Some Aspects of Carbohydrate Metabolism of Bacteria.

A Thesis.

presented in part fulfilment of the requirements for the Degree of Doctor of Philosophy of the University of Adelaide by

CONTENTS

PREFACE 1

INTRODUCTION

Glucose Stimulation of Bacterial Enzymes 2
The Nature of Pyruvate Metabolism in Lactobacillus arabinosus 5

CHAPTER 1.

Pyruvate Metabolism in Whole Cells of Lactobacillus arabinosus 7

Methods 7
Decarboxylation of Pyruvate 7
The Effect of Inhibitors on Activity and Stimulation 10
The Effect of Glycolytic Intermediates 11
Adaptation Experiments 11
Experiments with Pentose-Adapted Cells 13
The Effect of Substrate Concentration on Glucose Stimulation 14
The Effect of Prolonged Pre-incubation with Glucose on 14
Stimulation
Decarboxylation of α-Acetolactate 17
Pyruvate Metabolism in Vacuum Dried Cells 19
Discussion 20

CHAPTER 2.

Pyruvate Metabolism in Cell-Free Extracts of Lactobacillus 24

arabinosus 17-5

Methods and Preparations 25
Pyruvate Decomposition 26
The Effect of Disintegration Time on Extract Activities 28
Stoichiometry of the Reaction 31
Relationship Between Cell Activity, Glucose Effect and Extract 31
Activity
Relation of Activity and pH. 41.
Reaction Time Curves and the Effect of Substrate Concentration. 41.
Effect of Added Coenzymes and Acceptors. 46.
The Effect of Furacin. 49.
Nature of "Acetoin" Formed and the Metabolism of Diacetyl in Whole Extracts. 50.
Fractionation of Whole Extracts. 52.
Distribution of Enzyme Activity in the Extracts. 52.
Separation of Oxidative and Acetoin Forming Enzymes. 55.
The Acetoin Forming Fraction. 55.
Nature of Pyruvate Oxidation in Gel Treated Extracts. 60.

CHAPTER 3.
The Metabolism of Glucose in Cell-Free Extracts.
Methods. 62.
Utilisation of Glucose and Glycolytic Intermediates by Whole Extracts. 64.
Distribution of Glycolytic Enzymes. 73.
Glucose Metabolism in Bicarbonate-CO₂ Media. 76.
The Oxidation of DPNH. 81.

CHAPTER 4.
The Nature of the Pyruvic Oxidase of Lactobacillus arabinosus 82.
Methods. 77-5.
Purification of the Enzyme. 82.
General. 83.
The Oxidation of Pyruvate. 85.
The Flavin Portion. 88.
Electron Acceptors and -SH Reagents on Pyruvate Oxidation. 88.
Chromatographic Identification of Acyl-Products. 89.
The Phosphorylation Reaction. 91.
Discussion 92.
Flavin Enzymes and Pyruvate Oxidation. 95.
The Role of Molybdenum. 95.
CHAPTER 5.

The Metabolism of Glucose \(-1-C^{14}\), Glucose \(-U-C^{14}\) and Pyruvate \(-2-C^{14}\) in Whole Cells.

Methods.
Radioactive Chemicals.
Measurement of Radioactivity.
Chromatography.
Celite.
Isolation of Lactic Acid and Acetoin.
Degradation of Acetoin.
Degradation of Lactic Acid.
Isooform Combustion.
General.
The Exchange Between Added and Glucose Pyruvate.
The Effect of Substrate Concentration.
Substrate Concentration and Time Reactions with Pyruvate \(-2-C^{14}\).
\(\alpha\)-Acetolactate and Diacetyl.
Studies with Glucose \(-1-C^{14}\).
Isotope Distribution and the Variation in Behaviour towards Pyruvate in the Presence of Oxygen.
Stoichiometry of Glucose Catalysis.
Stimulation of Decarboxylation by Other Carbohydrates and Derivatives.
The Distribution of C\(^{14}\) in the Reaction Products from Radioactive Glucose and Pyruvate in the Stimulated System.

CHAPTER 6.

The Cell Wall and Pyruvate Metabolism.
The Action of Lysozyme on Lactobacillus arabinosus 17-5.
Pyruvate Metabolism in Lysozyme Treated Cells.
Isotope Distribution with Pyruvate and Glucose \(-U-C^{14}\) in Lysozyme Treated Cells.
The Nature of Lysozyme Action.
GENERAL DISCUSSION

The Mechanism of Acetoin Formation in Lactobacillus arabinosus 150.
The High Yeast Extract Effect. 17-5. 151.
The Oxidation Mechanism. 153.
The Relationship Between Pyruvate Oxidation and Stimulation. 154.
Cellular Permeability and Metabolism. 155.
The Nature of Glucose Catalysis of Pyruvate Decomposition. 159.
The Glucose Effect in Other Systems. 162.

SUMMARY

PUBLICATIONS AND COMMUNICATIONS

REFERENCES 169.
INTRODUCTION.

Glucose Stimulation of Bacterial Enzymes.

Carbohydrates serve as a major source of carbon and energy for the growth of many microorganisms. Consequently the questions of their chemical properties and transformations and the pathways of carbon and energy liberation has been extensively investigated.

Many workers, however, have observed an unexplained stimulation of various enzymes in bacteria by glucose. Instances are formic hydrogenlyase activity in Eschericia coli (Lascelles, 1948), aspartase in Proteus vulgaris CX-19 (Trudinger, 1951), decarboxylation of malate and of pyruvate by malate adapted cells of Lactobacillus arabinosus 17-5 (Nossal, 1951) and a similar pyruvate system in Lactobacillus plantarum (Rowatt, 1951). Later, Broquist and Kohler (1953) reported that the stimulation of formic hydrogenlyase in Aerobacter aerogenes by yeast and liver extracts also was due to the content of reducing sugars in the extracts.

Washed suspensions of E. coli grown on peptone meat extract glucose broth showed large losses in formic hydrogenlyase activity when suspensions are diluted or stored. These losses were partly restored by addition of extracts of boiled cells or of amounts of fermentable sugars such as fructose, mannose and glucose -1-PO4. Thus a fresh suspension with a Q(H2) formate of 150, in the presence of glucose gave Q formate of 378 (10^-5M glucose). After storage of this suspension for 24 hours at 4°C the Q(H2) formate was 27 and in the presence of glucose was 285. Stimulation by glucose was obtained even when formate was added to the suspension after the glucose had been completely fermented. Glucose appeared to eliminate the initial lag period which was always observed in the course of hydrogen evolution from formate.

Formic hydrogenlyase activity of these suspensions was greatly decreased by incubation in oxygen for 30 min. or for longer periods in air. Some of this last activity was restored by addition of glucose.
Preliminary anaerobic incubation of cells with $10^{-2}$M glucose resulted in greatly increased formic hydrogenlyase activity of the cells after they had been washed free of glucose. This activity was not further increased by the addition of $10^{-3}$M glucose.

Trudinger (1951) demonstrated that cells of *Proteus vulgaris* OX-19 harvested under the experimental conditions used, have only 30-40% of their potential ability to deaminate aspartic acid anaerobically. Addition of some fermentable substances such as glucose, lactate etc. or adenosine and related compounds, could produce a marked increase in the deaminating activity of the cells. Glutathione stimulated deamination to a smaller extent, its effect being considered due to its reducing properties. Glucose and adenosine stimulation occurred only during the period of metabolism of these compounds. Adenosine was effective by providing a metabolisable form of pentose. Lichstein et al (1951) concluded stimulation of similar systems in *E. coli* was related to the biosynthesis of a coenzyme for deamination. Trudinger discounted the coenzyme theory, and proposed asparagase stimulation was a permeability phenomenon related to energy production within the cell, and maintenance of $-SH$ groups at the permeability barrier.

During an investigation of the decarboxylation of L-malate to lactate by cells of *Lactobacillus arabinosus* 17-2, Nossl (1951) observed that pyruvate also yielded CO$_2$. Like the decarboxylation of malate, the decomposition of pyruvate was found to be most rapid in the presence of glucose.

For a similar system in *Lb. plantarum*, Rowatt (1951) found that in the presence of glucose, more pyruvate was decomposed than without glucose. There appeared to be no stoichiometric relation between the glucose added and the effect produced. Glucose alone and in the presence of pyruvate was converted quantitatively to lactic acid. Incubation of the cells at 37°C in citrate buffer at pH 3.9 without substrate reduce their ability to attack pyruvate. At pH values above
4, the capacity could be restored by glucose.

Growth of Lb. plantarum in media deficient in thiamine, yielded cells inactive towards pyruvate even in the presence of glucose. Addition of thiamine alone did not increase the CO₂ formation from pyruvate, but on incubation with pyruvate, glucose and thiamine, deficient cells formed CO₂ and acetoin at the same rate as non-deficient cells.

Moat and Lichstein (1953) indicated the glucose effect in Lb. arabinosus was a permeability phenomenon. This was based on the fact that (a) adenosine triphosphate could replace glucose in reconstituted vacuum dried cells and (b) glucose stimulation was lost if these preparations were aged, considered due to loss of the permeability barrier. However, the extent of stimulation by A.T.P. was very low indeed compared with glucose in whole cells. Furthermore, although the glucose effect disappeared in aged dried cells, these workers failed to indicate whether or not glucose was metabolised in these preparations, a fundamental requirement for the appearance of stimulation in all glucose systems so far investigated.

For the malate system of Lb. arabinosus, Nossal concluded that there was a definite relation between the glucose effect and cozymase. In intact cells, glucose accelerated the decomposition of malate and added cozymase had no effect. In freeze dried cells (where glucose probably could not be metabolised), cozymase was necessary. Similarly, in cells which had become less active to malic acid on account of prolonged storage, addition of cozymase caused partial restoration of activity; alternatively incubation with glucose and nicotinic acid also brought about partial reactivation.

As part of a general programme of research in this laboratory, into the mechanism of glucose stimulation of these bacterial enzymes, a more detailed study of the pyruvate system in Lactobacillus arabinosus 17-5 was undertaken. The results of this investigation are presented in this thesis.
The Nature of Pyruvate Metabolism in Lb. arabinosus. 17-5.

Nossal (1951) demonstrated that distillates from reaction mixtures of bacteria plus pyruvate under optimum conditions gave the Voges-Proskauer test for diacetyl and acetoin. The product of pyruvate decarboxylation was subsequently identified as acetoin from the colour time curve with alkaline α-naphthol and creatine, and from the melting point of the osazone. The amount of pyruvate decomposed in the presence of glucose, KCl, and MnCl₂ was almost totally accounted for as acetoin and CO₂, the reaction complying with the overall scheme,

\[ 2 \text{CH}_3\text{CO COOH} \rightarrow 2\text{CO}_2 + \text{CH}_3\text{CO CHOCH}_3 \]

Both aerobically and anaerobically, addition of glucose to cells metabolising pyruvate caused an increase in the rate of CO₂ evolution. The effect of glucose appeared to be catalytic. The addition of 0.16 µmoles glucose was claimed to increase CO₂ evolution by 8 µmoles in the first hour. The rates of pyruvate decomposition were increasingly accelerated by increasing concentrations of glucose up to 10⁻³ M.

Although grown under apparently identical conditions, some washed suspensions of Lb. arabinosus showed a considerable oxygen uptake with pyruvate as substrate (QO₂=50), whereas others had a low QO₂ (10). QO₂ values did not alter appreciably in air or nitrogen. However, the magnitude of the glucose effect on the rate of pyruvate decomposition varied inversely as the QO₂. In suspensions having a high QO₂, glucose failed to increase the rate of CO₂ evolution aerobically. The amount of pyruvate decomposed in such systems could not be accounted for as acetoin. Glucose, however, tended to depress oxygen uptake and to restore acetoin formation.

On this basis pyruvate metabolism was represented as

\[ 2 \text{pyruvate} \rightarrow 2\text{CO}_2 + 2(\text{C}_2\text{X}) \leftrightarrow 2 \text{acetate} \]

The stipulated 2C intermediary ("nascent aldehyde") would be partly
oxidised in the absence of glucose, but not in its presence, where all of it would condense to acetoin. This theory however, is untenable on present knowledge of acetoin formation in bacterial systems. Consequently the whole question of pyruvate metabolism has been reinvestigated in the present study.