# Demonstration of Infectious Agents in Sewage

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*Three investigations demonstrate the effectiveness of the swab as a device for finding infectious agents that may occur intermittently in sewage and remind us that "acceptable" sewage treatment facilities do not always destroy pathogenic agents.*

The technic devised by Moore [1] for collecting sewage specimens to be examined for infectious agents, together with isolation methods developed within the last two decades, has placed a valuable tool in the hands of microbiologists, sanitarians, and epidemiologists. In the work reported here this procedure was used for sampling in efforts to demonstrate three types of infectious agents in sewage: Coxsackie viruses in connection with water pollution studies to evaluate present indexes of pollution; tubercle bacilli, to determine the effect of sewage treatment on survival; typhoid bacilli, in a cooperative study with the Bureau of Epidemiology and Communicable Disease Control of the New York State Department of Health to locate a typhoid carrier.

Moore's method of sampling, used successfully in England to locate sources of paratyphoid infection,[1] typhoid carriers,[2] and centers of poliomyelitis virus spread,[3] consists of exposing pads of gauze (swabs) to sewage and using the liquid expressed from the swab as the sewage sample. This differs from the traditional type of sewage sample, the catch sample, which is dipped from the source in an instant and is representative of the sewage only at that instant. The expressed liquid from the swab, on the other hand, is a composite of the sewage which flows through the swab during the exposure period.

In general, the technical details of his sampling procedure were followed: Each swab was made by folding a strip of gauze $6 \times 48$ inches into a square pad and fastening a length of stout string to one corner. They were sterilized by autoclaving in paper bags, in which they were stored. During the exposure periods, the swabs were immersed in flowing sewage at sewage treatment plants in below manholes and were held in place by tying the strings to manhole covers or other convenient objects. While the same technic was used for sampling sewage for each infectious agent sought, the sewage sources and subsequent isolation procedures differed for each agent.

## Viruses

In connection with improving methods for detecting viruses in sewage and water supplies, the value of swab sampling over that of catch sampling was ascertained. As reported previously,[4] swab samples of sewage taken from treatment plants in the Albany area frequently yielded Coxsackie viruses; whereas catch samples obtained at the beginning or end of the exposure period (48 hours) did not. This finding is consistent (Table 1) and has been ob-

**Table 1—Coxsackie Virus Recovery From Catch and Swab Samples—Catch Samples Taken During Swab Sampling Intervals**

| | Number | | | |
|---|---|---|---|---|
| | 1952 | | 1953 | |
| Sample | Total | Positive | Total | Positive |
| Catch | 4 | 0 | 4 | 1 |
| Swab | 4 | 3 | 4 | 4 |

served also by Melnick.[5] The expressed liquid (60–100 ml) from the swabs (and catch samples) was reduced to a small volume by adsorption on Dowex 1 ion-exchange resin and elution from it with 2–3 ml of beef extract broth.[4] That this step increased the frequency of virus demonstration in sewage samples is clear from Table 2. The eluates to which streptomycin and penicillin were added were injected into 16 zero to two-day-old mice within 24 hours or following several weeks' storage in a dry ice chest.

In LoGrippo's description of the application of ion-exchange resins to virus purification,[6] 10 per cent phosphate is recommended as an eluant, followed by dialysis prior to animal injection. In comparative tests, beef-extract broth[7a] was found to elute the viruses as effectively as phosphate. This preparation does not require dialysis, since it is not toxic upon injection into mice. No doubt other solutions containing exchangeable ions would also be useful eluants.

**Table 2—Coxsackie Virus Recovery in Untreated (U) and Resin-Treated (R) Sewage Samples**

| Virus Detection in Sample | | Number of Samples |
|---|---|---|
| U | R | |
| 0 | 0 | 7 |
| 0 | + | 7 |
| + | ++ | 11 |
| + | + | 3 |

Mice that became paralyzed or spastic during the 14-day observation period following injection were presumed infected with Coxsackie viruses; isolations were confirmed by further passages in suckling mice and failure of passage in adult mice and in many cases by serologic characterization.

With consistent use of these two new technics, sampling by the swab method, followed by sample treatment with ion-exchange resins, the normal distribution of Coxsackie viruses in raw sewage throughout the year was determined. Swabs were exposed to the influent of the following sewage treatment plants in the area for 48-hour periods, the Albany plant receiving chiefly domestic sewage at a flow rate of 30 mgd from a population of approximately 130,000, and the Colonie (Albany-Schenectady Road) plant receiving domestic sewage at a flow rate of 0.4 mgd from a population of approximately 3,000. They were transported to the laboratory in pint Mason jars and if not processed immediately, as described above, they were stored in a dry ice chest (— 45°C to — 65° C) for several weeks. Frequently, the expressed liquids from the swabs were frozen before resin treatment, in which case they were frozen in an electric deep freezer before transfer to a dry ice chest; this prefreezing reduced container breakage during storage. Sewage was sampled weekly

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This paper was presented before a Joint Session of the Engineering, Epidemiology, Food and Nutrition, and Laboratory Sections of the American Public Health Association at the Eighty-Second Annual Meeting in Buffalo, N. Y., October 13, 1954.

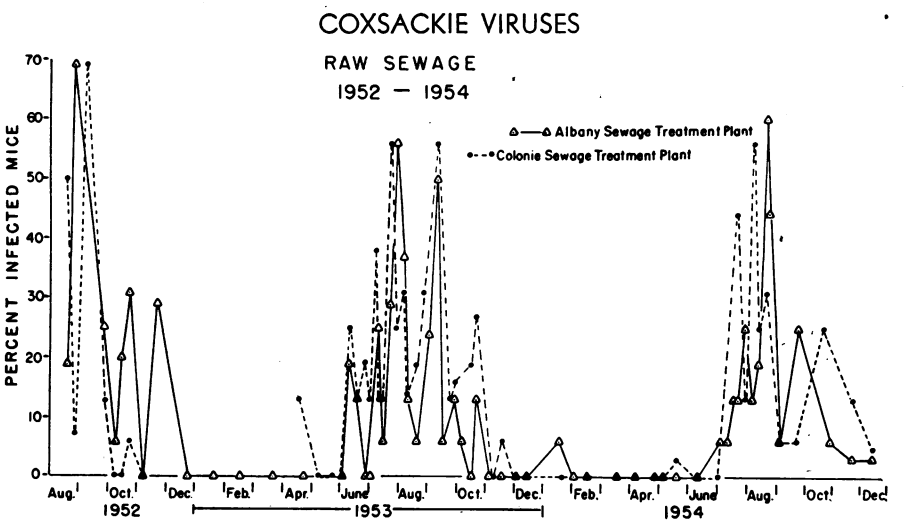


**Figure 1—Coxsackie Virus Occurrence in Raw Sewage Throughout the Year**

in months when high virus frequencies were anticipated[4] and biweekly or monthly in intervening periods.

Coxsackie viruses occur seasonally in sewage of the Albany area, being present continuously between June and November and only sporadically during the remainder of the year (Figure 1). This seasonal distribution of the viruses, indicated by a preliminary study,[4] has been reported since for other communities[8] and was found in earlier searches for poliomyelitis virus in Canada[9] and New York City.[10]

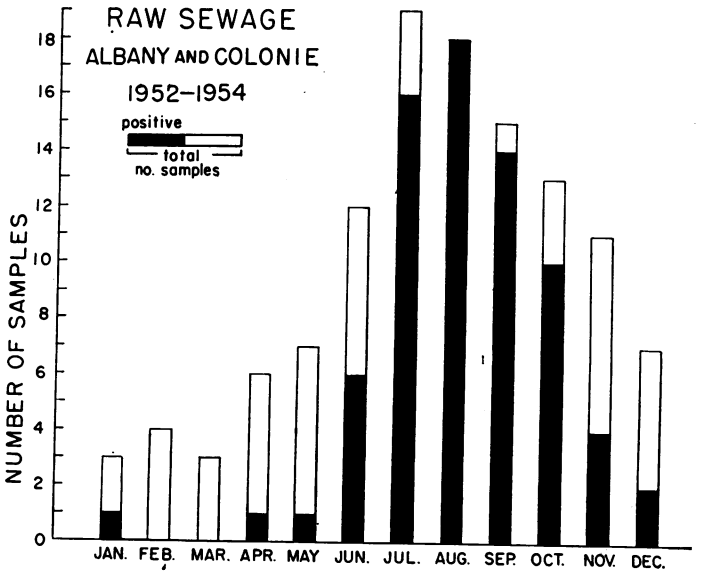


**Figure 2—Coxsackie Virus Frequency in Sewage Throughout the Year**

Quantitation of the amount of virus present was estimated by per cent of infected mice per sample. In the estimation, nonspecific deaths of mice were omitted from the tabulations. The quantitation shows that the viruses are present in greatest amount from late July to early September (Figure 1). Their frequency is highest in August (Figure 2). The fluctuations in amount found, within a short interval, may be owing to: (1) errors in sampling and subsequent treatment; (2) susceptibility differences in mice; and (3) actual fluctuations in the amount of virus in sewage even in the peak season. The rare appearance of these viruses out of season suggests they may be present in sewage continuously in small amounts and are undetectable only because of limitations in sampling and isolation procedures.

The amount of virus was found to fluctuate during the daily flow by testing the liquid from swabs exposed for one-hour periods in the morning and in the afternoon. Viruses were detected, for example, in sewage flowing through the plant in the morning but not in the afternoon. This finding coincides, perhaps, with the flow rate of domestic sewage which is greater in the morning.

Sewage treatment does not necessarily destroy the Coxsackie viruses. Sedimentation in an Imhoff tank having a retention period of two hours, as carried out at the Albany treatment plant, had no destructive action on virus. During the summer and fall viruses were recovered regularly from the effluent as well as from the influent of the plant. Secondary treatment on a trickling filter, followed by simultaneous sedimentation and chlorination, as practiced at the Colonie plant, was not always effective (Table 3). This plant suffers from overloading and frequent mechanical difficulties and as a result the residual chlorine often is zero.

Three other plants in the area were sampled several times during the summer months to determine the effect of sewage treatment on the viruses. Swab samples of raw sewage and plant effluent were taken from the following plants: Delmar, treating sewage from a population of approximately 9,000 by sedimentation in an Imhoff tank at a designed flow rate of 0.8 mgd; Schenectady, treating sewage from a population of approximately 92,000 by primary sedimentation at a designed flow rate of 16 mgd; Scotia, treating sewage from a population of approximately 7,800 by primary sedimentation at a designed flow rate of 1.2 mgd. In none of these plants did sewage treatment destroy these viruses (Table 3). This failure of certain phases of sewage

**Table 3—Coxsackie Virus Recovery From Various Stages of Sewage Treatment**

| Collection Period 1954 | Plant | Number of Samples | | | | | |
|---|---|---|---|---|---|---|---|
| | | Influent | | Filter Effluent | | Effluent | |
| | | Total | Positive | Total | Positive | Total | Positive |
| Jan.–Dec. | Albany | 20 | 10 | | | 20 | 10 |
| Jan.–Nov. | Colonie | 13 | 7 | 4 | 2 | 12 | 1 |
| July–Sept. | Delmar | 5 | 3 | | | 5 | 5 |
| July–Sept. | Schenectady | 5 | 4 | | | 5 | 4 |
| July–Sept. | Scotia | 4 | 4 | | | 4 | 3 |

treatment to destroy viruses was encountered also by Gear[11] studying poliomyelitis virus presence in sewage.

## Tubercle Bacilli

To determine whether present methods of sewage treatment are adequate to destroy tubercle bacilli that are discharged from tuberculosis hospitals, demonstration of the bacilli was attempted in sewage from several sewage treatment plants receiving wastes from large tuberculosis hospitals in New York State. Isolations from receiving streams were also attempted since they may constitute a source of infection. Sewage was sampled by catch and swab methods, and isolation technics developed chiefly with samples received from the Saranac Lake and Ray Brook sewage treatment plants. Sewage from two other plants, Oneonta and Perrysburg, was then examined for the bacilli by the procedures developed.

Sewage specimens were transported by parcel post, railway express, or messenger, usually arriving at the laboratory within 48 hours of collection. When swab sampling was carried out the swabs were immersed in sewage for 24 or 48 hours and were transported in pint Mason jars. Twenty-four-hour swab sampling came to be the practice since 48-hour specimens induced more intercurrent infection in inoculated animals. All sewage specimens were refrigerated upon being received at the laboratory and were usually processed within two days after arrival.

Isolation of the bacilli by cultural examination was unsatisfactory because of overgrowth with spore-forming bacilli and the presence of large numbers of saprophytic acid-fast rods, some of which resembled tubercle bacilli in their cultural characteristics. Demonstration by guinea pig inoculation gave much more satisfactory results and was used as the sole test in most of the examinations.

At the Saranac Lake sewage treatment plant gallon-catch samples were collected from the Saranac River at the plant outfall and at various distances down the river. One swab sample was taken at the outfall. This plant treats sewage from a population of approximately 6,900 by primary treatment at a designed flow rate of 1.8 mgd and discharges its effluent into the Saranac River, used at present for fishing and bathing.

Of 56 guinea pigs injected with catch samples from the outfall, 29 showed evidence of tuberculosis at autopsy. Of five injected with a sample collected 100 feet downstream, all had evidence of tuberculous infection. Samples collected approximately one-half mile and one mile downstream had no demonstrable tubercle bacilli. All the animals injected with liquid from the single sewage swab died of intercurrent infection, as did a number injected with the catch samples.

These initial sewage examinations indicated: (1) tubercle bacilli can be demonstrated in sewage more readily by animal tests than by cultural examination; (2) sampling by the catch method may be inadequate; (3) treatment of catch samples by centrifugation is laborious and time-consuming; (4) if swab sampling is used the liquid expressed requires further treatment before animal injection; (5) primary sewage treatment does not destroy tubercle bacilli; and (6) the bacilli may be present in streams receiving effluents from such plants. To improve methods and obtain further information about tubercle bacillus survival, sampling was extended to a secondary treatment plant at the Ray Brook Tuberculosis Hospital receiving wastes from a population of approximately 500 at a designed flow rate of 0.09

mgd. Secondary treatment facilities at this plant consist of a trickling filter, secondary clarifier, and chlorinator. Both catch and swab samples of clarified effluent before and following chlorination were examined for the bacilli. Tubercle bacilli were isolated from 18 of 35 guinea pigs inoculated with samples collected before chlorination, and in seven of 35 inoculated with specimens collected following chlorination. During the sampling period in this plant, there was a dosage of eight pounds of chlorine per day with an estimated contact time of 10 minutes and no residual chlorine. This degree of chlorine treatment decreased the number of tubercle bacilli demonstrable but by no means inhibited them completely.

Prior to animal inoculation, swab samples were treated as follows: They were suspended in 4 per cent NaOH, shaken in a machine, and centrifuged. This produced a bulky sediment which was neutralized with phosphate buffer to pH 6.6. Guinea pigs were inoculated with the sediment, but the presence of a large number of spore-forming bacilli caused a high mortality even when 200 units of penicillin per milliliter of inoculum were added. Heating the specimen to about 37° C for an hour while in contact with the NaOH, a procedure used to digest sputum specimens in routine examination for tubercle bacilli, decreased this mortality rate, and increased the percentage of animals showing evidence of tuberculosis. Because the volume of the sediment was large, only a portion was used for inoculum. As a means of separation of the tubercle bacilli from spore-forming bacilli, lactose to give 10 per cent by weight was added when the sediment was neutralized, and the mixture was then shaken.* On further centrifuging, the suspended solids and spore-forming bacteria were sedimented while the tubercle bacilli were found in the top layer, a portion of which was used for guinea pig inoculation. This lactose-treated fraction was less bulky to handle than was the heat-treated specimen and was used therefore in later examinations.

The gallon catch samples were concentrated by one of the following methods: (1) flocculation with such reagents as zinc acetate, aluminum sulfate, tannic acid, or ferric sulfate, (2) centrifugation at 2,400–2,800 rpm, or (3) filtration of a 100–200 ml aliquot on a "coli 5" membrane filter. Catch samples concentrated by flocculation and by membrane filtration, when inoculated into guinea pigs, gave higher mortalities and less evidence of tuberculous infection than did the heat-treated or lactose-treated swab samples (Table 4). Catch samples concentrated by centrifugation gave evidence of tuberculous infection comparable to that given by the treated swabs; the ease and safety with which the swabs were collected and handled, however, were decidedly in their favor for routine use.

Using centrifuged catch samples and lactose-treated swab samples, further studies were made of the survival of tubercle bacilli in sewage of the Oneonta treatment plant into which wastes from the Homer Folks Tuberculosis Hospital are discharged. This plant receives sewage from a population of 13,500 at a designed flow rate of 3.0 mgd and treats it by primary sedimentation. Twenty-seven guinea pigs were inoculated with specimens of raw sewage from the hospital, primary settling tank influent of the treatment plant, and tank effluent. As indicated in Table 5 sedimentation had little

* The addition of lactose to increase the density of the suspending medium is a procedure followed in the preparation of blood fractions by differential centrifugation in the University Laboratory of Physical Chemistry Related to Medicine and Public Health, Harvard University.

**Table 4—Comparison of Sample Treatments for Demonstration of Tubercle Bacillus in Sewage**

| Treatment | Number of Guinea Pigs Inoculated | | | | | |
|---|---|---|---|---|---|---|
| | Catch | | | Swab | | |
| | Total | Positive | Per cent Positive | Total | Positive | Per cent Positive |
| Centrifugation | 96 | 56 | 58 | | | |
| Flocculation (ferric sulfate) | 34 | 6 | 18 | | | |
| Filtration | 20 | 3 | 15 | | | |
| None | | | | 73 | 21 | 29 |
| Heat | 34 | 16 | 47 | 8 | 4 | 50 |
| Density change (lactose) | | | | 35 | 16 | 46 |

destructive action on the bacilli. Dilution of sewage by the town's population probably accounts for the difference in tubercle bacillus recovery in the hospital sewage and the tank influent.

A year later, at the secondary sewage treatment plant of the J. N. Adam Tuberculosis Hospital in Perrysburg, additional examinations for tubercle bacilli were made. This plant receives sewage from a hospital population of about 500 at a designed flow rate of 0.18 mgd, treats it by sedimentation, filtration on a trickling filter, and secondary clarification followed by chlorination and discharges the effluent into a tributary of Cattaraugus Creek, used at present for fishing. Twelve guinea pigs each were inoculated with specimens collected from various stages of sewage treatment. As shown in Table 5 secondary treatment was apparently effective in destroying the bacilli. Of 36 animals injected with catch samples, collected at various distances downstream from the outfall, one, however, showed evidence of tuberculosis. This positive sample was collected 1,000 feet from the outfall; while those collected 100 and 500 feet

**Table 5—Tubercle Bacillus Demonstration in Various Stages of Sewage Treatment**

| Stage of Treatment | Number of Guinea Pigs Inoculated (Tuberculous/Total) | | |
|---|---|---|---|
| | Homer Folks Hospital, Oneonta | | J. N. Adam Hospital, Perrysburg |
| | 1953 | 1954 | 1954 |
| Hospital sewage | 18/27 | 14/16 | |
| Primary tank influent | 7/27 | | 7/12 |
| Primary tank effluent | 5/27 | | 1/12 |
| Secondary clarifier influent | | | 3/12 |
| Secondary clarifier effluent | | | 0/12 |
| Plant effluent | | | 0/12 |

downstream on four occasions failed to produce tuberculosis. This discrepancy suggests that catch sampling is inadequate, since it reflects only a momentary state of the stream or sewage. It also indicates that failure to recover the pathogenic agent may mean, not that it is necessarily absent, but that the efficiency of the sampling and demonstration methods is the limiting factor in successful isolation.

The observation that four of the infected animals inoculated with sewage from the Perrysburg plant had lesions of the type induced by isoniazid-resistant strains led to speculation about the effect of the current widespread use of antibiotics on tubercle bacilli survival in sewage. As the use of isoniazid had greatly increased since specimens were first collected from the Homer Folks Hospital, an additional series of specimens were collected from that hospital and tubercle bacilli were found to be abundant. This indicates that the use of antibiotics has little, if any, influence on the recovery of the bacilli from sewage.

In the course of shipment of sewage specimens from the Homer Folks Hospital to the laboratory, one set was delayed in transit by railway express for eight days. Ample evidence of tuberculous infection was noted in guinea pigs inoculated with the set of specimens, suggesting that survival in sewage is not transitory.

## Salmonella

Five cases of typhoid fever occurred in three successive years in three families which lived within a mile of each other along a creek into which the effluent of the sewage treatment plant of a western New York village is discharged. The first case was a child who admitted drinking water from the stream below the outfall. The other patients lived below the outfall. The families did not know or mingle with each other.* The typhoid bacilli isolated from specimens from all the patients were phage Type $D_4$.

Gauze swabs were immersed in the influent and effluent of the secondary sewage treatment plant and in the sewers of the village at points selected to provide information on the occurrence of typhoid bacilli in sewage from specific streets or from lateral sewers. Following four days' immersion, each swab was placed in 200 ml buffered 30 per cent glycerol solution [7b] and was received at the laboratory within 24 hours after collection. The suspension was diluted 1:10 and 1:100 with buffered 30 per cent glycerol solution. The undiluted suspension and the two dilutions were streaked on bismuth sulfite agar [7c] and a modification of Endo's agar.[7d] One and 5 ml amounts of the undiluted suspension were added also to 12 ml and 50 ml, respectively, of tetrathionate medium [7e] and incubated for 18–20 hours at 35° C–37° C. The latter was then streaked on bismuth sulfite agar plates, which were examined after incubation at 35° C–37° C for 18–20 hours and again after 40–48 hours.

Salmonella typhosa, phage Type $D_4$, was isolated from 10 of 29 specimens collected, including one from the sewage drainpipe of a nursing home, but not above this point in the sewer system. The search for the carrier was therefore focused on this home and a carrier of S. typhosa, phage Type $D_4$, was discovered there.

It is of interest that S. montevideo was isolated from five specimens collected from the influent and effluent of the sewage treatment plant, but no attempt was made to determine the source of this microorganism.

* The epidemiologic data were furnished by Dr. Robert M. Albrecht, acting director of the Bureau of Epidemiology and Communicable Disease Control.

In six instances S. typhosa was found on plates streaked with one or both dilutions of the original suspension, but not on those inoculated with the undiluted material, while the reverse was true in only two. Twice it was found only on plates streaked with the 1:10 dilution, once only on those from the 1:100 dilution, and three times on those from both. It was never found on plates inoculated with tetrathionate medium. On the other hand, S. montevideo was found only on plates inoculated with tetrathionate medium.

In addition to illustrating the practicability of locating a typhoid carrier, the observations described here indicate that sewage treatment may not destroy Salmonella.

## Discussion and Summary

The results of these three investigations illustrate the suitability of the swab as a sampling device, and in combination with appropriate isolation technics, demonstrate the practicability of isolating infectious agents which may occur intermittently in sewage. The ease and safety with which the swab may be handled by the collector and laboratory technicians are additional factors that cannot be ignored. As suggested by the British workers, these procedures offer a practical approach to the heretofore frustrating problem of searching for Salmonella carriers among groups of food handlers.

More accurate information about the presence of infectious agents in sewage should now be obtainable, during periods when outbreaks or epidemics are nonexistent, as well as when they are recognized. The three studies offer ample evidence also of the disturbing fact that sewage treatment, while complying with existing standards, may not control pathogenic agents to the extent that receiving streams can be ignored as potential sources of infection.

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