FURTHER EXPERIENCE OF THE BISMUTH SULPHITE MEDIA IN THE ISOLATION OF *BACILLUS TYPHOSUS* AND *B. PARATYPHOSUS* B FROM FAECES, SEWAGE AND WATER.

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CONTENTS.

INTRODUCTION.

In two previous papers (1927 and 1928) we have described and applied a new medium for the isolation of *B. typhosus* from faeces and sewage. Favourable results on the use of the medium for the isolation of the *B. typhosus* from the stools of patients and "carriers" have been reported by Allison (1927–8), Mazzetti (1928) and D'Antona (1929), for the isolation of *B. typhosus* from the droppings of seagulls by Adams (1928), for the isolation of *B. typhosus* from sewage and cockles by Wilson (1928), for the isolation of *B. typhosus* from

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water by A. C. Houston (1928 and 1929) and Franco (1929), and for the isolation of *B. paratyphosus* B from Edinburgh sewage by Gray (1929) and by Begbie and Gibson (1930). The present paper is divided into three parts. Part I deals with further experience with the same media as described by us in 1927. In Part II modifications of these media are described and tested; and in Part III a description is given of a fluid bismuth sulphite enrichment medium which promises to be even more efficient than our solid media for the isolation of typhoid bacilli from the stools of convalescent typhoid fever cases.

PART I.

No modification of the media has been found necessary since our original publication (1927), but we may supplement our description of the media with the following remarks: (1) No filtration of the 3 per cent. agar is required; the medium is made in 12-litre amounts in a preserving pan in an autoclave and, after cooling and solidifying, the pan being inverted, the agar is turned out, and the lower layer containing precipitated matter is cut away from the clear upper layer. (2) The 1 grm. of exsiccated sodium phosphate which is added to each 100 c.c. of the medium is dissolved in 10 c.c. of boiling water. (3) Instead of a 20 per cent. solution of exsiccated sodium sulphite a 40 per cent. solution of sodium sulphite crystals may be used.

The original description of the media was:

Medium A. To 100 c.c. of a melted 3 per cent. nutrient agar are added 5 c.c. of a 20 per cent. solution of glucose, 10 c.c. of a 20 per cent. solution of sodium sulphite (anhydrous), 5 c.c. of a standard bismuth solution. After boiling for 2 minutes an addition is made of 1 grm. of exsiccated sodium phosphate and 1 c.c. of an 8 per cent. solution of ferrous sulphate crystals.

Medium B. The same as medium A with the addition of 0·5 c.c. of a 1 per cent. watery solution of brilliant green. The standard liquor bismuthi is prepared by mixing 60 grm. bismuth citrate with 50 c.c. of distilled water, and then with 20 c.c. liq. ammonii sp. gr. 0·880, and finally making the volume up to 500 c.c. with distilled water.

The following details in making the liquor should be followed as they ensure the action to the full of the ammonia employed. A glass-stoppered bottle is almost filled with 500 c.c. of distilled water, and the level of the fluid is marked on the side of the vessel. The water is poured out, 60 grm. of bismuth citrate are introduced through a wide funnel and followed by 50 c.c. of distilled water. By means of a glass rod the citrate is made into a thin paste with the water, and then 20 c.c. of liq. ammonii are poured in. The mixture is stirred with the glass rod and a chemical reaction occurs with the development of heat. The glass stopper is inserted, the bottle shaken, and as soon as the bismuth citrate is almost completely dissolved, distilled water is added until the level reaches the 500 c.c. mark.

The principle of the medium rests (1) on the positive property of the *B. typhosus* being able to reduce a sulphite to a sulphide in the presence of

glucose; (2) on the inhibitory action on the growth of *B. coli* and of many other bacteria, of a bismuth sulphite in the presence of a certain excess of sodium sulphite.

Isolation of *B. typhosus* from faeces.

Since the publication of our last paper we have had an opportunity of examining the stools of 50 different cases of typhoid fever, and in 44 we have

Table I.

No.	Date	Patient's identification sign	No. of typhoid colonies found on MacConkey medium	MacConkey plus brilliant green	Bismuth medium (A)	Bismuth plus brilliant green (B)
1	28. v. 27	McQ.	0	—	6	10
2	28. v. 27	B.	0	—	24	24
3	28. v. 27	F.	5	—	2000	1500
4	31. v. 27	L. J.	0	—	200	400
5	31. v. 27	M. J. P.	5	—	100	350
6	9. vii. 27	McB.	0	400	1000	1000
7	9. vii. 27	I.	3	5	148	132
8	13. vii. 27	M. D.	5	—	600	600
9	25. vii. 27	S. P.	0	3	6	6
10	28. vii. 27	R. S.	0	—	5	9
11	29. viii. 27	M. McR.	—	—	2	3
12	7. ix. 27	G.	6	—	6000	6000
13	16. ix. 27	R.	0	—	2	2
14	19. ix. 27	M. D.	0	200	200	200
15	20. x. 27	M.	2	6	300	200
16	8. xi. 27	F. R.	12	180	180	110
17	28. xii. 27	W. A.	0	—	500	400
18	18. i. 28	K. E.	30	90	600	500
19	21. ii. 28	A. S.	0	—	2	2
20	21. ii. 28	T. McM.	8	—	600	600
21	18. ix. 28	J. M.	0	2	0	20
22	22. ix. 28	E. McM.	0	20	300	300
23	10. x. 28	McB.	80	100	600	400
24	16. iii. 29	M. B.	0	—	0	Hs.*
25	21. iii. 29	B. T.	0	—	Hs.	Hs.
26	4. v. 29	M. E.	0	—	57	120
27	20. vi. 29	E. McG.	0	—	9	5
28	2. vii. 29	A. T.	0	—	0	5
29	7. ix. 29	J. O'H.	0	—	20	1000
30	13. ix. 29	M. D.	0	1	0	500
31	30. i. 30	N. D.	500	500	500	500
32	17. ii. 30	D. H.	0	0	70	60
33	20. iii. 30	J. A.	20	60	60	33
34	29. iii. 30	M. B.	0	0	16	24
35	1. iv. 30	J. Ma.	1	0	200	200
36	9. iv. 30	J. M.	36	40	500	500
37	12. iv. 30	H.	0	0	0	0
38	12. iv. 30	M. By.	2	0	20	3
39	12. iv. 30	C. M.	0	0	0	0
40	24. v. 30	P. R.	200	1000	1000	1000
41	27. v. 30	N. P.	1	500	500	500
42	9. v. 30	N. Bi.†	0	0	0	0
43	9. v. 30	Mrs Bi.†	0	0	0	0
44	14. vi. 30	A. McI.	0	0	0	0
45	14. vi. 30	F. McI.	0	0	0	0
46	13. vii. 30	J. R.	0	Hs.	Hs.	Hs.
47	1. vii. 30	M. R.	0	Hs.	Hs.	Hs.
48	1. vii. 30	M. S.	0	Hs.	0	Hs.
49	26. vii. 30	M. E.	0	24	100	300
50	19. viii. 30	E. I.	0	—	—	30

* Hs. = hundreds. † Convalescent.

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been successful in isolating the *B. typhosus* by means of the bismuth media. In most of the tests we also employed MacConkey's medium and MacConkey's medium containing brilliant green. Table I shows the approximate numbers of typhoid bacilli which developed on plates of the different media when inoculated with a drop of an emulsion of the typhoid stool.

With the MacConkey bile salt lactose neutral red agar medium on 17 occasions out of 49 attempts, *B. typhosus* was isolated, *i.e.* 34·7 per cent.; with MacConkey media containing in each 100 c.c. 0·3 c.c. of a 1 per cent. solution of brilliant green, 20 out of 30 attempts were successful, *i.e.* 66·6 per cent.; similarly with bismuth medium A, 38 out of 49, *i.e.* 77·5 per cent., and with bismuth medium B, 44 out of 50 attempts, *i.e.* 88 per cent. were successful.

Table I also shows that, as a rule, the typhoid colonies developing on the bismuth plates were several hundred times more numerous than those developing on the MacConkey medium. This is undoubtedly due to the suppression of the *B. coli*. Where the growth of this bacillus is suppressed by brilliant green, then the number of colonies on the MacConkey brilliant green plates and on the bismuth plates is much the same. With media depending for the differentiation between colonies of *B. typhosus* and *B. coli* on the presence of lactose, the dice are loaded against *B. typhosus*, since an extra supply of energy in a utilisable form is being supplied to the *B. coli*. It is also known (Nissle, 1916; Smith, 1923; Hashimoto, 1927; Vignati, 1928) that certain strains of *B. coli* are actively antagonistic to the growth of *B. typhosus*, as well as being able by the rapidity of their growth to occupy by their colonies nearly all available territory on a plate.

ISOLATION OF *B. PARATYPHOSUS* B FROM FAECES.

In Table II are tabulated the results of the examination of stools from 36 cases of paratyphoid fever. In all these the infective agent was the *B. paratyphosus* B.

The growth of *B. paratyphosus* B is for about 48 hours suppressed on the bismuth media, and as at the end of that time colonies of *B. coli* will in many cases have developed on bismuth medium A, it is better to use bismuth medium B containing brilliant green.

After 48 hours the colonies of *B. paratyphosus* B may appear black and not unlike those of *B. typhosus*, but slightly more raised and a little moister; more frequently they appear as moist, raised, clear, sticky colonies which, after another 24 hours, become black and also blacken the surrounding medium. On further incubation the colonies continue to enlarge until they may be 0·6 to 1 cm. in diameter. At this stage the colony is dark green in colour, the margin is raised, and the appearance resembles that of a motor-wheel disc surrounded by its tyre.

For prompt isolation of the *B. paratyphosus* B the bismuth media are not suitable, but, from our experience, we consider that medium B should always be employed in the examination of enteric stools as, occasionally,

B. paratyphosus B will be obtained with it where failure has occurred with the brilliant green MacConkey medium, by means of which, as a rule, the isolation is quick and satisfactory. With the MacConkey medium 11 out of 19 tests were positive, *i.e.* 58 per cent.; with MacConkey brilliant green medium 22 out of 26 tests were positive, *i.e.* 84·6 per cent. With the bismuth medium A, 19 out of 22 tests were positive, *i.e.* 86·3 per cent. and, with bismuth medium B, 22 out of 27 were positive, *i.e.* 81·5 per cent.

Table II.

No.	Date	Patient's identification sign	No. of paratyphoid colonies found on MacConkey medium	MacConkey plus brilliant green	Bismuth medium (A)	Bismuth plus brilliant green (B)
1	7. vii. 27	P. N. T.	Hs.	—	Hs.	Hs.
2	20. vii. 27	D. McC.	0	—	1	33
3	20. vii. 27	E. Y.	5	—	5	300
4	23. iii. 28	D. B.	0	17	21	—
5	1. iv. 28	C.	—	200	200	200
6	27. vii. 28	S.	—	100	—	6
7	16. iv. 29	J. K.	0	—	1	—
8	18. vi. 29	F. R.	—	20	1	0
9	19. vi. 29	E. J.	—	Hs.	—	—
10	19. vi. 29	J. C.	—	Hs.	1	—
11	19. vi. 29	A. C.	—	0	4	12
12	19. vi. 29	M. J.	—	20	45	2
13	19. vi. 29	W. A.	—	20	—	—
14	26. vi. 29	K.	—	—	0	4
15	26. vi. 29	H.	—	20	20	6
16	26. vi. 29	R. B.	1	—	20	—
17	26. vi. 29	J. B.	—	20	20	20
18	19. vii. 29	B.	—	Hs.	—	—
19	23. vii. 29	S. McB.	—	Hs.	—	Hs.
20	23. vii. 29	C.	—	0	20	—
21	24. vii. 29	S. T.	20	Hs.	10	0
22	29. vii. 29	M. W.	0	3	0	0
23	10. viii. 29	M. S.	0	1	0	0
24	10. viii. 29	R.	15	—	20	—
25	29. viii. 29	M. M. D.	0	—	—	70
26	29. viii. 29	M. M.	20	—	—	200
27	29. viii. 29	M. D.	—	—	—	100
28	3. ix. 29	S. B.	—	160	—	150
29	21. ix. 29	S. M. Y.	0	0	—	1
30	21. ix. 29	M. D.	Hs.	Hs.	—	0
31	21. ix. 29	M. Morg.	0	2	—	34
32	21. ix. 29	A. R.	50	50	—	100
33	22. x. 29	M. H.	—	0	—	81
34	11. i. 30	S. H.	20	50	50	50
35	8. iv. 30	E. C.	24	Hs.	Hs.	Hs.
36	24. iv. 30	M. B.	Hs.	Hs.	Hs.	Hs.

Hs. = hundreds.

In our earlier tests the great delay in the appearance of *B. paratyphosus* B colonies on the bismuth media was not fully appreciated, and this fact no doubt explains some of our failures with the media. If the stools are stale and *B. proteus* is abundant this organism may interfere with the development of *B. paratyphosus* B. The bismuth media inhibit the growth of *B. pyocyaneus*, which sometimes renders difficult the isolation of *B. paratyphosus* B on the MacConkey or MacConkey brilliant green plates.

Isolation of B. *typhosus* from sewage.

In the experiments fresh crude screened sewage was taken in sterile bottles as it flowed in open channels to sedimentation tanks. In the majority of the tests the sewage was from the City of Belfast, but in a few it was obtained from Lisburn, a neighbouring town. On an average about ten plates containing the bismuth medium B were inoculated on their surface with about $\frac{1}{4}$ or $\frac{1}{2}$ c.c. of the sewage. The surface was allowed to dry and the plates were inverted. Occasionally the sewage was mixed with water and then incorporated with the medium, which was then poured out into the plates. After 24–36 hours in the incubator isolated black colonies were subcultured on to a modified Endo medium. This medium was prepared by dissolving 1 grm. of lactose and 1 grm. of saccharose in about 15 c.c. of boiling water, adding melted 3 per cent. nutrient agar up to a volume of 200 c.c., and then making a final addition of 0·5 c.c. of alcoholic solution of fuchsin (2 grm. in 60 c.c. alcohol) and of 3 c.c. of a 20 per cent. solution of sodium sulphite.

The addition of 0·5 or 1 c.c. of liquor bismuthi prevents any *Proteus* bacilli which are subcultured from forming a spreading growth over the plates. On the dry surface of this medium each black colony was planted over an area about the size of a sixpence. Often within 8 hours, and always within 15 hours, it was possible to determine whether the colonies consisted of fermenters or non-fermenters of the lactose and saccharose. The acid formers nearly all appeared to belong to a group of organisms to which Wilson (1928) has given the name *B. effluviei.*

The agglutinability of the non-fermenting colonies was tested by removing a little of the growth on a platinum loop, and rubbing it up in a drop of a 1 in 60 dilution of a typhoid serum on a slide. The slide was then rocked to and fro over an electric lamp and the presence or absence of agglutination noted. If agglutination occurred, the colony from which the bacilli had been taken was subcultured into mannite broth containing Andrade's indicator, and then about an equal volume of melted agar (cooled to 50° C.) added. On incubation, if acid but no gas was formed, pure cultures were obtained from the colony, and their action on glucose, dulcite, saccharose and lactose was studied. Cultures in peptone water were tested for indole formation. The identification was completed by seeing whether the organism was agglutinated to full titre by a typhoid-agglutinating serum. In a considerable number of cases the capacity of the organism to remove agglutinins from a typhoid serum was examined. The organisms were motile and did not liquefy gelatin or inspissated serum.

Planted on Fleming's "influenza" medium the *B. typhosus* causes no clearing of the haemoglobin derivatives, whereas a zone of clearing occurs around the saccharose and lactose fermenting colonies of *B. effluviei*, and also with certain non-fermenters which probably are closely allied to the *B. effluviei* group.

As we discarded the majority of the organisms which were agglutinated by a strong anti-typhoid serum and which were subsequently found to produce acid and gas in glucose shake cultures, it is very probable that we missed many *B. paratyphosus* B strains, especially where the cultures were made after 48 hours' incubation of the bismuth media plates.

In Table III the results of experiments carried out since 30. viii. 28, the date of a former report by one of us (Wilson, 1928), are summarised.

Table III.

No. of exp.	Origin of sewage	Date	Amount of sewage examined in c.c.	Black colonies tested	No. of sac-charose or lactose fermenters	No. of non-sac-charose or lactose fermenters	No. of typhoid colonies found
1	Belfast	30. iii. 28	4·5	93	77	16	0
2	Belfast	17. v. 28	7·5	97	67	30	11
3	Belfast	5. vi. 28	7·5	93	72	21	9
4	Lisburn	22. viii.28	5·0	19	7	12	18
5	Belfast	3. ix. 28	5·0	150	150	0	0
6	Belfast	8. ix. 28	5·0	150	150	0	0
7	Belfast	20. ix. 28	4·0	10	9	1	1
8	Belfast	9. xi. 28	4·0	115	108	7	1
9	Belfast	21. xii. 28	6·0	119	98	21	7
10	Belfast	14. i. 29	6·0	83	45	38	33
11	Belfast	18. ii. 29	1·0	15	0	15	11
12	Belfast	8. iii. 29	2·0	52	38	14	4
13	Lisburn	8. v. 29	2·0	37	34	3	2
14	Belfast	30. vii. 29	6·0	69	53	16	5
15	Belfast	12. ix. 29	5·0	70	60	10	6
		Total	70·5	1172	968	204	108

It is seen that 12 out of the 15 experiments resulted in the isolation of the *B. typhosus*. The total volume of the sewage examined was 70 c.c. and from it 108 typhoid bacilli were isolated. There is, of course, irregularity in the distribution of bacilli in the sewage but, on an average, there is at least one typhoid bacillus in every c.c. of the sewage taken at any time the whole year round. The failure of experiments 5 and 6 was due to the large numbers of black colonies consisting mainly of saccharose fermenters.

In July, August and September there is a seasonal multiplication of sulphite-reducing organisms in sewage and in water, and at this season the isolation of the *B. typhosus* is rendered more difficult. Wilson (1928) has referred to this, and it has also been noted by A. C. Houston (1928).

Isolation of *B. paratyphosus* B from sewage.

As already observed, no attempts to isolate *B. paratyphosus* B were made in the earlier experiments. It is not improbable that *B. paratyphosus* B is as widely disseminated in Belfast sewage as is *B. typhosus*. Here we will refer to two occasions on which it was isolated from Belfast sewage. Of the 14 non-fermenters of lactose and saccharose isolated from the sewage of 8. iii. 29, seven were agglutinated on a slide by typhoid serum. Of these seven, four proved to be *B. typhosus* and two *B. paratyphosus* B.

On 12. iii. 29 1 c.c. of the sewage of 8. iii. 29 was mixed with 10 c.c. of water and then mixed with an equal volume of double strength bismuth medium B and poured out into a plate. In all 15 c.c. of the sewage were utilised in this way, and 15 cultures of *B. paratyphosus* B were obtained.

The sample of Belfast sewage taken on 12. ix. 29 and which, as we have noted, yielded 10 black colonies consisting of non-fermenters of lactose and saccharose, and nine of these were agglutinated by a strong typhoid serum. Subsequent tests showed that six of the nine were typhoid bacilli, and that three were paratyphoid B bacilli.

VIABILITY OF *B. TYPHOSUS* IN SEWAGE.

On 5. vi. 28 four Winchester Quart bottles, labelled *A*, *B*, *C* and *D*, were taken of Belfast crude screened sewage.

We have already seen that from 7·5 c.c. of this sewage nine typhoid bacilli were cultivated. The sewage contained 500,000 *B. coli* and 3000 spores of *B. welchii* per c.c. The bottles were stoppered and kept at room temperature.

On 13. vi. 28 bottle *B* was shaken and 1 c.c. was planted over the surface of each of four plates containing the bismuth brilliant green medium. Seven black colonies were studied, and five proved to be typhoid bacilli.

On 15. vi. 28 one c.c. on the surface of each of four plates yielded eight black colonies, two of which proved to consist of typhoid bacilli. On the same date four plates were poured out after 1 c.c., 5 c.c., 5 c.c. and 5 c.c. of sewage had been added to the melted and cooled medium. Fifty black colonies were studied; 48 were lactose and saccharose fermenters and two proved to be typhoid bacilli.

On 18. vi. 28 and on 22. vi. 28 we were unsuccessful in isolating any typhoid bacilli.

On 23. vi. 28 ten c.c. of the sewage in bottle *B* were mixed with an equal volume of the bismuth brilliant green medium and poured into plates. Fourteen black colonies developed, six were non-fermenters of lactose and saccharose, and of these I gave all the usual cultural characters of the *B. typhosus*, and was agglutinated up to full titre. This bacillus had survived for 18 days, *i.e.* from 5. vi. 28 till 23. vi. 28.

On 26. vi. 28 seven plates were inoculated with bottle *B* sewage which had, during the latter experiments, been exposed to the air and fairly well aerated; no black colonies of any kind developed.

On 26. vi. 28 ten c.c. of sewage of 5. vi. 28 contained in bottle *D* which was stoppered and undisturbed were planted out; 52 colonies were studied, 31 being fermenters and 21 non-fermenters of saccharose and lactose. Nine of the 21 were agglutinated by a typhoid serum but seven formed acid and gas and only two proved to be typhoid bacilli. Those which were agglutinated in low titre by the typhoid serum but which formed acid and gas in glucose may have been paratyphoid bacilli, but this point was not determined.

On 28 vi. 28 the deposit in bottle *D* was stirred up and 10 c.c. of the

contents were planted out: 51 black colonies were examined, 20 being non-fermenters of lactose and saccharose, and of these two were agglutinated to full titre with a typhoid serum and gave the cultural characters of *B. typhosus*.

On 10. vii. 28 thirty c.c. from bottle *D* were examined with negative results, but, on 12. vii. five black colonies developed from inoculations made on that date, and one proved to be *B. typhosus*.

On 13. vii. 28 the top liquor was poured off from bottle *D* leaving 100 c.c. at the bottom containing most of the larger solids; this was mixed with 100 c.c. of sterile water and 10 c.c. amounts mixed with melted bismuth brilliant green medium B, and poured out into each of five plates; 20 black colonies were found, of which nine were non-fermenters of lactose and saccharose and of which one proved to be the typhoid bacillus. This bacillus produced acid but no gas in glucose, maltose and mannite, had no action on saccharose, lactose and dulcite, formed no indole, and was agglutinated to full titre with an anti-typhoid serum. This result showed that there was at least one typhoid bacillus alive in the sewage after storage in bottle from 5. vi. till 13. vii. 28.

Further tests of the watery deposit in bottle *D* were made on 13. vii., 17. vii. and on 23. vii., but no typhoid bacilli were isolated. On 25. vii. supernatant liquor which smelled strongly of sulphuretted hydrogen was poured off from bottle *C* and 50 c.c. of the fluid at the bottom of the bottle were planted out in bismuth medium *B*; 36 black colonies developed and of these 29 were non-fermenters of lactose and saccharose but proved not to be typhoid bacilli.

From this experiment we may conclude that it is possible for the typhoid bacillus, which is in sewage under natural conditions, to survive for over 5 weeks (38 days) when kept in a Winchester Quart bottle at room temperature and exposed to light. Originally there were at least 500,000 *B. coli* present in each c.c. of the sewage, and there was no doubt a bacteriophage for the *B. typhosus* present in the sewage. We may observe that the bacilli seemed to disappear from the supernatant liquor and to survive in the sediment. Dodgson (1928) remarks: "An important consideration in connection with water directly polluted by crude sewage is the possibility of the presence of undisintegrated faeces, etc. Even if such material be merely particulate any typhoid bacilli which it may contain may be protected against adverse conditions to which they might be exposed if free in the water. Gaertner and Rubner and Ohlmüller have investigated this aspect of the question and claim to have shown that envelopment in particles of faeces definitely favours the survival of the bacilli." We have no doubt from our experiment that such is the case.

A second experiment showed that typhoid bacilli could be isolated from crude sewage at the end of 16 days. The sewage of 14. i. 29, of which 33 typhoid bacilli had been isolated, was re-examined on 30. i. 29, and of 52 black colonies examined seven proved to be non-fermenters of lactose and saccharose, and of these two consisted of typhoid bacilli.

VIABILITY OF *B. TYPHOSUS* AND *B. PARATYPHOSUS* B IN SEWAGE.

The sewage of 8. iii. 29 was poured out into 8 oz. bottles, which were filled and corked and kept at room temperature in the laboratory in a dark press, on the roof or in a Frigidaire cupboard.

Cultures were made from the deposit in these bottles on 19. iii. 29 on the surface of bismuth brilliant green plates. The results were: from the bottle at room temperature four typhoid bacilli and two paratyphoid B bacilli were isolated. The corresponding numbers for bottles in dark press and Frigidaire and on the roof were: 2 and 1, 1 and 3, 0 and 2 respectively.

On 29. iii. 29 seven plates were inoculated on the surface with deposit from bottle kept at room temperature, and resulted in the isolation of two paratyphoid but no typhoid bacilli. On the same day, 29. iii. 29, similar cultures were made from the deposit in bottles kept in dark press and resulted in the isolation of one paratyphoid bacillus but no typhoid bacilli.

In this experiment typhoid bacilli survived in considerable numbers from 8. iii. 29 till 19. iii. 29, *i.e.* 11 days. The paratyphoid bacilli survived equally well, and a few were found alive on 29. iii. 29, *i.e.* after 21 days.

The older work on the survival of typhoid bacilli in sewage consisted in adding large numbers of typhoid bacilli to sewage or in exposing bacilli contained in celloidin bags (Russell and Fuller, 1906) to the action of the sewage.

Dodgson quotes from MacConkey's experiment as follows: "That although *B. typhosus* (8,000,000 per 1 c.c. added) might disappear from crude sewage within six days, it might persist in similar sewage in significant numbers for 13 days (reduction from 200,000 to 1000 per c.c.). In the latter case the reduction in the *B. typhosus* population was in about the same ratio as that of the sewage organisms in general."

Russell and Fuller state that the survival period of *B. typhosus* subjected to the action of sewage is from 3 to 5 days.

We think that our work gives laboratory support for the possibility of the survival of *B. typhosus* in the outer world sufficiently long to render possible water-borne epidemics, the occurrence of which has been definitely established by epidemiological investigations, but for the possibility of which laboratory investigators could produce no evidence.

ATTEMPTS TO SUPPRESS *B. EFFLUVIEI.*

It was soon appreciated that, if the bismuth media could be modified in such a way as to inhibit the growth of organisms other than *B. typhosus* which formed black colonies on the plates and which we may consider as belonging to the group of *B. effluviei* (Wilson), the isolation of *B. typhosus* from sewage and water could be facilitated especially during July, August, and September when the organisms appear to show a seasonal increase in numbers. In faeces, as far as our experience indicates, *B. effluviei* is never present.

As the reduction of sulphites is probably connected with the respiration

of the bacteria and, as we had learned from Meyerhof's *Chemical Dynamics of Life Phenomena*, that narcotics inhibit the rate of oxidation, we were led to try the effects of the addition of certain narcotics to the bismuth media.

We may say at once that it was found possible in this way to inhibit the growth of certain strains of *B. effluviei* whilst allowing that of *B. typhosus*, but that in practice no great advantage occurred when we were dealing with the various strains met with in sewage.

In our experiments we made use of acetamide, urethane, chloral hydrate, barbitonum, chlorobutol, phenyl-urea, di-ethyl-urea, methyl-urea, di-ethyl-diphenyl-urea, acetone, chloroform, sulphonal and methyl-sulphonal.

The following concentrations of a few of these narcotics appeared to yield promising results for our purpose. If to 100 c.c. of agar the usual constituents of the bismuth media were added, it was found that the subsequent addition of 16 c.c. of a 16 per cent. watery solution of acetamide, of 0·7 c.c. of a 10 per cent. watery solution of chloral hydrate, 8 c.c. of a 10 per cent. solution of urethane, and 3 c.c. of a 5 per cent. alcoholic solution of barbitonum, did not prevent the development of *B. typhosus*, did inhibit some strains of *B. effluviei*, but in actual tests with sewage the modifications had no advantage over our standard media.

The results of our experiment carried out with sewage of 14. i. 29 are given in Table IV.

Table IV.

Medium	Amount of sewage c.c.	Total black colonies tested	Sac-charose fermenters	Non-saccharose fermenters	Typhoid bacilli isolated
Standard bismuth B	1	25	17	8	4
+ Acetamide	1	16	5	11	9
+ Urethane	1	28	12	16	11
+ Barbitonum	1	7	5	2	1
+ Di-ethyl-di-phenyl-urea	1	2	2	0	0
+ Chloral	1	15	4	11	8

Our experiments, which need not be given here, showed that narcotics showed a decided selective action on the growth of various micro-organisms—a result to be expected from their action on the surface of membranes and, in the case of bacteria, it is known that specific differences are in many cases associated with the chemical constitution of their capsules.

Isolation of *B. typhosus* and *B. paratyphosus* B from water.

Encouraged by Sir Alexander Houston's success in isolating the *B. typhosus* from Thames water by means of our media, we have commenced the testing of the River Lagan.

Here we shall report the results of our two first experiments. The technique was similar to that employed in the investigation of sewage, but to concentrate the bacteria 2·5 c.c. of a 10 per cent. solution of aluminium sulphate were added to each litre of water, the *p*H being adjusted to about 7. The precipitate

was allowed to settle, the supernatant water aspirated off and the lower layer containing about 100 c.c. was put into tubes and centrifugalised. Finally the precipitate in the bottom of the centrifuge tubes was spread over the surface of the bismuth medium B, or was incorporated in its substance when in the liquid condition.

Exp. 1. 24. ix. 29. Four litres of water from the River Lagan were taken about 2 miles above Belfast. Forty-nine black colonies were examined; 26 were lactose-saccharose fermenters and 23 non-fermenters, but none proved to be the *B. typhosus*, but were organisms capable of liquefying gelatin and of digesting inspissated serum.

Exp. 2. 7. x. 29. Four litres of water were taken from Lagan Canal close to the entrance of a stream into which the effluent of Lisburn sewage works discharged. The sewage of Lisburn is treated on contact-beds and then on land, and eventually the effluent reaches a stream which, after a course of about 200 yards, enters the Lagan Canal. The sewage is well purified and does not cause secondary decomposition in the canal. The water in our sample looked like water, but it contained a considerable amount of effluent, as *B coli* were present in 0·001 c.c. and there were 12 spores of *B. welchii* per c.c. From the aluminium coagulum from this water planted on or in the usual bismuth medium B, and in the same medium with the addition of chloral, a considerable number of black colonies developed. Ninety-three of these were studied: 74 fermented lactose and saccharose; 19 were non-fermenters and of these four proved to be typhoid bacilli and two were *B. paratyphosus* B.

Identification of *B. typhosus* and *B. paratyphosus* B.

For routine work we regarded a bacillus as the *B. typhosus* if it formed a characteristic dry black colony on our bismuth media, gave a clear growth on Endo's medium containing lactose and saccharose, formed acid and no gas in agar shake cultures containing mannite and glucose (Andrade's Indicator) and was agglutinated to full titre by an anti-typhoid serum. In most instances specimens were examined for absence of liquefaction of gelatin and absence of indole formation.

In a considerable number of the strains isolated we not only tested their agglutinability by a typhoid serum, but also by absorption tests proved that they were able to remove from the serum the agglutinins not only for themselves but also those for typical *B. typhosus*.

Similar tests were employed in the identification of the *B. paratyphosus* B. We satisfied ourselves that the bacilli failed to ferment lactose and saccharose and that they formed acid and gas in mannite and glucose, formed no indole, did not liquefy gelatin and were agglutinated to full titre by a specific serum. Absorption tests were also employed.

In the case of four typhoid strains and two paratyphoid B strains isolated from the Lagan Canal, rabbits were inoculated and their sera tested on the

homologous strains and on strains isolated from enteric fever patients. Absorption tests proved that genuine typhoid and paratyphoid bacilli B removed the agglutinins both for themselves and for the suspected organisms from the serum of the rabbits; also absorption of specific anti-typhoid and paratyphoid B sera with the strains removed the agglutinins for the strains and also those for genuine typhoid and paratyphoid bacilli. In the case of a few strains additional tests were employed.

In the differential medium of Jordan and Harmon (1928) containing sodium-potassium tartrate (Rochelle salts) 18 typhoid cultures were tested; all gave an acid reaction and of the 18, 5 were isolated from patients and 13 from sewage.

Thirty-three paratyphoid strains, 21 from patients and 11 from sewage, all gave an alkaline reaction, whilst a laboratory culture of *B. aertrycke* (Mutton) gave an acid reaction.

Rhamnose Fermentation.

According to Bitter, Weigmann and Habs (1926), when paratyphoid B cultures or *aertrycke* (Breslau) cultures are sown in a saline peptone solution containing 0·5 per cent. of rhamnose and, after a growth of 15 or 16 hours at 37° C., two drops of methyl red indicator (0·5 grm. methyl red dissolved in 100 c.c. of alcohol) are added to each culture, the paratyphoid bacilli will show a distinct yellow or orange colour, whereas the *aertrycke* (Breslau) will become distinctly red.

This we can confirm to a large extent from our observations as regards cultures of *B. paratyphosus* B recently isolated from cases of paratyphoid fever. In an experiment, where we employed 23 strains of *B. paratyphosus* B recently isolated from paratyphoid patients, 15 strains of *B. paratyphosus* B isolated from sewage, one old stock laboratory culture of *B. paratyphosus* B and one culture of *B. aertrycke*, it was found after 22 hours when the first observation was made that 12 of the cultures were acid in reaction, and that these consisted of the *aertrycke* culture, the old laboratory stock culture of *B. paratyphosus* B and 10 sewage strains of *B. paratyphosus* B; the 28 others including all the strains isolated from patients were alkaline. After 72 hours eight (including two sewage strains) became acid, and, after 96 hours, all gave the red reaction. We find that Andrade's indicator can show the changes more conveniently than the use of methyl red.

In ordinary peptone water containing rhamnose, the same differences in the rate of fermentation are manifested, but the changes in reaction occur more quickly.

In an experiment where rhamnose peptone water was inoculated with 20 paratyphoid B cultures recently isolated from patients, one old laboratory stock culture of *B. paratyphosus* B, one *aertrycke* bacillus, and five strains of *B. paratyphosus* B isolated from sewage, we found six tubes acid at the end of 16 hours' incubation at 37° C., and that most of the others showed slight

change of reaction at the end of 28 hours, going on to complete reddening at the end of 40 hours. The six cultures bringing about the rapid fermentation in this experiment consisted of our old stock culture of *B. paratyphosus* B, the *aertrycke* bacillus and four sewage paratyphoid bacilli.

These experiments would seem to indicate that, as regards fermentation of rhamnose, the majority of our sewage strains of *B. paratyphosus* B behaved more like the *aertrycke* bacillus than those of the strains of *B. paratyphosus* B we were finding in the stools of cases of paratyphoid fever. We may observe, however, that the 28 strains isolated from the River Lagan were slow fermenters and that it was in County Down, in which county the river rises, that most of our enteric fever patients resided.

That our sewage cultures were genuine *B. paratyphoid* B is probable from our agglutination and absorption experiments, even though we did not attempt a receptor analysis. Moreover, they behaved in Jordan and Harmon's medium like the *B. paratyphosus* B and not like *B. aertrycke*.

ISOLATION OF *B. ENTERITIDIS* GAERTNER FROM STOOLS.

On Wednesday, 16. x. 29, an outbreak of food poisoning occurred in Ballymena—a town about 30 miles distant from Belfast. The outbreak was not thoroughly investigated, but it is almost certain that the infection was conveyed by milk, and that the milk acquired the *B. enteritidis* Gaertner from a sick cow.

Through the kind services of Dr John Stuart (Ballymena) and Mr J. C. Loughridge F.R.C.S. (Belfast) we had an opportunity of examining 14 specimens of stools from 14 patients. The faeces of one patient (*D*) were taken on 18. x. 29, and were planted on MacConkey's medium containing brilliant green, and on our old standard bismuth brilliant green medium. On the MacConkey medium no members of the Salmonella group developed, but on the bismuth medium, after a delay of 60 hours, 200 colonies of *B. enteritidis* Gaertner appeared. On 21. x. 29 13 other stools were similarly tested with results shown in Table V.

Table V. *Colonies of* B. enteritidis *Gaertner.*

Patient's no.	MacConkey + brilliant green	On old standard bismuth brilliant green medium on fourth day
1	Negative	20
2	,,	2
3	,,	Negative
4	,,	100
5	,,	6
6	,,	Negative
7	,,	,,
8	,,	,,
9	100	Several hundred
10	Several hundred	,,
11	,,	,,
12	,,	,,
13	,,	,,

The 14 stools, therefore, yielded positive results ten times with the bismuth medium and five times with the MacConkey medium. The examination was made when many of the patients were convalescent about 5 days after the acute illness. In this outbreak, in addition to human beings, cats and dogs suffered. It is worthy of record that, in spite of the large number of bacilli in the stools of the patients, no secondary cases occurred. Immediately after the start of the outbreak the sick cow was slaughtered.

PART II.

Numerous experiments were carried out using the sulphite and bismuth solutions in different proportions. It was found that it was possible to obtain black surface colonies of *B. typhosus* in a medium free from iron. This new medium will be referred to in this paper as the New Standard bismuth medium to distinguish it from the older iron-containing medium, which we shall designate Old Standard medium B.

New Standard bismuth sulphite medium.

This medium is prepared in the usual way and contains: 100 c.c. nutrient agar, 2·5 c.c. of 20 per cent. glucose, 5 c.c. of 20 per cent. sodium sulphite, 2·5 c.c. liq. bismuthi, 0·5 grm. sodium phosphate (exsiccated), 0·5 c.c. of a 1 per cent. solution of brilliant green.

On this medium the *B. typhosus* forms black colonies, as does also the *B. paratyphosus* B, the latter growing better when only 3 c.c. of sodium sulphite instead of 5 c.c. are used.

The New Standard medium, if mixed with an equal volume of water containing a few typhoid bacilli and the mixture poured out into plates, allows the formation of black colonies in its depth as well as on its surface.

For the development of typhoid colonies in the depths of a tube or plate we have found the following formulae useful.

For deep colonies in tubes.

(1) 100 c.c. nutrient 3 per cent. agar, 10 c.c. glucose 20 per cent., 2 c.c. sulphite 20 per cent. (for plates use 3 c.c.), 3 c.c. liq. bismuthi, 1 c.c. 8 per cent. ferrous sulphate solution, 1 c.c. of a 1 per cent. solution of brilliant green.

(2) 100 c.c. nutrient 3 per cent. agar, 5 c.c. glucose 20 per cent., 1·5 c.c. sulphite 20 per cent., 3 c.c. liq. bismuthi, 0·5 c.c. of a 1 per cent. solution of brilliant green.

For the typhoid bacillus 2 c.c. of sodium sulphite with 2 c.c. of liq. bismuthi may be employed, for *paratyphosus* B, 2 c.c. of liq. bismuthi to 1 c.c. of sodium sulphite gives better results.

For deep colonies in plates.

100 c.c. nutrient agar 3 per cent., 2·5 c.c. glucose 20 per cent., 5 c.c. sulphite 20 per cent., 2·5 c.c. liq. bismuthi, 1 grm. exsiccated sodium phosphate,

0·5 c.c. of a 1 per cent. solution of brilliant green. The above is very suitable for *B. typhosus*, less suitable for *paratyphosus* B; for the latter use 2 c.c. sulphite and 3 c.c. liq. bismuthi.

STANDARDISATION OF THE MEDIA.

We have employed the media for over 3 years and never have had any trouble with them. Others would appear to have found the results inconstant. Buonomini (1929) found that the blackening of the colonies depended on the brand of bismuth citrate employed. We had employed bismuth citrate obtained from Baird and Tatlock, London, and from the British Drug Houses, Ltd. The secretary of the latter company has informed us that their bismuth citrate is prepared "by mixing bismuth subnitrate and citric acid with distilled water, and heating on a water bath with frequent stirring until a drop of the mixture yields a clear solution with ammonia: then adding water, allowing the salt to deposit, washing the precipitate so obtained until the washings are tasteless, and drying at a gentle heat." After the appearance of Buonomini's paper we obtained samples of bismuth citrate from Merck and from Kahlbaum: solutions of liq. bismuthi prepared in our usual way from these samples were compared with those that we were using. The appearance of the liquors differed, some being clear, some slightly turbid, but in our tests all gave satisfactory results—good black colonies.

For the guidance of those employing the medium for the first time, we would emphasise the importance of inoculating the plates with water containing a very few typhoid bacilli, so that discrete colonies may be obtained, as, where the growth is confluent, no darkening may occur.

As regards the solution of sulphite, it is a matter of indifference whether a 20 per cent. solution of sodium sulphite (exsiccated) or a 40 per cent. solution of sodium sulphite crystals is employed.

As regards the iron solution, we employed an 8 per cent. solution of ferrous sulphate crystals, but Buonomini prefers an 8 per cent. solution of ferrous ammonium sulphate $FeSO_4(NH_4)_2SO_46H_2O$.

It had been our practice to use Fairchild's peptone, and we always found it suitable for our media. We then tested Bacto peptone and found it equally good, but with Witte's peptone the colonies of typhoid did not come up well, and in many cases did not blacken. In the preparation of the media, therefore, Witte's peptone should be avoided.

For making nutrient agar, we employ 3 per cent. powdered agar No. 1 quality, 1 per cent. peptone (Fairchild's or Bacto), and 0·5 per cent. Lemco (Liebig's Extract of Meat). Ordinary Lemco does very well, but the special preparation Lab-Lemco is to be preferred.

We have used ordinary tap-water, but distilled water in many parts of the world may be necessary, as there may be salts in the water which interfere with the reduction process. We have found that the presence of 0·05 per cent. of potassium nitrate in the old standard medium prevents the development

of jet black colonies. The addition of brilliant green to the medium not only aids the inhibition and destruction of *B. coli* but promotes blackening. Blackening is also promoted by the addition of 2 c.c. of a 5 per cent. solution of tartar emetic to every 100 c.c. of the Old Standard medium.

We have already emphasised the importance of the addition of 1 grm. of exsiccated sodium phosphate dissolved in 10 c.c. of distilled water to each 100 c.c. of the Old Standard medium.

Stock bismuth sulphite mixtures.

Experiments made to ascertain if it were possible to get equally good results with stock solutions containing a mixture of the constituents, instead of adding them separately, showed that this was the case.

For example, the addition of 16 c.c. of the following mixture to 100 c.c. of hot nutrient agar plus 5 c.c. of a 20 per cent. solution of glucose, provides a medium yielding excellent black surface colonies of *B. typhosus*: 100 c.c. sodium sulphite 20 per cent., 50 c.c. liq. bismuthi, 10 c.c. of an 8 per cent. solution of ferrous sulphate crystals and 10 grm. of exsiccated sodium phosphate. These reagents boiled together give a mixture which has been found to remain active for over a month.

The mixture for the New Standard medium is made by boiling together 100 c.c. of a 20 per cent. sodium sulphite solution with 50 c.c. liq. bismuthi and 10·5 grm. of sodium phosphate (exsiccated). Of this mixture (warmed if necessary in cold weather to secure solution of the phosphate) 7 c.c. are added to 100 c.c. hot nutrient agar plus 2·5 c.c. of a 20 per cent. solution of glucose. In the case of both, the media are completed by the addition of 0·5 c.c. of a 1 per cent. solution of brilliant green.

Selective action of alcohols.

Experiments were made with different amounts of the mixtures in glucose and lactose agar, and the growth thereon of different intestinal bacteria was studied.

In the course of the investigation use was made of an alcoholic solution of phenol red as an indicator. It was found that the addition of the alcoholic solution of phenol red suppressed the growth of certain sewage organisms which developed on the bismuth plates, and which rendered the isolation of typhoid bacilli more difficult. Further work showed that it was the alcohol and not the phenol red which tended to suppress the sewage organisms, whilst allowing the development of the *B. typhosus*.

From numerous tests we conclude that, for the isolation of *B. typhosus* from sewage, the addition of 2 c.c. of absolute alcohol to every 100 c.c. of nutrient agar in the Old Standard bismuth sulphite brilliant green medium, and of 4 c.c. or 5 c.c. to the New Standard brilliant green medium is an advantage.

We have also studied the action of methyl alcohol, propyl alcohol and amyl alcohol, and recommend the addition of 1 c.c. and 1·6 c.c. of propyl alcohol to the Old and the New Standard bismuth sulphite brilliant green media respectively.

The addition of ethyl alcohol tends to prevent the development of *B. paratyphosus* B in the New Standard medium, but this does not result from the addition of propyl alcohol.

For the development of *B. typhosus* in the depth of the medium we recommend the following medium to be mixed when fluid with an equal volume of water containing typhoid bacilli and to be poured out into Petri dishes: 100 c.c. nutrient agar, 2·5 c.c. glucose (20 per cent.), 7 c.c. from a mixture consisting of 100 c.c. of 20 per cent. sodium sulphite (anhydrous), 50 c.c. liq. bismuthi and 10·5 grm. sodium phosphate (exsiccated), 1 c.c. of a 1 per cent. solution of brilliant green and then 3·5 c.c. of propyl alcohol or 6 or 7 c.c. of absolute ethyl alcohol.

The propyl alcohol is suitable either for *B. typhosus* or *B. paratyphosus* B, and the ethyl alcohol for *B. typhosus* only.

The addition of the alcohols tend to suppress the growth of the *B. effluviei*, dark colonies of which are especially abundant in sewage in July and August.

SELECTIVE ACTION OF MERCURY SALTS.

As *B. proteus* grew quite as well as *B. typhosus* on the bismuth media, an effort was made to discover a modification of the medium which would allow the growth of *B. typhosus* and suppress that of various strains of the *proteus* group. In this connection experiments were made with salts of antimony, copper, silver and mercury. It was found that approximately 1 in 400,000 of perchloride of mercury in the bismuth brilliant green media suppressed to a large extent the growth of many *proteus* strains, whilst allowing the growth of *B. typhosus*.

The addition of 4 c.c. of a 1 in 10,000 watery solution of perchloride of mercury to a medium which has been prepared from 100 c.c. nutrient agar, 2·5 c.c. glucose 20 per cent., 7 c.c. of the mixture (100 c.c. sod. sulphite 20 per cent., 50 c.c. liquor bismuthi, 10·5 grm. sodium phosphate exsiccated), 0·5 c.c. of a 1 per cent. solution of brilliant green afforded a substrate on the surface of which, when poured out in Petri dishes, colonies of *B. typhosus* developed in normal fashion, whilst those of most strains of the *proteus* group failed to appear. It was also found that the mercury solution could be used in conjunction with alcohol, and that, in some instances, the above medium was improved by the addition of 4 c.c. of absolute alcohol.

After our preliminary experiments, we proceeded to try out our new media by inoculating them with sewage.

ISOLATION OF *B. TYPHOSUS* OR *B. PARATYPHOSUS* B FROM SEWAGE BY MEANS OF VARIOUS MEDIA.

I. Old Standard glucose bismuth sulphite brilliant green medium.

(*a*) Sewage	(*b*) Date	(*c*) Amount of sewage in c.c.	(*d*) No. of black colonies	(*e*) Fermenters of lactose and saccharose	(*f*) Non-fermenters of lactose and saccharose	(*g*) No. of typhoid or paratyphoid isolated
Belfast	15. xii. 29	1	30	24	6	3 typhoid
,,	12. ii. 30	1	13	5	8	5 paratyphoid
,,	24. ii. 30	1	16	2	14	13 typhoid
,,	8. iii. 30	1	5	4	1	0
,,	28. iv. 30	1	20	20	0	0
,,	19. v. 30	1	11	9	2	0
,,	29. vii. 30	1	9	9	0	0

II. Old Standard medium plus 2 c.c. of absolute alcohol to every 100 c.c. nutrient agar.

(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)
Belfast	8. iii. 30	1	1·4	11	3	3 typhoid

III. New Standard medium.

(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)
Belfast	15. xii. 29	1	6	6	6	5 typhoid
,,	12. ii. 30	1	20	15	5	1 typhoid and 3 paratyphoid B
,,	24. ii. 30	1	20	7	13	10 typhoid
,,	6. vi. 30	1	5	5	0	0
,,	29. vii. 30	1	3	3	0	0

IV. New Standard containing for every 100 c.c. of nutrient agar: (1) 3 c.c. absolute alcohol; (2) 4 c.c. absolute alcohol; (3) 5 c.c. absolute alcohol; (4) 4 or 5 c.c. of $HgCl_2$ 1 in 10,000; (5) 3 c.c. absolute alcohol plus 4 c.c. of $HgCl_2$ 1 in 10,000; (6) 4 c.c. absolute alcohol plus 4 c.c. of $HgCl_2$ 1 in 10,000.

(*a*)		(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)
(1)	Belfast	6. vi. 30	1	4	0	4	3 typhoid
(2)	,,	28. iv. 30	1	5	1	4	3 ,,
	,,	6. vi. 30	1	10	0	10	6 ,,
	,,	29. vii. 30	1	6	3	3	3 ,,
(3)	,,	12. ii. 30	1	13	1	12	10 ,,
	,,	28. iv. 30	1	0	0	0	0
	,,	29. vii. 30	1	5	3	2	2 ,,
(4)	,,	12. ii. 30	1	6	5	1	1 paratyphoid
(5)	,,	6. vi. 30	1	6	1	5	4 typhoid
(6)	,,	6. vi. 30	1	5	0	5	2 ,,
	,,	29. vii. 30	1	11	11	0	0

Deep colonies.

The New Standard medium was mixed with an equal volume of water inoculated with 1 c.c. of sewage and poured out into plates. By this technique the sewage of 15. xii. 29 yielded several colonies of *paratyphosus* B. The same medium plus 3·5 c.c. of propyl alcohol per 100 c.c. of nutrient agar with sewage

of 8. iii. 30 which was employed on 17. iii. 30 yielded four black colonies, two of which proved to be typhoid and one of which was *paratyphosus* B.

A medium consisting of 100 c.c. nutrient agar, 5 c.c. glucose, 1 c.c. liq. bismuthi, 1 c.c. sulphite and 0·5 c.c. brilliant green mixed with an equal volume of water and poured into tubes, yielded with 1 c.c. of sewage of 15. xii. 29 numerous black colonies: 17 were examined, and of four which failed to ferment lactose and saccharose, two proved to be *paratyphosus* B.

PART III.

A FLUID ENRICHMENT BISMUTH MEDIUM.

We have employed fluid enrichment media containing sulphite, bismuth and brilliant green, and have succeeded in securing multiplication of typhoid bacilli and, at the same time, the inhibition or even destruction of *B. coli*. It is only recently that we have obtained encouraging results, as in our earlier experiments the *B. typhosus* was very frequently outgrown by various strains of the *proteus* and *alcaligenes* groups. Now by the addition of alcohol and perchloride of mercury and possibly also by the use of mannite instead of glucose it is possible to suppress these strains.

As a basis nutrient broth is employed, and for the formulae suggested by us it will be found necessary to use Fairchild's peptone and not Bacto or Witte's peptone. No doubt for these two brands formulae could be devised, but so far we prefer to use Fairchild's, as better growth of the *B. typhosus* occurs in broth made from it when our reagents and especially alcohol and perchloride of mercury are added.

The broth is made in the ordinary way and is filtered through filter paper to render it quite clear. It contains: distilled water 1000 c.c., Fairchild's peptone 10 grm., Lab-Lemco 5 grm., and its reaction is made faintly alkaline to litmus paper.

To the broth mannite is added to form a 0·5 per cent. solution. The enrichment medium is made by taking 100 c.c. mannite broth, adding to it 6 c.c. of a 20 per cent. solution of sodium sulphite (exsiccated) and 0·2 liq. bismuthi and by heating the mixture to the boiling point. A flocculent precipitate separates out and should be shaken through the mixture. Finally 0·5 c.c. of a 1 per cent. watery solution of brilliant green is added.

We may mention that in the preparation of the liquor bismuthi we have employed various brands of bismuth citrate, *e.g.* Baird and Tatlock's (London), British Drug Houses, Merck and Kahlbaum, and have found them all satisfactory.

This medium we shall designate Standard bismuth broth (*A*), and of it we have made several modifications by the addition to every 100 c.c. of 1 c.c. of absolute ethyl alcohol (*B*), 1 c.c. of propyl alcohol (*C*), 2 c.c. of methyl alcohol (*D*), 1 c.c. absolute alcohol plus 4 c.c. of 1 in 10,000 solution of perchloride of mercury in distilled water (*E*).

ISOLATION OF *B. TYPHOSUS* FROM STOOLS BY BISMUTH FLUID ENRICHMENT MEDIA.

Table VI.

No.	Designation	Date	Standard broth A	B	C	D	E
1	H.	12. iv. 30	+	+	−	...	...
2	J.	12. iv. 30	+	+	+	...	...
3	M. B.	22. iv. 30	+	+	+	+	...
4	C. M.	18. iv. 30	+	−	−	−	...
5	Ma. Bi.	9. v. 30	+	−	−	−	...
6	M. Bi.	9. v. 30	+	−	−	−	...
7	P. R.	24. v. 30	+	...	...	...	+
8	N. P.	27. v. 30	+	...	...	...	+
9	D. B.	29. v. 30	+	...	...	...	+
10	A. McI.	14. vi. 30	...	...	...	...	+
11	F. McI.	14. vi. 30	...	...	...	...	+
12	M.	16. vi. 30	+	...	...	...	+
13	T. R.	1. vii. 30	+	...	...	...	+
14	M. R.	1. vii. 30	+	...	...	...	+
15	M. S.	1. vii. 30	+	...	...	...	+
16	McE.	26. vii. 30	+	...	...	...	+
17	E. I.	19. viii. 30	+	...	...	...	+

From Table VI we see that the stools of 17 typhoid fever patients have been tested in the enrichment media and that 15 were tested in the Standard broth with 15 successes. Eleven were tested in the Standard broth containing in every 100 c.c. 1 c.c. of absolute alcohol, and 4 c.c. of a 1 in 10,000 $HgCl_2$ solution and in all 11 cases *B. typhosus* was isolated.

Subcultures from the broth tubes were made on to plates containing the Old Standard B medium and on to MacConkey plates. In this connection it is advisable to use MacConkey plates instead of Endo plates, as on the latter the bacilli are liable to be inagglutinable.

The use of alcohol and perchloride of mercury in the medium tends to the destruction of *proteus* strains. We may add that, in many cases, subcultures taken at intervals of 24 hours, 48 hours and 60 hours showed that there was a considerable multiplication of *B. typhosus* in the medium, and that the *B. coli* was not only inhibited in its growth, but was actually killed. Frequently on the MacConkey plates the subcultures afforded a pure growth of *B. typhosus*.

ISOLATION OF *B. TYPHOSUS* FROM SEWAGE BY MEANS OF STANDARD BISMUTH BROTH.

For this purpose tubes containing 25 c.c. of the broths were inoculated with 0·1 and 0·5 c.c. of the sewage. The results are given in Table VII.

Table VII.

Deposit of sewage of 21. iv. 30 Sewage	Date	Standard bismuth broth A	B	C	D	E
Belfast	16. v. 30	−	...	...	...	+
„	19. v. 30	−	−	...	...	+
„	26. v. 30	...	...	...	...	+
„	6. vi. 30	...	...	...	...	−
„	29. vii. 30	−	...	...	...	−

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It is, therefore, possible by means of a fluid bismuth sulphite enrichment medium to cultivate *B. typhosus* from sewage; whether as good results with this method could be obtained as with the Old and New Standard media containing alcohol, could only be shown by further investigations.

For the isolation of *B. typhosus* from faeces it is probable that where very few bacilli are present as in the case of some "carriers" and convalescents, the use of the fluid medium has advantages. At any rate on four occasions we were successful in demonstrating the presence of *B. typhosus* by its means where failure had occurred with the solid media.

DISCUSSION.

At the present stage it would be premature to attempt to explain the action of our bismuth sulphite media. One may hazard a guess that the inhibition of the growth of *B. coli* is concerned with the respiration of the bacillus. In fluid media and in deep cultures in solid media the growth of *B. typhosus* is best marked close to the surface. It would seem that our reagents interfere with the growth of *B. typhosus* unless a certain amount of oxygen is present. It is not improbable that the selective action on the growth of bacteria is determined by the varying permeability of the outer membrane of different species.

Whatever the explanation may be, it is an undoubted fact that a certain bismuth sulphite combination can kill *B. coli* and allow *B. typhosus* to grow, and this observation may find an application in the field of Chemotherapy.

It is possible that, along these lines, a substance may be found which would allow the growth of normal cells in the body, and at the same time inhibit the growth and destroy the cells of a malignant tumour, or those of a parasitic organism.

SUMMARY.

PART I.

1. By means of the Old Standard bismuth sulphite medium *B. typhosus* was cultivated 44 times out of 50 examinations of the stools of 50 typhoid fever patients and convalescents.

2. By means of the same medium *B. paratyphosus* B was cultivated 22 times out of 27 examinations of the stools of 27 different cases of paratyphoid fever.

3. In 13 examinations of Belfast sewage in different months during 1928 and 1929, by means of the Old Standard sulphite media, *B. typhosus* was isolated on 10 occasions. In two examinations of Lisburn sewage *B. typhosus* was isolated. Although no special search for *B. paratyphosus* B was undertaken, this organism was isolated on two occasions from Belfast sewage. The viability of *B. typhosus* and *B. paratyphosus* B in sewage is longer than was suggested by the work of previous observers. It has been possible to cultivate *B. typhosus* and *B. paratyphosus* B from the deposit of sewage stored in a

bottle at room temperature for 3 weeks. On one occasion *B. typhosus* was found alive at the end of 5 weeks.

4. On two occasions water from the River Lagan was examined, and on one of these *B. typhosus* and *B. paratyphosus* B were cultivated.

5. For the isolation of the *B. enteritidis* Gaertner the media gave good results in the examination of faeces from cases of food-poisoning.

Part II.

6. Instead of adding the reagents separately in the preparation of the media, it has been shown that stock solutions containing sodium sulphite, liq. bismuthi, ferrous sulphate and sodium phosphate can be employed.

7. A New Standard bismuth medium is described. It is prepared by adding to 100 c.c. of hot 3 per cent. nutrient agar, 2·5 c.c. of a 20 per cent. solution of glucose and 7 c.c. of a mixture previously boiled consisting of 100 c.c. sodium sulphite (anhydrous) 20 per cent. solution, 50 c.c. liq. bismuthi, and 10·5 grm. of sodium phosphate (exsiccated). The medium is completed by the addition of 0·5 c.c. of a 1 per cent. solution of brilliant green.

8. The bismuth media are rendered still more selective for the isolation of *B. typhosus* from sewage by the addition of 2 c.c. of absolute alcohol to 100 c.c. of the Old Standard medium, and of 4 or 5 c.c. of absolute alcohol to the New Standard. For the isolation of *B. paratyphosus* B 2 c.c. of propyl alcohol should be employed in the New Standard medium.

9. From December 1929 to August 1930, seven specimens of Belfast sewage have been examined at monthly intervals, and in all cases *B. typhosus* has been isolated by means of the Standard media or of their modifications. On four occasions *B. paratyphosus* B was also isolated. The 10 successes out of 13 attempts recorded in Part I, the seven out of seven recorded in Part II together with the four out of four already reported in 1928, making 21 in all, show the almost constant presence of *B. typhosus* over a period of 3 years in Belfast sewage. 1 c.c. of the sewage as a rule contains at least one typhoid bacillus. Though not specially looked for, the *B. paratyphosus* B was isolated on six occasions.

Part III.

10. In the preparation of the bismuth media Witte's peptone should not be employed, but Bacto or Fairchild's.

11. The addition of 4 c.c. of a 1 in 10,000 solution of perchloride of mercury to every 100 c.c. of the bismuth media tends to inhibit the growth of *proteus* strains and certain other organisms which develop from sewage, and whose presence interferes with the isolation of *B. typhosus*.

12. A fluid enrichment bismuth medium is described consisting of 100 c.c. mannite broth (Fairchild's peptone), 6 c.c. sodium sulphite 20 per cent. solution, 0·2 c.c. liq. bismuthi. To the boiled mixture 0·5 c.c. of a 1 per cent. solution of brilliant green is added. A useful modification of this medium is secured by the addition to every 100 c.c. of the mannite broth of 1 c.c. absolute

alcohol and 4 c.c. of a 1 in 10,000 solution of perchloride of mercury. By means of these fluid media the *B. typhosus* has been cultivated 17 times out of 17 stools from typhoid fever patients, and three times out of five specimens of sewage.

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