ADAPTIVE ENZYMES IN THE ESTIMATION OF GLUCONATE,
D-ARABINOSE, AND D-RIBOSE*

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(Received for publication, February 4, 1950)

The small amount of carbohydrate available in our recent studies (1)
of pentose formation from 6-phosphogluconic acid has led us to examine
microbiological analytical tools. In earlier studies on uronic acid metabo-
lism in an Escherichia coli strain (2), it had become clear that the adaptive
synthesis of new enzyme systems in response to the presence of specific
substrates is extraordinarily specific. Cells adapted to uronic acids were
incapable of fermenting d-ribose, d-arabinose, or d-xylose. Cells adapted
to any one of these pentoses were incapable of fermenting the other two.

Furthermore, the adaptive method provided a true control for the micro-
biological analysis of a mixture of sugars, since the strains examined are
capable of using glucose and certain other sugars non-adaptively (i.e. with
constitutive enzymes), whereas the adapted strain can utilize these sub-
strates plus the new substrate. Therefore, non-adapted cells could be
used to exhaust the substrate of fermentable material, with the exception
of the substance to be determined on addition of the adapted cells. Glu-
conate, d-arabinose, and ribose were susceptible of analysis by this method
with E. coli.

Of additional interest is the analysis of the pathway of gluconate metabo-
lism provided by these studies. Since cells adapted to gluconate were
unable to utilize 2-ketogluconate, d-arabinose, or d-arabonate, we may
conclude as a function of the postulates of simultaneous adaptation (3)
that these substances as such were not intermediates in the degradation
of gluconate.

Materials and Methods

Compounds—Gluconate was obtained from Merck and Company as the
monohydrate of the Ca salt. Ca was quantitatively removed by sodium
oxalate. It was found to be free of substances fermentable by non-adapted
bacteria. d-Arabinose was a Pfannstiehl product, and d-ribose came from

*The work described in this paper was conducted in part under a grant-in-aid
from the Commonwealth Fund, and in part under an Office of Naval Research Con-
tact N6ori-188, Task Order 1, NR 135-055.
the preparations of the late Dr. P. A. Levene or from the Schwarz Laboratories, Inc. These substances were apparently free of other sugars, as was determined on paper chromatograms in various solvent mixtures. Dr. Peter Regna of Charles Pfizer and Company generously gave us brucine 5-ketogluconate, Ca 2-ketogluconate, 3 H₂O, and Ca d-arabonate. Ca 2-ketogluconate was also kindly given to us by Dr. Lockwood of the Regional Research Laboratory. Crystalline K ribonate was prepared by the hypiodite oxidation of ribose (4).

Isolation and Handling of Bacteria—Three *E. coli* strains were used. The first strain, strain B (2), became adapted to gluconate readily, but was unable to adapt for growth on either d-ribose or d-arabinose. The simple medium containing glucose and ammonium salts has been described (2). The inoculated culture was aerated at 37° and a glucose concentration of 1 gm. per liter limited growth in the exponential phase at 1.3 × 10⁸ bacteria per cc. The turbidity of the cultures was measured in a Klett colorimeter with a No. 420 filter. The turbidity-viable count curve for this system is described elsewhere (5).

Mutants utilizing d-arabinose and d-ribose, henceforth to be designated as strains Bₐ and Bᵢ respectively, were selected from the parent strain B by growth in the mineral medium containing d-ribose or d-arabinose as the sole carbon sources. The cultures were plated on a solid medium containing 1.5 per cent agar in the synthetic medium, in which the pentose was the only available carbon source. Single colonies were selected and inoculated to agar slants. We have been unable to obtain mutants for d-ribonate, d-arabonate, or 2-ketogluconate by this method.

Growth curves were determined turbidimetrically for the various isolated products in standardized Klett tubes in aerated media containing glucose, glucose plus the desired substrate, or the substrate alone. Whereas strain B showed only a short latent period on gluconate after overnight exhaustion of glucose, strains Bₐ and Bᵢ had prolonged latent periods on transfer from exhausted glucose to the media containing pentose. Hence the demonstration that growth of these mutants on the pentoses was an adaptive process rather than a result of selection was furnished by an analysis of diauxic growth in glucose plus the pentose (6). The derived strain of Bₐ and Bᵢ showing the shortest latent period and most rapid growth rate in the homologous pentose was chosen as the analytical tool.

Manometry—Bacterial suspensions were collected during the exponential phase of growth, centrifuged, and washed twice with 0.85 per cent NaCl. They were suspended at about 5 × 10⁸ bacteria per cc. in the appropriate buffer. O₂ consumption in a phosphate buffer at pH 7.0 in air, or CO₂ production in a bicarbonate buffer at pH 7.0 saturated with 5 per cent
CO₂-95 per cent N₂ at 38°, was tested in Warburg manometers on appropriate substrates.

EXPERIMENTAL

Assimilation of Gluconate—To 2.5 cc. aliquots of the synthetic medium free of a carbon source and containing the salts at twice their usual concentration were added gluconate in known or unknown amounts of measured autoclaved aliquots and enough H₂O to make a total volume of 5.0 cc. The sterile Klett tubes were inoculated with 0.1 cc. of a 24 hour culture of strain B maximally grown in medium containing gluconate at 1 mg.

![Graph showing bacterimetric estimation of gluconate](http://www.jbc.org/)

**Fig. 1.** The bacterimetric estimation of gluconate per cc. Standard curves were set up in triplicate, in the range of 25 to 500 γ of gluconic acid per cc. Tubes were incubated for 18 hours at 37° in an upright position without aeration. The tubes were read in the Klett photoelectric colorimeter with a No. 420 filter, or their contents in a Beckman spectrophotometer at 2600 A. The ultraviolet absorption spectrum of strain B in these media has a pronounced nucleoprotein peak with a maximum at 2600 A (5).

In Fig. 1 are presented the readings on these instruments of these cultures as a function of gluconate concentration. It may be seen that the curves are linear but not proportional to concentration in the range, 50 to 500 γ per cc. The greater precision of the Beckman spectrophotometer and
the steeper character of the curve of ultraviolet absorption as a function of concentration make this a more sensitive instrument for estimations of gluconate as a function of total bacterial growth. Under conditions of aeration, the Klett readings almost double and approach proportionality to gluconate concentration.

This assay has been used on occasions in the estimation of gluconate under conditions whereby no other carbohydrate source was available, as in the analysis of eluates of gluconate and 2-ketogluconate mixtures from anion exchange columns. These analyses have been found to agree with estimations of gluconate by the method of fermentative analysis described below.

Fig. 2. The oxidation of various substrates by gluconate-adapted and non-adapted E. coli.

Adaptive Properties of Strain B—That the adapted organism in the analytical pair of adapted and non-adapted bacteria has synthesized only a limited set of specific enzymes and does not represent genetically altered cells, as in selected mutants, may be demonstrated in three ways (2).

We have shown by a turbidimetric analysis of the kinetics of growth that the growth of our strains on the new substrates is adaptive. Growth of strain B at about $2.5 \times 10^7$ per cc. on glucose from an exhausted 24 hour culture showed a very slight lag period and a mass doubling time of 51 minutes (2). From the same culture to gluconate the lag was about 40 minutes longer, followed by exponential growth with a mass doubling time of 58 minutes.

Cultures of strain B grown in glucose or gluconate at 1 mg. per cc. were harvested at a turbidity of about 110 and sedimented. Washed cells were resuspended in 0.1 M phosphate at pH 7.0. The specificities of the oxida-
tion of various substrates by these adapted and non-adapted organisms of E. coli are presented in Fig. 2. It may be seen that, after a 20 minute incubation of unadapted, washed strain B with gluconate, the cells can oxidize gluconate quite well, whereas adapted cells oxidize gluconate immediately. Neither organism oxidized n-arabonate or 2-ketogluconate.

The adaptation to substrates in air in Warburg vessels has been noted by several workers. Previously, however, it had been observed in studies with strain K-12 on the uronates and the pentoses (2) that adaptation in the vessels did not occur anaerobically in bicarbonate buffers. This is true for these strains as well and neither strain B, B,, nor B, has been observed to adapt under these conditions. In Fig. 3 are presented the data on the fermentative specificity of strain B adapted to gluconate. It can be seen that adapted cells produce acid from glucose and gluconate but not from n-ribose, n-arabinose, p-ribonate, p-arabonate, 2-ketogluconate, and 5-ketogluconate. Comparably prepared cells were used in the estimation of gluconate eluted from anion exchange columns in experiments on the degradation of 6-phosphogluconate (7).

Adaptive Properties of Strains B, and B,,-The most active isolated strain of B, showed typical diauxic growth (2, 6, 8) when grown on a mixture of glucose and D-arabinose. Its mass doubling times on these substrates were 60 and 120 minutes respectively. The shape of the growth curves demonstrated that the kinetics of growth on the mixture were not those of selection during growth and that the presence of glucose inhibited the formation of adaptive enzymes for arabinose. This strain was unable to grow on D-ribose.

The same was noted for strain B,. In addition, however, strain B,,
selected on ribose from an organism incapable of using d-ribulose, *i.e.* strain B, was capable of forming adaptive enzymes separately for ribose and arabinose, although the method of selection never involved exposure to arabinose. It would, therefore, appear that this mutation affected the complex adaptive process rather than only the enzyme essential for ribose fermentation.

In Figs. 4 and 5 is presented the fermentative behavior of adapted and non-adapted pairs of strains B<sub>r</sub> and B<sub>s</sub> respectively. It may be seen that an unadapted organism on incubation with a mixture of 400 γ each of glucose and pentose will metabolize only the glucose (Curve B). On
addition of the adapted organism, only the homologous pentose was metabolized. Thus adapted strain Bₐ will ferment D-arabinose, but not D-ribose, and adapted strain Bᵣ will ferment D-ribose and not D-arabinose. Unadapted strains Bₐ and Bᵣ produced 2.1 and 2.3 moles of CO₂ respectively per mole of glucose. Adapted strains Bₐ and Bᵣ produced 2.0 moles of CO₂ per mole of D-arabinose and D-ribose respectively.

The method of analysis of unknown fractions of dephosphorylated sugars is also presented in Figs. 4 and 5. The figures indicate the importance of exhausting the material with non-adapted organisms prior to the use of adapted cells.
SUMMARY

The preparation and properties of strains of *Escherichia coli* capable of the specific analysis of gluconate, D-arabinose, and D-ribose have been described. Spectrophotometric and turbidimetric analyses of growth on gluconate in the absence of other available carbon sources have been described. Specific manometric techniques for the three substrates utilizing pairs of adapted and non-adapted bacteria and capable of analyzing about 200 γ of the carbohydrate have been presented.

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