

## Interaction Between Endotoxic Lipopolysaccharides (LPS) and the Complement (C) System: Solubilization of C-Consuming Substances During Brief Absorbptions at 0°<sup>1</sup> (35311)

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While endotoxic lipopolysaccharides (LPS) have little effect upon hemolytic C activity during brief incubations with guinea pig serum at 0°, they are known to effectively consume large amounts of C-component activity during 1-hr incubations at 37°. Concomitantly, characteristic "lesions" are formed on the LPS surface and biologically active peptides are generated (1-6). This reaction has occurred in all vertebrate sera tested in which hemolytic C activity has been detected (7). It has also been seen, although at lesser intensities, in sera markedly deficient in immunoglobulins taken from animals with experimental and developmental agammaglobulinemia and humans with agammaglobulinemic syndromes (8). It is still not clear whether small amounts of immunoglobulin are so efficient in initiating this interaction that their participation is partially masked, or whether there are alternate means, not requiring immunoglobulin at all, to account for the C consumption initiated by endotoxins. To further explore the role of immunoglobulins in this interaction, attempts were made to remove potential antibodies by pretreating fresh normal guinea pig serum with brief absorption at 4°.

**Materials and Methods.** Endotoxic lipopolysaccharide was prepared from *Veillonella alcalescens* strain V5 by the phenol-water extraction procedure of Westphal and Lüderitz (9) and used in the desiccated state. In some experiments the parent organisms, washed three times in normal saline prior to desiccation, were used.

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Guinea pig serum was purchased from Texas Biologicals, Fort Worth, Texas.

Absorbptions were performed by adding 5 mg of dry LPS to 1.0 ml of guinea pig serum prechilled to 0°, agitating for 10 min in a 0° water bath, and centrifugating the mixture 18,000 rpm for 30 min at 0°. This procedure was repeated three times, with the clear test aliquots and control samples frozen at -70° for further study.

Total complement hemolytic activity was measured according to Mayer (10). In certain experiments, 0.1 ml of test serum and test reactivities were preincubated in a total volume of 1.0 ml at 37° for 1 hr prior to assay.

Antiserum against *Veillonella* organisms was prepared as previously described (11).

**Results and Discussion.** Unabsorbed serum and sera which were absorbed three times at 0° were found to have equal amounts of total hemolytic C activity (approx 10 units/0.1 ml). Hence, the absorption interval itself did not seem to remove hemolytic C activity. When these sera were allowed to incubate at 0° for 1 hr, no change in hemolytic activity was seen in either absorbed or unabsorbed samples. However, when these sera were incubated at 37° for intervals up to 2 hr, a time- and temperature-dependent depletion of hemolytic C activity was observed selectively in the absorbed samples (Figs. 1 and 2). Since it previously was found that a preincubation interval is necessary for LPS to display its anti-C activity in guinea pig serum (3-5), (*i.e.*, LPS does not "deviate" nor consume C activity when admixed simultaneously with the test serum and indicator sensitized erythrocytes), it can be concluded that the depletion of C observed in these experiments occurred during the prein-

## LPS AND C-SYSTEM INTERACTION

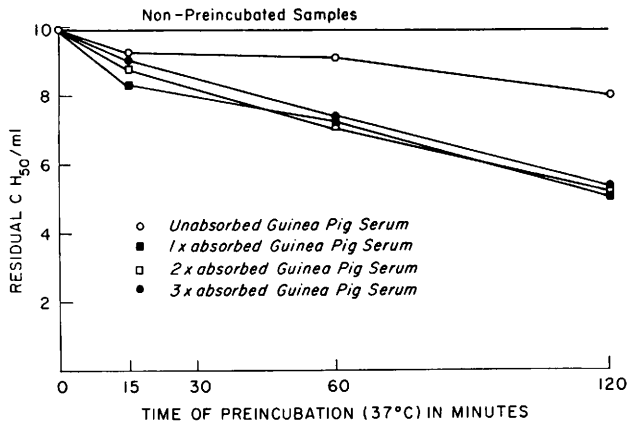


FIG. 1. Effect of brief (10 min at 0°) absorptions of 1.0 ml of guinea pig serum with 5 g of *V. alcalescens* LPS on subsequent stability of hemolytic C activity during preincubation intervals at 37° up to 120 min. The residual C of each sample at each time interval is shown. Ten CH<sub>50</sub> units were available in the incubation mixtures. Since LPS requires a preincubation period to display its anti-C activity (3-5), the solubilized LPS was expressing its anti-C activity during the preincubation or "fixation" step of this assay, rather than during the subsequent indicator "hemolytic" step.

cubation interval rather than during the subsequent hemolytic step of the assay. Additional absorptions did not increase either the rate or extent of the subsequent C depletion (Fig. 1). This raised the possibility that small amounts of endotoxin, solubilized during the 0° absorption procedures, exercised their C-consuming activity when the temperature was raised to 37°.

To test this possibility, both the absorbed test serum and unmodified control samples were preincubated at 37 and 0°, respectively, in the presence of hyperimmune rabbit anti-*Veillonella* serum. At both temperatures substantial consumption of C was seen selectively in the absorbed samples. The immune serum established the presence of antigenic constituents of *Veillonella* LPS in the absorbed serum during 0° preincubations, and magnified their presence during preincubations at 37° (Fig. 2). The ability of LPS to initiate C consumption in fresh guinea pig serum at 0° only upon the addition of hyperimmune serum had been previously described (3, 4). Hence these experiments established that antigenic C-consuming constituents of LPS are solubilized during brief absorption periods at 4°.

The ability of LPS to consume C was

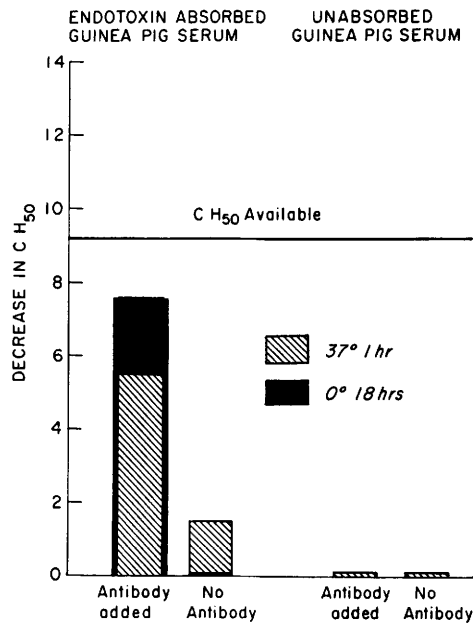


FIG. 2. Stability of C in both guinea pig serum absorbed four times at 0° with LPS, and unabsorbed serum. Depression of C was seen only in the absorbed samples. In absence of antibody, modest depression was seen only at 37°. In the presence of anti-*Veillonella* marked depression was seen at both 37 and 0°. This confirmed the solubilization of bacterial antigens during the absorption procedure,

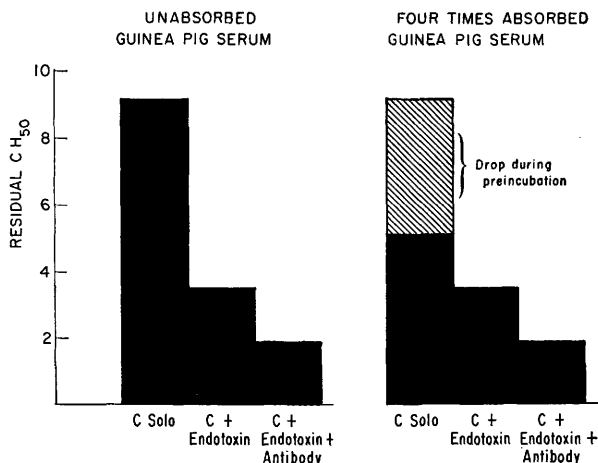


FIG. 3. Effect of 25  $\mu$ g of *Veillonella* endotoxin upon hemolytic activity in unabsorbed sera and serum preabsorbed four times with this endotoxin at 0°. Tests were performed both in the presence and absence of additional hyperimmune rabbit sera. The residual amounts of C were identical in each case; the samples differed only in the previously described C depletion which occurred selectively in the absorbed serum preincubated at 37° in the absence of additional LPS.

compared in preabsorbed and in control unmodified sera. However, identical amounts of C activity were found in each sample at the end of the preincubation period, whether or not hyperimmune serum was added (Fig. 3). The samples differed only in the selective loss of C in the absorbed sera as described above. Therefore the solubilized LPS components did not interfere with C consumption by the additional LPS.

In subsequent experiments it was found that a similar solubilization of bacterial antigens occurs when normal sera are absorbed with intact lyophilized parent bacteria. It should be noted that the absorptions at 4°, whether with LPS or intact bacteria, regularly led to loss of bactericidal activity against unsensitized *Veillonella* organisms, presumably by removal of certain "natural" antibodies, even as the ability of LPS to consume C in these sera persisted.

We conclude that C-consuming constituents of LPS are solubilized in fresh guinea pig serum during brief absorption periods at 0°, a phenomenon that interferes with attempts to evaluate the role of antibody in LPS-induced C consumption.

**Summary.** Attempts were made to remove antibodies and other serum factors involved in the interaction between endotoxic lipopoly-

saccharides (LPS) and the complement system by brief absorptions of guinea pig serum with LPS at 0°. However, in addition to removal of serum factors, this procedure also resulted in the solubilization or "imbibition" of LPS constituents into the fluid phase, such that when the absorbed sera were subsequently incubated at 37°, or hyperimmune rabbit anti-LPS serum was added to them at either 0 or 37°, LPS constituents which initiated C consumption were found. C consumption upon addition of LPS to absorbed sera persisted, even as bactericidal activity against the parent *Veillonella* organisms was reduced. While the importance of solubilization of LPS to its interaction with the C system is not yet known, these experiments show the difficulty of evaluating the factors in serum which initiate C consumption upon addition of LPS and their relationship to "natural" bactericidal antibodies.

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