# Effect of Carbohydrates on Aspartic Acid Deaminase Activity of Bacteria.* (20018)

WILLIAM L. BOYD AND HERMAN C. LICHSTEIN

*From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis.*

The observation that glucose, when incorporated into growth media, exerts an inhibitory effect upon bacterial deaminase activity is established, but the mechanism of action has been obscure(1). Recently evidence was presented in support of the concept that the glucose effect is not primarily on apoenzyme production, but rather the coenzyme of certain bacterial deaminases is resolved during glycolysis(2).

The present paper is concerned with the effects of other carbohydrates and the variation in the mechanism of action in different strains of microorganisms.

*Materials and methods.* The microorganisms studied were strains of *Escherichia coli, Aerobacter aerogenes, Proteus vulgaris,* and *Bacterium cadaveris.* Two types of media were employed. Medium A consisted of 1% each of yeast extract and pepticase and 0.5% $K_2HPO_4$ adjusted to a final pH between 6.5 and 7.0. Medium B was composed of 0.1% each of $KH_2PO_4$, $K_2HPO_4$, and NaCl; 0.4% $(NH_4)_2SO_4$; 0.07% $MgSO_4 \cdot 7H_2O$; 0.05% sodium citrate; and 0.5% vitamin-free acid-hydrolyzed casein, adjusted to a final pH between 6.5 and 7.0. It was necessary to employ casein in medium B to furnish carbon sources for those control cells which were grown in the absence of a carbohydrate. The carbohydrates were added aseptically from sterile 20% solutions to yield the concentrations desired. The cells were incubated for a period of from 16 to 18 hours at 30°C, harvested by centrifugation, washed once with distilled water, and resuspended in distilled water to give a cell concentration of 0.5 to 1 mg of bacterial nitrogen per ml. The deamination experiments were performed at pH 7 in M/20 phosphate buffer at 37°C, using adequate controls without added aspartic acid. The reaction was terminated by the addition of 0.2 ml of 25% trichloroacetic acid, the tubes centrifuged, and an aliquot of the supernatant fluid removed for ammonia determination by Nesslerization. Color was measured in a Klett-Summerson photoelectric colorimeter using a blue filter (400-465 mμ).

*Results.* Since previous data on the inhibitory effect of glucose had been obtained by growing bacterial cells in the presence of 2% glucose(3), it was desirable to determine the effect of other concentrations of this sugar on aspartic acid deaminase activity. Flasks of medium B containing from 0.02 to 2% glucose were inoculated with *E. coli,* strain 86 G, incubated for 18 hours, and the aspartate deaminase activity of the washed cell suspensions determined. Fig. 1 presents the results of this experiment. A concentration of 2% glucose was the most effective, whereas 0.02% exhibited essentially no inhibition. Since 0.2% was relatively as effective as 2%, this concentration of glucose was employed in subsequent experiments.

These findings led to studies of the effects of other sugars and related compounds on

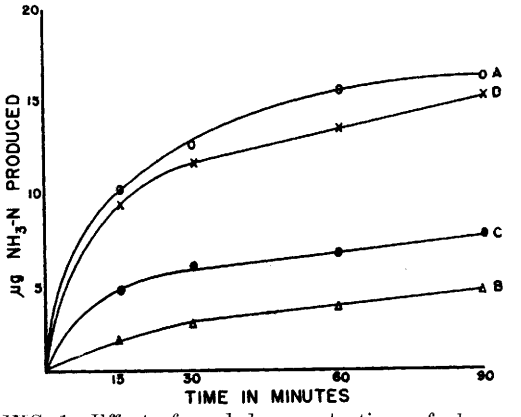


FIG. 1. Effect of graded concentrations of glucose in growth medium A on the aspartic acid deaminase activity of *Escherichia coli* (.085 mg bacterial nitrogen per tube). A = no carbohydrate; B = 2% glucose; C = .2% glucose; D = .02% glucose.

* This investigation was supported in part by the National Institutes of Health, Public Health Service.

TABLE 1. Effects of Presence of Glucose in Synthetic and Complex Media, as well as of pH, on Aspartic Acid Deaminase Activity of Several Bacteria.

| | | μg ammonia-nitrogen produced in 60 min. | | | | | |
|---|---|---|---|---|---|---|---|
| | | Medium A | | | Medium B | | |
| Organism | Cell N/tube (mg) | Basal, pH 7 (6.9-7.2)* | Basal, pH 5 (5.2-5.7) | Basal + glucose, pH 7 (5.2-5.9) | Basal, pH 7 (6.3-6.6) | Basal, pH 5 (5.2-5.7) | Basal + glucose, pH 7 (4.8-5.9) |
| *Bact. cadaveris* | .07 | 52.8 | 33.4 | 13.9 | 48.1 | 38.6 | 14.8 |
| *E. coli* (Tenn.) | .085 | 45 | 50.5 | 14.8 | 53.4 | 45.8 | 29.6 |
| (Crookes) | .11 | 53.7 | 21.2 | 62.9 | 21.8 | 18.9 | 49.3 |
| (Gratia) | .2 | 87 | 29 | 89.3 | | | |
| (Texas) | .085 | 51 | 51 | 23.8 | 67.6 | 64.4 | 21.8 |
| *A. aerogenes* (Tenn.) | .07 | 46.4 | 26.2 | 4.9 | 39.2 | 22 | 4.6 |
| *P. vulgaris* | .11 | 31.3 | 32.6 | 16.8 | 36.5 | 36 | 22.3 |

* Figures in parentheses represent final pH range at time of harvest of cells.

deaminase activity in order to ascertain the specificity of the so-called glucose effect. Representative members of the triose, pentose, hexose, and disaccharide groups were chosen for investigation using *A. aerogenes* as the test organism. The individual carbohydrates were incorporated singly into growth medium A at a concentration of 0.2%. The flasks of media were inoculated with *A. aerogenes,* incubated for 18 hours, and the aspartate deaminase activity of the washed cell suspensions determined. In Exp. 1 (initial pH of media adjusted to 6.5) using 0.085 mg of cell nitrogen per tube, the ammonia produced in 30 minutes was the following: no carbohydrate (final pH 6.5), 14.4 μg $NH_3$-N; glucose (final pH 4.9), 5.8 μg $NH_3$-N; xylose (final pH 4.9), 7.8 μg $NH_3$-N; glycerol (final pH 6.0), 9.9 μg $NH_3$-N; sucrose (final pH 6.5), 17.4 μg $NH_3$-N. In Exp. 2 (initial pH of media adjusted to 6.7) using 0.18 mg of cell nitrogen per tube the ammonia produced in 30 minutes was the following: no carbohydrate (final pH 6.7), 62.1 μg $NH_3$-N; glucose (final pH 4.6), 8.7 μg $NH_3$-N; citrate (final pH 7.1), 31.3 μg $NH_3$-N; inositol (final pH 5.3), 29.0 μg $NH_3$-N; maltose (final pH 5.1), 9.0 μg $NH_3$-N. It is clear from the changes in pH during growth that this strain of *A. aerogenes* utilized each of the compounds tested save sucrose. It is equally manifest that all of the compounds utilized had an adverse effect on the aspartic acid deaminase activity of the harvested cells, although the degree of inhibition varied considerably among the carbohydrates tested. On the basis of these data it may be concluded that the effect on deaminases attributed to glucose is common to other carbohydrates utilized by the organisms during growth. The results with citrate were particularly revealing for, although this carbohydrate was less effective than others, it did exert a definite inhibitory action on the aspartate deaminase activity of the cells, in spite of the fact that there was an increase in pH during growth rather than a decrease as is noted with the other carbohydrates utilized by this organism.

Even though the foregoing results supported the hypothesis that the adverse effect of the presence of carbohydrate during growth on certain enzyme systems is not due to the resultant drop in pH(1), nevertheless the effect of increased hydrogen ion concentration was reinvestigated and the effect of other medium constituents on the carbohydrate action was determined. Seven strains of microorganisms were employed in order to ascertain whether or not strain variation exists. Medium A and B were used with and without the addition of 0.2% glucose at an initial pH of 7.0. For control purposes, a flask of each medium without added carbohydrate and adjusted to pH 5 was included in order to simulate the increase in hydrogen ion concentration anticipated when the carbohydrate was present.

The results of these experiments (Table I) reveal several points of interest. It is noted that the nature of the medium had little effect on the deaminase activity of the cells or on the inhibitory action of the added carbohydrate.

More significant, however, was the variation in response exhibited by different strains to altered pH and/or the presence of glucose. On the basis of these responses, the 7 strains studied may be divided into 3 groups. Group 1. composed of *E. coli,* strains Tennessee and Texas, and *P. vulgaris,* exhibited no pH effect, while a definite glucose effect was apparent. Group 2, composed of *Bact. cadaveris* and *A. aerogenes,* was affected by both the increased hydrogen ion concentration and by the presence of glucose, but the carbohydrate effect was far greater than that of the altered pH. Group 3, composed of the Gratia and Crookes strains of *E. coli,* exhibited a reduced aspartic acid deaminase activity when grown at acid pH, but no reduction in enzyme activity when grown in the presence of glucose. Growth in the glucose medium was associated with an increase in hydrogen ion concentration.

*Discussion.* It is manifest from the results presented that the presence of glucose or other fermentable carbohydrates in the growth medium exerts an adverse effect on the aspartic acid deaminase activity of the harvested cells of many, but not all, bacterial species studied. In those strains which are adversely influenced by the presence of glucose the effect is much greater than can be accounted from the resultant increase in hydrogen ion concentration. As noted earlier, the results with citrate speak strongly for an action not at all related to the concurrent pH change. This is also supported by the observation that the 2 strains not affected by the presence of glucose in the growth medium exhibited a lowered deaminase activity when cultured at a pH close to that which resulted from the utilization of the carbohydrate.

It is pertinent here to comment that in sharp contrast to the carbohydrate effects noted in this study and others where the sugar had been incorporated into the growth medium, the addition of such substances to resting cell suspensions resulted in a pronounced stimulation of the rate of deamination of aspartic acid(4).

*Summary.* 1. The reduction in aspartic acid deaminase activity in cells harvested from a medium containing glucose has been shown not to be specific to this sugar, but rather is shared by other fermentable carbohydrates. 2. The effect of the carbohydrate varies among the several strains studied, and is not associated with the increase in hydrogen ion concentration due to the production of fermentation acids.

1. Gale, E. F., *Bact. Rev.,* 1943, v7, 139.
2. Boyd, W. L., and Lichstein, H. C., *J. Bact.,* 1951, v62, 711.
3. Epps, H. M. R., and Gale, E. F., *Biochem. J.,* 1942, v36, 619.
4. Trudinger, P. A., *Austral. J. Exp. Biol. Med. Sci.,* 1951, v29, 117.

Received November 12, 1952. P.S.E.B.M., 1953, v82.

## Uric Acid Production in Normal and Gouty Subjects, Determined by $N^{15}$ Labeled Glycine.* (20019)

ALEX F. MULLER† AND WALTER BAUER.‡ (Introduced by Louis Dienes.)
*From the Mass. General Hospital, the Department of Medicine, Harvard Medical School, and the Mass. Department of Public Health, Boston.*

By measuring the isotopic composition of

* The studies in this clinic pertaining to articular structures and joint disease are made possible through a generous grant from the Commonwealth Fund, New York City.

This is publication No. 134 of the Robert W. Lovett Memorial Foundation for the study of crippling diseases, Harvard Medical School.

A grant from the Armour Laboratories, Chicago, Ill., made possible the purchase of the potassium phthalimide from which the glycine labeled with $N^{15}$ was synthesized.

† Fellow, Arthritis and Rheumatism Foundation, New York City.

‡ Jackson Professor of Clinical Medicine, Harvard Medical School, Chief of the Medical Services, Mass. General Hospital, Boston.