ISOLATION OF *BACT. TYPHOSUM* BY MEANS OF BISMUTH SULPHITE MEDIUM IN WATER- AND MILK-BORNE EPIDEMICS

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In this paper are given (*a*) brief accounts of outbreaks of enteric fever in which it has been possible by means of the bismuth sulphite medium to demonstrate the way in which the *Bact. typhosum* travelled from the body of the "carrier" to other hosts, (*b*) some details with regard to my present methods of preparation of the medium and of my use of some other media in the examination of water.

Prior to 1928 when I isolated *Bact. typhosum* from Belfast sewage, the belief that this micro-organism existed in sewage was based on faith and not on demonstration. Since that time the value of the medium in the isolation of the typhoid and paratyphoid bacilli from faeces, sewage and water has been confirmed.

Gray (1929) and Begbie & Gibson (1930) isolated paratyphoid bacilli from Edinburgh sewage, whilst Houston (1928, 1929) isolated typhoid bacilli from the river Thames.

In 1931 Wilson & Blair reported the almost constant presence of *Bact. typhosum* during two years in Belfast sewage, and also the isolation of *Bact. typhosum* and *Bact. paratyphosum* B from the river Lagan. In a paper (1933*a*) I referred to the isolation of typhoid, paratyphoid and food-poisoning organisms from sewage by Houston in London, by Scott at Ipswich, Daventry, Stoke and Wroxall, by Stewart & Ghosal (1932–3) at Calcutta, and by Fleming (1933) at Hereford. Since then Harold (1934–6) has continued to find paratyphoid bacilli in the effluent at Epping.

Ruchhoft (1935), with a modified bismuth sulphite medium, has isolated typhoid bacilli from sewage at Chicago.

Joós (1934, 1935) from the sewage of Budapest, and from the river Danube, has cultivated paratyphoid and food-poisoning organisms. Ruys (1936) in the canals near Amsterdam, and Dinger (1937) in the surface water of Batavia, have repeatedly found the *Bact. typhosum*. Peeters *et al.* (1936), Dinger (1937) and Van Loghem (1937) have considered the epidemiological bearing of these bacteriological findings.

COUNTY ARMAGH

The County Mental Hospital in Armagh was free from enteric fever for 30 years up to August 1931. The institution contains 605 patients, and a staff of ninety-two, distributed over three main buildings.

In 1931, the farm manager contracted enteric fever, and in 1932 there were four cases. In 1933 the occurrence of ten cases in March and April prompted by Dr T. W. H. Weir, the assistant medical officer to search for "carriers". Two patients, B. L. and G. J., who handled milk, were found to have enormous numbers of *Bact. typhosum* in their faeces. With their removal from the dairy the outbreak terminated. In 1933 another case, B. L., occurred, and no more until May 1934, when five female patients contracted the disease; in August and October of the same year, one female and two male patients developed typhoid fever.

B. L., who developed the disease late in August 1933, and whose condition was notified to the local Sanitary Authority on 6 September, presented points of great bacteriological and epidemiological interest. This man, 30 years of age, had been found by me to be a "carrier" on 31 March 1933, a drop of an emulsion of his stools at that time yielding 350 colonies on the bismuth plate. How long he had been a "carrier" it is impossible to state—no history of an attack of enteric fever was obtainable, and he had been an inmate of the institution for 2 years. In the middle of August this man developed pyrexia, and on 21 August 1933 his blood serum agglutinated *Bact. typhosum* in a dilution of 1 : 500. His stools examined on 11 September 1933 contained enormous numbers of *Bact. typhosum*. The interesting point is that this man for $4\frac{1}{2}$ months, and perhaps for a much longer period, was a "carrier" of the germ, and was probably the source of infection of the ten cases which developed the disease in March and April 1933, when it was probable that milk handled by this man was the vehicle. This man had a severe attack; his temperature fell by lysis at the end of the third week, remained normal for 1 week, and then rose again and remained up for another 2 weeks. After his recovery, examinations of his stools were negative. This was an instance of a "precocious carrier" of the *Bact. typhosum*.

Ledingham & Arkwright (1912), in their work on *The Carrier Problem in Infectious Diseases* state in reference to diphtheria: "By a precocious carrier is meant a person in whom *Bact. diphtheriae* is found for a longer or shorter time, without any symptoms of disease appearing, but who eventually develops diphtheria." They mention that Wesbrook *et al.* (1898) and Scheller (1906) isolated diphtheria bacilli from the throats of persons 2 months and 1 month before an attack of the disease. It is known that *Bact. typhosum* has been isolated from the stools in the incubation period of typhoid fever.

Ledingham & Arkwright (1912) refer to Prigge's classification of typhoid carriers, the first group including persons in whom the bacillus was found

before clinical symptoms arose, i.e. primary carriers (Conradi) or *porteurs précoces* (Sacquépée) and mention that three such carriers were discovered in whom the symptoms did not supervene till 18, 19 and 20 days afterwards.

These may be regarded as cases in which the bacillus was isolated from the stools in the incubation period. Mayer (1910) states that in 1903, 1905 and 1907 he had three such cases, the bacilli being isolated 8 days before the symptoms appeared. Conradi (1907) records two cases.

I have, in 1935, isolated typhoid bacilli from the stools of a nurse, who, a week later, showed symptoms of the disease. The isolation of typhoid bacilli from the faeces of typhoid fever patients in the incubation period is not surprising, but the case of B. L. belongs to quite a different category.

This case, B. L., shows that a person may harbour typhoid bacilli for at least $4\frac{1}{2}$ months before developing the disease and may be a source of infection for others. The "precocious carrier" in typhoid fever is probably of epidemiological importance, and will be referred to in connexion with the Bournemouth outbreak in 1936.

In 1935 there were fifteen cases in Armagh Mental Hospital, in 1936 eleven cases, and in most of these the source of infection was attributed to contact with carriers, or with cases in the early stages of the disease.

In connexion with the cases in the Mental Hospital over 339 stools were examined bacteriologically, and an opportunity was afforded of testing various techniques.

The major part of the sewage of the Mental Hospital discharges into the river Callan, a tributary of the Blackwater which enters Lough Neagh at its southern shore. The danger to health of residents on the banks of the river Callan, due to the presence of typhoid bacilli in the water, is shown by an investigation made by me at Loughgall in 1935.

DISTRICT OF LOUGHGALL

On 4 March 1935 I received from Dr W. McKenzie a sample of water taken from a tank at Summer Island containing a supply pumped up to it from the river Callan. Summer Island is situated on the river Callan about $4\frac{1}{2}$ miles below Armagh. At Armagh the Callan river receives the sewage of Armagh city, and, by a separate outfall, that of the Mental Hospital. This water sample afforded evidence of contamination with sewage showing on an average thirty-four *Bact. coli* and three *Cl. welchii* in each cubic centimetre.

The precipitate from 100 c.c. of the water, to which 0·5 c.c. of 10% alum solution had been added, when mixed with bismuth sulphite glucose agar medium showed two black colonies. One of these was a lactose fermenter, but the other was a non-fermenter of lactose and sucrose. Subcultures of this bacterium on agar yielded very tiny colonies or a delicate confluent growth with a tendency to the formation of discrete larger secondary colonies in its midst. The cultures formed acid but no gas in mannitol and glucose, formed no

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indole, and were agglutinated to full titre by a specific typhoid-agglutinating serum.

An agglutinating serum prepared by inoculating a rabbit with the water strain of *Bact. typhosum* agglutinated it and genuine typhoid strains in similar dilutions. The water strain, for convenience named Z, was grown on agar plates, and the culture mainly consisted of the fine delicate growth, but no doubt contained some of the larger secondary colonies.

Grown on Endo's medium, the growth of Z was similar to that of an ordinary typhoid culture.

	Z serum					
	40	80	160	320	640	1280
Z agar	+ + +	+ + +	+ + +	+ + +	+ + +	+
901 H	+ + +	+ + +	+ + +	+ + +	+ + +	+
901 O	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Z Endo	+ + +	+ + +	+ + +	+ + +	+ + +	+

An agglutinating serum prepared by means of a known typhoid bacillus gave a practically similar result. The saturation test and its mirror showed that Z was a genuine typhoid bacillus.

Using 901 H and 901 O, saturation tests seemed to indicate that Z grown on agar was identical antigenically with 901 O, and that Z Endo (i.e. Z grown on Endo) was identical with 901 H.

Strain	Z serum 1 : 10 saturated with				Typhoid serum saturated with			
	Z agar	901 H	901 O	Z Endo	Z agar	901 H	901 O	Z Endo
Z agar	—	—	—	—	—	—	—	—
901 H	Floccules	—	Floccules	—	Floccules	—	Floccules	—
901 O	—	—	—	—	—	—	—	—
Z Endo	—	—	—	—	Floccules	—	Floccules	—

Jacobsen (1910) would seem to have been the first to describe typhoid bacilli forming tiny colonies on ordinary agar but giving normal-sized colonies on ascitic agar or agar to which sodium sulphite or sodium thiosulphate had been added. Fromme (1911), Eisenberg (1914) and Baerthlein (1918) also describe such peculiar strains of the *Bact. typhosum,* and the dwarf colonies were named "Zwergkolonien" by Eisenberg.

These writers also noted that in an agglutinating serum these strains did not reach nearly as high a titre as ordinary strains. Kauffmann (1935) found that these Z strains grown on agar were agglutinated by O serum and not by H or Vi serum, but grown on ascitic agar or on agar with thiosulphate, were promptly agglutinated by H and Vi serum.

The Z strain isolated by me at Summer Island contained mainly O antigen, but even on agar, secondary colonies developed which behaved antigenically like ordinary typhoid bacilli. This Z strain of *Bact. typhosum* was isolated from a water tank on 4 March 1933 in a house where there was at the time no case of enteric fever and no known "carrier".

On 19 March 1935 an inmate of the house developed pyrexia and had a

typical attack of enteric fever, and from her stools on 24 March 1935 typhoid bacilli of the Z type were cultivated. Here we have a unique instance of typhoid bacilli being isolated from a water supply just before a consumer of the water contracted the disease and infection with a strain which, from its peculiar growth, was able to be identified with that found in the water.

On 13 March 1935 the stools of two enteric patients in the County Mental Hospital were found to contain bacilli of the Z type; probably other specimens previously examined contained Z type but were missed, as our subcultures were made on Endo, a medium on which the Z strains form normal-sized colonies. In the Mental Hospital at this period there were twelve recent cases of typhoid fever.

It is probable that the typhoid bacillus infecting the patient at Summer Island, Loughgall, was conveyed from Armagh by the river Callan for a distance of $4\frac{1}{2}$ miles. The same explanation is offered for the occurrence of seven cases in the first week of September 1934, and of two cases in April 1935 at Greenhall, Loughgall, living in a row of houses with a standpipe supplied with water from the river Callan.

To find support for this theory, I visited Armagh on 2 April 1935 and procured, with the assistance of Dr T. W. H. Weir, a sample of the sewage of the Armagh Mental Hospital as it entered the river Callan. From 20 c.c. of the sewage about 400 black colonies developed in the bismuth glucose sulphite propyl alcohol brilliant-green agar: of these black colonies about 300 were genuine typhoid bacilli and of them 50% formed normal and 50% formed *Zwerg* colonies on agar. On the same day, Dr Weir and I followed the course of the Callan from Armagh to its junction with the Blackwater, and then the course of the Blackwater until it entered Lough Neagh. At Loughgall the river showed no black colonies with 20 c.c., and 400 c.c. showed only one black colony which proved not to be typhoid. At the junction of the Callan and Blackwater, no black colonies developed, and the same is true of the Blackwater entering Lough Neagh.

On 13 May 1935 Dr Weir sent me samples of Armagh town sewage and Armagh Mental Hospital sewage, collected at the outfalls into the river Callan, and from both typhoid bacilli were isolated, as also from the river Callan several hundreds of yards below. On 10 June 1935 numerous typhoid bacilli were found to be present in the sewage of Armagh and of its Mental Hospital.

County Fermanagh

On 27 April 1936 I received from Dr Henry, Lisnaskea, a water sample from a well supplying the Lisnaskea Infirmary in which an outbreak of enteric fever had occurred. This sample was collected on 25 April 1936, so that 2 days elapsed before its examination. It was not packed in ice.

In each c.c. of the water there were, on an average, one typhoid bacillus and forty-eight *Bact. coli*.

On 30 April 1936, three typhoid bacilli were isolated from 100 c.c. of the

water; some of the bacilli had survived for 5 days in the water at room temperature. A second sample of the water was sent on 4 May 1936, and tested on 10 May 1936, when three typhoid bacilli were again isolated from 100 c.c. A third sample taken on 13 May 1936 and examined on 15 May 1936 contained 940 *Bact. coli* and one *Cl. welchii* in 20 c.c.

One hundred c.c. of the water were tested quantitatively for typhoid bacilli. This was done by making pour-plate cultures with the new standard bismuth sulphite brilliant-green medium. To 100 c.c. melted nutrient agar there were added 10 c.c. bismuth sulphite phosphate glucose mixture and 0·5 c.c. of a 1% brilliant green. In short wide test-tubes, 20 c.c. of the water were mixed with 20 c.c. of the medium, and the mixture poured out into a Petri dish. In the five plates prepared on 15 May 1936, no black colonies were observed on 16 May 1936, but on 17 May 1936 there were several colonies, and on 18 May 1936 plates 1, 2, 3, 4, 5 contained respectively 15, 17, 19, 19, and 17 black colonies. These were subcultures on lactose-sucrose Endo and found to have no action on these sugars. In mannitol agar Andrade agar shake cultures, acid, but no gas, was formed. They were promptly agglutinated by a typhoid agglutinating serum.

A fourth sample from the well taken on 28 May 1936 and examined on 29 May 1936 contained in 100 c.c. 400 *Bact. coli*, sixty *Cl. welchii* and thirty typhoid bacilli.

It has thus been shown that typhoid bacilli persisted in this water for a month. As the well was being pumped daily for the supply of the institution, the bacilli were probably drawn in from the contaminated ground-water. In a bottle after a week three bacilli were still alive in every 100 c.c.

When viability of typhoid bacilli in water is being considered, the question of continuous reinfection of the water from the ground contaminated by the leakage of sewage from a drain has to be taken into account.

This outbreak afforded a unique opportunity of studying the viability of typhoid bacilli in water under natural conditions. For a month there was no other supply of water available to the institution, and as the water in the well was not chlorinated, but chlorination was carried out in the storage tanks into which it had been pumped, the opportunity was given of testing the water during this period.

The cultural characters of the Lisnaskea water typhoid bacillus and of the bacilli isolated from enteric patients infected by the water, were the same, and corresponded to those of typical typhoid bacilli.

On isolation, the water bacillus was agglutinated to full titre 1 : 10,000 by specific typhoid serum.

The serum of a guinea-pig immunized with cultures of the water bacillus which had been heated to 55° C. agglutinated it and a typhoid bacillus from one of the patients to a titre of 1 : 1280. A commercial agglutinating serum agglutinated the water bacillus completely at 1 : 6400, but a freshly isolated strain from a patient only at 1 : 3200.

Absorption tests carried out on these sera proved conclusively that the Lisnaskea water bacillus and the typhoid bacillus from a Lisnaskea enteric patient were antigenically identical.

Number of cases

The total number of inmates of the institution at Lisnaskea in which the outbreak occurred was seventy, and of these twenty-three contracted the disease. The outbreak followed the admission into the institution of a patient supposed to be suffering from puerperal fever, but who, in reality, was an enteric case.

The well and its water

The well is situated in the centre of an open field, at a considerable distance from the buildings, and 30 yards from the main drain. The level of the water in the well is 10 ft. below the surface of the ground. The stratum consists of limestone. It is probable that the water was contaminated from sewage leaking into the ground from a defective drain. The physical characters of the water were good—it was bright, clear and sparkling.

A sample of water taken on 4 May 1936 gave the following figures expressed as parts per 100,000: total solids 58, total hardness 44 (temporary 33, permanent 11), chlorine as chloride 3·8, nitrogen as nitrate 0·24, nitrite absent, saline ammonia 0·004, organic ammonia 0·017, oxygen absorbed from permanganate 0·116.

BELFAST 1935

In September 1935, a sudden outbreak of typhoid fever occurred in Belfast and was traced by Dr Barron, the Acting Medical Officer of Health, to an infected milk supply.

Over 100 cases were admitted to Hospital. Two "carriers" were found at the dairy, but these were temporary "carriers" and were free from bacilli in 5 weeks. It is probable that these "carriers" acquired their bacilli at the same time when others developed the disease, and that they were not the *fons et origo* of the outbreak.

The water supply at the dairy was from a well which at times showed an excessive number of *Bact. coli*. No typhoid bacilli were isolated from the well, but, in a stream passing through the yard of the dairy, Dr Blair and I, on one occasion, found over 300 typhoid bacilli in each c.c. The pollution of the stream was traced to the escape of sewage into it from a broken drain coming from a row of adjoining houses. It is probable that this stream, subject to intermittent specific pollution, was, in some way, casually related to the outbreak.

BOURNEMOUTH-POOLE EPIDEMIC 1936

In this outbreak, in which there were 718 cases, raw milk from one supplier was the factor common to all the primary cases.

The late W. Vernon Shaw (1937), in his *Report* on the outbreak, associated

it with the presence of typhoid bacilli discharged from a cesspool into a stream at a point half a mile above the residence of Mrs A. from which the infected milk was supplied.

On 16 October 1936 Dr Chesney, Assistant Medical Officer of Health, Poole sent me a sample of sewage effluent in which I found 100–200 typhoid bacilli per c.c. Dr Scott, of the Ministry of Health, examined another sample with the same result. I had examined an effluent from the same place on 25 September 1936, but was unable to detect typhoid bacilli, either because they were absent, or because the presence at that season of large numbers of *Bact. effluviei* rendered the isolation too difficult.

On 26 October 1936 another sample of effluent was examined, and about 160 typhoid bacilli were present in each c.c.

On 2 November 1936 I examined 8 oz. of stream water taken well below the effluent inlet, and isolated nine typhoid bacilli from its contents. How the milk became infected from the stream is not clearly demonstrated.

A boy living close to Mrs A.'s house developed typhoid fever in 1934. There is no evidence that the water supply to the dairies was specifically contaminated. The cows had access to the stream, and their udders might have been soiled with the polluted water, and this might have led to infection of the milk, but it would hardly explain the continued infection of the milk over a period of 3 or 4 weeks. The question is asked: Can a cow become a "carrier" and excrete typhoid bacilli in her milk? No evidence can be produced to support such an hypothesis.

Mrs A., from whose house the infected milk was derived, died from typhoid fever, but an objection to her being the source of the outbreak is that "Mrs A.'s onset of illness was not before 10 August, by which time twenty-nine other patients had fallen ill, seven Bournemouth residents, eight visitors, and fourteen Poole residents, and, in this respect, she belongs to the Bournemouth, Poole, Christchurch series. Certain assumptions would be necessary to justify the conclusion that Mrs A. was the primary source of infection. These are that she was infective from the very beginning of her incubation period; that she infected the milk practically at once, and consistently for several days immediately thereafter, and that the incubation period of the early Bournemouth and Poole cases was not more than seven days. Such a combination of circumstances is very unlikely although it is admitted that infectivity during the incubation period is possible. It is not rare to find the specific organism in the faeces in this stage, and indeed Conradi (*Dtsch. Med. Wschr.* 1907, **33**, 1684) considered infection by patients in the incubation period to be an important factor in the spread of enteric fever" (Shaw, 1937).

Here I would suggest that it is possible that Mrs A. was a "precocious carrier", such as B. L. was in the first milk-borne outbreak in the Armagh Mental Hospital.

Technique in the use of the bismuth sulphite medium

During the 10 years that have elapsed since the introduction of the bismuth sulphite medium of Wilson & Blair in 1927, I have made some modifications which render its preparation very simple and very quick.

Some observers had some difficulty in the preparation of liquor bismuthi by the solution of bismuth citrate in ammonia. This is overcome by the use of bismuth-ammonia-citrate scales. To prepare a stock solution which keeps for many months, dissolve 6 g. bismuth-ammonia-citrate scales in 50 c.c. boiling distilled water, and mix this with a solution obtained by boiling 20 g. sodium sulphite anhydrous in 100 c.c. of water and then, whilst the mixture is boiling, there is added 10 g. of sodium phosphate anhydrous (Na_2HPO_4) or 25·2 g. $Na_2H.PO_4 12.H_2O$.

To the sulphite-bismuth-phosphate mixture, when cool, add a solution of glucose obtained by dissolving 10 g. of commercial glucose in 50 c.c. of boiling distilled water (Wilson, 1933 a).

Surface colonies

Add to 100 c.c. of hot melted 2% nutrient agar 20 c.c. of stock mixture, then 1 c.c. of an 8% solution of ferrous sulphate crystals in water, and 0·5 c.c. of a 1% solution of brilliant green in distilled water; pour into Petri dishes, and when the medium has set, inoculate the surface.

Isolated colonies of *Bact. typhosum* and *Bact. paratyphosum* B are black, the typhoid colonies usually appearing within 24 hr., and the paratyphoid within 48 hr.

For some time past I have been using instead of 1 c.c. ferrous sulphate, 4·5 c.c. of 1% solution of iron citrate to every 200 c.c. of which there have been added 25 c.c. of a 1% solution of brilliant green in distilled water. Ruys (1936) recommends the use only of ferrous sulphate which has been precipitated in alcohol, giving a perfectly clear solution free from ferric sulphate.

The emulsion of faeces can be made in water, but I prefer to emulsify a large loopful of faeces in 0·5 c.c. of the stock bismuth sulphite glucose phosphate solution. One-half of the plate is heavily, and one-half lightly, smeared with the faecal emulsion. In the case of most peptones with the above concentration of the sulphite bismuth mixture *Bact. typhosum* forms black colonies where the colonies are discrete, but where confluent, the growth is greenish. With Witte's peptone the colonies are greenish, but black colonies develop when the amount of the sodium sulphite is reduced by one-half, the other constituents remaining the same.

An occasional strain of *Bact. typhosum* is found which causes little darkening of the medium. I have found such strains which have been described by Ruys (1936). Such strains, in my experience, are of rare occurrence; they are able to reduce sulphites like ordinary strains in glucose iron sulphite agar and probably would form black colonies in the depth of the sulphite bismuth medium.

Deep colonies

Black colonies develop when an emulsion of *Bact. typhosum* is mixed with an equal volume of agar, containing the usual amount of the bismuth sulphite glucose phosphate mixture.

My experience is, that the best results are obtained by reducing the dose of the stock mixture by one-half, and by leaving out the iron. For instance, to 100 c.c. of melted nutrient agar there are added 10 c.c. of the stock bismuth sulphite glucose phosphate mixture and 0·5 c.c. of 1 % brilliant-green solution. Twenty c.c. of the diluted faecal emulsion are mixed with 20 c.c. of the above melted agar cooled to 50° C. and poured out into a Petri dish. Black colonies of *Bact. typhosum* appear in the inverted Petri dishes in 24–48 hr. and can be subcultured on Endo or MacConkey, or better on the ordinary bismuth sulphite glucose phosphate medium, containing iron and brilliant green. The addition of 2 c.c. of propyl·alcohol to the 10 c.c. of the stock bismuth mixture when the medium is being mixed, causes the typhoid colonies to be delayed in their appearance, but tends to suppress the growth of *Bact. effluviei*, and re-ducing coliform organisms, which sometimes occur in faeces, and are at certain seasons found in large numbers in sewage.

For ordinary purposes I use surface inoculation of plates, but there is a great advantage in using one plate for surface inoculation and another plate for deep colonies. The deep plates enable one to employ large volumes of a faecal emulsion or of water or of dilute sewage and to isolate the organism when very few are present.

The advantages of the deep method were shown by the examination of eighty-six consecutive stools derived mainly from contacts and convalescents. By the surface method thirty-two, and by the deep method forty were positive. On two occasions the surface method yielded hundreds of typhoid colonies, and no dark colonies appeared in the "deep" plates. In these cases the enormous numbers of typhoid bacilli present prevented the colonies developing to a size visible to the naked eye.

The objection to the deep method is that it is more tedious and involves more labour. For the examination of water it has, however, great advantages.

Some prefer an enrichment broth medium for the isolation of the *Bact. typhosum*. For this purpose Dr Blair and I suggested (1931) the use of 100 c.c. nutrient broth containing 0·5 % of mannitol with the addition to each 100 c.c. of 6 c.c. of 20 % sodium sulphite anhydrous 0·2 c.c. liquor bismuthi (or 0·2 c.c. of a solution of 6 g. of bismuth ammonia citrate scales in 50 c.c. boiling distilled water) and 0·5 c.c. of 1 % brilliant-green solution.

A broth with these constituents has been employed at Asmara and Milan with excellent results according to Ciantini (1937). The paper of Ciantini gives an excellent review of the experience of different observers with regard to the value of media proposed for the isolation from faeces of bacilli of the typhoid-

paratyphoid group, and also the results of the testing of 160 stools with six different media.

In Italy, the bismuth sulphite⁻media since their introduction into that country by Mazzetti (1928) are extensively used, some observers preferring the solid agar medium and others the broth enrichment. Whether the broth enrichment medium is superior to the poured plate deep cultures has yet to be determined. I am inclined to favour the poured plate method, as the typhoid bacilli form a characteristic black colony and can readily be picked out; moreover, there is less likelihood of the typhoid bacillus being outgrown by various *Proteus* strains. Whatever method is employed, I recommend that subcultures should be made on the surface of bismuth sulphite glucose phosphate iron agar plates, as the black typhoid colonies can then be readily picked out even when large numbers of *Proteus* and coliform organisms are present in the inoculum. Horgan (1935) at Khartoum found the bismuth plates much superior to enrichment in brilliant-green broth.

Whether Muller's broth or its modification by Kauffmann will prove superior to the bismuth sulphite broth and bismuth solid media is still uncertain although Ciantini found the bismuth medium slightly more selective.

Suppression of Bact. effluviei

The greatest obstacle to the easy isolation of typhoid-paratyphoid bacilli from sewage is the frequent presence at certain seasons of an organism which forms dark colonies and which I named *Bact. effluviei* (1928). I have found that this organism will not grow at temperatures at which growth of the typhoid-paratyphoid group occurs. On the bismuth media at 41–42° C. typhoid-paratyphoid bacilli grow, and many, but not all, strains of *Bact. effluviei* are inhibited.

Quantitative estimation of Bact. coli *and* Cl. welchii

The number of *Bact. coli* in water can be readily determined by use of a medium introduced by me in (1933b), but apparently not, so far, widely adopted.

This medium consists of distilled water 1000 c.c., peptone (Difco) 10 g., lactose 10 g., sodiumcitrate 10 g., sodium taurocholate 10 g., agar (Difco) 20 g. The medium after autoclaving is very clear, requiring no filtration, and its reaction is adjusted to about pH 8. For convenience I have called this the W.A. (water) medium. Before use, 1 c.c. of 1 % watery solution of neutral red is added to each 100 c.c. of the medium.

A supply of sterile short wide test-tubes of about 50 c.c. capacity is very useful for the bismuth pour plates and for the estimation of the *Bact. coli* and *Cl. welchii* content of a water or other fluid. Varying quantities, usually 20 c.c., of the water are taken in the test-tubes into which are poured an equal volume of the W.A. medium melted and cooled to 55° C.; rapid admixture occurs, and the contents are poured into a Petri dish. After setting, the plates are inverted, and half of them are incubated at 38° C. and half at 44° C.

I was led to adopt this latter temperature as the result of G. S. Wilson's *Report* (1935). The citrate and bile salt combined favour the development of deep colonies of *Bact. coli* and *Bact. lactis aerogenes* at 38° C. but at 43·5–44° C. generally only true excretal *Bact. coli* develop.

As a rule there is no difficulty in picking out the deep red lenticular colonies of the *coli-aerogenes* group. Occasionally there develop tiny reddish colonies which can be distinguished from genuine lactose fermenters, and which, in my experience, have proved to be of the *alcaligenes* group.

Cl. welchii

My present practice is to keep a stock sodium sulphite glucose solution consisting of 200 g. anhydrous sodium sulphite dissolved in 1000 c.c. boiling water, mixed when cool with 500 c.c. of a 20% solution of commercial glucose in water. To 100 c.c. of melted nutrient agar are added 15 c.c. of this stock solution and 1 c.c. of a stock solution of 8% ferrous sulphate crystals in distilled water.

Twenty c.c. of the water to be tested are taken in a wide test-tube and an equal volume of the medium (cooled to 55° C.) added and then the mixture poured out into a Petri dish. After solidification, the medium is incubated at 44° C. Large black colonies develop, and in most cases, these are genuine *Cl. welchii*.

SUMMARY

1. An account is given of enteric fever in a Mental Hospital, and the discovery of a "precocious carrier" of the *Bact. typhosum*, a man who, $4\frac{1}{2}$ months before he showed symptoms of the disease, was a "carrier" and probably the source of infection in a milk-borne outbreak.

2. Description of a strain of *Bact. typhosum* forming dwarf colonies which was isolated (*a*) from a river, (*b*) from a patient who had drunk the water, (*c*) from the sewage of a mental hospital discharged into the river $4\frac{1}{2}$ miles higher up, and (*d*) from patients in the mental hospital.

3. Isolation of the *Bact. typhosum* from a well of an institution in which an outbreak of enteric fever had occurred.

4. Isolation of *Bact. typhosum* from a stream adjoining a well in a farmyard in Belfast at a time when the milk was the source of infection of over 100 cases of the disease.

5. Isolation of *Bact. typhosum* from a cesspool discharging into a stream near Bournemouth, and the suggestion that Mrs A. from whose premises milk was obtained, which was the infective vehicle in the Bournemouth-Poole epidemic, may have been a "precocious carrier".

6. An account of methods employed in the bacteriological examination of water and sewage.

REFERENCES

BAERTHLEIN, K. (1918). *Zbl. Bakt.* I. Abt. Orig. **81**, 369.

BEGBIE, R. S. & GIBSON, H. J. (1930). *Brit. Med. J.* **2**, 55.

CIANTINI, F. (1937). *Boll. Sez. ital. Soc. int. Microbiol.* **9**, 7.

CONRADI, H. (1907). *Klin. Jb.* **17**, 297.

DINGER, J. E. (1937). *Geneesk. Tijdschr. Ned.-Ind.* p. 1090. (Reprint.)

EISENBERG, P. (1914). *Zbl. Bakt.* I. Abt. Orig. **73**, 449, 466.

FLEMING, G. W. T. H. (1933). *Brit. Med. J.* **1**, 412.

FROMME, Dr (1911). *Zbl. Bakt.* I. Abt. Orig. **58**, 445.

GRAY, J. D. A. (1929). *Brit. Med. J.* **1**, 142.

HAROLD, C. H. H. (1934, 1935, 1936). *29th, 30th and 31st Annual Reports of the Metropolitan Water Board.*

HORGAN, E. S. (1935). *J. Hyg., Camb.,* **35**, 38.

HOUSTON, A. C. (1928–31). *23rd, 24th, 25th* and *26th Annual Reports of the Metropolitan Water Board.*

JACOBSEN, K. A. (1910). *Zbl. Bakt.* I. Abt. Orig. **56**, 208.

JOÓS, ILONA (1934). *Zbl. Bakt.* I. Abt. Orig. **132**, 352.

—— (1935). *Zbl. Bakt.* I. Abt. Orig. **135**, 266.

KAUFFMANN, F. (1935). *Z. Hyg. InfektKr.* **116**, 617.

LEDINGHAM, J. C. G. & ARKWRIGHT, J. A. (1912). *The Carrier Problem in Infectious Diseases.* London: Arnold.

VAN LOGHEM, J. J. (1937). League of Nations Health Organization. *CH./Hyg. rur. Typh./*11. Geneva. 2nd Nov. 1937. (Cyclostyled circular.)

MAYER, G. (1910). *Zbl. Bakt.* I. Abt. Orig. **53**, 234.

MAZZETTI, G. (1928). *Communiaz. del R. Accad. dei Fisiocritici di Siena,* **25**, 528. (Reprint.)

PEETERS, H., RUYS, A. C. & EPHRAIM, C. H. (1936). *Ned. Tijdschr. Geneesk.* **2**, 2353.

RUCHHOFT, C. C. (1935). *J. Bact.* **29**, 25.

RUYS, A. C. (1936). *Proc. K. Akad. Wet. Amst.* **39**. (Reprint.)

SACQUÉPÉE, E. (1910). *Bull. Inst. Pasteur,* **8**, 1–15, 49–62.

SCHELLER, R. (1906). *Zbl. Bakt.* I. Abt. Orig. **40**, 1.

SHAW, W. VERNON (1937). *Report on an Outbreak of Enteric Fever, Ministry of Health Report on Public Health and Medical Subjects.* No. 81.

STEWART, A. D. & GHOSAL, S. C. (1932–3). *Indian J. Med. Res.* **20**, 341.

WESBROOK, F. F., WILSON, L. B., McDANIEL, O. & ADAIR, J. H. (1898). *Brit. Med. J.* **1**, 1008.

WILSON, G. S. (1935). *The Bacteriological Grading of Milk. Spec. Dep. Ser. Med. Res. Coun., Lond.,* No. 206.

WILSON, W. J. (1928). *Brit. Med. J.* **1**, 1061.

—— (1933*a*). *Brit. Med. J.* **2**, 560.

—— (1933*b*). *J. Hyg., Camb.,* **33**, 404.

WILSON, W. J. & BLAIR, E. M. McV. (1927). *J. Hyg., Camb.,* **26**, 374.

—— —— (1931). *J. Hyg., Camb.,* **31**, 139.