Plasma lipoprotein changes attending the intravenous administration of Triton WR-1339 in normolipidemic dogs: preferential effect on high density lipoproteins

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Abstract The nonionic detergent Triton WR-1339 was injected intravenously into normolipidemic dogs in a single dose of 150 mg/kg body weight followed by three other injections (75 mg/kg) on days 2, 6, and 12. The Triton produced a significant elevation of the plasma cholesterol of these animals, but not of their triglyceride levels, and profound changes of their plasma lipoproteins, particularly of the high density lipoprotein class. These changes were dependent on the concentration of Triton attained in plasma; when the levels were above 1.5 mg/ml, density gradient ultracentrifugation, electrophoretic, and chemical analyses indicated that an interaction between Triton and HDL had occurred. This interaction was attended by a gradual loss of the surface components of HDL, namely apoA-I, phospholipids, and unesterified cholesterol, and by the appearance of two cholesteryl ester-rich lipoproteins of d 1.019–1.024 g/ml and d 1.038–1.058 g/ml containing apoA-I and proteins with electrophoretic mobilities of apoB, apoE, and apoA-IV. At the time that these changes had occurred, the activities of the enzymes lecithin:cholesterol acyltransferase and post-heparin lipase were unaffected. When ^{125}I-labeled apoA-I was injected intravenously into animals receiving Triton, the residence time of the radiolabeled protein in plasma increased from a control value of 3.1 days to 7.2 days. However, the apparent half-times of the radiolabeled apoA-I varied among the lipoprotein fractions it was associated with: d 1.119–1.159 g/ml, 5.28 days; d 1.019–1.024 g/ml, 7.55 days, and d 1.038–1.058 g/ml, 5.39 days. The structural alterations of HDL observed in vivo were similar to those we previously reported in vitro (K. Yamamoto et al. 1984. J. Lipid Res. **25:** 770–779) at comparable plasma detergent concentrations and were no longer present 3 weeks after the final administration of the detergent. Taken together, the in vivo results indicate that within the concentration of Triton WR-1339 used, HDL is the primary target of action by this detergent based on the observed structural changes and metabolic behavior of these lipoprotein particles and also on the apparent residence time of apoA-I, their major apolipoprotein component. —**Edelstein, C., R. E. Byrne, K. Yamamoto, C. Zarins, and A. M. Scanu.** Plasma lipoprotein changes attending the intravenous administration of Triton WR-1339 in normolipidemic dogs: preferential effect on high density lipoproteins. *J. Lipid Res.* 1985. **26:** 351–359.

Supplementary key words hyperlipidemia • HDL structure

Triton WR-1339 is a nonionic detergent, which has been shown to interact in vitro with plasma lipoproteins and to produce concentration-dependent physicochemical alterations in them (1, 2). We have shown that in vitro this detergent interacts preferentially with canine high density lipoproteins (HDL), which in this animal species is the major lipoprotein component in plasma (3). Previous in vivo studies (4) had established that dogs became hyperlipemic when injected intravenously with this detergent. Thus, in the present work we wanted to establish whether, in vivo, HDL is the primary target of Triton WR-1339 and, if so, how these structural changes affect the metabolism of this lipoprotein class. The results of these studies are the subject of this report.

MATERIALS AND METHODS

Blood was obtained by venipuncture from healthy normolipidemic male mongrel dogs maintained on a regular Purina Chow diet. The blood was collected into bottles containing 0.28% sodium citrate as an anticoagulant. Plasma was separated from the red cells by centrifugation at 4°C for 30 min at 1000 *g*.

Abbreviations: HDL, high density lipoproteins, d 1.063–1.21 g/ml; LDL, low density lipoproteins, d 1.006–1.063 g/ml; apoA-I, apolipoprotein A-I derived from the high density lipoproteins; SDS, sodium dodecylsulfate; LCAT, lecithin:cholesterol acyltransferase; EDTA, ethylenediamine tetraacetic acid; apoE, apolipoprotein E derived from the very low density lipoproteins of d 1.006 g/ml; apoA-IV, apolipoprotein A-IV derived from chylomicrons; apoB, apolipoprotein B derived from low density lipoproteins of d 1.006–1.063 g/ml; CMC, critical micelle concentration.

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Preparation of HDL

Canine HDL of d 1.063–1.21 g/ml was separated from plasma by ultracentrifugal flotation as described previously by Edelstein, Halari, and Scanu (5). The purity of HDL was assessed by agarose electrophoretic gels (6). As determined by sodium dodecyl sulfate (SDS)-gel electrophoresis (7), apolipoprotein A-I was the only protein component. Before use, HDL was dialyzed extensively against 0.15 M NaCl, 1 mM ethylenediamine tetraacetic acid (EDTA), pH 7.2.

Isolation of canine apoA-I

Canine apoA-I was prepared from delipidated HDL (8) by high performance liquid chromatography utilizing TSK 3000 columns (Varian Associates, Palo Alto, CA) as previously described (9). The preparation gave a single band by SDS-polyacrylamide gel electrophoresis and a single line of precipitation against its monospecific polyvalent antibody.

Iodination of canine apoA-I

Canine apoA-I was iodinated with carrier-free $Na^{125}I$ by a lactoperoxidase method as described by Edelstein et al. (5). Approximately 0.8–1.0 atom of iodine was incorporated per molecule of apoprotein.

Preparation of canine [^{125}I-apoA-I]-HDL

Canine HDL (d 1.063–1.21 g/ml) in 0.15 M NaBr, 0.05% EDTA at pH 8.0 was incubated with 100 μCi of ^{125}I-labeled canine apoA-I at room temperature for 30 min. Reisolation of [^{125}I-apoA-I]-HDL and removal of the lipid-free apoA-I were accomplished as described previously (5). Briefly, the incubation mixture was adjusted to a volume of 1 ml with 0.15 M NaBr and layered on top of a discontinuous NaBr density gradient (density limit 1.078–1.298 g/ml) and centrifuged for 66 hr at 14°C using a Beckman SW 40 rotor. After centrifugation, gradient fractionation was performed with an ISCO density gradient fractionator. The fractions were monitored at 280 nm and their radioactivity was measured in a gamma counter. The fractions containing [^{125}I-apoA-I]-HDL were pooled and dialyzed against 0.15 M NaCl.

Triton injection protocol

Triton WR-1339 was dissolved up to a concentration of 20%, w/v, in 0.05 M phosphate buffer, pH 7.2, and sterilized through a 0.4-μm filter (Millipore Corp., Bedford, MA). An intravenous injection of Triton 150 mg/kg body weight was given followed by three successive injections (75 mg/kg each) on days 2, 6, and 12. Based on the ^{125}I-labeled Triton residence time of 4 days (4) and the concentration of this detergent in plasma (**Fig. 1**), the injected Triton was in a micellar form, i.e., above 0.08 mg/ml (3) during the course of the study.

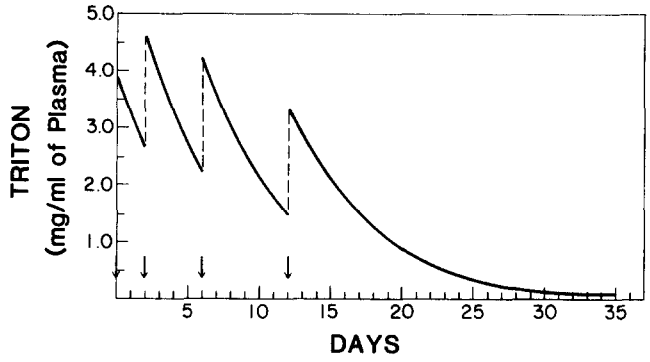

Fig. 1. Calculated plasma Triton WR-1339 concentration following its intravenous injection into a dog. Triton WR-1339 was dissolved to 20% w/v in 0.05 M phosphate buffer, pH 7.2, and filter-sterilized through a 0.4-μm filter (Millipore Corp., Bedford, MA). An initial injection of 150 mg/kg body weight was administered followed by three successive doses of 75 mg/kg at 2- to 6-day intervals. The arrows indicate the times of Triton injection which coincide with the dashed lines. The concentration of Triton was determined based on a dog's weight of 14 kg, plasma volume of 546 ml, and a residence time of 4 days for the detergent (4).

Turnover of ^{125}I-labeled canine apoA-I in plasma

Four dogs weighing 12 to 16 kg were placed in metabolic cages and fed a commercial dog chow diet with free access to food and water. Three days before the experiment and throughout the entire turnover study, 1 ml of water containing 5 drops of saturated potassium iodide solution (Upsher-Smith Laboratories, Minneapolis, MN) was added to the food in order to prevent the uptake of radioiodine by the thyroid gland. Following an 18-hr fast, 400 μg of canine ^{125}I-labeled apoA-I or [^{125}I-apoA-I]-HDL (70 μCi per mg of protein) in 0.15 M NaCl was injected intravenously. Blood samples (5 ml) were withdrawn in tubes containing EDTA (0.05 ml of 10% EDTA, pH 7.0) before (control), 10 min after injection (taken as the initial radioactivity), and daily for 5 weeks. Plasma radioactivity measurements were carried out in a Tracor Analytic Model 1190 automatic gamma counter (Tracor Analytic, Elk Grove Village, IL) having an efficiency of 70% for ^{125}I. The decay of radioactivity in plasma was expressed as:

$$\text{percent of injected dose} = (\text{cpm/ml})_t/(\text{cpm/ml})_i \times 100$$

where t was the radioactivity at a given time corrected for isotopic decay after injection and i was the initial radioactivity. The log of the percent of injected dose was plotted against time and the half-time ($T_{1/2}$) of apoA-I in the intravascular compartment was determined from the slope of the linear portion of this curve.

Distribution and turnover of ^{125}I-labeled apoA-I among plasma lipoproteins

Plasma samples were fractionated by a single-step density gradient ultracentrifugation technique as previously described (10). Briefly, a discontinuous gradient was constructed in a 13.2-ml centrifuge tube; the gradient consisted of 500 mg of sucrose, 5 ml of 4 M NaCl, 1 ml of

plasma in 0.15 M NaCl, and 6.8 ml of 0.67 M NaCl solution containing 0.05% EDTA, pH 7.0. Centrifugation was conducted at 14°C in a Spinco titanium swinging-bucket rotor, model SW 40 at 39,000 rpm for 66 hr. A curvilinear gradient was formed with density limits of 1.037 to 1.20 g/ml. The absorbance of the effluents was measured at 280 nm and collected (400 μl/fraction) in an ISCO Model 640 fractionator (Instrumentation Specialties, Co., Lincoln, NE) and their radioactivity was determined. The turnover of the radiolabeled apoA-I in each individual lipoprotein in plasma was determined from specific radioactivity (cpm/mg of protein) measurements in fractions separated by density gradient ultracentrifugation.

Quantitative electroimmunoassay of apoproteins

Antisera against canine plasma apoA-I were raised in goats by injecting, intramuscularly, 1 mg of the purified preparation dissolved in 0.5 ml of 0.9% NaCl, emulsified in an equal volume of complete Freund's adjuvant (Difco Lab., Detroit, MI). Three more injections of the antigen emulsified in incomplete Freund's adjuvant were given at 2-week intervals. Ten days after the fourth injection, the goat was bled and the serum was separated by centrifuging the blood at 3000 rpm for 20 min at 4°C. This antiserum gave a single precipitin line against either the purified canine apoA-I or canine plasma by double immunodiffusion in a 1% agarose system (11). The immunoassay for apoA-I was carried out by the rocket immunoelectrophoretic procedure described by Laurell (12). ApoA-I was determined in the presence of 7 M urea as described by Kashyap, Hynd, and Robinson (13).

Measurement of lecithin:cholesterol acyltransferase (LCAT) activity

LCAT (EC 2.3.1.43) activity in plasma was assayed by measuring the production of radioactive cholesteryl ester from [4-^{14}C]cholesterol-labeled HDL_3 as described by Piran and Morin (14). The activity was expressed in terms of units, where one U equals the quantity that catalyzes the esterification of 1 nmol of free cholesterol/hr at 37°C, pH 7.1. Activities were mean values of duplicate samples.

Measurement of lipase activity

Lipase activity in the plasma was determined 10 min after the intravenous injection of heparin (50 μ/kg body weight). The substrate used was gum arabic-stabilized [^{3}H]triolein emulsion as described by Enholm et al. (15). The reaction mixture in a total volume of 100 μl contained: 0.25 μmol of triolein, 500 μg each of BSA (fatty acid-free), and gum arabic in 0.2 M Tris-HCl buffer, 0.15 M NaCl, pH 8.2. After incubation at 37°C for 30 min, the liberated ^{3}H-labeled free fatty acids were extracted with benzene–chloroform–methanol 10:5:12, saponified with NaOH, and the upper phase was further extracted with hexane. The hexane phase was transferred to counting vials, evaporated under N_2 at 50°C and counted in 10 ml of scintillation fluid.

SDS-Polyacrylamide electrophoresis

Electrophoretic analyses of apoproteins in the presence of SDS were performed by using 10% acrylamide (w/v) as described by Weber and Osborn (7).

Agarose gel electrophoresis

Electrophoresis of plasma samples or isolated lipoproteins was carried out essentially as described by Noble (6) using Agarose Universal Electrophoresis film (ACI-Corning, Palo Alto, CA) and an ACI electrophoresis apparatus. Following electrophoresis, lipoproteins were fixed and stained with Fat Red 7B or Amido Black.

Gradient gel electrophoresis

Gradient gel electrophoresis was carried out on a Pharmacia Electrophoresis apparatus GE-4 loaded with gradient gels PAA 4/30 as described previously (5).

Chemical analyses

Protein content was determined by a modification (16) of the method of Lowry et al. (17) using 0.5% SDS added to the reagent. Total and free cholesterol were determined enzymatically according to the modified procedure of Allain et al. (18) and Gallo et al. (19). Phospholipid content was measured as lipid phosphorus according to the method of Bartlett (20), using a conversion factor of 25. Triglyceride was determined using the enzymatic kit supplied by Bio-Dynamics (Boehringer Mannheim).

RESULTS

The time course of response of plasma cholesterol, triglyceride, and apoA-I concentration after the intravenous injection of Triton is presented in **Fig. 2**. The plasma cholesterol levels progressively increased from a control value of 170 mg/dl to a maximum of 520 mg/dl 14 days after the initial Triton injection; they fell back to the control value 3 weeks after the last injection. The triglyceride levels, on the other hand, remained in a normal range during this time interval, in keeping with the normal measurements of post-heparin plasma lipase activity. The plasma apoA-I levels decreased by 33% immediately after the detergent injection and reached normal values 6 days after. The LCAT activity decreased immediately after Triton injection; the specific activity before injection was 2.2 U/ml and after 10 min, 1 hr, and 2 hr, the activities were 1.03, 1.56, and 1.95 U/ml, respectively. Additional LCAT measurements were made on days 2, 5, 7, 26, 28,

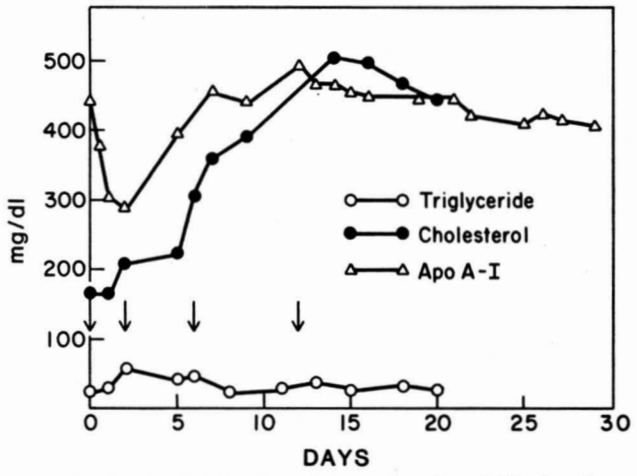

Fig. 2. Levels of triglyceride, cholesterol, and apoA-I in dog plasma before and after Triton injection. The arrows indicate time of Triton injection.

and 36. The mean LCAT activity (± SD) for these determinations was 2.35 ± 0.55 U/ml which was in the range of the pre-Triton values.

The agarose electrophoretic patterns of canine plasma were examined before, 10 min, and 5, 20, and 33 days after the first injection of Triton (**Fig. 3**). Control plasma before the Triton injections (lane a) displayed a typical pattern characterized by lipid-stainable bands of α and β mobility corresponding to HDL and low density lipoprotein (LDL), respectively. Ten minutes after Triton injection (lane b), the lipid-stained band of α mobility seen in the control gel was no longer visible and was replaced by a diffuse band in the pre-β region. Five days after the injection (lane c) there was a more intense lipid and protein-stained band in the pre-β region (c and c', respectively); the latter had a migration rate equal to that of apoA-I. The patterns remained unchanged up to 16 days after the first Triton injection. Signs of regression were noted by the appearance of an α migrating band 8 days after the last injection (lane d), and the patterns essentially normalized after 3 weeks (lane e and e').

The alterations noted on agarose electrophoretic gels were more clearly observed by subjecting the serum to density gradient ultracentrifugation. **Fig. 4A thru G** shows the lipoprotein absorbance profiles from 10 min to 33 days after the first Triton injection. Compared to the control pattern (panel A), HDL 10 min after the first injection (panel B) banded at a lower density and its composition resembled that of control HDL (**Table 1**). The gradient electrophoretic gels (**Fig. 5**, lane 4) exhibited a band (mol wt 220,000) which corresponded in mobility to that of the control HDL (Fig. 5, lane 2) and a band with a position intermediate between that of control HDL fractions 16–18 and fractions 20–24 (lanes 1 and 2, respectively). In addition, there were bands of faster mobility which we attributed to represent apoA-I and its self-associated forms. Thus, the diffuse lipid-staining band in the beta-migrating position observed on agarose gels (Fig. 3b) was probably a consequence of a complex of Triton with the lipoprotein molecule and its attending disruption. Two days after Triton injection (Fig. 4C), the HDL peak became broader and appeared multimodal. The gradient electrophoretic gel pattern (Fig. 5, lane 5) appeared diffuse although the 220,000 mol wt component was still discernible. The chemical composition was grossly similar to that of control HDL (Table 1). After 5 days (panel D), two major peaks were resolved; one had the same position as that of normal HDL, and the other was in a lighter density region. By compositional analysis (Table 1), the low density fraction (11–17) had a low protein content, was enriched in cholesteryl esters, and exhibited two high molecular weight components one of which, 990,000 mol

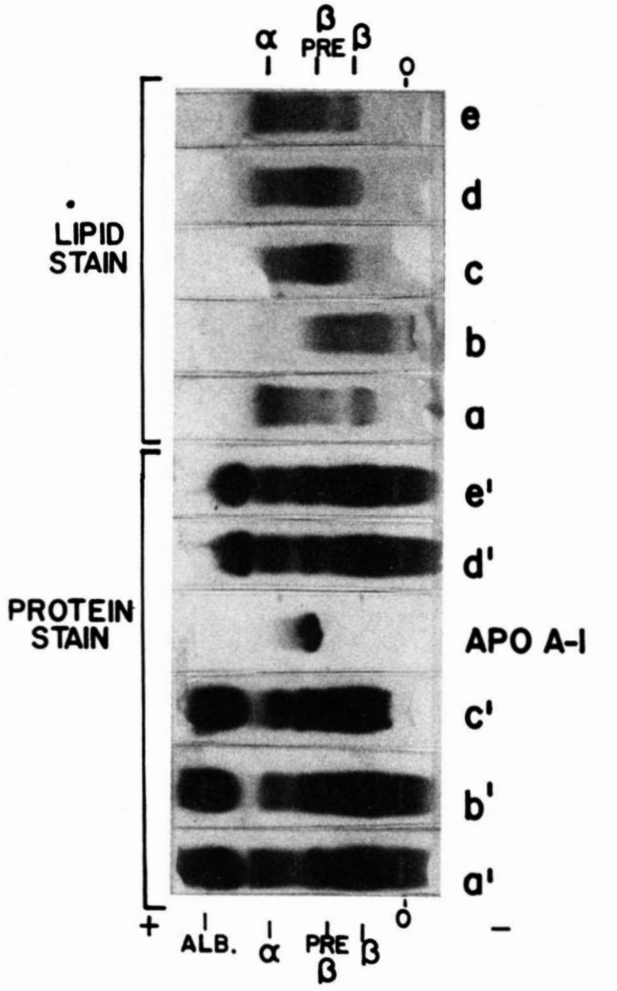

Fig. 3. Agarose electrophoretic patterns of canine plasma. One microliter of plasma was applied to prepared agarose plates as described in Methods and electrophoresed. Lipoproteins were fixed and stained with fat red 7B (a–e) and amido black (a'–e'). a, a', Control plasma before Triton injection; b, b', plasma 10 min after Triton injection; c, c', plasma 5 days after the first Triton injection; d, d', plasma 20 days after the first Triton injection; e, e', plasma 33 days after the first Triton injection.

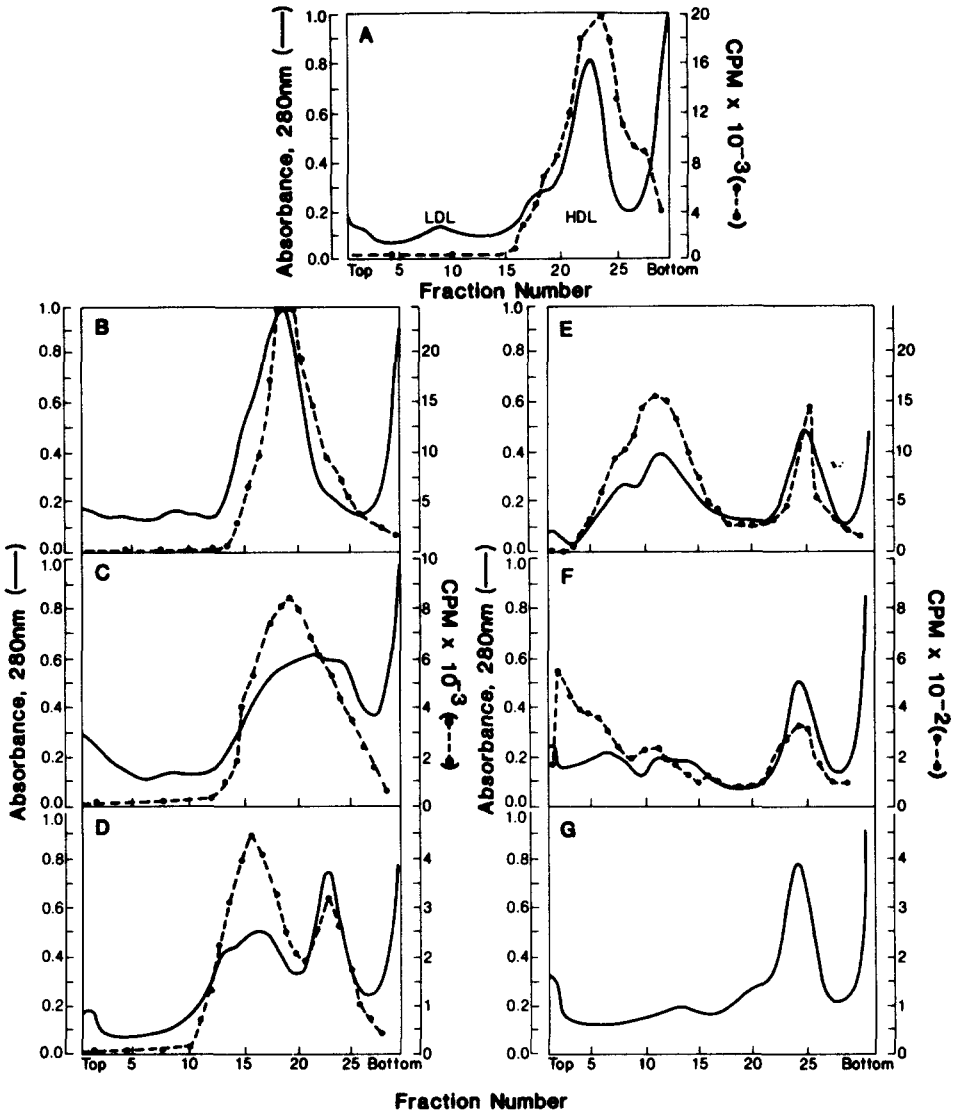

Fig. 4. Density gradient profiles of canine plasma before and after Triton injection. Plasma (1 ml) was applied to a salt density gradient (see Methods) and centrifuged at 15°C for 66 hr at 39,000 rpm. The absorbance profiles were recorded at 280nm. A, Control plasma before Triton injection; B, plasma 10 min after first Triton injection; C, plasma 2 days after first Triton injection; D, plasma 5 days after first Triton injection; E, plasma 16 days after first Triton injection; F, plasma 24 days after first Triton injection; G, plasma 33 days after first Triton injection; (—) absorbance; (• - •) radioactivity profile of ^{125}I-labeled apoA-I which was injected at time of initial Triton injection.

wt, was in the size range of an LDL which was obtained from normal dog serum. The chemical composition of the HDL fraction (21–25) was similar to that of controls except for a small increase in cholesteryl ester content. Sixteen days after the first injection (panel E), three components were seen. HDL (fractions 23–27), which banded in a more dense region (Table 1) was enriched in protein and poor in phospholipid and had an apparent molecular weight of 187,000, a value somewhat lower than that exhibited by the HDL fractions at earlier times after injection. The LDL fraction (11–15) was enriched in cholesteryl esters and fractions 5–7 represented a protein-poor, phospholipid-rich complex of an apparent molecular weight of 940,000. **Fig. 6** shows the SDS-electrophoretic patterns of the protein moieties obtained from these fractions. ApoA-I was the major component in all fractions; however, proteins with a mobility corresponding to apparent molecular weights of 43,000 and 48,000 were seen in the low density fractions (lanes 4–5) and to a lesser extent in the HDL fraction (lane 6). These proteins appeared 5 days after the first Triton injection and disappeared during the regression phase. Panels F and G show the regression patterns which returned to normal 24 days after the fourth or last injection (panel G).

TABLE 1. Physico-chemical properties of lipoprotein fractions before and after Triton injection

Time After 1st Injection	Fraction Number	P	FC	CE	PL	Density Range	Calculated[a] Density	Molecular Weight ($\times 10^{-3}$)[b]
				Progression				
			%			g/ml	g/ml	
Control	20–24	40.2	3.2	21.4	35.3	1.093–1.128	1.125	200
10 min	15–25	37.8	4.0	20.6	37.6	1.058–1.138	1.119	220, 325
2 days	15–25	35.3	3.9	22.2	38.5	1.058–1.138	1.110	230
5 days	11–17	22.5	4.7	37.3	35.5	1.038–1.071	1.060	990, 560
	21–25	39.4	2.5	26.6	31.5	1.101–1.138	1.118	212
16 days	5–7	16.9	40.3[c]		42.7	1.019–1.024	[d]	940
	11–15	22.9	5.0	39.8	32.2	1.038–1.058	1.059	920–730
	23–27	52.0	2.3	22.3	23.4	1.119–1.159	1.161	187
				Regression				
Time after 4th injection								
16 days	21–25	43.3	2.2	22.5	32.0	1.101–1.138	1.134	200
24 days	20–24	40.1	3.6	21.4	34.8	1.093–1.128	1.125	200

[a]Calculated density = $(\Sigma\%)/(\%CE/0.958 + \%C/1.033 + \%P/1.372 + \%PL/1.033)$ (21) where P = protein, FC = free cholesterol, CE = cholesteryl esters, and PL = phospholipids; the protein density was taken as the reciprocal of the partial specific volume (22).

[b]The molecular weights were estimated from a plot of the log of molecular weight of standards versus their migration distance. The standards were those described in the legend to Fig 5. The molecular weight estimates for fractions 5–7 and 11–17 were based on their mobility in 2–16% gradient gels; human LDL with a molecular weight of 2.3×10^6 was used as marker in addition to the standards already described.

[c]Total cholesterol is represented.

[d]The density could not be calculated because the CE composition was not determined.

Distribution of radioactivity in plasma following the intravenous injection of canine [^{125}I-apoA-I]-HDL

Following the intravenous injection of either 25 μCi of ^{125}I-labeled apoA-I or [^{125}I-apoA-I]-HDL into control animals most of the radioactivity (90%) was found in the HDL fraction (control, Fig. 4A). In turn, when the radiolabeled material was injected into a dog that had previously received Triton intravenously (150 mg/kg body weight), the radiolabeled apoA-I was found entirely in a lipoprotein fraction of a slightly lighter density (d 1.058–1.138 g/ml) than normal HDL (Fig. 4B). Two days after the Triton injection, ^{125}I-labeled apoA-I banded as a broad peak in a lighter density region (Fig. 4C). After 5 days, there were two peaks of radioactivity, one corresponding to the HDL fraction proper and the other in a lipoprotein banding at lower density (Fig. 4D). Sixteen days after the injection, ^{125}I-labeled apoA-I was distributed in HDL as well as in two lipoprotein fractions of lighter density (Fig. 4E, fractions 5–7 and 10–16, respectively). Finally, 5 weeks after the Triton injection, no radioactivity was detected in plasma. Moreover, the density gradient absorbance profile appeared normal.

Effect of Triton WR-1339 on the turnover of canine [^{125}I-apoA-I]-HDL

The kinetics of clearance in whole plasma of intravenously injected [^{125}I-apoA-I]-HDL in the presence or absence of Triton are shown in **Fig. 7**. Between 5 and 16 days after the initial Triton injection, the slope of the decay curve was linear. The $T_{1/2}$ value for this interval was significantly higher (7.20 days) than in the control animal (3.10 days). The latter value agrees with that reported previously by Nakai and Whayne (23) for the half-life of canine apoA-I. The $T_{1/2}$ value of ^{125}I-labeled apoA-I in the control animals was unaffected whether the radiolabeled apoA-I injected was originally incorporated into

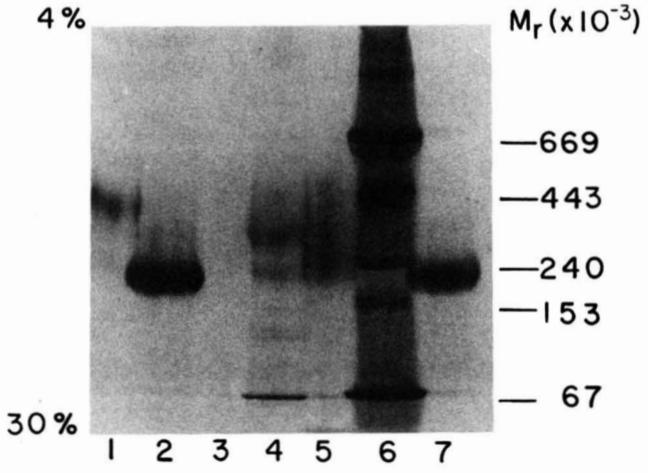

Fig. 5. Gradient gel electrophoresis pattern of ultracentrifugal fractions obtained from control plasma and after injection of Triton WR-1339. Lanes 1 and 2 refer to fractions from Fig. 4A (before Triton injection); lane 1, fractions 16–18; lane 2, fractions 20–24; lane 3, empty; lane 4, fractions 15–25 (see Fig. 4B, 10 min after Triton injection); lane 5, fractions 15–25 (see Fig. 4C, 2 days after Triton injection); lane 6, mixture of standard proteins: thyroglobulin, M_r = 669,000; apoferritin, M_r = 443,000; catalase, M_r = 240,000; globulin, M_r = 153,000; bovine serum albumin, M_r = 67,000; lane 7, canine HDL obtained by ultracentrifugal flotation. The slab gels contained a gradient of polyacrylamide in the range of 4% (top of gel) to 30% (bottom of gel).

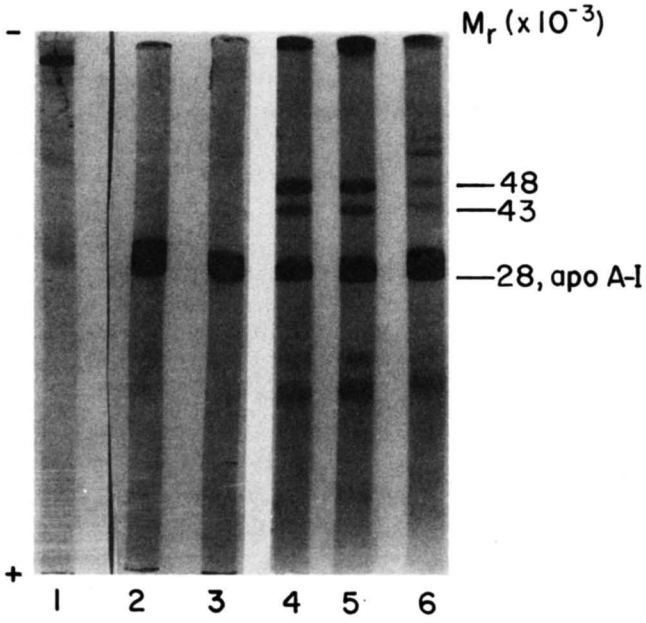

Fig. 6. SDS-10% polyacrylamide gel electrophoretic pattern of ultracentrifugal fractions obtained from plasma before and after injection of Triton WR-1339. Lanes 1–3 refer to fractions from Fig. 4A (before Triton injection); lanes 4–6 refer to fractions from Fig. 4E (16 days after Triton injection); lane 1, fractions 7–10; lane 2, fractions 16–18; lane 3, fractions 20–24; lane 4, fractions 5–7; lane 5, fractions 11–15; and lane 6, fractions 23–27.

canine HDL or was injected as a lipid-free apoprotein. Between 20 and 26 days after the initial Triton injection, the slope of the decay curve was again linear. However, the $T_{½}$ of ^{125}I-labeled apoA-I in plasma was 2.90 days for this interval, a value similar to that observed in the control dog or when the animal was reinjected with [^{125}I-apoA-I]-HDL after the regression phase ($T_{½}$ = 2.92 days). Since it was evident from the data presented in Fig. 4 that ^{125}I-labeled apoA-I was found in lighter density fractions as well as in HDL, the log of the specific activity versus time of fractions 5 to 7, 10 to 16, and 20 to 27 was plotted (see **Fig. 8**). The slopes of all three plots were linear and the apparent $T_{½}$ values for the disappearance of ^{125}I-labeled apoA-I from each fraction were calculated. The specific decay of fraction 20–27 exhibited a half-time of 5.28 days between days 8 and 24 (Fig. 8c). Similarly, the $T_{½}$ value of fraction 10–16 was 5.39 days (Fig. 8b), whereas, the half-time of apoA-I in fraction 5–7 was 7.55 days (Fig. 8a). The $T_{½}$ values obtained for individual fractions were considered to be apparent values since it was possible that apoA-I exchanged or transferred among the lipoprotein fractions.

DISCUSSION

Our studies have shown that the intravenous injection of Triton WR-1339 (75–150 mg/kg body weight) into normolipidemic dogs causes nearly a twofold increase of plasma cholesterol (Fig. 2) and significant structural alterations of plasma HDL, as shown by changes in electrophoretic mobility, buoyant density, and a redistribution of apoA-I among plasma lipoproteins after ultracentrifugation. From the amount of detergent injected and the plasma volume of the recipient animals, we calculated that the lipoprotein changes occurred at an initial Triton concentration in plasma above 1.5 mg/ml, a value several fold higher than that determined (8) for the CMC (critical micelle concentration) of Triton WR-1339 in solution (about 0.008 g/dl). It may be noted that in previous studies we found that the structure of canine plasma lipoproteins is altered at concentrations of Triton WR-1339 above 1 mg/ml serum. Thus, Triton in a micellar form can induce structural changes in HDL in a concentration-dependent manner. The $T_{½}$ value of Triton WR-1339 in the plasma of dogs injected intravenously with this detergent has been reported to be 4 days (4). In keeping with those observations, we found that the levels of this detergent in the plasma of our dogs dropped progressively from the time of injection to a negligible concentration (approximately 0.008 g/dl) 21 days after the final injection. From the data in Table 1 and Figs. 4E–G and 7, it is evident that the structural alterations of HDL decreased progressively as a function of detergent concentration and this may be taken to support further the direct action of the Triton micelles on this lipoprotein class.

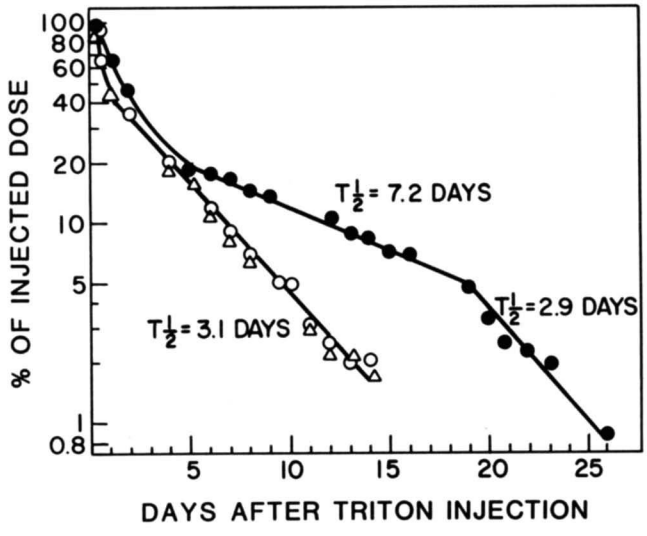

Fig. 7. Turnover of ^{125}I-labeled apoA-I in plasma before (△–△) and after (●–●) injection of Triton in the dog. After an 18-hr fast, 400 μg of canine [^{125}I-apoA-I]-HDL (70 μCi per mg of protein) in 0.15 M NaCl were injected intravenously, followed by Triton injection as described in Fig 1. Blood samples (5 ml) were collected in tubes containing EDTA (0.05 ml of 10% EDTA, pH 7.0) and plasma radioactivity measurements were carried out as described in Materials and Methods. The reversibility of Triton action in vivo was assessed by reinjection of [^{125}I-apoA-I]-HDL in the same dog 90 days after the last Triton injection (○–○).

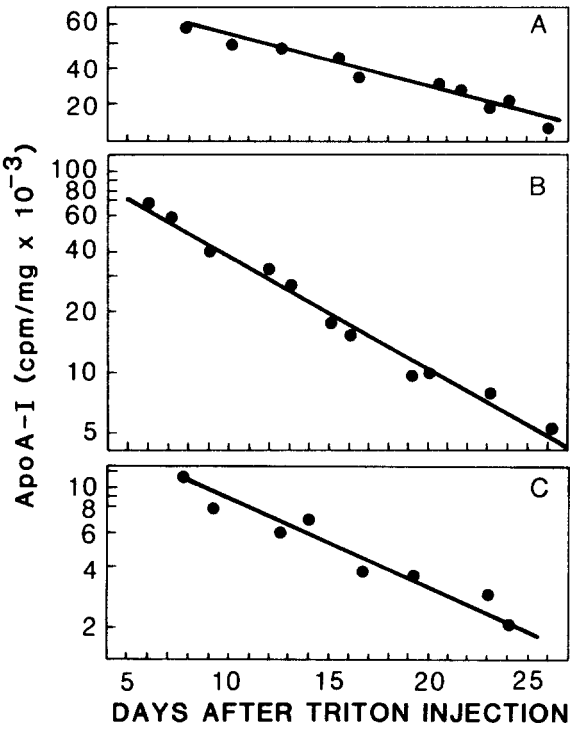

Fig. 8. Turnover of individual lipoprotein fractions after injection of Triton in the dog. The decay of specific radioactivity (cpm/mg of protein) of pooled fractions separated by density gradient ultracentrifugation was determined as described in Materials and Methods: A) fractions 5–7; B) fractions 11–15; C) fractions 23–27.

In addition to the concentration-dependent alterations in HDL, time-dependent changes were also noted, as exemplified by the lipoprotein patterns in days 2 and 5 (Fig. 1 and Fig. 4C and 4D) and by the 43,000 and 48,000 molecular weight electrophoretic bands which first appeared on day 5 and increased in intensity during the progression stage. Moreover, we observed the formation of light density lipoprotein fractions several days after Triton injection. These lipoproteins contained a protein resembling apoB, significant amounts of apoA-I, and other apoproteins having a mobility on SDS polyacrylamide gel electrophoresis similar to human apoE and apoA-IV. Since these apoproteins were not observed in control animals, they must have been secondary to the Triton administration, likely representing metabolic products secondary to the primary effect of Triton on the lipoproteins. Triton administration also affected the turnover of apoA-I which appeared to be dependent on the type of lipoprotein complex this apoprotein was associated with. This might have been due either to changes in apoprotein conformation, the presence of Triton WR-1339 on the lipoprotein particles, or both.

It should be noted that the amount of injected Triton, which was sufficient to cause hypercholesterolemia and to disrupt the structure of HDL, caused neither changes in the plasma triglyceride levels (Fig. 2) nor of the activities of LCAT and lipoprotein lipase. The observed lack of effect of Triton on post-heparin plasma lipase may appear to be in conflict with previous reports showing an inhibitory action of this detergent on this enzyme activity in the rat (24) and in the mouse (25). Moreover, an inhibitory action of Triton on LCAT activity in the rat was reported by Soler-Argilaga, Russell, and Heimberg (26). The discrepancy, however, may only be apparent since the amounts of detergent used were significantly different. In those studies, the dose of Triton administered was approximately 400–500 mg/kg body weight, whereas in our study the dose was much less, 75–150 mg/kg. It should also be noted that different animal species were used.

In summary, our data have shown that Triton can markedly affect the structure of HDL and that this effect is observed at concentrations of the detergent well above its CMC. We have also shown that these structural changes not only modify the metabolic fate of the HDL particles, probably due to a primary effect of Triton on these particles, but also cause secondary metabolic changes expressed by the appearance in the plasma of components not present before Triton injection. From these studies, it is apparent that the Triton effects are complex and that, in describing the in vivo response to Triton injection(s), one must take into account the variability of the recipient animal species, dose, and length of administration of the detergent.

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