

6634

Delayed Sugar Utilization by Bacteria.

ARNOLD G. WEDUM. (Introduced by A. A. Day.)

From the Department of Bacteriology, Northwestern University Medical School.

From one of the variants (C16) obtained by Nungester and Anderson¹ in a study of a recently isolated *B. coli*-like organism, Nungester² has in later work obtained 7 additional variants. These agglutinate in similar high dilutions of the parent immune serum but differ from each other in their reactions on lactose, maltose and sucrose Andrade agar as indicated in the table.

TABLE I.
Carbohydrate Reactions of C16 and its Variants.

Variant	Source	Lactose	Maltose	Sucrose
C16	—	—	—	—
C17	C16	+	—	—
C19	C16	—	+	—
C20	C16	—	—	+
C18	C17	+	+	—
C21	C19	—	+	+
C23	C20	+	—	+
C22	C18	+	+	+

In carbohydrate broth medium 2 types of reactions occurred: (1) in broths containing the same kind of sugars as in the indicator plates which gave positive reactions, acid was produced in one day; (2) in broths containing the same kind of sugars as in the indicator plates which gave negative reactions, acid production was delayed and appeared after one to 10 days.

Jones, Orcutt and Little³ have reported atypical slow lactose fermenting colon bacilli which appear somewhat similar to those described above. Alkalinity was maintained in broth for several days before acid was evident. In all cases, however, quantitative analysis showed that lactose was being consumed from the first day of growth. The authors postulate that although lactose is used from the start, sufficient alkali is produced to mask the fermentation.

It therefore seemed advisable to determine whether the same mechanism was operative in the case of C16 and its variants or whether this delay in acid production corresponded to a postponement of sugar utilization. For this purpose, two basic mediums

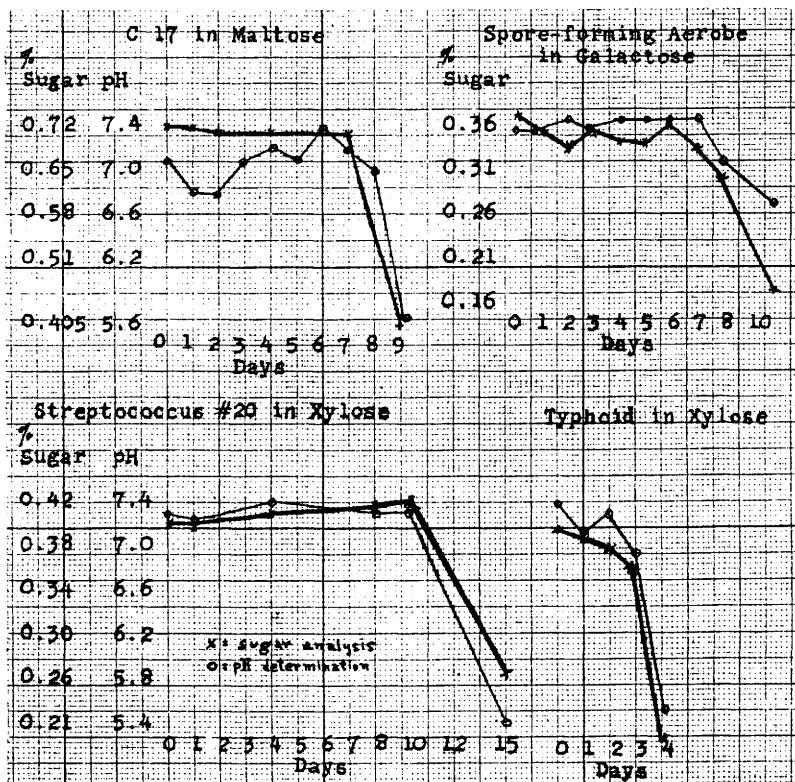
¹ Nungester, W. J., and Anderson, S. A., *J. Inf. Dis.*, 1931, **49**, 455.

² Nungester, W. J., *J. Bact.*, 1933, **25**, 49.

³ Jones, F. S., Orcutt, M., and Little, B. B., *J. Bact.*, 1932, **23**, 267.

were used: (1) sugar-free beef infusion broth and (2) 2% peptone 0.5% sodium chloride solution. To each was added 10% lactose or maltose solution, sterilized by filtration, to make a final concentration of about 0.8%. These mediums were then tubed (10-15 cc. per tube), incubated several days for sterility, and inoculated either from agar slants or from 24-hour carbohydrate indicator agar plate cultures. The daily hydrogen ion concentrations were taken by means of a colorimetric La Motte Hydrogen Ion Testing Set. Quantitative sugar determinations were made daily or every other day by the Stiles, Peterson, and Fred⁴ copper reduction method. Two or more tubes were used for each organism and determinations were repeated several times with different batches of medium.

C16, 19, 20, and 21 in lactose, and C16, 17, 20, and 23 in maltose all showed delayed sugar utilization and a correspondingly delayed development of acidity. The reaction would remain neutral or become slightly alkaline; then over night there would be a shift to the



⁴ Stiles, H. R., Peterson, W. H., and Fred, E. B., *J. Bact.*, 1926, **12**, 427.

acid side accompanying a sudden use of the sugar. The length of the latent period in a given medium varied with the different variants from one to 10 days but was fairly constant with each variant. The graph for C17 records the hydrogen ion concentration and the percentage of sugar at different incubation intervals and is representative of the curves obtained for all the variants.

The graph shows that for a number of days no sugar was utilized and that sudden utilization of sugar was accompanied by a sudden shift to acidity. When such tubes were plated out, the cultures were found to be without contamination. Also subcultures from the acid broths (in which sugar had been utilized) to the appropriate carbohydrate indicator agar plates often failed to show any acid production. This is in agreement with Nungester's studies on these organisms which will be reported at a later date.

In addition to C16 and its variants, 2 other coli-like organisms, freshly isolated, gave the same type of delayed reactions in lactose, although they produced acid in maltose and sucrose in one day. From these 2 organisms could be developed variants which produced acid in lactose broth in one day.

In the course of quantitative determinations of sugar use by various bacteria, 3 other microorganisms were found to give this same type of delayed utilization. The Rawlins strain of *B. typhosus* carried for a number of years in the laboratory produced, in xylose peptone medium, curves of pH and sugar use similar to those of C16. A streptococcus did the same. A spore-forming aerobe was found to act similarly in galactose media. The graphs are illustrative.

It therefore seems that certain organisms are capable of growing in carbohydrate liquid media for several days without using any of the sugar. When suddenly sugar begins to be used, there is a corresponding increase in acidity. These observations do not necessarily question those of Jones, Orcutt and Little, for there are at hand in this and in other laboratories several instances of slow sugar used by bacteria either without appreciable alteration of pH or with increasing alkalinity.