

THE FERMENTATION OF MUCIC ACID BY SOME INTESTINAL BACTERIA

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In the course of a study of the fermentation of some sugar derivatives by bacteria, it was found that various intestinal bacteria showed interesting results with mucic acid. *Escherichia coli*, *Shigella dysenteriae*, Sonne-Duval, *Salmonella enteritidis*, and *S. schottmülleri* fermented this compound with the production of acid. *Aerobacter aerogenes* and *Salmonella aertrycke* produced acid and gas. *Eberthella typhi*, *Shigella dysenteriae*, Flexner, *Salmonella cholerae-suis*, and *Salmonella paratyphi* did not ferment the compound (Sternfeld and Saunders, 1937). Inasmuch as the differentiation of these organisms is of interest it was decided to extend the original observation by testing various strains of each organism.

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Mucic acid, the dicarboxylic acid of galactose, was prepared by the usual method from lactose, the sugar being hydrolyzed and oxidized by treatment with concentrated nitric acid. On cooling, the water-insoluble mucic acid precipitates, and after filtration is recrystallized several times from 50 per cent ethyl alcohol. The pure product is obtained in the form of white crystals, melting at 212° and optically inactive.

For fermentation tests, the acid was neutralized with sodium

hydroxide and added in 0.5 per cent concentration to ordinary nutrient broth, pH 7.0 to 7.2. Brom-cresol-purple (pH range 5.2 to 6.8) was added as indicator and the medium tubed with inner inverted fermentation vials. Sterilization was carried out by autoclaving, using fifteen pounds of steam for ten minutes. It had previously been found that sterilization of the sugar acid by filtration through a Seitz filter yielded results that in no way differed from those obtained by using the ordinary sterilizing procedure. An incubation temperature of 37°C. was employed

TABLE 1
Fermentation of mucic acid

ORGANISM	ACID	ACID AND GAS	NEGATIVE
<i>A. aerogenes</i> (5).....	4	1	0
<i>E. coli</i> (5).....	3	1	1
<i>E. typhi</i> (9).....	0	0	9
<i>S. paratyphi</i> (4).....	0	0	4
<i>S. Schottmülleri</i> (4).....	3	1	0
<i>S. aertrycke</i> (21).....	9	12	0
<i>S. cholerae-suis</i> (7).....	0	0	7
<i>S. enteritidis</i> (12).....	3	9	0
<i>S. suipestifer</i> (4).....	0	0	4
<i>S. dysenteriae</i> , Shiga (2).....	0	0	2
<i>S. dysenteriae</i> , Flexner (4).....	0	0	4
<i>S. dysenteriae</i> , Sonne-Duval (8).....	6	0	2

in all the tests. The controls in each experiment consisted of a non-inoculated tube of medium. In some experiments a control was used consisting of tubes of ordinary nutrient broth (without the sugar acid) inoculated with each organism. In this fashion, acid production due to a spontaneous breakdown of the sugar or due to abnormal bacterial breakdown of the constituents of the nutrient broth could be detected.

Readings were taken once a day for five successive days and then every third day for a total period of two to three weeks. In table 1 are presented the results of the fermentation of mucic acid.

All positive fermentations except two strains of *Shigella*

dysenteriae, Sonne-Duval, occurred within forty-eight hours, many fermenting within twenty-four hours. The two exceptions showed positive fermentation after nine days. An interesting observation was that most of the strains exhibited an alkaline reaction from forty-eight to seventy-two hours after they had shown a positive fermentation, indicating in all probability a further utilization of the intermediary acid products resulting from the breakdown of the mucic acid.

It is of some interest to compare the results obtained by the use of the mucic acid with those obtained when *d*-tartaric acid was used in fermentation tests for differentiation of some types within the *Salmonella* group (Jordan and Harmon, 1928). *S. paratyphi* and *S. schottmülleri* did not ferment the tartrate, whereas *S. aertrycke*, *S. enteritidis*, *S. cholerae-suis*, *S. abortivo-equinus*, and *S. herschfeldii* produced acid. Koser (1923) showed that *E. coli* and *A. aerogenes* would, in most cases, grow on a synthetic medium where the only source of carbon was mucic acid. Kendall and Gross (1930) reported that mucic acid was not fermented by *B. paratyphosus*.

Barker (1936) showed that *A. aerogenes* would ferment *d*-tartaric, *l*-malic, and fumaric acids but not *d*-malic, maleic, or succinic acids.

SUMMARY

The fermentation of mucic acid by various intestinal bacteria was tested, using eighty-two strains. With the exception of three strains, the results were consistent in that all the strains of one species either fermented or did not ferment this compound. *Aerobacter aerogenes*, *Escherichia coli*, *Salmonella schottmülleri*, *Salmonella aertrycke*, *Salmonella enteritidis*, and *Shigella dysenteriae*, Sonne-Duval, ferment mucic acid; whereas, *Eberthella typhi*, *Salmonella paratyphi*, *Salmonella cholerae-suis*, *Salmonella suipestifer*, *Shigella dysenteriae*, Shiga and *Shigella dysenteriae*, Flexner, do not ferment mucic acid. Inasmuch as mucic acid is very readily obtained, we believe that this compound will be of help to the bacteriologist working with intestinal bacteria.

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