DIFFERENTIATION OF THE PARATYPHOID-ENTERITIDIS GROUP, II

LEAD ACETATE AGAR

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Variations in rapidity and intensity of hydrogen-sulfid production have been suggested by several observers as a means of bacterial differentiation. Orlowski[1] found that B. typhosus produced a black precipitate, lead sulfid, in lead acetate medium, while B. coli did not. Sacquépée and Chevrel[2] confirmed these results and showed further that a culture of B. paratyphosus B blackened lead acetate medium even earlier than did B. typhosus, while one of B. paratyphosus A was negative, thus resembling B. coli. Burnet and Weissenbach,[3] applying this method to 517 cultures isolated in the French army during the present war, found that the results obtained with lead acetate medium corresponded in all cases with the results of the agglutination tests. B. paratyphosus B blackens the medium in 18 hours; B. typhosus blackens the medium a little more slowly and less intensely; B. paratyphosus A grows either without blackening at all or only after several days. Hollande and Beauverie[4] advocate the use of test papers of various sorts, among them one with lead acetate, for the differentiation of this group, and report results essentially the same as those of earlier authors.

Strains of B. enteritidis and B. suipestifer do not seem to have been tested by any of the investigators, and no comparative series of cultures with definitely ascertained characters has been worked out. For these reasons, we have used a lead acetate medium for comparing the behavior of 74 strains already studied in considerable detail by Jordan.[5]

Preliminary tests showed the most favorable medium and mode of procedure to be as follows:

Three per cent. Witte's peptone was dissolved (by boiling) in fresh meat broth (1 pound lean beef to 1 liter water). After filtering this broth, 1.5% agar was dissolved in it, the reaction brought to 1% acid on the phenolphthalein scale, and the medium tubed and sterilized. Then the tubes were cooled to 43 C.,

Received for publication July 12, 1917.

[1] Beitrag zur Kenntniss der biologischen und pathogenen Eigenschaften der Bacterium coli communis. Diss., St. Petersburg, 1897.

[2] Compt. rend. Soc. de biol., 1905, 59, p. 535.

[3] Ibid., 1915, 78, p. 565.

[4] Ibid., p. 722.

[5] Jour. Infect. Dis., 1917, 20, p. 457.

and 2 drops (0.1 c.c.) of a 10% lead acetate solution made from recently sterilized water were added to each tube and the tube well shaken. On cooling, the tubes were inoculated by sliding the needle in between the agar and the wall of the tube (duplicates for each organism). These tubes were then incubated at 37 C. for 18-24 hours.

A positive reaction is indicated by a blackening of the medium along the needle track, often spreading over the whole surface. This is generally apparent in 6-8 hours, but the sharpest results are obtained in 18-24 hours.

The 74 strains tested in the lead acetate agar have given the following results:

20 strains of B. paratyphosus A	Negative
28 strains of B. paratyphosus B	Positive
20 strains of B. suipestifer	Negative
6 strains of B. enteritidis	Positive

These are the same strains described in detail in an earlier paper.[5]

The hydrogen sulfid reaction shows an exact correspondence with the agglutination and fermentation reactions there described. Some slight irregularities are occasionally observed, as in the case of the fermentation reactions, but the principal group distinctions are quite as sharp as those based on other characteristics. It must be especially noted that the 5 strains of the B. paratyphosus type, which are of porcine origin (Nos. 62, 115, 161, 169 and 175, for the full description and history of which see Footnote 5), have shown more irregularity than any other strains, occasionally yielding a negative reaction when plated and subcultures from individual colonies are tested. Thus in 1 instance, 1 colony of 10 of No. 169 gave a negative reaction. The variability of these strains corresponds with the variability which they have shown in other respects, and which has been recorded in the first paper. It is perhaps significant that the 5 strains of B. paratyphosus B, which are of porcine origin, should in this respect show some affinity to the B. suipestifer type.

SUMMARY

In lead acetate agar, all typical paratyphoid A strains (20) fail to blacken the medium in 18-24 hours. All strains of B. enteritidis (6) give a positive reaction. The great majority of B. paratyphosus B strains (23 of 28) give a consistently positive reaction while all B. suipestifer strains (20) are negative. Five strains of porcine origin, belonging to the B. paratyphosus B type, are not constant in their reactions, but these are the same strains that in Jordan's earlier study have been found variable and irregular in other respects.