A SURVEY BY THE SEWAGE SWAB METHOD OF LATENT ENTERIC INFECTION IN AN URBAN AREA

By B. Moore, B.Sc., M.B., B.Ch., B.A.O., Public Health Laboratory, Exeter, Col. E. L. Perry, D.S.O., M.R.C.S., L.R.C.P. (Medical Officer of Health) and S. T. Chard, M.S.I.A., Public Health Department, Sidmouth

(With 4 Figures in the Text)

A simple technique for the location of enteric carriers in towns by means of serial sewage examinations has been described in two earlier papers by Moore (1948, 1950). The principle underlying this method is essentially that some form of continuous sampling of the sewage passing along a particular sewer should be a more sensitive index of the passage of enteric organisms than the examination of bulk samples of sewage, taken from the sewer at times which could not be related in advance to the personal habits of a hypothetical excreter of enteric organisms proximal to the point of sampling. It was found that a gauze swab immersed in the flowing sewage for 48 hr. was an effective trap for enteric organisms and that, when suitable cultural techniques were used, this method gave consistently good results and was moreover far easier in practice than the awkward procedure of collecting sewage in bulk from various points on a sewerage system. It was also shown that when enteric organisms, had been isolated from a swab, it was possible to trace the organism back to an individual household by systematic sampling from key manholes on the sewerage system in the area concerned.

In both of the papers cited, reference was made to investigations carried out at Sidmouth during the past few years. This work was undertaken mainly to develop methods for the location of enteric carriers, and the techniques which proved satisfactory in Sidmouth were then successfully applied to the solution of an unexplained paratyphoid outbreak at another place. Our investigations were also relevant to local problems, however, as many sporadic cases of both typhoid and paratyphoid fever have been notified from Sidmouth during the past 15 years and its record in this respect has been far worse than that of any other town of similar size along the South Devon coast. It was hoped that our investigations might throw some light on these infections, the majority of which had not been satisfactorily explained by the usual methods of epidemiological inquiry. Unfortunately, a long enforced leave of absence of one of us has curtailed the scope of the investigations originally planned, and the survey of Sidmouth has in consequence not been completed to our satisfaction. Such results as we have obtained are here presented, and various problems posed by the survey are discussed. Some suggestions are also made on the possible application of the methods we have used to various epidemiological problems.

J. Hygiene

Epidemiological background

Sidmouth, a flourishing seaside resort with a population of about 10,000, lies on the coast of South Devon 15 miles east-south-east of Exeter. It is situated at the mouth of the River Sid and occupies a deep roughly semicircular valley surrounded on the east, north and west by hills which rise to a height of 500-600 ft.

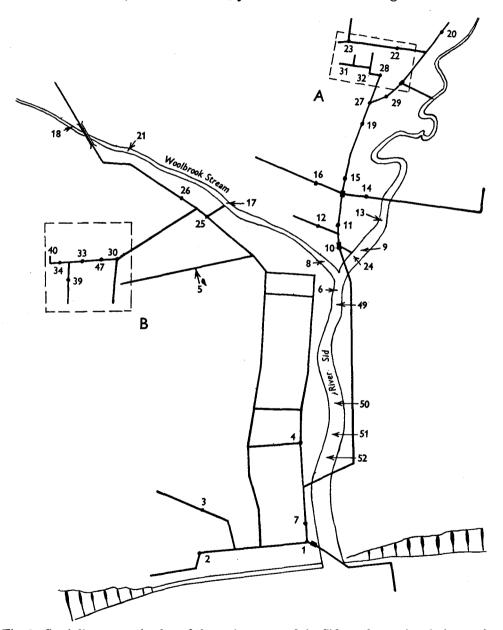


Fig. 1. Semi-diagrammatic plan of the main sewers of the Sidmouth area in relation to the River Sid and the Woolbrook stream. The distance from point no. 20 at the top of the figure to the sea is approximately 2 miles. The areas A and B are shown in greater detail in Figs. 2 and 3. Numbers refer to sewage sampling points.

The urban district of Sidmouth, in which our investigations were conducted, is an area of approximately 11,500 acres whose boundaries skirt the tops of the adjacent hills. The Sid enters the sea at the easternmost end of the town after receiving a number of tributary streams in its short course from its source in the hills within the urban boundaries. It flows at first through agricultural land, then through parkland and finally through a suburban area with numerous gardens and allotments. Reference will be made in a later section of the paper (pp. 151, 154) to the common practice of drawing water from the river to water the allotments on the river bank; at one point a pipe draws water from the river to a pump on an allotment site.

The village of Sidford, to which some of our investigations extended, is on the river $2\frac{1}{2}$ miles inland from Sidmouth, and Sidbury is a mile farther upstream.

The sewerage system of Sidmouth is shown diagrammatically in Fig. 1, with numerical references to important sampling points which will be mentioned in the account of our investigations. The sewer draining Sidbury enters the northeast corner of this area and is joined at manhole no. 27 by the sewage of Sidford. The common sewer then runs south on the right bank of the river, and at point no. 10 is passed through a large tank, from which a storm-water overflow enters the river itself. From tank no. 10 the sewer continues its southward course across the river bed, to run for some distance on the left bank of the river, and eventually re-crosses to the right bank to join the sewer which runs down the main shopping street of Sidmouth.

The main part of the town lies to the west of the river, in the angle between the Sid and the Woolbrook. Sewage from this built-up area is drained through three main sewers, which pass through manholes nos. 2, 3 and 25 respectively. Only a few of the smaller sewers are shown in Fig. 1; Figs. 2 and 3 are enlarged diagrams of the areas A and B of Fig. 1 in which individual houses were investigated.

The sewage of the whole area is finally disintegrated mechanically and discharged out to sea on the ebb-tide from a large storage tank on the sea front.

Table 1 is a list of the notifications of enteric fever in Sidmouth or in people infected at Sidmouth since 1938, with the Vi-phage type of the infecting strain where this was known. It will be seen that both typhoid and paratyphoid fever are endemic in the town.

In marked contrast to these figures it may be mentioned that Exmouth, for example, with a population one and a half times that of Sidmouth, has had no indigenous notification of enteric fever during the same period.

METHODS

Choice of sampling points

The great majority of swabs taken at Sidmouth were collected either from manholes on the sewerage system of the town and its adjacent villages or from the River Sid and its main tributary. When an enteric strain had been traced to a small group of houses, swabs were then taken from the individual house drains.

A necessary preliminary step to the swabbing of a particular area was local inspection to check the accessibility of suitable manholes and to ensure that

Table 1. Enteric infections notified from or associated with Sidmouth, 1930-50	Table 1.	Enteric in	<i>ifections</i>	notified	from or	associated	with	Sidmouth,	1930-50
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	No. of		Tone of accordance with Stantown, 1900 of
Year	cases	Diagnosis	Remarks
1930	3	Enteric fever	•
1936	1	Enteric fever	
1937	1	Enteric fever	•
1938	3	Typhoid fever	Single cases in June, July and September. No common factor established. Two had some association with river, viz. one keen swimmer, one angler
1939	2	Typhoid fever	Single cases March and April. No common factor found, except again possible association with river. In one household river water used for domestic purposes. Other case was a small boy who played near river
1941	2	Typhoid fever	Single cases August and October. Both Viphage Type F1. No common factor found
1942	4	Typhoid fever	Family episode. Vi-phage Type E1. Probably caused by carrier in household
1946	4 [.]	2 Typhoid fever	Both Vi-phage Type F1. No common factor found
		2 Paratyphoid B	Both Vi-phage Type 2. Two girl visitors at same hotel
1947	4	3 Typhoid fever	All Vi-phage Type F1. Three visitors to same hotel, including mother and son. Source of infection not found
		1 Paratyphoid B	Vi-phage Type 2. Day visitor who had ice- cream at a café. No further cases notified. Member of staff found to have unnotified paratyphoid B infection
1949	1	Paratyphoid B	Vi-phage Type 2. Child visitor to Sidmouth. See text on point no. 119
1950	3	Paratyphoid B	All Vi-phage Type 2. Single cases August, November, December. No common factor found

manhole covers could be raised readily when swabs were being laid. We had very little difficulty in this respect in Sidmouth, but have had some experience elsewhere of out-of-date sewer maps, and of manhole covers which were rusted firmly in position or had even been covered over during road-surfacing operations. On a few occasions we have wanted to take samples from a particular point on a certain sewer but found no suitable manhole through which swabs could be laid. This difficulty has been overcome by inserting a swab through the inspection chamber of the drain from a neighbouring house and pushing it right through into the sewer itself.

The course of the investigations described in this paper was dictated largely by the site of our first positive results, obtained from a swab placed in the River Sid. A rapid survey of the whole area carried out at a later stage was made by taking swabs from important junctional points on the sewerage system, i.e. where two or three sewers met at one manhole.

Collection of swabs

The swabs used in the early stages of our investigations were made from strips of gauze 4 ft. long and 6 in. wide, folded into a compact pad and trailed in the flowing sewage at the end of a length of stout twine, which was attached to the under-surface of the manhole cover or to some other convenient point in the manhole. These swabs caused some obstruction in small sewers, and we have since found that swabs of only one-quarter of the original length apparently give equally good results. Similar swabs were used to detect sewage pollution of the river; the attached twine was tethered to a low-lying branch by the river's edge and was suitably weighted to keep the swab in mid-stream.

The swabs were as a rule left in position for 48 hr. and then sent to the laboratory in sterile jars. Care was taken to prevent cross-contamination, particularly during the terminal stages of locating carriers.

Cultural methods

The techniques used for the isolation of enteric organisms from infected swabs were described in the earlier papers, but are again discussed here, partly for convenience and also to amplify certain points. The isolation of paratyphoid B bacilli was far easier than that of typhoid bacilli, and different cultural methods were required for each organism.

Isolation of paratyphoid B bacilli. During the earlier part of the survey, swabs were incubated overnight in a modified Leifson's selenite-F medium (Hobbs & Allison, 1945). Later, swab washings were found more satisfactory. After overnight incubation, the selenite cultures were plated on to a modified Hynes's (1942) deoxycholate-citrate medium containing, in addition to the normal ingredients, 0.5% mannitol and 0.02% lead acetate ('M.L.A.' medium). Paratyphoid B colonies had a characteristic morphology on this medium, well-isolated colonies being 2-3 mm. in diameter and showing a dark brown centre, grey or greyish pink periphery and a brown halo in the surrounding medium. When plates were kept for 24 hr. on the bench after initial overnight incubation at 37° C., paratyphoid B colonies became quite distinctive owing to the production of the 'mucoid wall' effect, which made the colonies larger in size and now showing a greyish white periphery and a dark brown centre.

It was found in practice that salmonella colonies were sometimes detected more readily on crowded M.L.A. plates by inspection of the undersurface of the medium for colonies with a dark-brown base.

Suspicious colonies were picked from the M.L.A. medium and confirmed biochemically and serologically.

Not all batches of the M.L.A. medium gave the characteristic colonial appearances described, and some batches found empirically to be unsatisfactory had to be discarded. We have not been able to define precisely the conditions necessary to ensure a greater uniformity in the performance of successive batches. Batch variations in the agar, peptone and lead acetate used and slight differences in the

pH of the final medium all contributed to the variations in colonial morphology found from time to time during the survey.

Isolation of typhoid bacilli. A combination of direct plating of appropriate dilutions of swab washings on to Wilson & Blair's medium with subculture of a selenite-F enrichment on to the same medium gave satisfactory results in the culture of typhoid bacilli from sewage. Each swab was washed with 20 ml. of nutrient broth. Three fivefold dilutions of the broth washings were then made and 0·1 ml. of each dilution plated on to Wilson & Blair's medium and spread with a glass spreader. The plating of successive dilutions was necessary to ensure adequate separation of the colonies on the medium, as typhoid colonies were otherwise not of characteristic appearance. Similar dilutions of the selenite enrichment cultures were also plated on to Wilson & Blair's medium. After 48 hr. incubation, all black colonies were spotted on to a Hynes medium containing sucrose as well as lactose. Confluent growth of typhoid bacilli on this medium was of characteristic appearance and gave slide agglutination with typhoid O serum. Strains were confirmed biochemically and serologically.

Bacteriophage typing of enteric strains

Although our primary concern in the Sidmouth survey was the elaboration of suitable cultural techniques for the isolation of enteric organisms from sewage, it was realized that we could not hope to interpret the epidemiological implications of results obtained without recourse to Vi-phage typing of the typhoid and paratyphoid B strains isolated. Accordingly, at least one strain from almost every sampling point which had yielded a positive culture was typed through the kindness of Dr A. Felix. The typing of Salmonella typhi was done according to the scheme of Craigie & Felix (1947), and that of Salm. paratyphi B according to the scheme published by Felix & Callow (1943, 1951).

PRELIMINARY INVESTIGATIONS

The first swabs were laid at Sidmouth in November 1946, into a manhole just proximal to the storage tank on the sea front. During the ensuing weeks further swabs were placed in other manholes so that samples from all the main sewers draining to the outfall were being examined. In April 1947, it was decided that an attempt should also be made to isolate enteric organisms from the River Sid, and a swab was therefore taken regularly from the river at point no. 6 (Fig. 1), some 50 yards below the point of discharge of the storm water overflow from tank no. 10, on the suspicion that sewage was regularly gaining access to the river along this pipe.

Between November 1946 and October 1947, forty-one batches of swabs, taken from one to four sites at a time, at intervals varying from 2 days to 4 weeks as opportunity offered, were examined bacteriologically. We used in these early trials a tetrathionate enrichment medium (Rolfe, 1946). None of 140 such cultures yielded an enteric strain. Parallel work in relation to another investigation then suggested that the technical methods already described for the isolation of paratyphoid bacilli might be more fruitful. Within a week of adopting the new

technique we isolated Salm. paratyphi B from a swab from the river sampling point no. 6, after an unbroken series of eighteen negative swabs at this point during the previous 6 months. The immediate success which followed the use of the selenite enrichment technique led to its exclusive use during the greater part of the investigation. Work done at a later stage showed, however, that although this method was satisfactory for the isolation of paratyphoid organisms, it was not suitable for the culture of typhoid bacilli from sewage (Moore, 1950). The greater part of the Sidmouth survey was therefore in effect concerned with paratyphoid B bacilli. Results obtained in this part of the investigations are for this reason discussed independently of the more restricted investigation which followed the fortuitous isolation of a typhoid strain from the sewage at one point and the subsequent discovery of better methods for the consistent isolation of this organism.

PARATYPHOID B SURVEY

Chronological sequence of investigations

The long series of negative swabs taken between November 1946 and October 1947 has already been described, and needs no further comment. With the isolation of paratyphoid B bacilli from the river at sampling point no. 6 (Fig. 1), a more deliberate planning of further specimens became possible. It was thought probable that the organisms had entered the river through the storm-water overflow from sampling point no. 10, and the first swab taken from this tank yielded paratyphoid B bacilli, thus indicating a paratyphoid B excreter in Sidford or Sidbury, or in the suburbs of Sidmouth which were drained through manholes nos. 14 and 16 (Fig. 1). While these possible lines of infection were being investigated, control swabs were maintained at point no. 6, and, in all, seven out of eight successive swabs from the river at this point yielded paratyphoid B bacilli.

Before any progress had been made in tracing the paratyphoid bacilli from tank no. 10 northwards, the position was complicated by the isolation of paratyphoid B bacilli from the Woolbrook, a tributary of the Sid, at point no. 8 (Fig. 1). Later Vi-phage typing was to show, however, that the organisms isolated from point no. 8 fell into paratyphoid B Vi-phage Type 1, whereas the strains isolated from points nos. 6 and 10 belonged to Type 2—a remarkable demonstration of the necessity for phage-typing in investigations of this kind.

The isolation of paratyphoid bacilli from three successive swabs at point no. 15, with negative swabs at points nos. 14 and 16, showed that the paratyphoid excreter north of no. 10 was apparently living in either Sidford or Sidbury. A series of negative swabs from manholes nos. 20, 22, 23 and 29, and seven successive positives from no. 28, narrowed the search for a paratyphoid excreter to a relatively small number of houses, and it was soon established that paratyphoid bacilli were reaching the sewers we had been investigating from a small council estate of twenty houses (Fig. 2). As these houses were council property there was no difficulty in examining specimens from the drains on a pretext of 'drain testing'. It was found that the north side of the estate was the source of infection, as a series of seven successive swabs from point no. 31 (Fig. 2) all yielded

paratyphoid B bacilli, while specimens from nos. 36–38 were negative. All that remained was the identification of a single household on the estate as the infective focus, and the location of a paratyphoid excreter by personal approach to the occupants. We found to our surprise, however, that all five of the manholes nos. 42–46 yielded two or three positive results in series, and that these results could not be attributed to cross-contamination of the swabs used. This finding

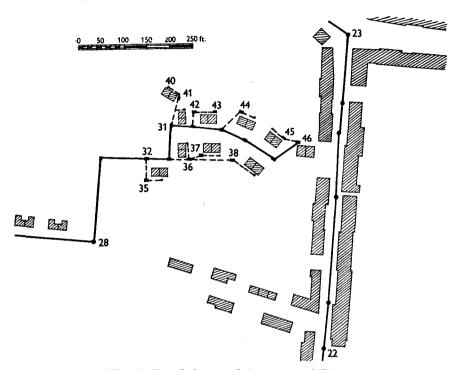


Fig. 2. Detailed map of the area A of Fig. 1.

was later shown to be due to the fact that the drains from the housing estate were very sluggish and that slight partial blockage at the lower end was sufficient to cause a backward flow of sewage to the higher points. It seemed very unlikely that we should find paratyphoid B excreters in so many adjacent households, and more detailed investigation of the area was almost abandoned at this stage. It was decided eventually to make a tactful approach to the residents on the estate, who were most co-operative and agreed to submit samples of faeces for examination. One paratyphoid B excreter was found at a house draining into sampling point no. 43. Details of this excreter are given in a later section of this paper (p. 147).

Meanwhile, an attempt was made to trace back to its source the paratyphoid B strain found in the Woolbrook stream. Sampling point no. 17 yielded four consecutive positives, while two points further up the stream, nos. 18 and 21 (Fig. 1), remained negative. It was found that just above point no. 17 a small trickle of water entered the stream from a storm-water overflow pipe coming from the parallel sewer at point no. 25. Swabs were therefore placed in this manhole, and

a series of ten out of eleven swabs from this point yielded paratyphoid B organisms. We assumed at the time that paratyphoid B bacilli were gaining access to the overflow pipe from the sewer passing through point no. 25 and contaminating the Woolbrook stream. Phage typing once again showed that this assumption was incorrect, as the strains from point no. 25 belonged to Vi-phage Type 2. The source of the Woolbrook strain remains unknown at the present time.

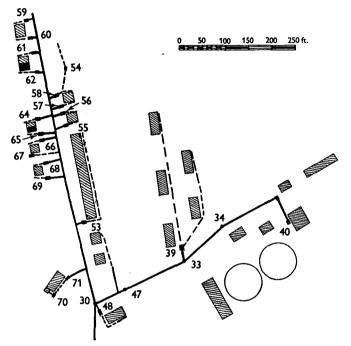


Fig. 3. Detailed map of the area B of Fig. 1.

The succession of positive results at manhole no. 25 led to further investigations in this part of Sidmouth (see Figs. 1 and 3). It soon became clear that at least the greater part of the infection reaching point no. 25 was coming down a sewer leading from point no. 30, which over a period of 2 months yielded twenty-two successive positive cultures for paratyphoid B bacilli. Point no. 30 was the junctional point of two subsidiary sewers, one of which drained a small number of private houses and also the sanitary conveniences at the local gasworks; this sewer could be swabbed readily through three manholes. The other sewer drained several houses, but was less easy of access as no manhole could be found; swabs had therefore to be taken from the inspection chambers on the drains from individual houses draining into this sewer.

The first investigation of the sewers leading to point no. 30 was done on the shorter sewer leading from the gasworks. This area yielded four positives in a fortnight's intensive swabbing, including one positive out of nine swabs at point no. 34 (Fig. 3), one out of eleven at point no. 39 and two successive positives out of seven samples taken from point no. 40, the men's lavatory at the gasworks.

Apart from the single positive at point no. 39, which was possibly due to the chance visit of a paratyphoid B excreter to one of the ten houses drained through this inspection chamber, the other positives were presumably from an excreter on the gasworks staff. We were unable to secure specimens of excreta from the persons concerned, so that this focus of infection was not identified more precisely.

It was clear that the consistently positive cultural results at point no. 30 could not be explained by the sporadic positives at points nos. 34, 39 and 40, and that there must be a profuse excreter along the course of the other sewer draining through point no. 30. Accordingly, each inspection chamber of drains leading into this sewer was swabbed twice, and positive results were obtained from points nos. 60 and 65. The results of further inquiry at these households are described below (pp. 147, 148); a chronic paratyphoid B carrier was found at no. 65.

By this time it was clear that the primary purpose of the survey had been realized; the feasibility of locating paratyphoid B carriers by serial examinations of sewage had been demonstrated. Circumstances made it impossible to continue our original policy of following each positive back until we found a paratyphoid B excreter, although we had so far failed to link any carrier with previous enteric cases in Sidmouth. In the limited time left for the completion of the survey we decided to make some attempt to estimate the number of individual paratyphoid B foci in Sidmouth. The map of the urban area was therefore arbitrarily divided into squares, each of which represented approximately 70 acres; and samples were taken from junctional sewer points in each square. Each swab was examined for paratyphoid B bacilli, but a special attempt was also made to find a typhoid strain, which had so far eluded us in this survey. The final investigation undertaken on these lines continued as opportunity offered from August 1948 to June 1949, and 330 swabs from over one hundred manholes were examined during this time. In December 1948, we isolated Salm. typhi from a certain manhole, and followed up this finding with investigations described in a later section of this paper (p. 149), and also previously in the paper by Moore (1950) on the cultural methods found suitable for the isolation of typhoid organisms from sewage. In addition to finding typhoid bacilli, we found evidence of at least three paratyphoid B foci within the urban area other than those already described. One of these unidentified foci, detected at sampling point no. 119, was possibly associated with a later case of paratyphoid B infected at Sidmouth; this episode will be referred to later (p. 148).

Vi-phage typing of paratyphoid B strains isolated

An arbitrary decision was made on the number of strains which should be sent to Dr Felix for Vi-phage typing, partly because we were more particularly concerned with cultural techniques than with epidemiological problems. But we were also faced with the difficulty that any one swab might well harbour paratyphoid B bacilli of more than one Vi-phage type, e.g. it seems very probable from the results quoted above that the swabs from the sampling point no. 6 in the River Sid frequently contained paratyphoid B bacilli of Types 1 and 2. To prove this would have required the testing of multiple paratyphoid colonies from

all cultures of swabs from no. 6. The application of such a policy to all swab cultures would have produced a massive collection of enteric strains. In the event, a series of forty-four strains, including at least one paratyphoid B strain from the great majority of positive sampling points, was submitted to Dr Felix, to whom we are much indebted for his report on this material. Forty of the strains examined fell into Vi-phage Type 2 and four into Type 1.

An estimate of the number of paratyphoid B foci in Sidmouth

Correlation of the results of Vi-phage typing with the location of positive cultural results on the sewer map of Sidmouth, and its adjacent villages, indicated the probable existence of at least two Type 1 and six Type 2 foci in the area. Only two of these foci were investigated adequately, and in each a chronic paratyphoid B carrier was discovered. The possibility that two apparently distinct foci of infection represented only a single excreter, living in one part of the town and working in another part, could not be excluded owing to the incomplete follow-up of positive results obtained in several areas in the town.

Results of investigation of single households

When a positive cultural finding required a personal visit to a particular household from which it appeared that paratyphoid B bacilli were being excreted, one of us (B.M.) who was not known in Sidmouth called at the household and made tactful inquiries. The following information on the results of such visits is given under the numbers of the various sampling points, the cultural results from which led to these further investigations being made.

Point no. 43. This inspection chamber drains the first house in which a paratyphoid B excreter was found by the sewage swab method. The house was occupied by the X family, comprising a father, mother and five children. They agreed to submit specimens of faeces for examination and Mrs X was found to be a profuse paratyphoid B excreter. Her strain fell into Vi-phage Type 2.

Mrs X gave no history of enteric fever nor of any other gastro-intestinal disturbance. Although she prepared all meals for her family, and the hygiene of the household was not particularly good, there was no evidence that she had at any time infected another member of the family. She had not worked away from home, and could not be linked in any way with previous paratyphoid cases in the district.

Paratyphoid B bacilli were first isolated from Mrs X's stools in February 1948, i.e. $3\frac{1}{2}$ months after the isolation of paratyphoid B bacilli from the River Sid at point no. 6 had started the investigation which led to her discovery. We were unable to obtain a specimen of blood to confirm her carrier state, but she was still excreting paratyphoid organisms in large numbers over $2\frac{1}{2}$ years later, so that there is little doubt that she is a chronic paratyphoid carrier.

The X family was offered, and accepted, T.A.B. inoculation as a protective measure against intrafamilial infection with the mother's strain.

Point no. 60. The positive cultural findings at this sampling point led to inquiries at a house occupied by an elderly widow and one lodger, a middle-aged

woman employed in a store in Sidmouth not concerned with the food-handling trade. Unfortunately, the owner became very alarmed when told that certain organisms in which we were interested had been isolated from the drain leading from her house. She refused to allow any discussion of the subject with her lodger, and when one sample of faeces submitted by herself had failed to yield paratyphoid bacilli, refused any further co-operation. She could at first give no relevant history, but then volunteered the interesting information that her son had stayed with her for some days 5 weeks previously and had suffered a severe attack of gastro-enteritis during his visit. It seemed possible that he had been an undiagnosed case of paratyphoid fever, but his refusal to submit any specimens of excreta for examination prevented any further investigation.

Point no. 65. This household was visited after two successive swabs from its drains had yielded paratyphoid B bacilli in large numbers. It was occupied by a young married couple, Mr and Mrs Y and their baby son of 18 months. Mrs Y gave a history of having had paratyphoid fever 7 years previously while serving in the A.T.S. during the war. She had been treated in an isolation hospital for 6 weeks and was still excreting paratyphoid bacilli on her discharge to an army hospital. An army laboratory had shortly afterwards reported a series of three clearance specimens of faeces as negative on culture, and she was then sent back to her unit.

A specimen of faeces from Mrs Y yielded paratyphoid B bacilli of Vi-phage Type 2. She had thus been a carrier for 7 years. A follow-up specimen examined recently, $2\frac{1}{2}$ years later, still yielded paratyphoid bacilli in large numbers.

Mrs Y was a woman of considerable intelligence and of good personal hygiene. She had not been connected with the food-handling trade in Sidmouth, and had apparently not infected her family although she cooked all their meals.

Point no. 119. It is convenient to discuss at this point the isolation of paratyphoid bacilli from a manhole sampled shortly before the end of our survey, although it did not lead to the investigation of a single household nor to the finding of an enteric carrier. The sewage sampled at this point came from sixty-eight houses, and no attempt could be made to locate an enteric carrier in one of them. Five months later, a child living in North Devon who had just spent a fortnight's holiday in Sidmouth went down with paratyphoid fever. Inquiry showed that she had stayed at one of the houses in the group draining through no. 119. Unfortunately, circumstances have so far prevented us from determining whether this child might have been infected by the carrier of the strain isolated from point no. 119.

Isolation of paratyphoid organisms from river water

As the isolation of enteric organisms from river water is generally considered to be technically difficult, a few words on our experiences in this investigation may be justified. Without knowing the degree or the regularity of pollution, we could not assess whether our results in isolating paratyphoid bacilli from the River Sid and the Woolbrook stream were completely satisfactory or not. From some sampling points, however, consistently positive cultures were obtained. Thus,

after the adoption of a selenite enrichment technique, seven out of eight successive swabs from point no. 6 (Fig. 1) yielded paratyphoid bacilli; swabbing at this point was then discontinued. Our positives were less consistent at point no. 8, the lowest sampling point on the Woolbrook stream. The first two swabs taken at this point were negative. We then had nine successive positive swabs over a period of 3 weeks, followed by intermittent success with only four out of eighteen swabs positive over a period of 7 weeks. Point no. 17, the other sampling point in the Woolbrook stream at which paratyphoid bacilli were isolated, was swabbed four times and all four swabs yielded paratyphoid B bacilli.

Finally may be mentioned a small experiment done on the initiative of Mr R. W. Pinney, Assistant Sanitary Inspector. It had been decided to divert the sewage from tank no. 10 (Fig. 1) into the River Sid between midnight and 4 a.m. of one night in February 1948, so as to test for infiltration of surface water into the sewerage system in this area. Swabs were placed in the River Sid at points nos. 49–52, and sent for culture on the day after the sewage diversion. Salm. paratyphi B of Vi-phage Type 2 was isolated from points nos. 49, 51 and 52; and Salm. typhi-murium of Vi-phage Type 4 from point no. 50.

LOCATION OF A TYPHOID CARRIER

As already mentioned, the final rapid survey of the whole of Sidmouth which was undertaken to estimate the number of distinct paratyphoid foci in the town, and also to find typhoid carriers if possible, lasted from August 1948 to June 1949. The isolation of a typhoid strain from one sampling point, and the subsequent investigation of suitable methods for the consistent isolation of typhoid bacilli from sampling points from which the organism had been cultured, have been previously described (Moore, 1950). This brief section describes the course of these investigations.

Salm. typhi Vi-phage Type F 1 was first isolated from sampling point no. 137 (Fig. 4), on an old sewer running along the river bank and draining the sewage from approximately 120 houses. Suitable cultural techniques enabled us to follow this result back to a single household, point no. 153, from which the organism appeared to be coming and where a typhoid carrier was found. The course of this work is summarized in Table 2 and Fig 4. When the laboratory findings suggested that a typhoid excreter would be found in the terrace 148–55 (Fig. 4), a swab was passed through the inspection chamber no. 148 into the main sewer, and the culture of typhoid bacilli from this swab confirmed that the organism was coming from some house in the terrace. Two subsequent swabs placed in inspection chamber no. 148 so as to sample sewage coming from the house served by this drain alone and not the main sewer as well were negative for typhoid organisms. These findings confirmed the usefulness of the method of sampling sewers by passing swabs into them through the inspection chambers on drains of individual houses.

Results of investigation of point no. 153

The house from which it had been shown that typhoid bacilli were being excreted was occupied by the Z family, which comprised a father, mother and four children.

This family was most co-operative and agreed to submit specimens of excreta for examination. The father was found to be an excreter of typhoid bacilli of Vi-phage Type F 1, the endemic type in Sidmouth.

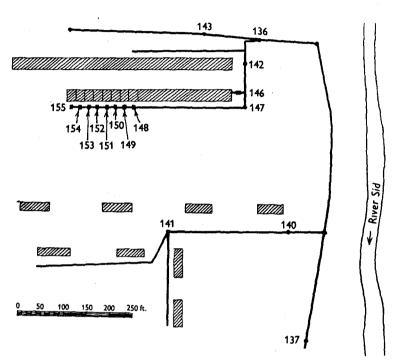


Fig. 4. Map showing the sewer points tested in locating a typhoid carrier.

Table 2. Location of a typhoid carrier
(Results of examination for Salm. typhi of swabs taken from various manholes.)

Date of sampling

														_
Sampling point, no.	1948			1949										
	9. xii	14. xii	20. xii	29. xii	4. i	6. i	10. i	12. i	17. i	20. i	24. i	27. i	31, i	3. ii
136			+	+	+	+	+	+	+	+	+	+		
137	+		·	_	+	+	+	+	+					
140			_	_		_								
141		•	-	_	_	_								
142				•			+	+	+	+	+	+	•	+
143							_	_	_					
146										_	•	•		•
147										+	+	+		•
148		•										+*		
149									•			•	-	_
150									•			•		
151					•				•			•	\	-
152						•			•	• ,	•	• .	-	-
153									•	•	•	•	+	+†
154				•			•		•	•	•	•	-	-
155					•						•	•	-	-

^{*} Swab pushed through house drain into main sewer.

[†] Drain of house where typhoid carrier found.

Mr Z was a man aged 45, employed in the transport services and not connected in any way with the food-handling trade. He had lived in Sidmouth since 1936. He could give no history of enteric infection, nor could he remember having at any time suffered from an undiagnosed disease of enteric type. He had spent 3 years in Brazil as a boy and may have been infected during that time.

Samples of blood from Mr Z could not be obtained for serological examination, but a follow-up specimen of faeces examined 2 years after the initial positive still yielded typhoid bacilli.

We were unable to link Mr Z in any way with the F 1 typhoid cases which occurred in Sidmouth between 1941 and 1949, and it is of course possible that he himself was infected with typhoid after coming to live in Sidmouth and became a chronic carrier without undergoing any clinical attack of enteric fever.

A recent finding which may conceivably link Mr Z with the endemic incidence of typhoid in this area has been the discovery that the old sewer, sampled at point no. 137 to give our first typhoid positive and running along the river bank, is made up of unjointed lengths of piping placed in apposition and that some seepage of sewage occurs which may possibly contaminate the river. As mentioned in an earlier section (p. 139), river water is extensively used to water the allotments by the river side below this point, and it is therefore possible that vegetables occasionally become contaminated with Mr Z's typhoid strain.

DISCUSSION

The development of a method for locating excreters of pathogenic organisms without widespread individual examination of the whole population of a given area has furnished an epidemiological tool of potential value for the investigation of any infection the causative organism of which is excreted in sewage.

By far the greater part of the time spent on the survey described in this paper was devoted to the preliminary work of finding suitable cultural methods for the isolation of paratyphoid and typhoid bacilli from sewage, a problem which other experiments had shown us could not be satisfactorily solved by artificial inoculation of sewage in the laboratory with stock cultures of enteric strains. We were thus hampered for almost a year by lack of a satisfactory method for culturing paratyphoid bacilli from sewage, and typhoid bacilli were isolated for the first time over 2 years after the beginning of the survey. Circumstances then prevented the completion of an adequate study of the Sidmouth area with the cultural methods found to be suitable. Hence only a very tentative discussion is yet possible of the techniques used at Sidmouth and of the results of the survey in relation to the long record of enteric infections in the town.

The only criterion by which the efficiency of the methods used in the Sidmouth survey can so far be judged is that of success in the location of enteric carriers. Three previously unsuspected carriers were found, and the detection of enteric carriers by these methods has also been described by Moore (1948) and by Lendon & Mackenzie (1951). No serious attempt has yet been made, however, to judge whether considerable improvement in the methods used may not be possible. The isolation of enteric organisms from a swab taken from any given sampling

point probably depends on many factors besides the presence of an enteric carrier in the area under investigation, such as, for instance, the size and composition of the swabs used, the laboratory cultural methods, the size of the population represented by the swab sample, whether the carrier excretes profusely or intermittently, and the possible effect of the presence of disinfectants or other toxic products in the sewage examined. Apart from the cultural side, none of these factors has so far been adequately investigated. It would be useful, for instance, to examine frequent specimens of faeces from a known carrier in parallel with swab samples taken at increasing distances from his home, so as to check the consistency of swab positives in relation to excretion of the enteric strain by the carrier, and also to assess more critically the value of the cultural methods used. Little information is available on these points.

Analysis of our results in relation to the population drained through manholes from which enteric organisms were isolated showed that regular isolation was possible from communities of up to about 100 houses harbouring a single carrier, and that the paratyphoid B strain traced back to a single carrier in Sidford was isolated from the sewage of about 1500 to 2000 people. Obviously, swabs placed in larger sewers may yield false negative results for particular sampling points merely through failure of contact between the swab and the enteric organisms passing along the sewer. In surveying communities of moderate size, therefore, we should now prefer to start if possible by swabbing peripheral groups of 50–100 houses at a time rather than depend on the findings on swabs from larger main sewers.

The results of sewage sampling may be affected by the presence of disinfectants or other toxic substances in the sewer. Thus, when on one occasion we had negative cultural results from swabs taken from previously positive manholes, it was shown that gas liquors had been discharged into the sewer during the time when the swabs were present.

The cultural methods found suitable for the isolation of paratyphoid and typhoid bacilli from sewage can certainly not be considered ideal, although they have served their purpose adequately in this survey. Each swab required painstaking investigation, and the methods used were time-consuming and could not be readily applied to more than a small number of samples at a time. On the solid media used, the colonial appearances of paratyphoid and typhoid bacilli were fairly characteristic, so that little screening of colonies was done. As we were not particularly concerned about the lag in isolation of enteric strains after receipt of the swab in the laboratory, likely colonies were either picked on to agar slopes for slide agglutination or on to the lactose-sucrose deoxycholate-citrate medium. These methods worked satisfactorily. During the course of the survey the papers of Cook (1948) and of Elek (1948) on the use of the urease test as a screening procedure in the isolation of intestinal pathogens appeared, but for the reasons stated this test was not used by us, useful though it might have been if we had wished to test a large number of swabs at a time. The main surprise about the cultural methods used was the relative inefficiency of the methods of fluid enrichment culture. A reliable fluid medium for the enrichment culture of enteric organisms from sewage would make surveys of the type here described very much easier to conduct.

Before considering the relationship between the survey findings and our previous knowledge of enteric fever in Sidmouth, it should be stressed that, though we have so far discussed only the factors affecting the successful culture of enteric organisms from sewage, no valid epidemiological interpretation of the findings of a survey can be made without the further technical procedure of Vi-phage typing of all typhoid and paratyphoid B strains isolated. The practical value of Vi-phage typing will then depend to some extent on the number of Vi-phage types demonstrated in the community investigated. At Sidmouth, for instance, Vi-phage typing of the paratyphoid B strains isolated there has shown that, although Type 2 is the predominant type according to both our survey findings and the typing of case strains, Type 1 bacilli are present in at least two areas of the town. In any further work at Sidmouth, therefore, apart from the question of the incursion of strains of other Vi-phage types into the area, it will always be necessary to determine if a given paratyphoid B strain falls into Type 1 or Type 2. On the other hand, we have demonstrated so many probable foci of Type 2 infection in the district that it can no longer be assumed that two or more paratyphoid B strains isolated from this area and shown to fall into phage Type 2 necessarily bear a direct epidemiological relationship to one another. Vi-phage typing of enteric strains has been of the greatest value in the epidemiological investigation of enteric fever in the county of Devon as a whole (Felix, 1943; Cruickshank, 1947). It cannot be expected to play such an important role in attempting to solve the epidemiological patterns in more localized areas where strains of one Vi-phage type have become as dominant as have paratyphoid B bacilli of Type 1 in North Devon or of Type 2 in Sidmouth.

We come now to discuss the relationship of the survey findings to the history of endemic infections in Sidmouth. It has long been known that after an epidemic of enteric fever in any area there is likely to be residual infection due to persistent carriage of the enteric strain in the population or to a continued cycle of subclinical infections. Thus, after the paratyphoid B epidemic in Epping in 1931, every Annual Report of the Ministry of Health between 1931 and 1938 recorded that paratyphoid B bacilli were still being discharged in the sewage effluent of the town. We do not know of any previous attempt to obtain a more comprehensive picture of latent infection in an endemic area. In this survey we have shown that in an urban area of about 10,000 inhabitants in which sporadic cases of enteric fever have occurred during the past 15 years, there are at least nine apparently distinct foci of infection. Only three of these have been precisely located and in each case we have found a chronic paratyphoid or typhoid carrier. The two paratyphoid carriers have been found to excrete paratyphoid B bacilli of Vi-phage Type 2 and the typhoid carrier harboured typhoid bacilli of Vi-phage Type F 1. These types of enteric organisms have also been the types associated with cases of paratyphoid and typhoid infection in the Sidmouth area. It is possible that we have missed several typhoid excreters in the area, because the appropriate cultural methods were evolved at a late stage of the survey and were applied to only a small number of sewage swab samples.

We have unfortunately not yet succeeded in tracing by the sewage swab method

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the source of infection of any of the enteric cases which have occurred in Sidmouth in recent years. It is hoped that investigations to this end may be resumed in due course.

It is interesting to speculate on why Sidmouth has been so much more unfortunate in its experience of enteric fever during the past 10-15 years than other Devon towns of similar size. Is this associated with the proximity of a contaminated river? The evidence on which this suggestion is founded may be briefly considered. First of all, if one excludes those larger enteric outbreaks which at once point to the infection of some food vehicle by a known or unknown enteric carrier, we have been impressed by the association of sporadic cases of enteric fever in Devon with areas where the population is not fully protected against the likelihood of contact with enteric organisms. The gap in the defences may be an infected river, as at Sidmouth, or an unsatisfactory sewage outfall on to a populous beach, or a tidal estuary on a river into which crude sewage is being discharged. Only once, in a North Devon case of typhoid fever in a boy of 6, was the evidence of direct infection from such a source fairly conclusive. Martin (1947), however, traced infections with paratyphoid B bacilli of Vi-phage type 'Beccles' to pollution of the river Waveney with sewage from septic tanks in Beccles. More recently, Lendon & Mackenzie (1951) have given an interesting description of a river shown to contain typhoid bacilli and associated with a number of cases of typhoid fever in persons who had drunk the river water. It would seem possible that in areas where such contact with enteric organisms can occur there may be a continued circulation of minor undiagnosed enteric infections. The sporadic notified case of enteric infection and the occasional epidemic should then be considered as only outcrop indications of a substratum of subclinical infections by which the enteric organisms continue to circulate in the community. We have obtained suggestive evidence in the Sidmouth survey of this extensive prevalence of infection with enteric organisms in the absence of notified cases of enteric fever.

At Sidmouth, contact with enteric organisms in the river might occur either directly, along the course of the river or where it enters the sea, or indirectly, as for example, through vegetables watered with river water. It may be recalled that long before the demonstration of enteric organisms in the River Sid it had been noted that a series of typhoid cases in 1938 and 1939 seemed to have a common link in some association with the river, through fishing, bathing or the use of river water for domestic purposes. The record of sporadic enteric infections in Sidmouth is also of some interest in this regard. Since 1938, there have been eleven notified typhoid episodes in the area. One of these was a family outbreak, due to Vi-phage Type E 1, caused by a chronic carrier in the family itself. Another episode involved three visitors to Sidmouth staying at a certain hotel. The remaining nine were isolated single cases. Similarly, of six paratyphoid B cases notified in Sidmouth during the past 5 years, two were related cases in friends staying at the same hotel; the remaining four were single cases. Only in the family outbreak of typhoid fever due to a carrier in the family itself did investigation of household contacts reveal an excreter of enteric organisms. It is possible that this long sequence of minor enteric episodes, with no serious epidemic so far, is associated with exposure of the population to some relatively minor hazard such as the pollution of the River Sid which, although not a serious risk at any one time, causes occasional enteric infections directly or indirectly. If further investigation at Sidmouth should tend to confirm the importance of contamination of the River Sid as a factor in the causation of sporadic enteric infections in the area, the question would then arise whether steps to block the sources of pollution should be taken. It should be easy enough by swabbing to determine all points of entry of enteric organisms into the River Sid and its tributaries, and to identify in due course all the enteric carriers concerned. A much more difficult problem would then be how to prevent access of enteric organisms from these excreters to the river. The obvious solution of requiring enteric carriers to use chemical closets would be very difficult to enforce. Disinfection of excreta coming from particular households by some modification of the house drains might be feasible. Much work remains to be done at Sidmouth before the clearing of the river from pollution with enteric organisms needs serious consideration.

In this paper we have merely described the preliminary application of a technique which may prove of some value in the investigation of intestinal infections. Its main practical use in relation to enteric fever may well be the investigation of enteric outbreaks which have not been explained by the usual methods of epidemiological inquiry, and particularly of sporadic cases in an endemic area with no obvious common source of infection. A number of other possible applications of the sewage swab method on which preliminary work has already been done may be mentioned very briefly. F. O. MacCallum (personal communication) is already using the swab method for the detection of the poliomyelitis virus in bulked sewage in an attempt to determine the distribution of infection throughout the population. The use of the swab method is also being studied in relation to the epidemiology of bacillary dysentery and of sporadic salmonella infections. Finally, the experimental use of regular sewage swab examination as a check on the prevalence of intestinal infections among members of the staffs of large food-handling establishments is being considered.

SUMMARY

By a combination of a swab sampling method, modern cultural techniques and Vi-phage typing, a survey for enteric organisms has been made of the sewerage system of a town of about 10,000 inhabitants.

The location of typhoid and paratyphoid B carriers by systematic swabbing of sewers in accordance with cultural findings has been proved feasible.

Two paratyphoid B carriers and one typhoid carrier were discovered by this method. Evidence was obtained of the presence of at least six other foci of paratyphoid infection in the town.

Paratyphoid bacilli were repeatedly isolated from a river flowing through the town. The possible role of river pollution in the maintenance of endemic enteric infection in the area is discussed.

The methods used in this survey might be applied with advantage to the

elucidation of unsolved enteric outbreaks, to the control of food-handling establishments and to epidemiological studies of other infections in which the causative organism gains access to sewage.

We are indebted to Mr R. W. Pinney and Mr W. E. Coles, who did by far the greater part of the work of laying the sewage swabs and sending them to the laboratory; to Mr L. M. Blanchard, Surveyor, Sidmouth U.D.C., for his assistance with the preparation of maps of the area; to Dr A. Felix for his kindness in typing the enteric strains isolated; and to Mr R. N. Brown and Mr S. Barlow for much assistance in the development of cultural techniques.

REFERENCES

- COOK, G. T. (1948). Urease and other biochemical reactions of the Proteus group. *J. Path. Bact.* **60**, 171-81.
- Craigle, J. & Felix, A. (1947). Typing of typhoid bacilli with Vi bacteriophage. Suggestions for its standardization. *Lancet*, 1, 823-7.
- CRUICKSHANK, J. C. (1947). Typhoid fever in Devon. The value of phage-typing in a rural area.

 Mon. Bull. Minist. Hlth Publ. Hlth Lab. Serv. (directed by Med. Res. Council), 6, 88–96.
- ELEK, S. D. (1948). Rapid identification of Proteus. J. Path. Bact. 60, 183-92.
- Felix, A. (1943). Experiences with typing of typhoid bacilli by means of Vi bacteriophage. *Brit. med. J.* 1, 435–8.
- Felix, A. & Callow, Bessie R. (1943). Typing of paratyphoid B bacilli by means of Vi bacteriophage. *Brit. med. J.* 2, 127–30.
- Felix, A. & Callow, Bessie R. (1951). Paratyphoid-B Vi-phage typing. Lancet, 2, 10.
- Hobbs, Betty C. & Allison, V. D. (1945). Studies on the isolation of *Bact. typhosum* and *Bact. paratyphosum* B. *Mon. Bull. Minist. Hlth Publ. Hlth Lab. Serv.* (directed by Med. Res. Council), 4, 63–8.
- HYNES, M. (1942). The isolation of intestinal pathogens by selective media. J. Path. Bact. 54, 193-207.
- LENDON, N. C. & MACKENZIE, R. D. (1951). Tracing a typhoid carrier by sewage examination.

 Mon. Bull. Minist. Hlth Publ. Hlth Lab. Serv. (directed by Med. Res. Council), 10, 23-7.
- MARTIN, P. H. (1947). Field investigation of paratyphoid fever with typing of Salm. paratyphi B by means of Vi bacteriophage. Mon. Bull. Minist. Hlth Publ. Hlth Lab. Serv. (directed by Med. Res. Council), 6, 148-51.
- MOORE, B. (1948). The detection of paratyphoid carriers in towns by means of sewage examination. *Mon. Bull. Minist. Hlth Publ. Hlth Lab. Serv.* (directed by Med. Res. Council), 7, 241-8.
- MOORE, B. (1950). The detection of typhoid carriers in towns by means of sewage examination. *Mon. Bull. Minist. Hlth Publ. Hlth Lab. Serv.* (directed by Med. Res. Council), 9, 72-8.
- Rolff, V. (1946). A note on the preparation of tetrathionate broth. Mon. Bull. Minist. Hlth Publ. Hlth Lab. Serv. (directed by Med. Res. Council), 5, 158-9.

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