Reprinted from ENVIRONMENTAL SCIENCE & TECHNOLOGY, Vol. 23, Page 51, January 1989

Transport of Microspheres and Indigenous Bacteria through a Sandy Aquifer: Results of Natural- and Forced-Gradient Tracer Experiments

Ronald W. Harvey* and Leah H. George

U.S. Geological Survey, Water Resources Division, Menlo Park, California 94025

Richard L. Smith

U.S. Geological Survey, Water Resources Division, Arvada, Colorado 80002

Denis R. LeBlanc

U.S. Geological Survey, Water Resources Division, Boston, Massachusetts 02114

Transport of indigenous bacteria through sandy aquifer sediments was investigated in forced- and natural-gradient tracer tests. A diverse population of bacteria was collected and concentrated from groundwater at the site, stained with a DNA-specific fluorochrome, and injected back into the aquifer. Included with the injectate were a conservative tracer (Br- or Cl-) and bacteria-sized (0.2-1.3-μm) microspheres having carboxylated, carbonyl, or neutral surfaces. Transport of stained bacteria and all types and size classes of microspheres was evident. In the natural gradient test, both surface characteristics and size of microspheres affected attenuation. Surface characteristics had the greatest effect upon retardation. Peak breakthrough of DAPI-stained bacteria (forced-gradient experiment) occurred well in advance of bromide at the more distal sampler. Transport behavior of bacteria was substantially different from that of carboxylated microspheres of comparable size.

Introduction

Transport of bacteria through groundwater has long been a concern to Public Health officials (1) and is becoming an increasingly important issue in waste management. In addition to the biological contamination of water supply wells, transport of bacteria may result in the "seeding" of aquifer sediments downgradient of contamination sources with bacteria acclimated to and capable of degrading refractory organic compounds. Degradation of highly mobile and persistent organic contaminants in aquifer sediments may be further enhanced by cotransport with free-living (unattached) bacteria. Therefore, transport of bacteria may have implications not only to placement of water supply wells but to practices of groundwater recharge using direct injection of waste water, to on-land disposal of organic wastes, and to a number of proposals for in situ biological treatment of organically contaminated aquifers. Models have been developed that describe transport of bacteria through porous media (2, 3), but experimental data are scarce.

We describe here a study on the transport of indigenous groundwater bacteria through sandy aquifer sediments. Many aspects of this problem are best investigated in the field, since sediment columns in the laboratory cannot duplicate actual structure of aquifer sediments (4). Findings from an earlier investigation suggested that transport of bacteria affected bacterial distribution within a plume of organically contaminated groundwater (5). The first objective of this study was to examine transport of bacteria under more controlled conditions. To accomplish this, morphologically diverse populations of bacteria were collected and concentrated from the contaminant plume, labeled with a DNA-specific fluorescent marker, reintroduced into aquifer sediments, and monitored as they moved downgradient. Several types and sizes of well characterized, bacteria-sized fluorescent microspheres were employed to simultaneously investigate potential effects of surface characteristics and cell size upon transport of bacteria. A second objective was to assess the suitability of the microspheres as analogues for bacteria in transport experiments.

Table I. Test Parameters and Conditions for Small-Scale Transport Experiments

parameters	forced gradient sampler A	forced gradient sampler B	natural gradient
Groundwater Conditions			
ambient flow, m/day	0.3–0.5		0.3–0.5
temperature, °C	10 ± 2		11 ± 2
conductivity, μS	60		345
dissolved oxygen, mg/L	9		<0.1
DOC, mg/L	<1		1–2
Injection			
volume, L	200		75
rate, L/min	95		0.5
duration, h	0.03		2.5
bromide, mg/L	1525		
chloride, mg/L			1425
Tracer Breakthrough[a]			
bromide, mg/L	187	34	
chloride, mg/L			100
C/C_0	0.123	0.022	0.065
time, days	0.04	0.27	21
av velocity, m/day	41	12	0.33

[a] At peak concentrations.

Methods

Injection Tests. Two types of small-scale, groundwater tracer experiments were employed to examine transport of bacteria and bacteria-sized microspheres through a sandy, freshwater aquifer on Cape Cod, MA. A divergent (forced-gradient) test was run in June 1986 at U.S. Geological Survey well site F393, and a natural-gradient test was run in October 1986 at well site F347. The aquifer sediments, which were deposited in layers as glacial outwash, contain little clay and are composed largely of quartz and feldspar. Mean grain size, average porosity, and hydraulic conductivity are -0.5 mm, 0.38, and -0.1 cm/s, respectively (6). Groundwater conditions and test parameters are listed in Table 1.

In the forced-gradient experiment, a radially divergent flow field was formed by continuous pumping of groundwater from a supply well (50 m downgradient) into an injection well, which was screened 10.0-11.2 m below land surface (BLS) (Figure IA) in an area of uncontaminated groundwater (7). Depth to water was -5 m. The injectate

0013-936X/89/0923-0051$01.50/0 9 1988 American Chemical Society

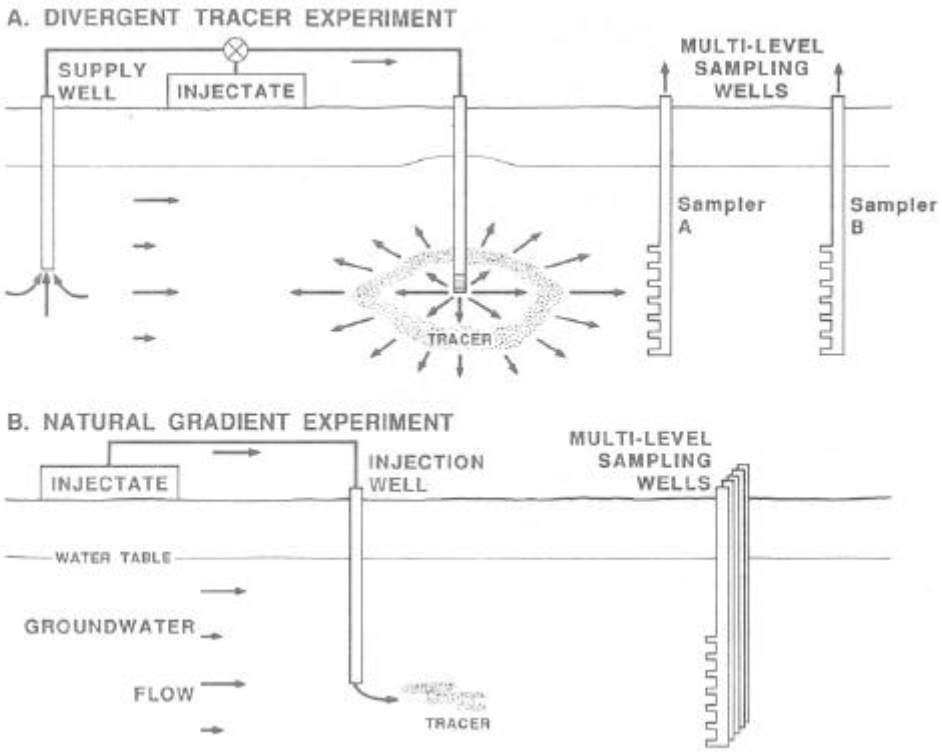

Figure 1. Schematic representation of the divergent (forced-gradient) (A) and natural-gradient (6) groundwater tracer tests.

was added as a short pulse to the injection stream. Stained groundwater bacteria, 0.2-, 0.7-, and 1.2-µm (diameter) fluorescent, carboxylated microspheres (Polysciences, Warwick, PA), and a conservative tracer (bromide) were monitored as they moved with the radial flow past multilevel sampling devices (MLSD) located 1.7 (sampler A) and 3.2 m (sampler B) from the injection well. The MLSD were constructed from 3.2-cm (diameter) poly(vinyl chloride) (PVC) pipe that encase a number of screened, 6.5-mm (diameter) polyethylene (PE) tubes. The PE tubes exit the PVC pipe into surrounding aquifer sediments along a vertical transect at -0.3-m intervals. Groundwater samples (500 mL from 11.3 in BLS) were taken every 5 min at sampler A and every 15 min at sampler B. The sampled layer was opposite the injection interval and was where bromide breakthrough was most significant in a previous test (7).

In the natural-gradient tracer experiment, fluorescent, bacteria-sized microspheres of differing types and diameters and chloride (used as a conservative tracer) were injected into the plume of contaminated groundwater (6) 500 m downgradient from a treated-sewage infiltration bed. Microspheres in the 0.23, 0.53, 0.91, and 1.35-µm (diameter) size class were composed of carboxylated latex. Microspheres in the 0.6-µm size class were plain latex and had uncharged surfaces. The 0.84-µm microspheres were polyacrolein and had carbonyl surface groups. The microspheres and chloride were added slowly to the aquifer at 8.5 and 9.1 in BLS and monitored as they moved with the natural groundwater flow past a row of MLSD (6.9 in downgradient) set perpendicular to the direction of groundwater (Figure 1B). Groundwater samples (500 mL) were collected at 1-day intervals from a sampling port 9.1 in BLS. Earlier tests with chloride (unpublished data) guided selection of the MLSD and sampling depth. During both natural- and forced-gradient tracer tests, measurements of conservative tracers at several ports ensured that samples were collected at appropriate times and depths to capture maximum breakthrough of microspheres and, in the forced-gradient test, bacteria.

Bacteria. A stainless steel submersible pump (Model SP81; Keck Geophysical Instruments, Inc., Okemos, MI) connected to Teflon tubing was used to collect groundwater from a screened, PVC observation well (5.0-cm diameter, 250-,um slot width) located 250 m (1.6 years groundwater travel time) downgradient from an on-land, treated-sewage infiltration bed. A morphologically diverse population of bacteria was concentrated on-site from 1000 L of collected groundwater into 1-2 L using a stacked sheet, tangential-flow filtration device (Pelicon model concentrator, Millipore Corp., Bedford, MA). Recovery of bacteria was somewhat low (18%), likely due to irreversible entrapment of bacteria on the filters. Recovered bacteria (largely rod-shaped, 0.2-1.6,uni long) were stained with the fluorochrome, 4,6-diamidino-2-phenylindole (DAPI; Sigma Chemical Co., No. D1388) at 5,uM (final concentration) for 3 h and added to 200 L of uncontaminated groundwater to dilute the stain below its threshold staining concentration. Stained bacteria were injected back into the aquifer after being held for 24 h at 4 *C. Samples collected downgradient were kept at 4 'C, and counts of DAPI-stained bacteria were made within 48 h.

Analyses. Bromide (forced-gradient tracer test) was measured in the field with a specific ion electrode and later in the laboratory by ion chromatography (Waters Model ILC-1 ion/liquid chromatograph; Waters ICP-A column with borate gluconate buffer at 1.2 mL/min and 25 °C. Chloride (natural-gradient tracer test) was measured with• specific ion electrode, which had been calibrated with a chlorimeter. Preparations for enumeration of DAPI-stained bacteria and fluorescent microspheres were made with 100-200 mL of sample in order to obtain good counting statistics. The DAPI-stained bacteria and carboxylated microspheres in these samples both fluoresced under incident UV and/or blue light and were differentially enumerated on black Nuclepore filters (0.2-µm pore size, 25-min diameter) with a Leitz Dialux 20 microscope, fitted for epifluorescence as described by Harvey et al. (8). DAPI-stained bacteria and 0.7-µm (diameter) carboxylated microspheres (forced-gradient test) and 0.91- and 0.5-µm

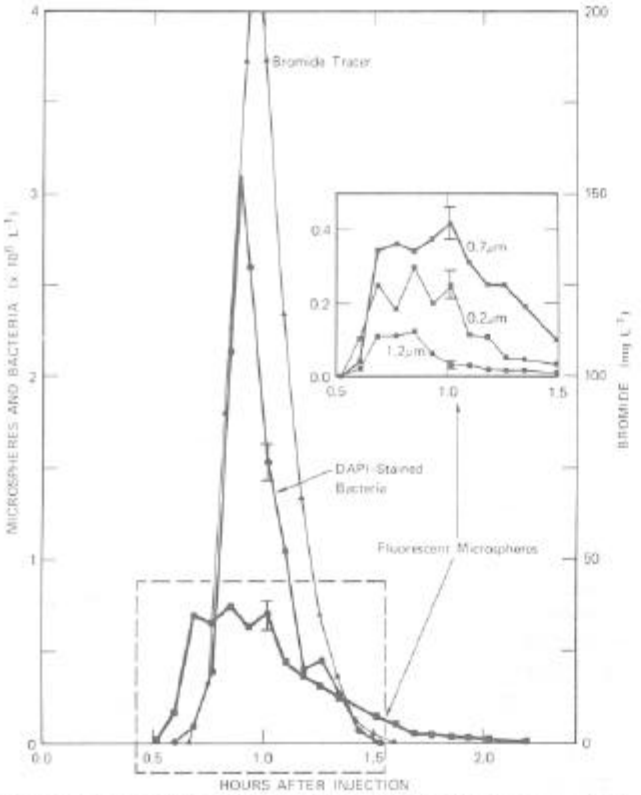

Figure 2. Concentration histories for bromide, DAPI-stained bacteria, and fluorescent microspheres (0.2, 0.7, and 1.2-μm diameter) at the closest sampler in the forced-gradient test.

(diameter) carboxylated microspheres (natural-gradient test) were counted under UV excitation (340-380 nm). Microspheres in other size classes were counted under incident blue light (390-490 nm).

Transport of bacteria and microspheres was evaluated from observed concentration histories (C vs time at sampling points downgradient) and from the following parameters: maximum dimensionless concentration, relative breakthrough, attenuation, and retardation. Maximum dimensionless concentration, $(C/Co)_{max}$ was calculated as the ratio of the highest concentration observed in samples collected downgradient to that present in the injectate. Relative breakthrough for bacteria and each class of microsphere was calculated as the integral of dimensionless concentration history normalized to that of the conservative tracer:

$$\% \text{ RB} = \left[\int_{t_0}^{t_f} \frac{C(t)}{C_0} \, dt \Big/ \int_{t_0}^{t_f} \frac{[\text{Tr}]_{lt}}{[\text{Tr}]_0} \, dt \right] \times 100$$

where Co and $[Tr]_o$ are microsphere and tracer (Cl- or Br-) concentrations in the injectate, $C(t)$ and $[Tr]_{lt}$ are concentrations at time t, and t_0 and t_f are elapsed times from injection to the beginning and end of breakthrough. The percentage of bacteria and microspheres that were immobilized during transport through aquifer sediments, referred to herein as attenuation, was calculated as 100 % RB. Retardation factors (RF) were calculated as ratios of time required to reach peak abundance for the microspheres or bacteria to time to peak concentration for the conservative tracer.

Results

Forced-Gradient Test. Concentration histories of carboxylated microspheres, DAPI-stained bacteria, and bromide for sampler A are shown in Figure 2. Concentration histories for DAPI-stained bacteria and bromide

Table II. Relative Breakthrough, Maximum Dimensionless Concentration, Retardation Factor, and Attenuation for Bacteria and Different Size Classes of Carboxylated Microspheres in the Forced-Gradient Test (Sampler A)

diam, μm	type	RB, %	$(C/C_0)_{max}$	RF[a]	atten, %
0.2	microsphere	0.01	2.7×10^{-6}	NC	99.9
0.7	microsphere	0.01	5.2×10^{-6}	NC	99.9
1.2	microsphere	0.01	7.8×10^{-6}	NC	99.9
0.2–1.6	bacteria	0.74	7.8×10^{-4}	~1.0	99.3
		2.61[b]	6.3×10^{-6}	0.8[b]	97.4

[a] NC, not calculated because time of peak breakthrough could not be determined accurately. [b] Sampler B.

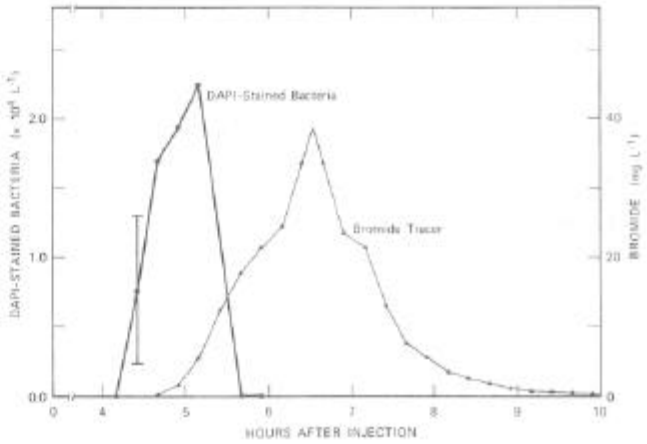

Figure 3. Concentration histories for bromide and DAPI-stained bacteria at the more distal sampler in the forced-gradient test.

were remarkably similar. Peak abundances of both bromide and stained bacteria occurred 0.9-1.0 h after injection. Also, both concentration histories exhibited single peaks and declines to undetectable levels -1.6 h after injection. However, the center of mass of bromide appeared to lag marginally behind that of the stained bacteria. The portion of total breakthrough occurring within the first hour following injection was 66% for DAPI-stained bacteria as compared to 55% for bromide.

Concentration histories for the microspheres differed substantially from those of bacteria and bromide. Carboxylated microspheres (0.2, 0.7, and 1.2 μm (diameter); Figure 2 inset] reached near-maximal abundance before bromide was detected and were present at least 30 min after bromide and stained bacteria had declined to undetectable levels. Microspheres appearing before and after detectable breakthrough of bacteria and bromide accounted for - 15 % of total breakthrough. Microspheres were attenuated by aquifer sediments to a greater degree than the bacteria (Table II). Values of $(C/Co)max$ were -100, 150, and 290-fold lower for the 1.2, 0.7, and 0.2-gm (diameter) microspheres, respectively, as compared with stained bacteria in spite of the overlap in size. Relative breakthrough of the microspheres was ~70-200-fold lower than for the bacteria.

Concentration histories of bacteria and bromide appearing at sampler B are depicted in Figure 3. Although numbers of DAPI-stained bacteria at this more distal sampler were near the lower level of detection, peak abundance of stained bacteria appeared to precede the maximum concentration of bromide by - 1-2 h. However, there was still substantial overlap in the concentration histories. Microspheres were detected in samples collected at sampler B, but were below levels required for quantification. Peak abundances of bromide and stained bacteria declined 5- and 113-fold, respectively, relative to break

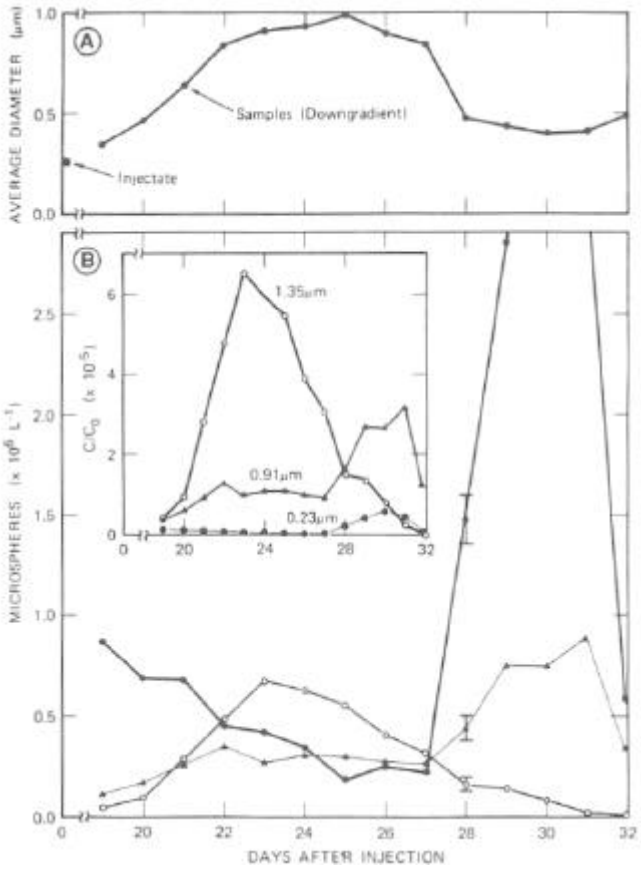

Figure 4. Average microsphere diameter (A) and concentration histories (13) for 0.23, 0.91, and 1.35-jum (diameter) carboxylated microspheres in the natural l-gradient test. Dimensionless concentration history is depicted in the inset.

Table III. Relative Breakthrough, Maximum Dimensionless Concentration, Retardation Factor, and Attenuation for Different Size Classes and Compositions of Microspheres in the Natural-Gradient Test

diam, μm	type	RB, %	$(C/C_0)_{max}$	RF	atten, %
0.23	carboxylated	0.01	5.9×10^{-6}	1.4	99.9
0.53	carboxylated	0.04	4.4×10^{-5}	1.4	99.9
0.91	carboxylated	0.06	2.7×10^{-5}	1.4	99.9
1.35	carboxylated	0.12	6.5×10^{-5}	1.1	99.8
0.6	uncharged	0.05	1.1×10^{-5}	~1.0	99.9
0.85	polyacrolein	3.11	2.3×10^{-3}	1.3	96.9

through at the closest sampler.

Natural-Gradient Test. Concentration histories for 0.23, 0.91, and 1.35-μm (diameter) carboxylated microspheres at 6.9 m downgradient from the injection well are shown in Figure 4B. Values of $(C/Co)_{max}$ and relative breakthrough for the carboxylated microspheres generally increased with sphere diameter and were, respectively, 11- 17-fold higher for the largest as compared with the smallest size class. However, attenuation in aquifer sediments between injection well and MLSD was >99.8% for all size classes. The increase in relative breakthrough with increasing microsphere diameter resulted in a large average microsphere size (up to 1.0-μm diameter) in samples collected downgradient as compared to the injectate population (0.3 μm (Figure 4A).

The effects of surface characteristics upon retardation and attenuation during transport for the 0.5-0.8 μm size classes of microspheres are depicted in Figure 5 and Table III. Peak abundance of uncharged latex microspheres came within 1 day of maximum chloride breakthrough. However, the maximum breakthrough of the polyacrolein

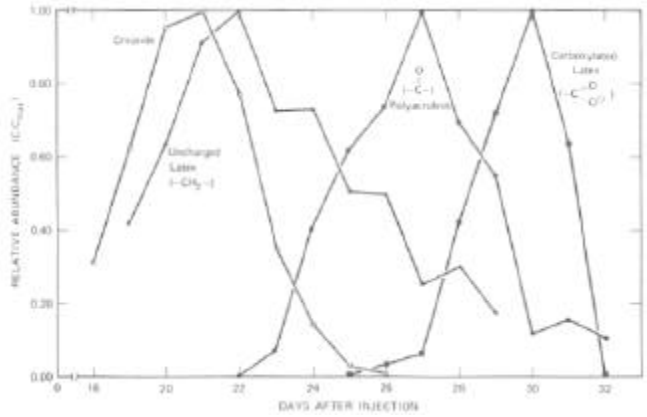

Figure 5. Concentration histories for chloride, uncharged latex (0.53-gm-diameter), polyacrolein (0.84-Am-diameter), and carboxylated latex (0.53-pm-diameter) microspheres in the natural-gradient test. Data have been normalized to maximum concentrations.

(carbonyl surface groups) and carboxylated latex microspheres was significantly retarded; peak abundances of the carboxylated latex and polyacrolein microspheres were retarded by 8 and 5 days, respectively, relative to the uncharged latex spheres. Little overlap in breakthrough of uncharged and carboxylated microspheres was observed. Retardation (relative to chloride) of peak breakthrough for the carboxylated microspheres was 9-10 days, with the exception of the 1.35-Am (diameter) spheres (2 days retardation). The difference in retardation between types of microspheres does not appear to result from size differences, because peak breakthrough of 0.23-, 0.53-, and 0.91-gm-diameter carboxylated microspheres occurred at approximately the same time.

Values of relative breakthrough, attenuation, and $(C/CO)_{max}$ also varied among types of microspheres in the 0.5-0.8-Am size classes (Table III). Relative breakthroughs for carboxylated and uncharged latex microspheres were similar (0.04 vs 0.05, respectively), but were ~60-80-fold lower than that for the polyacrolein microspheres. Attenuation of uncharged and carboxylated microspheres was >99%, but ~97% for the polyacrolein microspheres.

Discussion

Bacteria. The appearance of DAPI-stained bacteria at sampler B in the forced-gradient experiment (Figure 3), in spite of the high degree of forced dispersion resulting from radially divergent flow, suggests that transport of bacterial populations through porous aquifers is possible. Earlier breakthrough of the DAPI-stained bacteria relative to bromide further suggests that this transport can be relatively rapid and that there may be some sort of chromatographic effect. Size exclusion chromatography involving preferential exclusion of bacteria from smaller, more tortuous pores between sediment particles would result in a more direct average path of travel for the unattenuated (nonadsorbed and unfiltered) bacteria. Hydrodynamic chromatography may also play a role, since bromide, on the basis of size, would be subjected to the effects of particle surface roughness to a greater degree than the stained bacteria.

Bacterial motility was not a factor in the transport and early breakthrough of DAPI-stained bacteria. This is because the short duration (10 h) of the forced-gradient test would not have allowed for chemotaxis to account for the differences in breakthrough relative to bromide. Also, bacterial motility would be expected to be random in the absence of chemical gradients. However, it has been demonstrated that in the presence of nutrient gradients

some bacteria can move by chemotaxis fairly quickly (up to 0.5 cm h-') through porous rock of low permeability (9). Therefore, motility may be an important process in long-term transport of bacteria in highly contaminated porous aquifers. Our results here indicate that even in the absence of strong chemical gradients unattenuated bacteria may be transported more quickly than a conservative tracer, simply on the basis of size.

Faster transport relative to a chemical tracer has also been observed in several tracer experiments involving microorganisms not indigenous to aquifers. For example, hydrogen sulfide producing strains of *Escherichia coli* were found to move through a New Zealand aquifer more quickly than rhodamine WT dye (10), and it has recently been observed that nonadsorbed viruses appear to travel through aquifer sediments about 1.5-1.9 times faster than halide tracers (C. Gerba, personal communication). Peak breakthrough of 2-3-,um-diameter yeast cells, *Saccharomyces cerevisiae,* injected into a sand and gravel aquifer in Florida, occurred before that of bromide and iodide (11), and it was believed that many of the yeast cells traveled through channels in the sand and gravel aquifer rather than through intergranular pores. The potential importance of secondary pore structure in the enhancement of microorganism transport through the subsurface can be inferred from results of column experiments, where up to 100-fold greater recovery of *E. coli* traveling through intact soil columns as compared with columns of repacked soil were observed (12).

The degree to which indigenous bacterial populations are transported through contaminated aquifers may be substantially greater than for nonindigenous populations. This is because growth of native bacteria during transport would, in part, compensate for their removal due to sorption/ biological adhesion to particle surfaces, filtration (straining), predation by protozoa, and lysis. The effect of growth rate upon transport was not assessed in these experiments, since DAPI intercalates among the nucleic acid bases within the bacteria and thus impairs cell metabolism. However, measured in situ bacterial growth rates in the 5-km-long contaminant plume where the natural gradient experiment was run range from 0.005 ± 0.002 h^{-1} in the more distal portion of the plume to 0.042 ± 0.005 h^{-1} near the source of contamination (13). At the higher growth rate, average generation time would be less than 17 h and would more than offset expected rates of attenuation occurring during transport. DAPI-stained bacteria were not employed in the natural-gradient experiment, since the stability of intracellular DAPI over the duration of the experiment was not known. However, if attenuation of bacteria relative to 1-ttm-diameter carboxylated microspheres were the same in the natural-gradient experiment as in the forced-gradient experiment, total attenuation at 6.9 m downgradient in the former experiment would account for losses of -90% of the bacteria injected. This estimate assumes no growth of the stained bacteria. Since this rate of attenuation would preclude a nongrowing bacterial population from being transported more than 100 in through aquifer sediments at Cape Cod, abundance of readily degradable dissolved organic carbon (DOC), which controls growth, may be one of the most important determinants of transport of the indigenous populations.

Abundance of DOC in highly contaminated aquifers may also affect bacterial size and propensity for solid surface attachment. Several studies suggest that, under severe nutrient limitation, a number of bacteria exhibit decreases in cell size and an increased adhesion to solid surfaces, where organic matter is more abundant (14-16). Therefore, increased levels of utilizable organic matter should be accompanied by enhanced transport of bacteria in aquifer sediments. This is because larger, less surface active bacteria would have a lesser tendency to contact and sorb onto particles. Transport of indigenous bacterial populations would appear to be greatest in heavily contaminated aquifers where growth rates are relatively high and attenuation in aquifer sediments relatively low.

Microspheres. The lower relative breakthrough and greater dispersion of the 0.2-1.2-μm (diameter) carboxylated microspheres relative to the fluorochrome-labeled bacteria of similar size in the forced-gradient injection experiment (Figure 2, Table 11) suggest greater interaction between the microspheres and aquifer sediment particles. Interactions with sediment particles may also explain retardation in peak breakthrough, relative to chloride, for neutral latex and polyacrolein microspheres in the natural-gradient experiment (Table III). The greater dispersion of carboxylated microspheres relative to bacteria suggests carboxylated microspheres behave quite differently during transport through the aquifer than bacteria. However, since these microspheres are stable over time and for a given size class have a fairly tight size distribution and uniform and well-characterized surface characteristics, they can be used to obtain information involving abiotic transport processes.

The inverse relationship between size of the carboxylated microspheres and attenuation within permeable aquifer sediments in both natural and forced-gradient experiments can be predicted from colloid filtration theory (17). This model predicts that smaller microspheres within the bacterial size range should contact and therefore sorb to stationary surfaces in porous media with greater frequency because of their higher rates of diffusion or Brownian motion. Although there are a number of uncertainties in applying this model developed for wastewater-filter applications to bacteria removal in porous aquifers, there does appear to be an optimal size range for transport in the micron class. Our most recent natural gradient tracer experiments (unpublished data) with neutral latex microspheres in the 1-, 3-, and 6-μm size classes suggest that optimal size for transport at our site may be as much as several microns. This has important implications for transport through aquifer sediments of at least the smaller protozoa (18) that prey upon groundwater bacteria.

Within the bacterial size range, microsphere size (0.2-1.4 μm) did not appear to be the primary determinant of retardation in these experiments. The only substantial difference in retardation among size classes of carboxylated microspheres occurred in the natural-gradient experiment (Figure 4) where peak breakthrough of 1.35-μm microspheres occurred first. However, there is evidence to suggest more rapid transport of larger microorganisms through aquifer sediment than smaller microorganisms. For example, it has been reported that E. coli injected simultaneously with coliphage f2 into an aquifer in a forced-gradient tracer test broke through at an observation well 150 in downgradient well in advance of the smaller virus (19), and a capsulated strain of *Klebsiella aerogenes* was observed to be transported through the aquifer more rapidly than a smaller noncapsulated strain (20). However, it cannot be assumed that differences in retardation of microbial transport through aquifer sediments can be explained totally on the basis of cell size, since the influence of surface characteristics was not known.

Surface characteristics of microspheres had a marked effect upon retardation during transport in the natural

gradient experiment (Table III, Figure 5). The relationship between microspheres' composition and retardation is complex, since microspheres can interact with both dissolved organic material (DOM) and aquifer sediment particles. That carboxylated latex microspheres were retarded the most and neutral latex microspheres the least may be attributed to differences in surface charge; carboxylated latex spheres have a stronger net negative surface charge at groundwater pH (6-7) and, consequently, should be influenced to a greater degree by surface charges (both positive and negative) on sediment particles. The neutral latex microspheres are likely to acquire a slight surface charge, due to adsorption of DOM. However, levels of DOC in groundwater at the test site are low (Table 1). Surfaces of neutral spheres would be expected to be less reactive than those of the moderately retarded (RF = 1.3) polyacrolein spheres, which have carbonyl surface groups.

In general, there appeared to be no clear relationship between attenuation (immobilization within aquifer sediments) and retardation (slowing down of transport). In some cases, microspheres that were transported more slowly were subject to less attenuation (Table III). In the forced-gradient injection experiment with E. coli and f2 coliphage described by Gerba and Bitton (19), attenuation was substantially greater for the bacterium than for the virus even though the virus took longer to arrive at the sampling well downgradient. The reasons for this are unclear and the in situ decay rates for the two nonendemic microorganisms were likely quite different. However, it is clear that increased retardation does not necessarily lead to a greater removal of microorganisms. Although increased contact with aquifer sediments occurring as a result of increased retardation should allow for greater opportunities to sorb onto solid surfaces, our results with bacteria-sized microspheres suggest that attenuation and retardation can vary independently. It also appears that bacterial surface characteristics may play a bigger role in retardation in some highly porous aquifer sediments than the size of the microbe.

In summary, transport of bacteria through organically contaminated aquifers appears to be possible. Bacterial growth rate would appear to be an important factor in determining how far indigenous bacteria may be transported. At the higher in situ growth rates (0.03-0.04 h-1) reported for the contaminant plume at Cape Cod (13), some bacteria may be transported over 1000 in. None of the fluorescent, bacteria-sized microspheres that we tested appeared to be useful as tracers of bacteria in groundwater injection experiments. Although they are easy to detect, and reasonably well-defined, their transport behavior differed substantially from that of bacteria. Also, they do not account for bacterial growth, which can be substantial in highly contaminated aquifers. We are presently modifying the surfaces of selected microspheres so that the degrees of retardation, dispersion, and attenuation that occur during their transport through porous media will be closer to those observed for bacteria. The effects of nutrient and geohydrologic conditions upon transport of indigenous bacteria and how this transport affects degradation of organic compounds in contaminated aquifers have not been well studied and are subjects worthy of further study.

Acknowledgments

We thank C. Quadri, K . Hess, S. Garabedian, J. Duff, and M. Ceazan (U.S. Geological Survey) and B. Howes (Woods Hole Oceanographic Institute) for their assistance with the tracer tests and J. Rubin and T. Rees for their helpful comments on the manuscript. Discussion with P. Roberts (Stanford U.) and C. O'Melia (Johns Hopkins U.) concerning filtration theory is also gratefully acknowledged.

Literature Cited

(1) Keswick, B. H. In *Groundwater Pollution Microbiology*; Bitton, G., Gerba, C. P., Eds.; Wiley: New York, 1984; pp 59–64.
(2) Yavuz Corapcioglu, Y.; Haridas, A. *J. Hydrol. (Amsterdam)* **1984**, *72*, 149.
(3) Yavuz Corapcioglu, Y.; Haridas, A. *Adv. Water Resour.* **1985**, *8*, 188.
(4) McDowell-Boyer, L. M.; Hunt, J. R.; Sitar, N. *Water Resour. Res.* **1986**, *22*, 1901.
(5) Harvey, R. W.; George, L. H.; Smith, R. L.; LeBlanc, D. R.; Garabedian, S. P.; Howes, B. L. In *Open-File Report 87-109*; U.S. Geological Survey: Reston, VA, 1987; pp B29–31.
(6) LeBlanc, D. R. In *Open-File Report 84-475*; U.S. Geological Survey: Reston, VA, 1984; pp 1–46.
(7) Garabedian, S. Ph.D. Dissertation, Massachusetts Institute of Technology, Cambridge, MA, 1987.
(8) Harvey, R. W.; Smith, R. L.; George, L. *Appl. Environ. Microbiol.* **1984**, *48*, 1197.
(9) Jenneman, G. E.; McInerney, M. J.; Knapp, R. M. *Appl. Environ. Microbiol.* **1985**, *50*, 383.
(10) Pyle, B. H. *Lincoln College Department of Agricultural Microbiology Tech. Publ. No. 2*; Canterbury, New Zealand, 1979.
(11) Wood, W. W.; Ehrlich, G. G. *Ground Water* **1978**, *16*, 398.
(12) Smith, M. S.; Thomas, G. W.; Ritonga; D. *J. Environ. Qual.* **1985**, *14*, 87.
(13) Harvey, R. W.; George, L. H. *Appl. Environ. Microbiol.* **1987**, *53*, 2992.
(14) Fletcher, M.; Marshall, K. C. *Adv. Microb. Ecol.* **1982**, *6*, 199.
(15) Dawson, M. P.; Humphrey, B. A.; Marshall, K. C. *Curr. Microbiol.* **1981**, *6*, 195.
(16) Kjelleberg, S.; Humphrey, B. A.; Marshall, K. C. *Appl. Environ. Microbiol.* **1982**, *43*, 1166.
(17) Yao, K. M.; Habibian, M. T.; O'Melia, C. R. *Environ. Sci. Technol.* **1971**, *5*, 1105.
(18) Sinclair, J. T.; Ghiorse, W. C. *Appl. Environ. Microbiol.* **1987**, *53*, 1157.
(19) Gerba, C. P.; Bitton, G. In *Groundwater Pollution Microbiology*; Bitton, G., Gerba, C. P., Eds.; Wiley: New York, 1984, pp 66–88.
(20) Bitton, G.; Lahav, N.; Henis, Y. *Plant Soil* **1974**, *40*, 373.

Received for review December 29,1987. Accepted June 28,1988.