Sachs' "Enterobacterium A12" Is an Aerogenic Variant of *Shigella boydii* 14

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We studied the biochemical relationships of "Enterobacterium A12" of Sachs to *Shigella boydii* 14 and *Escherichia coli* O32. "Enterobacterium A12" differed from typical strains of *S. boydii* 14 only in its ability to produce gas from glucose. Otherwise, "Enterobacterium A12" and *E. coli* O32 were biochemically quite different. On the basis of these findings we propose that "Enterobacterium A12" should be regarded as an aerogenic variant of *S. boydii* 14, analogous to the aerogenic varieties of *Shigella flexneri* 6.

In 1943 Sachs (9) described 10 strains of an aerogenic, mannitol-negative, enteric organism that he designated "Enterobacterium A12." Sachs thought that this organism was a possible member of the group of *Shigella* species which do not ferment mannitol. Subsequent studies of this organism led other workers to take different positions with regard to its classification. In 1950, Ewing and Hucks (4) concluded that the biochemical reactions of "Enterobacterium A12" were like those of *Shigella* and that the O antigens of this organism were identical to those of *Escherichia coli* O32. For this reason, these authors considered "Enterobacterium A12" to be an aberrant member of *E. coli* group O32. This concept was discussed further by Ewing and Hucks (5) in 1952. In 1961, Carpenter (1) showed that "Enterobacterium A12," *E. coli* O32, and *Shigella boydii* 14 had identical O antigens and that "Enterobacterium A12" and *S. boydii* 14 had the same biochemical pattern, except that "Enterobacterium A12" strains produced gas from glucose. These findings were confirmed by Szturm-Rubinsten et al. (13) in 1962. Based on these data and on the fact that *Shigella flexneri* 6 biovars can also be aerogenic (2, 3), these authors proposed that "Enterobacterium A12" should be regarded as an aerogenic biovar of *S. boydii* 14. However, in 1972 Edwards and Ewing (3) stated that the microorganisms designated by Sachs as "Enterobacterium A12" should not be regarded as possible members of the genus *Shigella* because they were aerogenic and utilized citrate mutatively in Christensen citrate agar medium and because some strains showed mutative fermentation of lactose.

As far as we know, no agreement has been reached with regard to the classification of "Enterobacterium A12" of Sachs. Accordingly, in

Bergey's Manual of Determinative Bacteriology, 8th ed. (2), *S. boydii* is defined as a group composed of only anaerogenic serovars.

In this paper we compare the biochemical characteristics and virulence properties of "Enterobacterium A12" and *E. coli* O32. We also suggest a modification in the definition of the genus *Shigella* in order to include aerogenic variants.

MATERIALS AND METHODS

Bacterial strains. We studied eight strains of "Enterobacterium A12" (T1-66 Pe, 28-67 SP, T-68 SP, T4-68 SP, 1968-68 IAL, 7-77 Pe, 35-77 Pe, and 38001-79 LIAC), which were isolated from human feces in different areas of Brazil between 1966 and 1979. We also studied nine strains of *E. coli* O32; six of these strains (P6a, 5985-50 CDC, 3685-54 CDC, 6498-59 CDC, 1559-67 CDC, and 1913-72 CDC) were kindly provided by Betty R. Davis, Centers for Disease Control, Atlanta, Ga., and three strains (4-77 HC, 1191-77 CM, and 36-77 Pe) were freshly isolated strains identified in our laboratory.

Serological identification. Heated suspensions (100°C, 1 h) of the eight "Enterobacterium A12" strains agglutinated to the titer of an *S. boydii* 14 O antiserum (1:2,560). Furthermore, cross-absorption tests showed that the O antigens of "Enterobacterium A12" strain 35-77 Pe and *S. boydii* 14 were identical.

The serogroup of the *E. coli* O32 strains was determined by tube agglutination tests in O antiserum prepared with the standard strain of *E. coli* O32 (strain P6a). Slide agglutination tests were also performed with the same antiserum absorbed by the O antigen of *E. coli* O83, since this antiserum gave significant cross-reactions with strains of this serogroup.

We used the methods recommended by Edwards and Ewing (3) for preparing the antisera and for performing the serological tests.

Biochemical tests. The β -galactosidase test was performed by the method of Lowe (7). All of the other tests were performed by using the methods recom-

mended by Edwards and Ewing (3). However, the carbohydrate fermentation tests were observed for only 14 days.

Invasiveness assay. The ability of the strains to invade guinea pig eyes was tested by the method described by Serény (10). The "Enterobacterium A12" strains and the three *E. coli* O32 strains isolated in Brazil were tested within 1 week after isolation. The animals were observed for 7 days for evidence of keratoconjunctivitis.

RESULTS

Biochemical tests. The biochemical characteristics of the "Enterobacterium A12" and *E. coli* O32 strains tested are shown in Table 1. Table 1 also shows the biochemical pattern described by Ewing et al. (6) for 17 typical strains of *S. boydii* 14. This table shows that, with the exception of their ability to produce gas from glucose, the "Enterobacterium A12" strains could not be distinguished biochemically from typical strains of *S. boydii* 14. On the other hand, "Enterobacterium A12" and *E. coli* O32 strains had quite different biochemical patterns.

Invasiveness assay. All eight "Enterobacterium A12" strains yielded positive results in the Serény test. The keratoconjunctivitis which they caused was identical to that caused by the *Shigella* strains (10). None of the *E. coli* O32 strains (even the freshly isolated strains) gave positive results in this test.

DISCUSSION

The biochemical tests (Table 1) showed that "Enterobacterium A12" and *E. coli* O32 strains are quite different. Whereas all or the majority of the *E. coli* O32 strains tested gave positive results in the tests for production of indole and β -galactosidase, utilization of sodium acetate and mucate, motility, lysine decarboxylase, and fermentation of lactose, mannitol, salicin, rhamnose, and esculin, the "Enterobacterium A12" strains were negative in all these tests. On the other hand, the "Enterobacterium A12" strains differed from typical strains of *S. boydii* 14 only in their ability to produce gas from glucose. The inability of "Enterobacterium A12" strains to ferment mannitol no longer can be considered a difference between these strains and *S. boydii* 14, since most anaerogenic *S. boydii* 14 strains do not ferment this substrate either (6). On the basis of these results, it seems clear that "Enterobacterium A12" strains are much more closely related to *S. boydii* 14 than to *E. coli* group O32. The mutative utilization of sodium citrate and lactose by "Enterobacterium A12" strains, which was detected by the method of Edwards and Ewing (3), was not included in our study

TABLE 1. Biochemical reactions of *S. boydii* 14, "Enterobacterium A12," and *E. coli* O32^a

Test	% of positive strains		
	<i>S. boydii</i> 14 ^b	"Enterobacterium A12"	<i>E. coli</i> O32
Indole (Kovacs)	0	0	100
Methyl red	100	100	100
Voges-Proskauer	0	0	0
Christensen citrate	0	0	0 (11.1) ^c
Simmons citrate	0	0	0
Sodium acetate	0	0	77.8
Growth in KCN	0	0	0
H ₂ S (TSI) ^d	0	0	0
Christensen urea	0	0	0
Malonate	0	0	0
Mucate	0	0	88.9
Jordan tartrate	0	0	44.4
Phenylalanine deaminase	0	0	0
Arginine dihydrolase	7 (93)	37.5 (62.5)	0 (55.6)
Lysine decarboxylase	0	0	66.7
Ornithine decarboxylase	0	0	22.2 (22.2)
Motility	0	0	66.7
β -Galactosidase (ONPG) ^e	0	0	100
Glucose (acid)	100	100	100
Glucose (gas)	0	100	88.9
Lactose	0	0	100
Mannitol	45	0	100
Sucrose	0	0	22.2 (11.1)
Salicin	0	0	55.6 (44.4)
Dulcitol	0	0	44.4
Inositol	0	0	0
Adonitol	0	0	33.3
Raffinose	0	0	33.3
Sorbitol	60 (40)	75 (25)	100
Arabinose	100	100	100
Rhamnose	0	0	100
Xylose	7 (93)	0 (87.5)	100
Trehalose	100	100	100
Esculin	0	0	55.6 (33.3)
Glycerol	0 (100)	0 (100)	100
Cellobiose	0	0	0
Erythritol	0	0	0
Maltose	27 (73)	12.5 (87.5)	100

^a We tested 8 "Enterobacterium A12" strains and 9 *E. coli* O32 strains.

^b Results of 17 *S. boydii* strains from Ewing et al. (6).

^c Numbers in parentheses are percentages of positive strains after 3 or more days of incubation.

^d TSI, Triple sugar iron agar.

^e ONPG, o-Nitrophenyl- β -D-galactopyranoside.

since these tests have not been utilized recently as taxonomic criteria for enteric organisms.

If one considers that *S. flexneri* serovar 6 includes aerogenic biovars (2, 3), there is no reason for not accepting "Enterobacterium A12" as an aerogenic *S. boydii* 14 variant, as proposed by Carpenter (1) and Szturm-Rubinsten et al. (13). Furthermore, Rowe et al. (8) described an analogous situation for *S. boydii* 13 and concluded that the aerogenic variant could not be

excluded from the genus *Shigella* by currently accepted criteria. A variant of *Shigella dysenteriae* 3 which produced gas from glucose was also described by Stypulkowska (12).

We suggest a modification in the definition of the genus *Shigella* to include strains that produce gas from fermentable carbohydrates but otherwise have typical *Shigella* characteristics. Aside from the academic aspect of this suggestion, there are some important practical considerations. For example, we (14) and probably other authors (15) have considered "Enterobacterium A12" strains as invasive *E. coli* O32 strains in order to be in agreement with the current definition of *S. boydii*, which states that this species is composed of only anaerogenic members (2, 3, 6). Furthermore, according to several authors, the presence of gas in screening media, such as triple sugar iron agar and other media (3), should eliminate an enteric organism such as a *Shigella* unless the organism agglutinates in *S. flexneri* 6 antiserum (2, 3). It is possible that this recommendation has caused bacteriologists to miss aerogenic *Shigella* strains (other than *S. flexneri* 6) in routine diagnostic work or in epidemiological studies.

Very little is known about the geographic distribution of the aerogenic *S. boydii* 14 variant. However, this variant has also been isolated in other parts of the world (Betty R. Davis, Enteric Section, Centers for Disease Control, Atlanta, Ga., and Bernard Rowe, *Salmonella* and *Shigella* Reference Laboratory, Central Public Health Laboratory, London, England, personal communications). It is noteworthy that in Brazil we have not isolated the typical anaerogenic *S. boydii* 14.

The results of the Serény test show that "Enterobacterium A12" strains are invasive for guinea pig eyes, whereas *E. coli* O32 strains are not. Also, the biochemical reactions of *E. coli* O32 are different from those described for invasive *E. coli* strains (11, 14). Therefore, *E. coli* O32 must be regarded as a member of a typical noninvasive *E. coli* serogroup which has a somatic antigen identical to that of *S. boydii* 14.

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REPRINT REQUESTS

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