In Vitro and In Vivo Interactions of Triton 1339 with Plasma Lipoproteins of Normolipidemic Rhesus Monkeys

Preferential Effects on High Density Lipoproteins

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Triton WR-1339 was incubated in vitro in various proportions with plasma from normolipidemic rhesus monkeys or with ultracentrifugally purified lipoproteins, and the products were examined by isopycnic density gradient ultracentrifugation, agarose column chromatography, electrophoretic and immunochemical techniques, and electron microscopy. Some experiments used apo A-I, apo A-II, or Triton labeled with either $^{125}$I or $^{131}$I. At concentrations of less than 10 mg/ml plasma, Triton interacted preferentially with HDL, changing lipoprotein size and density; Triton was progressively incorporated into the HDL particles, displacing apo E, apo A-I, and apo A-II. At concentrations above 10 mg/ml plasma, Triton displaced all apo A-I from the particle, and much lipid was dissolved into the Triton micelles. When Triton-treated HDL particles were used as a substrate for the enzyme LCAT, enzyme activity decreased in parallel to the displacement of apo A-I. There was no displacement of apo B from LDL nor any loss of lipids; but the particles became deformed and formed rouleaux.

A single intravenous dose of Triton WR-1339 administered to a normolipidemic monkey (N) and to a hypercholesterolemic monkey (H) resulted in concentration-dependent HDL changes similar to those observed in vitro. LDL was less affected by Triton, with changes occurring only at high doses. After these structural changes, intravenously injected $^{131}$I apo A-I disappeared rapidly from the circulation; $^{125}$I apo A-II disappeared less rapidly. These increased clearances were accompanied by a drop in apo A-I plasma levels and the disappearance of HDL particles from plasma. The lipoprotein and apolipoprotein patterns returned to normal 14 days after Triton.

We conclude that Triton WR-1339, when exposed to rhesus plasma in vitro or in vivo, interacts preferentially with HDL in a dose-dependent manner. At low concentrations, Triton acts on surface components of the HDL particle; at higher concentrations, Triton penetrates the particle, causing structural disruption. Because of its high affinity for HDL, Triton WR-1339 is a useful reagent for study of HDL structure-function relationships. (Arteriosclerosis 4:418-434, July/August 1984)
sus monkey plasma and lipoproteins using a combination of physical, chemical, and immunological methods, aided by the use of radioactive compounds. We also followed the changes in plasma lipoproteins and apolipoproteins in their progression and regression phases following a single intravenous administration of Triton WR-1339 into one normocholesterolemic monkey and one animal made hypercholesterolemic by the administration of a diet containing 25% coconut oil and 2% cholesterol. The results obtained are the subjects of this report. It will be shown that there is a close parallel between plasma lipoprotein changes induced by Triton in vivo and in vitro.

**Methods**

**Materials**

For the in vitro studies, blood was collected by venous puncture into tubes containing 0.1 M EDTA as an anticoagulant from two fasting, healthy male rhesus monkeys weighing 13 to 15 kg, which were fed regular Purina Chow. Their plasma cholesterol levels were between 108 and 130 mg/dl and the triglycerides, between 18 and 42 mg/dl. For the in vivo studies, two male adult rhesus monkeys (body weight 13 to 15 kg, 13 years old) were used. One was fed a regular Purina Chow diet (Ralston Purina Chow Company, St. Louis, Missouri), the other, a diet of 70.5% modified, low fat Purina Primate Chow supplemented with 25% coconut oil, 2% cholesterol, 1% vitamin mix, and 1.5% gelatin. These monkeys were maintained on their respective diets for a period of 6 months. All of the experiments conformed with institutional guidelines on animal care.

The monkeys fasted at least 16 hours before the experiments began. They were sedated with 5 to 7 mg Ketaset i.m. (Ketamine HCl, Bristol Laboratories, Syracuse, New York) per kg of body weight. The iodinated homologous apolipoprotein A-I and A-II (each 20 μCi) were injected 30 minutes before injection with a 20% solution of Triton WR-1339 (Tyloxapol, Sigma) in 0.05 M phosphate buffer, pH 7.2 (60 mg/kg/body weight). This dose of Triton was purposely chosen because it was smaller than those previously used in experimental animals;2-4, 13 in preliminary experiments, this dose was sufficient to induce physicochemical changes in plasma lipoproteins without causing hemolysis. We conducted three independent experiments in each monkey at approximately 3-month intervals. In each case, we obtained similar results on plasma lipoproteins, apolipoproteins, and lipids. Thus, only representative experiments will be described.

Blood samples (4 ml) were withdrawn from the femoral vein immediately before and after intravenous injection of Triton at 0, 15, 60, 180, and 270 minutes and at 1, 3, 7, 10, and 14 days. The samples were collected in 0.1 M EDTA and the plasma was rapidly separated by low speed centrifugation (5000 g, 4°C).

**Incubation Conditions In Vitro**

Triton WR-1339 was dissolved in 0.05 M phosphate buffer (pH 7.2). Rhesus plasma was incubated with Triton in 10:1 ratio (vol/vol) so that the incubation mixture contained Triton at concentrations ranging from 0 to 10 mg/ml. In incubations of Triton with separated lipoproteins, the protein concentration was adjusted to be similar to the HDL concentration in whole plasma/Triton mixtures. The incubation was conducted at 37°C for 2 hours based on the results of preliminary observations.

**Ultracentrifugal Flotation**

Low density lipoproteins (LDL) of d = 1.019 to 1.063 g/ml and high density lipoproteins (HDL) of d = 1.063 to 1.21 g/ml were separated by ultracentrifugal flotation and washed free of other plasma proteins according to procedures established in this laboratory.17, 18 They were extensively dialyzed at 4°C against 0.15 M NaCl, 10⁻³ M EDTA (pH 7.2) before use.

**Density Gradient Ultracentrifugation**

After incubation, the reacted mixture was separated by a single-step density gradient ultracentrifugation using a technique previously described.19 The effluents were continuously monitored at 280 nm in an ISCO UA-5 (Instrumentation Specialty Company, Omaha, Nebraska) and collected as 0.4 ml fractions.

**Quantitative Immunoassay of Apoproteins**

Purified rhesus apo A-I and A-II were obtained as previously described.20 Rhesus apo B was obtained from normal rhesus serum according to the method of Karlin et al.21 Rhesus apo E was purified from hypercholesterolemic rhesus apo very low density lipoprotein (VLDL) by high performance liquid chromatography.21

Antisera against either apo A-I, B, or E were raised in New Zealand rabbits by injecting 100 μg of each antigen emulsified in Freund's adjuvant intramuscularly three times at intervals of 2 weeks. The first injection contained complete Freund's adjuvant; the other two, incomplete Freund's adjuvant. The immunoassays for rhesus apo A-I, B, and E were carried out by the rocket immunoelectrophoretic procedure described by Laurell.22 For plasma samples and lipoproteins containing Triton, no specific treatment was necessary to insure maximal antigenicity. In the samples not containing the detergent, apo A-I was determined in the presence of 7 M urea; apo B and E were determined in the presence of 0.1% Triton X-100.

**Radiolodination of Apo A-I, Apo A-II, and Triton WR-1339**

Apo A-I and A-II were iodinated with either ¹²⁵I or ¹³¹I using the lactoperoxidase method.24 The radiolabeled protein was separated from unreacted ¹²⁵I NaI.
or $^{131}$I Nal in a column packed with Sephadex G-75 superfine.

Triton WR-1339 in 0.05 M phosphate buffer (pH 7.2) was labeled with $^{125}$I by the method of McFarlane. Free iodine was removed from the radiolabeled protein by passage through a Sepharose 4B column.

**Radiolabeling of HDL In Vivo**

Thirty minutes after the intravenous injection of 30 μCi of both $^{131}$I apo A-I and $^{125}$I apo A-II for a rhesus monkey, blood was obtained. HDL of $d = 1.063-1.21$ g/ml containing the two radioactive apoproteins was separated by ultracentrifugal flotation and dialyzed against 0.15 M NaCl, 10⁻³ M EDTA (pH 7.2) before use.

**Molecular Sieve Chromatography**

Gel filtration of the incubated mixtures of lipoproteins and Triton WR-1339 was conducted in glass columns (2.5 x 70 cm) packed with Sepharose 4B (Pharmacia Fine Chemicals, Upsala, 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.

**Electron Microscopy**

Fractions from single spin ultracentrifugation were negatively stained with 1% sodium phosphotungstate (pH 7.0) after deposition onto a carbon film-coated 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. Average diameters in Å were obtained from the sizing of 100 particles.

**Chemical Analysis**

Before analysis, all samples were extensively diazylized against 0.005 M NH₄HCO₃ buffer (pH 8.2). The protein content was determined by the Lowry procedure, modified to include the addition of 0.5% sodium dodecyl sulfate to reagent A. Total and free cholesterol were determined enzymatically according to a slight modification of the procedure of Allain et al. Lipid phosphorus was measured according to the method of Bartlett.

Total triglycerides were determined in a Technicon Autoanalyzer II after isopropanol extraction of Zeolite-treated samples. Triton was measured after extraction with 10 volumes of isopropanol at 278 nm. Electrophoretic separation of apoproteins was performed in polyacrylamide gels containing 8 M urea or 0.1% SDS. Agarose gel electrophoresis was carried out on Agarose Universal Electrophoresis film (ACI-Corning, Palo Alto, California). After electrophoresis, lipoproteins were fixed and stained with either Fat Red 7B or Amido Black 10B.

**Gradient Gel Electrophoresis**

Gradient gel electrophoresis was carried out on a Pharmacia Electrophoresis Apparatus GE-4 loaded with gradient gels PAA 4/30 at 14°C at 125 V for 20 hours. Following electrophoresis, the gels were fixed and stained overnight in 0.04% Coomassie Brilliant Blue G-250 in 3.5% perchloric acid followed by destaining in 5% acetic acid. Molecular weight standards were run in each gel slab.

**Determination of LCAT Activity In Vitro**

LCAT activity was determined by the modified method of Stokke and Norum.

**Reagents**

All chemicals were reagent grade. Triton WR-1339 was purchased from Sigma Chemical Company, St. Louis, Missouri. Thyroglobulin, Catalase and Aldolase (Pharmacia Fine Chemicals, Piscataway, New Jersey) were used as the molecular weight standards. Na$^{125}$I and Na$^{131}$I (carrier-free) were purchased from Amersham Corporation, Arlington Heights, Illinois. Radioactivity measurements were carried out in a Tracor Analytic Model 1190 automatic gamma-counter (Tracor Analytic, Elk Grove Village, Illinois).

**Results**

**In Vitro Studies**

**Effect of Triton on Whole Plasma, HDL and LDL: Analyses by Agarose Gel Electrophoresis**

The effect of Triton was dose-dependent. In the case of whole plasma (Figure 1 A), as Triton increased in concentration, the lipid-stained band in the α₁ position decreased in mobility; at a Triton concentration of 5 mg/ml, the plasma remained close to the origin. The gels stained with Amido Black 10B (protein stain) showed that the α₁ band decreased in mobility to the β region (Figure 1 A, B), a region where purified A-I was found to move in other experiments (data not shown). When $^{125}$I Triton was used as a tracer, the peak of radioactivity at 5 mg was in the same area as the lipid band (Figure 2) indicating that Triton had caused a dissociation of the lipids from the serum carriers and particularly from the α₁ lipoproteins. This interpretation was supported by the studies of Triton-HDL mixtures (Figure 1 B) showing that the detergent caused the lipid-stained band to move away from the HDL protein. At 10 mg of Triton, this band was behind the origin, whereas the protein band was in the α₁ position. Contrary to HDL, the LDL band showed only a decrease in electrophoretic mobility even at high concentrations of Triton (Figure 1 C).
Figure 1. Agarose gel electrophoretic patterns of whole plasma (A), HDL2 (B), and LDL (C) after incubation with various concentrations of Triton WR-1339 for 2 hours at 37°C; HDL2 (3 mg protein/ml), and LDL (2 mg protein/ml). After incubation, 3 µl of each sample was applied in duplicate to the agarose film. For details see text. After electrophoretic separation, one sample was stained with Fat Red 7B and the other with Amido Black 10B. Lip = lipid staining; prot = protein staining; o = origin. The arrows indicate the position of the new band behind the α1 region.

Isopycnic Ultracentrifugal Profiles of Rhesus Plasma following Incubation with Triton

As shown in Figure 3, Triton in the concentration of 2 to 10 mg/ml of plasma caused a marked change in the 280 nm single-spin profile. The band corresponding to HDL (Peak a) was progressively shifted to lighter densities with an attendant increase in absorbance readings at 280 nm due to both Triton (maximal absorbance of Triton, 278 nm) and protein. We have named these bands: b (2 mg Triton); c,d (5 mg Triton); and e,f,g (10 mg Triton) with buoyant densities varying between 1.063 and 1.131 g/ml (Table 1).

Figure 2. Electrophoretic distribution of 125I-labeled Triton after incubation with whole plasma. Tracer amounts of 125I-labeled Triton were mixed with unlabeled Triton in the stated concentrations. After incubation with whole plasma, 3 µl aliquots of each mixture were applied in duplicate to agarose films. One sample was stained with Fat Red 7B, the other was cut into 0.5 cm segments and counted.
Figure 3. Distribution of apo A-I, apo E, apo B, $^{125}$I-labeled Triton, $^{131}$I-labeled apo A-I, and $^{125}$I-labeled apo A-II in ultracentrifugal fractions of plasma incubated with various concentrations of Triton (2 hours, 37° C). The labeling of plasma with $^{131}$I-apo A-I or $^{125}$I-apo A-II was carried out in vivo (see Methods). One milliliter of each incubated sample was separated by single-spin density ultracentrifugation. The effluents were continuously monitored at 280 nm in an ISCO UA-5 monitor and 0.4 ml fractions were collected for a total of 32 fractions. The concentrations of apo A-I, apo E, and apo B were quantified by electroimmunoassay. The amount of radioactivity in each tube was determined. The pooled HDL fractions were arbitrarily named from a through g (a = fraction number 18–26; b = 15–23; c = 11–18; d = 23–26; e = 11–18; f = 19–22; g = 23–26).

Table 1. Physicochemical Properties of the Main Peaks (a–g) Shown in Figure 3

<table>
<thead>
<tr>
<th>Peak</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
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<tbody>
<tr>
<td>Diameter (Å)</td>
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<td>116</td>
<td>124</td>
<td>72</td>
<td>140</td>
<td>82</td>
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<td>Hydrated density (g/ml)</td>
<td>1.115</td>
<td>1.089</td>
<td>1.063</td>
<td>1.140</td>
<td>1.063</td>
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<td>12</td>
<td>50</td>
<td>6</td>
<td>20</td>
<td>35</td>
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<td>Total cholesterol</td>
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<td>14</td>
<td>13</td>
<td>5</td>
<td>8</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>33</td>
<td>32</td>
<td>26</td>
<td>4</td>
<td>14</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Triton</td>
<td>0</td>
<td>28</td>
<td>49</td>
<td>41</td>
<td>70</td>
<td>69</td>
<td>57</td>
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</table>

Diameter was obtained by gradient gel electrophoresis.
From the analysis of the 0 mg Triton column in Figure 3, it is evident that authentic HDL contained essentially apo A-I and apo A-II, little apo E, and no apo B, and that Triton banded in the same density range as HDL. At 2 mg of Triton, there was some displacement of apo A-I from HDL, whereas all of the apo E was in the bottom fraction. There were no detectable effects on apo B. At 5 mg of Triton/ml plasma Peak C contained neither apo A-I (which was all found in Fraction d) nor apo E, and consisted of Triton, apo B, and apo A-II. At 10 mg Triton/ml plasma, three fractions were generated: Fraction e contained Triton and apo B, while Fractions f and g contained apo A-I, apo A-II, and Triton. In terms of apoproteins, Fraction f contained mainly apo A-II and Fraction g contained apo A-I. Thus, there was a marked difference in the effect of Triton on apo A-I, apo A-II, apo E, and apo B as a function of the amount of detergent added to plasma. Except for apo B, this detergent displaced the apolipoproteins for the HDL surface, although to a different degree.

The polypeptide distribution of Fractions a–g of the single spin profiles of Figure 3 as assessed by SDS and 8 M urea-polyacrylamide gel electrophoresis corroborated the data obtained by the immunochromic or radioactivity measurements (Figure 4). The general properties of Fractions a–g are shown in Table 1. Addition of Triton up to 2 mg/ml caused an increase in the size of the HDL-Triton complex which retained the same lipid content and composition as authentic HDL although undergoing a progressive loss of apoproteins (see data on Peaks a, b, and c). The other peaks (d–g) contained mainly Triton and apoproteins. More than 90% of the Triton that was present in the ultracentrifuged plasma was recovered in the major and minor peaks. By electron microscopy (Figure 5), the control HDL had a diameter of 105 ± 10 Å, and Peak b contained spherical particles with a diameter of 125 ± 15 Å. The particles corresponding to Peak e were heterogeneous and ranged in size from 50 to 220 Å; those of Peak g had an average diameter of 80 ± 8 Å.

Studies by Molecular Sieve Chromatography

As shown in Figure 6, the elution profile of HDL incubated with Triton yielded two major peaks labeled I and II. Peak I eluded before, and peak II after, the original HDL. The elution time of the Triton micelle peak almost coincided with that of Peak II. With the increment of Triton concentration, Peak II exhibited a higher absorbance reading at 280 nm; at the concentration of Triton of 5 to 10 mg/ml, it exceeded that of Peak I. The composition of these peaks varied according to the amount of Triton in the system (Figure 6). In each case, Peak 1 contained less Triton than Peak II. In the extreme case examined (10 mg Triton), about 80% Triton was present in the complex. When Peaks c and d from the ultracentrifugal experiments shown in Figure 3 were applied to this column, they eluted in the regions of Peaks I and II respectively.

![Figure 4](http://atvb.ahajournals.org/)

Figure 4. Polyacrylamide gel electrophoretic patterns of the ultracentrifugal peaks from the experiments summarized in Figure 3. After dialysis against 0.005 M NH₄HCO₃ buffer (pH 8.2), 100 μl of each sample was separated by SDS (1) or 8 M urea (2) polyacrylamide gel electrophoresis.
Electron micrographs of negatively stained preparations of incubated mixtures of HDL-Triton WR-1339 separated by density gradient ultracentrifugation (see Figure 3). The various fractions were dialyzed against 5 mM NH₄HCO₃ (pH 8.0) applied onto carbon film-coated copper grids and negatively stained by a 2% solution of Na phosphotungstate (pH 7.0). A. HDL control (Peak a). B. HDL plus 2 mg/ml Triton (Peak b). C. HDL plus 10 mg/ml Triton (Peak e). D. HDL plus 10 mg/ml Triton (Peak g). Bar = 1000 Å.

Triton Effect on LDL

Figure 7 shows the single spin ultracentrifugal profile of an incubated mixture of Triton and LDL (0–10 mg Triton/2 mg LDL protein). At a concentration of 5 mg/ml, Triton caused the appearance of two major peaks. Peak I contained Triton, protein, and lipids, whereas Peak II contained mainly Triton. By gradient gel electrophoresis, the incubated LDL-Triton complex was increased in size (about 20%) as compared to the control LDL. By electron microscopy, untreated LDLs were mainly spherical, whereas the ones incubated with Triton (2 mg Triton/2 mg LDL protein) appeared flattened with the tendency to form rouleaux. At a higher concentration of Triton (10 mg Triton/2 mg LDL protein), there was a marked decrease of rouleaux figures replaced by circular structures embedded in a uniform background of small granular elements of an unknown nature (Figure 8). The mean diameter of the control particles was 243 ± 10 Å and for the deformed particle 180 × 220 Å; in the presence of 2 mg Triton, the average diameter of the spherical particles was 285 ± 15 Å and for the flattened LDL, 330 ± 25 × 170 ± 25 Å. In the presence of 10 mg Triton, the spherical particles had a diameter of 241 ± 40 Å.

Effect of Triton on LCAT In Vitro

Triton WR-1339 caused an inhibition of LCAT. As shown in Figure 9, the activity of the enzyme in plasma decreased as a function of the concentration of the detergent. At a concentration of 1 and 2 mg Triton/ml plasma, LCAT activity was less than 30% and 15% of the control, respectively.
In Vivo Studies

Structural Changes of Lipoprotein Attending the Intravenous Injection of Triton WR-1339

Figures 10 and 11 show the changes in the single spin profile and distribution of iodinated apo A-I, apo A-II, and Triton as well as the concentration of apo A-I, apo B, and apo E before and 15 minutes after the injection of Triton (60 mg/kg body weight) in the normocholesterolemic (N) monkey and the hypercholesterolemic (H) monkey.

In the N monkey (Figure 10), the two major subsets of HDL (d = 1.080 g/ml, and d = 1.107 g/ml) shifted to a lighter density (d = 1.074 g/ml and d = 1.086 g/ml) and were increased in concentration as assessed by absorbance readings at 280 nm. However, the 280 nm peak comprised both the HDL protein and Triton since Triton when studied alone had a maximal absorbance at 278 nm. Moreover, 37% of apo A-I was found in the bottom fraction and had the same specific activity as the apo A-I in
Figure 7. Single-spin density ultracentrifugal profile and chemical analysis of incubated mixtures of LDL and Triton. Preparations of rhesus LDL (2 mg protein/ml) were incubated with various concentrations of Triton (final concentration of Triton; 0, 2, 5 and 10 mg/ml) for 2 hours at 37°C. The samples were separated by single-spin density gradient ultracentrifugation. After separation, the effluents were continuously monitored at 280 nm and 0.4 ml fractions. The two main peaks labeled I and II were analyzed for chemical composition and electron microscopy.

Figure 8. Electron micrographs of negatively stained preparations of incubated mixtures of LDL and Triton WR-1339. The samples were obtained by density gradient ultracentrifugation (see Figure 7). The samples were dialyzed against 5 mM NH₄HCO₃ buffer, (pH 8.0) applied onto carbon film-coated copper grids and negatively stained with a 2% solution of Na phosphotungstate (pH 7.0).  A. LDL control.  B. LDL plus 2 mg/ml Triton.  C. LDL plus 10 mg/ml Triton.  Bar = 1000 Å.
In vitro effect of Triton on LCAT activity of whole plasma. After preincubation of whole plasma (100 μl) with albumin-3H-labeled cholesterol emulsion (30 μl) for 4 hours, various concentration of Triton (30 μl) were added to plasma and the mixtures were incubated for 2 hours at 37°C. The reaction was started by adding 20 μl of 0.1 M mercaptoethanol solution and incubating for 1 hour at 37°C. The LCAT activity is given as the percentage of labeled cholesterol acylated per hour.

HDL. In turn, radioactive apo A-II was only detected in the HDL fraction. Apo E, which in the control sample was distributed between VLDL, intermediate density lipoprotein (IDL), and LDL, was totally shifted to the bottom fraction after Triton injection. No change in density distribution and concentration was found associated with apo B. As shown in Table 2, the chemical analysis of pooled HDL from the single spin fractions before and after injection in the N monkey revealed a decrease in protein, the presence of Triton and no change in the lipid components. As assessed by gradient gel electrophoresis (Figure 12), Triton caused an increase in the size of the HDL particles. This finding was corroborated by electron microscopy studies showing that after Triton the HDL particles were larger and more heterogenous (before Triton a pooled HDL sample had an average diameter of 95 ± 5 Å and after Triton 180 ± 40 Å). Polyacrylamide gel electrophoretic analyses in SDS and urea indicated a relative decrease of apo A-I compared to apo A-II (data not shown).

In the H monkey (Figure 11), the single spin profile before Triton showed a single broad LDL and a single dense HDL peak before Triton injection. After Triton, the HDL peak (d = 1.115 g/ml) shifted to a lighter density (d = 1.093 g/ml). The apoprotein redistribution seen in the N monkey was also seen with the H monkey. The physicochemical changes in the properties of HDL induced by Triton were similar in the N and H monkeys except that in the latter, the plasma concentrations of apo B and apo E were higher.

Progression and Regression of Plasma Changes with a Single Intravenous Administration of Triton

Apolipoproteins, Lipids, and Triton in Whole Plasma. As shown in Figure 13, the single intravenous injection of Triton into either the N or the H monkey caused a rapid fall of plasma apo A-I. This fall was particularly dramatic in the H monkey, where the apo A-I was no longer detected 1 day after the injection and was still absent 3 days later. By the seventh day, there was partial restoration of the apo A-I levels; these were back to the pre-Triton range after 14 days. Although the changes were less marked, apo A-I levels reacted similarly in the N monkey.

The changes in apo B and apo E were also more pronounced in the H than in the N monkey. For both apoproteins, the peak maximum was observed 1 day after the injection and returned to normal after 14 days. Elevation of serum cholesterol and triglycerides was also observed in the H monkey showing a marked inverse relationship with the drop in serum.

Table 2. Physicochemical Parameters of HDL Before and 15 Minutes After Injection of Triton

<table>
<thead>
<tr>
<th></th>
<th>Normolipidemic monkey</th>
<th>Hyperlipidemic monkey</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>15 min after</td>
</tr>
<tr>
<td>Diameter (Å)</td>
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<tr>
<td>Hydrated density (g/ml)</td>
<td>1.080, 1.107</td>
<td>1.074, 1.086</td>
</tr>
<tr>
<td>Chemical composition (weight%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>43</td>
<td>25</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>33</td>
<td>31</td>
</tr>
<tr>
<td>Triglyceride</td>
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<td>3</td>
</tr>
<tr>
<td>Triton</td>
<td>0</td>
<td>21</td>
</tr>
</tbody>
</table>

The analyses refer to a mixture of the two HDL subsets. The size of the particles was obtained by gradient gel electrophoresis.
apo A-I levels. The Triton plasma levels immediately after injection into the N and H monkeys were 1.46 and 1.39 mg/ml plasma, respectively. The levels of the detergent decreased at a much faster rate in the H monkey than in the N monkey. Overall, it took 2 weeks before the plasma was free of detergent and returned to normal.

Electrophoretic and Ultracentrifugal Changes. When post-Triton plasma was applied to 1% agarose films (Figure 14), the α, band stained by Fat Red 7B disappeared within 15 minutes from the injection. After 1 day, the lipid-stainable band was in the β region. After 1 week the α, band reappeared, and 2 weeks later the electrophoretic profile returned to its original pattern. Similar changes were observed with the H monkey except that after 1 day, a marked lipid-stainable material was seen between the origin and the β region. After 2 weeks, the pattern returned to normal. This total reversibility as a function of time was supported by the single spin ultracentrifugal

![Graph](image-url)

**Figure 10.** Alterations in density gradient profile and distribution of iodinated apo A-I, apo A-II, and Triton, and the concentration of apo A-I, apo B, and apo E before and 15 minutes after injection of Triton (60 mg/kg body weight) in the normocholesterolemic (N) monkey. Plasma (1.0 ml) was applied to a single-step density gradient and spun to isopycnic equilibrium (66 hours, 39,000 rpm, 15°C). The density profiles were all recorded at 280 nm. Each tube was monitored for radioactivity. Immunoquantification of apoproteins was performed on every other tube.
The ultracentrifugal profiles of the N monkey showed that the changes induced by Triton affected the HDL peak and that the changes were already prominent 15 minutes after Triton administration and reverted to the pre-Triton pattern after 2 weeks. As shown by the data in Figure 10 and our current studies in vitro, the observed density changes were caused by the incorporation of Triton into the HDL particles and also by the displacement of apo E and apo A-I to the bottom fraction. In the H monkey, the changes were more dramatic; after 1 day there was a marked elevation of the VLDL-IDL peak, whereas there were no peaks in the areas corresponding to LDL and HDL. After 14 days, the pattern was similar to that seen before Triton injection.

**Clearance of Iodinated Apo A-I, A-II and Triton from Plasma Before and After Injection of Triton WR-1339**

The results of these studies are shown in Figure 16. The disappearance curves of intravenously injected iodinated apo A-I, apo A-II, and Triton were bi-exponential. After 24 hours, about 90% of the radioiodinated apo A-I had disappeared from the...
Figure 12. Polyacrylamide gradient gel electrophoresis of pooled HDL obtained from the single-spin ultracentrifugation studies reported in Figures 10 and 11. 100 μl of HDL was applied to gradient gels PAA 4/30 and electrophoretic separation was carried out at 14°C at 125 V for 20 hours. a = pooled HDL of the hypercholesterolemic (H) monkey before injection (tubes 19–25); b = pooled HDL of the H monkey 15 minutes after injection (tubes 16–22); c = pooled HDL of the normocholesterolemic (N) monkey before injection (tubes 16–24) and d = pooled HDL of N monkey 15 minutes after injection (tubes 13–21). The average size of the particles was calculated from the calibration curve of reference proteins. Their radii were: thyroglobulin (85.0 Å), apoferritin (61.0 Å), lactate dehydrogenase (40.8 Å), and bovine serum albumin (35.5 Å).

Figure 13. Time changes in the levels of plasma apo A-I, Triton, apo B, apo E, total cholesterol, and triglycerides after a single intravenous injection of Triton (60 mg/kg) into the normocholesterolemic (N) and the hypercholesterolemic (H) monkey.
plasma of the H monkey compared to about 50% in the N monkey (A). This was also the case with apo A-II (B). The $t_\frac{1}{2}$ value of radiolabeled apo A-I from the die-away curve during the first 270 minutes after the Triton injection was 13 hours in the N monkey and 6 hours in the H monkey. For apo A-II, the $t_\frac{1}{2}$ value was 24 hours for N and 8 hours for H. From the slow phase of the curve of the specific activity values, the $t_\frac{1}{2}$ of apo A-I was 3.8 days and that of apo A-II, 4.8 days. Similar values were obtained after Triton. When radiolabeled Triton was injected in tracer amounts during the first day (C), its clearance was faster in the H monkey than in the N monkey. After 3 days, about 90% of the injected Triton had disappeared from the plasma of the H monkey as compared to 60% in the N monkey. Between 3 days and 14 days, the slopes of the curves were comparable, with an estimated $t_\frac{1}{2}$ of 4.1 days.

Discussion

By combining isopycnic density gradient ultracentrifugation, column chromatography, electrophoresis, electron microscopy, immunochemical and radiolabeling techniques, we showed that when plasma of rhesus monkeys interacts in vitro with the nonionic detergent Triton WR-1339 above the critical micellar concentration (cmc) of this detergent (unpublished data), changes in both LDL and HDL particles occurred. However, within the concentration ranges of Triton used, the effects on HDL were more marked and began to occur at low detergent levels (1–2 mg Triton/mg HDL protein). At these levels, there was a progressive replacement of apo A-I and apo E by the detergent at the HDL surface without a detectable effect on the lipids or the other apolipoproteins, particularly apo A-II. Even at higher concentrations of Triton, this apoprotein was more resistant to displacement by Triton than apo A-I, an observation which is in keeping with the previously reported differences in affinity of these two apolipoproteins at the HDL surface. The mass of Triton added to the HDL particles caused an increase in their particle size until the detergent mass reached a level at which the lipids separated into the detergent micellar phase from the protein moiety. Thus, depending on concentration, Triton could act either as a stabilizer of the lipoprotein particle by substituting for apo A-I at its surface or cause disruption by competing for the lipids normally interacting with the apoproteins. At this stage, mixed micelles of detergent and lipids were formed which could absorb apo A-I at their surface, generating a Triton lipid-apoprotein complex. However, not all the apo A-I displaced from the HDL surface followed this fate; some may have associated with detergent monomers as described by Reynolds and Simon for the interaction of human apo A-I and A-II with the anionic detergent sodium dodecyl sulfate and as Makino et al. described cationic detergent tetradeccyltrimethyl ammonium ions. The displacement of apo A-I from the HDL surface by Triton may partially account for the results of our LCAT studies, which showed an inverse relationship between enzymatic activity and the amount of apo A-I displaced from the surface in keeping with previous studies by this laboratory and in vivo studies in the rat.

The in vivo studies also showed a great affinity of Triton WR-1339 for the HDL particles. The single intravenous injection of the detergent into either a normolipidemic rhesus monkey or a monkey which was made hypercholesterolemic by an atherogenic diet was followed by important structural changes in these lipoproteins, which were dependent upon the levels of Triton in the plasma. These in vivo changes were both quantitatively and qualitatively similar to those elicited by the detergent when incubated in vitro with whole plasma, at least within the limits of the initial plasma levels of Triton produced by the injection of 60 mg/kg Triton. We avoided injecting intravenous doses of the detergent above 60 mg/kg to avoid red blood cell hemolysis. Scanu et al. reported that in the dog, Triton has a hemolytic action on canine red blood cells in vitro and in vivo.

As to the preferential effect of Triton WR-1339 on HDL, Portman et al. reported an immediate (after 5 minutes) 70% drop in HDL plasma levels of $d = 1.063$ to 1.21 in squirrel monkeys injected intravenously with Triton. Moreover, a disruptive action of
Triton WR-1339 on HDL in the rat was reported by Ishikawa and Fidge\textsuperscript{15} after a single intravenous injection of Triton (250 mg/kg). As in our studies, the most striking biochemical lesion induced by Triton was the dissociation of apo A-I from the HDL particles, the extent of which was dependent on the plasma concentrations of Triton. Since apo A-I dissociation also occurs in the dog (unpublished observations), it is probable that such a dissociation may have occurred in all of the previous studies where Triton was used in doses between 300 mg/kg and 500 mg/kg/body weight to produce a hyperlipidemic state.\textsuperscript{2,4,5,10,13,15} Based on calculations of the ratio between blood volume and body weight, these doses correspond to a detergent level of 8 to 13 mg/ml of plasma, which our results indicate would cause the total displacement of apo A-I from HDL. The interaction of Triton with HDL had important metabolic consequences indicated by the rapid drop of plasma HDL and the rapid clearance of apo A-I and apo A-II from the circulation. Several factors may have contributed to these events. Possibly once the HDL particles became coated by Triton, they could no longer interact physiologically with other plasma lipoprotein systems, enzymes, or membrane receptors\textsuperscript{16} and so they underwent cellular uptake by nonspecific mechanisms. The involvement of the reticulo-endothelial system in dogs receiving Triton WR-1339 has been documented.\textsuperscript{13} In those studies, a marked accumulation of lipid-laden macrophages in the spleen, liver, lymph nodes, and arteries was observed after the chronic administration of Triton. Still used electron microscopy to demonstrate\textsuperscript{40} the presence of Triton-lipid complexes in the endothelium of the arterial intima in rats and rabbits given several intravenous injections of Triton WR-1339. In our studies, the clearances of apo A-I and apo A-II were more rapid in the animal that was on an atherogenic diet.

![Figure 15](http://atvb.ahajournals.org/)

**Figure 15.** Time changes of the plasma lipoprotein distribution after a single intravenous injection of Triton (60 mg/kg) into normo- and hypercholesterolemic monkeys. One milliliter of plasma was separated by single-spin density gradient ultracentrifugation and the eluates from each tube were continuously recorded at 280 nm.
and we speculate that this diet acted as an activator for the reticulo-endothelial system, favoring the phagocytic process of the Triton-containing HDL. This interpretation is in keeping with the observation of a rapid plasma clearance of radiolabeled Triton in the hypercholesterolemic monkey. Moreover, a structural alteration of HDL may account for the hyperlipidemia following the intravenous injection of Triton. An accumulation of VLDL in animals treated with Triton WR-1339 has been noted in dogs, rats, and squirrel monkeys and has been attributed to abnormalities in VLDL-LDL conversion secondary to changes in HDL structure. The displacement of apo E from the HDL particles may also have contributed to the alteration in HDL catabolism. Ishikawa and Fidge showed an abnormality in apo C-II metabolism in the rat after Triton administration. At higher concentrations, the Triton-induced metabolic abnormalities become more complex because of the potential involvement of the lipolytic enzymes and other plasma lipoproteins. This would be true in animals subjected to repeated injections of Triton. We reason that once HDLs are no longer present in plasma as intact particles, Triton may associate with the other plasma lipoproteins; and that once those sites are saturated, Triton produces structural changes at the cell membrane level.

The structural changes of LDL induced by Triton were small compared with those observed with HDL. This was probably due to the structural characteristics of LDL and the fact that less than 10% of the added Triton associated with LDL. Triton did increase the size and the hydrated density of LDL, but even with the high concentrations of detergent used (10 mg/2 mg of LDL protein), there was no displacement of apo B or lipid from the lipoprotein particle. In early studies on dogs, Scanu and Oriente demonstrated that with a concentration of 40 mg of Triton/mg LDL protein, some disruption of LDL occurs. However, these are exceptionally high doses which can produce hemolysis.

Many years ago, Hirsch and Kellner suggested that the action of Triton WR-1339 was confined to the blood alone and that it somehow "coated" fat particles, thus altering their physiologic and metabolic states. Based on past and current studies in this laboratory and studies by Ishikawa and Fidge, we can state that the early Triton lesion is confined to the HDL class, which is known to play several functional roles in lipid metabolism. In this context, Triton WR-1339 appears to be a very useful probe for studying the structure and function of HDL both in normo- and hyperlipidemic animals.

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