An Organism Differing from *Shigella boydii* 13 Only in Its Ability to Produce Gas from Glucose

B. ROWE, R. J. GROSS, AND E. VAN OYE

Salmonella and Shigella Reference Laboratory, Central Public Health Laboratory, London NW9 5HT, England, and Institut d'Hygiene et d'Epidemiologie, B-1050 Bruxelles, Belgium

It is generally accepted that, except for two biotypes of *Shigella flexneri* 6 (the Manchester and Newcastle biotypes), all members of the genus *Shigella* are anaerogenic. Two bacterial isolates are described which have O antigens identical to those of *S. boydii* 13 and which cannot be distinguished biochemically from typical strains of that biotype except by their ability to produce gas from glucose and other fermentable substances. The relationship between these strains and the typical *S. boydii* 13 is analogous to that between strains of the Manchester biotype and the typical *S. flexneri* 6.

According to the 1958 report of the *Enterobacteriaceae* Subcommittee (7), members of the genus *Shigella* do not produce gas from glucose. However, the report recognized that some biotypes of *S. flexneri* 6 possess this character. These biotypes were known previously as the Newcastle bacillus, described by Clayton and Warren (2), and the Manchester bacillus, described by Downie et al. (4).

The present study describes two isolates which possess the O antigen of *S. boydii* 13 and which cannot be distinguished biochemically from typical strains of that serotype except by their ability to produce gas from glucose and other fermentable substances. Therefore, the relationship between these strains and biochemically typical *S. boydii* 13 is analogous to that between strains of the Manchester biotype and the typical biotype of *S. flexneri* 6, which is anaerogenic.

Strain E.2054/71 was isolated from the stool of an adult female patient from Nigeria, who was admitted to a hospital in the United Kingdom as the result of an attack of asthma. The patient did not give a history of diarrhea, and the strain was isolated during a routine examination for parasites.

Strain BR.1639 was isolated in Brussels from the stool of a patient with diarrhea, who had recently returned from India; *S. flexneri* 2b was isolated from the same fecal specimen.

The strains were examined biochemically by the methods of Cowan and Steel (3). They were examined further by the methods of Ewing et al. (11) for mutative fermentation of lactose, sucrose, and salicin and by a similar technique for mutative utilization of citrate in Christensen medium.

O antisera for strains E.2054/71 and BR.1639

were prepared in rabbits, and the two strains were tested for O antigen identity by using reciprocal absorption techniques.

O antigen suspension prepared from strain E.2054/71 was tested for agglutination in O antisera for all *Shigella* serotypes and for *Escherichia coli* O antigens 01 to 0163. Where reactions occurred, the agglutination titers were determined and the serological relationships were examined by absorption techniques.

O antigen suspensions of all *Shigella* serotypes and of *E. coli* O antigens 01 to 0163 were tested similarly for agglutination in O antiserum for strain E.2054/71.

Both strains gave the general biochemical reactions of the family *Enterobacteriaceae*. Their additional reactions (Table 1) differed from those of typical *S. boydii* 13 in the ability to produce gas from glucose. Both strains were ornithine decarboxylase positive, an important observation because in the genus *Shigella* only *S. boydii* 13 and *S. sonnei* are known to have this character (8).

Mutative fermentation of sucrose, salicin, and lactose was not found, and mutative utilization of Christensen citrate was not demonstrated.

Serological identity of the O antigens of strains E.2054/71 and BR.1639 was demonstrated by reciprocal absorption techniques. Agglutination tests showed that strain E.2054/71 gave reciprocal cross-reactions with *S. boydii* 13 and *E. coli* 0123; *S. flexneri* 5 was agglutinated by E.2054/71 antiserum; and E.2054/71 was agglutinated by antiserum for *S. boydii* 9 and *E. coli* 028ac, 098, and 0120. Reciprocal absorption studies showed that the O antigen of strain E.2054/71 was identical to that of *S. boydii* 13 but failed to show signifi-

TABLE 1. *Biochemical reactions*

Determination	E.2054/71 ^a	BR.1639 ^a
Motility (Craigie)	-, 14	-, 14
Methyl red test	+, 2	+, 2
Voges-Proskauer reaction	-, 5	-, 5
Simmons' citrate	-, 7	-, 7
Christensen citrate	-, 7	-, 7
Sodium acetate	-, 7	-, 7
Mucate	-, 14	-, 14
Malonate	-, 1	-, 1
Growth in potassium cyanide	-, 2	-, 2
Indole (Kovacs)	+, 2	+, 2
H ₂ S (triple sugar iron)	-, 7	-, 7
Gluconate	-, 2	-, 2
Christensen urea	-, 7	-, 7
Phenylalanine deaminase	-, 1	-, 1
Gelatin (stab)	-, 28	-, 28
Arginine decarboxylase	-, 4	-, 4
Lysine decarboxylase	-, 4	-, 4
Ornithine decarboxylase	+, 1	+, 1
β -galactosidase (ONPG) ^b	-, 2	-, 2
Glucose (acid)	+, 1	+, 1
Glucose (gas)	+, 1	+, 1
Lactose	-, 14	-, 14
Mannitol	+, 1	+, 1
Sucrose	-, 14	-, 14
Salicin	-, 14	-, 14
Dulcitol	-, 14	-, 14
Inositol	-, 14	-, 14
Adonitol	-, 14	-, 14
Raffinose	-, 14	-, 14
Sorbitol	+, 2	+, 1
Arabinose	+, 2	+, 1
Rhamnose	-, 14	-, 14
Xylose	-, 14	-, 14
Trehalose	+, 2	+, 1
Inulin	-, 14	-, 14
Glycerol	+, 7	+, 7
Cellobiose	-, 14	-, 14
Sorbose	+, 7	+, 3
Maltose	-, 14	-, 14

^a Numerals indicate days of incubation.^b ONPG, *o*-Nitrophenyl- β -D-galactopyranoside.

cant serological relationships between E.2054/71 and any other cross-reacting O antigens.

Typical strains of *E. coli* may be easily differentiated from *Shigella* by their motility and by their range of activity in fermentable carbohydrate substrates. However, even in routine work, isolates are frequently encountered that have a fermentation spectrum indistinguishable from that of *Shigella*. In these circumstances, additional biochemical tests are required; for example, Ewing (8) has shown that strains which decarboxylate lysine cannot be classified as *Shigella*. Ewing and his colleagues studied over over 2,000 *Shigella* strains and re-

ported that these organisms failed to utilize citrate in Christensen medium. Most biochemically atypical *E. coli* strains may be easily differentiated from *Shigella* strains by these two tests. Strains E.2054/71 and BR.1639 are nonmotile and have the general biochemical properties of the genus *Shigella* (7). Furthermore, these strains fail to decarboxylate lysine and do not utilize citrate in Christensen medium.

Serologically, strains E.2054/71 and BR.1639 possess an O antigen that is identical to that of *S. boydii* 13. Both strains showed some antigenic similarity to *E. coli* 028ac, 098, 0120, and 0123, but absorption studies did not show complete antigen identity. It is recognized that many of the O antigens of *E. coli* are identical or closely related to those of *Shigella* (6). It is for this reason that in the identification of *Enterobacteriaceae* biochemical classification must always be considered in addition to the serological findings.

Freshly isolated shigellae invade epithelial cells; this property may be demonstrated by the Sereny test (13) using the guinea pig eye conjunctivum. However, there is abundant evidence that this property is not restricted to shigellae and that certain strains of *E. coli* are invasive and give positive results in this test (5). For this reason, tests for cellular invasiveness are of little value in the differentiation of *E. coli* and *Shigella*.

Sachs (12) described an aerogenic organism known as enterobacterium A12, and subsequent studies by Carpenter (1) and Szturm-Rubinstein et al. (15) showed that strains of this organism gave the general biochemical reactions of *Shigella*. These workers considered the A12 organism to be an aerogenic biotype of the *S. boydii* subgroup and reported that these organisms possessed the O antigens of *S. boydii* 14. However, Ewing and Hucks (9) showed that strains of enterobacterium A12 differed from typical strains of *S. boydii* 14 in that they failed to ferment mannitol. The same authors (10) showed that the O antigens of A12 strains were identical to those of *E. coli* 032 and they regarded the A12 strains as aberrant members of the genus *Escherichia*. Edwards and Ewing (6) subsequently reported that A12 strains utilized Christensen citrate mutatively and could, in their view, no longer be considered as strains of *S. boydii*.

Stypulkowska (14) described a variant of *S. dysenteriae* 3 which differed from typical strains in that it fermented mannitol and produced gas from glucose.

Strains E.2054/71 and BR.1639 resemble typical strains of *S. boydii* 13 in their ability to ferment mannitol and their failure to utilize Christensen citrate mutatively. These strains cannot be excluded from the genus *Shigella* by currently accepted criteria.

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REPRINT REQUESTS

Address reprint requests to: Dr. B. Rowe, Salmonella and Shigella Reference Laboratory, Central Public Health Laboratory, Colindale Ave., London NW9 5HT, England.

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