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# BACTERIAL CARBOHYDRATE METABOLISM

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## Title of Project and Objectives

Comparative biochemical study of the intermediary carbohydrate metabolism of *Clostridium perfringens* (BP6K), a strict anaerobe, and *Bacillus subtilis* (Marburg), a strict aerobe; elucidation of the mechanisms of fermentation and respiration in both organisms; characterization of the effect of nutrition on both types of metabolic mechanisms.

## Introduction

The work accomplished since the last report (Report No. 1, 1 March - 1 June, 1952) is described below. These reports represent, therefore, the first six months' work on this contract.

## Personnel involved

In addition to the principal investigator (R. C. Bard), a research assistant was involved, the latter on a full-time basis. The assistant's position was held by J. K. Martin, B.S., M.S., until 15 August 1952 when he resigned to return to his home in New Zealand. A replacement was obtained subsequent to the period of this report. Pursuing related problems are two graduate students, one working with *C. perfringens* (K. Shankar, B.S., A.M.) and one with *B. subtilis* (R. E. Klausmeier, A.B.). Although the latter personnel do not constitute part of the project, some of their data are included herein since these relate to the basic problems being studied.

The radioisotope data described in this report were obtained by R. C. Bard while conducting research as a Research Collaborator in the laboratory of Dr. Martin Gibbs, Biology Department, Brookhaven National Laboratory, Upton, New York, from 16 June to 1 September 1952. Gratitude to the installation mentioned is recorded here since training in the field of isotope research was thereby obtained and certain aspects of the problem under investigation were studied which otherwise could not have been pursued.

## Summary of Results

### Part I. Clostridium perfringens

Studies pertaining to the fermentation and respiration of this organism were continued.

Inoculum. As indicated previously (see Report No. 1), the history of the inoculum plays an important role in determining the vigor of fermentation and respiration of cultures derived from various types of inoculum. Accordingly, the procedure adopted for subsequent work involved use of a 12 hours old, heart infusion broth culture inoculum for the Medium A cultures used to study fermentation and respiration. This procedure assures resting cells with high fermentative and respiratory activities.

Dissimilatory balances. A number of additional balances, using glucose as the substrate, were performed. Although moderate quantitative variations in the amounts of end-products formed, either by fermentation or respiration, were noted the patterns of dissimilation described previously (see Report No. 1) do not change in any significant manner. Anaerobically, the major end-products are hydrogen, carbon dioxide, ethanol and acetic acid with traces of lactic and butyric acids. Aerobically, hydrogen formation is replaced by oxygen consumption (though fermentation does proceed in air if large quantities of cells are used), while carbon dioxide formation remains essentially the same as in fermentation; less ethanol is formed aerobically with a roughly corresponding increase in acetic acid formation; only traces of lactic and butyric acids are found. If instead of using 5 hours old resting cells -- as was employed routinely for the above balances -- 14-15 hours old resting cells are used, considerable amounts of pyruvic acid accumulate, and traces of methylglyoxal are also found.

Substrates other than glucose. Neither young (5 hours old), nor old (14 hours old) cells, grown in the presence of glucose, oxidize mannitol, sorbitol, galactose, gluconic acid, ribose, xylose or arabinose; slight respiratory

activity was noted with sucrose and lactose; with fructose and maltose, respiration does occur but only between 1/5 to 1/2 the rate noted with glucose.

Inhibitors. Study of the effect of inhibitors on glucose fermentation, and respiration was initiated. Sodium fluoride (up to 0.04 M) does not affect glucose fermentation nor (up to 0.01 M) glucose oxidation. Sodium iodoacetate, at a level of 0.001 M, completely inhibits fermentation whereas 0.01 M is required to impede respiration significantly. Sodium azide (up to 0.1 M) impedes fermentation and respiration only moderately. The metal chelating agent $\alpha, \alpha'$-dipyridyl, at a level of 0.003 M, completely inhibits fermentation and respiration, as would be expected from the previous knowledge that this organism contains a metallo-aldolase (Bard and Gunsalus, 1950).

Radioisotope data. Using glucose-1-$C^{14}$ as substrate, it was found that none of the carbon dioxide formed during fermentation and respiration contains $C^{14}$. The methyl carbon of the acetic acid isolated from such reaction mixtures contains $C^{14}$, the carboxyl carbon is unlabeled. Using glucose-3, 4-$C^{14}$, only one-half the $C^{14}$ was isolated in the carbon dioxide formed during fermentation and respiration. These data, still far from complete, are adequate, however, to indicate that this organism possesses a pathway of glucose dissimilation unlike any previously described.

Metallic ion nutrition (by K. Shankar). Continued study of $Mg^{++}$-deficient cells has revealed that such cells ferment glucose with the same production of hydrogen and carbon dioxide as cells grown in medium containing optimal quantities of $Mg^{++}$. Addition of $Co^{++}$ to resting cells, $Mg^{++}$ -sufficient or -deficient, does not affect gas production. However, growth of $Mg^{++}$ -deficient cells in the presence of $Co^{++}$ impedes growth considerably and yields cells incapable of gaseous fermentation; under these conditions, glucose is fermented to lactic acid. Such an effect suggests that $Co^{++}$ interferes with the synthesis of the enzyme system responsible for the formation of hydrogen and carbon dioxide.

Part II. Bacillus subtilis (by R. E. Klausmeier). It was previously reported (see Report No. 1) that several amino acids were capable of inducing fermentation of glucose by cells grown in a simple inorganic nitrogen medium and which are incapable of fermentation. Subsequent study revealed that pyruvic acid, acetylmethylcarbinol or diacetyl induce fermentation as do certain amino acids, suggesting that when the latter substances are used as stimulants, their effect is attributable to the pyruvic acid formed therefrom. Indeed, pyruvic acid is probably stimulatory because acetylmethylcarbinol is formed and is the actual stimulatory substance. It would appear that fermentation is induced by these compounds because the latter serve as hydrogen acceptors. The passage of hydrogen during fermentation may be the rate limiting step and the addition of a hydrogen acceptor overcomes this deficiency.

Plans for Future

(1) Continue radioisotopic studies, in collaboration with Dr. Martin Gibbs, Brookhaven National Laboratory. At present, facilities here are inadequate for such studies but steps are being taken to remedy this situation.

(2) Investigate further the effect of metabolic inhibitors on the fermentative and respiratory mechanisms of C. perfringens.

(3) Continue study of the effect of $Co^{++}$ on the mechanism of gas formation in C. perfringens.

(4) Determine the effect of hydrogen acceptors on glucose fermentation by B. subtilis.

References cited

BARD, R.C., and GUNSALUS, I.C. 1950 Glucose metabolism of Clostridium perfringens: existence of a metallo-aldolase. J. Bact., 59, 387-400.

Reports and Publications

None for this report period.