

LEGIONNAIRES' DISEASE

Isolation of a Bacterium and Demonstration of Its Role in Other Respiratory Disease

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Abstract To identify the etiologic agent of Legionnaires' disease, we examined patients' serum and tissue specimens rickettsiae for toxins, bacteria, fungi, chlamydiae, rickettsiae and viruses. From the lungs of four of six patients we isolated a gram-negative, non-acid-fast bacillus in guinea pigs. The bacillus could be transferred to yolk sacs of embryonated eggs. Classification of this organism is incomplete. We used yolk-sac cultures of the bacillus as antigen to survey suspected serum specimens, employing antihuman-globulin fluorescent antibody. When compared to con-

trols, specimens from 101 of 111 patients meeting clinical criteria of Legionnaires' disease showed diagnostic increases in antibody titers. Diagnostic increases were also found in 54 recent sporadic cases of severe pneumonia and, retrospectively, in stored serum from most patients in two other previously unsolved outbreaks of respiratory disease. We conclude that Legionnaires' disease is caused by a gram-negative bacterium that may be responsible for widespread infection. (*N Engl J Med* 297:1197-1203, 1977)

AN outbreak of severe respiratory illness occurred in the summer of 1976 in Pennsylvania, chiefly among persons who attended a state American Legion Convention. An estimated 182 cases of pneumonia occurred, and 29 people died. A detailed description of the outbreak, including its clinical presentation, is reported in the companion paper.¹ An extensive laboratory investigation was undertaken; techniques for detection of a variety of toxins and for identification of infections caused by bacteria, chlamydiae, fungi, mycoplasmas, parasites, rickettsiae and viruses were used. A bacterium was isolated in guinea pigs from the lung tissues in four fatal cases. The etiologic role of this organism was demonstrated by indirect fluorescent-antibody tests of survivors' serum specimens.

MATERIALS AND METHODS

Tissue samples were analyzed for abnormal concentration of more than 30 metallic elements by one or more of the following techniques: atomic absorption spectrophotometry; neutron activation; electron-induced x-ray fluorescence; and proton-induced x-ray fluorescence. Appropriate tissue and urine samples were also examined for a broad spectrum of organic toxic substances. Techniques employed were high-pressure liquid chromatography, gas chromatography and mass spectroscopy, with various combinations of sample extraction and preparation. In addition, specific assays were performed for organic compounds suggested by the Legionnaires' symptoms — for example, 1,1'-dimethyl-4,4'-dipyridilium (Paraquat).*

The microbiologic and serologic methods used and the specimens examined in the search for an etiologic agent are summarized in tables on deposit.[^] These tests involved attempts by fluorescent-antibody and eight other methods to visualize the agent, isolation attempts on 14 bacteriologic and mycologic mediums and 13 virologic host systems, and tests of patients' serums against 77 infectious agents.

The rickettsiologic techniques employed were those in regular use in the Leprosy and Rickettsia Branch. Adult male guinea pigs (weighing approximately 600 g) were inoculated intraperitoneally with 1 ml of 10 per cent suspensions of patients' tissues prepared in sucrose-phosphate-glutamate buffer, pH 7.2.² Fresh autopsy materials from patients with Legionnaires' disease were used in three isolation attempts, and tissues that had been stored at -70°C for four

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months in three other attempts. Animals were monitored by daily rectal temperatures and clinical observation, and those that became ill were killed on the second or third day after the onset of fever. Pieces of guinea-pig spleen, liver and lung were ground separately with alundum in a mortar and pestle, and 10 per cent suspensions of each were prepared in sucrose-phosphate-glutamate buffer. Aliquots of these suspensions were tested for the presence of bacteria by inoculation onto trypticase soy agar and sheep-blood agar and into thioglycollate broth. Half-milliliter aliquots of the tissue suspensions were inoculated into the yolk sacs of embryonated hens' eggs six to seven days old from antibiotic-free flocks. The eggs were incubated at 35°C and candled daily. Those that died before the third day were considered to be contaminated with extraneous bacteria and were discarded. Yolk sacs from those that died after the third day were smeared, stained by the Gimenez method,⁵ and examined microscopically.

Indirect fluorescent-antibody tests were carried out on smears of 5 per cent suspensions of infected yolk sacs prepared in phosphate-buffered saline, pH 7.2. Microhematocrit tubes were used to place microdrops of the suspensions (one drop of each of two isolates, side by side) in each 5-mm well of a 12-well microscope slide. Slides were prepared fresh daily from small aliquots stored at -70°C, and, after the smears had dried, they were fixed in acetone at room temperature for 15 minutes. Twofold dilutions of each patient's serum, made in 10 per cent normal yolk sac in phosphate-buffered saline, were placed on the smears and incubated at 37°C for 30 minutes in a humid atmosphere. The slides were washed with phosphate-buffered saline and then incubated for an additional 30 minutes with the optimal dilution of rabbit antihuman conjugate capable of staining human IgG and IgM. The titer of the serum was taken as the reciprocal of the highest serum dilution that gave distinct staining of the organism. Nearly all serums were tested as coded unknowns, and a positive control serum was included in each test. The microscope used epi-illumination, which greatly facilitates accurate estimation of the degree of fluorescence. Serum end points tend not to be sharp with indirect fluorescent antibody, so that whenever feasible, serial dilutions of the serum specimens from the same patient were run in the same test, to ensure accurate comparison of the brightness of staining at particular dilutions.

RESULTS

Toxicologic Studies

Because the clinical symptoms exhibited by the patients with Legionnaires' disease were nonspecific and could have been caused by a variety of agents, toxicologic studies were initiated along with microbiologic studies on the first specimens having sufficient quantities. Comparison of case and control data failed to show the consistent presence of any unusual components or elevated levels of toxins that might be related to the epidemic.

Microbiologic Studies

Inoculation of specimens on bacteriologic and mycologic mediums resulted in the isolation of a number of organisms commonly found in normal flora or in the flora of patients under treatment with broad-spectrum antibiotics, and otherwise formed no particular pattern of isolation. Cultures for mycoplasma and spiroplasma were negative.

With the exception of one herpesvirus, no agent was isolated in the cell cultures, eggs or mice. Primary

monkey-kidney, human embryonic lung fibroblasts, HEp-2, human embryonic kidney and Vero-cell cultures inoculated with specimens from the respiratory tract were challenged in the terminal (fourth or fifth) blind passage with Coxsackie A-9 virus in an effort to detect an agent that might be demonstrated by viral interference. None was found.

Thin-section electron-microscope studies were carried out on lung tissues in 10 fatal cases. Thin-walled bacteria were found in specimens from seven of 10 patients. No other microbial agents were observed. Direct fluorescent-antibody staining of lung tissue with antisera to 13 known agents failed to show the presence of any microbial agents, with the exception of specimens from one patient with a secondary *Candida* infection whose lung stained positively for a *Candida* species by the fluorescent-antibody technic*.

Serum specimens from serial bleedings were examined by complement fixation, indirect fluorescent-antibody, immunoprecipitin, and indirect hemagglutination tests for antibodies to a large variety of antigens.* Substantial (fourfold or greater) increases in antibody titers were detected for *Mycoplasma pneumoniae* (in one patient), herpesvirus (in two patients), and *Blastomyces dermatitides* — in one patient, who had evidence of disseminated candidiasis at death.

Isolation and Preliminary Description of the Agent

An agent was isolated in guinea pigs from four of six lung specimens collected on autopsy. Three isolates were from patients classified epidemiologically as having Legionnaires' disease. The other isolate was from a patient who had Broad Street pneumonia¹ — that is, the patient had not attended the convention and had not entered the hotel about which the epidemic appeared to center, but had been within one block of the hotel during the epidemic period. In the four successful isolation attempts, a febrile illness characterized by watery eyes and eventual prostration developed in guinea pigs. With two isolates, fever (temperatures ranging from 39.5° to 41.0°C) developed in guinea pigs as early as 18 hours after inoculation, and with the two other isolates similar fever developed after an incubation period of one or two days. Impression smears of guinea-pig liver and spleen obtained on the second day of fever, stained by the Gimenez method, contained scattered bacilli. When the disease was allowed to progress in guinea pigs, the animals became moribund three to six days after the onset of fever. An exudate containing numerous bacilli was observed in the peritoneum, especially on the liver and spleen of animals killed when moribund.

Spleen, liver and lung-tissue suspensions from the affected guinea pigs, when inoculated into embryonated eggs, caused death in four to seven days. Eggs inoculated with spleen tissue died earliest, and eggs

*Further information is on file with the National Auxiliary Publications Service. See footnote on page 1197.

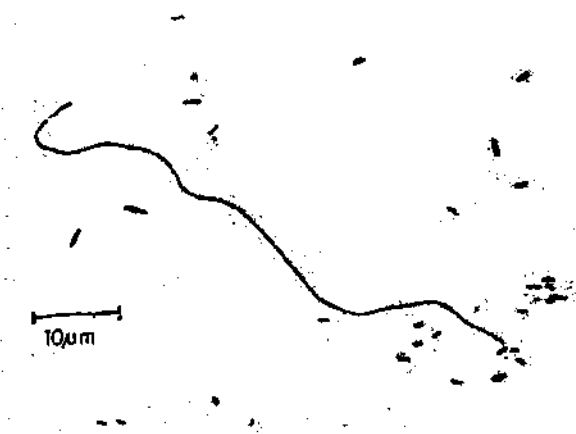


Figure 1. Photomicrograph of the Bacteria of Legionnaires' Disease in Yolk Sac Stained by the Gimenez Method.

inoculated with lung tissue died last, indicating, as did smears and histologic sections, that the spleen contained a greater number of organisms than the lung tissue. Smears of yolk sacs from embryos that died, when stained by the Gimenez method (Fig. 1), showed many bacilli. The bacilli were 0.3 to 0.4 μm in width and usually 2 to 3 μm long, but bacilli 8 to 20 μm long were often observed, and forms longer than 50 μm were occasionally seen (Fig. 1). The sides of the bacteria were sometimes not parallel, and the ends often were somewhat pointed. We found the organism to be gram-negative and not acid fast. By thin-section electron microscopy of infected yolk sacs we found the bacteria to have structure typical of gram-negative rods. The organism grew in primary chick-embryo cell cultures and was nonpathogenic for mice.

The organism did not grow on trypticase soy or blood agar or in thioglycollate broth, but as described in another report,⁴ it has been successfully cultured on Mueller-Hinton agar containing 1 per cent hemoglobin and 1 per cent Isovitalex (BBL) in 5 per cent carbon dioxide. (Supplemented Mueller-Hinton agar was not used in the initial isolation attempts.) We subsequently isolated the organism on supplemented Mueller-Hinton agar directly from human lung tissue in two of six attempts. The latter isolates reacted with

convalescent-phase serums from patients' with Legionnaires' disease in indirect fluorescent-antibody tests. Also, they produced the characteristic pattern of disease when inoculated into guinea pigs and were reisolated from infected guinea-pig tissues both by cultivation in embryonated eggs and by direct inoculation onto supplemented Mueller-Hinton agar. Serums from convalescent guinea pigs specifically stained the organism in indirect fluorescent-antibody tests; preinfection serums from the same guinea pigs did not. Guinea-pig convalescent-phase serum did not react in complement-fixation tests with standard rickettsial antigens prepared from *Rickettsia rickettsii*, *R. prowazekii*, *R. mooseri* (typhi) or *Coxiella burnetii*.

Etiologic Role of the Yolk-Sac Isolates in the Pennsylvania Outbreak

Serologic evidence for the etiologic role of the bacterium was obtained by indirect fluorescent-antibody staining. Results with some of the first serum specimens tested are shown in Table 1. With certain patients, antibody rises were observed; in others, the first specimen appeared to have been taken too late for an antibody rise to be demonstrated, but high titers were obtained. The brightness of staining and the changes in titers were very similar to those observed in other infectious diseases — for example, Rocky Mountain spotted fever, with which we have had extensive experience.

Most patients from whom suitable serum specimens were available could be classified as having "seroconversion" (with titer increases ranging from fourfold to 128-fold and reaching a minimum titer of 64) or as having "positive" results (minimum titer of 128). The titers observed in these two groups are shown in Figure 2. Titers for a few patients were elevated six or seven days after onset. Their titers rose rapidly in the next two to three weeks to a maximum at about the fifth week. The proportion of serums with titers greater than 64 was largest in the interval from 22 to 60 days, and titers decreased somewhat during the next few months. The trends for individual patients were similar, but the time at which elevated titers appeared varied.

Table 1. Representative Results of Indirect Fluorescent-Antibody Tests with Bacterial Agent on Serum Specimens from Patients with Legionnaires' Disease.

CASE No.	DAYS AFTER ONSET	RECIPROCAL DILUTION OF SERUM*							Titer	INTERPRETATION
		16	32	64	128	256	512	1024		
1	31		1/1	\pm/\pm	0/0	0/0	0/0	0/0	32/32	Negative
	163		1/1	\pm/\pm	0/0	0/0	0/0	0/0	32/32	
2	1	1/1	\pm/\pm	0/0	0/0	0/0	0/0		16/16	Conversion
	23	3/3	3/3	2/3	1/2	$\pm/1$	0/0		128/256	
3	13	3/3	3/3	3/3	2/3	1/2	1/1	0/ \pm	512/512	Positive
	30	3/3	3/3	3/3	2/3	1/2	1/1	0/ \pm	512/512	

*Numbers indicate brightness of fluorescence From 0 (no staining) through \pm (questionable staining), 1+ (minimal but definite staining) to 4+ (maximally bright). Staining with isolate 1/staining with isolate 2. †Highest dilution of patient's serum giving definite staining. Titer with isolate 1 /titer with isolate 2.

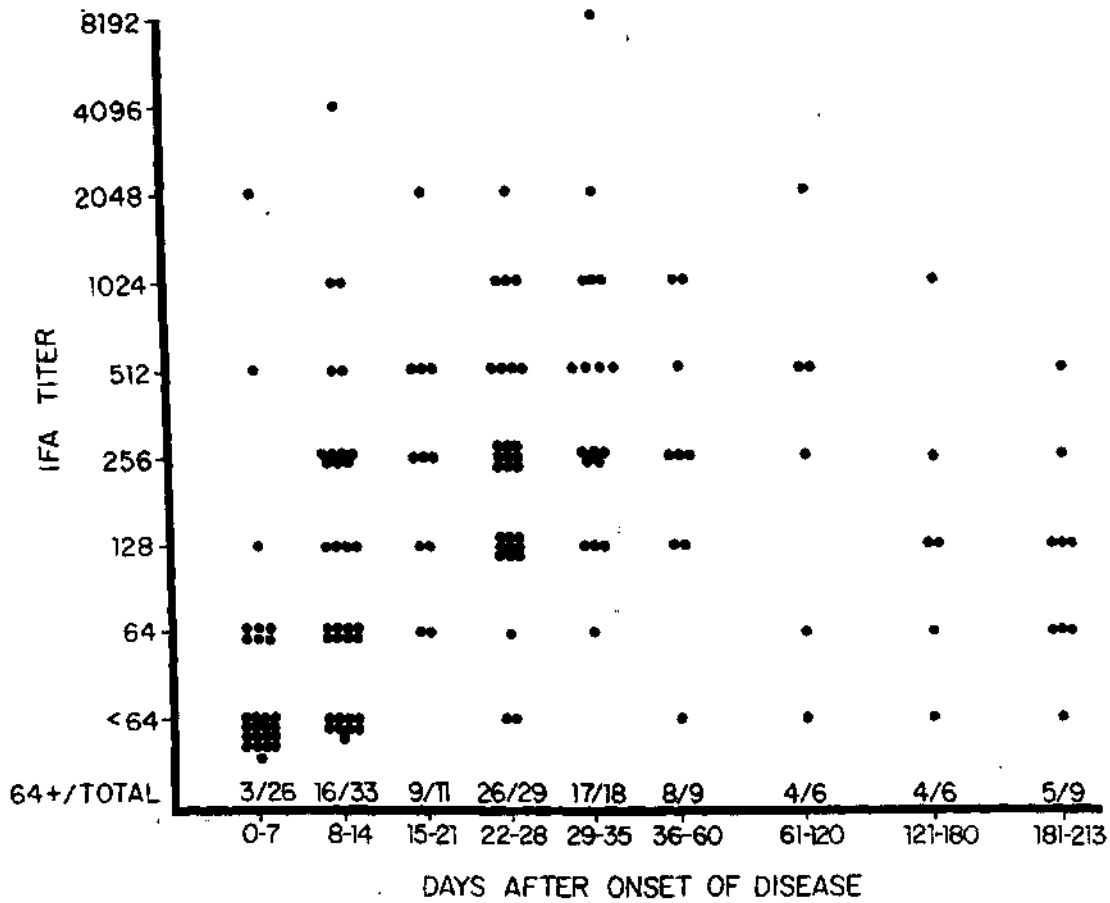


Figure 2. Results of Indirect Fluorescent-Antibody (IFA) Tests with Bacterial Agent on Serum Specimens from Patients with Legionnaires' Disease Classified as Having Seroconversion or a Positive Result.

Table 2 shows the results of the serologic tests of case serums. Of the 111 patients with suitably timed specimens, 62 could be placed in the group with seroconversion and 39 in the group with positive results. The maximum indirect fluorescent-antibody titers obtained with the various patient categories are also shown in Table 2.

Table 2. Results of Indirect Fluorescent-Antibody Tests with Bacterial Agent of Serum Specimens from Patients with Legionnaires' Disease.

SEROLOGICAL CATEGORY	No. OF PATIENTS	HIGHEST TITER OBSERVED FOR EACH PATIENT									
		<32	32	64	128	256	512	1024	2048	4096	8192
Seroconversions*	62			4	11	14	16	9	5	2	1
Positive only**	39				10	14	13	1	1		
Negative***	10	4	2	4							
Questionable****	25	9	7	9							
Total patients	136										

*Increase in titer of at least 2 dilutions with highest titer 64 or greater.

**No seroconversion but highest titer >128.

***Did not meet qualification as seroconversion or positive only even though specimen was available from interval 22-60 days after onset.

****Did not meet qualification as seroconversion or positive only but specimen was not available from interval 22-60 days after onset.

The basis for the selection of the minimum titer requirement for serologic diagnosis is given in Table 3, in which results with various control groups are shown. In Group 3, a titer of 256 was found in convalescent-phase serum from a patient with pneumonia who came from Michigan and in another from Vermont; acute-phase serums showed titers of <16 and <32, respectively. In Group 4, one patient from California had a titer of 2048; the acute-phase serum titer was 16. The convalescent-phase serums from these three patients are considered below, along with other isolated cases of pneumonia presumed to be due to the agent of Legionnaires' disease. The Pennsylvania health-department personnel (Groups 5 and 6) were tested shortly after they had begun handling specimens associated with the epidemic of Legionnaires' disease and again several months later. No clinical illness had been observed in this group,¹ and the results provided no evidence of inapparent infections. Serum from a psittacosis outbreak (Groups 7 and 8) provided a stringent test for observation of antigenic relation of the agent of Legionnaires' disease to *Chlamydia psittaci*. Even though all the specimen pairs tested

table 3- Results of Indirect Fluorescent-Antibody Tests with Bacterial Agent on Various Control Serums.

LINE	Group	TITER				
		<64	64	128	256	2048
1	Rickettsia, serologic test negative ^a	38	2			
2	Pneumonia, ^b serologic test negative	81	10	2		
3	Pneumonia, ^b serologic test positive	68	5	2	2*	
4	Q fever negative ^d	41	6	1		1 ^c
5	Health Dept personnel. ^e Aug. 1976	30				
6	Health Dept personnel ^e Feb. 1977	31				
7	Psittacosis outbreak, S 1	20		1		
8	Psittacosis outbreak, S 2	19	2			
9	Psittacosis, serologic test positive**	7	8			
10	Rheumatoid arthritis & SLE ^f	10				
11	Legionnaires. not attendee ^g	9	2			
	Totals ^{§§}	304	35	5	2	1

^aConvalescent-phase serum with request for rickettsial tests. Rickettsial serologic reaction was negative.

^bConvalescent-phase aenurt specimens from patients reported to have pneumonia. Specimens were tested against viral ft mycoplasmal antigens, ft they are separated here according to the serologic results with these antigens.

^cConvalescent-phase serum specimens from patients with pneumonia, very probably caused by the agent of Legionnaires' disease (see Table 5).

^dConvalescent-phase serum submitted Tor Q-fever serologic test; all were negative with this antigen.

^eHealth Department personnel handling specimens from Philadelphia outbreak.

^fAcute-phase A convalescent-phase serum specimens from patients with diagnostic rises in complement-fixing antibody to chlamydia group antigen during outbreak of psittacosis in turkey-processing plant.

^gConvalescent-phase serum specimens from other patients with diagnostic rises in complement-Fixing antibody to chlamydia-group antigen.

^hGroup of serum specimens with rheumatoid factor ft antinuclear antibody.

ⁱPennsylvania Legionnaires who did not attend convention in Philadelphia.

^{§§}Not including lines 5 ft 7

had distinct antibody rises to psittacosis antigen, none had any noteworthy change in titer to the agent of Legionnaires' disease. One patient had a titer of 128 in the first specimen and 64 in the second. Another had a titer of 32 in the first specimen and 64 in the second.

On the basis of the results presented in Table 3, a minimum titer requirement for serologic diagnosis by indirect fluorescent antibody was set at 64 for patients who had a fourfold rise in antibody titer and 128 for patients who did not have such a rise. The data also indicate that with a titer of 128, the expected number of false-positive results would be about five in 344. With a titer of 256, the expected number of false-positive results would be less than one in 344. If, however, a titer of 256 was set as the minimum requirement, in the absence of a fourfold or greater rise in titer, 10 of 39 patients with Legionnaires' disease in the group with positive results only (Table 2) would have been called seronegative.

Causes of Two Previous Outbreaks of Respiratory Disease

Epidemiologically, the outbreak of Legionnaires' disease was similar in many respects to two large outbreaks of febrile disease, one in 1965 (District of Columbia)⁵ and the other in 1968 (Pontiac, Michigan),⁶ Despite intensive investigation, the cause of these two outbreaks had not been determined before the present investigation. Serum specimens stored in the serum bank at the Center for Disease Control from these earlier investigations were examined for evidence of antibody to the bacterium

from Legionnaires' disease.

The District of Columbia outbreak involved patients in a large psychiatric hospital in July, 1965, in which there were 81 cases and 12 deaths.⁵ The clinical picture was similar to that observed in Pennsylvania Legionnaires in 1976. Appropriately timed acute-phase and convalescent-phase serum specimens from 23 patients were tested against the agent of Legionnaires' disease (Table 4). Of the 23 patients, 21 had serologic results characteristic of Legionnaires' disease.

The Pontiac outbreak of acute febrile illness involved personnel and visitors in an office of the county health department in July, 1968.⁶ There were 144 cases and no deaths. Typically, there was an acute onset of fever, with chills, myalgia and minor respiratory symptoms. Pneumonia was not seen. Paired serum specimens from patients and controls were tested for antibodies to the Legionnaire isolate (Table 4). Of 37 cases, 31 had seroconversion and one was seropositive only. All 10 control serums collected from workers in an unaffected county health-department building were negative. The titers observed and the brightness of staining at low dilutions of positive serums were similar to those observed with patients in the pneumonia outbreaks in Pennsylvania and the District of Columbia.

Table 4. Results of Indirect Fluorescent-Antibody Tests with Bacterial Agent on Paired Serum Specimens from Patients Involved in Other Outbreaks.

INTERPRETATION OF TITERS	OUTBREAK	
	WASHINGTON, DC	PONTIAC, MI
Seroconversions*	17	31
Positive only**	4	1
Negative	2	5 ^a
Total patients tested	23	37

*Increase in titer of at least 2 dilutions with highest titer >64.

**No seroconversion, but highest titer >128.

^aControl serums collected at the same time as case serums, but from 10 workers in an unaffected country health-department building, were negative.

Isolated Cases of Pneumonia Presumed to be Due to the Agent of Legionnaires' Disease

Serologic results with individual cases found thus far have been arranged chronologically in Table 5 according to date of onset of illness. Cases 5 and 8 had apparently not been out of their home state during the estimated incubation period of two to 10 days. Case 6 was a merchant seaman who became ill aboard ship en route to Alaska; the ship had left southern California nine days before the onset of his illness. Case 7 had been on a Caribbean cruise and had returned to Tennessee eight days before onset. Case 9 was a truck driver who became sick and collapsed at the wheel while driving from California to Ohio. The results with serum specimens from Cases 1, 2 and 6 were obtained in the process of screening serum from 170 patients with a clinical diagnosis of pneumonia and 49

Table 5. Sporadic Cases of Pneumonia with Seroconversions to the Bacterial Agent of Legionnaires' Disease.

CASE No.	AGE(YR)	SEX	STATE OF RESIDENCE	DATE OF ONSET	SERUM 1	SERUM 2		CLINICAL COURSE/iter*	
						day	titer*		
1	31	F	Vermont	8/10/76	<32/<32	8	256/256	31	Respiratory failure; death on 35th day.
2	62	M	Massachusetts	8/18/76	32/32	7	>1,024/>1,024	23	Fever (of 40.6°C) for 11 days
3	34	M	Michigan	8/19/76	<16/<16	3	>512/>512	21	Fever(to41.7°C); respiratory-distress syndrome; recovery.
4	55	F	Massachusetts	9/07/76	<32/<32	3	512/512	50	Fever (of 40.6°C) for 5 days; respiratory & cardiac arrest.
5	32	M	Indiana	10/05/76	16/16	10	64/128	14	Pneumonia; respiratory failure; death on 14th day.
6	60	M	California**	1/03/77	16/16	10	2,048/2,048	20	Diffuse interstitial pneumonia, with renal failure; recovery.
7	60	M	Tennessee**	2/11/77					Respiratory & renal failure; death on 10th day.
8	31	M	Vermont	3/06/77	64/256	4	>1,024/>1,024	17	Fulminant broncho-pneumonia; temperature of 40.6°C; recovery with ventilatory support.
9	43	M	California**	4/01/77	64/64	5	1,024/1,024	15	Gastroenteritis; trilobar pneumonia; mechanical ventilation for 3 days; recovery.

*Titer against isolate 1/titer against isolate 2.

**Because these patients traveled during their incubation periods, they could have acquired the infection outside state of residence.

{Bacterium isolated from lung specimen.

patients with a clinical diagnosis of Q fever (presumably, many of them also had pneumonia); thus, the proportion of the pneumonias caused by the bacterium in such patients was only 1 to 2 per cent. With the other serums, the bacterium of Legionnaires' disease was suspected as the cause because of the severity of the illness.

DISCUSSION

The etiologic role of the organism in Legionnaires' disease seems proved by its isolation from the tissues of four of six patients who died and by the very high proportion of seroconversion to the agent among cases. The importance of these findings is greatly increased, however, by the negative results obtained in very extensive investigations in this institution and elsewhere of the possible role of other infectious agents and toxic materials. The bacterium isolated from Legionnaires' disease was indistinguishable on electron microscopy from that initially observed in the lungs in seven fatal cases. Although initial conventional bacterial and fungal stains did not consistently demonstrate an agent in paraffin-embedded lung sections, more recent studies with a silver-impregnation stain⁷ have shown the bacterium to be easily detected and present in large numbers in the alveoli, both within inflammatory cells and extracellularly.⁸ These histologic findings and the early observations by electron microscopy confirm the presence of extensive numbers of bacteria in the lungs of patients who died of Legionnaires' disease. The organisms were morphologically identical to those seen in the peritoneal exudate of inoculated guinea pigs.

At this initial stage of investigation, the agent of Legionnaires' disease has been distinguished by the following properties: the characteristic disease that it produces in guinea pigs; the characteristic death pattern of chick embryos after yolk-sac inoculation; morphology of the organism in smears of infected yolk sacs and guinea-pigs peritoneal exudates; gram-negative characteristics; failure to grow on ordinary bac-teriologic mediums such as trypticase soy agar, blood agar, and thioglycollate broth; and specific immuno-fluorescent staining with convalescent-phase serums of patients with Legionnaires' disease. As mentioned above, the organism has since been cultivated on supplemented Mueller-Hinton medium, and studies of its classification are in progress.⁴ We cannot say as yet whether the organism has previously been isolated from other sources, but it seems clear that this is the first demonstration of its role in human disease. The properties listed above differentiate it from other known microbes causing human disease.

Several clinical and epidemiologic manifestations of infection appear to be involved. The most dramatic one is severe pneumonia with 15 to 20 per cent mortality, as exemplified by the epidemic associated with the Legionnaires' convention in Philadelphia in 1976 and the epidemic in the psychiatric hospital in the District of Columbia in 1965.⁵ The clinical picture suggested severe viral pneumonia.

Another entity is sporadic severe pneumonia, as represented by the nine isolated cases in Table 5. In addition, there was an isolation directly on chocolate agar from the lung tissue in a fatal case of pneumonia in Michigan in December, 1976⁹ This organism appears to be the same as the

isolates in Legionnaires' disease, since it is stained specifically in the indirect fluorescent-antibody test with serum from patients with Legionnaires' disease. As with the outbreaks in Philadelphia and the District of Columbia, in none of these isolated cases was there evidence of secondary spread. Moreover, the clinical picture appeared to be the same as that in those two outbreaks. Three of the sporadic cases were found in a survey of 170 paired serum specimens submitted for virologic and mycoplasmal diagnosis, from patients with clinical pneumonia (Table 3); thus, the bacterium appears to have been the cause of only 1 to 2 per cent of cases of such pneumonia.

Finally, there is the entity of a sharp outbreak of acute febrile illness without pneumonia, as represented by Pontiac fever.⁴ Convalescent-phase serum specimens from the Pontiac outbreak reacted with the Philadelphia organism to the same degree as serum from patients with Legionnaires' disease did, and this finding suggests that the etiologic agent of Pontiac Fever is antigenically similar, if not identical, to the Legionnaire bacterium. The differences in the clinical picture do not necessarily suggest that the agent of Pontiac fever has a different pathogenicity for man, but rather that it could be related to dose response or to host factors. The organism that apparently caused Pontiac fever has recently been isolated from lung tissues preserved at -60°C from guinea pigs exposed to the air in the health department. Those isolates are now being compared to the isolates obtained in the fatal cases of Legionnaires' disease.

Since the manuscript was first submitted for pub-

lication, the indirect fluorescent-antibody test has been used for the laboratory diagnosis of a total of 54 isolated cases of pneumonia in the United States, two cases of pneumonia in Scots who had vacationed in Benidorm, Spain,¹⁰ and in outbreaks of pneumonia in Ohio, Vermont and Tennessee that are still being investigated.

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