Type-Specific Indirect Hemagglutinating Antibody in Patients with *Pseudomonas aeruginosa* Infection

SHIRO SHIGETA,* YOSHIO YASUNAGA, AND MASAHIRO OGATA

Department of Bacteriology and Central Clinical Laboratory, Fukushima Medical College, Fukushima, Japan

Received for publication 3 August 1978

Lipopolysaccharide antigens were extracted from heated cell extracts of several serotypes of *Pseudomonas aeruginosa*. Indirect hemagglutination with the extracts indicated specific reactivity with sera from rabbits immunized with homologous serotypes of *P. aeruginosa*. Sera from healthy adults and patients infected with *P. aeruginosa* were studied subsequently and shown to possess antibodies against *P. aeruginosa*. In patients infected with *P. aeruginosa* type E, indirect hemagglutination antibody against type E was resistant to 2-mercaptoethanol (2-ME) and classified as immunoglobulin G. In patients infected with any other type of *P. aeruginosa* and in healthy adults, it was sensitive to 2-ME and classified as immunoglobulin M. The antibody in the seven patients infected with *P. aeruginosa* was titrated during the course of infection with lipopolysaccharide antigen prepared from the infecting strain. As a result, all patients either possessed 2-ME-resistant antibody or showed antibody rise to homotypic antigen during the infection. However, no patient showed 2-ME-resistant antibody against heterotypic lipopolysaccharide antigens. In hospitalized patients, the incidence of antibody against type E, G, and I *Pseudomonas* strains was greater than that against type A. In particular, 2-ME-resistant antibody to all four serotypes was detected at a higher rate in hospitalized patients than in healthy adults.

Several epidemiological assays for *Pseudomonas aeruginosa* infection revealed that some particular types of *P. aeruginosa* were prevalent in the hospital environment and caused nosocomial infection (9, 16, 21). By using an agglutination test, *P. aeruginosa* was divided into seven serotypes by Fisher et al. (5), 13 by Homma (8), 11 by Verder and Evans (18), 12 by Habs (7), 13 by Lanyi (10), and 17 by Meitert (13).

As a result of bacterial studies of specimens from hospitalized patients (9, 16), it was reported that types 5 and 8 in Homma's serogroup were prevalent in the hospital environment in Japan. For the epidemiological study of *P. aeruginosa* infection, a survey based on the frequency of isolation is inadequate to prove the prevalence of infection by a particular type of this bacterium. The frequency and the site of sampling during the infection may bias the results. Thus, some serological diagnostic methods should be combined to confirm infection with *P. aeruginosa*.

In the present report, we demonstrate the results of titration of type-specific indirect hemagglutination (IHA) antibody to *P. aeruginosa* in human sera. In particular, we emphasize the diagnostic value of the detection of type-

specific immunoglobulin G (IgG) antibody in patients infected with *P. aeruginosa*. In addition, the incidence of antibody against four serotypes of *P. aeruginosa* was investigated among patients hospitalized in Fukushima Medical College Hospital in Japan.

MATERIALS AND METHODS

***P. aeruginosa* strains.** All strains were isolated from clinical specimens of patients admitted to Fukushima Medical College Hospital. Grouping of *P. aeruginosa* was performed serologically, following the slide agglutination procedures of Homma (8). All strains were cultured on a slant of Dorsett egg yolk agar and kept at 4°C until use.

Preparation of LPS antigen. *P. aeruginosa* was inoculated on brain heart infusion agar (Nissui Pharmaceutical Ltd., Tokyo), and the culture was incubated at 37°C for 24 h. After the incubation, the culture was harvested, and approximately 2 g (wet weight) of organisms was suspended in 5 ml of 0.067 M phosphate-buffered saline (PBS; pH 7.2). The suspension was heated at 120°C for 90 min, diluted with distilled water, sonically treated for 90 s with a Tomy Sonifier (Tominaga Seiki Ltd.), and then centrifuged at 3,000 rpm for 10 min. The supernatant was used for the preparation of lipopolysaccharides (LPS) antigen, following the method of Westphal and Luderitz (19). For the LPS antigen preparation, *P. aeruginosa* types A, E, G, and I were used. The specificity of LPS

antigen was confirmed by IHA and agar-gel precipitation tests based on homologous and heterologous antibody titration or detection of precipitin bands, using type-specific anti-*Pseudomonas* rabbit sera. These LPS antigens absorbed only the type-specific antibody from immune rabbit sera (17).

Antisera. Type-specific anti-*Pseudomonas* rabbit sera, prepared against the 13 serotypes of *P. aeruginosa* classified in Homma's revised schema (8), were purchased commercially from Toshiba Biochemical Ltd. (Niigata, Japan). All antisera were absorbed with sheep erythrocytes before use.

Sampling of patients' sera. Three to five milliliters of blood was collected from patients with or without *Pseudomonas* infection who were hospitalized in, or who visited the outpatient clinic of, Fukushima Medical College Hospital. From each blood sample, serum was separated by centrifugation at 3,000 rpm for 10 min and frozen at -20°C until use. Before antibody titration, all sera were heated at 56°C for 30 min and then absorbed with sheep erythrocytes.

Antibody titration. Type-specific antibody against each serotype of *P. aeruginosa* was titrated by IHA by a slight modification of the method of Middlebrooke and Dubos (14). Briefly, a 2.5% suspension of washed sheep erythrocytes in PBS (pH 7.2) were mixed with an equal volume of LPS antigen, which was diluted in PBS to contain 4 IHA units. The mixture was incubated at 37°C for 1 h, and then the sensitized erythrocytes were washed three times with PBS and diluted to a 0.5% cell suspension with the specially prepared PBS, which contained a trace amount of bovine serum and sheep erythrocyte membrane (Fujizaki Pharmaceutical Ltd., Tokyo). Serum was diluted in PBS by the microtiter method, as follows. Into each well of a microplate containing 25 μl of diluted serum was added a 50- μl sensitized cell suspension, and then the microplate was shaken on a vibrator for 5 min and allowed to stand overnight at 20°C . The reciprocal of the highest dilution of serum which showed apparent agglutination of erythrocytes was designated the antibody titer.

Treatment of serum with 2-ME. To detect the IgG antibody, 0.2 mol of 2-mercaptoethanol (2-ME) was added to the patient's serum, and the mixture was incubated at 37°C for 2 h before antibody titration. For IHA assay, dialysis of 2-ME was not necessary if the serum was diluted 1:4 or greater.

DEAE-cellulose column chromatography. Diethylaminoethyl (DEAE)-cellulose chromatography was used for the separation of serum immunoglobulins, following the method of Leonard et al. (11). Briefly, a 10-ml column (1 by 15 cm) packed with DEAE-cellulose was washed with phosphate-buffered (PB) solution (0.01 mol, pH 8.0) for 24 h. A 1.0-ml serum sample, which had been dialyzed overnight against 0.01 mol of dibasic sodium phosphate (pH 9.5), was layered on the column, and serum fractions were eluted by a stepwise procedure, using the following four PB solutions successively: 0.01 mol, pH 8.0; 0.05 mol, pH 7.0; 0.1 mol, pH 6.0; 0.3 mol, pH 4.5. Each eluted fraction was concentrated by dialysis against PBS (pH 7.2), and the protein was adjusted to 500 mg/dl. The protein concentration in the fraction was estimated by the method of Lowry et al. (12).

Immunodiffusion. For the quantitative analysis of immunoglobulins in human serum, a single radial immunodiffusion was employed, using agar-gel plates containing anti-IgG or anti-IgM rabbit serum (Behringwerke).

RESULTS

Antibody titration of human serum to *P. aeruginosa* type E. Twenty-two sera collected from 19 patients infected with *P. aeruginosa* were tested for IHA antibody to LPS antigens of *P. aeruginosa*. In Table 1 the IHA antibody titers to type E and to the strains isolated from the patients are given. All 22 sera possessed antibody to type E antigen, with titers ranging from 10 to 320. When these sera were treated with 0.2 mol of 2-ME, all seven sera from four patients infected with type E *P. aeruginosa* showed antibody titers of 32 to 160 to type E (Table 1). On the other hand, the antibody titers in 15 sera from 15 patients infected with types of *P. aeruginosa* other than type E were reduced to less than 10 after 2-ME treatment. When 10 of these 15 sera were examined for antibody against the LPS antigen prepared from the patient's homological strain, 9 sera had titers ranging from 16 to 160 and 6 had 2-ME-resistant antibody titers varying from 8 to 80. Ten healthy

TABLE 1. Effect of 0.1 mol of 2-ME on IHA antibody to *P. aeruginosa* LPS antigens

Serum	Sero-type of isolate	IHA antibody titer (reciprocal) to:			
		Type E		Patient's strain	
		Un-treated	Treated with 2-ME	Un-treated	Treated with 2-ME
R-40	E	32	32		
R-58	E	56	112		
R-59	E	80	40		
R-62	E	64	32		
R-64	E	320	160		
R-65	E	256	64		
R-73	E	128	64		
R-41	A	8	<4	NT ^a	NT
R-12	B	64	<4	NT	NT
R-51	B	32	4	NT	NT
R-71	B	128	6	NT	NT
R-88	B	20	<10	40	<10
R-76	C	160	<10	160	20
R-90	C	40	<10	160	10
R-70	D	64	<4	64	8
R-82	F	80	<10	<10	<10
R-69	G	256	<4	16	<4
R-75	G	128	<4	32	32
R-86	G	10	<10	40	<10
R-84	H	320	<10	320	80
R-92	I	80	<10	80	40
R-80	M	20	<10	NT	NT

^a NT, Not tested.

adults were examined for antibodies to type E LPS antigen, and all had titers ranging from 8 to 64, decreasing to less than 4 after 2-ME treatment.

Fractionation of sera from patients and healthy adults by DEAE-cellulose chromatography. Fractionation of serum by DEAE-cellulose chromatography showed that fraction 1, which was eluted by pH 8.0 PB solution, and fraction 2, which was eluted by pH 7.0 PB solution, contained only IgG. In fraction 3, eluted by pH 6.0 PB solution, no remarkable IgG or IgM was detected. In fraction 4, eluted by pH 4.5 PB solution, a considerable amount of IgM and a trace of IgG were detected. Results of the frac-

tionation of four human sera are illustrated in Table 2. When two sera from patients infected with type E *P. aeruginosa* were fractionated and anti-E antibody in each fraction was titrated, antibody activity was detected mainly in fractions 1 and 4. However, in the case of two sera from healthy adults who possessed anti-E antibody, the antibody was detected only in fraction 4.

Homologous and heterologous antibody titration of patients' sera with *P. aeruginosa* isolated from the same patients. To investigate the diagnostic value of serological tests for *P. aeruginosa* infection, patients' sera were examined for antibody during the course of infection. Of these patients, five infections were urinary, one was respiratory, and one was septic. Paired or tripled sera were sampled from seven patients infected with *P. aeruginosa*, and both 2-ME-sensitive and 2-ME-resistant antibodies were titrated reciprocally, using LPS antigens from the seven strains of *P. aeruginosa* isolated from the patients.

When the sera were examined before 2-ME treatment, six of seven patients demonstrated a type-specific antibody rise (twice), and three (A.K., K.Y., and Z.T.) demonstrated a significant rise ($\geq 4\times$) in titer during the infection (Table 3). A significant rise was observed as early as 16 days after infection in the case of patient A.K. All of the patients possessed heterotypic antibody in the range of 10 to 640, but no significant rise of titer was observed during the infection. When these sera were treated with 2-ME, heterotypic antibody titers were reduced to less than 10 in all seven patients (Table 4). However, five patients (M.U., T.H., K.K., K.W., and Z.T.)

TABLE 2. *Immunoglobulins and specific antibody in sera of type E-infected patients and healthy adults*

Serum	Infection with <i>P. aeruginosa</i> type E	Fraction ^a	Immunoglobulins (mg/dl)		Anti-E titer (reciprocal)
			IgG	IgM	
R-62	Yes	1	500	(-) ^b	8
		2	128	(-)	<4
		4	34	107	16
R-64	Yes	1	158	(-)	128
		2	366	(-)	16
		4	24	138	64
H-23	No	1	360	(-)	<4
		2	90	(-)	<4
		4	14	138	16
H-24	No	1	200	(-)	<4
		2	210	(-)	<4
		4	(-)	152	16

^a In fraction 3 no remarkable immunoglobulins and antibodies were observed.

^b Precipitin ring was not detectable.

TABLE 3. *Antibody titration of patients' sera with LPS antigens of homologous and heterologous strains of *P. aeruginosa* (untreated sera)*

Patient	Serotype of isolate	No. of strains isolated	Days after 1st isolation of organisms	Titer with LPS antigen:						
				4122-B	1863-C	3991-C	3572-E	4060-G	3920-H	4132-I
A.K.	4122-B	2	4	10	20	40	40	10	160	80
			16	40	40	40	20	10	160	160
M.U.	1863-C	7	7	40	80	80	40	20	320	160
			47	40	160	80	80	20	320	320
T.H.	3991-C	10	150	<10	160	80	40	40	640	80
			165	<10	160	160	40	40	320	80
K.K.	3752-E	10	96	10	NT ^a	NT	64	<10	NT	<10
			153	10	NT	NT	64	<10	NT	<10
			325	10	NT	NT	128	<10	NT	<10
K.Y.	4060-G	3	8	<10	10	10	10	<10	160	80
			23	<10	20	20	10	40	160	80
K.W.	3920-H	7	25	10	20	20	320	<10	320	80
			40	<10	20	20	320	<10	320	80
Z.T.	4132-I	8	10	20	20	10	80	<10	80	10
			145	20	20	10	80	<10	80	80

^a NT, Not tested.

TABLE 4. Antibody titration of patients' sera with LPS antigens of homologous and heterologous strains of *P. aeruginosa* (2-ME-treated sera)

Patient	Serotype of isolate	No. of strains isolated	Days after 1st isolation of organisms	Titer with LPS antigen:						
				4122-B	1863-C	3991-C	3752-E	4060-G	3920-H	4132-I
A.K.	4122-B	2	4	<10	<10	<10	<10	<10	<10	<10
			16	<10	<10	<10	<10	<10	<10	<10
M.U.	1863-C	7	7	<10	<10	<10	<10	<10	<10	<10
			47	<10	20	<10	<10	<10	<10	<10
T.H.	3991-C	10	150	<10	<10	<10	<10	<10	<10	<10
			165	<10	10	10	<10	<10	<10	<10
K.K.	3752-E	10	96	<10	NT ^a	NT	64	<10	NT	<10
			153	<10	NT	NT	32	<10	NT	<10
			325	<10	NT	NT	64	<10	NT	<10
K.Y.	4060-G	3	8	<10	<10	<10	<10	<10	<10	<10
			23	<10	<10	<10	<10	<10	<10	<10
K.W.	3920-H	7	25	<10	<10	<10	<10	<10	80	<10
			40	<10	<10	<10	<10	<10	80	<10
Z.T.	4132-I	8	10	<10	<10	<10	<10	<10	<10	<10
			145	<10	<10	<10	<10	<10	<10	40

^a NT, Not tested.

demonstrated 2-ME-resistant antibody against homotypic antigen, and in the case of patient K.W. the antibody was detected on day 25 after infection.

Immunodiffusion of LPS antigens with immune rabbit sera and patients' sera. Twelve LPS antigens were prepared from three strains each of *P. aeruginosa* types A, E, G, and I. These antigens were examined for precipitin bands against homotypic and heterotypic immune rabbit sera. Only one precipitin band against homotypic immune serum and none against heterotypic immune sera were observed. A fused precipitin band appeared in gel between three homotypic antigens and homotypic immune serum. In the case of type E and I antigens, a precipitin band was observed against patients' sera which had IHA antibodies to each LPS antigen. This precipitin band against patients' sera was shown to be fused with the band which appeared in LPS antigen and homotypic immune serum (Fig. 1).

IHA antibody-positive incidence in hospitalized patients against type A, E, G, and I *P. aeruginosa*. One hundred twenty-five patients hospitalized at Fukushima Medical College Hospital and 73 patients who visited the outpatient clinic of the hospital were examined for the presence of IHA antibody to type A, E, G, and I *P. aeruginosa* in comparison with 27 healthy adults. Each of the three antigens of the serogroups was used for IHA antibody titration. These antigens were previously shown to have homologous antigenicity by immunodiffusion and reciprocal IHA antibody titration against immune serum (Table 5). For 2-ME-sensitive antibody, a 1:16 serum dilution was determined to be the positive level for antibody. However,

a 1:8 serum dilution was taken as the positive level for 2-ME-resistant antibody. In all 12 antigens used, the level of 2-ME-sensitive antibody was similar among hospitalized, outpatient, and healthy groups (Fig. 2). However, against each of the three strains of types E and G, antibody levels were higher than those against types A and I. In the case of 2-ME-resistant antibody, hospitalized patients had a higher rate of positive incidence of antibody against almost all antigens than did outpatients and healthy adults, the exceptions being some strains of type G in which the positive rate was similar in hospitalized and outpatient groups. Among hospitalized patients, a high incidence of detectable antibody was observed against types E, G, and I compared with type A.

DISCUSSION

Serological diagnosis of *Pseudomonas* infection is difficult because of the chronic process of infection and the presence of antibody in healthy persons (6). Diaz et al. observed IHA antibody rises in patients with neoplastic disease and cystic fibrosis (37.5 and 71%, respectively) during the course of infection with *P. aeruginosa* (3, 4). When LPS vaccine of *P. aeruginosa* was given to normal volunteers and cancer patients, remarkable IHA antibody response was shown, although the bulk of antibody was IgM (20).

In our study, IHA antibody to type E *P. aeruginosa* was detected not only among the patients infected with type E, but also among those infected with other types of *P. aeruginosa* and in healthy adults. Thus, it is not likely that the detection of IHA antibody in serum is useful for the diagnosis of *P. aeruginosa* infection. However, the type-specific antibody was de-

FIG. 1. Immunodiffusion pattern between LPS antigens and patient's serum or immunized rabbit serum. LPS antigens of type E (3752E, 1883-E) reacted against a rabbit serum immunized with type E (A-E) and 3752-E reacted against a patient's serum (#65). Three precipitin bands were fused with each other. LPS antigens from other serotypes (1839-A and 3704-I) did not react against A-E and #65. The well designated N contained serum from a healthy adult without anti-E antibody.

TABLE 5. Specificity of LPS antigens prepared from 12 strains of various serotypes of *P. aeruginosa*

Antigen	Antigen titer ^a for:			
	Anti-A	Anti-E	Anti-G	Anti-I
1839-A	160	— ^b	—	—
1861-A	160	—	—	—
2018-A	1,280	—	—	—
1882-E	—	80	—	—
1891-E	—	80	—	—
3752-E	—	160	—	—
1932-G	—	—	160	—
1987-G	—	—	640	—
2034-G	—	—	160	—
1856-I	—	—	—	40
3704-I	—	—	—	40
4132-I	—	—	—	40

^a Reciprocal of maximum dilution of LPS preparation which showed positive reaction by IHA.

^b —, <10.

tected solely in patients infected with the same type of *P. aeruginosa* after 2-ME treatment of the serum. By DEAE-cellulose chromatography,

these 2-ME-resistant antibodies detected in patients' sera seemed to be IgG. Reyes et al. also detected 2-ME-resistant antibody in patients with *P. aeruginosa* endocarditis (15). We also tested IHA antibody in paired or tripled sera from patients with urinary, respiratory, and septic infections. As a result of homologous and heterologous antibody titration with LPS antigens of *P. aeruginosa* isolated from the same patients, the 2-ME-resistant antibody was detected in five of seven patients, using homotypic antigens, but in none of the seven patients, using heterotypic antigens. Antibody titers ranged from 16 to 320 in 2-ME-sensitive antibody and from 8 to 160 in 2-ME-resistant antibody. These titers were lower than those found by Reyes et al. (15) in patients with *Pseudomonas* endocarditis, whose IHA titers ranged from 8 to 8,192 (15). The reason for low IHA titer in our cases is not known, but the site of infection seemed to influence the antibody production. The site of infection was intravascular in Reyes's cases (endocarditis), and it was extravascular in most of

FIG. 2. Incidence of IHA antibody to type A, E, G, and I *P. aeruginosa*. Three strains of each serotype were used as LPS antigen. The sera were collected in January and July 1977 at Fukushima Medical College Hospital. Symbols: (▨) hospitalized patients, 125 examined; (▤) outpatients, 73; (□) healthy adults, 27.

our cases. The amount of LPS antigens able to reach the immune system through the blood stream may determine the production of type-specific antibody.

Most Japanese workers have reported that types 5 and 8 of Homma's serogroup are dominant in hospital-acquired *P. aeruginosa* infection. In Homma's extensive study, these serotypes corresponded to types E and G of his own revised serogroup and to types 2 and 1 of Fisher's serogroup. The study by Young and Moody during 1970 to 1973 demonstrated that Fisher's type 1 (Homma's type 8) was predominant in the isolates from clinical specimens (21). Recently, Brokopp et al. (2) examined serological typing of *P. aeruginosa* by using commercial antisera and live antigens. In their report, O antigen group 6 (corresponding to Habs' type 6 and Homma's type 8) was most prevalent in four hospitals in the United States, one in Brazil, and one in Spain (2). On the other hand, in Baltimore's report of a survey at Walter Reed Army Hospital in Washington, D.C., Fisher's type 2 (Homma's type 5) was most frequently isolated (1). In our previous study of serogrouping of *P. aeruginosa* isolated from clinical specimens

from patients admitted to Fukushima Medical College Hospital, Homma's types 5 and 8 were most frequently detected (16). In the present report, we have demonstrated that the 2-ME-resistant, type-specific antibody was present only in patients who were infected with the same type of *P. aeruginosa* as the antigen used. Thus, the detection of type-specific IgG antibody in serum may indicate past infection with *P. aeruginosa* of the same serotype. We examined 125 hospitalized patients and 73 outpatients at Fukushima Medical College Hospital for a positive incidence of antibody against types A, E, G, and I of Homma's revised subgroup. In this report, we have confirmed serologically similar levels of positive incidence of 2-ME-sensitive antibody among hospitalized, outpatient, and healthy groups. However, the highest positive incidence of 2-ME-resistant antibody was obtained in hospitalized patients against all four serotypes of *P. aeruginosa*. Moreover, the strains of types E, G, and I (Homma's previous types 5, 8, and 10) showed higher positive incidences of 2-ME-resistant antibody than did type A (Homma's previous type 1) among hospitalized patients. These results also indicate that

type E, G, and I *P. aeruginosa* were more predominant than type A in hospital-acquired infection.

LITERATURE CITED

1. Baltimore, R. S. 1974. Clinical and epidemiological correlates of pseudomonas typing. *J. Infect. Dis.* **130**:s53-s59.
2. Brokopp, C. D., R. Gomez-Lus, and J. J. Farmer II. 1977. Serological typing of *Pseudomonas aeruginosa*: use of commercial antisera and live antigens. *J. Clin. Microbiol.* **5**:640-649.
3. Diaz, F., L. Mosovich, and E. Neter. 1970. Serogroup of *Pseudomonas aeruginosa* and immune response of patients with cystic fibrosis. *J. Infect. Dis.* **121**:269-274.
4. Diaz, F., and E. Neter. 1970. *Pseudomonas aeruginosa*: serogroups and antibody response in patients with neoplastic diseases. *Am. J. Med. Sci.* **259**:340-345.
5. Fisher, M. W., H. B. Devlin, and F. J. Ghabasik. 1969. New immunotype schema for *Pseudomonas aeruginosa* based on protective antigens. *J. Bacteriol.* **98**:835-836.
6. Gaines, S., and M. Landy. 1955. Prevalence of antibody to *Pseudomonas aeruginosa* in normal human sera. *J. Bacteriol.* **69**:628-633.
7. Habs, J. 1957. Untersuchungen über die O-Antigen von *Pseudomonas aeruginosa*. *Z. Hyg.* **114**:218-228.
8. Homma, J. Y. 1976. A new antigenic schema and live-cell slide-agglutination procedure for the intrasubspecific, serologic classification of *Pseudomonas aeruginosa*—report of the serotyping committee of the Japan *Pseudomonas aeruginosa* society. *Jpn. J. Exp. Med.* **46**:329-336.
9. Homma, J. Y., H. Shionoya, H. Yamada, and Y. Kawabe. 1971. Production of antibody against *Pseudomonas aeruginosa* and its serological typing. *Jpn. J. Exp. Med.* **41**:89-94.
10. Lanyi, B. 1967. Serological properties of *Pseudomonas aeruginosa*. I. Group specific somatic antigens. *Acta Microbiol. Acad. Sci. Hung.* **13**:295-318.
11. Leonard, L. L., R. Allen, and S. E. Sulkin. 1968. But immunoglobulins formed in response to experimental Japanese B encephalitis (JEB) virus infection. *J. Immunol.* **101**:1168-1175.
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
13. Meitert, T. 1964. Contribution a l'etude de la structure antigenique des B. pyocyaniques (*Pseudomonas aeruginosa*). Individualisation des groupes serologiques au moyen des antigens "O." *Arch. Roum. Pathol. Exp. Microbiol.* **23**:679-688.
14. Middlebrooke, G., and R. J. Dubos. 1948. Specific serum agglutination of erythrocytes sensitized with extracts of tuberculi bacilli. *J. Exp. Med.* **88**:521-528.
15. Reyes, M. P., M. W. Fisher, and L. Martin. 1974. *Pseudomonas* endocarditis; hemagglutinating antibodies to Fisher-Devlin-Ghabasik immunotypes in sera. *J. Lab. Clin. Med.* **83**:845-852.
16. Shigeta, S., and Y. Yasunaga. 1974. An epidemiological study of *P. aeruginosa* infection in hospitalized patients. (In Japanese.) *Rinshobyori* **23**:507-520.
17. Shigeta, S., and Y. Yasunaga. 1976. Studies of *Pseudomonas aeruginosa* infection. 4. Type specific antibody in human serum. (In Japanese.) *Jpn. J. Bacteriol.* **31**:621-628.
18. Verder, E., and J. A. Evans. 1961. Proposed antigenic schema for differentiation of strains of *Pseudomonas aeruginosa*. *J. Infect. Dis.* **109**:183-193.
19. Westphal, O., and O. Ludertiz. 1954. Chemische Erforschung von lipopolysacchariden gramnegativer Bakterien. *Angew. Chem.* **66**:407-416.
20. Young, L. S., R. D. Meyer, and D. Armstrong. 1973. *Pseudomonas aeruginosa* vaccine in cancer patients. *Ann. Intern. Med.* **79**:518-527.
21. Young, V. M., and M. R. Moody. 1974. Serotyping of *Pseudomonas aeruginosa*. *J. Infect. Dis.* **130**:s47-s52.