Salmonellae as an Index of Pollution of Surface Waters

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Screening enrichments of surface water specimens by means of a polyvalent fluorescent antibody reagent for the salmonellae yielded approximately 60% more positive specimens than was obtained by cultural procedures. It is not known what fraction of the excess of fluorescent antibody-positive over culturally positive specimens represents staining of non-salmonellae or non-arizonae as opposed to the staining of non-cultivatable organisms of these two genera. Cotton gauze and rayon-polypropylene fiber swabs were equally sensitive for collecting salmonellae from the streams examined. Tetrathionate enrichment incubated at 41.5 C appeared to be superior to selenite-cystine for isolation of salmonellae from surface waters. Twenty-eight serotypes of Salmonella and two serotypes of Arizona were identified in the 121 positive specimens. In water rated moderately polluted, 65% of all specimens tested were positive; in minimally polluted waters, 38% were positive; and in unpolluted streams, 44% were positive.

Salmonellae are frequently isolated from surface waters. Various techniques using a variety of media and incubation temperatures have been used. Spino (15) described a procedure which combined selective media with an elevated temperature of incubation to increase the frequency of isolation of Salmonella from polluted river water. Selenite-Brilliant Green (BG)-sulfa broth and tetrathionate broth were the enrichments into which Spino placed Moore swabs (14) of river water. These cultures were incubated at 41.5 C and streaked on Brilliant Green agar plates which also were incubated at 41.5 C. Salmonellae were recovered consistently, whereas they were not detected under the same conditions when the enrichment incubation temperature was 37 C.

Fair and Morrison (5) examined the "unpolluted" Poudre River in Colorado for salmonellae and arizonae by using membrane filter techniques followed by selenite enrichment with plating on four selective media. They tested 463 samples consisting of a total of 268 liters of water which yielded 11 isolates of salmonellae and 51 of arizonae. The Arizona cultures were derived from 23 individual water samples. The mean coliform count in the river during three consecutive years of sampling was 30/100 ml. The authors concluded that, regardless of the apparent lack of pollution, the existence of naturally occurring potable surface water is a myth.

Hendricks and Morrison (9), also studying the Poudre River, found that several enteric bacteria, including a strain of S. senftenberg, multiplied in dialysis sacs placed in the river both above and below a sewage outfall. Washed resting cells of the test species were suspended in saline and placed in the sacs to serve as inocula. Growth of the cells was generally better at 16 C than at 10 C; no multiplication occurred at 5 C.

Hooper (10) placed fine muslin swabs into two rivers in Wales when he surveyed the water for S. dublin over a period of 12 mo. With two exceptions, S. dublin was the only Salmonella serotype isolated, but its presence in the streams could not be correlated with clinical outbreaks of S. dublin infection in cattle that occurred during the survey. The author concluded that surface waters draining cattle pastures and barns were the probable source of the organisms in the rivers. Since Hooper used an unfavorable method for recovery of salmonellae—selenite enrichment

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with plating on deoxycholate agar—he probably obtained a minimal number of positive swabs and serotypes of salmonellae.

Claudon et al. (3) used the Moore swab and membrane filter techniques to analyze Wisconsin's Lake Mendota and its tributaries for salmonellae. They found these organisms to be distributed in the lake up to 0.5 mi (ca. 0.81 km) from the point of entry of a stream presumed to be the source of the salmonellae. Elevated fecal coliform counts usually were associated with Salmonella recoveries, but in two sites on three sampling dates Salmonella were isolated in the absence of detectable fecal coliforms.

Hendricks (8) reported higher recovery rates for Salmonella from stream bottom sediments than from surface water in a polluted river of Eastern Georgia. The methods used, however, were not favorable for isolation of these organisms, i.e., selenite enrichment followed by plating on MacConkey's agar.

Wells et al. (18) developed a cotton gauze swab method for the examination of milk for salmonellae. When the swab cultures were incubated at 43 C, as few as one Salmonella organism per liter was recovered from inoculated raw milk. The new method was much more sensitive than the conventional modified North method with which it was compared.

During the spring of 1971, William Burbanck and his class in advanced ecology at Emory University carried out an ecological survey of Peavine Creek which rises in the city of Decatur and flows through suburban DeKalb County, Georgia. A portion of the survey, done under the supervision of personnel of the Center for Disease Control (CDC), consisted of an analysis of the water for salmonellae. These studies were continued during the following summer and were extended to encompass a survey of some rural streams of a completely different nature. All of the surface water samples were from the state of Georgia. Both cultural and fluorescent antibody (FA) techniques were used to test for the presence of salmonellae. The use of FA techniques with polyvalent conjugates was reported recently by Thomason and Wells (17) and by Thomason (16). The objectives of the study were: (i) to compare FA with cultural techniques for sensitivity in the detection of salmonellae in surface waters, (ii) to evaluate the efficiency of two types of fiber swabs in the recovery of salmonellae from water, and (iii) to determine the numbers and variety of salmonellae serotypes present in these waters.

MATERIALS AND METHODS

Collection of specimens. The Moore (14) swab technique was used to collect salmonellae from streams. The fiber swabs were of two types: ordinary cotton gauze and a nonwoven synthetic material consisting of 75% rayon and 25% polypropylene. Pieces of gauze two layers in thickness and of dimensions 6 by 12 inches were cut lengthwise to within 1 inch of the end to make four strips each 1.5 inches in width. The gauze then was doubled over lengthwise, and a string was tied around the intact end. Swabs of the same dimensions were prepared from a single thickness of polypropylene (lot no. H-756, Kendall Fiber Products Division, Walpole, Mass.). All swabs were packaged in paper bags and sterilized.

To collect organisms, one swab of each type was suspended in the stream current at each sampling site. The two swabs were tied close together but in a manner to prevent their becoming entangled. The period of time the swabs were exposed varied from 30 min to 3 days.

At the end of the sampling period, swabs were removed from the water, drained for a few seconds while being held by the string, and deposited individually either in plastic bags or directly into jars of tetrathionate or selenite-cystine broth by cutting the string just above the swab. With few exceptions, swabs in plastic bags were transported to the laboratory and placed in the enrichment media within 2 hr of collection. When longer periods of transportation were involved, the swabs were kept on ice during transport to the laboratory. The amount of water retained by the drained swab varied between 10 and 15 ml. Tetrathionate cultures were kept at ambient temperature during transport to the laboratory, a period seldom exceeding 2 hr.

Processing of specimens. For isolation of salmonellae, tetrathionate enrichment broth was prepared from tetrathionate broth base (Difco) to which was added 10 ml of a 1 : 1,000 aqueous solution of Brilliant Green dye per liter (7). Approximately 150 to 200 ml of the finished medium was placed in wide-mouth jars of 400-ml capacity which were fitted with screw caps. Selenite-cystine broth (Difco) was prepared according to directions and distributed in the same manner.

In the first experiments, duplicate sets of swabs were incubated at both 37 and 41.5 C as recommended by Spino (15). Subsequently, all specimens were incubated at 41.5 ± 1.0 C because slightly better results were obtained at the higher temperature.

After approximately 24 and 48 hr of enrichment in tetrathionate or selenite broths, the swab specimens were routinely subcultured to both bismuth sulfate (BS) agar (Difco) and to BG agar (Difco) and incubated at 36 ± 1 C. Colonies were fished from BG plates after 24 hr and from BS after 48 hr of incubation. In a few experiments the second subculture was delayed until the third day. In early experiments, selenite-cystine broth was used as an enrichment, but, because most specimens were positive from te-
tetrathionate broth, the use of a second medium was discontinued. In some experiments, 1.0 ml of the original enrichment broth was subcultured into a tube of 9.0 ml of tetrathionate broth and incubated for 24 hr at 41.5 C before plating. This procedure also was discontinued because most specimens were positive from the original enrichment broth. The BG agar was supplemented by the addition of 5 ml of a 1.6% solution (80 mg) of sodium sulfadiazine in distilled water per liter (6). This prevented the swarming of Proteus cultures and inhibited the growth of Pseudomonas.

Characterization of cultures. Colonies typical of salmonellae were screened by transferring them from BG or BS agar to Triple Sugar Iron (TSI, Difco) agar, to lysine iron (LI, Difco) agar, and to urea agar slants (Difco). Cultures that produced alkaline slants (Difco). Cultures that produced alkaline slants, acid butts, and H$_2$S in TSI, and that gave an alkaline reaction throughout the LI medium with evidence of H$_2$S production, and which were urease negative were subjected to serologic testing. Alcohol-treated O antigens (4) were used in slide agglutination tests with Salmonella grouping sera, and formalinized broth cultures were screened with a CDC polyvalent H serum for flagellar antigen factors by the methods of Edwards and Ewing (4). The flagellar serum contained antibodies for 39 flagellar antigens (a through z$_{39}$) plus antibodies for phase 2 factors (1,2; 1,5; and 1,6). Representative cultures that agglutinated in O or H antisera were definitively serotyped. When several cultures which agglutinated in the same O grouping serum were obtained from a single specimen, only one was selected for definitive serotyping. Since some O groups contain hundreds of different serotypes, the number of serotypes reported in this paper is a minimal estimate of those present in the specimens.

FA studies. Smears were made from either tetrathionate or selenite enrichments, fixed for 2 min in Kirkpatrick’s solution (13) consisting of absolute ethanol, chloroform, and Formalin (60:30:10), rinsed in 95% ethanol, and air dried. They were stained, mounted, and examined as described by Thomason and Wells (17). The FA reagent was the CDC polyvalent OH II conjugate described by Thomason and Wells (17). It was prepared with Formalin-killed, motile broth cultures of salmonellae representing O groups A through I plus groups K, L, and O, and it contained labeled antibodies for both somatic and flagellar antigen factors.

FA tests on enrichments were read as positive when at least 10 well-stained organisms of typical morphology were seen in the smear. Usually each field contained one or two to several fluorescent bacteria resembling Salmonella. Observing in situ stained flagella significantly increased our confidence in interpreting results.

RESULTS

Comparison of FA and cultural procedures. Table 1 includes data on the 159 specimens which were studied. FA tests indicated the presence of salmonellae in approximately 63% more specimens (145) than were positive by culture (91). Fifty-seven percent (91) of the specimens examined (159) were culturally positive and 91% (145) were FA positive. Similar results (60% and 94%) also are evident in Table 2 in which FA and cultural test results are given for specimens paired according to the type of sampling swab used. The data of Table 2 indicate that surgical gauze and polypropylene are equally effective in sampling water for salmonellae. The results given by the two fibers are comparable whether measured by FA or by cultural tests.

Comparison of enrichment and plating media for isolation of salmonellae from water. Preliminary experiments indicated that, when paired Moore swab specimens were enriched in tetrathionate broth and in selenite-cystine broth and plated on both BG and BS agar, superior results were obtained from the tetrathionate broth (Table 3). Samples positive in selenite were not always positive in tetrathionate and vice versa. Thus, the use of a single enrichment decreases the number of culturally positive specimens. In practice, however, one compromises between the intensity of the search for salmonellae in a few specimens and a somewhat less-thorough search for these organisms in a larger number of specimens. The last approach was compatible with

### Table 1. Results of FA and cultural examination of surface water specimens for Salmonella

| Staining reaction | Culture  | Total |
|-------------------|----------|-------|
| -                 | +        |       |
| FA +              | 90*      | 145   |
| FA -              | 1        | 14    |
| Total             | 91       | 159   |

*Three specimens yielding A. hinshawii are included because these organisms are considered to have the same significance as do the salmonellae.

### Table 2. Results of FA and cultural examination of surface water specimens collected with gauze and polypropylene swabs

| Swab            | FA | Culture |
|-----------------|----|---------|
| Gauze           | 49 | 2       |
| Polypropylene   | 47 | 4       |
| Total (102)*    | 96 | 6       |

* Only 102 (51 pairs) of the 159 specimens of Table 1 were compared in respect to the fiber on which they were collected.
our objectives.

Salmonellae detected in water specimens from various sources. The watercourses sampled were divided into three groups based on an evaluation of the degree of pollution: (i) moderately polluted, (ii) minimally polluted, and (iii) unpolluted (Table 4). The headwaters of the first stream, Peavine Creek (6,070 m in length), are in an urban area bordered by light industry, but the remainder of the creek passes through residential areas. Specimens were taken at seven stations located along the length of the stream from its source to its mouth. Analyses for total coliforms, fecal coliforms, and fecal streptococci were carried out on three different dates in April and May 1971 at all sampling sites. Enumeration was based on direct plating procedures performed according to Standard Methods for the Examination of Water and Wastewater (1). The corrected mean values of all three groups of bacteria were extremely variable at different sites on the different dates. On one date, the fecal coliform count ranged from $10^9$ to $1.6 \times 10^4$ at the seven different sites. On another sampling date it ranged from $5.4 \times 10^2$ to $4.7 \times 10^5$; the last figure was the highest fecal coliform count recorded. Total coliform counts ranged from approximately $1 \times 10^4$ to $4 \times 10^4$ at different sites on different dates. The results of these tests indicated that the stream was moderately polluted with sewage along its entire length. Some industrial (chemical) pollution also was present.

In the second category (Table 4) the Chattooga River is probably the least polluted large stream in Georgia. Our samples were taken at the bridge on State Highway 76 where the river was judged to be minimally polluted. The Chattahoochee River above the Atlanta sewage outfalls probably is the cleanest and most beautiful stream within the city limits of any large metropolitan area in the United States. Although in a suburban area, Thompson Creek is a relatively clean stream that rises in a heavily wooded section and flows through a park. Callaway Gardens Lake is a small, clean, recreational lake. Savannah Beach is, of course, on salt water.

The third category (Table 4) of streams is interesting because at least two of them (Barnum and Cherry Creeks) were judged to be completely free from human, domestic animal, and industrial pollution. Both rise in and flow through heavily wooded areas. The parts of the streams sampled were at least 1 mi (ca. 1.6 km) from the nearest human or domestic sources of pollution. Furthermore, the nearest barns and houses were downstream from the sampling areas. Fecal pollution from an occasional human being or farm animal was possible, but the only apparent source of pollution was terrestrial or aquatic wildlife.

Salmonellae were isolated from 65% of the samples taken from Peavine Creek (Table 4), the moderately polluted stream which was the source of the majority of our specimens. Thirty-eight percent of the samples from minimally polluted waters and 44% of those from unpolluted streams yielded salmonellae. The number of culturally positive specimens obtained from Peavine Creek was not a true representation of the degree of contamination of

### Table 3. Surface water specimens yielding positive results for Salmonella listed by enrichment and plating media

| Enrichment media | No. of specimens positive on | Total |
|------------------|------------------------------|-------|
|                  | Brilliant Green | Bismuth sulfate |       |
| Tetrathionate    | 28              | 21              | 49    |
| Selenite-cystine | 13              | 13              | 26    |
| Total            | 41              | 34              | 75    |

### Table 4. Results of FA and cultural examination of surface water from various sources for Salmonella

| Source                        | No. of specimens | Percent culturally positive |
|-------------------------------|------------------|-----------------------------|
| Moderately polluted water    |                  |                             |
| Peavine Creek                 | 72               | 60a                         |
| FA + ; culture +              | 37               | 0                           |
| FA + ; culture -              | 1                | 1                           |
| FA - ; culture +              | 5                | 0                           |
| Culture only                  | 5                | 4                           |
| Total                         | 120              | 65                          |
| Minimal pollution             |                  |                             |
| Chattooga River               | 12               | 75                          |
| Thompson Creek                | 2                | 100                         |
| Chattahoochee River           | 2                | 50                          |
| Callaway Gardens Lake         | 3                | 0                           |
| Savannah Beach                | 10               | 0                           |
| Total                         | 29               | 38                          |
| Unpolluted streams            |                  |                             |
| Barnum Creek                  | 4                | 50                          |
| Moss Creek                    | 3                | 33                          |
| Cherry Creek                  | 9                | 44                          |
| Total                         | 16               | 44                          |
| Sum total                     | 165              | 58a                         |

* Based on total of 120 specimens in the category.
* Represents 96 culturally positive specimens.
that stream by salmonellae because many of the water specimens were taken after very short sampling periods. Many of these specimens were negative by culture but positive by FA tests (Table 4). Swabs left in Peavine Creek for 6 hr or longer were, with few exceptions, culturally positive. Frequently, specimens were culturally positive after 30 min of exposure; that was the shortest period tested.

In Cherry Creek, a small but permanently flowing stream, we attempted to determine how quickly the water became contaminated after it emerged from the earth. The results of this experiment are shown in Table 5. By using culture techniques, we found the stream to be contaminated with salmonellae within 350 ft (ca. 106.7 m) of its origin. Minimal numbers of organisms stained by the Salmonella polyvalent conjugate were observed in enrichments from specimens of water sampled at 20 ft (ca. 6.9 m) and at 150 ft (ca. 45.7 m) from the origin of the stream; this finding suggests the water may be contaminated within a much shorter distance of its source than was demonstrated by culture.

Swabs placed in Barnum Creek, another stream judged unpolluted, yielded S. give from one swab and A. hinshawii from the other. No salmonellae were recovered from a concrete-enclosed spring nor from the branch 7 ft (ca. 2.13 m) below the spring. S. muenchen was recovered from one of two swabs placed in a third small stream judged unpolluted by inspection of the water, stream bed, and the watershed.

**Serotypes of Salmonella and Arizona isolated.** Table 6 contains a listing of the serotypes of salmonellae isolated from the 165 specimens examined. Note that 21 specimens yielded two serotypes and two specimens three serotypes each, for a total of 121 isolations of salmonellae or arizonae from the 96 positive specimens (Table 4). Arizona cultures were included with the salmonellae because their significance as pathogens is similar. The serotypes of salmonellae reported represent a minimal number of those present because usually

| Serotype    | Antigenic formula | No. specimens yielding serotypes |
|-------------|------------------|----------------------------------|
| Group B     |                  |                                  |
| S. typhimurium | 1.4,5,12:i:1,2   | 5                                |
| S. typhimurium var. copenhagen | 1.4,12:i:1,2 | 1                                |
| S. heidelberg | 1.4,5,12:r:1,2  | 4                                |
| S. san-diego | 4.12:e.h.e.n.z,1s | 12                              |
| S. derby     | 1.4,5,12:f,g    | 1                                |
| S. agona     | 4.12:f.g.s      | 1                                |
| S. saint-paul | 1.4,5,12:e.h:1,2 | 3                              |
| S. bredenev  | 1.4,12,27:l.v:1,7 | 1                              |
| Group B Salmonella | Not typed |                                  |
| Group C1     |                  |                                  |
| S. thompson  | 6.7:k:1,5       | 13                               |
| S. infantis  | 6.7:r:1,5       | 6                                |
| S. oranienburg | 6.7:m,t       | 1                                |
| S. braenderup | 6.7:e.h.e.n.z,1s | 1                              |
| S. montevideo | 6.7:g.m.s     | 11                               |
| S. bareilly  | 6.7:y:1,5       | 1                                |
| S. eimsbuettel | 6.7(14):d:1,w  | 1                                |
| Group C2     |                  |                                  |
| S. albany    | (8,20):z,zz     | 1                                |
| S. muenchen  | 6.8:d:1,2       | 5                                |
| Group D      |                  |                                  |
| S. enteritidis | 1.9,12:g.m   | 2                                |
| Group E1     |                  |                                  |
| S. anatum    | 3,10:e.h:1,6    | 6                                |
| S. weltevreden | 3,10:r:z       | 1                                |
| S. amager    | 3,10:y:1,2      | 4                                |
| S. give      | 3,10:l.v:1,7    | 9                                |
| Group E Salmonella | Not typed |                                  |
| Group F      |                  |                                  |
| S. rubislaw  | 11::e:n,x       | 3                                |
| Group G      |                  |                                  |
| S. cubana    | 1,13,23:z,zz   | 4                                |
| S. mississippi | 1,13,23:b      | 9                                |
| Further groups |            |                                  |
| S. redlands  | 16::e.n,z,zz    | 1                                |
| S. cerro     | 18::z,zz       | 1                                |
| S. minnesota | 21::e:n,x      | 1                                |
| S. bern      | 40a,40b::z,zz  | 5                                |
| Arizona hinshawii | 20:23-21 | 2                                |
| Arizona hinshawii | 12:27-28  | 1                                |
| Total positive specimens | 121*  |                                  |

*Compare with number culturally positive in Table 4. Difference due to 21 specimens which yielded two types each and two specimens containing three types each; 121–25 = 96.

**Table 5. Results obtained from sampling surface water of Cherry Creek for Salmonella**

| Distance from source of stream (ft) | Type of swab | Salmonella | FA | Culture |
|------------------------------------|--------------|------------|----|---------|
| 20 (ca. 6.1 m)                     | G            | +a         |    |         |
| 150 (ca. 45.7 m)                   | PP           | +a         |    |         |
| 350 (ca. 106.7 m)                  | G            | +a         | S. give |         |
| 500                                | G            | +a         | S. bern, S. give |     |
| 670                                | G            | +a         | S. bern |     |

*Only a few microscope fields contained organisms. G, Gauze; PP, polypropylene; water sampled one time with each swab as indicated.
only one culture of each O group from each water sample was serotyped. Some of the serotypes isolated were rather rare. S. weltevreden, which we recovered once from Peavine Creek water, is very common in Hawaii but has been found only rarely in the continental states (2). We also isolated S. bern, another rare type, from five specimens (Table 6). Kauffmann et al. (11) first reported the isolation of this serotype from a snake (Natrix natrix). Cultures of S. bern liquefy gelatin and may ferment salicin or grow in KCN medium. They fail to ferment lactose and dulcitol and to utilize d-tartrate, mucate, and malonate. Although the antigenic formula of S. bern was given by Kauffman et al. (11) as 1,40:z4,232:-, they recognized the complexity of the O antigens. It was shown that both the O and H antigens of the original culture of S. bern were identical to those of the 10:1,2,10 serotype of A. hinshawii. Thus, biochemically, cultures of this serotype are intermediate in characteristics between Arizona and typical Salmonella.

The first cultures of S. bern were received by the National Salmonella Center at the CDC in 1968, 8 yr after the original isolation of the serotype in Switzerland. At the present time, 15 cultures have been typed, including the five isolated from surface water during our study. One culture was isolated from pond water, two from human feces, one from a bovine animal, and six from feral animals (five of these six were from opossums). The O antigens of all these cultures are expressed as 40a, 40b.

Other rare types isolated were S. albany, S. agona, S. amager, S. cerro, S. eimsbuettel, S. minnesota, and S. rubislaw. S. redlands was not found among 11,653 Salmonella cultures reported from nonhuman sources in 1970 nor was it found in 1969 (2). This type was last reported from a human source in 1964 when it occurred one time (14). It did not appear among the 24,216 cultures of human origin which were reported in 1970 (2). We isolated it one time from Chattooga River water.

**Most probable number (MPN) experiments.** Two experiments were conducted during the summer of 1971 to determine the MPN of salmonellae in Peavine Creek. In the first, five 100-ml, five 10-ml, and five 1-ml samples of water were filtered through Gelman C.A. membranes (0.45 μm pore size) as described by Kenner et al. (12). The filters were incubated in tetraionate broth and plated on both BG and BS agar after 24, 48, and 96 hr of incubation at 41.5 C. Salmonella (two serotypes) were isolated from three of the 100-ml samples. Based on MPN tables (1), these results indicated 0.8 cells (95% confidence limits 0.1-1.9 cells) of salmonellae per 100 ml of water. In another experiment, double-strength tetraionate broth was prepared, and equal amounts of creek water were added for the MPN determination. The samples consisted of five 500-ml, five 100-ml, and five 10-ml samples. Salmonellae were cultured as described above. The MPN of salmonellae was calculated to be 0.1 cells (95% confidence limits 0.05 to 0.2 cells) per 100 ml of water. The pH of Peavine Creek water in July and August of 1971 ranged between 6.7 and 7.2, and the mean water temperature was 24.8 C. Intermittent discharge of pollutants and variation in rainfall, water temperature, and pH would be expected to influence the MPN of salmonellae in the Creek.

**DISCUSSION**

FA techniques indicated the presence of salmonellae in about 60% more specimens than did culture techniques. Knowing the degree of pollution of many of these water samples, one assumption which can be made is that FA-positive enrichments were frequently culturally negative because of overgrowth of salmonellae by other bacteria. This assumption should be confirmed by intensive efforts to culture salmonellae from FA-positive, culture-negative enrichments. Also, the flora of these enrichments should be examined for possible cross-staining by the polyvalent conjugate. An alternative assumption is that the excess of FA-positive over culture-positive specimens represents false-positive staining of antigenically related, morphologically similar bacteria. Previous workers (17) showed that 12% of the Escherichia coli and Citrobacter freundii strains and 36% of the Arizona strains studied were stained by the polyvalent Salmonella conjugate. Data are not available, however, on the frequency of occurrence of serogroups of these species in the surface waters which we tested.

Contrary to the findings of Dockstader and Gromes in the recovery of salmonellae from milk chocolate (Bacteriol. Proc., p. 7, 1971), we did not find any advantage of polypropylene fiber over cotton gauze for isolation of salmonellae from surface waters. The synthetic fiber also is considerably more expensive and less available. We were, however, impressed with the sensitivity of both of these fibers in the recovery of small numbers of salmonellae from water.

In our studies, more surprising than the
number and types of salmonellae in polluted Peavine Creek was the ease of recovering these organisms within 350 ft (ca. 106.7 m) of the origin of supposedly "unpolluted" mountain streams where the only reasonable source of pollution appeared to be terrestrial or aquatic wildlife. The frequency of occurrence of salmonellae in such streams substantiates the conclusion of Fair and Morrison (5) that naturally occurring potable surface waters do not exist.

Hendricks and Morrison (9) showed that Salmonella and other enteric bacteria could multiply in both polluted and unpolluted river water at temperatures as low as 10 C. This fact may help to explain the ease with which a large number of Salmonella types were isolated from Peavine Creek, a stream which was moderately polluted and which had a relatively high temperature. This facet of the problem deserves further investigation.

We have always thought that the only habitat of the salmonellae is the intestinal tract of animals. The possibility should now be entertained that these bacteria may exist as free living organisms multiplying under natural conditions. Regardless of their ecology, salmonellae and arizonae are potential animal pathogens. Their widespread occurrence in surface waters of various quality constitutes a public health problem the magnitude of which is obscured by inadequate diagnosis and under-reporting of diarrheal diseases. Comprehensive epidemiologic studies relating disease to exposure to polluted water are needed. The Moore swab technique coupled with the screening of enrichments with polyvalent FA reagents is a highly sensitive tool for more intensive analysis of the natural environment for salmonellae. Simple means for detecting these organisms and their significance as pathogens may make them superior to coliforms as indexes of water quality from the viewpoint of public health.

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