Blockade of intestinal lipoprotein clearance in rabbits injected with Triton WR 1339–ethyl oleate

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Abstract Although Triton WR 1339 has been used to block triglyceride or cholesterol removal from plasma, no data are available on the extent to which Triton WR 1339 administered to rabbits blocks clearance of newly absorbed dietary lipids. In the present study, we have measured the efficiency of this blockade during a 24-hr interval. After the Triton WR 1339 administration, plasma $S_f > 400$ and $d < 1.019$ g/ml lipoprotein lipid concentrations increased greatly, but the concentration of $d > 1.019$ g/ml lipids decreased. In the rabbits fed 0.5% cholesterol for 1 week, the increase in $d < 1.019$ g/ml and the decrease in $1.019 < d < 1.063$ g/ml lipoprotein fractions 24 hr after the Triton WR 1339 injection were much greater than in the chow-fed Tritonized rabbits. After the Triton treatment, 50% of intravenously injected LDL-^{125}I-labeled apoB disappeared in 24 hr, but little or no apoB appeared in other lipoprotein fractions and no VLDL apoB was converted to LDL. Labeled cholesterol and retinol were fed to rabbits and 24-hr increments in plasma cholesteryl- and retinyl-ester label and mass were measured. In chow-fed Tritonized rabbits about one-half of the absorbed oral doses of both labeled lipids was recovered in plasma, indicating that Triton WR 1339 does not completely inhibit the clearance of intestinal lipoproteins. When rabbits were injected with Triton and an ethyl oleate emulsion, the blockade of dietary lipid removal from plasma was substantially improved and chylomicron cholesterol uptake by extrahepatic tissues was completely abolished.—**Nagata, Y., and D. B. Zilversmit.** Blockade of intestinal lipoprotein clearance in rabbits injected with Triton WR 1339–ethyl oleate. *J. Lipid Res.* 1987. **28:** 684–692.

Supplementary key words chylomicron clearance • dietary lipid • cholesterol • retinol

Since Kellner, Correll, and Ladd (1) showed that the injection of Triton WR 1339 resulted in hyperlipidemia in rabbits, many investigators have used Triton WR 1339 to block the clearance of triglyceride-rich lipoproteins and to measure triglyceride (2–8) and cholesterol (9, 10) secretion by the liver. Triton WR 1339 reduces lipolysis of TG-rich substrates which interferes with VLDL and chylomicron removal (11). Thus, lipoproteins accumulating in plasma of Tritonized animals have been considered as newly secreted lipoproteins from the liver and possibly the intestine. Triton WR 1339 has also been used to study apoprotein metabolism (12–14) and to determine the interaction of lipoproteins with cells (15, 16). Although the effect of Triton WR 1339 on plasma lipids has been studied in different species (17), little information is available on the efficiency with which Triton WR 1339 blocks the clearance of different lipoproteins from plasma in the rabbit. Information on this issue is presented in our study.

Although Otway and Robinson (18) demonstrated that the disappearance of injected radiolabeled chylomicrons from plasma in rats is greatly slowed down after Triton WR 1339 injection, they did not quantitate the degree of blockade induced by Triton. Lipkin, Cooper, and Shipley (19), on the other hand, showed that hepatic triglyceridemia in Tritonized rats may underestimate hepatic triglyceride secretion.

The experiments reported here were part of a series of tests to determine whether one could measure the relative contributions of liver and intestine to plasma lipids of normal and the cholesterol-fed rabbits with Triton. To estimate the 24-hr average secretion rates of cholesterol and triglyceride by intestine and liver from the accumulated plasma lipids after Triton WR 1339 treatment, it is important to determine to what extent Triton WR 1339 inhibits the clearance of lipoproteins over a prolonged period of time. In this study, we have focused primarily on the clearance of intestinal lipoprotein particles from plasma.

It has been shown that the initial transport of chylomicrons can be monitored by the presence of retinyl ester in plasma (20). If chylomicron removal was blocked completely by Triton WR 1339, one would expect to recover all of the absorbed retinol in the total plasma volume. In the present study we have shown that, in the rabbit, Tri-

Abbreviations: TG, triglyceride; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HPLC, high performance liquid chromatography.

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ton WR 1339 partially blocks the removal of dietary lipoprotein particles during a 24-hr period. In confirmation of earlier findings (20), we observed that a significant amount of dietary lipoprotein was removed by non-hepatic tissues. The additional injection of ethyl oleate emulsions produced a nearly complete blockade of dietary particles and abolished their uptake by peripheral tissues.

MATERIALS AND METHODS

Animals and diets

Female New Zealand white rabbits (Beckens Research Animal Farm, Sanborn, NY), weighing 2–3 kg, were caged individually and consumed 100 g/day of Purina Laboratory Rabbit Chow (Ralston Purina, St. Louis, MO). A 0.5% cholesterol diet was prepared by adding 500 mg of cholesterol (U.S.P., Nutritional Biochemicals, Cleveland, OH), dissolved in 2.7 g of Wesson Oil (Hunt Wesson, Fullerton, CA) at 120°C, to 100 g of basal ration.

On the day of the blockade experiment, animals were fed a test meal of 50 or 100 g of chow, supplemented with 2.5 g of oil and 500 mg of cholesterol, with or without 9 mg of all-*trans*-retinol (Eastman Kodak, Rochester, NY). Radioactive retinol, cholesterol, and triolein were also added to some of the diets.

Isotopes

Radioactive lipids were mixed with Wesson Oil, nonradioactive cholesterol and retinol, dissolved in diethyl ether, and added to chow. The solvent was evaporated under vacuum. [1,2(n)-^{3}H]cholesterol (60 Ci/mmol), [4-^{14}C]cholesterol (54.8 mCi/mmol), [1-^{14}C]oleic acid (59.9 mCi/mmol), and glycerol tri[9,10(n)-^{3}H]oleate (470 mCi/mmol) were purchased from Amersham (Arlington Heights, IL). Before use, radiopurity was determined by thin-layer chromatography on silica gel (Type 60, EM Science, Gibbstown, NJ) in hexane–diethyl ether–acetic acid 80:20:1 (v/v/v). All-*trans*-retinyl-11-[^{3}H]acetate (1.41 Ci/mmol) was obtained from SRI International (Menlo Park, CA). Radioactive retinyl acetate was saponified, and radiopurity of the resulting retinol was determined to be more than 96% by HPLC (Waters Associates, Milford, MA) on a Supelco (Bellefonte, PA) C-18 ODS 50 × 4.6 mm column with absolute ethanol as the eluting solvent (0.5 ml/min). Calibrated [^{3}H]- and [^{14}C]toluene (Du Pont NEN Products, Boston, MA) were used as standards to determine counting efficiencies in two channels of an LS 8100 Beckman scintillation counter (Palo Alto, CA). Carrier-free ^{125}I was purchased from Amersham and was counted in a Beckman Gamma 8000 counter.

Plasma volume

In representative animals in each experiment, plasma volumes were determined with intravenously injected albumin (21) (rabbit serum albumin, Miles, Elkhart, IN), iodinated with ICl (22). Radioactivity in plasma at zero-time was calculated by extrapolating the log of plasma radioactivities at 15 and 30 min. The average value of plasma volume for 33 rabbits was 4.20 ± 0.43 (SD) % of body weight.

Triton and ethyl oleate

In experiments where the plasma retention of orally administered lipid doses was tested, Triton WR 1339 (designated in text as Triton; obtained from Ruger Chemical, Irvington, NJ or from Sterling Organics, Rensselaer, NY) was injected intravenously (200 mg/kg body weight) in 0.9% NaCl immediately before feeding the test meals. A 10% ethyl oleate–0.9% NaCl (Nu-Chek-Prep, Elysian, MN) emulsion was prepared by modifications of the method of Packard, Slater, and Shepherd (23). In the beginning, the emulsion was prepared with a Polytron (Type PT 10 OD, Brinkmann, Westbury, NY) at a setting of 6 for 15 min. A dose of 10 ml of emulsion/kg body weight was infused intravenously at approximately 2 ml/min, 28 hr and 4 hr before feeding the test meal lipids. In later experiments, the emulsion was prepared by sonication with a metal probe at 120–130 watts for 15 min (Sonifier cell disruptor, Heat Systems, Melville, LI, NY) and infused (3.3 ml/kg body weight) 4 hr before feeding the labeled lipids and injecting Triton.

Synthesis of labeled ethyl oleate

[^{14}C]oleic acid-labeled ethyl oleate was synthesized from 10 mg (40 μCi) of [1-^{14}C]oleic acid heated at 60°C for 1 hr in 5 ml of 2% H_2SO_4 in absolute ethanol. Ethyl oleate was then extracted into 5 ml of hexane after the addition of 5 ml of H_2O. Radiopurity was determined by thin-layer chromatography as described under Isotopes. Radioactive ethyl oleate emulsions were prepared either by sonication or by Polytron emulsification, and were infused intravenously. Thirty minutes later, the rabbits were killed and radioactivities in plasma and tissues were determined after the extraction of lipid by the method of Thompson et al. (24).

Absorption of lipids

The absorption of cholesterol, retinol, and triolein was measured 24 hr after feeding labeled lipids. The entire gastrointestinal tract and feces were homogenized with water in a 1-gallon capacity Waring blender and a weighed aliquot of the homogenate was combusted in a Biological Material Oxidizer (Harvey Instruments, Hillsdale, NJ). $^{14}CO_2$ and $^{3}H_2O$ were counted in Oxifluor-CO_2 and Oxifluor-H_2O, respectively (Du Pont NEN Products). Lipid absorption was calculated by subtracting the radioactivity in feces and gastrointestinal tract from that in the fed dose.

Blockade factor

To estimate the blockade of intestinal lipoprotein removal from plasma, a blockade factor was calculated from the labeled retinyl ester or cholesteryl ester retained in plasma 24 hr after lipid feeding. The blockade factor for retinol,

(radioactivity of esterified retinol in total plasma volume)/(radioactivity of retinol absorbed from the diet), is based on the observation that essentially all dietary retinol is absorbed from the rabbit intestine in the esterified form (20). The blockade factor for cholesterol, (radioactivity of esterified cholesterol in total plasma volume)/(radioactivity of cholesterol absorbed from the diet × 0.85), is based on the observation that, in the rabbit, 85% of the absorbed cholesterol enters the circulation in the esterified form (20).

Lipid and Triton analyses

Nonpolar lipids in plasma and lipoprotein fractions were extracted with hexane–ethanol according to the method of Thompson, et al. (24). Liver was homogenized with five volumes of 0.9% NaCl and nonpolar lipids were extracted quantitatively as above. The presence of Triton in plasma or lipoprotein fractions did not interfere with the quantitative extraction of cholesteryl and retinyl esters. Aliquots of the extract were separated into unesterified and esterified retinol by HPLC as described under Isotopes. Retinyl palmitate (Sigma, St. Louis, MO) was used as a standard. Eluted unesterified and esterified retinol were dried with N_2, and radioactivity was determined in a liquid scintillation counter (Beckman, LS 8100). Unesterified and esterified cholesterol were separated by thin-layer chromatography as described under Isotopes. Unesterified and esterified cholesterol fractions were eluted from silica gel scrapings with chloroform–methanol 9:1 (v/v). Cholesterol was determined after saponification (25) by the colorimetric method of Zak et al. (26). The concentration of Triton in plasma was measured according to the method of Schurr, Schultz, and Parkinson (3).

Lipoprotein lipase activity

Lipoprotein lipase activity of postheparin plasma was measured by the method of Corey and Zilversmit (27). This assay is insensitive to hepatic lipase. Postheparin plasma was collected 5 min after intravenous injection of heparin (20 U/kg body weight). Heated rabbit serum (1 hr, 56°C) was used as a source of cofactor. To measure the effect of Triton on lipoprotein lipase activity, appropriate quantities of Triton were added to the assay medium.

Labeled LDL

Lipoprotein classes were fractionated by ultracentrifugation according to Hatch and Lees (28). Isolated LDL was iodinated by the method by Bilheimer, Eisenberg, and Levy (29). ^{125}I-labeled LDL was injected into rabbits treated with Triton and specific activities of apoB in VLDL and LDL were determined. ApoB of these lipoproteins was selectively precipitated with butanol–isopropyl ether (30). This procedure was not affected by the presence of Triton and ethyl oleate emulsion.

RESULTS

Triton–lipoprotein interactions in vitro

In all in vivo experiments involving Triton, 200 mg/kg body weight was injected. Triton concentrations in plasma were maintained at approximately 4 mg/ml for 24 hr following a single injection. In order to validate the ultracentrifugal separation procedures, we examined whether the addition of Triton to plasma from cholesterol-fed rabbits, at a concentration of 4 mg/ml, altered the densities of plasma lipoproteins. In one experiment, plasma from a cholesterol-fed rabbit with a total cholesterol concentration of 381 mg/dl was incubated with Triton for 3 hr at 37°C. The percentages of cholesterol in the $d < 1.006$, $1.006 < d < 1.019$, $1.019 < d < 1.063$, and $1.063 < d < 1.21$ g/ml fractions of the plasma incubated without Triton were 38.8, 22.4, 32.2, and 6.6, respectively. The samples incubated with Triton were quite similar: 37.6, 20.5, 36.0, and 5.9% for the respective fractions. No cholesterol was present in the $d > 1.21$ g/ml fraction. The lack of effect of Triton on the densities of lower density lipoproteins has also been observed in normolipidemic monkey (31) and rat plasma (32).

Triton-mediated lipoprotein alterations in vivo

The effects of intravenously administered Triton on lipoprotein cholesterol of rabbit plasma, shown in **Table 1**, are for animals given a test meal of 100 g of Purina rabbit chow containing 2.5 g of Wesson Oil, 500 mg of labeled cholesterol, and 9 mg of labeled retinol just before Triton administration. Two normal rabbits, 24 hr after this test meal, showed a 282 mg/dl increase in plasma cholesterol. Two animals that had been fed a 0.5% cholesterol diet for 7 days prior to this test showed an increase of 514 mg. Because each rabbit received the same amount of cholesterol in the test meal, the large difference probably resulted from increased cholesterol secretion by the livers of the 7-day cholesterol-fed animals.

In the normal rabbits given the above test meal (Table 1), the increase in plasma cholesterol after Triton injection was about equally divided between $S_f > 400$ and the rest of the $d < 1.019$ g/ml fraction. In contrast, the cholesterol concentrations of the LDL ($1.019 < d < 1.063$ g/ml) and HDL ($d > 1.063$ g/ml) fractions decreased to approximately one-third of the zero-time values. In the rabbits that had been fed a 0.5% cholesterol diet for 7 days prior to the experiment, the increase in $S_f > 400$ cholesterol was nearly 1.5 times that for the rest of the $d < 1.019$ g/ml fraction. Simultaneously, the LDL fraction decreased to about one-third of the zero-time value.

The distribution of labeled cholesterol and retinol in various lipoprotein fractions is shown in **Table 2.** These data illustrate that 24 hr after feeding labeled cholesterol and retinol

TABLE 1. The effect of Triton on lipoprotein total cholesterol[a]

Pre-Test Diet	Time	Total Cholesterol				
		Lipoprotein Fraction[b]				Total Plasma
		$S_f > 400$	$d < 1.019$ g/ml	$1.019 < d < 1.063$ g/ml	$d > 1.063$ g/ml	
	hr			*mg/dl*		
Chow	0	2	12	53	26	93
Chow	24	161	186	18	10	375
Difference		+ 159	+ 174	− 35	− 16	+ 282
Cholesterol	0	155	543	327	21	1046
Cholesterol	24	587	838	106	29	1560
Difference		+ 432	+ 295	− 221	+ 8	+ 514

[a]Triton (200 mg/kg) was injected intravenously immediately after the 0-hour plasma sample. Two rabbits were fed 100 g of chow/day. Two rabbits were fed 100 g of 0.5% cholesterol diet for 7 days prior to the 0-hour plasma sample. All animals were fed a test meal of 100 g of chow containing 2.5 g of Wesson Oil, 500 mg of labeled cholesterol, and 9 mg of labeled retinol at 0-hour. At 0-hour, rabbits were 16 hr postprandial. All values are averages of two rabbits.
[b]Ultracentrifuge fractions obtained after centrifugation for 10^6 *g*-min/cm in a Beckman SW 27 rotor ($S_f > 400$) and for 1.58×10^8 *g*-min at d 1.019 g/ml and d 1.063 g/ml sequentially in a Beckman 40.3 rotor. The $d < 1.019$ g/ml column represents $d < 1.019$ g/ml minus $S_f > 400$ cholesterol.

to Tritonized rabbits, more than 95% of both labels in plasma (90% of each present in the esterified form) accumulated in the $S_f > 400$ and $d < 1.019$ g/ml density fractions.

The observation in Table 1 that LDL cholesterol, in both the normal and cholesterol-fed rabbit, decreased markedly within 24 hr after the Triton injection raises the question as to whether this cholesterol had been removed from plasma or had been transferred to the $d < 1.019$ g/ml fraction. This is not easily determined because of the known exchangeability of free and esterified cholesterol in rabbit plasma. Thus we investigated the disappearance of ^{125}I-labeled LDL in Tritonized animals. **Table 3** shows that 24 hr after injecting ^{125}I-labeled LDL from a cholesterol-fed rabbit into three cholesterol-fed recipients, 50% of the LDL apoB had disappeared, with little apparent transfer of apoB to other lipoproteins. In these animals, during the same time interval, 58% of the LDL cholesterol had disappeared, suggesting that the LDL particle had been removed in toto. Table 3 also illustrates that, during the disappearance of LDL radioactivity, the specific activity of LDL apoB remained essentially constant. This suggests that no significant amount of VLDL had been converted to LDL.

Blockade factor

In previous studies (10), Triton was used to block plasma lipid clearance in fasting rabbits. However, data are not available on the degree to which Triton blocks plasma clearance of dietary lipoprotein particles. In **Table 4** we have calculated the 24 hr retention of dietary lipids in plasma after Triton injection. The data show that in a normal rabbit, blockade factors, calculated from absorbed labeled cholesterol, labeled retinol, and retinol mass, agree within 11%. This is representative of the results obtained in subsequent experiments. The close agreement between calculated blockade based on three different measurements suggests that the results based on exchangeable labeled lipids (cholesteryl ester and retinyl ester) are as valid as those based on the increment in lipid mass (retinyl ester).

The agreement between the factors, calculated from labeled retinol and from retinol mass (49.1 vs. 47.1%), implies that the specific activity of the retinyl ester increment in plasma is indistinguishable from the specific activity of the retinol present in the diet. However, this is not the case for the cholesteryl ester increment in plasma chylomicrons,

TABLE 2. Percent distribution of labeled total retinol and cholesterol in plasma lipoproteins 24 hr after feeding labeled lipids to rabbits injected with Triton[a]

Pre-Test Diet	$S_f > 400$		$d < 1.019$ g/ml		$1.019 < d < 1.063$ g/ml		$d > 1.063$ g/ml	
	Retinol	Cholesterol	Retinol	Cholesterol	Retinol	Cholesterol	Retinol	Cholesterol
				%				
Chow	62.0	52.3	35.4	44.0	0.9	2.3	1.7	1.4
Cholesterol	45.0	39.2	49.3	53.9	3.9	5.8	1.9	1.2

[a]These data are from same experiment as Table 1.

TABLE 3. Fate of intravenous ^{125}I-labeled LDL in Tritonized cholesterol-fed rabbits[a]

Time	% of LDL Dose[b]			LDL ApoB Specific Activity
	d < 1.019 g/ml	1.019 < d < 1.063 g/ml	d > 1.063 g/ml	
hr				*(cpm/mg) 10^{-3}*
0.08	4.5 ± 0.96[c]	93 ± 0.8	2.0 ± 0.1	192 ± 17
3	5.6 ± 0.75	75 ± 0.8	1.7 ± 0.07	178 ± 17
12	5.8 ± 0.27	60 ± 0.3	1.7 ± 0.08	211 ± 4
24	5.9 ± 1.03	50 ± 1.0	0.6 ± 0.04	199 ± 33

[a]Three rabbits, fed a 0.5% cholesterol diet for 3 months, were injected with Triton (200 mg/kg). One hour later, ^{125}I-labeled LDL from a cholesterol-fed rabbit (1.7 mg of protein) was injected.
[b]Percent of dose is based on total ^{125}I; in the 0.08-hr 1.019 < d < 1.063 g/ml fraction, 94% of ^{125}I was in apoB.
[c]Mean ± SD.

which showed specific activities ranging from 10 to 40% of those in the cholesterol-supplemented test meal. This lowering of cholesterol specific activities is the result of possible cholesterol exchange and secretion by liver and of exchange with endogenous cholesterol in the intestinal lumen and mucosa, plasma, and other tissue sites. The exchange of labeled cholesterol in the intestinal mucosa not only lowers the specific activity of the absorbed cholesterol, but also delays the appearance of labled cholesterol in plasma. Thus, for a 24 hr experiment (Table 4), the "absorption" of labeled cholesterol, as measured by the difference between the fed dose and that still present in the entire gastrointestinal lumen and wall, was 58% of that for labeled retinol. Some of this difference was probably due to the fact that the absorption was measured during a relatively short period. Although most of the labeled retinol absorbed from the lumen had been transported to plasma, a significant quantity of labeled cholesterol was still residing in the intestinal mucosa, due to exchange of labeled cholesterol molecules from the diet with the large pool of mucosal cholesterol during the absorptive process.

For the calculation of a "blockade factor" we have used the retention of esterified retinol and esterified cholesterol rather than the retention of total retinol and cholesterol in plasma. The nonesterified form of the retinol was not included, because it is normally present in plasma in combination with retinol binding protein (33). This fraction bears little or no relation to newly absorbed lipid. For nonesterified cholesterol, the exchange with other nonesterified cholesterol pools might invalidate the assumption that newly absorbed labeled lipid is retained in plasma in the presence of a blocking agent. Even though we have consistently used the retention of esterified lipids in plasma during 24 hr as a measure of "blockade", it is of interest to note that in 13 normal rabbits injected with blocking agents, the retention of *total* labeled retinol or cholesterol did not differ by more than 6% from that of the percent blockade calculated from the *esterified* labeled lipids.

Triton-ethyl oleate blockade

Six normal rabbits were injected with Triton and fed a test meal containing labeled, low specific activity cholesterol and retinol (**Table 5**). Mean blockade factors, calculated from plasma cholesteryl ester and retinyl ester label or mass, were 59, 53, and 55%, respectively. The close agreement between results from labeled lipids and retinol mass (as in Table 4) suggests again that exchangeability of labeled lipids did not invalidate the calculations. However, the mean degree of blockade was less than 60%.

In three rabbits (not shown) we repeated the blockade test with labeled retinol and labeled triolein, the latter to label the triglyceride of dietary lipoproteins. These rabbits were fed a high-fat test meal containing 20 g of Wesson Oil. The animals fed the extra fat showed about the same amount of labeled retinyl ester in plasma (49 ± 3% of the absorbed dose) as animals with much lower dietary fat loads. The recovery of labeled triglyceride in the same animals was only 30 ± 14% (mean ± SD).

A separate set of experiments was carried out in normal rabbits that were given the test meal and were injected with Triton and with emulsions of ethyl oleate prepared by a Polytron (see Methods). In two experiments of four nor-

TABLE 4. Blockade factor calculated for rabbits fed cholesterol or retinol after Triton injection

Test Meal[a]	Absorption[b]	Label or Mass in Total Plasma[c]	Blockade Factor[d]
	%	*% of dose*	%
Cholesterol (label)	43.3	19.5	53.0
Retinol (label)	74.1	36.4	49.1
Retinol (mass)		34.9	47.1

[a]One normal rabbit was fed a test meal of 100 g of chow, 500 mg of labeled cholesterol, 9 mg of labeled retinol, and 2.5 g of Wesson Oil immediately after intravenous injection of Triton, 200 mg/kg.
[b]Absorption of labeled lipids, measured 24 hr after feeding, by analysis of the gastrointestinal tract plus feces (see Methods).
[c]Plasma volume was calculated by the dilution of intravenously injected ^{125}I-labeled rabbit albumin. Label or mass increment in esterified cholesterol or esterified retinol was divided by the fed dose. Contribution to 24 hr retinyl ester mass increment from retinol and carotene in Purina chow was approximately 800–900 nmol, which is equivalent to about 2–3% of that in the test meal.
[d]For labeled cholesteryl ester in plasma 19.5/(43.3 × 0.85) = 0.53. The factor 0.85 represents the fraction of absorbed labeled cholesterol transported to plasma as cholesteryl ester (20). For retinol this factor is close to unity (20).

TABLE 5. Effect of Triton and ethyl oleate (EO) on blockade factor and recovery of labeled lipids in plasma and liver

Label	Treatment[a]	Pre-Test Diet[b]	Absorption	Blockade factor[c]	Recovery		
					Plasma	Liver	Total
			% of dose			% of absorbed dose	
Cholesterol	Triton	Chow (6)[d]	44 ± 5[e]	59 ± 10	57 ± 9	8.2 ± 1.3	65 ± 10
Cholesterol	Triton + EO	Chow (8)	37 ± 10	92 ± 13[f]	86 ± 13	8.6 ± 1.7	94 ± 14
Cholesterol	EO	Chow (2)	45, 37[g]	32, 30	33, 30	37, 35	70, 65
Cholesterol	Triton + EO	Cholesterol (4)	37 ± 11	70 ± 12	68 ± 11	12 ± 2.1	80 ± 13
Retinol	Triton	Chow (6)	61 ± 11	53 ± 5 55 ± 6[h]	56 ± 5	19 ± 2.1	75 ± 4
Retinol	Triton + EO	Chow (8)	61 ± 12	79 ± 9[f]	85 ± 9	12 ± 1.3	97 ± 10
Retinol	EO	Chow (2)	74, 72[g]	5.3, 7.9	8.2, 11	51, 54	59, 64
Retinol	Triton + EO	Cholesterol (4)	56 ± 5	77 ± 5	80 ± 6	30 ± 5.9	110 ± 5

[a]EO, ethyl oleate (Polytron) 10% emulsion (10 mg/kg) was injected intravenously 28 and 4 hr before the 0-hr plasma sample. Triton (200 mg/kg) was injected intravenously immediately after the 0-hr plasma sample.

[b]Chow, 100 g/day, or cholesterol, 100 g of 0.5% cholesterol diet/day, for 15 days. A test meal was fed at 0-hr (50 g of chow containing 9 mg of labeled retinol, 500 mg of labeled cholesterol, and 2.5 g of Wesson Oil). Animals were fed 50 g of chow 9 hr after the test meal. Top and bottom part of table represent the same animals.

[c]Absorption, blockade factors, and recovery were calculated from 24-hr plasma and liver data and the analysis of the gastrointestinal tract plus feces at 24 hr. Calculations as in Table 4.

[d]Number of animals

[e]Mean ± SD.

[f]Significantly different from animals on the same feeding protocol but receiving Triton only ($P < 0.001$).

[g]Individual values for two animals.

[h]Retinyl ester mass.

mal rabbits each (combined in Table 5), the blockade factors calculated from labeled cholesteryl ester and retinyl ester were 92 and 79%, respectively. This was a marked improvement over the retention after the injection of Triton alone. However, in a third experiment on four normal rabbits (not shown), the blockade factors for labeled cholesteryl ester averaged only 54 ± 8%. In this case, twice the normal amount of ethyl oleate emulsion was prepared by Polytron, which prompted the question as to whether poor emulsification could have reduced the blocking effect of ethyl oleate on chylomicron or chylomicron remnant clearance (see Sonicated ethyl oleate emulsions, below). To ascertain that the observed blockade after Triton plus ethyl oleate injections did not result from the effect of ethyl oleate alone, two rabbits were injected with an ethyl oleate emulsion. The data in Table 5 show blockade factors of only about 30% for cholesteryl ester and less than 10% for retinyl ester. In a group of four rabbits, which were fed cholesterol for 15 days prior to the injection of Triton and ethyl oleate, the blockade factors for cholesteryl ester and for retinyl ester were 70 and 77%, respectively (Table 5).

Lipid recovery in plasma and liver

Because the blockade factors were less than 100%, even in the presence of Triton and ethyl oleate, we assumed that the uptake by liver was not completely eliminated. Table 5 shows the percentages of absorbed labeled cholesterol and retinol in total plasma volume, in liver and their sum as total percent recoveries. The recoveries are reported for total cholesterol and retinol label, rather than for the esterified label employed in the calculation of blockade factors. The percent recovery, therefore, includes the fraction of the absorbed labeled esterified lipid that is hydrolyzed (up to 80% for cholesterol and 30% for retinol in liver). Total recovery of label in plasma plus liver was 65% for cholesterol and 75% for retinol when Triton alone was injected into normal rabbits. This suggests that in Tritonized animals, 25% to 35% of absorbed lipids might have been removed from plasma by extrahepatic tissues. In normal rabbits injected with Triton–ethyl oleate, the total recovery of labeled cholesterol and retinol was close to 100%. The mean value for total labeled cholesterol recovery in four animals fed cholesterol for 15 days and given Triton–ethyl oleate was 80%, and for total labeled retinol, 110%. It seems likely that, at least in normal rabbits, the addition of the ethyl oleate to the Triton injections may have blocked the uptake of labeled cholesterol and retinol by extrahepatic tissues.

Lipoprotein lipase

To account for the relatively poor 24 hr retention of dietary lipoprotein particles produced by Triton and because it is thought that the inhibition of lipoprotein lipase by Triton is responsible for the slow clearance of triglyceride-rich lipoproteins (11), we investigated the degree of inhibition of lipoprotein lipase by Triton. In one experiment, heparin (20 units/kg body weight) was injected intravenously into a normal rabbit and a blood sample was obtained 5 min later. After the addition of about 0.4 mg Triton/ml of assay mixture, which is equivalent to 1/10th the Triton

concentration in plasma from Tritonized rabbits, lipoprotein lipase activity was reduced by 88%.

Sonicated ethyl oleate emulsions

Up to this point in the investigation, ethyl oleate emulsions were prepared with a Polytron homogenizer. Since plasma clearance of emulsions may depend on the particle size (34), we investigated emulsification by probe sonication. As judged by filtration of the emulsions through polycarbonate membranes (Bio-Rad Laboratories, Richmond, CA), two-thirds of the ethyl oleate, emulsified with the Polytron, was retained by a 5-μm filter, whereas most of the particles after sonication passed through a 0.8-μm filter. A sonicated labeled ethyl [^{14}C]oleate emulsion was cleared from plasma with a half-time of 20 min, which was not affected by Triton. Thirty minutes after ethyl [^{14}C]oleate injection, much of the label was present in liver.

With the sonicated emulsions we tried to determine optimal dosages and dosage schedules. Since fatty acid ethyl esters have been shown to suppress phagocytic activity (34, 35), we measured the rate of colloidal carbon clearance as a possible index of ethyl oleate effectiveness (23). This approach was abandoned, because of the large variability in carbon clearance rates. We, therefore, used the increments in total plasma cholesterol of normal rabbits, after injection of standard doses of Triton and variable amounts of sonicated ethyl oleate at different times, as an empirical index of ethyl oleate effectiveness. As a result, we injected, in subsequent experiments, a single dose of 10% ethyl oleate (3.3 ml/kg body weight) followed 4 hr later by the standard dose of Triton. In the tests shown in **Table 6,** normal rabbits received a single test meal of 50 g of chow, containing 500 mg of labeled cholesterol and 2.5 g of Wesson Oil followed by 50 g of chow 9 hr later. After the injection of Triton only, the 24 hr blockade factor for dietary cholesterol in six rabbits was 62%. The injection of sonicated ethyl oleate-Triton in seven additional animals increased the blockade to 87% ($P < 0.05$). The blockade was similar to the blockade previously obtained with the larger dose of ethyl oleate emulsion prepared by Polytron. In contrast to some instances in earlier experiments, none of the animals injected with the lower dose of sonicated ethyl oleate emulsion showed any lethargy or other signs of malaise.

Table 6 also illustrates the recovery of dietary-labeled total cholesterol in plasma and in liver of normal rabbits 28 hr after sonicated ethyl oleate and 24 hr after Triton injection. The absorption of cholesterol during the 24-hr period was relatively low, but did not differ in the Triton and the ethyl oleate-Triton-treated animals. Significantly more of the total cholesterol label was present in the plasma of animals treated with ethyl oleate-Triton, but liver uptake of labeled cholesterol did not differ significantly in the two groups of animals. The recoveries of total labeled cholesterol in plasma and liver were 73% for the animals injected with Triton and 92% for those receiving the additional ethyl oleate injection, a difference significant at $P < 0.05$. These recoveries are similar to values shown in Table 5 for animals receiving the Polytron ethyl oleate preparation at the higher dosage.

DISCUSSION

Lipid accumulating in plasma after Triton injection has been considered as newly secreted, or absorbed, lipid (2-10). Our observation of decreases in LDL and HDL cholesterol, while the total d < 1.019 g/ml cholesterol increased, suggests that Triton blocks the removal of various lipoproteins to different extents. This is compatible with different roles of lipoprotein lipase in the clearance of plasma lipoprotein fractions. The differential effects of Triton on lipoprotein clearance also suggest that secretion rates of lipoprotein lipid after Triton or Triton-ethyl oleate injection should be estimated from increments of newly secreted lipids rather than of total plasma lipids. The fact that most of the radioactive lipids absorbed by the intestine accumulate in the d < 1.019 g/ml fraction suggests that this fraction may be an appropriate indicator for absorbed lipids as well as for lipids secreted by liver.

In the calculation of blockade factors for Triton-ethyl ole-

TABLE 6. The effect of Triton and sonicated ethyl oleate emulsion (EO) on blockade factor and recovery of labeled cholesterol in plasma and liver

Treatment[a]	Absorption	Blockade factor	Recovery		
			Plasma	Liver	Total
	% of dose		*% of absorbed dose*		
Triton (6)[b]	30 ± 7[c]	62 ± 14	62 ± 13	11 ± 2.2	73 ± 14
Triton + EO (7)	29 ± 7	87 ± 7[d]	84 ± 5[d]	8.0 ± 1.3	92 ± 5[d]

[a]Two hundred mg of Triton/kg was injected intravenously just before feeding the test meal. Sonicated ethyl oleate (EO), 10% emulsion; 3.3 ml/kg was injected intravenously 4 hr before the test meal. All rabbits were fed 100 g of chow per day. Animals were fed a test meal of 50 g of chow containing 500 mg of labeled cholesterol and 2.5 g of Wesson Oil at 0-hr. Animals were fed 50 g of chow 9 hr after the test meal. Details of calculations as in Table 5.
[b]Number of animals.
[c]Mean ± SD.
[d]Significantly greater ($P < 0.05$) than for Triton only.

ate, we have used the accumulation of labeled cholesteryl or retinyl esters in plasma. This calculation assumes that, without Triton–ethyl oleate, the accumulation of absorbed labeled cholesteryl or retinyl esters is insignificant. That this is indeed the case was shown by Thompson and Zilversmit (36) in normal rabbits fed a single meal of chow plus cholesterol and retinol, similar to that used in the present study. In the earlier study about 10% of fed labeled cholesterol and less than 5% of labeled retinol was present in plasma 24 hr after feeding.

Yamada et al. (17) observed that the degree of hyperlipidemia induced by Triton varied in different species. This might be partially explained by the finding that, during the first 24 hr after injection, Triton concentrations in plasma decline rapidly in monkeys (37), rats (3, and our unpublished data), and dogs (38), but not in rabbits. In rabbits injected with 200 mg of Triton/kg, the concentration of Triton in plasma remained quite constant for 48 hr (10, and present study). Despite this constancy, chylomicron cholesteryl and retinyl ester removal was not inhibited completely, since only about one-half of absorbed lipids was retained in the circulation. Although we might have been able to increase the dosage of Triton, toxic levels are approached rapidly (11). Even at the present dosages we have observed severe hemolysis in rats (Nagata, Y., and D. B. Zilversmit, unpublished data) and in ponies (8). Instead, we tried to improve the blockade factors by supplementary treatments. In one experiment, we attempted to improve the Triton-induced blockade by feeding a high-fat meal containing labeled triglyceride. The rationale for the addition of the triglyceride was: *1)* many tests for the efficiency of Triton in other species were done with triglyceride as a marker for lipoprotein clearance (2–8); *2)* if the low recovery of dietary retinol in plasma was the result of the binding of chylomicrons or their remnants to receptor sites on endothelial surfaces or hepatocytes, then the simultaneous presence of a large number of dietary lipoprotein particles might release the particles with the labeled marker; and *3)* if marginal amounts of lipoprotein lipase were still active in the presence of Triton, a large mass of dietary triglyceride in plasma might exhaust the available lipase activity. However, the addition of triglyceride to the diet did not improve the blockade nor was the retention of labeled triglyceride in plasma any greater than that for cholesteryl or retinyl esters.

Other mechanisms whereby Triton might affect the metabolism of lipoproteins have been proposed. Triton may interfere with the binding of chylomicrons and their remnants to rat liver membranes (16) or with the binding of LDL to fibroblasts (15). It has also been suggested that Triton might coat lipoproteins that would then be recognized as foreign bodies by the reticuloendothelial system (39). Taken together, it is likely that in Tritonized animals the removal of intestinal lipoprotein particles from plasma is blocked, in part, by the inactivation of lipoprotein lipase, but that a portion of the Triton effect may be mediated by one or more other pathways.

Little is known about the mechanism of action of ethyl oleate. Packard et al. (23) and Slater, Packard, and Shepherd (40) used an ethyl oleate emulsion to suppress phagocytic activity in rabbits and found that the clearance of modified LDL was greatly reduced. In the present study, the administration of an ethyl oleate emulsion before Triton injection significantly reduced the plasma clearance of dietary lipoprotein particles. Apparently, the injection of ethyl oleate in Tritonized rabbits abolished the uptake of dietary particles by peripheral tissues. A significant uptake of dietary particle lipid by extrahepatic tissues has also been demonstrated in rabbits (20) and in dogs (41) not treated with Triton. The principal mechanism whereby ethyl oleate reduces chylomicron or remnant clearance by peripheral tissues in Tritonized rabbits is still unclear, but it seems plausible that it may involve a suppression of phagocytic activity in these tissues. If macrophages in some peripheral tissues of the normal or cholesterol-fed rabbit also play a significant role in the removal of dietary lipid particles from plasma, then the magnitude of this phagocytic process could affect the contribution of dietary lipid particles to the development of arterial and xanthomatous lesions (42).

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REFERENCES

1. Kellner, A., J. W. Correll, and A. T. Ladd. 1951. Sustained hyperlipemia induced in rabbits by means of intravenously injected surface-active agents. *J. Exp. Med.* **93:** 373–383.
2. Recknagel, R. O. 1967. Carbon tetrachloride hepatotoxity. *Pharm. Rev.* **19:** 145–208.
3. Schurr, P. E., J. R. Schultz, and T. M. Parkinson. 1972. Triton-induced hyperlipidemia in rats as an animal model for screening hypolipidemic drugs. *Lipids.* **7:** 68–74.
4. Fiser, R. H., Jr., J. C. Denniston, R. B. Rindsig, and W. R. Beisel. 1974. Triglyceride secretion rates: use of Triton in the rhesus monkey. *J. Nutr.* **104:** 223–226.
5. Kalopissis, A-D., S. Griglio, M-I. Malewiak, and R. Rozen. 1980. Effect of a high-fat diet on rat very low density lipoprotein secretion. *Biochim. Biophys. Acta.* **620:** 111–119.
6. Holt, P. R., and A. A. Dominguez. 1980. Triton-induced hyperlipidemia: a model for studies of intestinal lipoprotein production. *Am. J. Physiol.* **238:** G453–G457.
7. Frost, S. C., W. A. Clark, and M. A. Wells. 1983. Studies on fat digestion, absorption, and transport in the suckling rat. IV. In vivo rates of triacylglycerol secretion by intestine and liver. *J. Lipid Res.* **24:** 899–903.
8. Morris, M. D., D. B. Zilversmit, and H. F. Hintz. 1972. Hyperlipoproteinemia in fasting ponies. *J. Lipid Res.* **13:** 383–389.
9. Zilversmit, D. B., L. B. Hughes, and M. Remington. 1972.

Hypolipidemic effect of pregnancy in the rabbit. *J. Lipid Res.* **13:** 750–756.
10. Klauda, H. C., and D. B. Zilversmit. 1974. Influx of cholesterol into plasma in rabbits with fasting hyperbetalipoproteinemia. *J. Lipid Res.* **15:** 593–601.
11. Schotz, M. C., A. Scanu, and I. H. Page. 1957. Effect of Triton on lipoprotein lipase of rat plasma. *Am. J. Physiol.* **188:** 399–402.
12. Scanu, A., and P. Oriente. 1961. Triton hyperlipemia in dogs. I. In vitro effects of the detergent on serum lipoproteins and chylomicrons. *J. Exp. Med.* **113:** 735–757.
13. Ishikawa, T., and N. Fidge. 1979. Changes in the concentration of plasma lipoproteins and apoproteins following the administration of Triton WR 1339 to rats. *J. Lipid Res.* **20:** 254–264.
14. Yamamoto, K., B. Shen, C. Zarins, and A. M. Scanu. 1984. In vitro and in vivo interactions of Triton 1339 with plasma lipoproteins of normolipidemic rhesus monkeys. Preferential effects on high density lipoproteins. *Arteriosclerosis.* **4:** 418–434.
15. Goldstein, J. L., G. Y. Brunschede, and M. S. Brown. 1975. Inhibition of the proteolytic degradation of low density lipoprotein in human fibroblasts by chloroquine, concanavalin A, and Triton WR 1339. *J. Biol. Chem.* **250:** 7854–7862.
16. Cooper, A. D., S. K. Erickson, R. Nutik, and M. A. Shrewsbury. 1982. Characterization of chylomicron remnant binding to rat liver membranes. *J. Lipid Res.* **23:** 42–52.
17. Yamada, K., F. Kuzuya, T. Oguri, M. Mizuno, K. Kuno, and M. Kitagawa. 1966. Species difference in hypercholesterolemia induced by a surface-active agent (Triton). *J. Atheroscler. Res.* **6:** 299–301.
18. Otway, S., and D. S. Robinson. 1967. The effect of a non-ionic detergent (Triton) on the removal of triglyceride fatty acids from blood of the rats. *J. Physiol.* **190:** 309–319.
19. Lipkin, E. W., C. Cooper, and R. A. Shipley. 1978. The contribution of serum triacylglycerol to hepatic triacylglycerol turnover in the starved rat. *Biochem. J.* **172:** 205–218.
20. Ross, A. C., and D. B. Zilversmit. 1977. Chylomicron remnant cholesteryl esters as the major constituent of very low density lipoproteins in plasma of cholesterol-fed rabbits. *J. Lipid Res.* **18:** 169–181.
21. Gregersen, M. I., R. A. Rawson. 1959. Blood volume. *Physiol. Rev.* **39:** 307–342.
22. MacFarlane, A. S. 1958. Efficient trace-labelling of proteins with iodine. *Nature.* **182:** 53–57.
23. Packard, C. J., H. R. Slater, and J. Shepherd. 1982. The reticuloendothelial system and low density lipoprotein metabolism in the rabbit. *Biochim. Biophys. Acta.* **712:** 412–419.
24. Thompson, J. N., P. Erdody, R. Brien, and T. K. Murray. 1971. Fluorometric determination of vitamin A in human blood and liver. *Biochem. Med.* **5:** 67–89.
25. Abell, L. L., B. B. Levy, B. B. Brodie, and F. E. Kendall. 1952. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem.* **195:** 357–366.
26. Zak, B., N. Moss, A. J. Boyle, and A. Zlatkis. 1954. Reactions of certain unsaturated steroids with acid iron reagent. *Anal. Chem.* **26:** 776–777.
27. Corey, J. E., and D. B. Zilversmit. 1977. Validation of a stable emulsion for the assay of lipoprotein lipase activity. *J. Lab. Clin. Med.* **89:** 666–674.
28. Hatch, F. T., and R. S. Lees. 1968. Practical methods for plasma lipoprotein analysis. *Adv. Lipid Res.* **6:** 1–68.
29. Bilheimer, D. W., S. Eisenberg, and R. L. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
30. Klein, R. L., and D. B. Zilversmit. 1984. Direct determination of human and rabbit apolipoprotein B selectively precipitated with butanol–isopropyl ether. *J. Lipid Res.* **25:** 1380–1386.
31. Portman, O. W., M. Alexander, N. Tanaka, and D. R. Illingworth. 1977. Triacylglycerol and very low density lipoprotein secretion into plasma of squirrel monkeys. *Biochim. Biophys. Acta.* **486:** 470–482.
32. Solar-Argilaga, C., A. Danon, H. G. Wilcox, and M. Heimberg. 1976. Effect of sex on formation and properties of plasma very low density lipoprotein in vivo. *Lipids.* **11:** 517–525.
33. Smith, J. E., Y. Muto, P. O. Milch, and D. S. Goodman. 1973. The effects of chylomicron vitamin A on the metabolism of retinol-binding protein in the rat. *J. Biol. Chem.* **248:** 1544–1549.
34. Blickens, D. A., and N. R. DiLuzio. 1965. Metabolism of methyl palmitate, a phagocytic and immunologic depressant, and its influence on tissue lipids. *J. Reticuloendthel. Soc.* **2:** 60–74.
35. Stuart, A. E., G. Biozzi, C. Stiffel, B. N. Halpern, and D. Mouton. 1960. The stimulation and depression of reticuloendothelial phagocytic function by simple lipids. *Br. J. Exp. Pathol.* **41:** 599–604.
36. Thompson, K. H., and D. B. Zilversmit. 1983. Plasma very low density lipoprotein (VLDL) in cholesterol-fed rabbits: chylomicron remnants or liver lipoproteins? *J. Nutr.* **113:** 2002–2010.
37. Illingworth, D. R., O. W. Portman, and L. E. Whipple. 1974. Metabolic interrelationships between the lipids of very low, low and high density lipoproteins in the squirrel monkey. *Biochim. Biophys. Acta.* **369:** 304–314.
38. Scanu, A., P. Oriente, J. M. Szajewski, L. J. McCormack, and I. H. Page. 1961. Triton hyperlipemia in dogs. II. Atherosclerosis, diffuse lipidosis, and depletion of fat stores produced by prolonged administration of the non-ionic surface-active agent. *J. Exp. Med.* **114:** 279–294.
39. Scanu, A. M. 1965. Factors affecting lipoprotein metabolism. *Adv. Lipid Res.* **3:** 63–138.
40. Slater, H. R., C. J. Packard, and J. Shepherd. 1982. Receptor-independent catabolism of low density lipoprotein. Involvement of the reticuloendothelial system. *J. Biol. Chem.* **257:** 307–310.
41. Melchior, G. W., R. W. Mahley, and D. K. Buckhold. 1981. Chylomicron metabolism during dietary-induced hypercholesterolemia in dogs. *J. Lipid Res.* **22:** 598–609.
42. Zilversmit, D. B. 1979. Atherogenesis; a postprandial phenomenon. *Circulation.* **60:** 473–485.