the administered tritium-cholesterol in the case of rat No. 4, and 3.01% in the case of rat No. 5. The liver and whole blood cholesterol of these 2 animals contained only insignificant amounts of activity. Control animal No. 6 had tracer cholesterol in the blood and liver after 48 hours to account for approximately 4.83% of the administered dose.

The specific activities of the free and esterified components of the lymph cholesterol (first 24 hours) was determined for rats No. 4 and No. 5. (Rat No. 4: Free cholesterol—SA $= 5.01 \times 10^{-2}$ μc/mg; esterified cholesterol—SA $= 6.80 \times 10^{-2}$ μc/mg. Rat No. 5: Free cholesterol—SA $= 3.74 \times 10^{-2}$ μc/mg; esterified cholesterol—SA $= 5.56 \times 10^{-2}$ μc/mg). The esterified cholesterol contains a significantly higher specific activity than the free cholesterol.

Discussion. In the experimental animals with thoracic duct fistulae the cholesterol recovered from the lymph had a specific activity more than 45 times as great as the specific activity of the serum or liver cholesterol. The intestinal lymph must therefore be quantitatively the most important pathway for the transport of absorbed cholesterol to the serum, and hence to the body organs. Since it has been demonstrated that the blood and liver

contain a large portion of the cholesterol absorbed following a labeled cholesterol meal (4), the overall data may be evaluated to show that at least 90% of the cholesterol absorbed as such during the experimental period studied was recovered in the lymph samples collected from rats No. 1, No. 2, No. 4, and No. 5. In view of the probable presence of anastamotic lymph channels draining lymph into the systemic circulation via routes which would not be shunted by a cannula in the thoracic duct, the data strongly suggest that all food cholesterol may be transported normally via the lymphatics to the systemic circulation.

Only a small fraction of the amount of cholesterol orally administered is absorbed in the intact animal and it has been demonstrated as well that cholesterol absorption occurs slowly over a period of many hours, even days(4,7). Thus, the low percentage of the administered tritium-cholesterol dose which was recovered in the lymph samples, bloods, and livers of the experimental animals was to be expected.

It is noteworthy that the highest specific activity of cholesterol recovered from the lymph, 5.98 x 10^{-2} μc/mg in the case of rat No. 4, is only approximately one-tenth the specific activity of the cholesterol fed. This large dilution must arise primarily from cholesterol of endogenous origin.

Summary. Exogenous cholesterol after its intestinal absorption was found to be conveyed into the systemic circulation by the lymph of the thoracic duct. Little or no transport occurred via the portal venous system.

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Effect of Primaquine on Experimental *Trypanosoma cruzi* Infection.* (19168)

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Since 8 - (isopropylaminoamylamino) - 6 methoxyquinoline, pentaquine or SN 13276, has some activity against *Trypanosoma cruzi* infections in mice(1,2), it seemed desirable to test the chemically related and less toxic 8-(4-amino-1-methylbutylamino)-6 methoxyquinoline, primaquine or SN 13272, against this same trypanosome. This drug has been recently tested against vivax malaria(3). Preliminary successful results in the early stages of *T. cruzi* infection in mice are presented in this paper.

Materials and methods. A highly virulent strain of *T. cruzi* (Tulahuén strain), isolated in 1945 from a human case in the northern part of Chile, was used. Following observations on the genetic constitution of mice in relation to their susceptibility to *T. cruzi* (4), the trypanosomes have been transferred regularly every 10 days for the past year into male mice (about 8 weeks old, weighing 18-21 g) of the genetically pure C3H strain (Jackson Memorial Laboratories). After a standardized intraperitoneal injection of about 200,000 trypanosomes in 0.4 ml citrated mouse blood, trypanosomes appear in the blood in 6 to 8 hours, remain scarce for several days, suddenly increase in number at 4 to 6

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