his faculties for the service of his profession and expected others to do the same. He gave all the remarkable energy, unsurpassed skill, and exquisite gentleness he possessed fully and freely to his work. He made no excuses for himself and would accept none from his subordinates. He was never completely satisfied with any operation, always thinking he might have done something a little better, ever striving after an ideal which none could reach. He often said that an operation was a religious rite which demanded sacrifice and attendant ceremonial from us all; he was the arch-priest, we his acolytes. In the operating theatre he was very stern and silent—no sound must be heard during the administration of the anaesthetic, and during the operation all must speak if at all in whispers—the gravest incident did not make him raise his voice or hurry his activity. . . . With it all he inspired us with such admiration as must be very rare. We loved him very dearly and would have liked to show it more than we dared do; but he knew more than we thought, and his letters reveal a depth of affection which he could not bring himself to show in any other way."

When the period of their close association was over Braithwaite presented the bust of Moynihan by Wilcoxon to his chief as a memento, and this was later given to the College.

The time had come (1910-14) when Moynihan could enjoy some of the rewards of success, to play as well as to work. He bought a house in Norway, and a yacht in which he took his family each year for their summer holiday. Just before the war he purchased Carr Manor at Meanwood, a few miles outside Leeds. It was a stately mansion situated in a beautiful garden and grounds, where he loved to entertain his friends.

(To be concluded)

THE ISOLATION OF TYPHOID, PARATYPHOID, AND DYSENTERY BACTERIA FROM FAECES AND URINE

A COMPARATIVE STUDY OF SOME CULTURE MEDIA

BY

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During the last few years several new media for the isolation of pathogenic enteric bacteria have been published, but no general opinion of their value for routine purposes is available. In order to find out which are the most reliable methods for daily work I made a comparative study of the following six media: the old ones of Endo and Müller and the newer ones of Wilson and Blair (1931; Wilson, 1933), Kauffmann (1931), Ruys (1934), and Leifson (1935).

Eberthella typhosa

In the isolation of *E. typhosa* from faeces and urine the results with Wilson and Blair's old standard medium (glucose-bismuth-sulphite-phosphate-iron-sulphate-agar) exceeded those obtained with the culture medium of L. Müller (tetrathionate broth), which is often spoiled by the growth of *proteus* bacteria. The Wilson medium is highly selective; so it is possible to make rather heavy inoculations, giving a better chance of success. Some difficulties in the preparation of the medium were over-

come when we used ferrous sulphate crystals precipitated in alcohol; the solution contained so little ferric salts that it was perfectly clear. Also, we employ only freshly prepared media. After pouring the mixture into Petri dishes it is allowed to evaporate and cool without covering. Without further drying the medium is ready for use; it should not be stored.

A few drops of the faeces suspension are smeared on the surface. Besides this we always mix some of the fluid medium in dishes with 1 c.cm. and 4 c.cm. of the faeces suspension, a method also recommended by Wilson (1938). In most cases these pour-plates are superfluous, but sometimes they give a positive result which could not be obtained otherwise. In one case, only once were both pour- and smear-plates positive, but the pour-plates alone yielded typhoid colonies on four occasions. Neither by the Endo plates nor by the Müller broth could typhoid bacilli be isolated.

TABLE I.—*E. typhosa* isolated from Faeces and Urine with Three Differential Media. (Total Number of Positive Specimens, 222)

With Endo's medium	positive	96	Only Endo positive	..	1
" Müller's "	"	133	" Müller "	..	14
" Wilson-Blair's "	"	198	" Wilson-Blair positive	..	57

Table I indicates that it is not sufficient to rely on Wilson and Blair's medium alone. As the tetrathionate broth was not very satisfactory I tried Kauffmann's medium, which consists of tetrathionate broth, bile, and brilliant-green. It is recommended by that author as an improvement on the plain Müller broth. The results are recorded in Table II.

TABLE II.—*E. typhosa* isolated from Faeces and Urine with Four Differential Media. (Total Number of Positive Specimens, 96)

With Endo's medium	positive	35	Only Endo positive	..	0
" Müller's "	"	49	" Müller "	..	6
" Kauffmann's "	"	47	" Kauffmann positive	..	4
" Wilson-Blair's "	"	86	" Wilson-Blair "	..	26

These figures show that the plain tetrathionate broth is not inferior to Kauffmann's medium. The increase in the numbers of positive results may be accounted for by the larger number of media employed.

Salmonella

The medium of L. Müller gives satisfactory results, but it also stimulates the growth of *B. proteus*, which from time to time spoils the cultures. In order to avoid the overgrowth with *proteus* bacteria I tried several chemicals and media, and finally found that the addition of 2 per cent. Esbach's reagent (1 per cent. picric acid and 2 per cent. citric acid) to brilliant-green broth (1:100,000) gives a good culture medium for *Salmonella* which inhibits the growth of *B. proteus* to a large extent.* The results of several years' routine practice are recorded in Table III.

TABLE III.—*Salmonella* isolated from Faeces and Urine with Three Differential Media. (Total Number of Positive Specimens, 616)

With Endo's medium	positive	..	..	..	..	151
" Müller's "	"	..	..	..	..	419
" Ruys's "	"	..	..	..	..	551

For over a year I also tried Kauffmann's medium in comparison with the previously mentioned media, but,

* In experiments on the swarming of *B. proteus* in agar media the inhibiting effect of Esbach's reagent was first pointed out by Dimitrijevic-Speth (1929).

as shown in Tables IV and V, it did not improve the results. For the isolation of *Salmonella* Wilson and Blair's old standard medium gives rather poor results, so after a short series of experiments I omitted it. Wilson's other media were not tested.

TABLE IV.—*S. schottmülleri* isolated from Faeces and Urine by Four Differential Media. (Total Number of Positive Specimens, 93)

With Endo's medium positive	17	Only Endo positive	1
" Müller's "	71	" Müller "	6
" Kauffmann's "	44	" Kauffmann positive	3
" Ruys's "	80	" Ruys positive	14

TABLE V.—*S. typhimurium* and *S. enteritidis* isolated from Faeces by Four Differential Media. (Total Number of Positive Specimens, 66)

With Endo's medium positive	19	Only Endo positive	1
" Müller's "	35	" Müller "	1
" Kauffmann's "	45	" Kauffmann positive	5
" Ruys's "	54	" Ruys positive	9

The brilliant-green-Esbach broth (Ruys) proved to be the most effective in all groups of *Salmonella* infections, though it did not give the full 100 per cent. Next best were: for *S. schottmülleri*, the plain tetrathionate broth; for *S. typhimurium* and *S. enteritidis*, the more complicated medium of Kauffmann. The larger the number of media employed the greater was the number of positive results.

Shigella

The isolation of the aetiological agent in bacillary dysentery is not at all satisfactory, partly because of the lower resistance of the bacteria, which may be killed when too much time elapses between defaecation and the inoculation of the material. Another factor is the insufficiency of the Endo plate, the medium almost exclusively employed. A few years ago Leifson advocated a more selective medium, especially for the isolation of *Sh. paradysenteriae* (Flexner group); this consists of desoxycholate-citrate-agar. While Leifson himself thought it less satisfactory for the isolation of *Sh. sonnei*, others—such as Hardy, Watt, De Capito, and Kolodny (1939)—obtained favourable results also in infections with the *Sonne* strains. The ordinary intestinal flora is almost entirely inhibited, so even undiluted faeces can be inoculated, and one plate suffices. In the acute phase of the disease often both media show a positive result, but in convalescents the Leifson agar gives more positives. Sometimes, however, the Leifson agar is less good than the Endo plate, perhaps because of the very heavy inoculations on the former. *Sh. sonnei* grows on the Leifson medium only in smooth colonies. I got the impression that the rough form is inhibited. In cases where the stools contain a majority of rough forms this property may be one of the reasons for the failure of the medium.

From 142 patients with bacteriologically proven bacillary dysentery, in thirty the infective agent was found only by means of the Leifson agar. In one case the presence of *Sh. sonnei* in the stools six times was demonstrated only by means of Leifson's agar and never by Endo's. So even if the Leifson medium sometimes fails, it does considerably improve the bacteriological diagnosis in bacillary dysentery.

TABLE VI.—*Sh. sonnei* isolated from Faeces with the Media of

Endo positive and Leifson positive	126
" negative " " negative	37
" negative " " positive	51
Total	214

TABLE VII.—*Sh. paradysenteriae* (Flexner Group) isolated from Faeces with the Media of

Endo positive and Leifson positive	8
" negative " " negative	1
" negative " " positive	12
Total	21

Summary

This comparative study indicates that in cases of typhoid fever it is advisable to use the media of Wilson and Blair and of L. Müller, in paratyphoid B those of Müller and Ruys, and in the enteric form of the disease those of Kauffmann and Ruys. Endo's agar should not be neglected, because it may give a positive result one day earlier than any of the others.

In bacillary dysentery both Leifson's desoxycholate-citrate-agar and Endo's agar should be used.

Formulae

The formulae of the media employed are as follows:

Wilson and Blair (1931) and Wilson (1933)

- 100 c.cm. nutrient 3 per cent. agar, pH 7.
- 20 c.cm. glucose-sulphite-phosphate mixture (a solution of 6 grammes bismuth-ammonium-citrate in 50 c.cm. distilled water is neutralized and added to 100 c.cm. of a 20 per cent. exsiccated sodium sulphite solution, then 10 grammes sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 4\text{H}_2\text{O}$), and after cooling 10 grammes glucose in 50 c.cm. distilled water are added).
- 1 c.cm. 8 per cent. ferrous sulphate solution, freshly dissolved in distilled water.
- 0.5 c.cm. 1 per cent. solution of brilliant-green.

Müller (1925)

- 180 c.cm. nutrient broth is poured into a flask with 9 grammes chalk.
- 20 c.cm. sodium thiosulphate solution (50 grammes sodium thiosulphate in 100 c.cm. water, sterilized).
- 4 c.cm. Lugol's solution (25 grammes iodine, 20 grammes iodide of potassium, in distilled water 100 c.cm.).

Kauffmann (1931)

- 500 c.cm. Müller tetrathionate broth.
- 5 c.cm. brilliant-green solution in water (1:1,000).
- 25 c.cm. sterile ox bile.

Ruys (1934)

- 1,000 c.cm. nutrient broth, pH 7.2.
- 10 c.cm. brilliant-green solution (1:1,000).
- 20 c.cm. Esbach's reagent (1 gramme picric acid, 2 grammes citric acid, in 100 c.cm. water).

Leifson (1935)

- 1,000 c.cm. pork infusion.
- 10 grammes peptone (bacto, Fairchild ND. C. CB.).
- 20 grammes agar-agar.
- 10 grammes lactose.
- 25 grammes sodium citrate ($2\text{Na}_2\text{C}_6\text{H}_5\text{O}_7 \cdot 11\text{H}_2\text{O}$).
- 5 grammes sodium desoxycholate.
- 2 grammes ferric ammonium citrate (green scales).
- 20 mg. (1:50,000) neutral red, pH 7.4.

REFERENCES

- Dimitrijevic-Speth, V. (1929). *Zbl. Bakt.*, **113**, 524.
- Hardy, A. V., Watt, J., De Capito, T. M., and Kolodny, M. H. (1939). *Publ. Hlth. Rep.*, Wash., **54**, 287.
- Kauffmann, F. (1931). *Zbl. Bakt.*, **119**, 148.
- Leifson, E. (1935). *J. Path. Bact.*, **40**, 581.
- Müller, L. (1925). *C. r. Soc. Biol.*, Paris, **93**, 433.
- Ruys, A. C. (1934). *Zbl. Bakt.*, **132**, 349.
- Wilson, W. J. (1933). *British Medical Journal*, **2**, 560.
- (1938). *J. Hyg., Camb.*, **38**, 507.
- and Blair, E. M. M. (1931). *Ibid.*, **31**, 138.

Some years ago the International Labour Office published its two-volume *Encyclopaedia of Industrial Hygiene (Occupation and Health)*. In view of new discoveries and developments that have been made since then the I.L.O. has decided to publish a *Supplement* which will appear not as a new volume but as a series of pamphlets issued periodically in loose-leaf form. Articles dealing with sixteen different subjects have already been published, including such matters as allergy and occupation, brucellosis, constitutional factor and occupation, and silicosis. Further information may be obtained on application to the Branch Office, International Labour Office, 12, Victoria Street, London, S.W.1.