New Serogroup of *Legionella pneumophila*, Serogroup 8

MARJORIE L. BISSETT,* JADE O. LEE, AND DAVID S. LINDQUIST

California Department of Health Services, Berkeley, California 94704

Received 20 September 1982/Accepted 25 January 1983

A strain of *Legionella pneumophila* (designated Concord 3) was isolated from a postmortem lung specimen of a patient with pneumonia. Direct fluorescent-antibody and immunoelectrophoresis tests, using absorbed and unabsorbed reagents prepared to this and other strains of legionellae, indicated that the Concord 3 strain represents a new serogroup of *L. pneumophila*, serogroup 8.

Several serogroups of *Legionella pneumophila* and six other species of *Legionella* have been described since *L. pneumophila* serogroup 1 was determined to be the cause of an outbreak of pneumonia in Philadelphia in 1976 (1-4, 7, 10-16). The laboratory diagnosis of legionellosis is dependent on the isolation and identification of the organism, demonstration of the organism in patients' specimens by direct fluorescent-antibody (DFA) tests, or, retrospectively, by serological tests utilizing indirect fluorescent-antibody techniques. The diagnostic value of these methods is directly related to the availability of reagents that will detect all species and serogroups of legionellae. In January 1981, an organism with the physiological and biochemical characteristics of *L. pneumophila* was isolated in this laboratory from an autopsy specimen from a patient with severe respiratory disease. The organism reacted weakly or not at all with conjugates currently available for identification of *Legionella* sp.

In this paper, we describe the relationship of this organism to already described serogroups of *L. pneumophila* and to other species of *Legionella*. We have been advised that a manuscript is in preparation describing *L. pneumophila* serogroup 7, which differs in its antigenic makeup from the Concord 3 strain and other serogroups of *L. pneumophila* (W. F. Bibb, B. M. Arnow, D. L. Dellinger, and S. R. Perryman, submitted for publication). We therefore propose that the Concord 3 strain represents a new serogroup, *L. pneumophila* serogroup 8.

MATERIALS AND METHODS

Source of organism. The organism, designated as the Concord 3 strain, was isolated from human lung tissue taken at autopsy from a 70-year-old man with multiple medical diagnoses. The patient was admitted to a Concord, Calif., hospital on 21 January 1981, after 2 days of severe coughing, sweats, and fevers up to 101.5°F (ca. 38.6°C). The diagnosis was one of severe respiratory distress with bilateral infiltrates of un-

known etiology. The patient had been diagnosed as having malignant lymphoma and thrombocytopenia when hospitalized between 26 December 1980 and 1 January 1981. He had had coronary bypass surgery and a permanent pacemaker implantation in May 1980. Before his final hospitalization he was being treated with high-dose steroids. He succumbed on 22 January 1981.

Cultural and biochemical characteristics. The organism was isolated from alundum-ground suspensions of lung tissue inoculated onto plates of buffered charcoal-yeast extract agar (19) incubated at 35°C in a 2.5% CO₂ atmosphere. Biochemical tests and growth studies were performed by previously described techniques (20). Beta-lactamase production was determined by the chromogenic cephalosporin technique (20). The method described by Hebert (8) was used to determine hippurate hydrolysis. Fatty acid analysis was performed by the techniques described by Moss et al. (17, 18). DNA homology studies were performed as previously described (3).

Antisera and DFA conjugate production. Antisera were prepared in rabbits by the method of Cherry and McKinney (5). Approximately 2.4×10^9 formalinized organisms in a 50% suspension of incomplete Freund adjuvant were inoculated intracutaneously into rabbits, followed by inoculation of an identical number of organisms intramuscularly at 21 days and intracutaneously at 30 days. The antisera were conjugated with fluorescein isothiocyanate (9). Conjugates were adjusted to contain 10 mg of immunoglobulin G per ml. The titer of the conjugate was defined as the highest dilution of conjugate giving 4+ staining. The working dilution was defined as 1 twofold dilution less than the titer. A highly concentrated dilution (1:10) of conjugate was used in tests for cross-reactivity. Conjugates were prepared to the Concord 3 isolate and to the individual type strains of *L. pneumophila* serogroups 1 through 6, *L. micdadei*, *L. bozemanii*, *L. dumoffii*, *L. gormanii*, and *L. longbeachae* serogroups 1 and 2. Conjugate to *L. jordanis* (4) was not available at the time these studies were done.

DFA procedure. DFA tests were done by the method of Cherry et al. (6). Organisms used in these tests are listed in Table 1.

Absorption of conjugates. Absorption of conjugates was performed essentially as described by McKinney et al. (15) except that a 1:10 ratio of cells/conjugate was used in the absorption. Concord 3 (serogroup 8)

TABLE 1. Strains of legionellae used in this study

Species	Serogroup	Strain	Source
<i>L. pneumophila</i>	1	Knoxville 1	CDC
	1	Philadelphia 2	CDC
	1	Bellingham 1	CDC
	1	Pontiac 1	CDC
	1	Long Beach 5	MDL ^a
	1	San Francisco 1	MDL
	1	Ventura 1	MDL
	1	Berkeley 3	MDL
	1	Concord 4	MDL
	1	Hayward	MDL
	1	Alameda 1	MDL
	2	Togus 1	CDC
	3	Bloomington 2	CDC
	4	Los Angeles 1	CDC
	5	Dallas 1E	CDC
	5	Orange 1	MDL
	6	Chicago 2	CDC
	6	Berkeley 2	MDL
<i>L. micdadei</i>	6	Concord 2	MDL
	8 ^b	Concord 3	MDL
<i>L. bozemanii</i>		Tatlock	CDC
		San Francisco 2	MDL
<i>L. dumoffii</i>		Wiga	CDC
<i>L. gormanii</i>		Tex-KL	CDC
<i>L. longbeachae</i>		LS-13	CDC
<i>L. jordanis</i>	1	Long Beach 4	CDC
	1	Concord 1	MDL
	1	Concord 5	MDL
	2	Tucker 1	CDC
<i>L. jordanis</i>		BL-540	CDC

^a MDL, California State Microbial Diseases Laboratory, Berkeley.

^b A strain of proposed serogroup 7 was not available for testing.

conjugate was absorbed with Dallas 1E (serogroup 5) cells, and Dallas 1E conjugate was absorbed with Concord 3 cells.

Immunoelectrophoresis (IE). Solubilized antigen extracts (SAE) of legionellae were prepared with cells from the Concord 3 and Dallas 1E isolates. One Roux bottle containing 180 ml of buffered charcoal-yeast extract agar was inoculated with Concord 3 organisms and one was inoculated with Dallas 1E organisms. The organisms were harvested after 72 h of growth at 35°C in a 2.5% CO₂ atmosphere by washing the growth from the agar with 0.85% NaCl. The cell suspensions were centrifuged at 1,200 × g for 30 to 60 min. The packed cells were resuspended in fresh 0.85% saline, vortexed to obtain a smooth suspension, and heated in a boiling-water bath for 15 min. The heated suspensions were centrifuged at 1,200 × g for 1 h, and the supernatant was dialyzed against distilled water overnight and lyophilized. The weighed lyophilized product was adjusted with distilled water to 10 mg (dry weight) per ml.

Agar plates for IE were prepared with 0.8% agarose in pH 7.4 barbital buffer. Concord 3 and Dallas 1E SAE were placed in the antigen wells. The antigens were then subjected to electrophoresis in a refrigerator (4°C) at 200 V for 90 min, using a Shandon Volkam power unit (Colab Laboratories, Inc., Chicago Heights, Ill.). Buffer used in the reservoir was Tris-barbital buffer, pH 8.8 (high-resolution buffer; Gelman Science, Ann Arbor, Mich.). After completion of electrophoresis, undiluted absorbed and unabsorbed conjugates prepared against the Concord 3 and Dallas 1E strains were added to the troughs and incubated for 48 h at room temperature. The development of antigen-antibody precipitation lines was observed at 24 and 48 h of incubation.

RESULTS

Isolation and characterization of Concord 3 strain. Small (0.5 to 1 mm) greyish-white colonies appeared after 48 h of incubation on buffered charcoal-yeast extract agar plates inoculated with lung suspensions. Smears of these colonies stained as gram negative and non-acid-fast rods. The organisms did not grow on heart infusion agar supplemented with 5% sheep blood. Growth on Feeley-Gorman medium produced a brown soluble pigment and dull yellow fluorescence when exposed to 366-nm UV light in the dark. The organism was nitrate negative and catalase positive, hydrolyzed hippurate, and produced gelatinase and beta-lactamase. Gas-liquid chromatographic analysis of the fatty acids of the Concord 3 strain produced profiles that were similar to those obtained with other *L. pneumophila* strains. Concord 3 DNA was 89 and 93% related to tritium-labeled DNA from *L. pneumophila* Philadelphia 1 in two reactions done at 60°C. (DNA homology tests were performed by D. J. Brenner and A. G. Steigerwalt in the Molecular Biology Laboratory at the Centers for Disease Control [CDC], Atlanta, Ga.) This high relatedness is similar to that seen between other serogroups of *L. pneumophila* (1, 3, 7, 12) and is indicative of strains from the same species (2-4, 10, 14, 16).

Immunofluorescent staining. DFA tests performed on formalinized and fresh lung tissue with a conjugate pool detecting serogroups 1 through 4 of *L. pneumophila* showed weakly staining (1+ intensity) organisms. DFA tests performed on the Concord 3 isolate, using individual conjugates to *L. pneumophila* serogroups 1, 2, 3, and 6, *L. micdadei*, *L. bozemanii*, *L. dumoffii*, *L. gormanii*, and *L. longbeachae* serogroups 1 and 2, were all negative. Tests with *L. pneumophila* serogroup 5 conjugate showed 2+ staining of the Concord 3 organism. Serogroup 4 conjugate stained approximately 1% of the organisms. The Concord 3 isolate was submitted to the CDC laboratories for confirmation. CDC reported that it resembles *L. pneumophila* and is

fluorescent antibody positive for *L. pneumophila* serogroup 4 (3 to 4+ staining intensity) and serogroup 5 (2 to 3+ staining intensity).

DFA tests on tissue specimens that had been stored at -70°C were performed with the Concord 3 conjugate and retested at the same time with conjugates to serogroups 4 and 5. A significantly larger number of organisms reacted with the homologous conjugate than were stained with either the serogroup 4 or 5 conjugates, and the intensity of the staining was much greater (very bright 4+).

The Concord 3 conjugate diluted 1:10 was used to test the Concord 3 isolate and other species and serogroups of legionellae (Table 1). A strong reaction occurred in the DFA test only with the homologous Concord 3 organism and with the two organisms belonging to serogroup 5. Table 2 shows the results obtained in titrations of this conjugate against the homologous isolate (Concord 3, serogroup 8) and Dallas 1E (serogroup 5) organisms. Titrations using the Concord 3 conjugates against Los Angeles 1 (serogroup 4) antigen showed approximately 1 to 5% of the organisms staining at a 2 to 4+ intensity through the 1:80 dilution.

Titrations indicated that the Concord 3 conjugate stained Dallas 1E organisms at a titer 1 dilution below that obtained with the homologous Concord 3 organisms (Table 2). When the Concord 3 conjugate was absorbed with Dallas 1E organisms to remove reactivity against Dallas 1E, the homologous titer was reduced by only 1 twofold dilution. Unabsorbed Dallas 1E conjugate stained homologous Dallas 1E organisms through a dilution of 1:160, but stained Concord 3 organisms with a 2+ intensity only at a 1:10 dilution. When this reactivity was removed by absorption with Concord 3 organisms, the titer to the homologous Dallas 1E organism was reduced by 1 twofold dilution to 1:80.

IE studies. IE studies with absorbed and unabsorbed conjugates gave patterns as shown in Fig. 1. Concord 3 unabsorbed conjugate tested against the homologous SAE of Concord 3 organisms produced a major precipitin arc around the well and three other arcs (one cathodic arc and two anodic arcs). Absorption of the Concord 3 conjugate with Dallas 1E organisms did not affect the major precipitin arc around the well and the anodic arc closest to the well, but did reduce the length and strength of the cathodic precipitin arc and of the other anodic arc, indicating that the Concord 3 organism shares some common antigens with Dallas 1E. Dallas 1E SAE reacted with unabsorbed Concord 3 conjugate, producing a strong cathodic arc and a weaker arc. No precipitin arcs were observed when Dallas 1E SAE was tested against Concord 3 conjugate which had been absorbed with

TABLE 2. Results of DFA studies on the relationship of Dallas 1E and Concord 3 conjugates with homologous and cross-reacting antigens of serogroups 5 and 8 of *L. pneumophila*

Antigen	Titer obtained with given conjugate ^a			
	Dallas 1E		Concord 3	
	Unabsorbed	Absorbed with Concord 3 organisms	Unabsorbed	Absorbed with Dallas 1E organisms
Serogroup 5 (Dallas 1E)	160	80	80	<10
Serogroup 8 (Concord 3)	<10 ^b	<10	160	80

^a Numbers represent the reciprocal of conjugate dilution at which staining intensity was at least 3+ to 4+ on a scale of 0 to 4+.

^b Serogroup 8 (Concord 3) antigen stained 2+ with unabsorbed Dallas 1E conjugate at a 1:10 dilution. Absorption removed this reactivity.

Dallas 1E cells. When Dallas 1E was tested with the homologous Dallas 1E unabsorbed conjugate, one strong cathodic precipitin arc and a weaker anodic arc were produced. Use of Dallas 1E conjugate absorbed with Concord 3 cells yielded essentially the same result. Tests of the Concord 3 SAE with unabsorbed Dallas 1E conjugate and with Dallas 1E conjugate absorbed with Concord 3 cells produced no precipitin arcs.

DISCUSSION

In this report we describe the isolation of an organism, Concord 3, typical of *L. pneumophila* species based upon DNA hybridization studies, cultural and biochemical characteristics, and gas-liquid chromatographic analyses. The organism, however, possesses antigenic differences that separate it from recognized serogroups of *L. pneumophila*.

As additional isolates of *L. pneumophila* continue to accumulate from both environmental and clinical sources, it is inevitable that unique variants should arise which, although having the growth, DNA, biochemical, and gas chromatographical characteristics of *L. pneumophila*, nonetheless differ serologically from currently recognized serogroups. Such differences have proven to be extremely useful tools for the clinical diagnosis and epidemiological study of legionellosis. However, it is essential that serological differences indicative of uniqueness be carefully evaluated to determine whether they represent variations of antigen expression within a currently recognized serogroup, and hence might best be classified as "serovariants" or "serotypes," or whether they are sufficiently

FIG. 1. Immunoelectrophoretic patterns of SAE of Concord 3 and Dallas 1E strains of *L. pneumophila* with unabsorbed and absorbed Concord 3 and Dallas 1E conjugates. Trough A contains Concord 3 conjugate; trough B, Concord 3 conjugate absorbed with Dallas 1E organisms; trough C, Dallas 1E conjugate; and trough D, Dallas 1E conjugate absorbed with Concord 3 organisms. Wells 1, 3, and 5 contain Dallas 1E SAE. Wells 2 and 4 contain Concord 3 SAE.

unique to justify the definition of new serogroups. To date, the designation of new serogroups has been demonstrated as necessary based on a combination of findings, including: (i) failure of existing DFA conjugates to adequately (3+ to 4+) stain the unique organism in tissue specimens, whereas a homologous conjugate does; (ii) results of reciprocal cross-absorption studies done on DFA conjugates prepared against the unique organisms and cross-reactive organisms from recognized serogroups, which indicate that the unique organism contains a distinct antigen set, not shared by the others; and (iii) demonstration by either double diffusion in gel or IE that the unique organism contains at least one antigen which is physically distinct and not cross-reactive with antigens found on strains from currently recognized serogroups. Since DFA staining is the widely used, specific method for detecting legionellae directly in clinical specimens, it is necessary to continue to describe antigenic variants that affect detection by this method.

In the present study, using DFA assays, serogroup 5 conjugates produced only weakly fluorescent (1 to 2+) reactions when used in tests of Concord 3 organisms and could not be relied upon to detect this or antigenically similar organisms in clinical material. When tested against panels of antisera to other legionellae not including antiserum against the Concord 3 strain, Concord 3 preparations reacted poorly or not at all. The reaction of a small number (approximately 1 to 5%) of fluorescing cells in DFA tests of cultures of the Concord 3 strain tested with serogroup 4 conjugate was similar to reactions observed by McKinney et al. in DFA test of serogroup 6 cells with serogroup 4 conjugate (15). The number of Concord 3 cells reacting with serogroup 4 conjugate varied in repeated

tests. At present, we have no explanation for this observation. It may represent staining of cells in a particular stage of the growth cycle. This reaction cannot be relied upon because of its transient and varying occurrence. Thus, detection of strains of Concord 3-like organisms in tissues by DFA requires the use of specific conjugates prepared against this strain. However, in DFA assays with legionella cultures, Concord 3 conjugate gave a significant cross-reaction (4+ intensity) with serogroup 5 organisms. It is not known whether cross-reactivity would reliably occur in stained tissue sections. In reciprocal cross-absorption studies, specificity of the Concord 3 conjugate could be obtained by absorbing the cross-reacting antibodies with the heterologous cross-reacting antigen (Dallas 1E) without affecting the homologous titer. Conversely, it was also possible to absorb the serogroup 5 conjugate with Concord 3 organisms without affecting the homologous titer. This finding suggests that Concord 3 and Dallas 1E organisms each contain physically distinct antigens not shared by the other. Moreover, this same behavior was exhibited with each of three different conjugates prepared in three different rabbits against the Concord 3 strain, suggesting that the difference represents a stable serological characteristic.

The results of electrophoretic studies done reciprocally with Concord 3 and Dallas 1E antigens against both the Concord 3 conjugate and the serogroup 5 conjugate show distinct antigenic differences between Concord 3 and Dallas 1E. Concord 3 conjugate detected two bands in Dallas 1E SAE, one migrating anodally and the other cathodally. When absorbed with Dallas 1E, the Concord 3 conjugate failed to detect either of these bands, but continued to detect the several Concord 3 bands migrating differently from the Dallas 1E bands. In the reciprocal experiment, the Dallas 1E conjugate failed to show any reactivity with the Concord 3 SAE, but did detect two bands in the Dallas 1E SAE, one migrating anodally and a spur migrating cathodally. Absorption with Concord 3, although removing all DFA reactivity against Concord 3, did not alter the position or intensity of the two bands seen in IE of Dallas 1E against the absorbed serogroup 5 conjugate. The anodally migrating band in this case was remarkably similar to a band of similar intensity and position detected in both Concord 3 and Dallas 1E SAE by the Concord 3 conjugate. Whereas the band could be eliminated by absorbing the Concord 3 conjugate with Dallas 1E organisms, reactivity against it was not removed from the serogroup 5 conjugate by absorption with Concord 3 organisms, indicating that, although related in the two organisms, the antigen in Dallas 1E must

contain additional determinants not found in Concord 3. The small cathodally migrating band detected in Dallas 1E SAE by serogroup 5 serum was distinct from anything detected in the Concord 3 SAE by Concord 3 conjugate and was unaffected by absorption of serogroup 5 conjugate with Concord 3 organisms. This finding indicates that Dallas 1E has at least one antigen set not shared by Concord 3. The results of these electrophoretic findings, together with the ease with which DFA and IE cross-reactivities may be reproducibly absorbed from both the serogroup 5 conjugates against Concord 3 organisms and the Concord 3 conjugates against serogroup 5 organisms without reducing working titers, suggest that both serogroup 5 organisms and Concord 3 organisms contain physically distinct major antigenic sets not shared by the other. On this basis, it seems appropriate to designate the Concord 3 strain as a new serogroup of *L. pneumophila*, serogroup 8 (ATCC 35096).

ACKNOWLEDGMENTS

We thank Yosh Inouye and his staff of the Biologics Unit of the Microbial Diseases Laboratory, California Department of Health Services, for preparation of conjugates and provision of equipment used in this study. We are indebted to Don J. Brenner and A. G. Steigerwalt of CDC for performance of the DNA analyses. We thank Roger McKinney and D. J. Brenner of CDC for their critical review of this manuscript. We gratefully acknowledge the invaluable support and helpful discussions provided by Edith Coffey, Paul Duffey, and Genevieve Nygaard of the Microbial Diseases Laboratory.

This study was supported in part by Public Health Service grant AI 6441 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

- Bibb, W. F., R. J. Sorg, B. M. Thomason, M. D. Hicklin, A. G. Steigerwalt, D. J. Brenner, and M. R. Wulf. 1981. Recognition of a second serogroup of *Legionella longbeachae*. *J. Clin. Microbiol.* 14:674-677.
- Brenner, D. J., A. G. Steigerwalt, G. W. Gorman, R. E. Weaver, J. C. Feeley, L. G. Cordes, H. W. Wilkinson, C. Patton, B. M. Thomason, and K. R. L. Sasseville. 1980. *Legionella bozemanii* sp. nov. and *Legionella dumoffii* sp. nov.: classification of two additional species of *Legionella* associated with human pneumonia. *Curr. Microbiol.* 4:111-116.
- Brenner, D. J., A. G. Steigerwalt, and J. E. McDade. 1979. Classification of the Legionnaires' disease bacterium: *Legionella pneumophila*, genus novum, species nova, of the Family Legionellaceae, familia nova. *Ann. Intern. Med.* 90:656-658.
- Cherry, W. B., G. W. Gorman, L. H. Orrison, C. W. Moss, A. G. Steigerwalt, H. W. Wilkinson, S. E. Johnson, R. M. McKinney, and D. J. Brenner. 1982. *Legionella jordanis*: a new species of *Legionella* isolated from water and sewage. *J. Clin. Microbiol.* 15:290-297.
- Cherry, W. B., and R. M. McKinney. 1979. Detection of Legionnaires' disease bacteria in clinical specimens by direct immunofluorescence, p. 92-103. *In* G. L. Jones and G. A. Hebert (ed.), "Legionnaires'," the disease, the bacterium, and methodology. Centers for Disease Control, Atlanta.
- Cherry, W. B., B. Pittman, P. P. Harris, G. A. Hebert, B. M. Thomason, L. Thacker, and R. E. Weaver. 1978. Detection of Legionnaires' disease bacteria by direct immunofluorescent staining. *J. Clin. Microbiol.* 8:329-338.
- England, A. C., R. M. McKinney, P. Skaliy, and G. W. Gorman. 1980. A fifth serogroup of *Legionella pneumophila*. *Ann. Intern. Med.* 93:58-59.
- Hebert, G. A. 1981. Hippurate hydrolysis by *Legionella pneumophila*. *J. Clin. Microbiol.* 13:240-242.
- Hebert, G. A., B. Pittman, R. M. McKinney, and W. B. Cherry. 1972. The preparation and physicochemical characterization of fluorescent antibody reagents. Centers for Disease Control, Atlanta.
- Hebert, G. A., A. G. Steigerwalt, and D. J. Brenner. 1980. *Legionella micdadei* species nova: classification of a third species of *Legionella* associated with human pneumonia. *Curr. Microbiol.* 3:255-257.
- McDade, J. E., C. C. Shepard, D. W. Fraser, T. R. Tsai, M. A. Redus, W. R. Dowdle, and the Laboratory Investigation Team. 1977. Legionnaires' disease. Isolation of a bacterium and demonstration of its role in other respiratory disease. *N. Engl. J. Med.* 297:1197-1203.
- McKinney, R. M., L. Thacker, P. P. Harris, K. R. Lewallen, G. A. Hebert, P. H. Edelstein, and B. M. Thomason. 1979. Four serogroups of Legionnaires' disease bacteria defined by direct immunofluorescence. *Ann. Intern. Med.* 90:621-624.
- McKinney, R. M., B. M. Thomason, P. P. Harris, L. Thacker, K. R. Lewallen, H. W. Wilkinson, G. A. Hebert, and C. W. Moss. 1979. Recognition of a new serogroup of the Legionnaires disease bacterium. *J. Clin. Microbiol.* 9:103-107.
- McKinney, R. M., R. K. Porschen, P. H. Edelstein, M. L. Bissett, P. P. Harris, S. P. Bondell, A. G. Steigerwalt, R. E. Weaver, M. E. Ein, D. S. Lindquist, R. S. Kops, and D. J. Brenner. 1981. *Legionella longbeachae* species nova, another etiologic agent of human pneumonia. *Ann. Intern. Med.* 94:739-743.
- McKinney, R. M., H. W. Wilkinson, H. M. Sommers, B. J. Fikes, K. R. Sasseville, M. M. Yungbluth, and J. S. Wolf. 1980. *Legionella pneumophila* serogroup 6: isolation from cases of legionellosis, identification by immunofluorescence staining, and immunological response to infection. *J. Clin. Microbiol.* 12:395-401.
- Morris, G. K., A. G. Steigerwalt, J. C. Feeley, E. S. Wong, W. T. Martin, C. M. Patton, and D. J. Brenner. 1980. *Legionella gormanii* sp. nov. *J. Clin. Microbiol.* 12:718-721.
- Moss, C. W. 1979. Analysis of cellular fatty acids of bacteria by gas-liquid chromatography, p. 117-122. *In* G. L. Jones and G. A. Hebert (ed.), "Legionnaires'," the disease, the bacterium, and methodology. Centers for Disease Control, Atlanta.
- Moss, C. W., R. E. Weaver, S. B. Dees, and W. B. Cherry. 1977. Cellular fatty acid composition of isolates from Legionnaires disease. *J. Clin. Microbiol.* 6:140-143.
- Pasculle, A. W., J. C. Feeley, R. J. Gibson, L. G. Cordes, R. L. Myerowitz, C. M. Patton, G. W. Gorman, C. L. Carmack, J. W. Ezzell, and J. N. Dowling. 1980. Pittsburgh pneumonia agent: direct isolation from human lung tissue. *J. Infect. Dis.* 141:727-732.
- Weaver, R. E., and J. C. Feeley. 1979. Cultural and biochemical characterization of the Legionnaires' disease bacterium, p. 20-25. *In* G. L. Jones and G. A. Hebert (ed.), "Legionnaires'," the disease, the bacterium, and methodology. Centers for Disease Control, Atlanta.