A LATE MANNITOL-FERMENTING STRAIN AND RAPIDLY FERMENTING VARIANT OF *SHIGELLA FLEXNERI* TYPE Z

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Shigella organisms are divided biochemically into two main groups distinguished by their ability to ferment mannitol. Non-mannitol-fermenting strains of *Sh. flexneri* type 88 (biochemical type *newcastle*) and of *Sh. flexneri* type 103 (Mumford and Mohr, 1944) are, however, well recognized, and it is probable that similar variants occur among other mannitol-fermenting dysentery bacilli.

The organism described in this paper is of interest not only as a late-mannitol-fermenting variant of *Sh. flexneri* type Z, but also because the strain itself gave rise to variants which fermented mannitol within 24 hours. The strain was originally isolated by Dr. Rickards at the Royal Lancaster Infirmary from a patient with a typical bacillary exudate. Dr. Rickards found that the organism was agglutinated by Flexner Z serum, though it failed to ferment mannitol. The culture was sent to the Dysentery Reference Laboratory, where its identification was confirmed serologically as *Sh. flexneri* type Z.

Behaviour of Parent and Variant Strain

On heart agar and MacConkey's medium the strain produced colonies whose appearance and smell were characteristic of *Sh. flexneri*. It was non-motile, indole-positive, and H₂S-negative. Acid was produced from glucose after 24 hours, but not from maltose, lactose, sucrose, salicin, or dulcitol after 14 days' incubation. Mannitol was fermented late, usually about the fifth day.

In view of the results obtained with *Sh. sonnei* and a number of late-fermenting paracolon bacilli (Cook, Knox, and Tomlinson, 1951), it was thought probable that the slow fermentation of mannitol by this strain was associated with the production of biochemical variants capable of rapid mannitol utilization. In an attempt to isolate these variants the parent culture was plated on to an agar medium containing mannitol and neutral red. After three or four days' incubation at 37° C. both single colonies and the area of confluent growth showed a number of fermenting papillae from which a pure growth of a rapid mannitol-fermenting strain was readily obtained. The biochemical and serological behaviour of this variant strain was identical with that of the parent culture, except for the ability of the variant to produce acid from mannitol within 24 hours both on a solid medium and in peptone water. The rapidly fermenting variant bred true over a period of many months on agar not containing mannitol and still retained its power of rapid mannitol fermentation.

The time-relation between the appearance of variant cells and fermentation of mannitol in a liquid culture was shown by the following experiment. The parent or late-fermenting culture was grown in 1% mannitol peptone water containing Andrade's indicator, and daily subcultures were made on to mannitol-agar plates containing neutral red. Rapidly fermenting colonies were first detected on sub-

FIG. 1.—Graph of growth of parent culture and mannitol-fermenting variant in buffered mannitol and glucose peptone water and in buffered peptone water. (1) Parent culture grown in buffered peptone water. (2) Parent culture grown in buffered mannitol peptone water. (3) Parent culture grown in buffered glucose peptone water. (4) Mannitol variant grown in buffered peptone water. (5) Mannitol variant grown in buffered mannitol peptone water. (6) Mannitol variant grown in buffered glucose peptone water.

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culture after two days' incubation of the peptone water ; on subculture the following day these had increased to approximately half the total number of colonies on the plate. No change of pH, however, was observed in the mannitol peptone water until the fifth day, by which time the fermenting colonies formed approximately 80% of the total colonies obtained on subculture.

FIG. 2.—Graph of growth of mannitol-fermenting variant in buffered mannitol and glucose peptone water after subculture on agar with and without mannitol. (1) Subcultured from agar and grown in buffered peptone water. (2) Subcultured from agar and grown in buffered mannitol peptone water. (3) Subcultured from agar and grown in buffered glucose peptone water. (4) Subcultured from mannitol agar and grown in buffered peptone water. (5) Subcultured from mannitol agar and grown in buffered mannitol peptone water. (6) Subcultured from mannitol agar and grown in buffered glucose peptone water.

The ability of the parent and variant cells to utilize mannitol was investigated by comparing the growth rates of each culture in buffered peptone water with 1% mannitol, in peptone water with 1% glucose, and in peptone water alone. The growth rates were measured by recording the turbidity readings in a "Hilger biochem" absorptiometer. Both cultures grew well in the presence of glucose and poorly in the basal medium without additional carbohydrate. The presence of mannitol conferred no advantage to the parent culture, but improved the growth of the variant cells after a delay of four or five hours (Fig. 1). These results suggested that the variant cells in a growing culture were able to utilize mannitol only after a period of enzymatic adaptation. Evidence supporting this was obtained by comparing the growth rates of the variant strain after subculture on media with and without mannitol (Fig. 2). Previous contact with the substrate almost completely abolished the "lag period" of several hours observed in mannitol fermentation, although it did not increase the rate of growth. Previous contact with mannitol also reduced the short lag period observed in glucose fermentation, though this reduction was not so marked as in the case of mannitol.

Discussion

The isolation from a case of bacillary dysentery of a strain of *Sh. flexneri* type Z which failed to ferment mannitol in 24 hours emphasizes once again the need for constant vigilance in the routine investigation of intestinal organisms (Nelson, 1947). Rapid mannitol-fermenting variants were obtained from this strain, and the behaviour of the parent and variant cultures proved of some interest in relation to the mechanism of delayed fermentation.

In the late fermentation of sucrose by *Sh. sonnei* Cook and his colleagues (1951) have demonstrated two distinct processes, viz. mutation, a hereditary change independent of the substrate, and substrate-induced adaptation: it would appear that both these processes are also involved in the fermentation of mannitol by the strain of *Sh. flexneri* described in this paper. The variant cells produced by this strain, having permanently acquired the property of rapid mannitol fermentation and being able to transmit this characteristic to succeeding generations in the absence of the substrate, can probably be regarded as true mutants of the parent culture. These cells were present in relatively small numbers during the early stages of growth of the parent culture in mannitol peptone water, though their proportion steadily increased until the substrate was fermented, the mannitol probably acting as a selective agent. This association of the production of rapidly fermenting mutant cells with delayed fermentation has been observed by a number of other workers (Lewis, 1934; Sears and Schoolnik, 1936; Cook *et al.*, 1951). Growth experiments with the variant culture showed that some degree of enzymatic adaptation occurred before the cells were able to utilize mannitol.

There is one difference, however, between this fermentative variant and those produced by *Bact. coli mutabile*, *Sh. sonnei*, and certain paracolon bacilli. The biochemical reactions of these parent cultures are typical of the species or group, while those of the variant cultures are abnormal. In the case of the mannitol-fermenting variant of *Sh. flexneri* the parent cells are themselves atypical in fermenting mannitol late, and it is in fact the variant culture whose biochemical reactions are characteristic of the species.

Summary

A late mannitol-fermenting strain of *Sh. flexneri* type Z causing rapid mannitol-fermenting variants is described. Rapid fermentation during growth of the variant was a stable characteristic unchanged by subculture in the absence of mannitol. It is concluded that in the late fermentation of mannitol by the parent strain two distinct processes, namely mutation and substrate-induced adaptation, were concerned.

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REFERENCES

- Cook, G. T., Knox, R., and Tomlinson, A. H. (1951). *Brit. J. exp. Path.*, **32**, 203.
Lewis, I. M. (1934). *J. Bact.*, **28**, 619.
Mumford, E. P., and Mohr, J. L. (1944). *Amer. J. trop. Med.*, **24**, Suppl.
Nelson, M. G. (1947). *J. Path. Bact.*, **59**, 316.
Sears, H. J., and Schoolnik, M. (1936). *J. Bact.*, **31**, 309.