The Distribution of Lipopolysaccharide in Normocomplementemic and C3-Depleted Rabbits and Rhesus Monkeys

John C. Mathison, PhD, Richard J. Ulevitch, PhD, J. Raymond Fletcher, MD, and Charles G. Cochrane, MD

To examine the role of complement (C3) in determining the fate of lipopolysaccharide (LPS) in vivo, the distribution of LPS was studied in normocomplementemic (NC) and C3-depleted animals (pretreated with cobra venom factor [CoF]) after intravenous injection of highly purified, radioiodinated Salmonella minnesota R595 LPS. After injection of a lethal (250 µg) or nonlethal (5 µg) dose of LPS in NC and CoF rabbits and a lethal (5 mg/kg) dose of LPS in rhesus monkeys, the LPS disappeared from blood in a biphasic manner. In all cases, a substantial portion of the dose was removed from blood in an initial disappearance phase (t1/2 < 15 minutes), which, in some cases, was accelerated in CoF-treated animals. LPS remaining in blood beyond 30 minutes persisted with a much increased half-life (>5 hours). Liver contained the major portion (40%) of tissue-bound LPS (determined by use of 125I-BSA blood marker) in animals killed 3–5 hours after injection. The distribution of LPS in rabbits was found to be dose-independent and only minimally changed by prior depletion of C3. In addition, the tissue distribution and cellular localization of LPS in monkeys was similar to that we have reported previously for R595 LPS in NC rabbits and was not substantially changed by prior CoF treatment. These results indicate that binding of C3 to intravenously injected LPS is not required for the initial rapid disappearance from blood. Further, the uptake of LPS by cellular targets, notably the hepatic macrophages (Kupffer cells), is not altered by in vivo decomplementation. (Am J Pathol 1980, 101:245-264)

THE INTRAVENOUS INJECTION of a single dose of highly purified gram-negative bacterial lipopolysaccharide (LPS) in experimental animals has been shown to produce pathophysiologic changes similar to those observed in humans with gram negative sepsis and shock. One experimental approach to define the mechanisms of host-LPS interaction has been to study the distribution of radiolabeled LPS in vivo. Immediately after injection, LPS becomes partitioned between plasma and blood cells (primarily platelets, neutrophils, and mononuclear phagocytes). Within 5 minutes, less than half of the LPS remains in blood and is contained almost exclusively in plasma in a form with markedly reduced molecular weight and buoyant density as a result of combination with high-

From the Department of Immunopathology, Research Institute of Scripps Clinic, La Jolla, California, and the Department of Experimental Surgery, Naval Medical Research Institute, Bethesda, Maryland.

This is publication No. 2049 from the Department of Immunopathology of the Research Institute of Scripps Clinic. Supported by the Office of Naval Research Contract NR 207-027 and by United States Public Health Service Grants AI-15136, AI-07007, and HL-16411.

Accepted for publication May 7, 1980.

Address reprint requests to John C. Mathison, PhD, Department of Immunology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037.

0002-9440/80/1110-0245$01.00
© American Association of Pathologists
density lipoproteins. As we have recently shown, as much as 40% of the LPS dose is taken up by liver and concentrated in phagocytic vacuoles of hepatic macrophages (Kupffer cells).

Factors that are responsible for the binding of LPS to cellular elements are largely unknown. Although the physical and chemical properties of the LPS determine in part its ability to interact with plasma constituents and cells, it is likely that host factors are also important. A number of host plasma components, including coagulation Factor XII, complement, and lipoproteins, have the potential to interact rapidly in the vascular compartment with intravenously injected LPS in what appears to be a highly competitive environment. If binding of LPS to critical targets is requisite for the development of pathophysiologic changes, then factors that influence the distribution of LPS in vivo could play an important role in determining the nature and extent of the endotoxic effects that are produced.

In order to determine whether proteins of the complement system, eg, C3 and the terminal components, play a role in the distribution of LPS in vivo, we examined the clearance, tissue distribution, and cellular localization of a single intravenous dose of LPS in two groups of experimental animals: those that were normocomplementemic and those that were depleted of C3 and terminal components of complement by prior treatment with the anticomplementary protein, cobra venom factor (CoF). These studies were done first in rabbits, and the results may be compared directly with distribution studies we have reported previously for normal rabbits. Additional experiments were performed with rhesus monkeys to examine the role of complement on LPS distribution in a model that may better approximate gram-negative endotoxemia in humans.

The studies described in this report demonstrate that prior treatment of both rabbits and rhesus monkeys with CoF neither interferes with the initial rapid LPS loss from the blood nor affects the tissue distribution and cellular localization of the highly anticomplementary LPS from Salmonella minnesota R595.

Materials and Methods

Experimental Animals

Male New Zealand white rabbits (2–2.5 kg) were obtained from Rancho de Conejo, Vista, California, and maintained on a standard pelleted diet and water. Adult male rhesus monkeys (6.5–7 kg) were also used in these studies.

Lipopolysaccharide

LPS was obtained from Salmonella minnesota R595 by the phenol–chloroform–petroleum ether extraction method of Galanos with a minor modification. Following the final
ether wash the dried R595 LPS was redissolved in 20 mM EDTA (pH 7.5) with a final concentration of 10 mg LPS/ml and subjected to sonic oscillation (Heat Systems–Ultrasonics, Inc., Model W-375 Sonicator) to effect dissolution of the LPS. The resultant LPS solution was first dialyzed against 3 l sterile water for 72 hours with a change of the dialysis bath every 12 hours and then lyophilized. This procedure resulted in an LPS preparation with increased solubility, so that 5 mg/ml solutions were easily prepared by sonication in saline without the necessity of adding triethylamine. The LPS for injection in monkeys was solubilized by sonication in 0.1% triethylamine with 10 mM EDTA, dialyzed against sterile saline, and stored at -20 C. 131I-R595 LPS was prepared by the method of Ulevitch 20 with the chloramine T labeling procedure of McConahey and Dixon. 21 Specific activities of 2–4 μCi/μg of LPS were obtained. Radioiodinated LPS prepared by this method has been shown to be indistinguishable from the parent LPS with respect to biophysical, immunologic, and biologic properties. 20

Depletion of C3

Cobra venom factor (CoF) was purified by DEAE Sephadex A50 chromatography as described by Ballow and Cochrane 15 and treated with p-bromophenacyl bromide to inactivate the small amount of phospholipase A2 present. 22 Levels of C3 and terminal components of complement were depleted in some of the animals by a series of intraperitoneal injections of the CoF. The regimen consisted of 4 200-unit injections for rabbits 2 and 5 500-unit injections for monkeys. 23 The extent of depletion was determined by radial immunodiffusion 24 with anti-C3 antiserum as previously described for rabbits. 2 Quantitation of complement depletion in rhesus monkeys was accomplished with radial immunodiffusion methods with the use of antiserum to human complement components generously provided by Dr. Hans J Müller-Eberhard (SCRF, La Jolla, Calif).

Eighteen hours after the final CoF injection, C3 levels in rabbits and rhesus monkeys were found to be less than 5–10% of the pretreatment levels. Data for C3 and other complement components for the two depleted monkeys are shown in Table 1. Baseline values for blood leukocytes, platelets, hematocrit, and mean arterial pressure for these rabbits and monkeys were within normal limits following CoF treatment as previously described. 2,23

Determination of Tissue Blood Volumes

Tissue blood volumes were determined in rabbits and rhesus monkeys using 131I-bovine serum albumin (BSA) as described previously, 6 with the exception that the monkeys received 10 ml 131I-BSA intravenously 20 minutes prior to being killed. Neither CoF nor LPS injection appreciably altered tissue blood volumes in monkeys (Table 2) or rabbits.

Injection of LPS

Rabbits and lightly anesthetized monkeys (ketamine-HCl, Vetalar) were secured in the supine position. A catheter was placed in the femoral artery to the level of the aorta for

Table 1—Effect of Cobra Factor Treatment on the Serum Concentration of Complement Components in Rhesus Monkeys*

<table>
<thead>
<tr>
<th>Animal</th>
<th>C1q</th>
<th>C4</th>
<th>C2</th>
<th>C3</th>
<th>C5</th>
<th>C6</th>
<th>C7</th>
<th>C8</th>
<th>C9</th>
<th>P</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>519†</td>
<td>126</td>
<td>116</td>
<td>100</td>
<td>4.8</td>
<td>ND</td>
<td>9</td>
<td>ND</td>
<td>58</td>
<td>120</td>
<td>77</td>
<td>79</td>
</tr>
<tr>
<td>542</td>
<td>97</td>
<td>120</td>
<td>98</td>
<td>5.6</td>
<td>ND</td>
<td>12</td>
<td>ND</td>
<td>100</td>
<td>100</td>
<td>75</td>
<td>58</td>
</tr>
</tbody>
</table>

* Determined by radial immunodiffusion with antiserum against human complement components.
† Animal numbers.
‡ Not determined.
Table 2—Calculated Blood Volume of Tissues * in Control and LPS-Treated Rhesus Monkeys

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control†</th>
<th>Normocomplementemic‡</th>
<th>C3-depleted‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.18, 0.23</td>
<td>0.15, 0.16</td>
<td>0.2, 0.20</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.14</td>
<td>0.11, 0.08</td>
<td>0.12, 0.14</td>
</tr>
<tr>
<td>Lung</td>
<td>0.55</td>
<td>0.43, 0.50</td>
<td>0.75, 0.56</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.14, 0.18</td>
<td>0.10, 0.11</td>
<td>0.12, 0.14</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0.12</td>
<td>0.14, 0.12</td>
<td>0.008</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.007</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td>Blood volume♂</td>
<td>286 (4.8)</td>
<td>245 (4.5)</td>
<td>331 (5.2)</td>
</tr>
<tr>
<td></td>
<td>362 (5.3)</td>
<td>329 (4.8)</td>
<td>320 (5.4)</td>
</tr>
</tbody>
</table>

* The data shown are from individual animals and are expressed as milliliters of blood per gram (wet weight) of tissue.
† Control animals received a bolus injection of 10 ml of sterile saline used as a diluent for the LPS.
‡ The normocomplementemic and C3-depleted monkeys received a bolus injection of 5 mg/kg of R595 LPS.
§ The data are shown as the total blood volume in milliliters and the ratio of milliliters of blood per 100 grams body weight in parentheses.

Collecting blood samples and measuring arterial pressure as described previously. After determining baseline hematologic and hemodynamic parameters, either a 5-μg or 250-μg bolus of LPS was rapidly injected into the marginal ear vein of rabbits, and 5 mg per kg of LPS was administered through a femoral vein catheter in the monkeys. In previous studies from this laboratory it was shown that a 50-μg intravenous dose of R595 LPS in rabbits was sufficient to produce a sustained 30% fall in mean arterial blood pressure over 5 hours. Acute neutropenia and thrombocytopenia were observed, and animals killed at 24 hours after injection showed histologic evidence of DIC in liver, lung, kidney, and spleen. Prior depletion of C3 abrogated only the acute evanescent fall in circulating platelets but did not affect the gradually developing thrombocytopenia, acute neutropenia, or other changes. In a similar manner, a 500-μg/kg dose of R595 LPS in rhesus monkeys was shown to produce neutropenia, thrombocytopenia, and hypotension. In the present studies fibrin deposits were detected in PAS-stained histologic preparations from normocomplementemic and C3-depleted monkeys killed 5 hours after injection of a 5-mg/kg dose of R595 LPS. The distribution of fibrin deposits was comparable to that reported in rabbits.

In order to monitor the disappearance of 125I-LPS from the circulation, 1-ml blood samples were collected in dry plastic tubes at timed intervals after injection of the LPS dose. Immediately after the collection of each sample, a 500-μl aliquot was pipetted into a glass tube containing 55 μl of 3.8% sodium citrate. Upon completion of the experiment, the radioactivity in the blood samples and aliquots of the LPS dose was measured in an automatic gamma counter.

Determination of Tissue-Bound LPS Distribution

The rabbits were killed 180 minutes after injection of LPS, and the monkeys were killed either 20 to 300 minutes after injection. In all cases, 131I-BSA was injected before the animals were killed, as described above. The amount of radioactivity in tissue samples and blood was determined, and the concentration of tissue-bound LPS (exclusive of that contained in blood) was calculated.

The percentage of the injected dose of LPS accounted for in blood and in the tissues examined in this study (liver, spleen, lung, kidney, adrenal, and skeletal muscle) ranged from...
50% to 65%. This was found to be the case for rabbits at the 5-μg and 250-μg dose as well as monkeys at the 5-mg/kg dose. In preliminary experiments we found that LPS contained in other tissues and fluids including skin, lymph nodes, fat (mesenteric), heart, brain, bone marrow, gut (and contents), bile, and urine of NC rabbits accounted for an additional 25–30% of the dose. However, concentrations of LPS in these sites were generally low, and considerable variation was observed.

**Autoradiography**

Tissues were fixed in neutral-buffered 10% formalin for histologic examination or in modified Karnovsky's fixative followed by OsO₄ fixation for electron-microscopic examination. Autoradiography was performed on 6-μ histologic sections and 0.5-μ plastic sections at the light-microscopic level and on thin sections with electron microscopy as described previously.²⁵-²⁷

**Results**

**Distribution of LPS in Normocomplementemic and CoF-Treated Rabbits (5-μg Dose)**

We first sought to examine the *in vivo* distribution of a dose of LPS below the minimal amount required to produce detectable injury. Therefore a single 5-μg bolus of radioiodinated *S. minnesota* R595 LPS (5–10 μCi ¹²⁵I-LPS) was used. This dose is well below the threshold required to produce detectable activation of complement (CH₅₀ reduction), hypotension, thrombocytopenia, or histologic evidence of DIC. However, acute marked neutropenia does occur at this dosage.

Blood samples were collected at 1, 2, 5, 10, 20, 50, 100, and 180 minutes after the injection of LPS to determine the rate of disappearance of LPS from the circulation. On the basis of the cpm per milliliter of blood and the specific activity of the injected dose (cpm/μg LPS), the concentration of LPS in blood was determined. As shown in Text-figure 1, LPS disappeared from the blood of normocomplementemic (NC) and complement-depleted (CoF) rabbits in a biphasic manner. Considering the blood volumes of the rabbits and the dose of LPS administered, the maximal theoretic LPS concentration expected (t = 0 minutes) was 36–43 ng/ml for NC animals (blood volume range 116–137 ml) and 35–40 ng/ml for CoF animals (blood volume range 126–142 ml). Therefore the percentage of the LPS dose remaining in blood 1 minute after injection was 30% for NC rabbits and 22% for CoF rabbits. Beyond 20 minutes the LPS remaining in blood disappeared at a much slower rate, with a half-life greater than 5 hours for both groups. Because animals were killed at 3 hours after the injection of LPS, the rate of disappearance of the second, slow phase of the clearance curve could not be more accurately determined.

In order to calculate the distribution of tissue-bound LPS, ¹³¹I-BSA blood marker was injected intravenously, and the animals were killed 5 minutes later (180 minutes after injection of LPS). Tissues and organs
TEXT-Figure 1—The disappearance of LPS from blood of normocomplementemic (NC) and complement-depleted (CoF) rabbits. The theoretical maximum LPS concentration expected (t = 0 minutes) was 36-43 ng/ml for NC and 35-40 ng/ml for CoF animals (see text). The number of animals in each group is shown in parentheses.

were weighed, and the amount of radioactivity present was determined as described in Materials and Methods and expressed as nanograms LPS per gram of tissue. The amount of the injected dose of LPS remaining in blood 180 minutes after injection was $13 \pm 1\%$ (SD) for NC animals and $11 \pm 1\%$ for CoF animals. The balance of the dose, designated tissue-bound LPS, was distributed as shown in Text-figure 2. Liver contained the major portion (approximately 40%) of tissue-bound LPS. Other organs and tissues, including spleen, lung, kidney, adrenal, and skeletal muscle tissues, contained individually less than 5% of the tissue-bound LPS. The distribution of tissue-bound LPS in CoF animals was essentially identical to that observed in NC animals. LPS contained in blood and in the tissues shown in Text-figure 2 accounted for $55 \pm 3\%$ of the injected dose of NC rabbits and $50 \pm 3\%$ of the dose in CoF rabbits.

Concentrations of tissue-bound LPS observed in various organs 180 minutes after injection of 5 µg of LPS are shown in Text-figure 3. Liver, spleen, and adrenal tissues contained the highest levels (16–26 ng/g). Intermediate levels were observed in lung and kidney (4–12 ng/g), and the lowest concentrations were found in skeletal muscle (0.13–0.16 ng/g). Tis-
sue-bound LPS concentrations in CoF rabbits were not significantly different from those observed in NC rabbits.

Thus, other than the apparent slight increase in the disappearance rate of LPS from blood in CoF rabbits (Text-figure 1), the distribution of a 5-µg dose of LPS was not affected by prior depletion of C3 and terminal components of complement.

**Distribution of LPS in Rabbits (250-µg Dose)**

Experiments were also done to determine whether the distribution of a high dose of LPS in rabbits would be affected by prior depletion of C3 and terminal components of complement. The 250-µg dose of R595 LPS used is well above the minimum amount required to produce hypotension and thrombocytopenia within 5 hours.²

After intravenous injection of a 250-µg dose of LPS (5–10 µCi $^{125}$I-LPS) blood samples were collected and analyzed as described above to determine the rate of disappearance of LPS from blood. As shown in Text-figure 4, LPS disappeared from the blood of NC and CoF rabbits in a biphasic manner with 27% of the dose remaining in blood 1 minute after injection. Based on the blood volume range of 120–160 ml for these animals and the dose of LPS injected, the theoretical maximal LPS concen-
TEXT-Figure 3—The concentration of tissue-bound LPS in NC and CoF rabbits killed 180 minutes after intravenous injection of 5 µg of LPS. Tissue-bound LPS was differentiated from LPS contained in blood by use of 131I-BSA blood marker. The number of animals in each group is given in parentheses.

TEXT-Figure 4—The disappearance of LPS from blood of NC and CoF rabbits. A theoretical maximum LPS concentration of 1.8 µg LPS/ml was expected at t = 0 minutes. The number of animals in each group is given in parentheses.
Concentration expected (t = 0 minutes) was 1.8 μg LPS/ml. Beyond 20 minutes the LPS remaining in blood disappeared with a half-life of greater than 5 hours in both groups of animals.

The distribution of tissue-bound LPS was determined in animals killed 180 minutes after injection in the same manner as described above. In both NC and CoF rabbits 18 ± 2% of the injected dose remained in blood. The balance of the dose, designated tissue-bound LPS, was distributed as shown in Text-figure 5. Liver contained greater than 40% of the tissue-bound LPS pool in both NC and CoF animals. Spleen, kidney, adrenal, and skeletal muscle tissues contained individually less than 5% of the tissue-bound LPS. Lungs of CoF rabbits contained 7% of the tissue-bound LPS, as compared with 3% in NC animals. The distribution of LPS among other organs was not affected by prior depletion of complement. LPS contained in blood and in the tissues shown in Text-figure 5 accounted for 66% of the injected dose in NC rabbits and 65 ± 2% of the dose in CoF rabbits.

Tissue-bound LPS concentrations observed in various organs 180 minutes after injection of 250 μg of LPS are given in Text-figure 6. Liver, spleen, and lung contained the highest concentrations of tissue-bound LPS (0.6–1.4 μg/g). Kidney and adrenal contained intermediate levels (0.3–0.9 μg/g).
TEXT-FIGURE 6—The concentration of tissue-bound LPS in various organs 180 minutes after intravenous injection of 250 μg of LPS in NC and CoF rabbits. Tissue-bound LPS was differentiated from LPS contained in blood by use of $^{131}$I-BSA blood marker. The number of animals in each group is given in parentheses.

According to the text, μg/g), and the lowest concentrations were observed in skeletal muscle (0.006–0.012 μg/g). CoF rabbits had increased LPS concentrations in lung and, to a lesser extent, adrenal tissue. The LPS concentration in other organs of CoF animals were comparable to those observed in NC animals.

**Distribution of LPS in Rhesus Monkeys (5-mg/kg Dose)**

The following experiments were done to determine if prior depletion of C3 and terminal components of complement would effect the distribution of a high dose of LPS in primates. A 5-mg/kg intravenous dose of *S. minnesota* R595 LPS in rhesus monkeys has been shown to produce a reduction of serum CH$_{50}$, neutropenia, thrombocytopenia, and hypotension over a 5-hour period.$^{23}$

The rate of disappearance of LPS from blood was measured in NC and CoF monkeys after intravenous injection of 5 mg/kg of LPS containing 200 μCi $^{125}$I-LPS. In both groups of animals LPS disappeared from blood in a biphasic manner. On the basis of the blood volumes of the monkeys and the LPS dose administered, theoretical maximal LPS concentrations expected (t = 0 minutes) were 97 μg/ml for NC animals and 95 μg/ml for CoF animals. As shown in Text-figure 7, the portion of the injected dose
TEXT-Figure 7—The disappearance of LPS from blood of NC and CoF rhesus monkeys. A theoretical maximum LPS concentration expected at t = 0 minutes was 97 μg/ml for NC animals and 95 μg/ml for CoF animals. Data from individual animals is shown for 2 CoF animals and 2 NC animals killed at 300 minutes. The data for early time points (1–10 minutes) from NC animals represent the mean from 4 animals.

remaining in blood 1 minute after injection was 85% for NC animals and 52% for CoF animals. LPS remaining in blood beyond 30 minutes disappeared, with a half-life of greater than 5 hours.

The distribution of tissue-bound LPS was determined in NC animals killed either 20 minutes or 300 minutes after injection and in CoF animals killed 300 minutes after injection. These time points were chosen to coincide with neutropenia and termination of the rapid disappearance of LPS from blood, which occur early after injection and with hypotension and DIC, which are present by 5 hours. Blood marker (50 μCi 131I-BSA) was injected intravenously 15 minutes before the animals were killed to permit differentiation between LPS contained in blood and that bound to tissues.

Twenty minutes after injection of LPS in NC monkeys, 27% of the injected dose was present in blood, whereas by 300 minutes this figure had decreased to 21%. In CoF animals 11% of the LPS dose remained in blood at 300 minutes. The balance of the LPS dose, referred to as tissue-bound LPS, was distributed as shown in Table 3. Liver contained the major portion of tissue-bound LPS in NC and CoF monkeys. Except for the lungs of NC animals killed 20 minutes after injection, the remaining tissues examined contained individually less than 10% of the tissue-bound LPS pool.
Table 3—Distribution of Tissue-Bond \(^*\) \(^{125}\text{I}-\text{R595}\) LPS in Rhesus Monkeys

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normocomplementemic</th>
<th>C3-depleted†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 minutes</td>
<td>300 minutes</td>
</tr>
<tr>
<td>Liver</td>
<td>37</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>0.87</td>
<td>0.8</td>
</tr>
<tr>
<td>Lung</td>
<td>14</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>8.0</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>5.6</td>
</tr>
</tbody>
</table>

\(^*\) Differentiated from blood-contained LPS with \(^{131}\text{I}-\text{BSA}\) blood marker. Animals were killed 20 or 300 minutes after injection of a 5-mg/kg intravenous LPS dose.

† Five intraperitoneal injections of CoF over a 36-hour period 18 hours prior to the experiment (C3 levels less than 5% of normal).

Including LPS contained in blood and that contained in tissues shown in Table 3, the portion of the injected dose accounted for was 65% for NC animals killed at 20 minutes, 56% for NC animals killed at 300 minutes and 65% for CoF animals killed at 300 minutes.

As shown in Table 4, the highest concentrations of tissue-bound LPS were observed in liver (66–97 µg/g), with somewhat lower levels in the spleen and kidney (16–82 µg/g). LPS concentrations in lung and adrenal tissue ranged from 5 to 31 µg/g, and the lowest levels were observed in skeletal muscle (0.34–0.96 µg/g). Tissue-bound LPS concentrations in NC animals remained unchanged between 20 and 300 minutes after injection, except for lung LPS levels, which were decreased at 300 minutes. CoF monkeys appeared to have increased lung and renal LPS concentrations and decreased splenic LPS concentration. LPS levels in the remaining organs examined were not affected by CoF treatment.

Cellular Localization of LPS in Rhesus Monkeys

The cellular localization of LPS was examined by autoradiography in the three groups of monkeys described above and was found to be comparable to that observed in NC rabbits killed 180 minutes after injection of LPS. CoF pretreatment appeared to have no effect on the cellular localization of LPS. As in rabbits, the most striking uptake of LPS was observed in the Kupffer cells (Figure 1) and in splenic macrophages. Although large numbers of leukocytes were present in the alveolar...
Table 4—Concentration of Tissue-Bound * ¹²⁵ I-R595 in Rhesus Monkeys

<table>
<thead>
<tr>
<th>µg LPS/g tissue</th>
<th>Normocomplementemic</th>
<th>C3-depleted†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 minutes</td>
<td>300 minutes</td>
</tr>
<tr>
<td>Liver</td>
<td>88</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>86</td>
</tr>
<tr>
<td>Spleen</td>
<td>52</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>50</td>
</tr>
<tr>
<td>Lung</td>
<td>31</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>13</td>
</tr>
<tr>
<td>Kidney</td>
<td>29</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>Adrenal</td>
<td>9.9</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>7.6</td>
<td>15</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.89</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>0.34</td>
<td>0.73</td>
</tr>
</tbody>
</table>

* Differentiated from blood-contained LPS with ¹³¹I-BSA blood marker. Animals were killed 20 or 300 minutes after injection of a 5-mg/kg intravenous LPS dose.
† Five intraperitoneal injections of CoF over a 36-hour period 18 hours prior to the experiment (C3 levels less than 5% of normal).

Discussion

The results from these studies demonstrate that prior depletion of C3 does not abrogate the initial disappearance of LPS from blood and does not result in a major change in the tissue-bound LPS distribution. Data to support these conclusions were obtained in experiments in which normocomplementemic and CoF-treated rabbits and rhesus monkeys received intravenously injected radioiodinated S minnesota R595 LPS. These observations were confirmed with a nonlethal dose (5 µg) in rabbits as well as with lethal LPS doses in rabbits (250 µg) and monkeys (5 mg/kg). In addition, the distribution and cellular localization of LPS in rhesus monkeys was found to be similar to that previously reported in rabbits and was minimally affected by prior depletion of C3.

After injection of LPS into rabbits and monkeys, an initial rapid disappearance of LPS from blood (t₁/₂ <15 minutes) was observed. This initial disappearance phase was not blocked by depletion of C3 by pre-treatment with CoF but, rather, appeared to be accentuated in rabbits at the 5-µg dose (Text-figure 1) and monkeys (Text-figure 7). In all of the animals, LPS remaining in blood beyond 20 minutes disappeared with a much longer half-life (>5 hours).

Determinants of the rate of disappearance of LPS from blood include
the physical and chemical properties of the LPS as well as host factors. For example, particulate LPS preparations are more rapidly removed from blood and taken up by macrophages than soluble preparations.\textsuperscript{6,13} The LPS subunit structure, including the ratio of polysaccharide (core and O antigen) to lipid A, has been shown to determine the complement activation properties of LPS and may also influence the rate of interaction of LPS with blood lipoproteins.\textsuperscript{9,12} Binding of complement to LPS would be expected to facilitate uptake of LPS by phagocytic cells, whereas interaction of LPS with blood lipoproteins, which results in formation of HDL–LPS complexes with a much smaller molecular weight than the parent LPS, has been shown to retard the uptake of LPS by tissues.\textsuperscript{6,10}

Intravenously injected LPS is rapidly brought into contact with plasma constituents, blood cells, tissue macrophages, and parenchymal cells of various organs. Interaction or binding of LPS to one system may facilitate or preclude its interaction with other systems. For example, binding of R595 LPS to rabbit platelets \textit{in vivo} appears to depend on activation of complement by the LPS and can be blocked by prior depletion of complement by CoF (Mathison JC, Ulevitch RJ, unpublished observations). However, if the LPS is first allowed to interact with blood lipoproteins \textit{in vitro} to form HDL–LPS complexes (low-density LPS),\textsuperscript{9,10} complement activation and binding of the LPS to platelets are blocked (Mathison JC, Ulevitch RJ, unpublished observations). In contrast to the parent LPS, low-density R595 LPS is avidly taken up by the adrenals.\textsuperscript{6} Thus the physical and chemical properties of the LPS determine its ability to interact with host systems, and the \textit{in vivo} distribution of LPS, in turn, is influenced by these interactions.

Results from the present study indicate that the \textit{in vivo} distribution of LPS is changed only minimally by depletion of C3. However, the early components of complement (C1, 4, 2) are not affected by CoF treatment. Therefore, we cannot exclude the possibility that these components could interact with R595 LPS and substantially influence the distribution of LPS. In previous studies we have demonstrated that \textit{Escherichia coli} 0111:B4 LPS, which does not activate the classic complement pathway,\textsuperscript{12} disappears from blood of rabbits in a biphasic manner, although the initial disappearance phase is not as pronounced as with R595 LPS.\textsuperscript{6} Further, the biphasic disappearance and distribution of 0111:B4 LPS in rabbits is not affected by prior depletion of C3 with CoF (Mathison JC, Ulevitch RJ, unpublished observations).

The apparent increased initial disappearance of LPS in C3-depleted rabbits (5-\(\mu\)g dose) and monkeys could be due to activation of macro-
phages during the CoF treatment, since certain complement components, notably Factor B and C3b have been implicated in macrophage activation. Because hematologic parameters in CoF-treated animals were within normal limits prior to LPS injection, it appears unlikely that the increased initial disappearance of LPS in these animals was due to uptake of LPS by blood cells that subsequently became marginated in vessels and localized in tissues.

Liver contained the greatest portion of tissue-bound LPS in rabbits (Text-figures 2 and 5) and monkeys (Table 3), and this uptake was not affected by CoF pretreatment, with the possible exception of somewhat increased hepatic LPS concentrations in C3-depleted monkeys. Substantial tissue-bound LPS concentrations were observed also in spleen, lung, kidney, and adrenal tissues in all of the animals studied (Text-figures 3 and 6, Table 4). Nevertheless, these organs contained individually, in most cases, less than 5% of the tissue-bound LPS. Lung LPS concentrations were increased in CoF-treated rabbits and monkeys after injection of a high LPS dose. However, this was the only consistent change in tissue-bound LPS concentration observed in rabbits and monkeys pretreated with CoF. Pretreatment of rabbits with CoF did not affect the distribution of a low dose of LPS.

The distribution profile of LPS in normocomplementemic rabbits after injection of a low dose of LPS was essentially identical to that observed in rabbits after a high dose. One minute after injection the portion of the LPS dose remaining in blood was 30% for the 5-μg dose and 27% for the 250-μg dose. LPS remaining in blood beyond 20 minutes disappeared with a half-life of >5 hours. The relative concentrations of tissue-bound LPS in liver, spleen, lung, adrenal, and skeletal muscle tissue were closely similar in both groups. These observations indicate that the distribution of LPS in vivo occurs independently of the amount injected over a wide dose range. Further, the development of endotoxic effects does not appear to require saturation of a clearance mechanism which would normally isolate LPS from sensitive tissues. Substantially greater percentages of the injected dose remained in the blood of NC and CoF monkeys 1 minute after injection (85% and 52%, respectively). Whether these high levels were due to the large dose (5 mg/kg) or species differences is not known.

The tissue distribution and cellular localization of R595 LPS in rhesus monkeys was closely similar to that reported previously in rabbits. By light-and electron-microscopic autoradiography, LPS was observed to be concentrated in phagocytic vacuoles of macrophages of the liver (Kupffer cells) and spleen. Silver grains were also occasionally observed over leuko-
cytes in hepatic and splenic sinusoids and pulmonary alveolar capillaries. Specific labeling with silver grains was not observed over parenchymal cells or vascular walls in liver, kidney, or adrenal tissue.

The results from these experiments provide additional evidence that C3 plays a minimal role in host–LPS interactions. Previous studies in this laboratory have demonstrated that C3 and terminal components of complement are not required for development of neutropenia, thrombocytopenia, hypotension, disseminated intravascular coagulopathy (DIC), shock, and death after intravenous injection of LPS in rabbits and rhesus monkeys.2,3,23 Although complement levels may be depressed after injection of a large LPS dose, severe endotoxic effects are produced by much smaller LPS doses that do not produce detectable depression of complement levels.2,3,23,30

It remains unclear how the endotoxic effects of LPS are mediated. Twenty-five years ago, Braude suggested that the cells that remove circulating LPS may release substances which initiate the physiologic changes produced by LPS.5 Recently, macrophages derived from peritoneal exudate cells of mice have been shown to respond to LPS challenge by the production of a number of substances, including plasminogen activator, prostaglandins, and procoagulant activity,14 and these activities appear to be produced by direct action of LPS on macrophages. Further, it has been shown that hepatic macrophages isolated from rabbits release endogeneous pyrogen when challenged with LPS.31 Studies in progress in this laboratory have demonstrated that hepatic macrophages isolated from rabbits produce 3–5-fold increased amounts of plasminogen activator, lysozyme, and lactic hydrogenase in response to parent LPS and HDL–LPS complexes but not latex beads (Maier RV, Ulevitch RJ, unpublished observations). Considering the large quantities of LPS sequestered in hepatic macrophages after intravenous injection of LPS, the direct response of these cells to LPS could play a major role in the initiation of shock and DIC.

References
4. Howard JG, Rowley D, Wardlow AC: Investigations on the mechanism of stimula-

Acknowledgments
We would like to acknowledge the technical expertise of Ms. Kathy Bangs Wesolak, who assisted in these studies. We also thank Ms. Betty Goddard for her assistance in preparing the manuscript.
Figure 1—Light-microscopic autoradiograph showing the cellular localization of LPS in liver of a CoF-treated rhesus monkey killed 500 minutes after injection of 5 mg/kg S. minnesota R595 (200 µCi 125I-LPS). Silver grains were observed over the cytoplasm of Kupffer cells and occasionally over leukocytes in the sinusoids. Similar results were obtained in normocomplementemic animals killed 20 minutes or 500 minutes after injection of LPS. (0.5 µ Epon section, toluidine blue, ×1100)
[End of Article]