Experimental Ground Water Pollution at Anchorage, Alaska

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UNDERGROUND WATER pollution is of prime importance to public health officials concerned with safeguarding the potability of water supplies.

A study by Stiles and associates (*1*) has been considered a classic in this field. An extensive inquiry into ground water pollution from experimental latrines was made by Caldwell and Parr (*2*). And more recently the sanitary engineering research laboratory of the University of California (*3, 4*) has investigated several aspects relating to the conservation of ground water by the reclamation of sewage effluents through direct recharge into the ground water.

In studying the lateral movement of simulated bacterial and chemical pollutants through ground water in an Alaskan area, our first task was to find suitable indicators which would persist during a winter in a subarctic climate. These indicators were to be traced at least 1 year to determine the limits of duration or survival.

The experimental site was a small plot of land about 2 miles south of Anchorage. The ground water table was shallow, with the top between 5 and 6 feet below the surface of the ground. Mechanical analysis of the soil at the ground water level indicated it had a sandy-gravel texture, representative of a considerable portion of the Greater Anchorage area (*5*).

The test agents, the dye uranin and bacteria, were introduced directly into the ground water.

The two bacterial types most commonly used for ground water pollution studies are coliform organisms and *Serratia marcescens*. But since some coliform organisms had been found sporadically in the soil of the test site, using these organisms as indicators, we felt, would cause confusion. We therefore tried, in separate tests, three chromogens in combination and an enterococcus.

In addition to the dye and bacterial tests were cultural and biochemical studies of organisms taken from the sampling wells and the dialysis of test organisms suspended in the ground water.

Tests With Dye

The dye of choice for ground water pollution studies is uranin, the sodium salt of fluorescein.

A dosing well was dug, and a galvanized pipe 10 inches in diameter was sunk into the ground water. A ring of 9 sampling wells was placed around the dosing well at a distance of approximately 3 feet. Eight- to ten-foot lengths of ½-inch pipe, with 12-inch wellpoints on one end and T-connections threaded onto the other, were sunk as sampling wells. The T-connections had solid metal plugs on top to facilitate pounding

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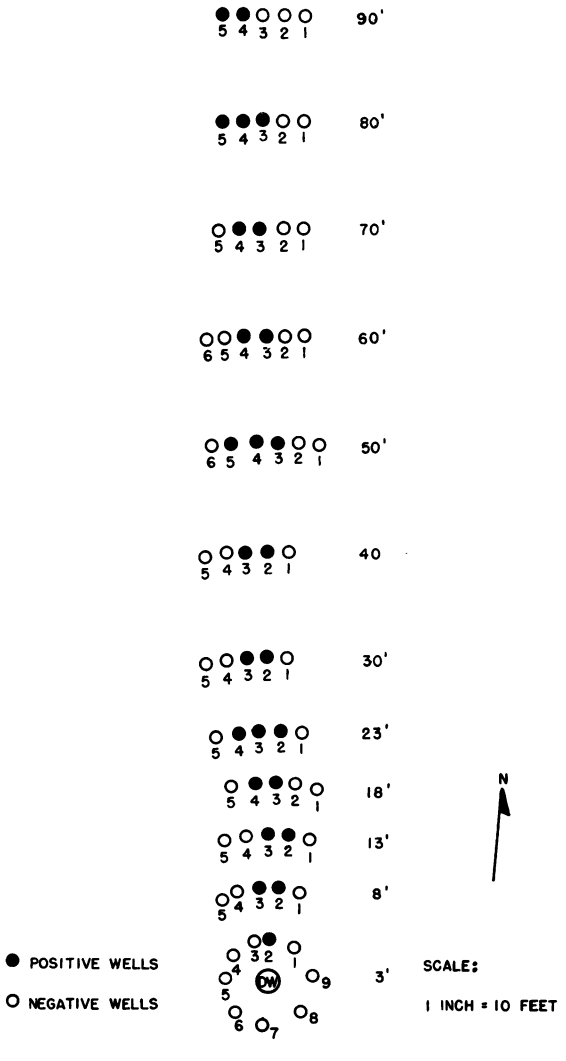
the pipe into the ground and ¾-inch openings on the side for connecting a suction sampling device.

The well was dosed once with 1 liter of a 10 percent concentration of uranin to determine how long this specific amount of the dye could be detected. The method of dye concentration for ultraviolet light examination recommended by the bureau of environmental sanitation of the New York State Health Department (*6*) was used.

On the second day after dosing, well No. 2 in the ring (fig. 1) became positive. Five or six sampling wells were then placed in arcs at 5- to 10-foot intervals in the direction of dye movement as wells became positive for the dye.

Figure 1. Original dye pattern, October and November 1951.



There appeared to be a relatively narrow channel of dye flow, between 1½ and 4 feet wide, with well-defined negative borders. This channel was apparent for approximately 2 to 5 weeks. The rate of movement of the dye through the ground water was about 2 feet per day.

Additional positive wells, west of the original direction of flow, were found 65 days after the original dosing. This indicates a primary and a secondary flow pattern. About 9 months after the dosing, a further shift of the dye westward was found in some of the wells. Three wells on the right border of the dye pattern had become negative.

About 2 years after the original dosing, the pattern of flow was wedge-shaped, extending from the apex at the dosing well in a northwest direction to about 40 feet in width 90 feet away. The outline of the wedge was marked by negative wells. Two years and ten months after the original dosing, dye was still evident in 38 of 48 wells examined. The dye pattern indicating all wells that were positive is shown in figure 2.

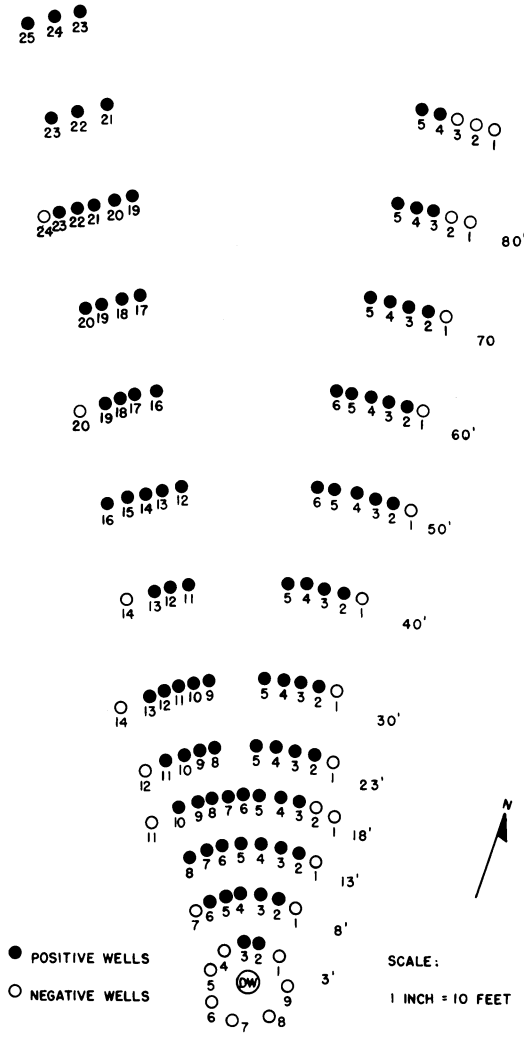
Tests With Chromogenic Bacteria

Prior to initiating the bacteriological field work, samples of the soil and ground water were examined in the laboratory for bacterial chromogens, and the effects of varying concentrations of uranin on the test organisms were compared. The first study showed that chromogens were present sporadically and in small numbers. We believed, therefore, that a group of selected bacterial chromogens together would be feasible as tracer organisms. In the second study, we found that uranin had no apparent inhibitory effect on the test organisms in concentrations that might be expected in the ground water.

The organisms used were *Serratia marcescens* (ATCC 6889), *Chromobacterium violaceum* (ATCC 553), and two strains of *Bacillus globigii* (ATCC 9372 and FDM). Cultures were obtained from the American Type Culture Collection, Washington, D. C., and the United States Army Biological Department at Fort Detrick, Md.

The dosing schedule for the bacterial chromogens was begun at the same time as the dye dosing. Nineteen 1-liter doses of 24- to 48-hour

Figure 2. Total dye pattern, October 1951 to August 1954.



nutrient broth cultures of each of the 4 chromogens were placed in the ground water over a period of 35 days.

The water was examined for 9 weeks. It was not possible to trace the movement of these organisms. Bacteriologically, there did not appear to be any noticeable difference from the predosing examinations. A complicating factor appeared to be the large numbers of natural soil bacteria found consistently in the ground water.

It was apparent at this point that we needed some definite criteria for the selection of test organisms. These criteria were: (*a*) the test organism should have some specific and permanent distinguishable property, cultural or biochemical, or both; (*b*) a laboratory test to distinguish this organism from soil bacteria should be available; and (*c*) the test organism should be capable of extended survival in the ground water.

Test With an Enterococcus

Certain enterococci appeared to meet the above requirements. Accordingly, a beta hemolytic, gelatin-liquefying strain, *Streptococcus zymogenes* (ATCC 4533), was obtained. (Although the culture is listed as *Streptococcus liquefaciens* in the ATCC catalog, it is designated here as *S. zymogenes* because it was beta hemolytic.) For the cultural and biochemical properties of the test organisms employed in this study, see Bergey's Manual (*7*).

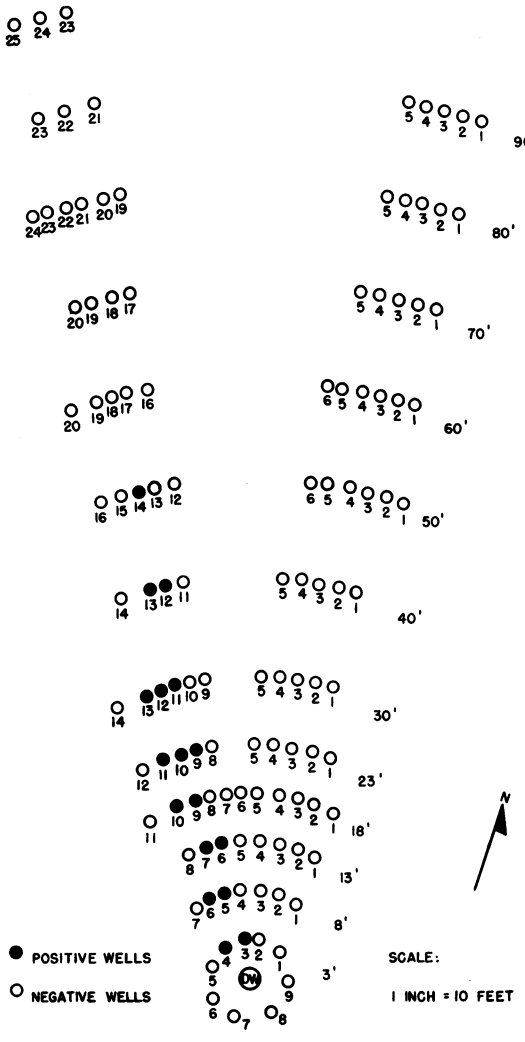
Prior to the use of this organism, 78 samples taken from the dosing well and from 26 of the experimental test wells were examined for enterococci. Four samples were positive for nonhemolytic enterococci only.

Ten liters of 48-hour nutrient broth cultures of 2 strains of the enterococcus test organism were introduced into the ground water over a period of 11 days. A limited number of test organisms were used to see how far such a number could travel and how long they could survive.

Either 1-liter or 1-gallon samples were taken over a period of 371 days. Bacterial population was estimated by planting serial quantities of 100 ml. and less, in tenfold dilution steps, into sodium azide glucose broth (*8*). The remaining portion of the liter or gallon was filtered through a membrane filter (*9*), and the filter pad was placed in the azide glucose medium. After incubation at 45° C., positive results, as indicated by growth in the liquid medium, were confirmed by streaking a loopful of the material onto blood agar plates. The need for confirmation was pointed out by Ostrolenk and Hunter (*10*), who showed that false positive as well as false negative reactions occur in this medium. Microscopic examinations were made of gram-stained smears taken from the blood agar plates. Representative cultures were picked for cultural and biochemical studies.

On the third day following the initial dosing,

Figure 3. Path of test organisms, August 1953 to August 1954.



the test organism was found in the 3-foot ring of wells, and on the seventh day, in the 8-foot arc. Although this indicated a rate of movement of about 1 foot a day, it held only at those two points. The rate of flow of the test organism was irregular, averaging about 0.48 foot per day. The organism was traced to a distance of 50 feet where it was detected on the 70th day after dosing.

The test organism described a definite path of flow with sharp and clear-cut boundaries (fig. 3). The width of the path ranged between 1½ and 4 feet.

Although the organism followed a definite path, its direction did not coincide with the path of the initial dye flow observed 2 years earlier since there had been a 26° change in the direction of the primary flow of the ground water. The path of the organism was still within the limits of the large wedge-shaped pattern formed by the dispersion of the dye. The reason for the change in direction of the ground water is not definitely known. However, there had been considerable housing construction in the area since the original dosing of the well with the dye, and the increased use of water from shallow wells may have contributed to the change in direction.

Large samples must be taken to show the test organism initially and at final sampling (see table). The bacteria gradually increased to a maximum number in most of the positive wells and then gradually decreased. This was most apparent in the wells nearest the dosing point.

Wells and samples positive for test organism

Wells [1] positive for test organism	Smallest amount of sample, in milliliters, showing test organism [2]		
	Initially	At highest concentration of organism	At final positive sampling
Dosing well	----------	0. 000001	10
3–3	1, 000	0. 0001	FP [3]
3–4	0. 01	0. 01	1, 000
8–5	10	0. 00001	100
8–6	1	0. 1	FP [3]
13–6	0. 1	0. 01	10
13–7	1	1	FP [3]
18–9	100	1	1, 000
18–10	10	10	FP [3]
23–9	1, 000	10	10
23–10	1, 000	10	100
23–11	1, 000	1, 000	1, 000
30–11	10	0. 01	1, 000
30–12	1	1	FP [3]
30–13	1, 000	10	FP [3]
40–12	10	1	100
40–13	FP [3]	10	100
50–14	10	1	100

[1] Wells are designated by position in feet and well No.

[2] Larger portions in the tenfold stepwise inoculation scheme were, in most cases, also positive while smaller portions were negative.

[3] 1-liter or 1-gallon sample filtered through sterile filter paper (FP) which was placed in azide glucose medium for incubation. This test gave the only positive result.

As progress was made away from the dosing well, fewer organisms were detected. The tabulation reveals that of the positive wells in each arc, one usually had a higher concentration of the test organism. This concentration ranged from a single tenfold dilution step to 4 tenfold steps.

Ninety-five days after dosing, test organisms were detected in all positive wells except 18–10. The latter became positive during the winter when sampling was incomplete because of frozen pipes and a lowered water table. The first positive sample from this well was taken on March 8, 1954. Regression of test organisms was first noted in May, about 280 days after dosing. After 295 days, the organism was not recovered beyond 30 feet; after 316 days, not beyond 13 feet; and after 327 days, not beyond 8 feet. At the end of the study period (371 days), the only well positive for the test organism was the dosing well. The complete regression is attributed mainly to bacterial die-off and to the effect of dilution. Although Caldwell and Parr (*2*) considered clogging of the filtration bed an important factor in bacterial regression, we do not believe it was a primary factor in this study since a limited amount of culture material was added to the ground water.

Cultural and Biochemical Studies

Further studies were made of 282 enterococcus cultures picked from blood agar plates. On the basis of hemolysis and gelatin liquefaction, these cultures were differentiated as follows:

Number of cultures	*Beta hemolysis*	*Gelatin liquefaction*	*Identity*
201	+	+	*S. zymogenes*
80	−	−	*S. faecalis*
1	−	+	*S. liquefaciens*

Fermentation studies detected acid production in sucrose, mannitol, sorbitol, glycerol, arabinose, and raffinose. The fermentation reactions of the *S. zymogenes* cultures were very constant. Acid was produced in 5 of the 6 sugars, the exception being raffinose. Only 1 of the 201 cultures tested varied by fermenting all 6 sugars.

The fermentation reactions of the *Streptococcus faecalis* cultures were variable and, along with the one *S. liquefaciens* culture, differed from those of the *S. zymogenes* cultures.

Dialyzing Culture Studies

One of the factors affecting the outcome of an experimental pollution study is whether or not the test organism can survive the environmental conditions to which it is exposed. Coincidental with the tracing of the movement of the test organism through the ground water, we studied the rate and extent of die-off of 5 strains of bacteria used in two phases of the experiment. The organisms were *S. marcescens*, 2 strains of *B. globigii*, and 2 strains of *S. zymogenes*.

Nutrient broth cultures of the organisms were placed in separate dialyzing membranous sacs, made from cellophane tubing, which were then suspended in the dosing well. Exposure times varied from 24 hours to 1 year. The semipermeable sacs retained the bacteria while permitting penetration and passage of substances in solution in the ground water into and through the membranous walls, thus simulating closely the conditions encountered by the test organisms in their travel through the ground water.

Although the rate and extent of bacterial die-off were quite pronounced for all five strains, an appreciable number of organisms survived after 1 year's exposure in the ground water. *S. marcescens* was reduced in numbers approximately 92 percent by the end of 28 days, but the surviving organisms were still well pigmented. At the end of 2 months, the decrease in numbers was 98.9 percent with some loss of pigment. After 4 months, there was no apparent further change in count, but there was a complete loss of pigmentation. Along with loss of pigment, biochemical changes, notably, a loss of fermenting powers, were also noted. The two strains of *B. globigii* showed similar losses in numbers and pigment-producing powers at the end of 28 days, and at the end of 4 months these strains were not recognizable.

The *S. zymogenes* cultures showed a progressive decrease in numbers from initial populations of 146 and 158 millions per milliliter for the two strains used to 5,800 and 4,900 per milliliter, respectively, after 1 year. Cultural and biochemical studies of isolated organisms

showed that the surviving organisms retained their original distinctive properties.

Discussion

The present study concerns methodology primarily, with emphasis on the selection of bacterial indicators. The need for such emphasis became apparent because of certain shortcomings of the several types of bacterial indicators first used in the study.

It has been shown that the enterococcus, *S. zymogenes*, is capable of long-time survival in ground water and possesses a high degree of permanency in its characteristic cultural reactions. Although there may be a pronounced reduction in numbers over a year's time, a sizable portion may be expected to survive if the initial or dosing numbers are sufficiently large.

The use of *S. marcescens* as a tracer organism has limitations because the pigmentation is a variable property. The failure of *B. globigii* may have been due to use of a mixture of vegetative cells and spores rather than a pure spore suspension.

In addition to those properties which favor the use of uranin as a chemical agent for tracing the movement of underground water, the dye was shown to have long-lasting properties in the soil and ground water, thereby facilitating prolonged studies of underground water flow. Because of the acid nature of the dye and the prevailing pH values of the ground water, it is believed that the dye could be used concurrently with the bacteria.

If dosing had been continuous, greater dye and bacterial concentrations would very likely have been found in the positive wells with possibly a resulting extension in the flow pattern of each agent.

The vertical depth to which the dye and test organisms penetrated was not determined.

The study has demonstrated that the rate and extent of die-off of bacterial test organisms in the ground water can be determined by dialyzing culture studies. The distance to which bacteria can travel through the soil via ground water is dependent upon the degree of survival of test organisms as well as the mechanical filtering action of the soil itself. The numbers of survivors will depend, in great part, upon the numbers of bacteria introduced into the ground water. The findings of this investigation suggest the inclusion of a dialyzing culture study concurrently with the introduction of the test organism into the soil or ground water.

No doubt there are other suitable bacteria for ground water studies. Other enterococci which should also be suitable are *Streptococcus durans* and *S. liquefaciens*. *S. faecalis* is not recommended because it is considered to be the most common of the enterococci. Through the use of large samples, this organism was isolated many times.

The coliform group as a whole is not considered suitable since it is made up of a number of different species. The property of lactose fermentation by itself is not sufficiently distinctive. Because of the widespread nature of these organisms, the findings of small numbers in ground water could easily be confused with contamination from the surface of the ground. If a member of the coliform group is especially desired as a test organism, there is a possibility that *Escherichia freundii*, a coliform intermediate, might be suitable. The production of hydrogen sulfide distinguishes this organism from the other coliforms.

Some applications of the procedure given in this report are: (*a*) formulation of safe standards for water supplies with respect to location from points of pollution; (*b*) providing a method, where pollution of a water supply is suspected, of determining whether or not it has occurred or is possible; and (*c*) showing the availability of two highly specific simulants, a dye and a bacterium, for detection of other forms of contamination.

Summary

This study has demonstrated a method for tracing the movement of simulated chemical and bacterial pollutants in ground water. A procedure for evaluating the effects of the underground environment on the viability and on the cultural and biochemical properties of bacterial indicators is given. Emphasis is placed on the necessity of selecting as bacterial indicators, test organisms with distinctive and stable characteristics.

Uranin was found to be very satisfactory for determining direction of flow of ground water. The dye was traced 100 feet from the point of dosing and was detected 2 years and 10 months later. The path of the dye varied from an initially narrow channel, 1½ to 4 feet in width, to a wedge-shaped expansion measuring 40 feet at the distal end after several years.

A member of the enterococcus group of bacteria, *Streptococcus zymogenes*, was found to be suitable as an indicator or tracer organism to determine the extent of travel through the ground water. It was possible to trace this organism for 50 feet. The width of the path of travel varied between 1½ and 4 feet.

The several bacterial chromogens used were not found suitable as test agents in this study.

The dialyzing culture study was found to be a necessary adjunct in determining the degree of survival of the test organisms in the ground water.

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McGuinness Appointed Special Assistant

Dr. Aims Chamberlain McGuinness has been appointed by President Eisenhower as the new Special Assistant for Health and Medical Affairs to the Secretary of Health, Education, and Welfare. Dr. McGuinness replaces Dr. Lowell T. Coggeshall, who resigned to resume his post as dean of the division of biological sciences at the University of Chicago.

Prior to his appointment, Dr. McGuinness practiced medicine in the field of pediatrics in Philadelphia. He has been a faculty member at the University of Pennsylvania School of Medicine since 1934.

In the period 1948–51, Dr. McGuinness was director of the Children's Hospital in Philadelphia. He served as dean of the University of Pennsylvania Graduate School of Medicine from 1951 to 1954, and subsequently as clinical director of the Miners Memorial Hospital Association of the United Mine Workers Welfare and Retirement Fund. In this capacity, he was instrumental in constructing and staffing hospitals in the mining areas of Kentucky.

During World War II, he served as assistant administrator for the Army Epidemiological Board, and was awarded the Legion of Merit.