Agar-Gel Precipitating Antibody in *Pseudomonas aeruginosa* Infections

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The human immune response to *Pseudomonas aeruginosa* infection was studied by using the double diffusion in agar-gel technique. Antigens from Fisher-Devlin-Gnabasik immunotypes were prepared by both trichloroacetic acid extraction and ultrasonic disruption. Serum from 72 of 168 patients (43%) from whom *P. aeruginosa* was isolated formed from one to eight precipitin bands. Precipitins were demonstrated in the sera of 60 of 66 (91%) patients recovering from bacteremia and deep infections; however, they were usually absent when *Pseudomonas* infection was fatal or when there was no clinical evidence of significant infection. Precipitating antibody was detectable at serum dilutions as high as 1:32, and appearance of single bands correlated with hemagglutinating antibody titers of ≥1:128. Antigen from sonically disrupted organisms usually resulted in stronger precipitin bands than trichloroacetic acid extracts, and antigen from the homologous infecting strain occasionally increased test sensitivity. None of 50 normal controls had *Pseudomonas* precipitins as was the case in patients convalescing from *Escherichia coli* (15 patients), *Klebsiella-Enterobacter-Serratia* (18), and *Proteus* (14) bacteremias. Measurement of agar-gel precipitins was useful and specific in evaluating the circulating antibody response to *P. aeruginosa* infections.

Infections caused by *Pseudomonas aeruginosa* are a significant cause of morbidity and mortality in patients with advanced neoplastic diseases (11), full thickness burns (18), cystic fibrosis (2), or recipients of immunosuppressive therapy (19). The incidence of *P. aeruginosa* infections appears to be increasing and has paralleled a general increase in severe, life-threatening infections caused by gram-negative bacilli (8). In recent years, these trends have been particularly evident at this institution despite the use of potent antimicrobial agents which have significant in vitro activity against most isolates of *P. aeruginosa* (22).

Understandably such developments have generated increased interest in the nature of the human immune response to *Pseudomonas* infection (1, 4). (*Pseudomonas* used alone in the text refers to *P. aeruginosa.*) However, a barrier to large-scale systematic studies has been the fact that *P. aeruginosa* is a serologically heterogeneous species (17, 21) and that cross-reactions with other bacterial antigens may occur (1).

The availability of an immunizing preparation derived from the seven immunotype strains of Fisher, Devlin, and Gnabasik (10) has made possible the study of the human circulating antibody response to *P. aeruginosa* infection by using purified lipopolysaccharide antigens with broad antigenic coverage. At the Memorial Center for Cancer and Allied Diseases during 1969, 88% of 546 isolates of *P. aeruginosa* and all 46 isolates from blood could be agglutinated with rabbit antisera prepared against the seven Fisher-Devlin-Gnabasik immunotypes.

We have used the Fisher-Devlin-Gnabasik immunotype antigens in a double diffusion in agar-gel system to detect precipitating antibodies to *P. aeruginosa*. For purposes of comparison, *Pseudomonas* antigens prepared by ultrasonic disruption of whole cells were also used. *Pseudomonas* antibodies detected by immunodiffusion have been correlated with the hemagglutinating antibody response in the same sera. Evidence for the specificity of the precipitating antibody reaction is detailed.

MATERIALS AND METHODS

Preparation of antigens. Lipopolysaccharide antigens prepared from the seven immunotype strains of
Fisher, Devlin, and Gnabasik (9) by trichloroacetic acid extraction (20) and purified by gel filtration were obtained from Myron W. Fisher and Henry B. Devlin of Parke, Davis and Co., Detroit, Mich. These were available as the pooled heptavalent antigen, hereafter referred to as P<sub>sv</sub>, and as individual immunotype antigens. Commercially available Boivin-type lipopolysaccharide antigens of <i>Serratia marcescens</i>, <i>Escherichia coli</i> O26:B6, and <i>Salmonella enteritidis</i> were obtained from Difco Laboratories, Detroit, Mich. Pseudomonas polysaccharide (Piromen), lot 1432-25, was donated by Travenol Laboratories, Morton Grove, III.

Antigens were prepared by ultrasonic disruption of the following strains of intact bacteria: (i) the seven Fisher-Devlin-Gnabasik immunotypes, (ii) P. acido- 
vorans, P. alcaligenes, P. fluorescens, P. maltophilia, <i>P. putida</i>, and <i>P. stutzeri</i> obtained from G. L. Gilardi of the Hospital for Joint Diseases, New York, N.Y.; (iii) <i>E. coli</i> O:14 from the American Type Culture Collection 19110; (iv) the homologous infecting strains from 28 cases of <i>P. aeruginosa</i> infection; (v) the clinical isolates of <i>E. coli</i> (six strains), <i>Proteus mirabilis</i> (six strains), <i>P. vulgaris</i> (three strains), <i>P. morgani</i> (three strains), <i>P. rettgeri</i> (three strains), <i>Klebsiella pneumonae</i> (three strains), <i>Enterobacter</i> species (three strains), nonpigmented <i>S. marcescens</i> (six strains), and <i>Aeromonas hydrophila</i> (two strains).

The method of preparation of sonically treated antigens was as follows: Eighteen-hour bacterial growth on Mueller-Hinton agar was removed with sterile cotton swabs and suspended in 0.5% formalized saline to a concentration standardized at an optical density of 1.3 at 610 nm as measured on a Coleman Jr. Spectrophotometer. Such suspensions contained 10<sup>9</sup> to 10<sup>10</sup> bacteria per ml. Bacteria were washed three times by centrifugation at 11,500 X g in a Sorvall angle centrifuge followed by resuspension in normal saline. After the third washing, 12 ml of the bacterial suspension was placed in the sealed chamber of a Branson ultrasonic cell disrupter (model 185; Heat Systems-Ultrasonics Inc., Plainview, N.Y.). Cell disruption was carried out at 100 to 125 w-sec for 30 min. The antigen suspension was then centrifuged at 2,000 rev/min for 10 min and the sediment was discarded.

A pooled, sonically treated antigen derived from the seven Fisher-Devlin-Gnabasik immunotypes and used in parallel with P<sub>sv</sub> for screening was prepared by individual ultrasonic disruption of types I through VII. Equivalent volumes were combined, and the suspension was concentrated seven times with Lyphogel (Gelman Instruments, Ann Arbor, Mich.); it will hereafter be designated P<sub>on</sub>. Total carbohydrate was determined by the indole test (reference 14, p. 527-528), and total protein was determined by the Lowry method (15).

For comparison purposes, a <i>Pseudomonas</i> antigen prepared by ultrasonic disruption of a single clinical isolate of <i>P. aeruginosa</i> reported in another study (4) was obtained through the courtesy of Arthur White of Indiana University (hereafter designated P<sub>2</sub>).

**Preparation of antisera.** Female albino New Zea-

land rabbits averaging 4 to 6 kg were immunized with either weekly intramuscular doses of purified lipopolysaccharide antigens or weekly intravenous doses of whole phenolized suspensions (10<sup>6</sup> to 10<sup>9</sup> organisms/ml) of Fisher-Devlin-Gnabasik immunotypes I and VII. Additional rabbit antisera to types I through VII were obtained from Henry B. Devlin.

For purposes of comparison, the following commercial rabbit antisera were obtained from Difco Laboratories, Detroit, Mich.: <i>P. pseudomallei</i>, <i>E. coli</i> poly A and <i>E. coli</i> poly B.

Human sera were obtained from venous blood which was allowed to clot and stand for as long as 48 hr at 4°C before centrifugation and separation of serum. In addition to sera from patients from whom <i>P. aeruginosa</i> was recovered, convalescent sera from patients with the following gram-negative rod bacteria were studied: <i>E. coli</i> (15 patients), <i>Klebsiella-Enterobacter-Serratia</i> (18 patients), and <i>Proteus</i> species (14 patients).

**Immunodiffusion technique.** A modification of the double diffusion in agar-gel technique of Macy et al. (16), which uses Lucite matrices containing conical wells to deliver antigen and serum, was employed (Armstrong and Yu, submitted for publication). On each matrix, six outer wells were positioned at a distance of 5 mm from the central well. Wells were filled with 30 µl of each of the reactants and were not refilled. A 2.5 ml amount of 0.9% agarose (Mann Research Laboratories, New York, N.Y.) was overlaid on glass slides [3 by 1.5 inches (7.62 by 3.8 cm)]; agar contained 0.02% sodium azide added to suppress microbial growth. All determinations were run in duplicate. The average periods of observation were 2 or 3 days. After the initial reactions were recorded, agar-gel slides were washed in saline, stained with Crowle’s triple stain, and compared with the initial impression.

**Hemagglutinating antibody measurements.** One hundred and fifteen serum samples from this study were tested for type-specific hemagglutinating antibody against the seven Fisher-Devlin-Gnabasik immunotypes by Henry B. Devlin. By using a microtiter modification of the method of Gaines and Landy (12), blood group O erythrocytes were sensitized with the individual purified lipopolysaccharide prepared from the seven Fisher-Devlin-Gnabasik immunotypes.

**Clinical states.** Clinical states were defined as follows: A diagnosis of <i>Pseudomonas</i> pneumonia was made when the organism was isolated in pure culture from the sputum or was the predominant organism in purulent sputum and unequivocal evidence of pulmonary infiltrates was detected radiologically. Respiratory tract colonization was diagnosed when the sputum contained <i>P. aeruginosa</i> in various numbers but was not associated with fever, dyspnea, rales, and radiological evidence of pulmonary infiltrates. Bronchitis was diagnosed when the isolation of the organism was associated with pulmonary symptoms or signs on physical examination but radiological evidence of pulmonary infiltrates was absent.

<i>Pseudomonas</i> pyelonephritis was diagnosed when two or more cultures of fresh urine yielded 10<sup>5</sup> or more organisms per ml, when five or more white cells per ml, or when two cultures yielded 10<sup>5</sup> or more colonies per ml.
high-powered field were seen on microscopic examination on the centrifuged urinary sediment, and when there were definite symptoms localizing to either flank or radiological abnormalities of one or more kidneys were noted. No attempt was made to localize the site of involvement within the urinary tract if patients had significant but asymptomatic bacteriuria.

A clinically significant wound infection was diagnosed when fever and localizing signs were present and P. aeruginosa was the sole or predominant organism on culture of the wound exudate. Simultaneously, gram-negative bacillary organisms and abundant polymorphonuclear leukocytes on stained smears of wound exudate were necessary for this diagnosis. If fever and localizing signs were absent, the wound was classified as being colonized by Pseudomonas.

RESULTS

Appearance of precipitating antibody in rabbits. Rabbits whose serum initially failed to react with P vac and P son were immunized with whole cell phenolized vaccines and purified lipopolysaccharide of Fisher-Devlin-Gnabasik types I and VII, respectively. After 4 weekly injections, serum from these rabbits showed three to four precipitin bands when reacted with the homologous sonically treated or purified lipopolysaccharide antigen. There was no significant difference in number or intensity of precipitin bands when sera from rabbits immunized by one method were reacted with the sonically treated or purified lipopolysaccharide antigen.

As many as three precipitin bands of identity were observed when rabbit and human sera (see below) containing antibody to the same Fisher-Devlin-Gnabasik types were placed in adjacent immunodiffusion wells and reacted with the type-specific antigen.

Precipitating antibody in human infections. Two hundred and seventy-one serum samples from 168 patients from whom P. aeruginosa was isolated were tested against P vac antigen. P vac and P son were used in parallel against 220 identical serum samples, and in 28 instances a third Pseudomonas antigen, prepared by sonic treatment of the homologous infecting strain, was also used.

In Table 1, the prevalence of gel-precipitating antibody in the serum of 168 patients is correlated with the clinical nature of their infections or circumstances relating to the isolation of P. aeruginosa. Serum from 72 or 43% of patients formed one to eight precipitin bands against one or more of the Pseudomonas antigens (P vac, P son, homologous). The appearance of precipitin bands in 24 patients was detected after an initial specimen was negative, and in 17 patients precipitin bands increased in intensity or in numbers, or in both, from the time of the initial specimen to the follow-up specimen. The remaining positive specimens were obtained during convalescence. Figure 1 demonstrates the strong immunodiffusion pattern when serum from a patient convalescing from Pseudomonas pneumonia was reacted with P vac, P son, and four enterobacteriaceal antigens. Eight precipitin bands are observed between the center well containing serum and P son in well 1. There are no lines of fusion between the Pseudomonas antigen-antibody reactions and the reactions between serum and the enterobacteriaceal antigens.

The results in Table 1 indicate a strong association between the presence of gel-precipitating antibody and recovery from clinically significant Pseudomonas infection. Of 66 patients, 60 (91%) recovering from clinically significant infections had gel-precipitating antibody in their sera. In contrast, only 6 of 63 patients (9%) with fatal Pseudomonas infections had gel-precipitating antibody, a highly significant difference ($P < 0.0005$). Furthermore, gel-precipitating antibody was not likely to be detected in the absence of evidence of significant infection. Asymptomatic bacteriuria, respiratory tract colonization, and isolation of P. aeruginosa from wounds in the absence of fever were not associated with development of gel-precipitating antibody.

Nature of antigen. P vac was found to contain 200 µg of total carbohydrate per ml and 1.65 µg of total protein per ml. P son contained 31 µg of total carbohydrate per ml and 21 µg of total protein per ml. There was no significant change in precipitin bands when P vac or any of the sonically treated antigens were boiled for 0.5 hr, treated with 3% trypsin, or concentrated 20-fold. Precipitin reactions could be abolished by treatment of any of the antigens with periodic acid (reference 14, p. 546).

Comparison of serum reactivity to various Pseudomonas antigens. P vac and P son were compared in 220 immunodiffusion tests (Table 2). In general, the sonically treated antigen yielded more intense precipitin bands and occasionally a greater number of precipitin bands. However, the use of one was not significantly more sensitive than the other, and the use of both antigens in parallel only increased the sensitivity of the gel-precipitin test by 3%.

Sonically disrupted antigens made from the homologous infecting strain usually produced more intense precipitin bands than the screening antigens. In 2 of 28 sera in which precipitins were not detected with the screening antigens, use of antigen from the homologous infecting strain resulted in faint precipitin bands. These
TABLE 1. Clinical findings associated with Pseudomonas agar-gel precipitating antibody

<table>
<thead>
<tr>
<th>Determination</th>
<th>Precipitins</th>
<th>Per cent with precipitins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Recovery from clinically significant infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With bacteremia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Wound infection</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Gastrointestinal abscess</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Without bacteremia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>Wound infection</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>Subtotal</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>Fatal infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With bacteremia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Wound infection</td>
<td>15</td>
<td>16</td>
</tr>
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<td>Gastrointestinal abscess, inflammation</td>
<td>4</td>
<td>4</td>
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<td>Source unknown</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Without bacteremia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Wound infection</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Subtotal</td>
<td>6</td>
<td>57</td>
</tr>
<tr>
<td><em>Pseudomonas</em> isolated, uncertain clinical significance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory tract isolate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Bronchitis&quot;</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>No symptoms, colonization</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Urinary tract isolate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asymptomatic bacteriuria (( \geq 10^8 ) colonies/ml)</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>&lt;10^6 colonies/ml</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Wound isolate, no systemic symptoms</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Subtotal</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>96</td>
</tr>
</tbody>
</table>

were from cases in which the organism was weakly typable by slide agglutination. In the remaining 26 cases, 12 of fatal infection and 14 in which the recovery of *P. aeruginosa* was of uncertain significance, use of antigen from homologous infecting strains did not result in precipitin reactions.

A sonically treated *P. aeruginosa* antigen used by Crowder and White (P\_I) in immunodiffusion studies with human serum (4) was compared with the antigens used in this study. Of 12 sera which reacted positively with P\_vac and P\_son, three were nonreactive with the P\_I antigen. In no instance did a serum react with the P\_I antigen and did not react with P\_vac or P\_son. In Fig. 2, convalescent serum from a patient who had *Pseudomonas* bacterial endocarditis due to a type II organism was reacted with P\_son, P\_vac, P\_I, type II lipopolysaccharide, and sonically treated antigen from the infecting strain. Two lines of identity were observed upon reaction of serum in the center well with the various antigenic preparations in the outer wells, indicating that these preparations have at least two common antigens.

No precipitin bands were detected when serum strongly positive with P\_vac, P\_son, and P\_I was tested against Piromen (*Pseudomonas polysaccharide*).

**Antibody in control sera.** No *Pseudomonas* gel-precipitating antibodies were detected in the sera of 25 normal individuals who were weekly donors of platelets at Memorial Hospital. An additional 25 sera from patients which were submitted for routine VDRL serology were found to be negative for *Pseudomonas* precipitins.
Fourty-seven patients convalescing from E. coli (15 patients), Klebsiella-Enterobacter-Serratia (18 patients), and Proteus (14 patients) bacteremias had serum specimens drawn between the second and fourth week after their initial positive blood cultures were obtained. Two of these patients had Pseudomonas gel-precipitating antibody. However, when serum from these two patients was used in an immunodiffusion system with Pseudomonas and enterobacteriaceal antigens, the precipitin bands which formed showed clear crossing rather than any lines of identity. Neither patient had a documented history of Pseudomonas infection although both patients had ileal bladders. Pseudomonas precipitins were not detected in commercial rabbit antiserum against P. pseudomallei, E. coli poly A, and E. coli poly B.

Reactions against other antigens in sera positive for P. aeruginosa antibodies. Sera from 30 patients which contained gel-precipitating antibody against P_vac and P_on were simultaneously reacted against each of the following: pooled E. coli (six isolates), Klebsiella-Enterobacter (six isolates), P. mirabilis (six isolates), and S. marcescens (six isolates) antigens prepared by ultrasonic disruption. The sera of six patients were tested against pooled P. vulgaris (three isolates), P. morgani (three isolates), and P. rettgeri (three isolates). The sera of four patients were tested against pooled sonically treated A. hydrophilia (two isolates) and individual sonically treated antigens of P. acidovorans, P. alcaligenes, P. fluorescens, P. maltophilia, P. putida, and P. stutzeri. Although 21 of 29 patients had precipitating antibody against one or more enterobacteriaceal antigens, no lines of fusion formed with their Pseudomonas precipitins.

One patient with P. aeruginosa bacteremia and a history of recurrent gram-negative bacillary...
urinary tract infections had serum which clearly reacted with \( P_{son} \) and the enterobacteriaceal antigens, forming two lines of identity. However, no lines of identity were noted when the purified lipopolysaccharide antigen (\( P_{vac} \)) was used.

No precipitin bands of identity were observed when serum from four patients containing \( P. \) aeruginosa gel-precipitating antibody was tested against the sonically treated \( A. \) hydrophilia antigen (two strains), the six sonically treated antigens of other members of the genus \( Pseu-
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domonas, and commercial lipopolysaccharide antigens of \( S. \) marcescens, \( S. \) enteritidis, and \( E. \) coli \( O26:B6 \).

Temporal appearance and persistence of \( Pseu-
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domonas gel-precipitating antibody. In one pa-
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tient with \( P. \) aeruginosa bacteremia, gel-pre-
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cipitating antibody was detected 5 days after the first positive blood culture was obtained. Twenty-four other patients whose serum was positive for precipitins were followed with serial measurements of antibody from the time of onset of infection. Seventy-five per cent de-
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volved precipitins between the second and third week of infection, and the remainder de-
}
volved precipitins within 30 days of onset of infection. Of 16 patients followed for 6 or more months after recovery from infection, 14 were found to have persistent gel-precipitating antibody. One patient followed for 18 months con-
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tinued to have serum which formed two pre-
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cipitin bands against \( P_{vac} \) and \( P_{son} \).

Intensity of precipitin bands and relationship to hemagglutination titers. The intensity and number of precipitin bands varied from single, faint lines to multiple, strong lines. Strongly reacting sera could be titered to a 1:32 dilution. There was poor correlation, however, between intensity of reaction and severity or duration of infection. Patients varied widely as to basic diagnoses, therapy, and antibiotic treatment.

Figure 3 demonstrates the relationship be-
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	ween the hemagglutination titer against the same type as the homologous infecting strain and the number of precipitin bands formed when serum was reacted against the individual (types I through VII) lipopolysaccharide antigens. In general, the detection of single bands correlated with hemagglutination titers of 1-128 or greater. The number of patients' sera showing two or more precipitin bands is too small to correlate with a range of hemagglutination titers. However, the reciprocal geometric mean antibody titer against the type of the infecting strain was significantly greater for patients with one or more precipitin bands than those who showed no precipitin bands (\( P < 0.0005 \)).

Type specificity of precipitating antibody response. By standard slide agglutination tech-
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niques, Fisher-Devlin-Gnabasik types I, II, and VII accounted for 26, 15, and 12\%, respectively, of all typable isolates. Each of the remaining four types as well as those organisms which agglutinated with more than one antiserum accounted for less than 10\% of the typable organisms.

Of the 72 patients with \( Pseu-
}
domonas precipitins against \( P_{vac} \) or \( P_{son} \), 62 (86\%) had specific pre-

![Fig. 3. Relationship between hemagglutination titer and the number of precipitin bands formed against the type of the infecting or colonizing \( Pseu-
}
domonas isolate.](http://iai.asm.org/)
cipitating antibody against the type of the infecting strain. Four of the 10 isolates from cases in which a correlation could not be made were autoagglutinatable. On the other hand, antibody to more than one Fisher-Devlin-Gnabasik type was frequently demonstrated in serum from patients whose isolates agglutinated strongly with only one antiserum. Table 3 summarizes these results, based on analysis of the 56 patients represented in Fig. 3 who had one or more precipitin bands in their sera against $P_{vac}$. When single precipitin bands were present against the type of the homologous infecting strain, single bands were also present against other immunotype antigens in 18 of 32 or 56% of the cases. With an increase in the number of precipitin bands against the type of the homologous infecting strain, the number of heterotypic reactions increased.

No lines of identity were detected when serum from rabbits immunized with lipopolysaccharide types I through VII inclusive were tested with the individual type-specific antigens. Similarly convalescing human sera formed precipitin bands of identity only when the sera being compared were from patients infected with the same Fisher-Devlin-Gnabasik type. Therefore, no species-specific antigen for $P. aeruginosa$ could be detected in $P_{son}$ and $P_{vac}$.

Figure 4 demonstrates that, when sera from different patients infected by organisms of the same Fisher-Devlin-Gnabasik type are compared (in this case type II), multiple lines of identity form against the antigen of the common infecting type. The sera were obtained from patients recovering from type II bacteremia, osteomyelitis, pneumonitis, endocarditis, wound infection and pyelonephritis.

**DISCUSSION**

Passive hemagglutination (5, 6, 12), mouse protection (4, 9), the serum bactericidal reaction (7), and agar-gel diffusion (2, 3) are four methods which have been employed to measure natural antibodies against $P. aeruginosa$ in man. The measurements of gel-precipitating antibody cannot be regarded as sensitive a measurement as hemagglutinating antibody, as Fig. 3 demonstrates. Since normal persons usually have hemagglutination titers of less than 16, a fourfold increase in titer is possible without the appearance of gel-precipitating antibody. However, immunodiffusion is a highly specific and reproducible method for demonstrating circulating antibodies to $P. aeruginosa$. None of 47 patients convalescing from other gram-negative rod bacteremias developed gel-precipitating $Pseudomonas$ antibodies. Only 1 of 30 patients studied who had $Pseudomonas$ antibody formed lines of identity when his serum was reagent against sonically treated enterobacteriaeal and sonically treated $Pseudomonas$ antigens; this line of identity was not detected when the lipopolysaccharide antigen was used. Burns and May (2) and Crowder, Fisher, and White (3), working with sonically treated antigens, also observed antigens shared between $P. aeruginosa$ and members of the family Enterobacteriaceae. Hobbs et al (13), using both sonically treated and lipopolysaccharide-extracted antigens in an Ouchterlony system, observed that $P. aeruginosa$ shared antigens with Aeromonas, Vibrio, "para-colon" species, and Achromobacter but failed to share antigens with the Enterobacteriaceae. Since preparation of sonically treated antigens

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**TABLE 3. Agar gel precipitin test with lipopolysaccharide Pseudomonas antigens: heterotypic reactions**

<table>
<thead>
<tr>
<th>No. of bands against immunotype of infecting strain</th>
<th>No. of other Fisher-Devlin-Gnabasik antigens which form precipitin bands with same serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1 2 3 4 5 6</td>
</tr>
<tr>
<td>One band (32) ........................................</td>
<td>14 10 6 1 1</td>
</tr>
<tr>
<td>Two bands (16) .........................................</td>
<td>0 5 4 6 1</td>
</tr>
<tr>
<td>Three or more bands (8) ................................</td>
<td>0 3 1 1 1 2</td>
</tr>
</tbody>
</table>

**FIG. 4.** Two lines of identity detected when the type II lipopolysaccharide antigen (c) was reacted against convalescent serum from six patients with infections due to type II organisms. Infections were (1) osteomyelitis, (2) pyelonephritis with bacteremia, (3) wound infection, (4) pyelonephritis, (5) endocarditis, (6) pneumonia.
from whole intact bacteria results in release of subcellular components, the possibility remains that lines of identity represent antibody to other than cell wall components, such as nucleoproteins. Regardless of the explanation, both this investigation and that of Crowder and White (4) indicate that the incidence of cross-reactions with organisms commonly encountered in nosocomial infections is not high; it may also be possible to minimize or abolish such cross-reactions by use of purified Pseudomonas lipopolysaccharides.

It is clear that to undertake general screening for Pseudomonas antibodies, a system with broad coverage of prevalent antigenic types is necessary. The Fisher-Devlin-Gnabasik schema currently meets this requirement at this hospital. This antigenic schema has its counterparts in both the Habs and Verder-Evans classification (M. W. Fisher, private communication) but was derived on the basis of cross-protection studies in animals rather than by slide agglutination techniques. It employs fewer typing antigens and antisera than other methods of classification, but, by comparison, one study which used the modified Verder-Evans system could measure the hemagglutinating antibody response in about 60% of cases by using only three antigens (5). At this institution, Fisher-Devlin-Gnabasik types I, II, and VII now comprise 53% of all typable clinical isolates. The experience in this study also indicates that use of antigens from homologous infecting strains seems justified only if the clinical isolates cannot be readily typed with Fisher-Devlin-Gnabasik antisera.

As screening antigens, both P\textsubscript{vac} and P\textsubscript{on} derived from the Fisher-Devlin-Gnabasik system appear to be of equal sensitivity, although stronger precipitin bands were more frequently noted with P\textsubscript{on}. The higher protein content of P\textsubscript{on} may be the explanation for this, although concentration of P\textsubscript{vac} 20-fold and trypsinization or boiling of P\textsubscript{on} did not alter the results in cases in which the sonically treated antigen seemed to give stronger precipitin bands. That reactive lipopolysaccharide was the common denominator of the various antigens employed by us in this study and in that of Crowder and White (4) is underscored in Fig. 2. There are at least two bands of identity in all reactions between convalescent serum and the purified lipopolysaccharide or sonically treated antigens. Furthermore, use of periodic acid to break polysaccharide bands abolished the precipitin reaction.

Consistent type—specific antibody responses in human and rabbit sera can be measured by immunodiffusion. When more than one precipitin band forms against the type of the infecting strain, it is likely that precipitating antibody against one or more of the other six types will be present (Table 3). These findings suggest at least two explanations. First, that the strongest serological reactions are to Pseudomonas strains containing a multiplicity of somatic antigens, or, secondly, that wild-type strains of P. aeruginosa may share a number of antigens in common with the Fisher-Devlin-Gnabasik groups and during a particularly intense infection antibody to a number of antigenic determinants will be produced.

Our data are in general agreement with those of Crowder and White (4) in regard to antibody formation and infection in specific organ sites. We failed to observe precipitins where Pseudomonas appeared to colonize or contaminate respiratory secretions, urine, or wounds, but when definite symptoms attributable to infection were present precipitins were then detected with a high degree of frequency.

The role of gel-precipitating antibody in Pseudomonas immunity is undetermined at this time. The association with recovery from clinically significant infection is striking as contrasted with fatal infection or lack of evidence of significant infection. Since antibody tends to appear after the first week of infection and most lethal Pseudomonas infections in cancer patients are rapidly fatal, the development of Pseudomonas antibody may merely accompany recovery from Pseudomonas infection rather than be a prime factor in contributing to clinical recovery. Patients in groups I and II of Table 1 are not comparable populations in terms of duration of exposure to infecting strains of Pseudomonas. On the other hand, 71% of patients in group II had localized infections with Pseudomonas before developing blood-borne dissemination of disease. In a smaller group of 21 patients observed prospectively, the appearance of antibody at the height of severe, clinical illness offered a highly favorable prognosis (L. S. Young and D. Armstrong, unpublished data). This suggests a protective role for antibody as does the finding of significant mouse protective activity in the sera of several of our subjects (M. W. Fisher, private communication). However, firm conclusions about the clinical significance of human antibody detected by agar-gel diffusion may be provided by controlled trials of Pseudomonas immunizing preparations and antisera in humans.

**LITERATURE CITED**

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