

MEDIA FOR THE ISOLATION AND DIFFERENTIATION OF PATHOGENIC *ESCH. COLI* (SEROTYPES O 111 AND O 55)

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The connexion between *Escherichia coli* and intestinal disease was observed for the first time by Adam (1923), who described the types of *Bact. coli* found in toxic intestinal diseases of infants. Later he named these bacteria "dyspepsicoli" (Adam, 1927) and divided them into six groups, A1-A6, according to their fermentative properties in relation to various sugars and alcohols. According to Adam, the two predominant types in clinical cases were A1 and A4, and he designated them *dyspepsicoli sensu strictiori*. Goldschmidt (1933) typed these strains and found that they formed a separate serological group.

Since then many workers (Bray, 1945; Giles and Sangster, 1948; Giles, Sangster, and Smith, 1949; Taylor, Powell, and Wright, 1949; Rogers, Koegler, and Gerrard, 1949; Drimmer-Herrheiser, and Olitzki, 1951; Dupont, 1951; Braun, 1951) have found in diarrhoea and vomiting (gastro-enteritis) in infants a predominant bacterial flora, *Escherichia coli*, which was named by Bray (1945) *Bact. coli neapolitanum*, by Giles and Sangster (1948) *Bact. coli* type alpha and beta, by Taylor *et al.* (1949) D 433, and by Rogers *et al.* (1949) B.G.T. These strains of *Esch. coli* behave as a distinct serological type. The classification was made by Kauffmann and Dupont (1950), who found that these bacteria belonged to the groups O 111 : B4, O 111 : B4 : H2, O 111 : B4 : H12, and O 55 : B5 : H6.

The growth properties and morphology of these strains are identical with those of other strains of *Esch. coli*, and till now the only means of differentiation has been based on their serological specificity. Even Adam and Aust (1950), who paid particular attention to their biochemical properties, stressed that "... as the fermentative properties of these bacteria are unstable and given

to fluctuations, it is safer to identify these strains of *B. coli* by means of specific agglutinating sera." A slide agglutination method for identification was also introduced by Bray and Beavan (1948).

We became interested in the possibility of a rapid biochemical differentiation of these pathogenic strains of *Esch. coli*, as it has always been necessary to test serologically all of the colonies diagnosed on McConkey medium as *Esch. coli* in order to be sure that no possible pathogenic colony had been omitted. This procedure, laborious and time-consuming, is impractical for diagnostic purposes.

Method

Some indications of a possible biochemical differentiation were found in previous investigations when Silberstein, Rappaport, and Kolmer (1932) encountered strains of *Esch. coli* that did not ferment sorbitol. Since the serotypes O 111 and O 55 of *Esch. coli* were found to ferment sorbitol slowly or not at all, we tried to include sorbitol in solid media of the type of McConkey. In our final medium the lactose of the McConkey medium was replaced by *d*-sorbitol according to the following formula:

Bacto peptone	17.0 g.
Proteose peptone	3.0 ..
<i>d</i> -Sorbitol	10.0 ..
Bacto bile salts No. 3	1.5 ..
Sodium chloride	5.0 ..
Bacto agar	13.5 ..
Neutral red	0.3 ..*
Crystal violet (0.1% solution)	1.0 ..
Distilled water	1,000 ml.

This medium was sterilized in an autoclave for 20 minutes at 15 lb./sq. in., poured into petri dishes, and left to solidify. A little faecal suspension in saline was streaked on the surface of the plate which was incubated for 18 to 24 hours at 37° C. Sorbitol-fermenters—that is, most of the non-pathogenic strains of *Esch. coli*—formed red colonies, but patho-

* This high concentration of neutral red (accidentally used in the first assays) was found to inhibit the spreading of *B. proteus*.

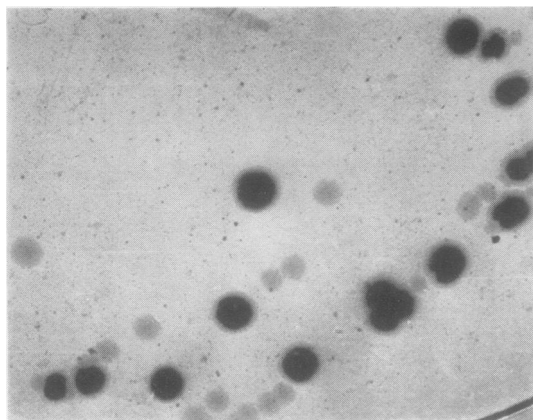


FIG. 1

genic *Esch. coli* grew in colourless colonies similar to those of non-lactose fermenters on McConkey medium (Fig. 1). As some strains of *B. proteus* and some *Shigellae* also grew on sorbitol-agar as colourless colonies, the final differentiation of the pathogenic *Esch. coli* was made on iron-sorbitol-agar slants of the type of Kligler's iron agar, the sugar again being replaced by sorbitol.

Iron-sorbitol-agar slants were prepared as follows:

Beef extract	3.0 g.
Proteose peptone (Difco)	15.0 ..
Sodium chloride	5.0 ..
Ferric ammonium citrate (green sea's)	0.5 ..
Bacto agar	20.0 ..

The mixture was dissolved in 1,000 ml. distilled water and the pH adjusted to 7.6 with NaOH. Finally it was distributed in 100-ml. quantities into bottles and sterilized in an autoclave.

A mixture was prepared by grinding in a mortar the following:

Phenol red (powder)	0.15 g.
<i>d</i> -Sorbitol	10.0 ..
Sodium thiosulphate	2.5 ..

Of this mixture, 0.25 g. was added to each 100 ml. of melted medium, stirred till dissolved, then distributed into small tubes, autoclaved, and left to solidify in a slanted position.

After 18 to 24 hours of incubation at 37° C. stab inoculation of colourless colonies into this medium gave a slight anaerobic fermentation without producing gas, while normal red colonies of *Esch. coli* grew with the formation of acid and gas. *B. proteus*, which also may form colourless colonies, blackens sorbitol-iron-agar slants due to the production of H₂S.

The colourless colonies of *Esch. coli* gave distinct agglutination with O 111 : B4 and O 55 : B5 sera, while no agglutination was observed in the case of red colonies from sorbitol-agar. As not all colourless colonies are *Esch. coli*, the serological types should be finally established by agglutination with specific sera and by such biochemical reactions as indol- and urease-production and behaviour on Kligler's iron agar and sorbitol-iron-agar. It is possible that new and hitherto undescribed types of *Esch. coli* may ferment sorbitol slowly or not at all. It remains to be seen whether such types, if they arise, are associated with infantile gastro-enteritis. After subcultures on laboratory media some strains of *Esch. coli* types O 111 and O 55 may develop the ability to ferment sorbitol, but this has never been found in the case of freshly isolated strains.

Finally it should be mentioned that Kauffmann and Dupont (1950) and Braun (1951) observed that sorbose was not fermented by the pathogenic strains of *Esch. coli*. We are now investigating the effects of the inclusion of sorbose as a differentiating sugar in solid media.

Summary

Strains of *Esch. coli* of the serotype O 111 and O 55 can be biochemically differentiated on sorbitol-agar medium and in sorbitol-iron-agar slants, due to their ability to ferment sorbitol only slowly or not at all. It is suggested that some of the sorbitol-non-fermenting strains of *Esch. coli* may belong to new and as yet undefined serological types.

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