In vitro effect of Triton WR-1339 on canine plasma high density lipoproteins

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Abstract We studied the effect in vitro of various concentrations of Triton WR-1339 on normolipidemic canine plasma and on the high density lipoproteins (HDL) isolated from this plasma by ultracentrifugation. As a preamble to this study, we established that Triton WR-1339 has a unimer molecular weight of 4,500, a micellar molecular weight of 180,000, and a critical micellar concentration (CMC) of 0.018 mM or 0.008 g/dl. Above its CMC, Triton WR-1339 in concentrations between 2 and 10 mg/ml induced concentration-dependent structural changes in HDL which were characterized by a progressive displacement of apoA-I from the HDL surface without loss of lipids. The addition of Triton WR-1339 to the HDL particles modified their electrophoretic mobility and caused an increase in size (95 ± 5 Å to 114 ± 7 Å). At the extreme Triton WR-1339 concentrations utilized in these studies (10 mg/ml) disruption of the HDL particles occurred; at this stage, the original, relatively homogeneous, spherical HDL particles were replaced by a heterogenous population ranging in size between 50 and 250 Å, representing complexes of Triton WR-1339 with lipids essentially free of apoA-I which could be sedimented by ultracentrifugation. The effects of Triton WR-1339 on whole plasma or isolated HDL were comparable.■ These studies indicate that Triton WR-1339 in vitro alters HDL in a concentration-dependent manner and that these changes vary from a displacement of apoA-I from the HDL surface to a state where all lipids are solubilized into the Triton WR-1339 micellar phase and are driven away from the protein moiety. These important structural changes in HDL may be responsible, at least in part, for the hyperlipidemia attending the intravenous administration of Triton WR-1339 into experimental animals.—**Yamamoto, K., R. Byrne, C. Edelstein, B. Shen, and A. M. Scanu.** In vitro effect of Triton WR-1339 on canine plasma high density lipoproteins. *J. Lipid Res.* 1984. **25:** 770–779.

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Although the intravenous administration of Triton WR-1339 is known to produce hyperlipidemia and plasma lipoprotein changes in the experimental animal, the mechanisms whereby these intravascular changes occur have not been clearly defined (1–9). In earlier in vitro studies, Scanu and Oriente (10) showed that Triton WR-1339 interacts with plasma lipoproteins and causes important physico-chemical changes in these particles, and

the HDL particles were recognized to be particularly sensitive to the action of the detergent both in vitro and in vivo (11). The early studies in the dog, and the more recent reports on the squirrel monkey (9) and the rat (12), have led to the suggestion that the physico-chemical changes in HDL are responsible for the development of the hyperlipidemia. However, studies by Portman et al. (9) in the squirrel monkey, have failed to show an in vitro effect of the detergent on HDL. Moreover, a primary effect of Triton WR-1339 on lipid-modifying enzymes has also been suggested (13–15). Because of these uncertainties, we considered it of interest to explore in more depth the nature of the interactions occurring between Triton WR-1339 and the plasma lipoproteins. For this purpose, we have used normolipidemic canine plasma since it contains mainly the HDL class and we also examined some of the physico-chemical properties of Triton WR-1339 in solution.

MATERIALS AND METHODS

Separation of plasma lipoproteins

Blood was obtained on several occasions by venous puncture from two fasting healthy male dogs (20–30 kg), which were fed a regular Purina Chow diet. Their serum cholesterol levels were 115 and 143 mg/dl, respectively, and their triglycerides were 15 and 41 mg/dl, respectively. The blood was collected into tubes containing sodium citrate as an anticoagulant (0.28% by weight) and the plasma was separated by centrifugation at 4°C for 30 min at 1000 g. The HDL of d 1.063–1.21 g/ml was separated by ultracentrifugal flotation as previously de-

scribed (16, 17) and extensively dialyzed against 0.15 M NaCl, 10^{-3} M EDTA, pH 7.2, before use.

Incubation of Triton WR-1339 with plasma or HDL

Triton WR-1339 (Tyloxapol, Sigma, St. Louis, MO) was dissolved in 0.05 M phosphate buffer, pH 7.2. Canine plasma was incubated in a 10:1 volume ratio with Triton WR-1339 so that the mixture contained the detergent at concentrations varying from 0 to 10 mg/ml. In the case of isolated HDL, the protein concentration was selected to be similar to that present in the incubation experiments with whole plasma. The incubations were conducted at 37°C for 2 hr. In some experiments, ^{125}I-labeled Triton WR-1339 was used and was prepared with [^{125}I]NaI by the method of McFarlane (18) and purified by passage through a Sepharose 4B column.

Separation of the incubation products

Density gradient ultracentrifugation. After incubation with Triton WR-1339, the plasma was separated by the single-step density gradient ultracentrifugation as previously described (19). The effluents were monitored at 280 nm and collected as 400-μl fractions.

Molecular sieve chromatography. Gel filtration was conducted in glass columns (2.5 × 70 cm) packed with Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden). The columns were eluted with 0.05 M phosphate, pH 7.2, at a flow rate of 20 ml/hr at 6°C. Eluates were monitored at 280 nm or at 278 nm.

Quantitative immunoassay of apoproteins

Pure canine apoA-I was obtained from canine apoHDL by high performance liquid chromatography (20). An antiserum against apoA-I was raised in the goat. Good antiserum titers were obtained after injecting the animal intramuscularly every 2 weeks for a total of four injections. For the first injection, the antigen was emulsified with complete Freund's adjuvant; incomplete Freund's adjuvant was used for the other injections. The immunoassay for apoA-I was carried out by the rocket immunoelectrophoretic procedure described by Laurell (21) in the presence of 7 M urea.

Determination of the critical micellar concentration of Triton WR-1339

The CMC was determined by measurements of the surface tension of Triton WR-1339 as a function of its concentration at 25°C as described elsewhere (22). The surface tension was determined from the maximum pull exerted on a du Nouy ring attached to the "B" loop of a Cahn Electrobalance when the ring was in contact with and was raised above the aqueous surface; the force was recorded as a function of time. The surface tension was calculated as the product of the weight times the ring constant, α, which was determined empirically with the use of pure liquids of known surface tension.

Unimer molecular weight of Triton WR-1339

The unimer[2] molecular weight of Triton WR-1339 was determined by high pressure liquid chromatography (IBM Model LC 9533) using an IBM GPC Type C column (7.0 × 250 nm; 5-μ pore size) equilibrated in either chloroform or tetrahydrofuran. Both of these solvents were HPLC grade (Burdick & Jackson Laboratories) with a cutoff of about 240 nm to allow readings at 278 nm. The retention time for Triton WR-1339 was determined from the elution time of its peak maximum. The detergent solution was applied to the column in a 10-μl volume (concentrations between 0.1 and 10.0 g/dl). The unimer molecular weight was obtained by comparing the retention time of the detergent to that of polystyrene standards of 1900 to 7600 daltons (gift from IBM Instruments, Inc.).

Electron microscopy

Fractions from the single spin ultracentrifugation of mixtures of Triton WR-1339 and plasma were dialyzed against 0.005 M NH_4HCO_3 buffer, pH 8.2, and then negatively stained with 1% sodium phosphotungstate, pH 7.0, after deposition onto a thin carbon film supported on a copper grid. The specimens were examined in a Phillips EM 300 microscope with condenser and objective apertures of 100 μm and 50 μm, respectively. The acceleration voltage was 80 kV and all specimens were examined at 55,000× magnification.

Electrophoretic analyses

Before electrophoretic analysis, all the samples were extensively dialyzed against 0.005 M NH_4HCO_3 buffer, pH 8.2. Agarose gel electrophoresis was carried out on Agarose Universal Electrophoresis film (ACI-Corning, Palo Alto, CA) using an ACI electrophoresis apparatus. After electrophoresis, the lipoproteins were fixed and stained with Fat Red 7B or Amido Black 10B. Electrophoretic separation of apoproteins was performed on 10% polyacrylamide gels containing 0.1% SDS (23). Gradient gel electrophoresis was carried out on a Pharmacia Electrophoresis Apparatus GE-4 loaded with gradient gels PAA 4/30 at 14°C, 125 V for 20 hr. The gels were then fixed and stained overnight in 0.04% Coomassie Blue G-250 in 3.5% perchloric acid followed by destaining in 5% acetic acid. Molecular weight standards were run in each gel slab.

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[2] Heterogeneous monomer.

Chemical and radioactivity analyses

Protein content was determined by the method of Lowry et al. (24) except that 0.5% sodium dodecyl sulfate was added to the reagents. Total and free cholesterol were determined enzymatically according to the modified procedures of Allain et al. (25) and Gallo et al. (26).

Lipid phosphorus was measured according to the method of Bartlett (27) and triglycerides were measured by the Technicon AutoAnalyzer II after isopropanol extraction of zeolite-treated preparations. Triton WR-1339 measurements were carried out in isopropanol extracts of plasma or lipoproteins at 278 nm (4). Radioactivity measurements were carried out in a Tracor Analytical Model 1190 Automatic Counter (Elk Grove Village, IL).

Reagents

All of the chemicals were reagent grade. Thyroglobulin, catalase, and aldolase (Pharmacia, NJ) were used as the molecular weight standards. [^{125}I]NaI (carrier free) was purchased from Amersham Corp. Triton WR-1339 (Tyloxapol) was purchased from Sigma. Its structure is shown in **Fig. 1.**

RESULTS

Properties of Triton WR-1339 in solution

Molecular weight in organic solvents. The HPLC elution profile of Triton WR-1339 solubilized in tetrahydrofuran in shown in **Fig. 2.** An identical profile was obtained with the detergent dissolved in chloroform. Some heterogeneity was noted, likely due to different numbers of ethylene oxide units in the ether side chain and/or in the
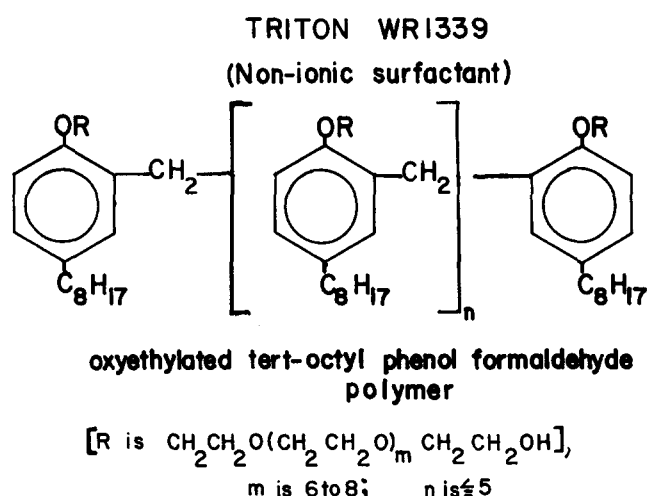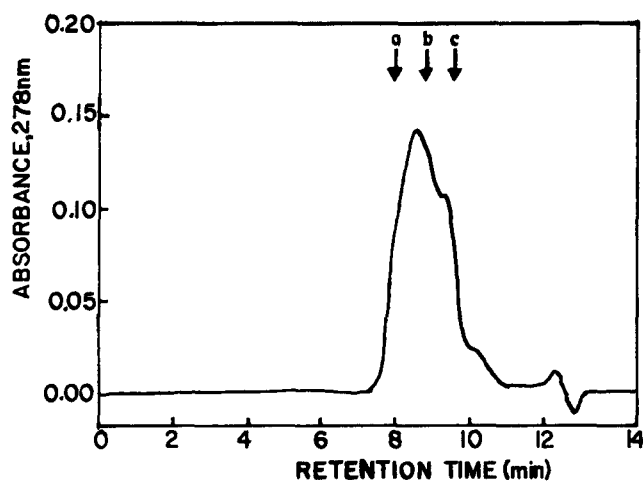
Fig. 2. HPLC elution profile of Triton WR-1339 (0.1 mg, injection volume, 10 μl). The eluting solvent was tetrahydrofuran, flow rate, 0.5 ml/min. The effluent was continuously monitored at 278 nm. Chart speed 1 cm/min. The temperature of fractionation was 23°C. The arrows indicate the elution position of polystyrene standards: a, 7600; b, 3650; c, 1900, which were run separately under identical column conditions. The negative peak eluting at 12.7 min is the buffer breakthrough.

number of octylphenol groups. The same elution profile was obtained with preparations of Triton WR-1339 diluted up to 200-fold in tetrahydrofuran and analyzed under the same chromatographic conditions. The major peak was attributed to unimeric Triton WR-1339 with some side chain heterogeneity leading to molecular weight polydispersity. Based on the retention times of the polystyrene standards (Fig. 2) and on the retention time of the major peak constituent of Triton WR-1339, the unimer molecular weight of the detergent was estimated to be 4500.

Molecular weight in phosphate buffer. Triton WR-1339 applied to a Sepharose 4B column at 5 mg/ml in 0.05 M phosphate solution, pH 7.4, eluted as a single symmetrical peak (data not shown). Aldolase, catalase, and thyroglobulin were applied to the same column. From the log molecular weight versus Kd plot it was estimated that the Triton WR-1339 micelle had a molecular weight of 180,000.

Critical micellar concentration. The plot of values for the surface tension, γ, of Triton WR-1339 in 0.05 M sodium phosphate, pH 7.2, against the natural logarithm of each concentration (mol/liter) generated a curvilinear relation whose break at 0.018 mM defined the CMC of the Triton WR-1339 in solution (**Fig. 3**). The data were further analyzed according to the equation describing the concentration of the detergent at the surface monolayer:

$$\Gamma = \frac{-1}{RT} \frac{\delta\gamma}{\delta \ln C} \qquad Eq. \ 1$$

where Γ is the surface concentration of Triton WR-1339

TRITON WR1339
(Non-ionic surfactant)

oxyethylated tert-octyl phenol formaldehyde polymer

$[R \ is \ CH_2CH_2O(CH_2CH_2O)_m CH_2CH_2OH]$,
m is 6 to 8;   n is $\leqq$ 5

Fig. 1. Structure of Triton WR-1339. This non-ionic surfactant is a p-(1,1,3,3-tetramethylbutyl) phenol polymer with ethylene oxide and formaldehyde. R = CH₂CH₂O(CH₂CH₂O)ₘCH₂OH; m = 6 to 8; n is not more than 5.
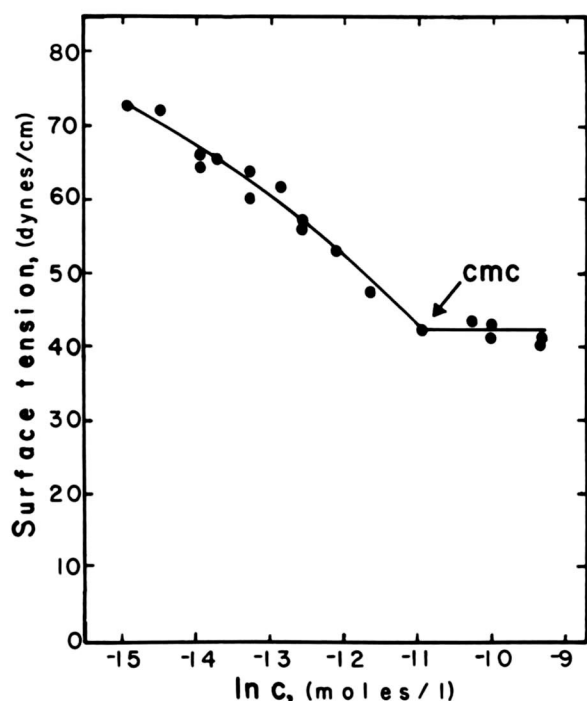
Fig. 3. Plot of surface tension of Triton WR-1339 versus concentration in moles/liter. The values of surface tension, γ, were determined using the du Nouy ring at 25°C as described in Methods. The solid line drawn from 3.0×10^{-7} to 1.8×10^{-5} moles/l represents the best fit to the experimental data ($\bullet$ —— $\bullet$) according to the equation: $Y = 157.53 + \dfrac{1264.16}{X}$.

in mol cm^{-2}, R is the gas constant in dyne-cm deg^{-1} mol^{-1}, T is the absolute temperature, γ is the surface tension in dynes cm^{-1}, and C is the molar concentration. From the value obtained for Γ, the area A, occupied at each concentration of Triton WR-1339, was calculated according to the relation:

$$A = \frac{1}{\Gamma}. \qquad Eq. \, 2$$

From the force-area curve, the straight line portion of the curve extrapolated to zero surface pressure gave a value of 62.5 Å^2 which defined the limiting area in Å^2 per molecule of Triton WR-1339 (data not shown). A summary of the physical properties of Triton WR-1339 is presented in **Table 1.** For comparison, literature data (28–31) for another non-ionic detergent (Triton X-100) are also tabulated.

Agarose gel electrophoresis of canine plasma and Triton WR-1339

The effect of Triton WR-1339 was dose-dependent. In the case of plasma (**Fig. 4A**), Triton WR-1339 caused the lipid-stained band in the α region to progressively decrease in mobility until at a concentration of Triton

TABLE 1. Physical properties of Triton WR-1339 and Triton X-100

Parameters	Triton WR-1339	Triton X-100
Molecular weight, unimer	4,500	643
Molecular weight, micelle	180,000	90,000[a] 81,250[b]
Number of unimers/micelle	40	140[a] 125[b]
CMC	0.008 g/dl 0.018 mM	0.016 g/dl[c,d] 0.249 mM
Limiting area, Å^2/molecule	62.5	53.0[d]

[a] Kushner, L. M., and W. D. Hubbard, (28).
[b] Biaselle, C. J., and D. B. Millar, (29).
[c] Ross, S., and J. P. Olivier, (30).
[d] Hsiao, L., H. N. Dunning, and P. B. Lorenz, (31).

WR-1339 of 10 mg/ml, it remained close to the origin or partly migrated toward the cathode. This shift in electrophoretic mobility was particularly evident in the studies with isolated HDL (Fig. 4B). As a consequence of the addition of Triton WR-1339, the Amido Black-stained
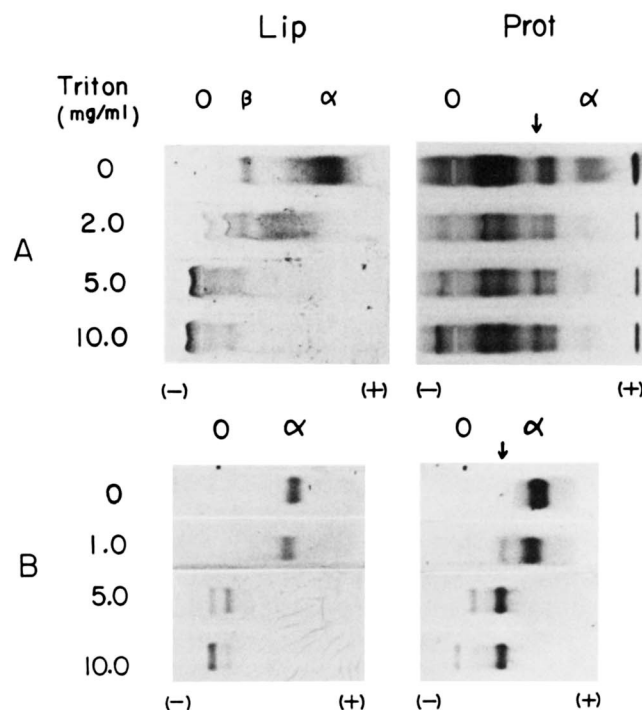

Fig. 4. Triton WR-1339 effect on the agarose gel electrophoretic profile of whole plasma (A) and HDL (B). After incubation of whole plasma or HDL (4 mg of protein/ml) with various concentrations of Triton for 2 hr at 37°C, 2 μl of each sample was applied to Agarose film in duplicate. After electrophoresis, one film was stained with Fat Red 7B and the other with Amido Black 10B. Lip, lipid staining; prot, protein staining; 0, origin. The arrow indicates the area where the new band appeared.

band in HDL decreased in mobility and became less stainable; at the same time the protein-stained band shifted from the α to the β position (Fig. 4B). As shown in **Fig. 5**, the β position is where apoA-I was found to migrate. The electrophoretic mobility of Triton WR-1339 was also affected by the amount of Triton WR-1339 applied to the agarose film (**Fig. 6**). When using radiolabeled detergent, the band that was detected both by staining with Amido Black and by radioactivity measurements decreased in mobility from the α position to the cathode where the Fat Red 7B-stained band of HDL treated with 10 mg of Triton WR-1339 moved (see Fig. 4B).

Effect of Triton WR-1339 on the distribution of plasma lipoproteins as assessed by density gradient ultracentrifugation

As shown in **Fig. 7**, Triton caused marked changes in the density gradient lipoprotein profile of whole canine plasma. The changes were concentration-dependent. Increasing the concentration of Triton WR-1339 from 2 to 10 mg/ml resulted in a progressive shift of the HDL peak to a lighter density peak (Fig. 7, peaks, a, b, c, d) associated with an increase in the absorbance at 280 nm which was partially contributed by Triton WR-1339. The distribution of radioactivity of ^{125}I-labeled Triton followed essentially the 280 nm absorbance readings, both in position and intensity. When this detergent was studied alone, its peak of maximal absorbance was at 278 nm. At a Triton WR-1339 concentration of 10 mg/ml of plasma, a new shoulder within the density range of d 1.10 and 1.15 g/ml appeared (Fig. 7, peak e). At this detergent concentration, the study of immunoassayable apoA-I showed the displacement of apoA-I from the HDL peak (Fig. 7, peak d) to a new position (Fig. 7, peak e). The apoprotein distribution, as assessed by SDS poly-
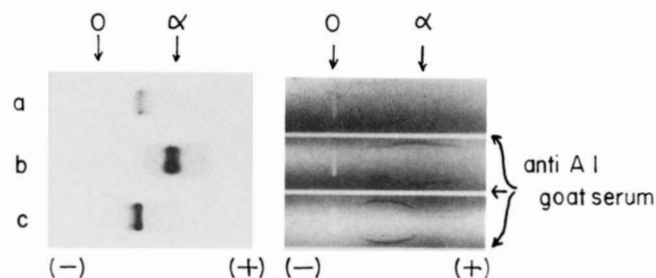

Fig. 6. Distribution of ^{125}I-labeled Triton WR-1339 in whole plasma. After incubation of ^{125}I-labeled Triton WR-1339 with whole plasma, the samples were applied to Agarose gel electrophoresis films in duplicate. One gel was stained with Fat Red 7B, the other was cut into 0.5-cm pieces and their radioactivity content was determined.

acrylamide gel electrophoresis, agreed with the immunochemical and radioactive results (**Fig. 8**). By gradient gel electrophoresis, HDL increased in size as a function of Triton concentration (**Fig. 9**). The chemical analyses of the ultracentrifugal peaks in Fig. 7 showed that peaks a through d had the same lipid composition as control HDL peaks (**Table 2**), indicating that Triton WR-1339 had no effect on the lipid matrix of the lipoprotein particle. In contrast, the minor peak e was composed mainly of Triton and protein. The chemical analyses also showed that over 90% of Triton WR-1339 that was subjected to ultracentrifugation was recovered with the lipoprotein peaks.

When ultracentrifugal peaks d and e were passed through a Sepharose 4B column (**Fig. 10**), peak d eluted as a major peak and a shoulder, whereas peak e was symmetrical eluting in the same position as the shoulder of peak d and the Triton WR-1339 peak when this detergent was run alone.

By electron microscopy, the control HDL particles had a diameter of 95 ± 5 Å (**Fig. 11a**). After incubation with

Fig. 5. Immunoelectrophoretic analysis of an incubated HDL–Triton WR-1339 mixture against an anti-canine apoA-I goat serum. Purified apoA-I and an incubated mixture of HDL and Triton WR-1339 (final concentration; Triton, 10 mg/ml, 2.5 μg of protein) were applied to Agarose films in duplicate. After electrophoresis, one (left column) was stained with Amido Black 10B, the other (right column) was reacted against a goat antiserum raised against canine apoA-I. a, apoA-I; b, HDL; c, HDL-Triton mixture.
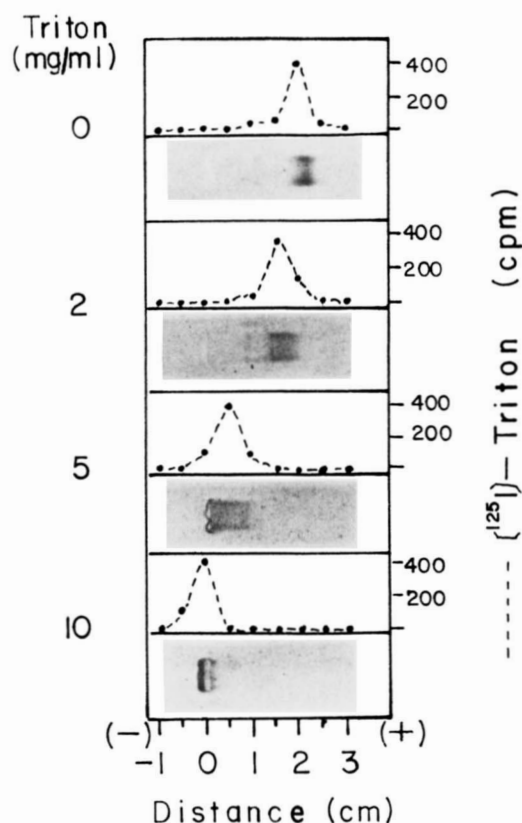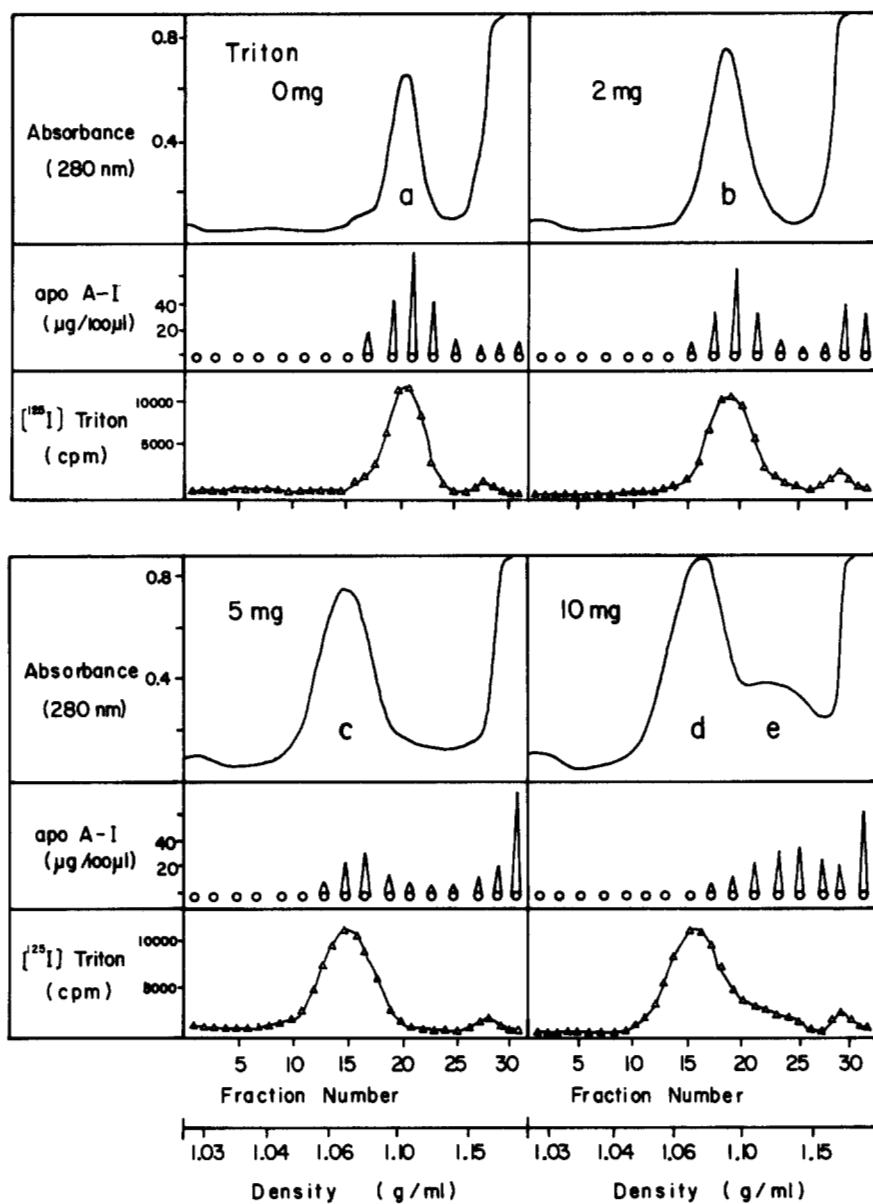
Fig. 7. Distribution of apoA-I and ^{125}I-labeled Triton WR-1339 after single-spin density gradient ultracentrifugation of whole plasma. Canine plasma was incubated in vitro with various concentrations of ^{125}I-labeled Triton WR-1339 (final concentration of Triton, 0, 2, 5, and 10 mg/ml) for 2 hr at 37°C. Then one ml of each sample was separated by single-spin density gradient ultracentrifugation. The effluents were continuously monitored at 280 nm in an ISCO UA-5 unit and collected in 0.4-ml fractions. The concentration of apoA-I was measured by electroimmunoassay and the radioactivity of ^{125}I-labeled Triton WR-1339 was measured in a gamma counter. The combined HDL peaks were named as a, b, c, d, e. The number of the tubes collected was a, tubes 19–23; b, 16–21; c, 12–18; d, 12–19; and e, 21–25.

Triton WR-1339 (2 mg of Triton WR-1339/ml), the HDL particles were spherical and larger (114 ± 7 Å) than control HDL (Fig. 11b). At 5 mg of Triton WR-1339/ml, the particles were heterogeneous varying in size between 50 Å and 250 Å (Fig. 11c). At 10 mg of Triton WR-1339/ml, there was a granular pattern with small particles in the 50 Å range (Fig. 11d).

DISCUSSION

The current studies have established that Triton WR-1339 forms micelles when dissolved in aqueous solutions at concentrations of 0.008 g/dl or above. These micelles are relatively homogeneous, have a micellar number of 40, and a molecular weight of 180,000 (Table 1). By

TABLE 2. Physico-chemical parameters of Triton WR-1339-HDL complex

	a	b	c	d	e
Radius (nm)[a]	4.40	5.25	6.10	6.10	3.85
Hydrated density (g/ml)	1.100	1.086	1.068	1.068	1.119
Molecular weight $\times 10^3$	200	276.8	425.6	n.d.[b]	n.d.
Triton WR-1339 molecules per particle	0	18.5	52.1	n.d.	n.d.
Composition			*weight %*		
Protein	40.2	22.4	6.8	1.8	27.7
Total cholesterol	30.9	25.7	21.1	14.4	6.3
Phospholipid	28.5	21.4	16.7	12.2	1.8
Triglyceride	0.4	0.4	0.3	0.3	0
Triton WR-1339	0	30.1	55.1	71.3	64.2

[a] Radii and molecular weights of molecules were obtained by gradient gel electrophoresis.

[b] n.d., Not determined.

comparison, the commonly used Triton X-100 has smaller unimer and micellar molecular weights and higher CMC values (Table 1). The current studies have also shown that at concentrations of Triton WR-1339 above the CMC, this detergent interacts with HDL and causes concentration-dependent structural changes in this lipoprotein class. This finding is in accord with earlier studies in the dog (10, 11) and other animal species (1, 3–5, 12). However, in this work we have defined more precisely the nature of the structural changes induced by Triton WR-1339. At low Triton WR-1339 concentrations (2 mg of Triton WR-1339/ml of plasma), the action of this detergent appears to be limited to the lipoprotein surface and to affect the specific displacement of apoA-I. Under these conditions an HDL–Triton WR-1339 complex is produced having all of the components of authentic HDL except that apoA-I is replaced by Triton WR-1339. This indicates that the apparent affinity of Triton WR-1339 for the HDL surface is greater than that exhibited by apoA-I. A quantitative assessment of the apparent affinity would require a thermodynamic treatment of the data which would require some unjustified assumptions (32) that *1*) micelles form a phase that is distinct from the aqueous solution; *2*) a minimum number of amphiphile molecules (m = 50) would have to associate with each other, which is a number larger than that determined for Triton WR-1339; and *3*) homologous aggregates would have to occur in aqueous media, which is not the case in our studies. Our results indicate that after HDL has reached maturation, apoA-I is not essential for the
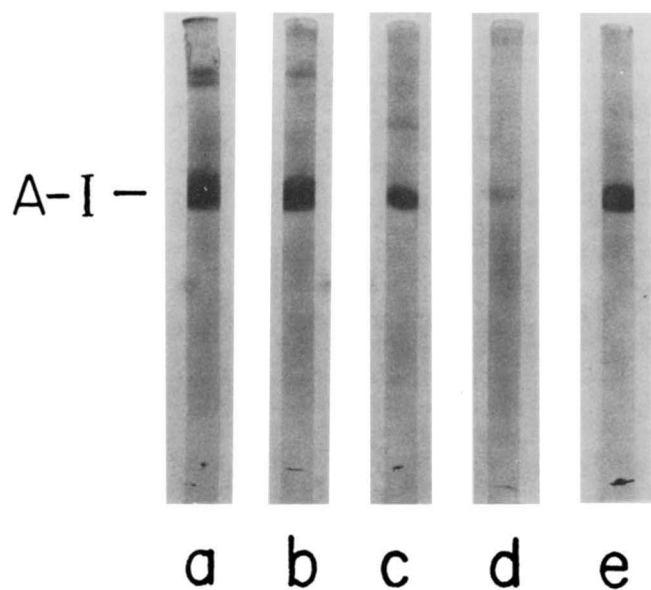

Fig. 8. SDS polyacrylamide gel electrophoresis of the ultracentrifugal peaks in Fig. 7. After dialysis of HDL-peaks (a–e, see Fig. 7) against 0.005 M NH₄HCO₃ buffer, pH 8.2, 100 µl of each sample was analyzed by SDS polyacrylamide gel electrophoresis.
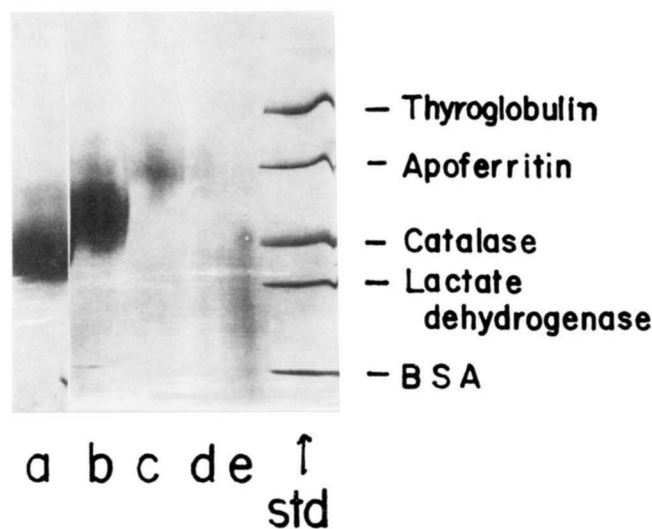

Fig. 9. Polyacrylamide gradient gel electrophoresis of ultracentrifugal peaks in Fig. 7. One hundred µl of each peak was applied to gradient gels at 14°C at 125 V for 20 hr. The radii of the particles were calculated from the calibration curve of standards: thyroglobulin (8.50 nm), apoferritin (6.10 nm), lactate dehydrogenase (4.08 nm), and bovine serum albumin (3.55 nm).
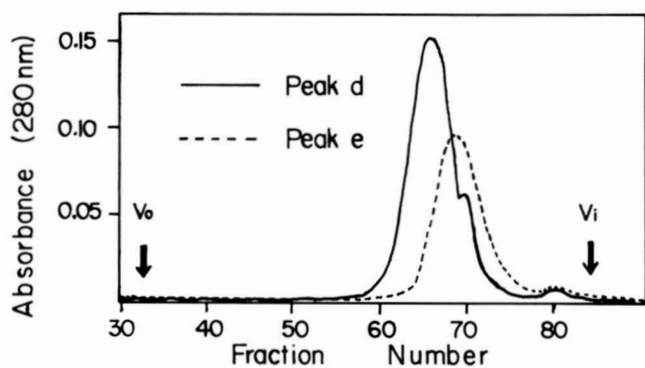
Fig. 10. Sepharose 4B column chromatography elution profile of the ultracentrifugal peaks in Fig. 7. Peaks d and e were separated by Sepharose 4B column chromatography using as an eluant 0.05 M phosphate, pH 7.2, at a flow rate of 20 ml/hr at 6°C. Eluates were continuously monitored at 280 nm and 4-ml fractions were collected. V_o and V_i indicate the elution volume of Blue Dextran 2000 and L-tyrosine, respectively.

maintenance of particle stability since other amphiphiles may take this role. This notion is in keeping with the results on the formation of apoA-I/apoA-II hybrids previously reported by this laboratory (17, 33). Those studies showed that, in the mature canine HDL particle, stability can be maintained by either apoA-I, human apoA-II, or mixtures of both. It is interesting that below 10 mg/ml plasma, the Triton WR-1339-containing HDL retained spherical shape in the face of an increase in size, suggesting that under relatively low Triton WR-1339 concentration, there is a mass addition of detergent to the lipoprotein particle, in excess to the mass contributed by naturally occurring apoA-I. However, when Triton WR-1339 is incubated with HDL at a concentration of 10 mg/ml plasma, there is a gross disruption of the HDL structure due to the formation of mixed lipid-detergent micelles readily separable from apoA-I. Thus, depending upon
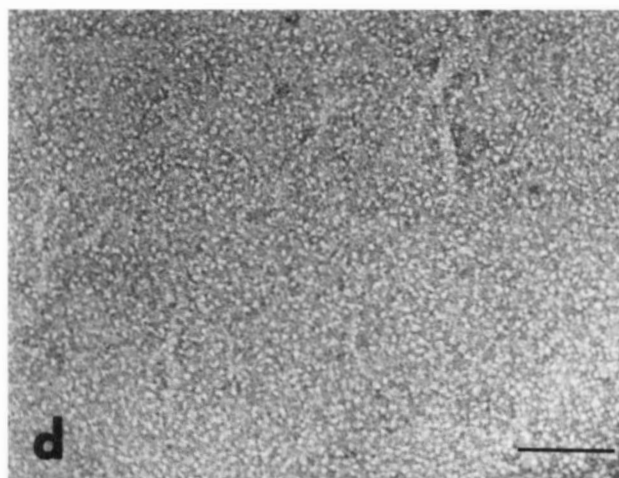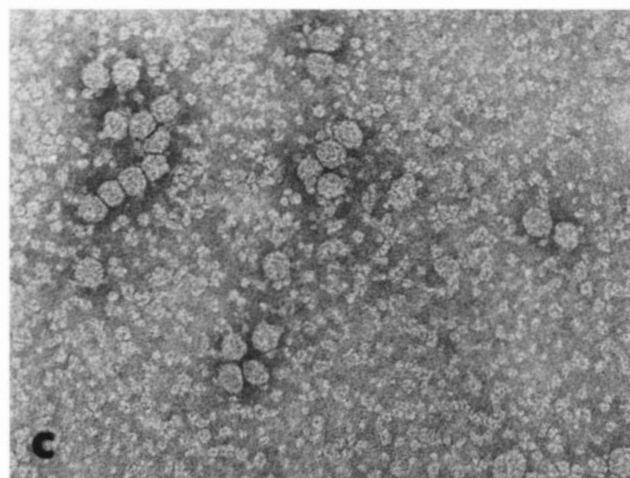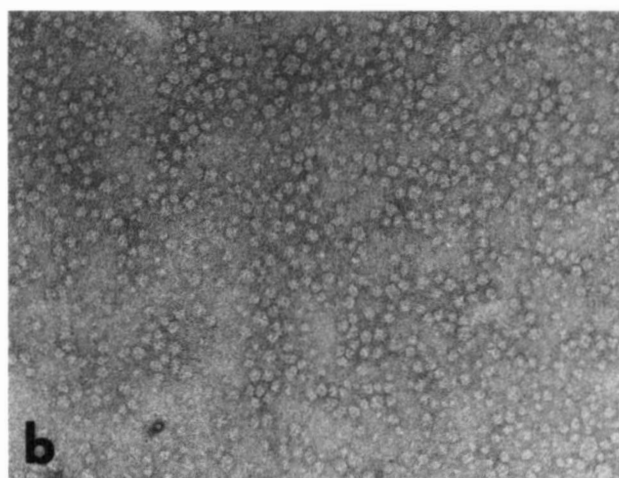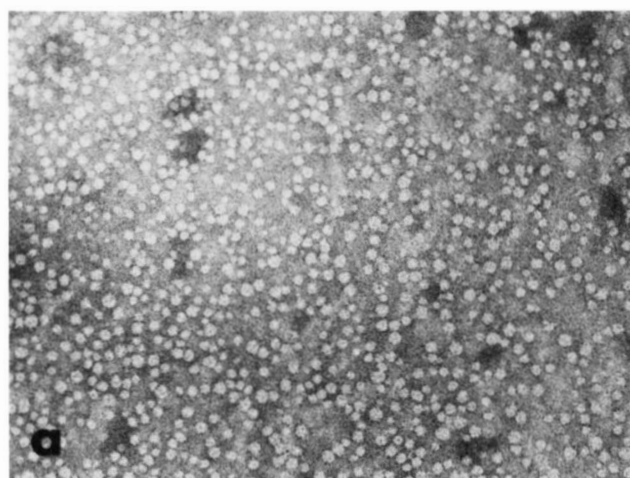

Fig. 11. Electron micrographs of negatively stained preparations of canine HDL before and after incubation with Triton WR-1339. The preparations examined were obtained by density gradient ultracentrifugation (see Fig. 7). Each peak was dialyzed against 0.005 M NH$_4$HCO$_3$, pH 8.2, applied to a thin carbon film supported on a copper grid and negatively stained with 1% sodium phosphotungstate, pH 7.0. Bar = 1000 Å. a, HDL-control (2 mg protein/ml), 95 ± 5 Å* (*, SD, 100 particles were measured); b, HDL + Triton (2 mg/ml), 114 ± 7 Å; c, HDL + Triton (5 mg/ml), 50 to 250 Å; d, HDL + Triton (10 mg/ml), amorphous pattern and small particles, average 50 Å.

its concentration, Triton WR-1339 can either act as a surface stabilizer of HDL or penetrate the HDL particle and cause its disruption. In this context, we would like to acknowledge that Portman et al. (9) reported no effect of Triton WR-1339 on the HDL of the squirrel monkey. Based on our results and as also suggested by those authors, it is likely that the mass ratio of detergent to lipoprotein was too low to elicit an action.

Overall, our studies show that Triton WR-1339 provides a useful probe for the study of the structure of HDL and for the production of HDL derivatives of defined physico-chemical properties. Moreover, the knowledge of the properties of the Triton WR-1339–HDL complexes in vitro should provide a better basis for understanding the effect of Triton WR-1339 in vivo. Triton WR-1339 has been shown to produce important changes in plasma lipoproteins when injected intravenously into animals (10–12). Because HDL plays a key role in the many lipoprotein interconversions occurring in plasma (34) and in view of the apparent high affinity of Triton WR-1339 for the lipoprotein particles, we may postulate that one of the primary events, if not the primary event, in Triton WR-1339-induced dyslipoproteinemia is at the level of HDL. The replacement of apoA-I by Triton WR-1339 at the HDL surface could lead to an inability by this lipoprotein to act as an acceptor of the surface lipids generated by the lipolysis of the triglyceride-rich particles, decrease the efficiency of the LCAT-cholesteryl ester transfer/exchange system, and also affect the uptake of HDL by tissues. Since HDL has also been implicated in the cellular efflux of cholesterol (35), an impairment of this process may also be anticipated. All of these possibilities are amenable to experimental testing.▣

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