THE FERMENTATION OF GLYCURONIC ACID BY CERTAIN BACTERIA

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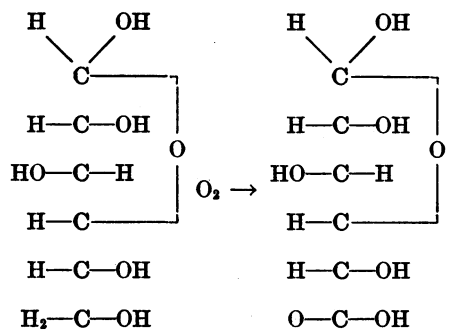
Although glycuronic acid is structurally very closely related to glucose, and is, furthermore, apparently widely distributed in both the animal and plant world, it has received little attention in the biological sciences outside of physiological chemistry. Glycuronic acid is a true sugar acid, for it possesses a carboxyl group and still preserves the essential carbonyl or aldehyde group of a sugar. Structurally, it differs from glucose only in that the terminal alcohol group has been oxidized to a COOH group as shown by the following formulae:



Glucose Glycuronic acid

The close chemical relationship that glycuronic acid has to glucose immediately raises the question as to the position and importance of this compound in the intermediary metabolism of glucose. The general consensus of opinion seems to be that glycuronic acid does not represent a step in the normal metabolism of glucose. In the first place, the free or uncombined glycuronic

acid does not appear to be handled readily by the animal organism, even though all laboratory animals, as well as man, can synthesize glycuronic acid in a conjugated form with ease and in relatively large quantities. In the second place, it appears that the introduction of the acid group into the glucose molecule has affected a profound change in its physiological behavior as illustrated by the fact that glycuronic acid is not fermented by yeast nor can it alleviate and cure insulin convulsions, according to Hurthle (1927).

In view of these facts it seemed interesting to investigate the action of bacteria on glycuronic acid. Such an investigation might not only furnish new information on the physiology of bacteria, but might also be valuable as an aid to evaluate metabolic studies in animals in which glycuronic acid was administered *per os*. Recently, Kay (1926) found that *B. coli* can utilize glycuronic acid and produce qualitatively the same end products as it does from glucose, but the relative amounts formed are markedly different. The author further states that glycuronic acid is fermented by a large number of bacteria of the colon-typhoid group but presents no experimental data nor specifies which particular organisms ferment it. It seemed desirable therefore to determine which particular bacteria can utilize glycuronic acid. Since glycuronic acid can readily be prepared in large quantities by a method recently developed by one of us (1927) it seemed worth while to investigate whether this compound might find a place in the list of sugars used in the differentiation of bacteria.

Glycuronic acid offers another point of interest to the bacteriologist. Recently, Heidelberger and Goebel (1927) found that glycuronic acid is one of the constituents in the serologically type-specific polysaccharide of the pneumococcus. The aldobionic acid which they obtained from the hydrolysis of the Type III specific carbohydrate was found to be a compound of glycuronic acid and glucose united in glucosidic linkage, and an isomeric compound of glycuronic acid and glucose was also obtained from the Type A Friedländer bacillus (1927). It thus appears that bacteria as well as the higher forms of life are capable of synthesiz-

TABLE 1

Fermentation of glycuronic acid by certain aerobic bacteria

ORGANISM	H-ION CONCENTRATION AFTER 48 HOURS INCUBATION
B. typhosus, No. 211	6.0
B. typhosus, Laboratory strain A	6.0
B. paratyphosus A, No. 235 M	6.2
B. paratyphosus B, No. 236 E	6.4
B. paratyphosus B, Laboratory strain S	6.4
B. coli-communis	6.1
B. coli-mutabili	6.3
B. coli-communior, Laboratory strain G	6.0
B. coli-communior, Laboratory strain 1 (a)	6.0
B. coli-communior, Laboratory strain 1 (b)	6.0
B. coli-communis	6.2
B. aerogenes, Laboratory strain No. 1	5.6
B. aerogenes, Laboratory strain No. 2	5.8
B. aerogenes, Laboratory strain No. 3	5.8
B. aerogenes, Laboratory strain No. 4	5.6
B. dysenteriae (Flexner)	6.0
B. enteritidis, No. 273	6.4
Vib. cholerae, No. 581	6.0
B. alkaligenes	7.1
B. proteus (Felix Weil)	6.1
B. proteus vulgaris, No. 23 ATE 2	6.5
M. zymogenes, Laboratory strain M	6.3
M. zymogenes, Laboratory strain S	6.2
B. fluorescens	7.2
B. pyocyaneus, Laboratory strain H	7.2
B. pyocyaneus, Laboratory strain No. 1	7.2
B. prodigiosus, No. 274	6.4
Staphylococcus aureus, No. 72	7.2
Staphylococcus aureus, No. 77	7.2
Staphylococcus aureus, Laboratory strain R	7.1
Staphylococcus aureus, Laboratory strain T	7.1
Staphylococcus albus	6.2
Streptococcus hemolyticus (Beta), from stool	7.1
Streptococcus viridans, No. P 7 H	6.5
B. subtilis	6.0
B. anthracis, No. 10	7.1
Oidium albicans	7.2
Control, sterile medium	7.2

ing glycuronic acid. The desirability of knowing more about the metabolism of this compound by the higher forms of life as well as by bacteria is apparent.

A series of aerobic and of anaerobic bacteria were planted in sugar free broth containing 1 per cent of glycuronic acid. This medium was adjusted to pH 7.2. In the case of the aerobic organisms the plants were made from twenty-four-hour growths in 0.5

TABLE 2

Fermentation of glycuronic acid by certain anaerobic bacteria

ORGANISM	H-ION CONCENTRATION AFTER 48 HOURS INCUBATION
B. welchii (Bull and Pritchett)	6.0
B. welchii, Type 1	6.0
B. welchii, Type 2	6.1
B. welchii, Type 3	5.8
B. welchii, Type 4	6.5
B. oedematiens	6.3
Vibrion septique	6.3
B. tetani (Pasteur)	6.5
B. aerofoetidis	6.6
B. sphenoides	6.0
B. putrificus (Meyer)	6.4
B. bifermentans	6.3
B. botulinus, Type A (U. S. P. H. S.)	6.0
B. botulinus, Type A (Burke)	6.3
B. sordellii	6.2
B. sporogenes	6.5
B. centro-sporogenes (Hall)	6.2
B. histolyticus	6.5
Control, sterile medium	7.2

per cent hormone agar. The anaerobic test cultures were planted from forty-eight-hour growths in casein digest broth and oxygen was excluded by use of the vaseline-seal boiling technic. Both anaerobic and aerobic test cultures were allowed to incubate at 37.5°C. for forty-eight hours, at the end of which time colorimetric tests were made to ascertain the hydrogen ion concentration for each individual culture.

SUMMARY AND CONCLUSIONS

The results obtained in the case of the aerobic organisms are summarized in table 1, and those for the anaerobes in table 2. In the case of the former it may be seen from this limited test that the members of the colon-typhoid-dysentery group are able to utilize glycuronic acid as a source of energy. Four strains of *Staphylococcus aureus* did not, in the presence of this substance, reduce the H-ion concentration to any appreciable degree, while a single strain of *Staphylococcus albus* produced acid, as evidenced by reduction of the H-ion concentration from pH 7.2 to pH 6.2. Before this substance can be used for separative purposes, however, a greater number of representatives of various bacterial species will have to be tested and it is hoped that other workers will be led to make further determinations with this substance in the possible differentiation of some of the above-mentioned and other bacterial types. Most of the anaerobic spore bearers which ferment glucose are also able to ferment glycuronic acid but seemingly less energetically so. In this group, also, a few members which do not ferment glucose, as evidenced by a reduction of the hydrogen ion concentration, feebly utilize glycuronic acid. These are *B. tetani* (Pasteur strain), *B. putrificus* (Meyers strain) and *B. histolyticus* (Barber-Weinberg strain).

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