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Iron Bacteria in Drinking-Water Distribution Systems: Elemental Analysis of *Gallionella* Stalks, Using X-Ray Energy-Dispersive Microanalysis

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"Iron" bacteria belonging to the genus *Gallionella* were observed by scanning electron microscopy in water samples and attached to pipe surfaces in a Southern California drinking-water distribution system. The cells were recognized by their characteristic elongated helical stalks composed of numerous intertwined microfibrils. Many of the stalks were partially coated with insoluble ferric salt deposits. Stalks recovered directly from water samples were analyzed for their elemental composition by using X-ray energy-dispersive microanalysis. Silicon, aluminum, calcium, and iron were the predominant elements present in the stalks. Smaller quantities of the elements phosphorous, sulfur, chlorine, copper, and zinc were also detected. Manganese, though present in measurable quantities in the water supply, was not detected in the stalks, suggesting that this organism is unable to utilize this element as an electron donor. This represents the first such analysis of *Gallionella* stalks recovered from environmental samples without prior subculturing in artificial laboratory media.

Fossilized "iron" bacteria belonging to the genus *Gallionella* were first discovered in ochre mineral deposits by Ehrenberg in 1836 (9). It was Cholodny in 1924 (5) and, later, Perfil'ev in 1926 (20), however, who provided the first detailed descriptions of the morphology and developmental cycle of the genus. A number of workers subsequently studied the physiology and ultrastructure of this extraordinary microorganism, and several recent literature reviews on these subjects have been published (8, 10, 13, 14, 16, 24). *Gallionella* organisms have been found in a variety of soil and aquatic habitats, always associated with iron (10, 23). The organism consists of a kidney-shaped mycoplasmodial cell body deficient in the usual peptidoglycan component which imparts rigidity to the bacterial cell wall (3). For this reason, Balashova (3) has suggested evolutionary kinship to the mycoplasmas and to *Metallogenium* sp., a related, wall-less polymorphic iron bacterium. A single elongated stalk constructed from numerous helically wound, uniquely mineralized fibrils extends outward from the convex side of the *Gallionella* cell body. The protein nature of the stalk fibrils was demonstrated by Balashova (2), who used proteolytic enzymes to actively digest them. Some workers believe that the stalk itself may constitute the principle living reproductive element of this microorganism and that the apical cell body develops only during transition to a motile stage in the life cycle (16). Hanert (12), however, contends that any *Gallionella* stalk growing slowly under natural conditions terminates in an apical cell. Though the exact function of the stalk remains obscure, it is sufficiently characteristic in appearance to readily enable microscopic identification of *Gallionella* sp. in crude preparations.

Very little is known about the biology of *Gallionella* organisms or other kinds of iron bacteria, despite their economically important roles in initiating and perpetuating biogeochemical corrosion processes in water distribution systems (8). Hanert (11) observed measurable fixation of $^{14}CO_2$ by *Gallionella ferruginea* grown axenically in a $^{14}CO_3$-FeS medium. These data and less direct physiological evidence (10, 15) suggest that *Gallionella* organisms and certain other iron bacteria (7, 22) derive their essential energy requirements through a strictly chemolithotrophic process, namely, the enzyme-mediated oxidation of ferrous (and sometimes also manganous) ions with the concomitant fixation of carbon dioxide. This typically results in the precipitation of ferric salts about the cell and stalk, usually the hydroxide form, which renders the cells brown or reddish-brown. Under favorable conditions, *Gallionella* organisms can rapidly multiply in water distribution systems, resulting in discoloration of the water and eventually impairment of the flow of water through the pipes due to the massive accumulation of insoluble iron salts. Such heavy growths of iron bacteria can alter the physicochemical properties of the pipe surfaces and serve as nutritional substrates for various chemoorganotrophic microorganisms. The metabolic activities of the latter un-

der anaerobic conditions can produce organic acids and hydrogen sulfide which, in turn, can lead to taste, odor, and corrosion problems. Reports of fouling and corrosion of wells and water distribution mains by *Gallionella* organisms have increased since the 1940s, and certain investigators have suggested that this is due to an increased occurrence of iron bacteria in these systems (19).

Extracellular precipitates formed in laboratory-grown cultures of *Gallionella* organisms have been specifically analyzed for iron in only a few instances (8, 17). However, a complete elemental analysis of the stalk encrustations has never been accomplished on cells recovered directly from the environment, where more complex physicochemical and biological interactions occur.

While investigating the environmental factors which influence microbial survival and regrowth in a Southern California municipal drinking-water supply, we obtained scanning electron microscope evidence indicating colonization of pipe surfaces by a variety of morphologically distinct microbial species (21). Among the most frequently encountered microorganisms was the iron bacterium *Gallionella* sp. To learn more about the nature and composition of the mineral encrustations on the surfaces of *Gallionella* cells and their possible involvement in microbial fouling and corrosion processes, we examined the microzonal distribution of this bacterium on pipe surfaces by scanning electron microscopy. In addition, the elemental composition of the mineral precipitates coating the surfaces of *Gallionella* stalks obtained directly from the distribution system was determined by X-ray energy-dispersive microanalysis.

MATERIALS AND METHODS

Sample collection and scanning electron microscopy. Water and pipe samples were obtained from the municipal water system of Garden Grove, Calif. The population of Garden Grove is supplied with untreated groundwater from a series of 30 wells located throughout the city. Pipe samples consisting of galvanized iron, copper, or asbestos-concrete, all of which had been in service for approximately 25 years, were obtained during routine maintenance operations. Upon removal from the ground, the pipes were sealed with plastic to prevent drying or contamination and placed on ice before transport to the laboratory. Rust-colored nodules and other distinct mineral deposits which protruded from the inner surfaces of the pipes were removed and prepared for scanning electron microscopy. The nodules were immersed in a solution of 2.5% (wt/vol) electron microscopy-grade glutaraldehyde (Ted Pella Co., Tustin, Calif.) prepared in a 5 mM dihydrogen potassium phosphate buffer, pH 7.0. After overnight fixation at 4°C, the samples were rinsed in two consecutive changes of distilled water and dehydrated stepwise in an increasing ethanol concentration series (30, 50, 75, 85, 95, and 100% × 3). The specimens were critical-point dried by the method of Cohen et al. (6), using Freon 113 as the intermediate transitional fluid, mounted on aluminum stubs with conducting collodial graphite, and then coated with either gold-palladium (60:40) for viewing in the scanning electron microscope or with evaporated carbon for X-ray energy-dispersive microanalysis.

Fire hydrants were flushed for 1 min at a rate of 800 liters/min, after which water samples were collected in clean, wide-mouthed, sterile, flint glass bottles containing 0.1 ml of 10% (wt/vol) sodium thiosulfate solution. The samples were placed on ice immediately after collection. A 5- to 20-ml portion (depending on the relative turbidity) of the water sample was filtered through a 0.2-μm pore size polycarbonate Nuclepore membrane filter (Nuclepore Corp., Pleasanton, Calif.). The particulate matter captured on the membrane surface was overlaid with 20 ml of the above glutaraldehyde fixative solution. After 30 min at 23°C, the glutaraldehyde was removed, and the membrane was washed by filtration in two changes of distilled water. The membrane was dehydrated by increasing ethanol concentrations. Critical-point drying was avoided to prevent loss of particulate matter from the membrane. The membrane was rapidly air dried from the final absolute Freon-113, cut into 1-cm squares, and mounted on aluminum stubs with collodial graphite. Specimens were coated with gold-palladium or carbon as previously indicated. For normal viewing, the gold-palladium–coated water and pipe samples were examined in a Hitachi model SU500 scanning electron microscope operated at an accelerating voltage of 15 keV.

X-ray energy-dispersive microanalysis. The carbon-coated specimens were examined in a Cambridge model S4 scanning electron microscope operated at 25 keV and equipped with an X-ray energy-dispersive microanalysis unit (Ortec, Delphi). A continuous X-ray energy spectrum from 0 to 10 keV was integrated over a minimum of 200 s for each elemental scan. The K_α and K_β energy lines were utilized for identifying the elements present in the sample. The limit of detection of any particular element in the sample was about 1,500 mg/liter. Control elemental scans were performed on background areas (e.g., the naked membrane surface) and on adjacent areas of the cell surface to eliminate possible artifacts introduced by topographical electron scattering. Quantification was achieved by interfacing a PDP-11 computer with an X-ray microanalysis multichannel processor. Ratios of the corrected areas under the peaks for the elements of interest were automatically computed and normalized to the amount of iron in the specimen.

Chemical analyses of water samples. Physical and chemical analyses of water samples were performed by standard procedures described in reference 1.

RESULTS AND DISCUSSION

When the surfaces of the rust-colored nodules from galvanized iron pipes were examined in the scanning electron microscope, they were seen to be coated with a superficial layer of flocculent precipitate (Fig. 1). Closer inspection of the precipitate at higher magnification revealed dense

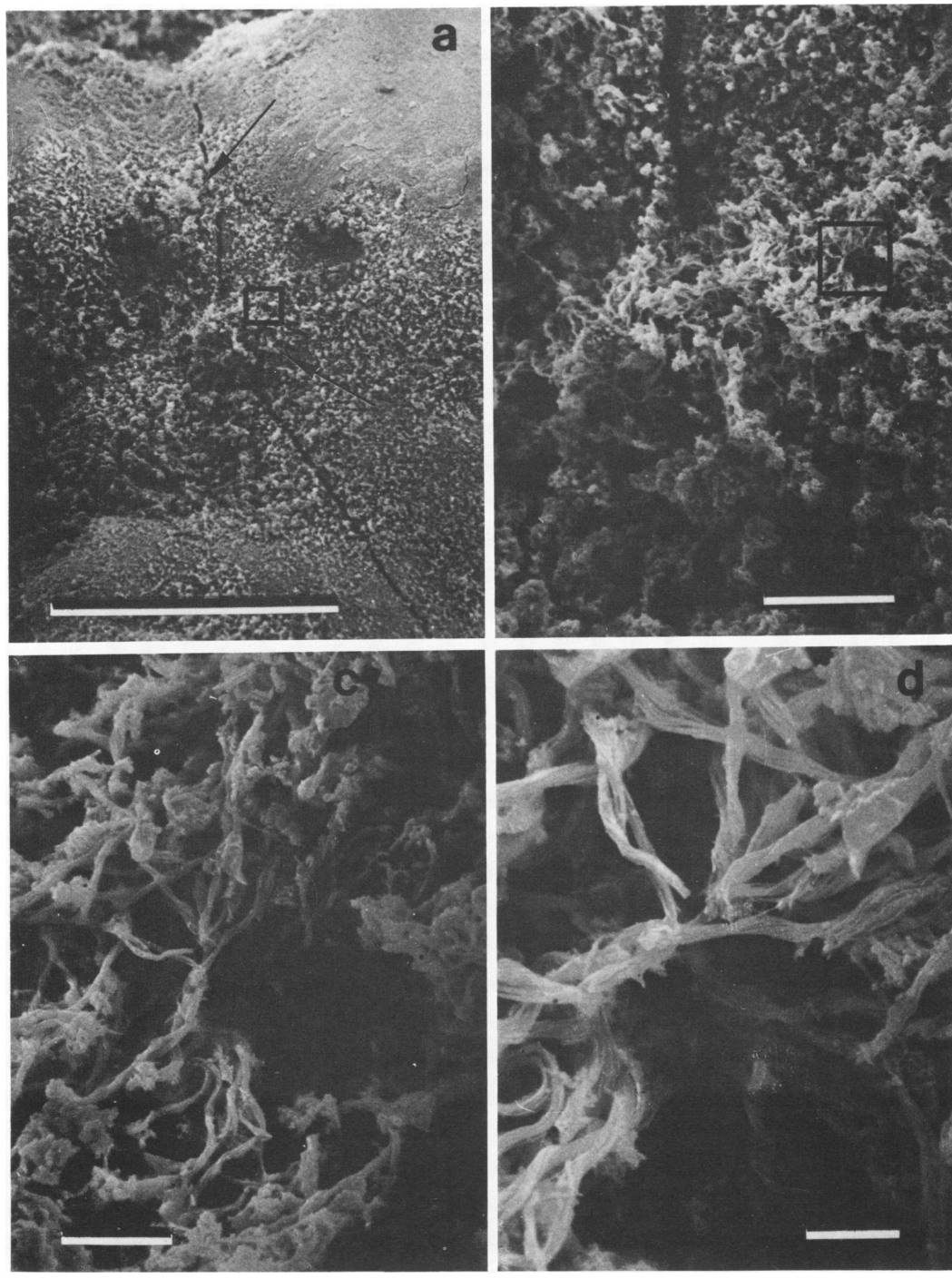

FIG. 1. *View of the surface of a rust-colored nodule from galvanized iron pipe showing flocculent-type precipitate and Gallionella microcolonies (a) (arrows). (b to d) increasingly higher-magnification views of a microcolony (box). Note typical striated helices of individual Gallionella stalks in (d). Bars, 500 μm (a); 50 μm (b); 10 μm (c); 5.0 μm (d).*

microcolonies of *Gallionella* sp. Such microcolonies were distributed over the nodule surface randomly, and in some areas the entire nodule surface was covered with a mat-like layer of cells up to several micrometers in thickness (Fig. 1a to d). *Gallionella* cell bodies were not observed, possibly having been obscured beneath the mineral deposits. Only the helical stalks, which tended to form intertwined masses, were clearly discernible. Neither the macroscopic rust-colored nodules nor *Gallionella* microcolonies were detected on connecting copper and asbestos-concrete pipes removed from a different, but proximal, location within the distribution system. Though an exhaustive search of the system was not made, *Gallionella* cells and microcolonies appeared to be predominantly associated with galvanized iron pipes which were in various stages of decay due to corrosion processes.

Individual *Gallionella* stalks were found throughout the year in various concentrations in water samples obtained from different wells or hydrant sites within the system. The relative abundance of *Gallionella* stalks and the corresponding physicochemical characteristics of the water at selected well and hydrant sites are shown in Table 1. No stalks were detected in water samples obtained from wells-reservoirs 6321, 239, or 6064, which collectively supply more than 90% of the water for the three hydrant locations shown in Table 1. The stalks were observed in greatest numbers in older, dead-end pipe sections where the water flow rate was reduced to a minimum and lower in situ oxygen tensions and redox potentials prevailed (e.g., Marshall Lane). It is probably also significant that these bacteria were most numerous in galvanized iron pipes where higher concentrations of soluble reduced-iron species might be expected to occur. McMillan and Stout (18) similarly found *Gallionella* stalks primarily associated with rusty pipes and dead-end mains in the Chicago water distribution system. This is in conceptual agreement with the findings of Hanert (11) who reported that growth of *C. ferrugiena* in laboratory culture was enhanced by low oxygen concentrations (0.1 to 0.2 mg of O_2 per liter), whereas higher concentrations (>2.75 mg of O_2 per liter) were inhibitory.

The above data suggest that physicochemical conditions prevailing at specific locations (or microhabitats) within the distribution system influence the survival and growth of *Gallionella* cells. The overall mineral composition of the distribution source water, which showed little variation over time, may be less influential in this regard. The concentrations of various ions in water from the three major source wells and

TABLE 1. *Occurrence of Gallionella stalks and physicochemical properties at sampling sites*[a]

Sample site	*Gallionella* stalks detected[b]	pH	Temp (°C)	Turbidity (NU)[c]	Dissolved O_2 (mg/liter)	Redox potential (mV)	Pipe material[d]	Maximum flow (gallons/min)	Date installed	Dead-end section
Well-reservoir										
6321	ND	7.28 ± 0.579	19.3 ± 1.36	0.246 ± 0.105	7.57 ± 1.12	114 ± 42.1	C		1969–1971	No
23/9	ND	6.83 ± 0.528	20.5 ± 2.68	0.300 ± 0.127	7.93 ± 0.731	170 ± 43.4	S		1966	No
6064	ND	7.46 ± 0.430	18.3 ± 0.985	0.223 ± 0.093	7.59 ± 0.698	133 ± 48.2	S		1954	No
Hydrant										
Rockinghorse	+	7.38 ± 0.414	20.5 ± 2.67	5.35 ± 22.9	7.20 ± 1.05	102 ± 71.9	AC	4,000 (15,140)[e]	1955	Yes
Harris	+	7.41 ± 0.462	19.6 ± 2.12	8.96 ± 31.5	7.23 ± 1.03	87.3 ± 108	AC	4,000 (15,140)	1955	Yes
Marshall	++++	7.46 ± 0.859	19.9 ± 2.38	8.78 ± 15.4	7.12 ± 1.14	60.4 ± 118	GI	60 (227.1)	1940	Yes

[a] Except for turbidity, parameters were measured in situ bimonthly from December 19, 1978 to November 8, 1979. Data for well 23/9 and the Harris site were collected from August 1979 to November 8, 1979. All data are shown as the mean ± the standard deviation.

[b] +, Some stalks observed; ++++, many stalks frequently observed; ND, no stalks detected in the scanning electron microscope after filtering 20 ml of sample through a 3.7-cm Nuclepore membrane filter (0.2-μm pore size).

[c] Water turbidity was measured in standard nephelometric units (NU) in a HIAC N-60 turbidimeter (1).

[d] AC, Asbestos-concrete; GI, galvanized iron; C, concrete; S, steel.

[e] Liters per minute is shown within parentheses.

from two of the hydrant sites in the distribution system are shown in Table 2. The minimum concentrations of iron reported in the literature which are able to support the growth of *Gallionella* (and *Leptothrix*) organisms in fast-flowing, well-aerated water, such as a pumping well, vary from about 0.2 to 0.5 mg/liter (13). The concentration of iron in the source water was, on the average, about one order of magnitude less than these values (i.e., approximately 0.02 mg of iron per liter) and may, therefore, explain the absence of *Gallionella* organisms from the well sites. Interestingly, the concentration of most ions, including iron, increased from the source water to the hydrant sites. As much as a 33-fold increase was observed in some instances in the total iron concentration from the source to the site. Iron concentrations at the hydrant sites were within the limits reported to support the growth of iron bacteria and may have been even higher where galvanized iron pipes were installed, especially in dead-end sections. Other substances required for the autotrophic growth of *Gallionella* organisms, such as dissolved oxygen, carbon dioxide-bicarbonate, ammonia, nitrogen, phosphate, etc., were generally present in sufficiently high concentrations as not to be growth limiting.

Examples of some of the waterborne *Gallionella* stalks obtained from the system are shown in Fig. 2a to d. These stalks were morphologically indistinguishable from those on pipe surfaces and consisted of at least 20 distinct, helically coiled microfibrils (Fig. 2b). Each fibril measured approximately 0.04 to 0.05 μm in diameter and apparently extended the length of the stalk (Fig. 2b). Contrary to the findings of Hanert (11), no clear indications of transverse fission or segmentation of the fibrils into "microcellulare reproduction units" were observed in the scanning electron microscope, although this may have been due to limitation in instrument resolution (about 10 nm). Intact stalks measured approximately 2 μm in diameter, though the overall lengths of the stalks exhibited considerable variation. Such variation in stalk length could have resulted from different-length filaments being shorn from the pipe surface by increased hydrodynamic stress during sampling.

Some stalks were coated to various degrees with small (0.2 to 0.5 μm) bud-like or nodular structures, possibly corresponding to the spherical bodies described earlier by Balashova and Cherni (4) in ultrathin sections of axenically-grown cultures of *Gallionella filamenta* (Fig. 2a). Most of the stalks, however, were clearly

TABLE 2. *Chemical analysis of water from well and hydrant sites*

Component	Method of analysis[a]	Amt of chemical (mg/liter)[b]		
		Well-reservoir sites[c]	Hydrant sites[d]	Hydrant/well ratio
Sodium	Flame photometry	40.1 ± 7.43	57.2 ± 6.41	1.4
Potassium	Flame photometry	3.82 ± 0.482	4.41 ± 0.330	1.2
Calcium	EDTA titration	80.8 ± 9.84	89.4 ± 19.8	1.1
Magnesium	EDTA (by calculation)	16.0 ± 2.62	23.3 ± 10.3	1.5
Manganese	Atomic absorption	0.004 ± 0.005	0.010 ± 0.010	2.4
Total iron	Atomic absorption	0.023 ± 0.035	0.186 ± 0.336	8.3
Silica	Colorimetry	20.7 ± 0.212	21.9 ± 0.919	1.1
Cobalt	Atomic absorption	0.002 ± 0.001	0.002 ± 0.001	1.1
Copper	Atomic absorption	0.035 ± 0.044	0.008 ± 0.006	0.2
Zinc	Atomic absorption	0.036 ± 0.055	0.007 ± 0.007	0.2
Bicarbonate	Potentiometric titration	216.0 ± 11.4	216.0 ± 28.6	1.0
Carbonate	Potentiometric titration	0.780 ± 0.425	0.639 ± 0.312	0.8
Carbon dioxide	Calculated from alkalinity	8.77 ± 8.94	9.48 ± 10.2	1.1
Chloride	Mercuric nitrate	62.7 ± 82.2	68.1 ± 5.12	1.1
Sulfate	Turbidimetry	91.5 ± 19.7	146.0 ± 27.3	1.1
Nitrate	Brucine colorimetry	17.0 ± 14.1	18.4 ± 8.20	1.1
Orthophosphate	Ascorbate	0.040 ± 0.032	0.041 ± 0.049	1.0
Kjeldahl-N	Kjeldahl	0.005 ± 0.001	0.068 ± 0.088	15.0
Ammonia-N	Phenate	0.022 ± 0.018	0.020 ± 0.013	0.9

[a] For a general description of analytical methods, see reference (1).

[b] Mean ± standard deviation. Data were accumulated bimonthly from December 19, 1978 to November 8, 1979.

[c] Arithmetic means for well-reservoir sites 6321, 23/9, and 6064. Data included for well 23/9 was collected from August 1979 to November 8, 1979.

[d] Arithmetic means for hydrant sites Rockinghorse and Harris. Due to incomplete data, the Marshall site was not included in this analysis. However, the Harris site is proximal to Marshall and therefore probably closely resembles it. Data included for the Harris site was collected from August 1979 to November 8, 1979.

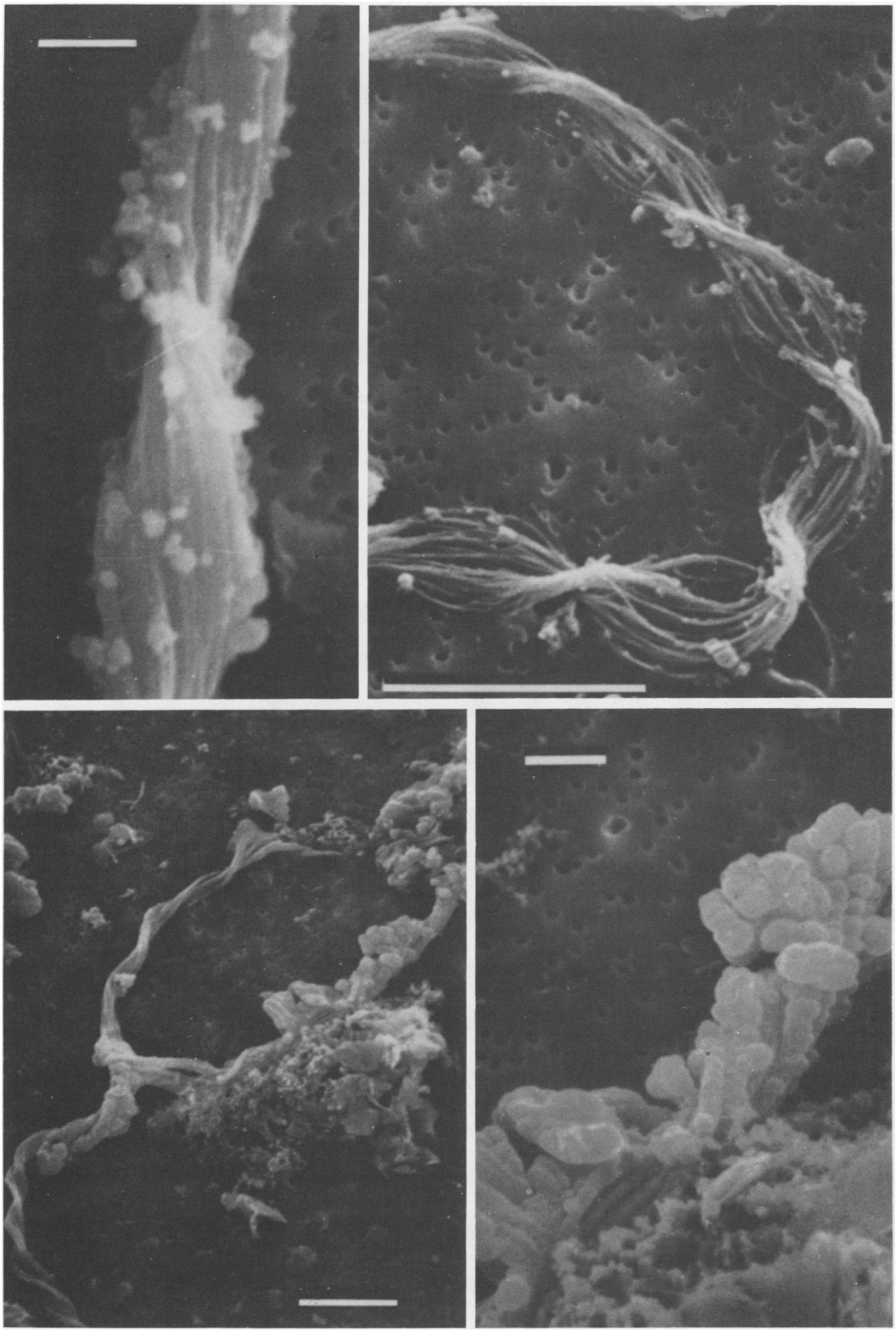

FIG. 2. *Selected views of Gallionella stalks recovered in water samples from the Marshall Lane site. Note individual microfibrils which comprise stalks (b). Bars, 0.5 μm (a); 5.0 μm (b); 10 μm (c); 5.0 μm (d).*

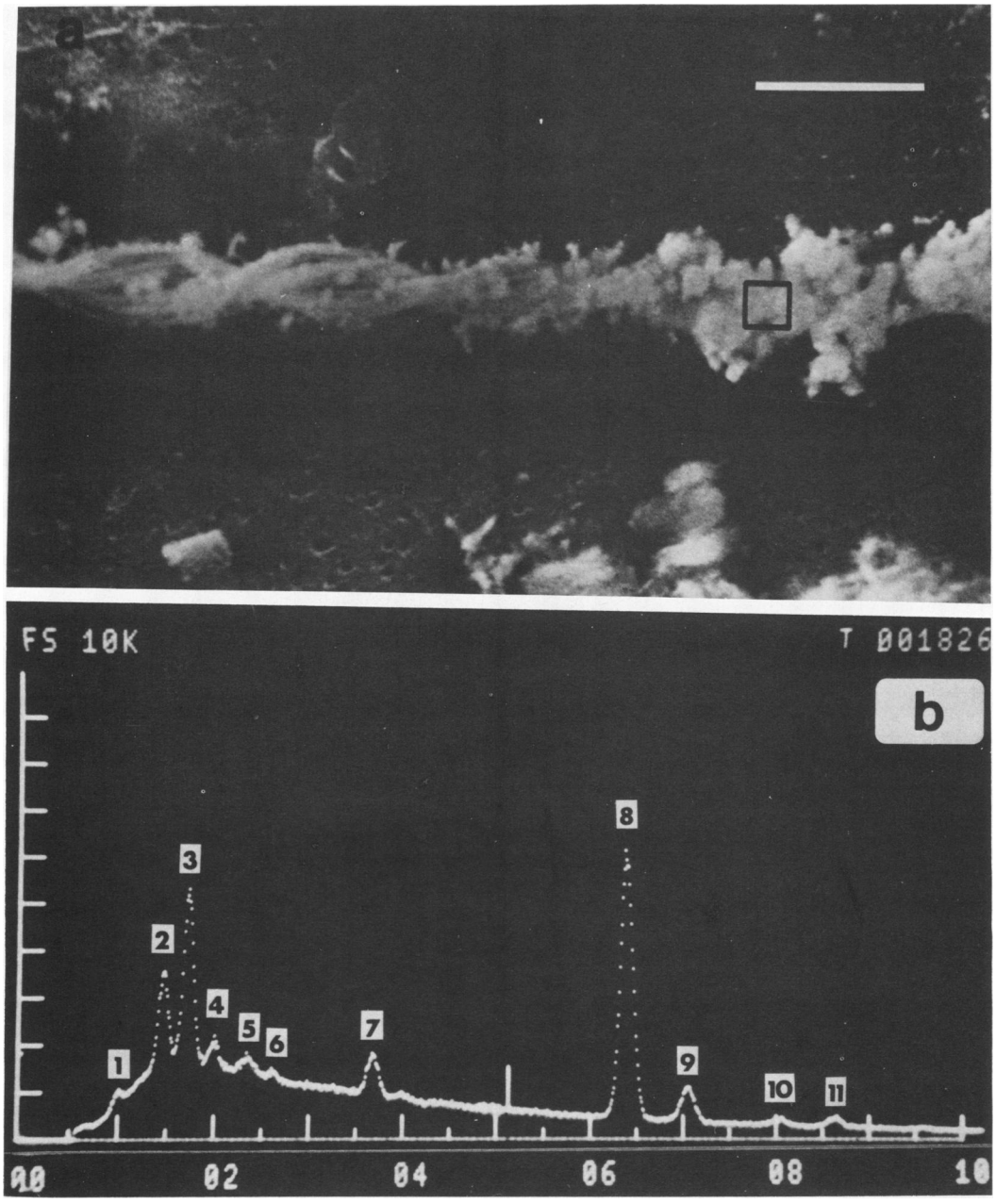

FIG. 3. *X-ray energy scan (b) of the region of the Gallionella stalk indicated in (a) (box). Peaks were identified as follows: peak 1, L_α line of zinc (1.001 keV); peak 2, K_α line of aluminum (1.49 keV); peak 3, K_α line of silicon (1.74 keV); peak 4, K_α line of phosphorous (2.01) keV); peak 5, K_α line of sulfur (2.31 keV); peak 6, K_α line of chlorine (2.62 keV); peak 7 K_α line of calcium (3.69 keV); peak 8, K_α line of iron (6.40 keV); peak 9, K_β line of iron (7.06 keV); peak 10, K_α line of copper (8.04 keV); peak 11, K_α line of zinc (8.64 keV). X-ray counts were integrated over a period of 1,826 s. Bar, 3.0 μm.*

encrusted with relatively large quantities of oxidized iron precipitates (Fig. 2c and d).

It was not feasible to perform X-ray energy-dispersive microanalysis on *Gallionella* cells attached to the pipe surface due to the complex microtopography of the sample, which can introduce significant electron scattering artifacts. Therefore, X-ray elemental analyses were per-

formed on selected *Gallionella* stalks isolated directly from water samples. In Fig. 3b is shown a continuous X-ray energy spectrum, from 0 to 10 keV, of the indicated portion of the filament shown in Fig. 3a. Four major peaks were observed corresponding to the K_α energy lines of aluminum (1.49 keV), silicon (1.74 keV), calcium (3.69 keV), and iron (6.40 keV). Manganese (K_α = 5.21 keV), though present in measurable quantities in the water supply (Table 2), was not detected in the stalks, thus confirming the apparent inability of this microbe to oxidize this element. Smaller peaks were identified as the L_α line of zinc (1.001 keV), the K_α lines of phosphorous (2.01 keV), sulfur (2.30 keV), chlorine (2.62 keV), copper (8.04 keV), and zinc (8.64 keV), and the K_β peak of iron (7.06 keV). As the major element present, the K_α peak of iron accounted for approximately 50% of the total X-ray counts collected in scans of *Gallionella* stalks (Table 3). None of the above elements were detected in significant quantities when an X-ray energy scan was performed on the naked membrane surface adjacent to a stalk.

In Fig. 4a is shown a high-magnification view of a portion of the *Gallionella* stalk shown in Fig. 3a, and in Fig. 4b an elemental map for iron is shown for the same section recorded between energy windows 6.25 and 6.55 keV (K_α for iron is 6.40 keV). The topological coincidence of the dots indicates the association of this element with the encrustations which envelope the stalk. A similar but lower-intensity map was obtained for silicon for the same section indicating a close association of these elements in the stalk.

X-ray energy scans were also performed at different locations along individual *Gallionella* filaments. The data for three such filaments are summarized in Table 3. There were no significant changes in the ratio of the elements aluminum, silicon, phosphorous, calcium, iron, and zinc between different locations along the stalk. Nor were any significant changes in the same elemental ratios noted between stalks. These data suggest that the metabolic capacity of different *Gallionella* filaments to precipitate oxidized iron compounds was relatively uniform and that the stoichiometry of mineral precipitation along a given filament was also uniform. Slight changes in the above elemental ratios might reflect possible chemical differences in the microhabitats which different *Gallionella* cells occupy in situ. Each of these microniches might be characterized by slightly different ionic concentrations of iron, silicon, and aluminum species. Conceivably, small changes in the in situ concentrations of specific ions might influence the overall stoichiometry of mineral oxidation and deposition on the cell surface.

This report is the first to describe the total elemental constitution of *Gallionella* stalks recovered directly from the environment without prior laboratory subculturing. Extracellular ferroaluminosilicate deposits coating the surfaces of *Gallionella* stalks have not been previously reported. Such complex mineral precipitates may not be formed on cells grown in relatively simple, chemically defined laboratory media. Only a few analytical studies have been done on the superficial encrustations of *Gallionella* cells grown in such media. Mardanyan and Balashova (17) investigated the state of iron on *G. ferruginea* stalks by using microdiffraction, X-ray diffraction, and nuclear gamma-resonance spectroscopy. They found that the oxidized iron (Fe^{3+}) was apparently actively and stably combined with the surfaces of the proteinaceous fibrils which comprise the stalk. Since no diffraction rings were ever observed, the Fe^{3+} detected was probably not organized into any kind of crystalline matrix, which is known to occur in simple $Fe(OH)_3$ deposits. They concluded, therefore, that the oxidized metal may be intimately associated with the protein fibrils in "a structure quite different from that of known compounds of oxidized iron." The precise chemical structure of the ferroaluminosilicate deposits described here is unclear. Nor is it known how closely the other elements, such as Si, Ca, and Zn, are associated with the protein compo-

TABLE 3. *X-ray microanalysis of selected elements in Gallionella stalks*

Element	Elemental ratio (% X-ray intensity) at following location of X-ray scan along stalk[a]		
	Left terminus[b]	Middle	Right terminus
Al	0.177 ± 0.039 (8.92 ± 1.97)	0.177 ± 0.001 (9.15 ± 0.825)	0.210 ± 0.094 (9.71 ± 3.95)
Si	0.474 ± 0.119 (23.6 ± 3.87)	0.440 ± 0.064 (22.6 ± 1.78)	0.449 ± 0.088 (22.0 ± 2.19)
P	0.099 ± 0.052 (4.86 ± 2.43)	0.103 ± 0.064 (5.15 ± 2.96)	0.101 ± 0.060 (4.91 ± 2.58)
Ca	0.112 ± 0.032 (5.58 ± 1.43)	0.097 ± 0.032 (4.92 ± 1.45)	0.125 ± 0.042 (6.13 ± 1.70)
Fe (K_α)	1.000 ± 0.0 (50.5 ± 4.77)	1.000 ± 0.0 (51.8 ± 4.12)	1.000 ± 0.0 (49.8 ± 5.73)
Fe (K_β)	0.1254 ± 0.0512 (6.302 ± 0.612)	0.121 ± 0.009 (6.24 ± 0.044)	0.129 ± 0.012 (6.48 ± 1.32)
Zn	0.013 ± 0.003 (0.680 ± 0.210)	0.012 ± 0.005 (0.599 ± 0.299)	0.020 ± 0.014 (0.902 ± 0.683)

[a] Arithmetic mean ± the standard deviation for three different stalks. All elemental ratios were obtained by normalizing the intensity of the element to the intensity of the K_α energy line of iron.

[b] Left and right ends of the stalks were arbitrarily selected.

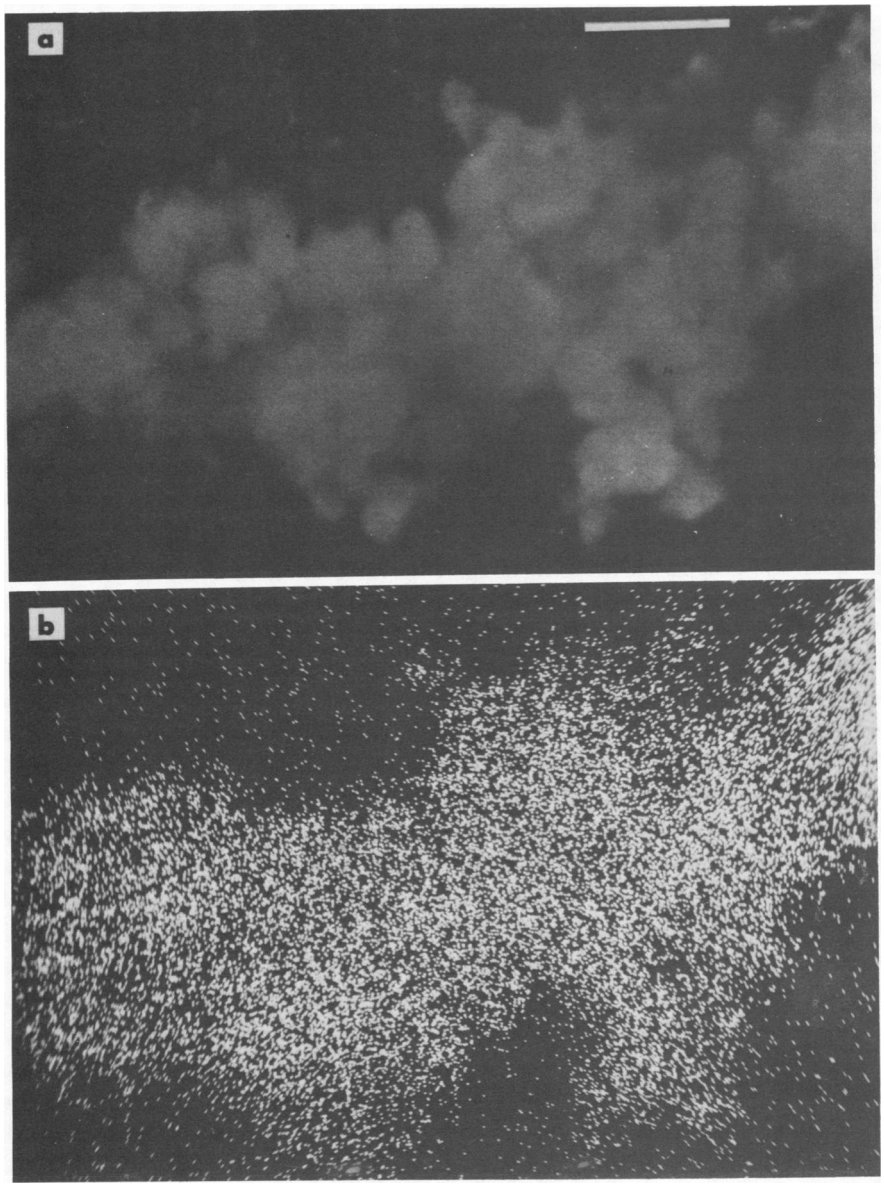

FIG. 4. *X-ray energy map for iron (b) for a portion of the Gallionella filament shown in (a). This is the same Gallionella stalk shown in Fig. 3a. X-ray counts were collected for 420 s between energy windows 6.25 and 6.55 keV (K_α energy line for iron 6.40 keV). Bar, 1.0 μm.*

nents of the stalks. Further analytical studies may ultimately help to elucidate the precise structure of these biologically mediated mineral complexes. Such information will be of value in understanding how these microorganisms chemically modify the pipe surface making it more susceptible to corrosion processes and fouling by other microorganisms.

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