

## AN OUTBREAK OF WATER-BORNE TYPHOID INVESTIGATED BY BACTERIOPHAGE TYPING AND 'SELECTIVE' SEWAGE EXAMINATION<sup>1</sup>

BY P. G. H. GELL, M.B., BETTY C. HOBBS, B.Sc., PH.D., DIP.BACT.  
AND V. D. ALLISON, M.D., D.P.H.

*From the Central Emergency Public Health Laboratory, Cardiff and the Cardiff  
and County Public Health Laboratory*

(With 1 Figure in the Text)

The application of the Vi bacteriophage method of Craigie & Yen (1938*a*, 1938*b*) to the differentiation of 440 strains of typhoid bacilli isolated in England, Wales and Scotland during 1940-2 has recently been described by Felix (1943), and evidence has been given of the reliability of the bacteriophage test for the investigation of outbreaks of sporadic cases of typhoid fever. Bradley (1943) has shown how phage typing has enabled a series of apparently sporadic cases of typhoid fever, widely separated in time and distance, to be linked up and traced to a common source.

This paper records an investigation which further confirms the value of routine typing of strains of *Bact. typhosum* in helping to determine the source of infection of sporadic cases of typhoid fever. It may also be of interest in demonstrating a method of locating carriers, especially in closed communities, by means of 'selective' sewage examination, and a technique used for the examination of samples of faeces from a large number of persons for the detection of individual carriers.

### PRIMARY CASES

The identification of the phage types of all the strains of *Bact. typhosum* isolated during the investigation from samples of water, sewage effluent, sewage, faeces and blood was carried out by Dr Felix. The cases of typhoid germane to the investigation were three in number, sporadic, and all occurred in the area of one local authority during 1942. The first case, Ju.S., a milliner's assistant aged 17 years, was admitted to a general hospital suffering from acute abdominal symptoms at the end of May 1942, where a diagnosis of typhoid fever was made and *Bact. typhosum*, type E.1, was isolated from her stool; her illness ran a fairly short

typical course and she was discharged from hospital on 30 July, samples of faeces and urine having been found negative on four consecutive occasions between 25 June and 27 July. The source of her infection was not traced, but, as will be seen later, it was different from that which gave rise to the succeeding cases.

The second case occurred in a farm worker, A.W., aged 15 years, who developed typhoid fever on 11 September and was admitted to the local isolation hospital on 23 September. His illness was straightforward although typical symptoms were rather slow in developing. *Bact. typhosum*, type C, was isolated from his stool. On inquiry it was found that he had been for a country walk on Sunday, 30 August, with another boy of his own age, when both had drunk water from a stream near a village and about 2 miles from a town. The other boy also developed gastro-intestinal symptoms about the same time, but he was not sent to hospital and no specimens could be obtained for laboratory examination. Inquiry showed that the stream was subject to pollution by the sewage from a large institution, the effluent of which, after treatment, discharged into it about 1 mile above the point at which the boys had drunk the water; further upstream the overflows from cesspools of houses and farms abutting on the stream were additional sources of pollution. Arrangements were therefore made to examine bacteriologically samples of the sewage effluent from the institution and of water from the stream.

While this was under way, specimens were sent for examination from Jo.S., aged 18 years, who was admitted to the isolation hospital on 25 September. Jo.S. was a nurse employed in the institution, the sewage effluent from which discharged into the stream. She first developed symptoms, malaise and headache, on 15 September, and *Bact. typhosum* was isolated from her faeces on 1 October, and from

<sup>1</sup> A Report to the Medical Research Council.

blood culture on 9 October, although agglutination tests had suggested that she was suffering from paratyphoid fever. This girl was a sister of Ju.S., the first case in the series, mentioned above, who had had typhoid in June. The infection in Jo.S. was at first naturally considered to be associated with that of her sister, with whom she was in contact from time to time, and it was assumed that the sister was still a carrier, or that the two girls had derived their infection from a common and still active source. However, the strain of *Bact. typhosum* isolated from Jo.S. was identified as type C, and as her sister had been infected with type E1, suspicion of the source of infection of the former was now diverted to the institution; attempts to investigate her home contacts were therefore deferred until samples of the stream water and sewage effluent from the institution had been examined bacteriologically.

#### THE SOURCE OF INFECTION

On 28 September samples of (a) the stream water where A.W. and his companion had drunk, (b) sewage effluent near the outfall to the stream, and (c) crude sewage from the institution, were examined bacteriologically. The samples were extremely dilute, as they had been taken after heavy rains, and as will be seen from Table 1 (nos. 1-3) all three yielded *Bact. paratyphosum* B, type 1, only. However, similar specimens (nos. 5-7) collected 7 days later on 5 October were all found to contain *Bact. typhosum*, and *Bact. paratyphosum* B was also isolated from the crude sewage. A specimen (no. 8) of water taken from the stream above the point where the effluent discharged was also examined, as it was here open to contamination from cesspools, but results were negative. A specimen of water (no. 4) taken some 4 miles downstream near the town was also negative, showing that either purification had taken place or dilution was such that the organism could not be isolated by the laboratory technique employed. The strains of *Bact. typhosum* isolated from the stream, sewage effluent and crude sewage were all identified as type C. It will be noted that *Bact. typhosum* was isolated from samples of the stream, sewage effluent and crude sewage taken 10 days after Jo.S. had left the institution to be admitted to the isolation hospital. The fact that Ju.S. had also had typhoid recently, due to type E1, must be regarded as purely coincidental, as there is ample evidence (Felix, 1943) to show that the types are fixed and not interconvertible. It should be emphasized that if the strain isolated from Ju.S. had not been typed as a routine procedure and recorded, it would not have been possible to show that the infections in the two sisters were quite unrelated.

Risk of further infection from the sewage effluent was eliminated by the construction by the institution authorities of an impromptu but simple and effective method of treatment, whereby the effluent was made to seep through a small metal chamber containing chloride of lime. The effluent was subsequently examined and was found to be sterile (no. 14).

Investigation of the source of infection in the institution was more difficult, as no clinical case of typhoid fever had been noted in the records for over 20 years, and no typhoid carriers were known or suspected. Moreover, the institution contained some 2000 individuals including inmates and staff. It was therefore decided to attempt to locate the source of the typhoid organisms by sampling the crude sewage from the various blocks of the institution. No complete plan of the drainage system was available, so the County Sanitary Inspector gave much time and valuable assistance in plotting the system by means of fluorescein tests and in finding the most suitable points at which to take specimens. Fig. 1 shows the drainage system and course of flow of the sewage effluent into the stream, and indicates the points at which the various samples of water, sewage effluent and crude sewage were collected.

The institution is divided into male and female sides, the former consisting of four blocks and the latter of five blocks with three floors to each block; the two upper floors each form a ward and the ground floor is a day room used by both wards jointly. The w.c.'s are in groups of eight on each floor, draining into a common soil pipe. Once, therefore, a block was implicated, it would be impossible to narrow the field further by the examination of sewage from the two wards separately. The method of sampling adopted was to block the inlet pipe from a ward block at the selected inspection chamber at 7 a.m. and to take a specimen at about midday. Numerous and repeated examinations of sewage from different blocks were carried out and the main findings are shown in the Table, from which it will be seen that *Bact. typhosum*, type C, was isolated from the sewage of wards 3 and 4 (no. 19) and *Bact. paratyphosum* B, type 1, from the sewage of wards 5 and 6 (no. 20), both on the female side. Samples of sewage from the remaining blocks on the female side and from all the blocks on the male side proved negative (nos. 11, 12, 16, 17, 18 and 21).

The sewage from female wards 7 and 8 was examined with negative results (no. 16) at an early stage, as owing to a misunderstanding it had been thought that Nurse Jo.S. had been working in wards 7 and 8 when she became infected at the end of August. It was later discovered that she had been working in wards 3 and 4 and was transferred

## Investigation on water-borne typhoid

Table 1. Result of bacteriological examination of samples of water, sewage effluent and sewage

No.	Date	Specimen	Place of sampling (see Fig. 1)	Result (organism isolated and phage type)	Comments
1	28. ix. 42	Stream water	A	Para. B, type 1	Taken at point where A.W. had drunk
2	28. ix. 42	Sewage effluent	B	Para. B, type 1	All specimens taken on this date very dilute owing to flooding with storm water
3	28. ix. 42	Crude sewage	C	Para. B, type 1	
4	5. x. 42	Stream near town	D	—	About 4 miles downstream from point of discharge of sewage effluent
5	5. x. 42	Stream near village	A	Typhoid, type C	Taken at point where A.W. had drunk about 2 miles downstream from point of discharge of sewage effluent
6	5. x. 42	Sewage effluent	B	Typhoid, type C	
7	5. x. 42	Crude sewage	C	Typhoid, type C, Para. B, type 1	
8	6. x. 42	Stream above inflow of sewage effluent	E	—	Taken about 100 yd. above point where effluent discharged into stream
9	8. x. 42	Do.	E	—	
10	8. x. 42	Sewage effluent	B	Typhoid, type C, Para. B, type 1	
11	8. x. 42	Male wards 5-8	F	—	
12	20. x. 42	Male wards 1-4	G	—	
13	27. x. 42	Sewage effluent	B	Typhoid, type C, Para. B, type 1	Taken before passing chlorinating filter
14	27. x. 42	Treated effluent	B	Sterile	Taken after passing chlorinating filter
15	27. x. 42	Female wards 5-6	H	Para. B, type 1	
16	27. x. 42	Female wards 7-8	I	—	
17	27. x. 42	Female ward 10	J	—	
18	3. xi. 42	Female wards 1-2	K	—	
19	3. xi. 42	Female wards 3-4	L	Typhoid, type C	
20	3. xi. 42	Female wards 5-6	H	Para. B, type 1	
21	3. xi. 42	Female wards 7-8	I	—	
22	9. xi. 42	Female wards 3-4	L	Typhoid, type C	Specimens from 158 inmates examined; six carriers of <i>Bact. typhosum</i> found (one type A, five type C)
23	9. xi. 42	Crude sewage	C	Typhoid, type C, Para. B, type 1	Taken prior to discovery of carriers in wards 5-6
24	26. i. 43	Female wards 3-4	L	—	Taken after carriers had been discovered and transferred
25	26. i. 43	Female wards 5-6	H	Typhoid, type C, Para. B, type 1	Specimens from 136 inmates examined; one carrier of <i>Bact. paratyphosum</i> B found and five carriers of <i>Bact. typhosum</i> (three type A, two type C)
26	26. i. 43	Crude sewage	C	Para. B, type 1	
27	17. iv. 43	Female wards 3-4	L	—	Taken after carriers had been discovered and transferred
28	17. iv. 43	Female wards 5-6	H	—	
29	11. v. 43	Effluent	B	—	
30	11. v. 43	Crude sewage	C	—	
31	21. vi. 43	Effluent	B	—	

to wards 7 and 8 at the beginning of September; as her first symptoms appeared on 15 September, she had therefore an incubation period of at least 15 days.

THE DETECTION OF CARRIERS

An investigation of specimens of stool from the 158 inmates in wards 3 and 4 was undertaken. Previous

inquiry had failed to reveal any suspicious history of typhoid, recent or remote, in a single case. It was therefore necessary to examine the stools of all the inmates, but for motives of economy of media, the specimens were bulked in batches of six or seven, and when *Bact. typhosum* was isolated from any one batch, the individual specimens comprising that batch were examined singly. The few days

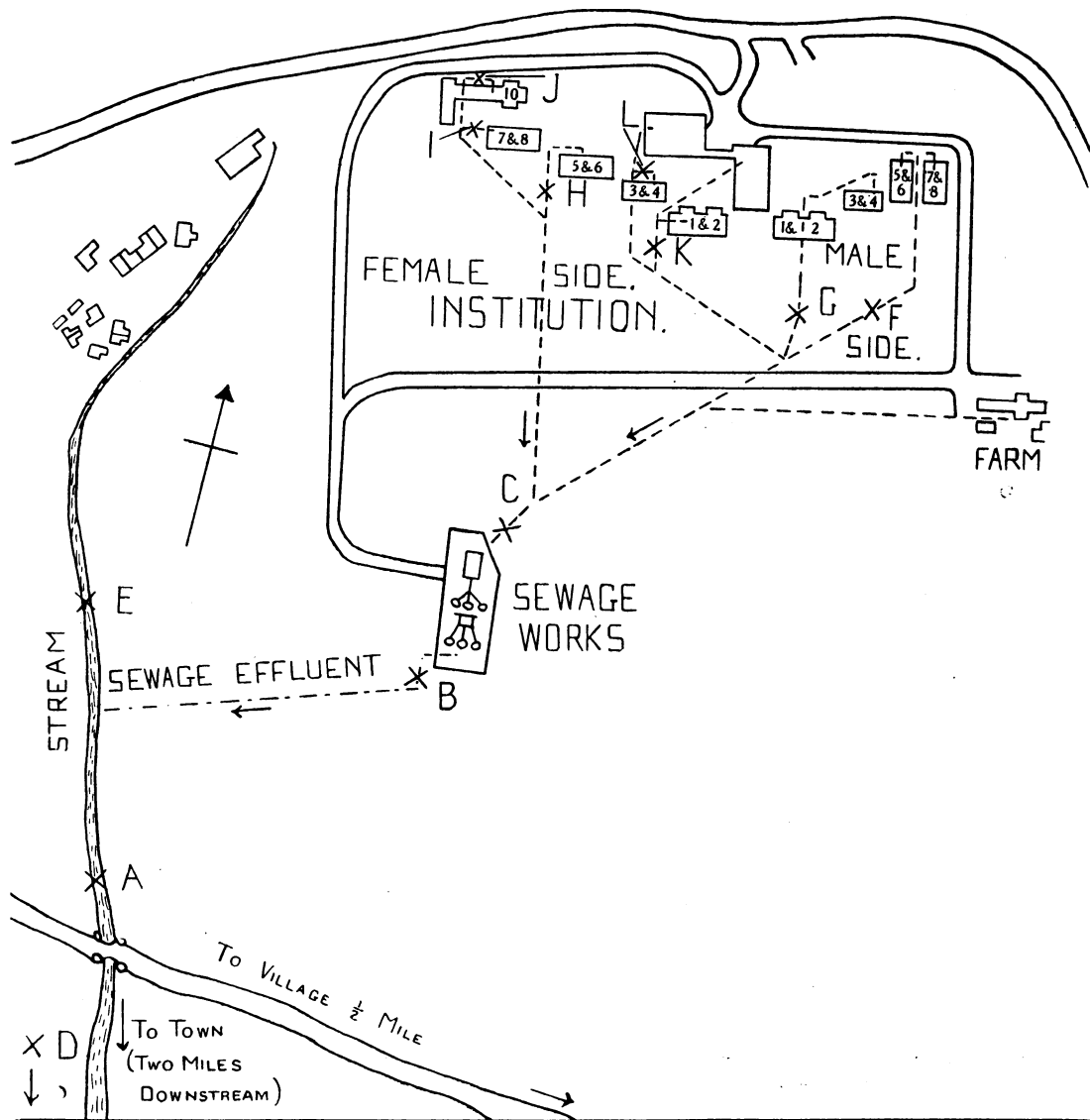


Fig. 1. Plan showing the course of flow of the sewage and sewage effluent from the institution with its relationship to the stream, and the points at which samples were taken for examination (see Table). A, point where A.W. drank water from stream. B, point at which samples of sewage effluent were taken. C, point at which samples of crude sewage from the institution were taken. D, sample of stream water taken near town four miles downstream from point of entry of sewage effluent into stream. E, sample of water taken from stream 100 yd. above point of entry of sewage effluent. F, G, points at which samples of crude sewage from blocks on male side were taken. H, I, J, K, L, points at which samples of crude sewage from blocks on female side were taken. - - - drains conveying sewage from institution buildings to sewage works. - . . . drain conveying sewage effluent to stream.

interval before re-examination of the specimens was not expected to prejudice the isolation of *Bact. typhosum* which can survive a considerable time in faeces at room temperature. By this method no less than six carriers of *Bact. typhosum* were found in wards 3 and 4; five of these were type C and one type A. The carriers were immediately isolated in another block and their stools were thereafter disinfected before disposal. Subsequent examinations of the sewage from wards 3 and 4 (nos. 24, 27) failed to reveal the presence of *Bact. typhosum*.

Samples of stool from the inmates in female wards 5 and 6 were examined in a similar manner. Before doing so, the crude sewage from these wards was re-examined (no. 25) to see if *Bact. paratyphosum* B was still present, with the unexpected result that *Bact. typhosum* and *Bact. paratyphosum* B were both isolated. The strain of *Bact. typhosum* was again type C, and the *Bact. paratyphosum* B was type 1. There were 136 inmates in wards 5 and 6, and specimens of stool from these were examined in a manner similar to that used for the specimens from wards 3 and 4; five of the inmates were found to be excreting *Bact. typhosum* (two type C and three type A), while one was found to be an excreter of *Bact. paratyphosum* B, type 1. These patients were also segregated and neither *Bact. typhosum* nor *Bact. paratyphosum* B was found in subsequent examinations of the sewage from these wards or of the untreated crude sewage and treated effluent from the whole institution (nos. 28, 29, 30, 31). When the crude sewage was re-examined precautions were taken to prevent the stools from the carriers entering the system for 1 week before samples were taken, in order to eliminate any possible disinfectant action of the phenolic anti-septic added to the stools of the carriers and to avoid the presence of typhoid or paratyphoid organisms which might have survived. The chain of evidence was therefore complete, linking the infections of A.W. and Jo.S. with the institution, one via the polluted stream and the other presumably by direct infection from one or more of the carriers in the course of her nursing duties.

#### DATA CONCERNING THE CARRIERS

We are indebted to the doctor in charge of the institution for data concerning the twelve inmates discovered to be excreting *Bact. typhosum* or *Bact. paratyphosum* B. Of the eleven patients who were carriers of *Bact. typhosum* (four type A and seven type C), three had been resident in the institution for 3-4 years, three for 7-8 years, three for 12-16 years and two for 22 and 29 years respectively, while the single patient who was found to be excreting *Bact. paratyphosum* B had been an inmate for 28 years. None of the carriers had at any time

during their life in the institution shown any signs or symptoms suggestive of enteric infection. Furthermore, there had been no cases of illness suggestive of typhoid among the other inmates. The last recorded case of typhoid occurring in the institution was a female member of the nursing staff who died in April 1920; she was diagnosed clinically, and there was no record of any bacteriological or serological examination. According to the records her attack was believed to have been contracted while off duty. The nursing staff had not been immunized against enteric infections. The results of phage typing postulate that there must have been originally at least three missed cases or carriers—one of *Bact. typhosum*, type A, one of *Bact. typhosum*, type C, and one of *Bact. paratyphosum* B, type 1. There is no routine contact between the inmates of blocks 3 and 4 and those of blocks 5 and 6, but the records show that one carrier who was an inmate of blocks 3 and 4 had previously been in blocks 5 and 6, while another carrier discovered in blocks 5 and 6 had been transferred some time previously from blocks 3 and 4. Thus there was probably ample opportunity for infection with types A and C to spread in each of the two blocks. That the majority of the typhoid carriers became infected in the institution seems likely, if the distribution of phage types of *Bact. typhosum* isolated from the inmates (four type A and seven type C) is compared with that of strains isolated from thirty-two cases and carriers in the county in which the institution is situated since August 1940. Of the twenty-one outbreaks from which the thirty-two strains were isolated, eight were due to type E1, six to type A, in four the strains of *Bact. typhosum* were 'imperfect' Vi forms, while the remaining three were due to type D2, type D4 and type J respectively; type C does not appear therefore to be common in this area, and apart from the present series of cases and carriers has not been met with before or since the present investigation. Moreover, in the institution the carriers were found in only two out of five female blocks, and no evidence of carriers was found on the male side. It may be considered strange that as many as eleven persons should have become infected with *Bact. typhosum* in the institution without exhibiting some clinical evidence, but they may have been 'missed' cases resulting from a mild infection or the failure to react symptomatically to infection which is sometimes a feature in certain classes of inmate resident in institutions. That the organisms were probably not avirulent is indicated by the fact that they all possessed Vi antigen and some of the type C organisms at least were able to produce clinical disease in A.W. and Jo.S.

One of the inmates (N.W., aged 30 years) who was found to be excreting *Bact. typhosum*, type C,

died from an intercurrent infection on 20 November 1942. Regular weekly examinations of stool samples from the remaining ten excretors of *Bact. typhosum* and from the single patient who was excreting *Bact. paratyphosum* B have been carried out over a period of 7 months. All except one of the typhoid carriers have continued to excrete the organism fairly regularly. The one exception has shown intermissions of 6-12 weeks during which *Bact. typhosum* was not found, followed by one or two successive weekly specimens in which *Bact. typhosum* was again isolated. The carrier of *Bact. paratyphosum* B has so far never failed to yield a positive culture.

Specimens of serum from the ten typhoid carriers were tested for the presence of Vi agglutinins using the typhoid Vi suspension issued by the Oxford Standards Laboratory. All were positive, the highest titre being 1/160 in one case, while in two cases the titre was as low as 1/5. The other titres were 1/80 and 1/40 each in one case, 1/20 in two cases and 1/10 in three cases.

#### SECONDARY CASES

As a pendant to this investigation, it should be mentioned that two further cases of typhoid occurred, both members of the nursing staff of the isolation hospital in which A.W. and Jo.S. were being nursed. The first was Sister C.M., who developed her earliest symptoms on 21 November 1942, and was admitted as a patient on 14 December; *Bact. typhosum*, type C, was isolated from a sample of her stool. The second case was Nurse M.J., who developed her earliest symptoms in hospital on 13 November 1942 and, while at home on leave, was admitted on 12 December to another isolation hospital following a positive Widal test. She experienced a typical mild attack of typhoid fever, but unfortunately *Bact. typhosum* was not isolated from either of the two specimens of stool submitted for examination. Although the evidence in Nurse M.J.'s case is purely circumstantial, there is no reason to doubt that both she and Sister C.M. were infected by A.W. or Jo.S., who were the only cases of typhoid fever in the hospital, and who were being nursed by their victims. Neither of these two nurses had been immunized.

#### LABORATORY TECHNIQUE

*Water, sewage and sewage effluent.* Cultures were made as follows: a suitable portion of the well-mixed specimen (approximately 0.1-0.2 ml.) was inoculated direct on to plates of Wilson & Blair (Difco) medium and usually of two other media, one of which was desoxycholate-citrate agar (Leifson, 1935; Hynes, 1942) and the other eosin-brilliant green-methylene blue agar (Knox, Gell & Pollock, 1942) or MacConkey. Fluid cultures were made by inoculating a large volume of the specimen into

enrichment and selective liquid media such as that devised by Wilson & Blair (1931, 1938) and tetrathionate broth (Knox *et al.* 1942, 1943); Ruys medium (1940) and a liquid desoxycholate-citrate medium were also used. Details of the liquid media used and their method of preparation are given in an appendix to this paper.

Direct plating was very seldom successful, and fluid cultures were found to be more reliable. Liquid Wilson & Blair medium appeared to be essential for the isolation of *Bact. typhosum* from water, crude sewage and sewage effluent, but usually failed to grow *Bact. paratyphosum* B, whereas tetrathionate broth proved an excellent enrichment medium for paratyphoid B organisms but was not always reliable for *Bact. typhosum*. During the course of the investigation a liquid desoxycholate-citrate medium was tried for the isolation of both pathogens and was finally included as a routine liquid medium as the results were so encouraging. For best results it was found to be essential to subculture from this medium on to a highly selective medium such as Wilson & Blair. The addition of brilliant green 1-100,000 to liquid desoxycholate-citrate increased its efficiency for the isolation of *Bact. paratyphosum* B, but made it too inhibitory for the growth of *Bact. typhosum*.

All liquid media were made up at five times strength, and to one part (50 ml.) of the concentrated solution were added four parts (200 ml.) of the fluid under test. By this means it was hoped that the inoculum would be large enough to isolate small numbers of pathogens without reducing the concentration and therefore the selectivity of the media.

The liquid cultures were plated out after 18-24 hr. incubation and again if necessary after 48 hr. incubation, on to two or more good selective media, such as Wilson & Blair, desoxycholate-citrate, or eosin-brilliant green-methylene blue agar. By increasing the amount of ferrous sulphate already present in the powdered Difco Wilson & Blair medium, it was found that typical black colonies of *Bact. typhosum* and *Bact. paratyphosum* B appeared within 18-20 hr. incubation, whereas without the addition of the extra iron salt, it was sometimes necessary to wait until the second day of incubation before typical black colonies appeared; it was observed repeatedly that even after 48 hr. incubation the type A strains of *Bact. typhosum* isolated in this investigation failed to blacken on the Difco medium, unless the amount of ferrous sulphate was increased (Hobbs, 1943). Desoxycholate-citrate agar proved a valuable medium for the growth of both typhoid and paratyphoid B organisms, but unfortunately on this medium and on MacConkey agar heavy growths of *Proteus* and coliform organisms often masked the pathogens. For the isolation of *Bact. paratyphosum* B eosin-brilliant green (Jones, 1936)

or eosin-brilliant green-methylene blue agar proved very satisfactory; the latter medium was found to be better than the former for *Bact. paratyphosum* B, but proved to be too inhibitory for *Bact. typhosum*.

*Faeces examinations; method of bulking specimens of faeces for examination of large numbers.* Each specimen was emulsified in about 5 ml. of saline to give a thick suspension. The gross particles were allowed to settle, and equal volumes (about 3 ml.) of the suspension from six or seven specimens were mixed together. Each mixture was then treated as a single specimen in a manner similar to that described above for sewage examination, inoculating suitable quantities on to direct plates and into liquid selective media. To one volume of the five times concentrated liquid medium was added one volume of 'bulked' mixture of faeces and 3 vol. of saline to make a total volume of 10–20 ml. Single specimens for the isolation of enteric organisms from individual carriers were cultured in the usual routine manner.

Out of twenty-one bulked batches of faeces from wards 5 and 6, five contained *Bact. typhosum* (three type A and two type C) and one showed *Bact. paratyphosum* B. The results showed that of the solid media Wilson & Blair was the most suitable for typhoid bacilli, both for direct plating and for subculture from liquid selective media. With liquid media similar results were obtained as have been described in the examination of sewage; tetrathionate broth, although excellent for the growth of paratyphoid organisms, sometimes failed to isolate typhoid organisms, particularly from those specimens containing type A bacilli. Subsequent observations suggested that the composition of the tetrathionate medium may be an important factor in determining success or failure in the isolation of certain strains of *Bact. typhosum*.

#### DISCUSSION

The preceding account illustrates the value of phage typing of *Bact. typhosum* in determining whether sporadic cases of typhoid fever in an area bear any relationship to each other. Type E1 with which Ju.S. was infected is the commonest type found in sporadic cases in this area, being the cause of about 36% of all outbreaks investigated during the last 3 years. Type C, which caused the infections in A.W. and Jo.S. has not before or since (with the exception of Sister C.M.) been encountered in this area. Had typing not been carried out Jo.S.'s attack of typhoid would undoubtedly and not unjustifiably have been attributed either to her sister or to a source common to her sister and herself. The finding of five carriers of type C among inmates in the ward in which Jo.S. had been working as a nurse at a material time settled this question unequivocally.

The method of approach to discover the source of A.W.'s infection, backwards via water, sewage effluent, selective sewage examination and 'bulked' specimens of faeces, proved unexpectedly successful and resulted in the discovery of eleven female typhoid carriers and one female carrier of *Bact. paratyphosum* B, all unsuspected and distributed in two blocks of the institution. In the block containing wards 3 and 4, six carriers of *Bact. typhosum* (one type A, five type C) were discovered and in the block containing wards 5 and 6 five carriers of *Bact. typhosum* (three type A, two type C) and one carrier of *Bact. paratyphosum* B (type 1) were found. No carriers were found on the male side.

It is of interest and possibly of importance in future investigations of this nature to note that on no occasion was *Bact. typhosum* type A isolated from the crude sewage, sewage effluent or stream water. This failure may be accounted for by several factors which, in short, are as follows: (a) the preponderance of type C carriers over type A carriers, seven of the former and four of the latter, suggests the dilution of the faeces in sewage, effluent and water as an important factor in the failure to isolate type A organisms; (b) an initial predominance of type C organisms in the sewage would be enhanced by multiplication overnight in fluid enrichment media, and (c) as already noted tetrathionate broth appeared to be more favourable to the multiplication of type C organisms than of type A organisms. On one occasion eighteen colonies of *Bact. typhosum* isolated from a sample of crude sewage, following plating from enrichment media, were all reported by Dr Felix to be type C, indicating a very high predominance of this type, if indeed type A was present at all.

It seemed curious that although there appeared to be only one paratyphoid carrier in the institution compared with eleven typhoid carriers, *Bact. paratyphosum* B was regularly isolated from the crude effluent and from the sewage from wards 5 and 6 while *Bact. typhosum* appeared infrequently in cultures. This can only be accounted for by the greater ease with which *Bact. paratyphosum* B can be isolated by means of the selective media used, because the subsequent examination of samples of stool from the typhoid carriers showed that the majority of them continued to excrete *Bact. typhosum* in very large numbers; it is probable therefore that there were many more viable typhoid bacilli than paratyphoid bacilli in the sewage effluent and stream, as the ability of these organisms to survive in sewage effluent and water is generally recognized.

The regular examination of specimens of faeces from eleven of the carriers over a period of seven months showed some degree of intermittency in the excretion of pathogens; for this reason it might

have been expected that other carriers, missed during the first investigation, might still be present in the institution, yet the examination of further samples of crude effluent and sewage from the incriminated wards failed to reveal the presence of either *Bact. typhosum* or *Bact. paratyphosum* B. The history of typhoid fever in some institutions has shown that the presence of unsuspected typhoid carriers is not an unusual phenomenon.

No dysentery organisms were found in samples of water, sewage effluent, crude sewage or faeces throughout the investigation, although a few clinical cases of dysentery due to *Bact. flexneri* Z occurred within the period of inquiry.

### SUMMARY

1. By means of phage typing of *Bact. typhosum*, the source of infection in a boy who drank water from a polluted stream was traced backwards via water, sewage effluent, crude sewage and faecal specimens to unsuspected typhoid carriers in a large institution.

2. A nurse in the institution contracted typhoid fever, caused by the same phage type (C) of *Bact. typhosum* as was found (a) in the stools of typhoid carriers in the wards in which she was on duty, (b) in the crude sewage and sewage effluent from the institution, (c) in the stream into which the sewage effluent discharged, and (d) in the stools of the boy who drank the stream water. The nurse's sister, who had had an attack of typhoid fever a few

months earlier, was infected with a different type (E1) of *Bact. typhosum* and was therefore not the source of her sister's infection.

3. The laboratory technique is described for the examination of (a) samples of water, sewage effluent and crude sewage, using different selective and enrichment media, (b) large numbers of faecal specimens for enteric organisms by a preliminary pooling of specimens in batches of six or seven, and further examination of the individual specimens comprising a positive batch, thus effecting a saving of time and economy of media.

We wish to thank the Medical Officers of Health, sanitary inspector and the medical and nursing staff of the institution for their enthusiastic co-operation in the investigation.

*Addendum.* Since the above was written King (1944), using similar methods, succeeded in tracking down a carrier of *Bact. paratyphosum* B during a search for enteric organisms in sewage effluent from an ordnance factory; the carrier had had an attack of paratyphoid B fever 18 months earlier in the area of another Sanitary Authority, and had been lost sight of after discharge from hospital.

As a result of further studies (Hobbs & Allison, 1945*a, b*; Hobbs, King & Allison, 1945) on the isolation of *Bact. typhosum* and *Bact. paratyphosum* B from faeces, the recommendations described above regarding the choice of media have undergone some modifications.

### APPENDIX

#### *Liquid enrichment media for sewage*

#### (1) *Basal nutrient medium:*

Peptone ... ..	80 g.
Lab. Lemco ... ..	40 g.
Tryptic meat digest broth (Hartley)	1000 ml.

Adjusted to pH 7.4-7.6 and sterilized by autoclaving.

#### (2) *Wilson & Blair liquid medium:*

Basal broth (as (1)) ... ..	50 ml.
Mannite ... ..	2.5 g.
Na. sulphite (anhyd.) ... ..	3.0 g.
Liq. Bis. et ammon. cit. B.P.C. (Boots) ... ..	0.5 ml.
or Bismuth ammonium citrate (12%)	0.5 ml.

Warm at about 50° C. for a few minutes until a precipitate appears.

Add (HgCl <sub>2</sub> 0.1%) ... ..	1.0 ml.
Ethyl alcohol ... ..	2.5 ml.
Brilliant green (1%) ... ..	1.25 ml.
Fluid under test ... ..	200 ml.

Not sterilized; pH not adjusted; incubated at 37° C. Subcultured after 18 hr. and again after 48 hr. if necessary.

#### (3) *Tetrathionate broth:*

Basal broth (as (1)) ... ..	50 ml.
Na. thiosulphate ... ..	12.5 g.
N/I Iodine (12.7 g. I <sub>2</sub> + 20 g. KI in 100 ml. aq. dist.) ... ..	10 ml.
Phenol red (0.04%) ... ..	1 ml.
Fluid under test ... ..	200 ml.

Not sterilized; pH adjusted to 7.6; incubated at 37° C. Subcultured after 18 hr. and again after 48 hr. if necessary.

#### (4) *Desoxycholate-citrate broth:*

Basal broth (as (1)) ... ..	50 ml.
Lactose ... ..	2.5 g.
Neutral red ... ..	0.5 ml.
'Solution A' ... ..	12.5 ml.
'Solution B' ... ..	12.5 ml.
Fluid under test ... ..	200 ml.

Not sterilized; pH not adjusted; incubated at 37° C. Subcultured after 18 hr. and again after 48 hr. if necessary. (For composition of solution 'A' and solution 'B' see Hynes, 1942.)



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