Interaction of Pneumococcal Antigens with Complement in Rats

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Complement activation with pneumococcal antigens was studied both in vitro and after injection of the antigens into rats. Whole pneumococci of various serotypes activated C3-C9 in rat serum treated with ethyleneglycol-bis (β-aminoethyl ether)-N,N'-tetraacetic acid, although serotypes differed greatly in the extent of activation. Some purified pneumococcal capsular polysaccharides also activated C3-C9 in rat serum, but only when the antigens were present in concentrations of 500 to 1,000 μg/ml. Much of the activation with capsular polysaccharides was eliminated by the use of ethyleneglycol-bis (β-aminoethyl ether)-N,N'-tetraacetic acid. Activation of C3-C9 by capsular polysaccharides did not correlate with the level of reactivity observed with whole organisms of the same serotypes. After injection of 5 × 10^8 pneumococci (type 3 or type 4) intravenously into rats, there was a transient decline in serum C3-C9 activity, but there was no decline in C3-C9 levels after intravenous injection of 1,000 μg of type 3 or type 4 capsular polysaccharides. As determined by immunofluorescence, circulating capsular polysaccharide was deposited in several tissues, including the vascular endothelium and glomerular mesangium of the kidney. C3 was not detectable in these deposits, and there was no histological evidence of an inflammatory response. Capsular polysaccharides appear to be only weak activators of complement. Other pneumococcal antigens may be more important in the pathogenesis of hypocomplementemia in pneumococcal infection.

Serum complement levels, and particularly alternate pathway activity, are decreased in some individuals with pneumococcal infection (6, 9, 18). It has been postulated that pneumococcal antigens may activate complement during infection (6, 18), perhaps in some cases to such an extent that there is depletion of the serum heat-labile opsonins. The studies of Winkelstein and Tomasz (20, 21) indicate that teichoic acid is the most important component of the pneumococcal cell wall for initiating alternate pathway activation. The capsular polysaccharides of several serotypes have also been reported to activate the alternate pathway (22). It has been suggested that capsular polysaccharide, which is often detectable in human body fluids in pneumococcal infection, may play a role in serum complement activation and depletion (G. S. Giebink et al., Clin. Res. 26:523, 1978), and may also be involved in the pathogenesis of pneumococcal glomerulonephritis (10).

In the present study, we evaluated pneumococcal capsular polysaccharides for complement activation in vitro and after injection of the antigens into rats. Our study indicates that some capsular polysaccharides can activate rat complement in vitro, but only at high concentrations of antigen. We could not detect complement activation after intravenous injection of the polysaccharides into rats.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) were used throughout. Serum or plasma was obtained by bleeding from the tail vein with light ether anesthesia.

Buffers. Isotonic Veronal-buffered saline (VB; pH 7.4) with 1.0 mM Mg^2+, 0.15 mM Ca^2+, and 0.1% gelatin (GVB^2+) was prepared as described earlier (17). Other buffers included GVB^2+ (pH 7.4) with glucose (Gl-GVB^2+) and VB with 0.1% gelatin and 0.01 M ethylenediaminetetraacetic acid (EDTA-GVB) (17).

Preparation of EA. Sheep erythrocytes were sensitized with hemolysin (Cordis Laboratories, Miami, Fla.) in GVB^2+ (17). The final suspension contained 5 × 10^8 sensitized erythrocytes (EA) per ml.

Preparation of cellular intermediates and assay of hemolytic C3-C9. Hemolytic C3-C9 was assayed with EAC142 cells prepared by a minor modification of the method of Rapp and Borsos (17). Purified C1^a was purchased from Cordis Laboratories, and fresh rat plasma diluted 1:10 in EDTA-GVB served as the source of C4^a to generate EAC1^aC4^a. C4^a was
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employed because of its superiority to C4 in reaction with terminal components of rat complement (2, 14). The cells were washed and suspended in a concentration of 1.75 x 10⁸/ml in G1-GVB². They were mixed with an equal volume of C9 (containing 250% hemolytic units, Cordis Laboratories) and incubated at 30°C for 12 min (T max). A 0.5-ml amount of EAC142 cells was mixed at 0°C with 1.0 ml of rat serum dilution and 2.0 ml of EDTA-GVB and incubated for 1 h at 37°C. Controls for stability of cellular intermediates and for 100% lysis were included. Free hemoglobin was determined spectrophotometrically at 412 nm. The hemolytic C3-C9 titer was calculated as the serum dilution yielding 50% lysis of cells. The C3-C9 titer of rat serum with seven different lots of EAC132 cells ranged from 194 to 266 U/ml with a mean of 232/ml. Serum C3-C9 titers of rat serum stored at -70°C were stable over a period of at least 3 months.

Antigens. Type-specific pneumococcal polysaccharides other than type 4 were generously provided by Eli Lilly and Co., Indianapolis, Ind. The polysaccharides had a mean molecular size of >200,000 as determined by Sephadex G-200 filtration, and they had less than 1% nucleic acid and less than 2% protein contamination, as indicated by the manufacturer. Type 4 polysaccharide (>200,000 molecular size) was kindly provided by Lederle Laboratories, Pearl River, New York. Polysaccharides were quantified by counterimmunoelectrophoresis as previously described (4).

Bacteria. Pneumococci were obtained from the American Type Culture Collection (Rockville, Md.). The organisms were passed in mice and stored in rabbit serum at 4°C. A portion from an overnight culture was inoculated into brain heart infusion broth containing 7% inactivated rabbit serum and incubated at 37°C. After 5 to 6 h of growth, the organisms were killed by heating at 70°C for 1 h. They were washed three times in VB and suspended to the desired concentration.

Other complement activators. Zymosan (Nutritional Biochemicals, Cleveland, Ohio) was boiled for 2 h in normal saline, washed, and brought to a concentration of 2 mg/ml. Cobra venom factor (CoVP) which had been purified by DEAE-cellulose chromatography was a gift of Jon Gockerman, University of Kentucky Medical School, Lexington.

Chelator. Stock solutions of 0.1 to 0.3 M ethylene-glycol-bis (β-aminoethyl ether)-N,N′-tetraacetic acid (EGTA) (Sigma Chemical Co., St. Louis, Mo.) were prepared in normal saline with adjustment of the pH to 7.45 before final dilution.

Complement consumption tests. Activation of C3-C9 was evaluated by mixing 5 volumes of pooled rat serum with 1 volume of VB and adding pneumococci, capsular polysaccharide, or other activators in 4 volumes of VB. Controls without activators were included in each experiment. After incubation of the mixtures for 1 h at 37°C, the tubes were chilled and centrifuged, and residual C3-C9 titers of the supernatants were determined. Samples with activators were compared with control samples without activators. In studies in which EGTA was used, 5 volumes of serum were mixed with 1 volume of EGTA, and the mixtures were incubated for 5 min at room temperature. Complement activators or diluent alone were then added in 4 volumes of VB, and C3-C9 consumption was measured. Spontaneous loss of C3-C9 during incubation at 37°C for 1 h averaged 15% of the initial C3-C9 level and was the same in serum treated with EGTA as in untreated serum.

Immunofluorescence studies. Pneumococcal polysaccharide and rat immunoglobulin G (IgG), C3, and IgM were detected in tissues by indirect immunofluorescence. Hyperimmune type-specific antisera, raised by injection of whole pneumococci into rabbits, was purchased from Statens Seruminstitut, Copenhagen, Denmark. Rabbit anti-rat IgG was obtained from Cappel Laboratories (Cochraneville, Pa.). Fluorescein-conjugated IgG fraction of goat anti-rabbit IgG (Cappel Laboratories) was used in the second step for detection of polysaccharide or rat IgG. Deposition of rat complement was evaluated by use of goat anti-rat C3 followed by fluorescein-conjugated IgG fraction of rabbit anti-goat IgG (both from Cappel). Rat IgM was detected by its cross-reaction with goat anti-human IgM (heavy-chain specific) (Cappel). The specificity of the latter reaction was indicated by the presence of a single heavy line of precipitin in double diffusion or in electrophoresis in agarose between rat serum (obtained after immunization with sheep red blood cells i.v.) (3) and goat anti-human IgM. Rat serum did not give a precipitin line with normal goat serum. All antisera were twice absorbed with 150 mg of prehydrated rat liver powder (Sigma Chemical Co., St. Louis, Mo.) per ml of antiserum before use in immunofluorescence.

Tissue was quick frozen in isopentane at -70°C, embedded (Tissue-Tek II, Lab-Tek Products, Naperville, Ill.) and sections of 4- to 5-µm thickness were cut on a cryostat. Sections were washed with phosphate-buffered saline (PBS; pH 7.2) and covered with an optimal dilution of antiserum for 45 min at room temperature in a moist box. After 3 washings of 15 min each with PBS, sections were layered with an optimal dilution of fluoresceinated antiserum, again incubated for 45 min at room temperature, washed, and read immediately. Controls included the use of rabbit antipneumococcal serum of a heterologous type, the use of normal rabbit and goat sera, and the use of fluoresceinated antisera singly without prior treatment of sections with antiserum. Normal rat tissue controls were also employed.

Histopathology. Tissue was fixed in neutral Formalin solution and embedded in butylcelranol glycol methacrylate. Sections of 1 to 2 µm in thickness were stained with hematoxylin and periodic acid-Schiff stain.

Urine. Rats were fed 5% sucrose water for 2 h, after which they were placed in metabolic cages, and urine was collected for 6.5 h. Urine proteins were measured with the biuret reaction (8).

RESULTS

Complement activation in EGTA-treated
rat serum. To evaluate alternate pathway activation by pneumococcal antigens in rat serum, it was necessary to determine optimal conditions for differential chelation of cations with EGTA. Buffers containing EGTA were added to fresh pooled rat serum, and the mixtures were incubated for 5 min before complement activators were added. Initially, 10 mM EGTA containing 2 mM MgCl₂ (Mg-EGTA) was evaluated because Mg-EGTA has yielded satisfactory results with human serum (6). With rat serum, however, Mg-EGTA did not adequately block the classical pathway. In various trials, 24 to 30% of added EA were lysed in 1 h at 37°C in rat serum treated with Mg-EGTA. The duration of incubation of Mg-EGTA with rat serum before addition of EA had no effect on the results, in that lysis of EA was essentially identical in sera which had been treated with Mg-EGTA at 1, 5, 10, or 30 min before the addition of EA. The pH of rat serum treated with Mg-EGTA did not fall below 7.35, which precluded a change in pH during chelation as a cause of the difficulty.

Results obtained with a series of buffers containing various concentrations of EGTA without added MgCl₂ are shown in Table 1. To completely eliminate lysis of EA, it was necessary to have 30 mM EGTA in the reaction mixture. The amount of C3-C9 consumed by zymosan in the presence of either 25 or 30 mM EGTA was only slightly lower than with untreated serum. Consumption of C3-C9 by CoVF was lower in serum containing 15 to 30 mM EGTA than it was in untreated serum, suggesting that the conditions required to completely block the lysis of EA may also have caused mild suppression of alternate pathway activity.

Complement activation by pneumococci. Pneumococci of different serotypes were incubated in rat serum at 37°C, and loss of hemolytic C3-C9 activity was measured. Rat serum treated with 30 mM EGTA was used to evaluate activation via the alternate pathway. The results (Table 2) indicated that each of a variety of pneumococcal serotypes could activate the alternate pathway, although complement consumption was consistently greater in unchelated than in chelated serum. Serotypes differed in the extent of activation. This latter point was further documented with studies of the rate of C3-C9 consumption in EGTA-treated serum by different pneumococcal serotypes (Fig. 1). Type 1 was the least reactive serotype, consuming less than 40% of the available C3-C9 activity during 60 min of incubation. In contrast, type 14, the most reactive serotype, almost decomplemented the serum during the first 15 min of incubation.

Complement activation by soluble capsular polysaccharides. Graded amounts of type-specific pneumococcal polysaccharides were added to rat serum, and consumption of hemolytic C3-C9 activity during incubation at 37°C was measured. The results are indicated in Fig. 2. Most of the different serotypes of capsular polysaccharide were able to activate complement, but only when the antigens were present in concentrations of 500 to 1,000 µg/ml. Three different lots of the type 6 and type 7 polysaccharides were evaluated, and in each case the antigens gave the same degree of activation.

Much of the complement consumption observed with the type-specific polysaccharides was eliminated by use of 30 mM EGTA and could not, therefore, be definitely ascribed to alternate pathway activation. The degree of activation of C3-C9 by the different serotypes of polysaccharides did not appear to bear any relationship to the level of reactivity observed with whole organisms of the corresponding serotypes. For example, type 1 pneumococci were the least reactive organisms, whereas type 1 polysaccharide was the most reactive of the capsular antigens tested.

In studies in which C-substance (C-polysaccharide) was incubated in quantities of 50 to 250 µg/ml with normal rat serum at 37°C, there was...
no consumption of C3-C9 beyond that observed in the control sample without antigen.

Activation of C3-C9 by *E. coli* lipopolysaccharide was evaluated for comparative purposes, using the same methods employed for the pneumococcal polysaccharides. Normal rat serum was completely complemented by 500 μg of the lipopolysaccharide, and 50 μg of lipopolysaccharide reduced the C3-C9 hemolytic titer to 39.4% of the control value during 1 h at 37°C.

To determine whether the preparations of pneumococcal polysaccharide might contain substances that would interfere with or mask C3-C9 consumption, we evaluated the effect of pneumococcal polysaccharide on C3-C9 consumption by lipopolysaccharide. *E. coli* lipopolysaccharide (50 μg) alone consumed 65% of the available C3-C9 activity, whereas 50 μg of the lipopolysaccharide combined with 100 μg of either type 6 or type 7 pneumococcal polysaccharide consumed 63 and 59%, respectively, of the C3-C9 activity. The pneumococcal polysaccharides alone consumed less than 6% of available C3-C9 activity. Thus, type-specific pneumococcal polysaccharides did not appear to inhibit or mask C3-C9 consumption by endotoxin.

**Complement activation by antigens injected into rats.** Type-specific pneumococcal polysaccharides (1.0 mg) or washed, killed whole pneumococci (5 × 10⁹) were injected into the tail veins of rats, and changes in the level of serum C3-C9 activity were compared with rats injected with sterile normal saline. The results (Fig. 3) showed a transient decrease in serum C3-C9 levels after injection of type 3 or type 4 pneumococci, but no decline after injection of type 3 or type 4 pneumococcal polysaccharides. The level of polysaccharide in the serum at 18 h after injection averaged 95.2 μg/ml for type 3 and 107.8 μg/ml for type 4 polysaccharide.

Results with *E. coli* lipopolysaccharide (Fig. 3), which was used for comparative purposes, resembled results obtained with pneumococcal organisms.

As another means of evaluating the possibility of complement activation by pneumococcal

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**Table 2. Effect of EGTA on the consumption of C3-C9 activity in rat serum by pneumococci**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>30 mM EGTA</th>
<th>No EGTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>47</td>
</tr>
<tr>
<td>4</td>
<td>88</td>
<td>&gt;95</td>
</tr>
<tr>
<td>6</td>
<td>67</td>
<td>82</td>
</tr>
<tr>
<td>7</td>
<td>39</td>
<td>&gt;95</td>
</tr>
<tr>
<td>14</td>
<td>&gt;95</td>
<td>&gt;95</td>
</tr>
<tr>
<td>25</td>
<td>35</td>
<td>63</td>
</tr>
</tbody>
</table>

* A total of 10⁸ pneumococci were added per ml of 1:2 dilution of rat serum, and the mixtures (and a control without pneumococci) were incubated for 1 h at 37°C. Results are expressed as a percentage of the control.

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**Fig. 1. Consumption of available C3-C9 hemolytic activity by pneumococci in rat serum treated with 30 mM EGTA.** A total of 10⁸ pneumococci were added per ml of a 1:2 dilution of chelated rat serum, and the mixtures (and a control without pneumococci) were incubated at 37°C. Residual C3-C9 was measured, and results are expressed as a percentage of the control.

**Fig. 2. Consumption of available C3-C9 hemolytic activity by pneumococcal capsular polysaccharides.** Each of the indicated concentrations of polysaccharide was added per milliliter of a 1:2 dilution of rat serum, and the solutions (and a control without polysaccharide) were incubated for 1 h at 37°C. Residual C3-C9 was measured, and results are expressed as a percentage of the control. All concentrations of polysaccharide were tested using normal rat serum (open bars) and 1,000 μg of polysaccharide per ml was also tested in rat serum with 30 mM EGTA (shaded bars).
polysaccharides in vivo, immunofluorescence and histological studies were performed with tissues from rats injected with polysaccharide. Preliminary studies established that type 1, 3, or 4 capsular polysaccharides, injected i.v., were rapidly deposited in several tissues including the liver, spleen and kidney. Systematic histopathological studies of the kidney were performed by injecting rats with 1,500 μg of either type 1, 3, or 4 polysaccharide and sacrificing subgroups of rats at various intervals thereafter. Blood and urine were obtained before and during the period of antigenemia. Virtually identical results were obtained with each of the polysaccharides; only the results obtained with type 3 are reported in detail here (Table 3).

As expected, capsular polysaccharide remained detectable in the circulation for a prolonged period. By use of immunofluorescence, capsular polysaccharide was detected in sections of kidney obtained between 1 and 20 days after antigen injection. The antigen appeared as small, discrete aggregates of fluorescence within the glomerular mesangium. Virtually all glomeruli contained deposits of the antigen. Aggregates of polysaccharide were also present in linear streaks of fluorescence, apparently situated within the capillary walls of the peritubular plexus and, more rarely, in the walls of the glomerular capillaries. The amount of polysaccharide deposited in the glomeruli appeared to increase during the first 2 to 3 days after injection of the antigen, giving some definition of glomerular loops in a few instances. In most sections, however, the fluorescence in glomeruli remained restricted to small mesangial deposits, with little definition of the glomerular capillary loops. Detectable antigen began to decrease by 6 days, and sections examined 6 weeks after antigen injection contained no detectable polysaccharide. In none of the sections was there any evidence of C3 deposition.

In three rats, small focal deposits of IgM were detected in the glomerular mesangium during the first week (Table 3), but IgG was not detected at any time.

All kidneys were examined by light microscopy of 1- to 2-μm sections stained with hematoxylin and periodic acid-Schiff stain. Rare polymorphonuclear leukocytes were seen within glomerular capillaries (1 leukocyte per 2 to 3 glomeruli), but no leukocytes were seen in the mesangium. Sections from control rats also showed rare leukocytes in glomerular capillaries. In no instance was there any increase in mesangial cellularity or matrix. Basement membranes were delicate, and tubules and arterioles appeared normal in all sections.

In keeping with the lack of any significant phlogistic response to deposits of capsular polysaccharide in the kidney, urine protein levels (Table 3) showed no increase over control levels after antigen injection (P > 0.05 by Student’s t test for paired, dependent data). Also, sediments of centrifuged aliquots of each of the urines did not show any change on microscopic examination from the control level of a rare leukocyte or a rare erythrocyte per high-power field.

**DISCUSSION**

These studies indicate that pneumococcal organisms contain antigens which can activate the terminal (C3-C9) complement sequence of rat serum. Each of the pneumococcal serotypes which we studied activated complement in serum treated with EGTA, presumably by acting through the alternate pathway. Serotypes varied in their level of reactivity, as has been described previously for human (7, 19) and guinea pig serum (22). Our results with EGTA-treated rat serum closely resemble the results of Winkelstein and Tomasz (22) who studied C3 activation by pneumococci in C4-deficient guinea pig serum. The reason for the type-associated variability of alternate pathway activation by pneumococci is not clear. Type-related differences have been correlated with the degree of nonimmunospecific binding of IgG by pneumococci of specific serotypes (19), but it has not been established that qualitative or quantitative variations
in the capsule itself are responsible for differences either in Fc binding or in alternate pathway activation by different serotypes. Variation in cell wall composition as a basis for differences in complement activation by pneumococci has not been excluded.

Our results with pneumococcal capsular polysaccharides indicate that these antigens can activate C3-C9 in rat serum in vitro, but activation occurred only at levels of 500 to 1,000 μg of polysaccharide per ml. Much of the consumption was prevented by use of EGTA and could not, therefore, be definitely ascribed to alternate pathway activation. The sera might have contained type-specific activators which could explain the results, or EGTA may have affected modulation of alternate pathway activation by antibody. In an early study, Pillemer et al. (15) detected activation of properdin by 3,000 μg of type 4 or type 14 pneumococcal polysaccharides per ml, but no activation was detected with type 8. In a recent study with C4-deficient guinea pig serum (22), there was complement activation by purified capsular polysaccharide types 1, 4, and 25 in concentrations of 400 to 800 μg per ml, but no activation by up to 800 μg of types 2, 3, 14, or 19. Other investigators (1) have reported marked activation of C3 in C4-deficient guinea pig serum by type 3 polysaccharide (500 μg/ml). Also, de-complementation of human serum chelated with Mg-EGTA by capsular polysaccharide (types 6, 7, 18, and 23) in concentrations of only a few micrograms per milliliter has been described (G. S. Giebink and P. G. Quie, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, Abstr. no. 297, 1977). Cryptococcal capsular polysaccharide (250 μg/ml) and Haemophilus influenzae type b polysaccharide (500 μg/ml) apparently do not activate complement (12, 16). Recent results in our laboratory with type 1, 3, 4, 6, and 7 polysaccharides incubated at 37°C for 1 h with human serum are essentially identical to our findings reported here with rat serum; consumption of more than 10 to 20% of available C3-C9 required 500 μg of polysaccharide or more per ml, and complement consumption was greatly decreased in serum treated with Mg-EGTA (J. D. Coonrod, unpublished data). Differences in results obtained by various investigators may be attributable in part to differences in methods or levels of type-specific or natural antibodies in the sera tested, or to variation in the purity or molecular size of the antigens employed. In general, however, the capsular polysaccharides appear to be only weak activators of the alternate pathway.

In the present study, pneumococcal polysaccharides were injected into rats to give levels of circulating antigen which were comparable to the levels observed in human infection (4), and serum C3-C9 levels did not decrease. In contrast, injection of E. coli polysaccharide into rats did decrease serum C3-C9 levels as did the injection of large numbers of killed whole pneumococci. The lack of any decrease in serum C3-C9 levels after injection of the soluble polysaccharides suggests that antigenemia with the capsular polysaccharides would not account for the reduction in serum complement levels which has been described in some instances of infection. Our present results are in agreement with an earlier study of patients with bacteremic pneumococcal pneumonia, in which we were not able to find a significant correlation between the presence of capsular polysaccharide antigenemia and the degree of reduction of complement levels or alternate pathway activity (6).

As another means of evaluating the effects of capsular polysaccharides in vivo, we examined the kidneys of rats by histological and immunofluorescence methods. We detected deposits of polysaccharide principally in mesangial cells of the glomeruli and to a small extent in capillary walls of the glomeruli and of the peritubular

### Table 3. Results of renal immunofluorescence studies and urine protein excretion in 19 rats injected with type 3 capsular polysaccharide

<table>
<thead>
<tr>
<th>Days after 1,500 μg of polysaccharide i.v.</th>
<th>Mean serum polysaccharide level (μg/ml)</th>
<th>No. of rats sacrificed</th>
<th>No. of rats with positive fluorescence</th>
<th>Mean urine protein (mg/6.5 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;0.05</td>
<td>3</td>
<td>0</td>
<td>1.8</td>
</tr>
<tr>
<td>1</td>
<td>76.8</td>
<td>3</td>
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<td>2.2</td>
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<tr>
<td>2</td>
<td>68.3</td>
<td>3</td>
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<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>21.3</td>
<td>3</td>
<td>3</td>
<td>2.6</td>
</tr>
<tr>
<td>6</td>
<td>3.2</td>
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<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>13</td>
<td>1.6</td>
<td>2</td>
<td>2</td>
<td>2.4</td>
</tr>
<tr>
<td>20</td>
<td>0.8</td>
<td>1</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>42</td>
<td>0.1</td>
<td>2</td>
<td>0</td>
<td>—a</td>
</tr>
</tbody>
</table>

*— Not tested.*
plexus. These findings are in agreement with the early studies of Kaplan et al. (11) who injected mice with type 3 pneumococcal polysaccharide and demonstrated rapid and widespread deposition of the antigen into reticuloendothelial cells, including the renal capillary endothelium. Small hemorrhages were noted in the kidneys and other tissues of their animals, which received very large doses of the polysaccharide (up to 4.0 mg). We did not detect any hemorrhages or other abnormalities in the present studies with 1.5 mg of antigen in rats. There was no evidence of C3 fixation in the deposits of the polysaccharide, and there was no inflammatory response to the antigens. Although many factors may affect the interaction of C3 with tissue-bound antigen, it has recently been shown that both immunoglobulins and C3 can enter the glomerular mesangium of rabbits (13). Failure to detect C3 deposition in conjunction with polysaccharide deposits in the mesangium in rats, therefore, may have biological significance.

Polysaccharide has been detected in the mesangium and, to a lesser extent, in glomerular capillary walls in biopsy material from a patient with type 14 pneumococcal infection complicated by nephritis (10). The presence of glomerular deposits of C3 and properdin with only minimal immunoglobulin deposition led to the suggestion that type 14 polysaccharide had activated the alternate pathway and caused nephritis. Capsular polysaccharides, however, are commonly present in body fluids during infection and, although these polysaccharides are readily detectable in blood and tissues, they may have little potential for producing nephritis. Other pneumococcal antigens, particularly antigens of the cell wall, have been detected in the bloodstream in pneumococcal infection (5) and could have a more important role in the pathogenesis of hypocomplementemia and other manifestations of pneumococcal infection.

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LITERATURE CITED