The Incorporation and Metabolism of Glucose by *Anabaena variabilis*

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SUMMARY

The incorporation and metabolism of glucose by the blue-green alga *Anabaena variabilis* is described. Experiments with [^{14}C]glucose indicated that this compound contributed up to 46% of the total dry wt of organism. Respiratory studies with [$1\text{-}^{14}\text{C}$] and [$6\text{-}^{14}\text{C}$] showed that the pentose phosphate pathway was the major route of glucose dissimilation. No evidence of alteration in enzyme concentration after growth in the presence of glucose was found in the ten examples of glycolytic and pentose phosphate pathway enzymes examined. Enzymes from both pathways were affected by the end-products. These results are discussed in relation to autotrophic metabolism in blue-green algae.

INTRODUCTION

In an earlier paper (Pearce & Carr, 1967*a*) we described the metabolism of acetate by two species of blue-green algae which until recently were considered to be strict photoautotrophs. Such metabolism, and that of other carbon compounds, by blue-green algae hitherto considered to be autotrophs, is now well established (Hoare & Moore, 1965; Hoare, Hoare & Moore, 1967; Carr & Pearce, 1966; Pearce & Carr, 1967*a*; Smith, London & Stanier, 1967). A noticeable feature of *Anabaena variabilis* is that even though glucose is incorporated and metabolized its addition to the growth medium produces no increase in growth rate and only slight increase in respiratory rate (Kratz & Myers, 1955*a*). In contrast, the growth of some species of blue-green algae is stimulated by glucose or other sugars, and indeed they may be maintained heterotrophically in the dark (Kiyohara, Fujita, Hattori & Watanabe, 1960; Fay, 1965). Studies on carbohydrate metabolism in blue-green algae have usually centred upon the synthesis of polysaccharide (Fredrick, 1954; Kindel & Gibbs, 1963) and on the operation of fructose diphosphate aldolase (Richter, 1959; Wildon & ap Rees, 1965; Willard, Schulman & Gibbs, 1965). An examination of [^{14}C]sugar metabolism in autotrophically grown *Tolypothrix tenuis* (a species capable of heterotrophic growth) led Cheung & Gibbs (1966) to conclude that the oxidative pentose phosphate cycle was the main pathway of carbohydrate dissimilation. Several of the enzymes necessary for this pathway were detected in extracts of *Anacystis nidulans* by Richter (1959). The work described in the present paper establishes the incorporation and metabolism of glucose by *A. variabilis* and examines some of the enzymes concerned in glucose breakdown in cell-free extracts prepared from organisms grown in the presence and absence of glucose.

METHODS

Growth of Anabaena variabilis. The blue-green algae used were maintained and cultured as previously described (Carr & Hallaway, 1965; Pearce & Carr, 1967a); *Anabaena variabilis* was used for most of the work; certain enzyme activities of *Anacystis nidulans* were also measured. The basic autotrophic growth medium was medium C (Kratz & Myers, 1955b) supplemented with 0.05% (w/v) sodium bicarbonate and gassed with air + carbon dioxide (95 + 5, v/v). Glucose was added to this medium, after separate sterilization, where indicated in the text.

Incorporation of [^{14}C]glucose. The growth medium (20 ml.) was supplemented with [^{14}C]glucose and incubated at 34° in boiling tubes illuminated with 30 W. warm-white strip lights at 9 in. distance. Vessels were gassed with air or air + carbon dioxide (95 + 5, v/v) as indicated. The extent of isotopic incorporation was measured in a harvested and thoroughly washed (6 times) suspension by counting, after drying, at infinite thinness in a Nuclear-Chicago Gas-Flow Automatic Planchette Counter of efficiency 34% and a background of 16 counts/min. Each sample was counted in duplicate to an accuracy of at least 2%.

Oxidation of [^{14}C]glucose. Bloom & Stetten (1953) devised a procedure for estimating the relative activities of the two main pathways of glucose breakdown by measuring the ratio of carbon dioxide production from different carbon atoms within the glucose molecule. Although the relative participation of the pentose phosphate pathway and glycolytic pathway cannot be absolutely determined (see Hollmann, 1964) the comparison of rate of [^{14}C]carbon dioxide evolution from the 1-C and 6-C positions of glucose indicate the extent of each pathway. The ratio of [^{14}C]carbon dioxide from [6- ^{14}C]glucose compared with [^{14}C]carbon dioxide from [1- ^{14}C]glucose ($\text{C}_6:\text{C}_1$ ratio) will be unity when glycolysis is the only operative pathway. $\text{C}_6:\text{C}_1$ ratios of less than unity indicate the contribution of the alternative pentose phosphate pathway.

Double side arm Warburg flasks contained a suspension of *Anabaena variabilis* in medium C in the main compartment, hyamine hydroxide (0.3 ml) in the centre-well (Snyder & Godfrey, 1961), with sodium dodecylsulphate (10%, w/v) in one side arm and [^{14}C]glucose in the other. At various times after the addition of glucose to the main compartment the respiration was terminated by adding sodium dodecylsulphate. After standing for 45 min. the carbon dioxide was totally absorbed by the hyamine hydroxide and this was transferred, by a syringe, to 5 ml. scintillation fluid. The centre-well was washed with toluene (0.2 ml.) and the washing added to the scintillation fluid. Samples were placed in glass vials together with 5 ml. scintillation fluid (in g./l. toluene:2,5-diphenyloxazole, 5; dimethyl-1,4-bis-2-(4-methyl-5-phenyloxazolyl-benzene, 0.3) and counted in a Packard Tri-Carb Liquid Scintillation Spectrophotometer Series 314E to an accuracy of at least 2%. The efficiency for counting [^{14}C] was 60% ($\pm 1\%$); the background counting rate was 20 counts/min.

Estimation of glucose. The colorimetric procedure of Roe (1955) was used; absorption at 620 m μ was measured in a Hilger and Watts Spectrochem and glucose concentration obtained from a standard curve.

Preparation of cell-free extract and protein estimation. These procedures were as described by Pearce & Carr (1967a).

Enzyme assays

In the assays described the amount of commercially prepared enzymes added are expressed as units of activity; one unit metabolized 1 μ mole substrate/min. Except where stated assays were done at room temperature (20°) and the spectrophotometer used was a Unicam SP700.

Hexokinase [EC2.7.1.1]. The method of assay (Slein, Cori & Cori, 1950) determined the rate of glucose-6-phosphate formation by measuring the rate of NADP reduction in the presence of glucose-6-phosphate dehydrogenase. The reaction mixture contained (μ mole): potassium phosphate buffer (pH 7.0), 200; $MgCl_2$, 10; ATP, 5; NADP, 0.5; cell-free extract containing 2 to 7 mg. protein; final volume 2.7 ml. The rate of NADP reduction at 340 m μ was determined after the addition of 10 μ moles glucose to the experimental cuvette. In this assay no coupling glucose-6-phosphate dehydrogenase was added as the extracts of *Anabaena variabilis* and *Anacystis nidulans* contained a relatively high glucose-6-phosphate dehydrogenase activity (see Table 1). During the first few minutes of the reaction the rate of glucose-6-phosphate formation was limiting and the constant rate attained was taken as the measure of hexokinase activity.

Glucose-6-phosphate dehydrogenase [EC1.1.1.43]. The reaction mixture was the same as above except that 5 μ mole glucose-6-phosphate were added to start the reaction (Kornberg & Horecker, 1955).

6-Phosphogluconate dehydrogenase [EC1.1.1.49]. The same reaction mixture was used as above but in this case 6-phosphogluconate (5 μ mole) was added to initiate the assay (Horecker & Smyrniotis, 1955).

Phosphoriboisomerase [EC5.3.1.6]. The reaction assay system was essentially that of Axelrod & Jang (1954) and contained (μ mole): potassium phosphate buffer (pH 7.0) 50; ribose-5-phosphate, 3; cell-free extract (3 to 5 mg. protein); total volume 1.0 ml. After incubation at 34° the reaction was stopped by adding concentrated H_2SO_4 . Ribulose was detected colorimetrically by the carbazole procedure of Dische & Borenfreund (1951).

Phosphohexoisomerase [EC5.3.1.9]. The formation of glucose-6-phosphate from fructose-6-phosphate was assayed by coupling the isomerase with glucose-6-phosphate dehydrogenase (commercial enzyme added). The reaction mixture contained (μ mole): potassium phosphate buffer (pH 7.0), 200; NADP, 0.5; 20 μ l. glucose-6-phosphate dehydrogenase (equiv. 3 units of activity); cell-free extract (3 to 6 mg. protein) in 2.7 ml. total volume. Fructose-6-phosphate (5 μ mole) was added to the experimental cuvette to start the reaction.

Phosphofructokinase [EC2.7.1.11]. The rate of production of fructose-1,6-diphosphate from fructose-6-phosphate and ATP was measured by coupling the kinase reaction with aldolase, triose phosphate isomerase and α -glycerophosphate dehydrogenase (Slater, 1953). The reaction mixture contained (μ mole): potassium phosphate buffer (pH 7.0), 200; ATP, 9; $MgCl_2$, 10; cysteine, 20; with 20 μ l. of a mixture of triose phosphate isomerase and α -glycerophosphate dehydrogenase (containing 14 units of each activity) + 50 μ l. aldolase (containing 5 units of activity) + cell-free extract containing 2 to 5 mg. protein; total volume 2.7 ml. $NADH_2$ (0.3 μ mole) was added to the experimental cuvette and after the measurement of the very slight $NADH_2$ dehydrogenase activity, 10 μ mole fructose-6-phosphate were added and the

decline in absorption at 340 m μ was recorded. When the algal extract was omitted from the assay system there was no decline in absorption on the addition of fructose-6-phosphate, indicating that the samples of commercial enzymes employed did not contain any phosphofructokinase activity.

Fructose diphosphate aldolase [EC4.1.2.13]. The formation of triose phosphate from fructose diphosphate was measured by coupling this reaction with triose phosphate isomerase and α -glycerophosphate dehydrogenase (Racker, 1947). Identical cuvettes contained 200 μ mole potassium phosphate buffer (pH 7.0) + 20 μ l. of a mixture of triose phosphate isomerase and glycerophosphate dehydrogenase (containing 14 units of each activity) + cell-free extract (3 to 7 mg. protein); 2.7 ml. final volume. NADH₂ (0.3 μ mole) was added to the experimental cuvettes and after the initial measurement of NADH₂ dehydrogenase activity in the extract, 5 μ mole fructose-1,6-diphosphate were added and the decrease in absorption at 340 m μ was determined. Control assays were made in which algal extract was omitted. No decrease in absorption at 340 m μ was found on adding fructose diphosphate in the absence of algal extract, indicating that the commercial enzymes did not contain contaminating aldolase activity.

Triose phosphate dehydrogenase ([EC1.2.1.12] NAD dependent; [EC1.2.1.13] NADP dependent). The rate of reduction of NAD or NADP resulting from the oxidation of glyceraldehyde-3-phosphate was followed at 340 m μ . The assay method was essentially that of Gibbs (1955). The reaction mixture contained (μ mole): potassium phosphate buffer (pH 7.5), 200; sodium arsenate, 10; cysteine, 12; NAD (or NADP), 0.5; cell-free extract containing 3 to 7 mg. protein; 2.7 ml. total volume. The reaction was initiated by adding 3 μ mole glyceraldehyde 3-phosphate to the experimental cuvette.

Pyruvate kinase [EC2.7.1.40]. The production of pyruvate from phosphoenolpyruvate was determined by measuring the rate of oxidation of NADH₂ in the presence of lactic dehydrogenase (Bücher & Pfeleiderer, 1955). Identical cuvettes were made up containing (μ mole): tris (pH 7.6), 100; MgCl₂, 10; KCL, 50; ADP, 2.5; 20 μ l. lactic dehydrogenase (containing 30 units of activity); cell-free extract, containing 3 to 8 mg. protein; 2.7 ml. total volume. NADH₂ (0.3 μ mole) was added to the experimental cuvette and after the initial measurement of NADH₂ dehydrogenase activity in the extract, 9 μ mole phosphoenolpyruvate were added and the decrease in absorption at 340 m μ determined. Controls showed that the sample of phosphoenolpyruvate used did not contain any contaminating pyruvate and also that the commercial lactic dehydrogenase did not have any pyruvate kinase activity.

Chemicals and commercially prepared enzymes. Unless otherwise stated, all chemicals were analytical grade or of the purest commercial grade available and were purchased from British Drug Houses Ltd., Poole, Dorset. NAD, NADH₂, NADP, ATP, AMP and glucose-6-phosphate dehydrogenase were from C. F. Boehringer (Mannheim, Germany). Phosphoenolpyruvate, triose phosphate isomerase, α -glycerophosphate dehydrogenase, aldolase and lactic dehydrogenase were from Sigma London Chemical Co. (12, Lettice Street, London, S.W. 6). Sodium dodecylsulphate was obtained from K and K Laboratories (177-10, 93rd Avenue, Jamaica 33, New York, U.S.A.) and hyamine hydroxide from the Packard Instrument Co. (10-12 St John's Road, Wembley, Middlesex). All radioisotope-labelled material was purchased from the Radiochemical Centre, Amersham, Buckinghamshire.

RESULTS

Growth and respiration

In agreement with the results of Kratz & Myers (1955*a*) the growth rate of *Anabaena variabilis* was not increased or retarded when glucose (10 to 50 mM) or sucrose (30 mM) was present in the autotrophic growth medium. When the available carbon dioxide was decreased by changing the gas phase from air + carbon dioxide (95 + 5, v/v) to air (about 0.05 %, v/v, CO₂) the growth rate was decreased, this decrease being unaffected by adding glucose (to 30 mM) to the medium. No growth of *A. variabilis* occurred in the complete absence of carbon dioxide and the presence of glucose. When *A. variabilis* was suspended in growth medium (see Methods) the respiratory rate was several times higher than that obtained with suspensions in 0.1 M-potassium phosphate (pH 7.0; Fig. 1*a*); in neither case did glucose stimulate the respiration rate.

Fig. 1. (*a*) Oxygen uptake by *Anabaena variabilis*. Washed organisms (equiv. 9.5 mg. dry wt) were incubated in growth medium + glucose (33 mM) (—●—●—); growth medium alone (○—○—); phosphate buffer (pH 7.0) 0.01 M + glucose (33 mM) (—●—●—); phosphate buffer (pH 7.0) 0.01 M alone (○—○—). (*b*) Oxygen uptake by starved *Anabaena variabilis*. Oxygen uptake in the presence of glucose (33 mM) (●—●—); endogenously (○—○—), by a starved suspension of *A. variabilis* (equiv. 7.4 mg. dry wt).

Starvation of organisms of carbon dioxide for 4 hr before harvesting decreased the endogenous rate of oxygen uptake; the addition of glucose or acetate largely restored this to that of the non-starved control (Fig. 1*b*). The lack of any permeability barrier to glucose metabolism in this organism was indicated by measurements of glucose utilization by a washed suspension of *A. variabilis*. Glucose was removed from the medium at a rate equal to 1.7 mμmole/min./mg. dry wt organism. (Fig. 2)

Experiments with [¹⁴C]glucose

The utilization of glucose by *Anabaena variabilis* was confirmed by measurement of incorporation of [U-¹⁴C]glucose into cell material during growth. When this organ-

ism was grown under the standard procedures in the presence of $2 \mu\text{C}$ [$U\text{-}^{14}\text{C}$]glucose (20 mM), 33 % of the total dry wt after 5 days of growth was derived from the [^{14}C]-labelled glucose (Table 1). This proportion increased to 46 % when air replaced air + carbon dioxide (95 + 5, v/v) as gas phase, and the addition of acetate (20 mM) to the medium decreased the extent of glucose incorporation to 20 % of the dry wt. The figures in Table 1 were calculated after measurement of isotope incorporation and

Fig. 2

Fig. 3

Fig. 2. Decrease in glucose concentration during incubation with *Anabaena variabilis*. *A. variabilis* (equiv. 1.2 mg. dry wt./ml.) was suspended in growth medium (100 ml.) containing glucose (300 μg./ml.) and illuminated by four 60 W. lamps at 34°. Organisms were kept in suspension by a magnetic stirrer. Samples were taken at intervals, the algae removed by centrifugation, and the glucose content determined (see Methods). ○—○, *A. variabilis*; ●—●, control of boiled organisms.

Fig. 3. Effect of ATP on phosphofructokinase activity in *Anabaena variabilis*. The assay was as in Methods except that ATP concentration was varied as shown in the absence (○—○) or presence (●—●) of AMP (0.3 mM).

Table 1. Incorporation of [$U\text{-}^{14}\text{C}$]glucose into growing cultures of *Anabaena variabilis*

Growth medium (20 ml.) was supplemented as indicated with glucose (2 μC) or acetate and organisms grown as in Methods. Results 1 and 2 are mean values; the range and number of experiments are given in parentheses.

Expt	Addition (20 mM)	Gas phase	% dry wt derived from glucose
1	Glucose ($U\text{-}^{14}\text{C}$)	Air + CO ₂ (95 + 5, v/v)	33 (29 to 34; 4 expts)
2	Glucose ($U\text{-}^{14}\text{C}$)	Air	46 (43 to 48; 4 expts)
3	Glucose ($U\text{-}^{14}\text{C}$) + acetate	Air	20

amount of cell material formed (as dry wt); they do not take into consideration any contribution to respiration by the [$U\text{-}^{14}\text{C}$]glucose during the growth period. As might be expected, the inclusion of glucose or acetate into the growth medium decreased the degree of incorporation of [^{14}C]carbon dioxide into the cell material of *A. variabilis* by an amount about equivalent to the [$U\text{-}^{14}\text{C}$]glucose incorporation (Table 2).

The relative contribution of carbon atoms 1 and 6 of glucose to respiratory carbon

dioxide has been used as an indication of the relative participation of glycolysis or the pentose phosphate pathway in glucose dissimilation (see Methods). A washed suspension of *Anabaena variabilis* was incubated with either [1-¹⁴C]- or [6-¹⁴C]glucose and the rate of [¹⁴C]carbon dioxide production measured (Table 3). The C₆:C₁ ratio obtained was low and indicated an extensive operation of the pentose phosphate pathway. This ratio was not altered by incubation in light or dark, nor by growth of the organism in the presence of glucose. Furthermore, *A. variabilis* grown with glucose did not exhibit higher rates of carbon dioxide production from the 1-C or 6-C positions.

Table 2. Incorporation of [¹⁴C] CO₂ by *Anabaena variabilis* in the presence of glucose or acetate

A. variabilis was grown in 20 ml. medium under conditions of illumination and temperature as described in the Methods section. The cultures were gassed by recycling a mixture of air + carbon dioxide. The carbon dioxide was released into the closed re-cycling system by the acidification of sodium [¹⁴C]carbonate. The [¹⁴C]carbon dioxide continually recycled through the cultures contained 100 µC, and the extent of incorporation was measured after incubation for 3 days.

Growth medium	Specific radioactivity (counts/min./mg. dry wt)	% inhibition of [¹⁴ C]CO ₂ assimilation
CO ₂ alone	108,000	0
CO ₂ + acetate (20 mM)	88,600	18
CO ₂ + glucose (20 mM)	52,000	51

Table 3. Relative release of [¹⁴C]carbon dioxide from 1-C and 6-C positions of glucose by *Anabaena variabilis*

Double side arm Warburg flasks contained *A. variabilis* (equiv. 8 mg. dry wt) in 1.7 ml. growth medium; hyamine hydroxide (0.3 ml.) in the centre-well, 10% sodium dodecylsulphate (1 ml.) in one side-arm and glucose ([1-¹⁴C] or [6-¹⁴C], 100 µmole containing 2 µC) in the other. The [¹⁴C]carbon dioxide evolved was dissolved by the hyamine hydroxide, and after termination of respiration by the addition of sodium dodecylsulphate the radioactivity in the hyamine hydroxide determined.

Carbon source for growth	Incubation	[¹⁴ C]carbon dioxide* derived from		C ₆ :C ₁ ratio
		1-C position	6-C position	
5% CO ₂	Dark	2830	390	0.14
	Light	2690	430	0.16
5% CO ₂ + glucose (20 mM)	Dark	2800	390	0.14
	Light	3100	410	0.13

* Expressed as counts/hr/mg. dry wt organism.

Enzymic activities

Many of the enzymes involved in glucose metabolism may readily be detected in extracts of *Anabaena variabilis* prepared either by extrusion through a French pressure cell or by ultrasonic disintegration. The specific activities of such enzymes are often low when compared with enzymes from heterotrophic bacteria, as is the growth rate of the blue-green alga under study. The most striking feature of the results presented in Table 4 is the similarity in activities found in extracts from organisms grown under different conditions. None of the enzymes examined was significantly altered by growth in the presence of glucose. Glucose-6-phosphate dehydrogenase and 6-phosphoglu-

conate dehydrogenase were specific for NADP. Triose phosphate dehydrogenase was active with either NAD or NADP. Recent work in this laboratory has indicated that these two enzymic activities are carried on the same protein and share the same active site (Hood & Carr, 1967). Triose phosphate formation by fructose diphosphate aldolase was measured by the reduction of dihydroxyacetone phosphate by NADH_2 to α -glycerophosphate with a commercial α -glycerophosphate dehydrogenase (see Methods).

Hexokinase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were also measured in extracts of *Anacystis nidulans*, after growth in the presence or absence of glucose. Inclusion of glucose in the growth medium resulted in enzyme activities very slightly higher (not more than 10 %) than those found in cultures grown with carbon dioxide alone.

Fig. 4

Fig. 4. *Anabaena variabilis*. Activation of pyruvate kinase by fructose-1,6-diphosphate. Assay procedure as described in Methods, with various concentrations of substrate as shown, in the presence (O—O) or absence (●—●) of fructose-1,6-diphosphate (1 mM).

Fig. 5

Fig. 5. Effect of octanoate of the activities of three glycolytic enzymes in extracts of *Anabaena variabilis*. Enzyme assays as in Methods except that octanoate was added as indicated. Results are expressed as the percentage activity of control without octanoate. ●—●, Pyruvate kinase; Δ—Δ, phosphofructokinase; O—O, and glucose-6-phosphate dehydrogenase.

In contrast to the lack of adaptive response that enzymes of glucose metabolism of *Anabaena variabilis* displayed, inhibition and activation of enzyme activity by end-products and associated molecules was readily demonstrated. The effect of increasing concentrations of ATP upon phosphofructokinase activity is illustrated in Fig. 3. Activity was maximal with 1.2 mM-ATP; above this concentration ATP became increasingly inhibitory. This inhibition was annulled by AMP (0.3 mM) and to a lesser extent by ADP (3 mM); inorganic phosphate did not annul the inhibition caused by excess ATP. Citrate (1 mM) inhibited phosphofructokinase activity completely and phosphoenolpyruvate (0.5 mM) did so to 44 % of the standard assay control. Pyruvate kinase was activated by fructose-1,6-phosphate (Fig. 4), there being no evidence of a

sigmoidal character to the reaction curve. This enzyme was similarly activated by fructose-6-phosphate and glucose-6-phosphate. Citrate, which may be considered to be an end-product of glycolysis, inhibited pyruvate kinase (Table 5). Free fatty acids inhibit the activity of glycolytic enzymes in rat liver preparations (Weber, Convery, Lea & Stamm, 1966). The inhibition by different concentrations of octanoate of glucose-6-phosphate dehydrogenase, phosphofructokinase but not of pyruvate kinase, activity in extracts of *A. variabilis* is shown in Fig. 5.

Table 4. *Glycolytic and pentose phosphate pathway enzymes in extracts of Anabaena variabilis grown autotrophically and in the presence of glucose*

Except where indicated activity is expressed as $\mu\text{mole/min./mg. protein}$; values are mean of several determinations.

Enzymes	Carbon source	
	CO ₂	CO ₂ + glucose (20 mM)
Hexokinase	1.2	1.2
Phosphohexoisomerase	5.4	5.3
Phosphofructokinase	8.1	7.8
Fructose diphosphate aldolase	3.6	3.6
Triose phosphate dehydrogenase (NAD)	8.3	7.5
Triose phosphate dehydrogenase (NADP)	4.2	3.7
Pyruvate kinase	13	11
Glucose-6-phosphate dehydrogenase	8.7	7.8
6-Phosphogluconate dehydrogenase	12	12
Phosphoriboisomerase*	0.35	0.32

* Activity expressed as $E_{540}/\text{min./mg.}$

Table 5. *Effect of metabolites on pyruvate kinase activity in extracts of Anabaena variabilis*

The pyruvate kinase assay was as in Methods except that 3 μmole phosphoenolpyruvate was used.

Addition	Concentration (mM)	Activity (%)
None	—	100
Citrate	0.1	81
	1.0	0
Fructose-6-phosphate	0.3	116
	3.0	144
	10.0	145
Glucose-6-phosphate	1.0	112
	10.0	155

DISCUSSION

The concept that autotrophic micro-organisms utilize only carbon dioxide for the biosynthesis of cellular material has undergone some revision in recent years. It is clear from the data presented here, and from those mentioned in the Introduction, that some species of blue-green algae (namely *Anabaena variabilis*, *Anacystis nidulans*) assimilate and metabolize exogenous organic compounds, even though carbon dioxide still serves as a major source of carbon. Similarly, species of chemosynthetic bacteria

which are 'obligate' autotrophs, can assimilate various reduced carbon compounds (see Kelly, 1967; Smith *et al.* 1967). The studies on incorporation of organic materials into autotrophs have led to interest in intermediary metabolism of these organisms. An incomplete tricarboxylic acid cycle has been found in blue-green algae and in chemosynthetic bacteria (Kelly, 1967; Pearce & Carr, 1967*b*; Smith *et al.* 1967). The latter workers suggested that the lack of NADH oxidase and of α -oxoglutarate dehydrogenase can account for obligate autotrophism.

The present results with *Anabaena variabilis* show no abnormalities of metabolic pathway in glucose metabolism. Although enzymes necessary for the glycolytic and pentose phosphate pathways are both present, the respiratory experiments with [1- 14 C]- and [6- 14 C]glucose indicate that the latter is the dominant pathway. The labelling pattern of polysaccharides, respiratory data and the use of inhibitors led Cheung & Gibbs (1966) to conclude that this was the main route of glucose dissimilation in *Tolypothrix tenuis*. Earlier work did not detect aldolase in blue-green algal extracts (Richter, 1959; Fewson, Al-Hafidh & Gibbs, 1962) but later experiments measured and examined this enzyme in extracts of the same species (Willard *et al.* 1965; Van Baalen, 1965; Willard & Gibbs, 1967). In *Anabaena cylindrica* experiments with isotope-labelled compounds indicated that the pentose phosphate pathway was the major but not the only operative pathway (Wildon & ap Rees, 1965).

The most striking feature of the enzymic activities measured in *Anabaena variabilis* in the present work is the failure of glucose in the growth medium to alter the activities of enzymes concerned in its metabolism (Table 4) or the over-all rate of glucose dissimilation (Table 3). Incorporation and respiratory studies showed that exogenous glucose was metabolized and could contribute up to 46% of the total cell material. The inclusion of glucose in the growth medium has been shown to increase, by up to three-fold, hexokinase activity in *Escherichia coli* (Scott & Chu, 1959) and *Clostridium perfringens* (Ivanov, 1959) and glucose-6-phosphate dehydrogenase in *Pseudomonas aeruginosa* (Hamilton & Dawes, 1960) and *E. coli* (Pakoskey, Leshner & Scott, 1965).

The control in *Anabaena variabilis* of enzymic activity by end-product inhibition and allosteric activation was readily demonstrated. The inhibition of phosphofructokinase by 5 mM-ATP and the annulment of inhibition when 0.3 mM-AMP was present is similar to that found in yeast (Ramaiah, Hathaway & Atkinson, 1964) and in *Escherichia coli* (Lowry & Passonneau, 1964). The activation of pyruvate kinase by fructose-1,6-phosphate found in *A. variabilis* has also been observed in rat liver preparations which show distinct sigmoidal character and is clearly an allosteric effect (Taylor & Bailey, 1967). The inhibition by octanoate of several liver enzymes involved in glucose metabolism, including glucose-6-phosphate dehydrogenase and phosphofructokinase, has been described (Weber *et al.* 1966). Octanoate inhibited these two enzymes in *A. variabilis* extracts (Fig. 5) but had no effect on pyruvate kinase which, in contrast, was markedly inhibited in the liver preparation of Weber *et al.* (1966).

The failure to control enzymic activity in *Anabaena variabilis* by repression or de-repression has been noted previously with respect to acetate metabolism, and its possible role in autotrophy has been discussed (Pearce & Carr, 1967*a*). The identical activities of glucose metabolizing enzymes, after growth in the presence and absence of glucose, is consistent with the view that *A. variabilis* does not adjust its metabolism as efficiently as many other microbial species and exerts control at the level of pre-formed enzymes rather than at enzyme synthesis. This may account, fully or in part,

for the inability of *A. variabilis* and other autotrophic blue-green algae to increase their growth rate in the presence of exogenous substrates. However, at least one example of enzyme repression, that of nitrite reductase by ammonium ion, exists in blue-green algae (Carr, 1967) and others may await detection.

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