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Survival and Multiplication of *Legionella pneumophila* in Municipal Drinking Water Systems

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Studies were conducted to investigate the survival and multiplication of *Legionella* spp. in public drinking water supplies. An attempt was made, over a period of several years, to isolate legionellae from a municipal system. Sampling sites included the river water supply, treatment plant, finished water reservoir system, mains, and distribution taps. Despite the use of several isolation techniques, *Legionella* spp. could not be detected in any of the samples other than those collected from the river. It was hypothesized that this was due to the maintenance of a chlorine residual throughout this system. To investigate the potential for *Legionella* growth, additional water samples, collected from throughout the system, were dechlorinated, pasteurized, and inoculated with *Legionella pneumophila*. Subsequent growth indicated that many of these samples, especially those collected from areas affected by an accumulation of algal materials, exhibited a much greater ability to support *Legionella* multiplication than did river water prior to treatment. Chemical analyses were also performed on these samples. Correlation of chemical data and experimental growth results indicated that the chemical environment significantly affects the ability of the water to support multiplication, with turbidity, organic carbon, and certain metals being of particular importance. These studies indicate that the potential exists for *Legionella* growth within municipal systems and support the hypothesis that public water supplies may contaminate the plumbing systems of hospitals and other large buildings. The results also suggest that useful methods to control this contamination include adequate treatment plant filtration, maintenance of a chlorine residual throughout the treatment and distribution network, and effective covering of open reservoirs.

In addition to natural habitats (12), *Legionella pneumophila* has been detected within the plumbing systems of homes, hospitals, and other buildings (8, 28, 32, 40). *Legionella* sp. has been shown to be capable not only of surviving in this environment, but also of multiplying in tap water, especially under conditions typical of those found in the hot-water systems of hospitals (42). In some cases the occurrence of *Legionella* sp. has been associated with disease, while in other cases it has not (13, 40).

A question arises concerning the source of the legionellae that contaminate internal plumbing systems. It has been suggested that this bacterium survives much of the drinking-water treatment process now in use across the United States (11). It has further been hypothesized that *Legionella* sp. may be introduced via the municipal water supply and that this supply seeds the hot-water and cooling systems of these buildings (17, 28, 31, 40). These suggestions are supported to some extent by our earlier findings that *L. pneumophila*, both agar (19) and non-agar (20) passaged, is more resistant to chlorine than are coliforms, the group commonly used to indicate sanitary quality of potable water.

The purpose of the present study was to investigate the potential for *Legionella* passage through a public water system. Several surveys were conducted within a large municipal system to detect the occurrence of *L. pneumophila*. The surveys were conducted both on a regularly scheduled basis and randomly and included a number of sampling locations. Also, the potential to support *Legionella* growth was examined by inoculating samples collected from various sites within the treatment and distribution network with legionellae originally isolated from the hot-water system of a building served by this municipal supply. Finally, an attempt was made to elucidate some of the factors contributing to the enhancement or inhibition of *Legionella* growth in the various niches within the municipal water system. A chemical analysis was performed on samples used for growth studies, and a statistical analysis was conducted to detect associations between growth and chemical parameters in the environment.

MATERIALS AND METHODS

Bacteria. A hot-water tank sample, collected from a gymnasium within this municipal water system, served as the initial source of the naturally occurring bacteria used in the growth studies. This sample contained 160 and 74,000 CFU of *L. pneumophila* and non-*Legionellaceae* bacteria per ml, respectively. Direct immunofluorescence testing indicated that the isolate of *L. pneumophila* in this sample belonged to serogroup 1. The most prevalent non-*Legionellaceae* bacteria were previously identified as a *Flavobacterium* sp. Details of their isolation have been presented elsewhere (19). Three other unidentified non-*Legionellaceae* bacteria which formed faint purple-, green-, and white-pigmented colonies on unsupplemented buffered charcoal yeast extract agar were also recovered from the water stock culture. The importance of non-*Legionellaceae* bacteria for support of

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legionellae has been demonstrated in a number of earlier studies (28, 38, 39).

These naturally occurring *L. pneumophila* and associated microbiota were maintained in the laboratory as a water stock culture by periodic transfer into membrane-filtered tap water which had been collected from a tap in the laboratory. A cellulosic nitrate membrane (Micro Filtration Systems, Dublin, Calif.) with a pore size of 0.20 μm was used to filter the tap water samples, which served as the growth medium for the bacteria. Serial transfers of the naturally occurring bacteria were made when the growth of *L. pneumophila* was found to be in the late exponential to early stationary phase. These cultures were diluted 1 to 100 in membrane-filtered tap water and incubated in polypropylene bottles in a room air incubator at 35°C. This system of maintaining water stock cultures of *L. pneumophila* permits study of the bacterium in the laboratory under conditions similar to those in the natural environment.

In this study *L. pneumophila* was enumerated by plating dilutions of water cultures on differential glycine-vancomycin-polymyxin B (DGVP) agar (37). Non-*Legionellaceae* bacteria were counted by plating dilutions of the culture on unsupplemented buffered charcoal yeast extract agar (40), which is prepared the same as buffered charcoal yeast extract agar (22), except that L-cysteine and ferric pyrophosphate are not added.

Samples. The municipal water system examined in this study was that of the City of Pittsburgh Water Department. This system, which serves approximately 500,000 customers and treats 65 million gallons (246,025,000 liters) of water daily, uses the Allegheny River as its source of raw water. From the raw intake, the water passes through a chemical treatment center and clarifier. As part of the clarification process, the water moves through a 120 million-gallon uncovered sedimentation basin. The clarified water then passes through a rapid sand filter composed of coal, sand, and gravel and into an underground finished water clearwell. From the clearwell, water serving the north side of Pittsburgh is pumped to Lanpher Reservoir. Lanpher Reservoir is an uncovered, finished water reservoir (capacity, 154 million gallons) located approximately 3 miles (ca. 4.8 km) from the treatment plant. From Lanpher Reservoir a portion of the water is subsequently pumped to Brashear Reservoir, an uncovered, secondary finished water reservoir (capacity, 11 million gallons). Another portion is pumped to McNaugher Reservoir, a vinyl-covered, secondary finished water reservoir (capacity, 3 million gallons). Water moves to distribution taps from all three reservoirs. This portion of the Pittsburgh system was chosen since it permits a direct comparison of a covered and uncovered secondary reservoir, both fed from the same uncovered primary finished water reservoir. The remainder of the water treated at the plant is pumped from the clearwell to uncovered primary and secondary reservoirs in the central and southern sections of the city.

Samples collected for detection of *Legionella* spp. within the municipal supply were collected as part of three independent surveys of the system. In the first survey, a total of 23 water samples were collected from four hospitals within the central section of the city. The collection period extended from September 1982 through June 1985. Water temperature during this period ranged from a low of 0.6°C in January 1984 to a high of 25.6°C in July 1983. Several of these hospitals have had documented sporadic cases of nosocomial legionellosis during the past 4 years. Also, *L. pneumophila* has been detected periodically within the hot-water plumbing systems and cooling towers of these buildings (40, 42). In this survey water samples were collected from the first valve located on the city water main just after the main enters the hospital buildings. For all 23 samples, 1.0 ml of water was cultured by the spread plate technique in which a bent glass rod was used to distribute the 1.0-ml aliquot over a DGVP plate. Thirteen of these samples were also cultured by using a membrane filtration method (R. Wolford, M.S. thesis, University of Pittsburgh, Pittsburgh, Pa., 1983). As part of this technique, a 100- to 250-ml sample of water was filtered through a 0.4-μm polyvinylidene fluoride membrane filter (Millipore Corp., Bedford, Mass.) held in a sintered-glass filter holder. The filter was then placed directly onto the surface of a DGVP agar plate, incubated at 37°C, and examined daily for 7 days for microbial growth. Also, five swab samples were collected from the interior piping and valve on the city water main, within one of the hospitals, when filters in the pipe were being changed.

In the second survey, monthly samples were collected, from April through December of 1984, from a corner of a secondary finished water reservoir serving the hospitals in the central section of Pittsburgh. Samples were also collected, on the same day, from the first tap on the main within the basement of a hospital supplied by this reservoir. A 1.0-ml volume of each sample was plated in duplicate onto DGVP medium for isolation of *Legionella* sp. Water temperature during this period ranged from a low of 1.1°C in December 1984 to a high of 23.3°C in August 1984.

In the third survey samples were collected from 19 separate locations extending from the river to the tap. These samples were collected for detection of *Legionella* sp., chemical analyses, and subsequent use in growth experiments. The samples were collected in July and again in August 1984, when algal and bacterial activity was greatest in the warm waters of the open distribution system. They were also collected a third time, in late October 1984, to evaluate the influence of the change of seasons and of leaf litter introduced into the open reservoirs during the autumn on *Legionella* standing crop and growth potential. Water temperature during these months averaged 21.1°C in July and August and 10.0°C in October and November 1984. The actual sites sampled varied to some extent in each of the three months and are listed in Table 1. Samples collected from various depths below the surface of the sedimentation basins and reservoirs were collected from a boat, using a Kemmerer sampler which had been sterilized at 121°C for 15 min. Subsamples (0.1 and 0.5 ml) from all samples were plated onto DGVP medium in an attempt to detect the actual presence of *Legionella* sp. within the system. In many cases samples were also concentrated by filtering 100-ml, 500-ml, and 1-liter aliquots through 0.45-μm mixed cellulose actate and nitrate filters (Millipore Corp.) and placing those filters directly onto DGVP plates. In the case of river water samples, membrane filtration was impractical due to elevated turbidities and high levels of non-*Legionellaceae* bacteria. Therefore, 20-liter volumes were concentrated to 10 ml by continuous-flow centrifugation. Subsequently, the concentrates were heated to 60°C for 2 min and acid treated for 5 min (pH 2.2) prior to plating 0.1 ml. Swab samples were collected from the top surface and undersurface of the vinyl covering of McNaugher Reservoir. Some of these swabs were streaked directly onto DGVP agar plates. Others were first placed in sterile tap water and then heat or acid treated or both, as described previously (20), to reduce the numbers of non-*Legionelloceae* bacteria.

Multiplication studies. Each of the samples collected in

TABLE 1. *L. pneumophila* and *Flavobacterium* sp. multiplication after inoculation in municipal water system samples

Source	Multiplication (log CFU/ml) in given mo of sample collection					
	July		August		October–November	
	L. pneumophila	*Flavobacterium* sp.	*L. pneumophila*	Flavobacterium sp.	*L. pneumophila*	*Flavobacterium* sp.
River	NG[a]	1.86	NG	0.62	NG	0.35
Treatment plant						
Clarifier	NG	NG	1.22	0.19	—[b]	—
Sedimentation basin surface	0.68	NG	NG	NG	NG	0.18
Sedimentation basin bottom	—	—	—	—	1.34	1.05
Sedimentation basin corner	—	—	—	—	0.51	1.29
Filter surface	—	—	1.40	0.52	NG	NG
Filter effluent	0.07	NG	NG	NG	NG	NG
Clearwell	NG	1.87	NG	0.08	—	—
Lanpher Reservoir (open)						
Surface	0.58	NG	1.10	NG	0.16	NG
Bottom	1.93	0.96	1.79	0.72	0.96	0.48
Corner	1.36	NG	0.88	NG	0.11	NG
Brashear Reservoir (open)						
Surface	0.77	NG	1.26	NG	0.12	0.91
Bottom	—	—	1.94	0.53	1.08	0.82
Corner	0.79	NG	1.24	NG	0.51	0.13
McNaugher Reservoir (covered)						
Surface	NG	1.46	0.37	NG	—	—
Bottom	—	—	1.09	NG	NG	0.26
Vinyl corner	0.28	0.85	NG	NG	2.02	1.02
Hatch	0.89	0.45	1.21	NG	NG	0.17
Tap samples originating from						
Lanpher Reservoir	0.82	NG	1.08	0.11	NG	0.87
Brashear Reservoir	1.08	NG	1.03	NG	NG	NG
McNaugher Reservoir	NG	NG	1.71	0.20	0.17	0.72

[a] NG, No growth (indicates an increase in counts of <15% over the original number inoculated).
[b] —No sample collected.

July, August, and October were evaluated for ability to support *Legionella* growth. *Legionella* and non-*Legionellaceae* growth was monitored in samples inoculated with the water stock culture. This experimental approach is similar to that used by van der Kooij et al. in studies of *Pseudomonas* and *Flavobacterium* spp. in public water systems (34, 35). Prior to inoculation, samples were stored in the dark at 4°C. At the beginning of the experiment, 100-ml unfiltered portions of each of the samples were dechlorinated by aeration, using a magnetic stirrer, and pasteurized at 60°C for 30 min in a water bath. Following pasteurization, each 100-ml sample was inoculated with 1.0 ml of the water stock culture containing *L. pneumophila* in late exponential phase. This resulted in an initial population density of approximately 1,000 CFU of *L. pneumophila* and 6,500 CFU of *Flavobacterium* sp. per ml. All inoculated samples were incubated in the dark in a room air incubator at 35°C. The samples were cultured weekly, for a 5-week period, on DGVP and unsupplemented buffered charcoal yeast extract media to monitor the growth of *Legionella* and non-*Legionella* populations. Plates were incubated for 6 days at 35°C in sealed plastic bags to prevent dehydration.

Chemical analyses. A chemical analysis was conducted on all samples collected as part of the multiplication studies in July, August, and October. Each sample was analyzed for organic carbon, using a DC54 total organic carbon analyzer (Dohrman Envirotech, Santa Clara, Calif.). The concentrations of nine metals (see Table 3) were measured by flame or graphite furnace atomic absorption spectrophotometry, using a model 503 spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.). Chloride, sulfate, alkalinity, pH, hardness, calcium, and magnesium were measured by mercuric nitrate, turbidimetric, sulfuric acid titrimetric, electrometric, or EDTA methods as described by the American Public Health Association (1).

RESULTS

System survey for presence of legionellae. In an attempt to isolate *L. pneumophila* from the municipal water supply, a number of water samples were cultured from a variety of locations including the river, treatment plant, reservoirs, mains entering hospitals, and taps in homes. Additional samples were collected from the vinyl covering of a finished water reservoir and from the interior piping and valve within a main entering a hospital. A combination of procedures were utilized including spread plating, concentration by membrane filtration, concentration by centrifugation, heat enrichment, and direct swabbing of environmental surfaces. *L. pneumophila* (serogroups 1, 3, and 6) was recovered only from the river when 20-liter samples were concentrated to 10 ml. Population counts were quite low, 15 to 30 CFU/liter. Legionellae were never isolated from any of the other

TABLE 2. Chemical parameters from municipal water system samples[a]

Sample source	pH	ppm (mg/liter)						Total dissolved solids (ppm)	Turbidity (NTU)
		Hardness	Ca	Mg	Alkalinity	CL	TOC		
River	7.33	100	31.0	5.3	27	13	3.21	184	9.87
Treatment plant									
Clarifier	8.53	126	38.4	7.3	38	16	2.32	221	4.97
Sedimentation basin surface	8.07	119	38.8	5.2	38	21	3.19	194	1.57
Sedimentation basin bottom	7.82	170	52.0	9.7	48	20	9.09	210	6.40
Sedimentation basin corner	7.60	162	53.6	6.8	45	19	6.35	221	9.70
Filter surface	8.23	143	42.5	9.0	40	11	2.12	196	1.73
Filter effluent	8.01	129	37.5	8.6	40	16	2.07	196	0.14
Clearwell	7.77	133	38.4	8.9	33	18	2.36	169	0.65
Lanpher Reservoir									
Surface	7.56	117	37.2	6.0	31	19	1.98	207	0.57
Bottom	7.54	118	37.4	6.0	31	20	1.99	205	6.47
Corner	7.58	117	37.2	6.0	31	20	3.22	204	1.25
Brashear Reservoir									
Surface	7.18	115	38.5	4.4	32	21	1.68	200	0.61
Bottom	7.33	131	24.4	6.6	33	20	2.56	217	11.60
Corner	7.54	121	37.5	5.8	30	21	2.42	203	0.91
McNaugher Reservoir									
Surface	7.36	118	40.1	5.9	30	21	1.49	178	0.93
Bottom	7.34	142	40.4	9.9	38	20	2.66	211	1.10
Cover	6.46	60	17.3	4.0	13	11	6.42	280	10.57
Hatch	7.38	131	41.2	7.6	28	20	2.83	177	1.02
Tap samples from									
Lanpher Reservoir	7.50	124	37.1	7.6	31	21	1.95	199	0.62
Brashear Reservoir	7.46	123	35.6	8.2	30	21	1.85	182	0.62
McNaugher Reservoir	7.49	123	37.8	6.6	30	21	1.59	181	0.84

[a] Values are averages of samplings conducted in July, August, and October–November 1984. NTU, Nephelometric turbidity units; TOC, total organic carbon.

locations with either unconcentrated samples or samples concentrated by membrane filtration.

Growth potential experiments. Table 1 depicts the growth of *L. pneumophila* during the 5-week period following inoculation into water samples collected from various parts of the municipal system. Values in the table represent maximum increases in bacterial concentration from an initial population density of approximately 10^3 CFU of *L. pneumophila* and $10^{3.81}$ CFU of *Flavobacterium* sp. per ml. The *Legionella* growth potential of water varies several times from the river to the tap. *L. pneumophila* did not multiply in river water. However, the *Legionella* population increased in inoculated samples from several locations within the treatment plant. This was especially true in locations where the water is pooled and nutrients accumulate, such as the bottom of sedimentation basins and the top of filters. *Legionella* growth potential decreased following passage through the filters, but increased once more in the distribution system. The increase in growth potential was more pronounced in the bottoms and corners of open primary and secondary reservoirs than in the covered McNaugher Reservoir. However, growth potential was also appreciable in stagnant water collected from the top of the vinyl cover of this reservoir. The data also suggest that the enhanced growth potential extends to samples collected from distribution taps.

The pattern of growth potential for *Flavobacterium* sp. differed from that of *Legionella* sp. (Table 1). In contrast with *L. pneumophila*, the inoculated *Flavobacterium* population grew to a greater extent in river water, in somewhat different locations within the treatment plant, in a similar pattern in the reservoir system, and more poorly in distribution tap samples. As was the case with *Legionella* spp., the growth potential was high in stagnant water collected from the top of the floating vinyl cover on McNaugher Reservoir.

Chemical survey and correlation. To explain the observed variation in the potential for bacterial growth, chemical analyses were performed on all samples collected. Tables 2 and 3 summarize the results of some of these analyses. A series of computer-assisted linear correlations were calculated to examine the relationship between the chemical environment and bacterial growth potential. A simple correlation coefficient was calculated between *Legionella* growth and each of the chemical parameters measured. A similar set of statistics was generated for the growth of *Flavobacterium* sp. Beginning with all chemical parameters whose simple correlation with bacterial growth was significant at the $P = 0.1$ level or better, a stepwise multiple linear regression program was used to generate the best possible multiple correlation between bacterial growth and a group of chemical parameters. The program selected the combination of independent parameters most closely associated with multiplication of *Legionella* or *Flavobacterium* sp. Statistical results for *Legionella* sp. appear in Table 4. Only correlation coefficients significant at the $P = 0.005$ level or higher are considered statistically significant in this study. Other coefficients (significant at the $P = 0.1$ level or higher) are included to indicate trends or demonstrate contrast with more significant coefficients. As the multiple correlation coefficients indicate, the potential for *Legionella* growth

TABLE 3. Concentrations of metals from municipal water system samples

Sample source	Avg concn (ppm [mg/liter])								
	Fe	Mn	Al	Zn	Cu	Pb	Ba	Na	K
River	0.723	0.443	0.051	0.027	<0.010	0.007	0.234	17.84	1.79
Treatment plant									
Clarifier	0.605	0.184	0.774	<0.001	<0.010	0.002	0.111	16.77	2.05
Sedimentation basin surface	0.155	0.069	0.778	<0.001	0.010	0.002	0.089	17.31	1.93
Sedimentation basin bottom	0.563	0.193	0.911	<0.001	0.012	<0.001	0.028	18.46	2.42
Sedimentation basin corner	0.129	0.060	0.776	<0.001	<0.010	<0.001	0.030	19.10	2.42
Filter surface	0.182	0.087	0.760	<0.001	<0.010	<0.001	0.066	17.45	2.04
Filter effluent	0.074	<0.002	0.721	<0.001	<0.010	0.002	0.063	17.77	2.15
Clearwell	0.093	<0.002	0.720	<0.001	<0.010	0.002	0.072	17.56	1.92
Lanpher Reservoir									
Surface	0.018	<0.002	0.382	0.001	0.147	0.001	0.066	17.12	1.87
Bottom	0.138	0.030	1.592	0.007	1.308	0.002	0.074	17.21	1.94
Corner	0.151	<0.002	0.272	0.015	0.151	<0.001	0.082	17.03	1.84
Brashear Reservoir									
Surface	0.014	<0.002	0.344	0.013	0.236	0.002	0.073	16.51	1.20
Bottom	0.214	0.065	1.350	0.024	2.343	0.005	0.056	17.55	2.05
Corner	0.014	<0.002	0.319	0.018	0.344	<0.001	0.062	16.97	1.81
McNaugher Reservoir									
Surface	0.015	0.002	0.266	0.032	0.125	<0.001	0.120	16.71	1.88
Bottom	0.013	<0.002	0.671	0.013	0.080	<0.001	0.057	17.33	1.99
Cover	0.088	0.028	0.040	0.047	1.038	0.011	0.038	6.29	1.15
Hatch	0.043	0.012	0.255	0.023	0.093	0.003	0.245	16.62	1.93
Tap samples from									
Lanpher Reservoir	0.014	<0.002	0.323	0.028	0.157	0.002	0.073	16.89	1.89
Brashear Reservoir	0.032	<0.002	0.209	0.028	0.122	0.003	0.086	16.80	1.88
McNaugher Reservoir	0.093	<0.002	0.225	0.025	0.101	0.009	0.070	16.81	1.87

generally appears to be significantly associated with the measured chemical environment. The individual parameters of importance, at various times, include copper, zinc, total organic carbon, and turbidity. In contrast to *Legionella* sp., *Flavobacterium* sp. did not appear to be closely correlated with the chemical factors measured in this study. In no instance was a multiple correlation coefficient significant at greater than the $P = 0.01$ level.

DISCUSSION

The detection of *L. pneumophila* in the plumbing systems of hospitals and other large buildings has raised the suspicion

TABLE 4. Correlation coefficients for chemical parameters correlated with greatest population size attained among municipal water system samples inoculated with *L. pneumophila*

Sampling mo	Simple correlation coefficients			Multiple correlation coefficients		
	Independent parameters	r	Significance of r	Independent parameters selected by SMLRP[a]	R	Significance of R
July	Cu	+0.566	0.05	Cu, Pb	0.554	0.01
	Pb	−0.552	0.05			
August	Turbidity	+0.461	0.05	Hardness, Cu	0.503	0.005
	Hardness	+0.479	0.05			
	pH	+0.453	0.10			
	Mg	+0.507	0.05			
	Cu	+0.591	0.01			
	Zn	+0.448	0.10			
	Al	+0.461	0.05			
October–November	Hardness	−0.470	0.05	Hardness, alkalinity, TOC, Cu, Zn, Na, Al	0.839	0.005
	Alkalinity	−0.494	0.05			
	TOC[b]	+0.647	0.005			
	Cu	+0.663	0.005			
	Zn	+0.651	0.005			
	Na	−0.546	0.02			
	Al	+0.449	0.10			
	Pb	+0.509	0.05			
	Turbidity	+0.694	0.005			

[a] SMLRP, Stepwise multiple linear regression program.
[b] TOC, Total organic carbon.

that *Legionella* sp. inhabits municipal drinking water supplies and that these supplies serve as pathways for contamination of buildings. In this study, a number of locations within the municipal water system were cultured for the presence of legionellae. While *L. pneumophila* could be isolated from river water, it was not detected in any of the other samples. This result is consistent with previous studies showing efficient removal of several different indicator bacteria during the various stages of treatment within a conventional water plant (23). It is also consistent with studies in which investigators were similarly unsuccessful in isolating viable or virulent *Legionella* sp. from water in treatment plants, finished water reservoirs, or distribution mains (10, 31). The inability to detect legionellae in these samples may be attributed to the possibility that *Legionella* sp. may only occur within municipal systems sporadically and in lower numbers. Also, in the municipal system examined in the present study, their absence might be related to the fact that a chlorine residual of 0.2 mg/liter is generally maintained throughout the distribution system, with even higher residuals detectable within some reservoirs and water mains. Alternatively, the failure to detect legionellae may be due to the absence of an environment suitable for maintenance and growth of *Legionella* populations.

To investigate the suitability of the environment for support of *Legionella* sp., samples collected from locations throughout the public water system were inoculated with *L. pneumophila* and non-*Legionellaceae* bacteria. The lack of detectable growth in river samples may be due to either the presence of growth-inhibiting materials or, as a result of the constant flow of the river, poor accumulation of growth-supporting substances. As water passes through the treatment plant, the ability to support *Legionella* growth increases. The removal of certain organic and inorganic compounds by treatment may remove inhibitory materials. Alternately, since the greatest growth potential in the plant occurs in areas of slow-moving water, growth-supporting materials can accumulate there. Passage of water through the rapid sand filters of the plant almost completely reduces growth potential. This suggests removal of growth-enhancing factors and is similar to the observations of Hoekstra et al. (16) on water passing through rapid and slow sand filters.

Within the distribution system, growth potential again increases and is most pronounced in the bottoms and stagnant corners of uncovered, finished water reservoirs. Earlier studies have demonstrated the algal and bacterial problems associated with storage of raw and finished water in open reservoirs (2, 18, 21, 36). Despite treatment with copper sulfate, these reservoirs typically support low-density algal populations during the warmer months. The bottoms and corners of reservoirs and sedimentation basins, and the tops of filters in the plant, are suspected of accumulating metabolites from these organisms as well as decaying organic matter from leaf litter. This in turn may support *Legionella* growth, as suggested by earlier studies of aquatic bacterial utilization of nutrients released by excretion and decomposition of algae (5, 41), and, more specifically, legionellae growth support by algae, amoebae, and protozoa (3, 9, 30, 33).

That growth potential is less within the covered secondary reservoir also indicates the importance of algae and leaf litter. The observation that *Legionella* growth potential is high in water collected from the top of the vinyl cover is expected due to the difficulty encountered in removing rainwater and debris from the deep folds of the cover. Enhanced growth potential in stagnant locations such as the covers, bottoms, and corners of reservoirs is similar to findings of enhanced *Legionella* growth in stagnant or obstructed areas in hospital plumbing systems (6).

That water collected from tap samples within the distribution system is also frequently growth supporting suggests the passage of growth-enhancing factors acquired from the reservoir system or water mains. Also, this may indicate the presence of growth-enhancing factors acquired within the building's internal plumbing system itself. Internally acquired support of *Legionella* growth has been previously demonstrated in studies of metallic components of hospital hot-water tanks (26) and sediment buildup and the use of rubber fittings within hospital plumbing systems (7, 27).

To identify the nature of the apparent growth-enhancing compounds, a chemical survey of the water system was conducted. Significant multiple correlations between *Legionella* growth potential and the measured chemical parameters of these same samples suggest that the combination of chemical parameters affects the suitability of water collected from these locations for support of *Legionella* sp. (Table 4). Identification of specific important compounds is difficult since no single environment factor has yet been described which accurately predicts environmental densities of legionellae (11). However, it is not surprising that total organic carbon and turbidity were positively associated with growth. These parameters are generally related to nutrient levels and their influence on bacterial growth has been noted previously (4, 15). The positive correlation of Zn with the ability to support *Legionella* sp. is consistent with earlier metal studies (24) and our work with hospital hot-water tanks (26). The observation that *Legionella* growth is positively correlated with Cu levels may be coincidental since the bottoms of open reservoirs and the top of the covered reservoir are areas where Cu accumulates due to algal treatment with copper sulfate and where *Legionella* growth potential may be appreciable due to the accumulation of nutrients. Cu concentrations on reservoir bottoms are typically high because of the extensive adsorption by clay minerals, humic acids, and bottom muds (25). However, the positive correlation between Cu and *Legionella* growth potential is also similar to earlier field observations of a correlation between $CuSO_4$ and standard plate count bacteria (14). It may also be consistent with observations of enhanced Cu tolerance by *L. pneumophila* (16) and with the generally high metal tolerance and metal requirements of legionellae (24, 26, 29).

The growth pattern of *Flavobacterium* sp. in the inoculation experiment differed from that observed for *L. pneumophila* (Table 1). Also, *Flavobacterium* sp. growth was not strongly correlated with the chemical parameters measured in this study. This indicates that the growth requirements of flavobacteria in the municipal water system differ from those of *Legionella* and suggests that, while *Flavobacterium* sp. has been shown to be capable of supporting legionellae growth (38), support may also be available from other sources.

This investigation, in general, indicates that although *Legionella* sp. is not readily isolated from public water systems, the systems are capable of supporting *Legionella* growth. Dechlorinated water from locations within the treatment and distribution chain appears to be more growth supporting than untreated river water. That *Legionella* sp. was not actually isolated from this particular municipal system is likely due to the presence of a chlorine residual throughout the system. These findings suggest that preven-

tion of *Legionella* growth is aided by maintenance of a free chlorine residual, especially in secluded sites such as on the bottoms of reservoirs. Rapid sand filtration also appears to effectively remove growth-supporting factors. Protection from *Legionella* regrowth may be enhanced by elimination of algae, protozoa, and leaf litter through covering of sedimentation basins and reservoirs. However, attention to the type of cover used is important to prevent potential contamination of finished supplies from nontreated water and debris that can accumulate on the top of flexible covers. The results of this study indicate that failure to control *Legionella* growth through these types of measures can result in the seeding of plumbing systems and cooling towers in hospitals and other buildings by legionellae supplied through the municipal water system.

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