MODIFICATIONS OF CULTURE MEDIA USED IN THE ISOLATION AND DIFFERENTIATION OF TYPHOID, DYSENTERY, AND ALLIED BACILLI.*

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A comparative study of some of the media proposed for the isolation of the typhoid group of bacilli led to the conclusion that the Endo and brilliant green plates are most satisfactory. Both of these media, however, possessed certain defects which impaired their usefulness. It is believed that the modifications reported below tend to eliminate these defects.

Modification of the Endo Medium.

It was observed that the Endo medium, as ordinarily prepared, with a phenolphthalein reaction of + 0.2, gave variable results with the dysentery group of bacilli, the Shiga type of *Bacillus dysenteriæ* being either markedly inhibited in its growth or failing to grow at all. This restraining effect was found to be due to the end-reaction, which, after the addition of the sulfite, reached a hydrogen concentration ranging from pH 8.6 to 8.8, a degree of alkalinity unfavorable to the development of the Shiga bacillus. By adjustment of the reaction of the medium to pH 7.8–8.0, the inhibiting effect is greatly reduced, and the plates are suitable for the isolation of all the pathogenic members of the typhoid-dysentery class of bacilli.

The agar is prepared in the usual manner, titrated to pH 7.4, flasked in 100 cc. or other suitable amounts, and sterilized.[1] Before the

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[1] This constitutes the stock medium from which all the special media are prepared.

plates are poured the reaction is adjusted to pH 7.8–8.0 by the addition of the requisite amount of N sodium hydroxide solution. The lactose and sulfite-fuchsin solutions are then added under sterile conditions and the plates poured. It was noted that it is sufficient to add 0.5 cc., instead of 1 cc., of the sulfite-fuchsin mixture to 100 cc. of agar, and this amount was found to be preferable in the isolation of dysentery bacilli. The sulfite-fuchsin is prepared by decolorizing 1 cc. of a 10 per cent alcoholic solution of basic fuchsin with 10 cc. of a 10 per cent solution of sodium bisulfite.

Modification of the Brilliant Green Medium.

The brilliant green plate as advocated by Krumwiede, Pratt, and McWilliams,[2] proved to be the most satisfactory of the various inhibitive, selective culture media tested. It lacked, however, the advantage of a sharp differential indicator. A number of indicators were tested and neutral red was found best to answer this purpose. It is not readily reduced by the action of the group of bacteria in question, it does not affect the activity of the dye, and it changes color at the acid zone, beginning at about pH 6.8. The differentiation between fermenting and non-fermenting types is the same as on Endo medium: the *coli* colonies are red or have a reddish center, while the typhoid and paratyphoid colonies are colorless or faintly brownish, owing to the brownish tinge of the plate.

The medium is prepared in the usual manner, care being taken to adjust the reaction to pH 7.0–7.2. This is important, because the reaction of the medium has a marked influence on the activity of the brilliant green, and this hydrogen concentration offers optimum conditions. The lactose, brilliant green, and neutral red solutions are added under sterile conditions just before plating. 0.25 cc. of a 1 per cent water solution of neutral red is added to 100 cc. of agar. The optimum concentration of dye should always be determined with known strains, as directed by Krumwiede. As a rule, 0.3 cc. of a 1: 1,000 solution of the dye added to 100 cc. of agar gives the desired concentration.

[2] Krumwiede, C., Jr., Pratt, J. S., and McWilliams, H. I., *J. Infect. Dis.*, 1916 xviii. 1

Modification of the Russell Double Sugar Tube Medium.

The Russell double sugar tube,[3] containing 0.1 per cent glucose and 1 per cent lactose with litmus or Andrade indicator, has proved highly serviceable for the rapid differentiation of suspicious colonies fished from Endo or brilliant green plates. The tube enables one to distinguish *coli*, typhoid, and paratyphoid bacilli from one another, but does not differentiate *Bacillus typhosus* from *Bacillus dysenteriæ* or *Bacillus paratyphosus* A from *Bacillus paratyphosus* B. In a previous paper[4] it was reported that such separation is rendered possible by the aid of basic lead acetate. It seemed, therefore, that a combination of the lead acetate with the Russell medium would yield a tube which simultaneously would differentiate all the members of this group. With some slight exceptions this proved to be the case, provided certain precautions, to be mentioned below, are taken in the preparation of the medium.

Preparation of the Medium.

Either meat infusion agar or beef extract agar may be used as a basis for this medium, although sharper reactions are, as a rule, obtained with meat infusion agar. The agar is adjusted to pH 7.4 or neutral to Andrade's indicator, 1 per cent by volume of this indicator is added, and the medium is tubed 5 cc. to a tube and sterilized. The lactose-glucose solution, containing 20 per cent lactose and 2 per cent glucose, is sterilized separately, and 0.25 cc. added in a sterile manner to each tube. The basic lead acetate solution, 0.25 per cent, is also sterilized separately and 1 cc. added to each tube. Both the lactose and the lead acetate solutions may be added before slanting and as soon as the agar is cooled to about 60°C.; otherwise the lead flocculates the peptone.

In order to obtain uniform results, certain details must be taken into consideration. The agar should preferably be sugar-free if meat infusion is used, and as nearly colorless as possible. The browner the medium the poorer are the results, partly because the reaction is

[3] Russell, F. F., *J. Med. Research*, 1911, xxv, 217.

[4] Kligler, I. J., *Am. J. Pub. Health*, 1917, vii, 1042.

masked, and partly because of the breaking down of some of the constituents of the medium itself. Care should be taken to use lactose free from glucose, for the obvious reason that an excess of the hexose vitiates the basic principle of the medium and leads to poorly defined reactions. Still another detail is the character of the slant and the mode of inoculation. The tube[5] should contain enough medium to allow for a butt of at least $\frac{1}{2}$ to $\frac{5}{8}$ inch and a slant of about $1\frac{1}{2}$ inches. The inoculation is made by stabbing the butt near the front and then streaking the slant as the needle is withdrawn from the stab.

If the precautions outlined are followed, it is possible to obtain clear-cut differentiation. *Bacillus coli* reddens the whole tube and breaks up the medium through the gas produced. *Bacillus typhosus* reddens the butt, leaves the slant colorless, and produces browning, particularly near the surface of the stab. The dysentery bacilli redden the butt, like *Bacillus typhosus*, but do not produce browning.

The *paratyphosus* bacilli differ from *Bacillus typhosus* and *Bacillus dysenteriæ* in that they produce gas. Further differentiation between the A and B types is obtained on the basis of the browning. *Bacillus paratyphosus* B and its allied bacilli, *Bacillus enteritidis* and *Bacillus murium*, produce browning, while the A type does not. Occasionally one finds strains of typhoid bacilli which do not cause browning, but they are the exceptions; *Bacillus paratyphosus* B, on the other hand, invariably gives a positive reaction.

CONCLUSION.

The three media as modified—Endo medium, neutral red-brilliant green, and the lead acetate Russell double sugar—have proved in our hands the best combination for the isolation and rapid differentiation of the various organisms belonging to the typhoid-dysentery group of bacilli.

[5] For economy and convenience the Wassermann tube is used instead of the large sized test-tube.