THE BIOLOGY OF BLUE-GREEN ALGAE

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THE BIOLOGY OF BLUE-GREEN ALGAE

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PREFACE

Although blue-green algae have had a long history of careful observation, both in the field and in the laboratory, the understanding of their biology has lagged behind that of some other groups of microorganisms. The advances made during the first few decades of this century in microbial physiology were hardly evident in the blue-green algae. Considerable attention was, however, paid to problems of taxonomy and morphology; our knowledge of the latter has now been recast and expanded with the advent of electron microscopy, but that of the former is in a scarcely better state than it was forty years ago. Critical studies on the mineral nutrition and laboratory culture of blue-green algae in the early 1950s made available certain species to general biochemical analysis. Since then, an increasing range of biologists have studied these microorganisms and we hope that the wide variety of disciplines employed is apparent from the chapters in this volume.

It was the detailed and often beautiful electron micrographs of blue-green algae, bacteria and other microorganisms that led to the widespread recognition that microbes, and indeed all living cells, fall into either prokaryotic or eukaryotic types. This morphological separation was argued persuasively by Stanier and van Niel in 1962 in their review, 'The Concept of a Bacterium'. The implication of the 'bacterial-nature' of blue-green algae, with the consequent anomaly of their possessing a higher-plant type photosynthetic system, has formed the framework of much of the increased attention devoted to these organisms. All subsequent information has confirmed this interpretation of bacteria and of blue-green algae.

New workers, coming fresh to the organisms which are the subject of this book, may be surprised to find such a wide range of names applied to them. This variety reflects their actual use in the literature, and we have made no attempt to standardize them. The name Myxophyta has historical precedence over Cyanophyta, although for a long period the latter was used more widely. Neither prefix is ideal, as not all of these organisms have a slime-sheath, nor are they all blue. Similarly, the name 'blue-green algae' is sometimes confusing to students in that it does not reflect the prokaryotic nature of the organisms.

The use of 'cyanophytes' as a semipopular term has recently been encouraged by some American workers.

The recent concern over environmental deterioration has spread from professional ecologists to a wider public, and the role of blue-green algae in eutrophic situations has become of more general interest. Aspects of this are touched upon by some of our contributors and it is clear that further investigations into the biology of these organisms are necessary. The purpose of the present volume is to give an account of most aspects of blue-green algal biology that are of general interest, or are currently the subject of particularly marked activity. The total literature on blue-green algae is considerably greater than that given in the bibliography, even if no allowance is made for purely taxonomic and floristic accounts. So, inevitably, we have had to omit any detailed account of some important topics, as for example, mineral nutrition, endosymbionts and toxins. We decided to ask our contributors to treat in depth a few ecological subjects, such as thermophiles and calcareous environments, at the expense of others, such as desert soils, rice fields and flowing waters.

We thank all our contributors, and the many others who have aided with advice and photographs, for their co-operation and encouragement. We acknowledge especially the interest and support of Professor J.H.Burnett and Professor G.E.Fogg. In order to minimize overlap, and for reasons of overall length, we have had, on occasions, to use editorial scissors, and for this we apologize. We have nevertheless allowed certain topics to be dealt with in more than one chapter, hoping thereby to preserve the arguments of the author and present differing views and interpretations.

We recognize that the still rudimentary state of knowledge concerning blue-green algae, together with the rapidly increasing research interest in them, excludes any sort of definitive account. Nevertheless, we hope that this volume will both form a useful basis for ensuing research and also make current work with these fascinating organisms known to a wider audience.

September 1972

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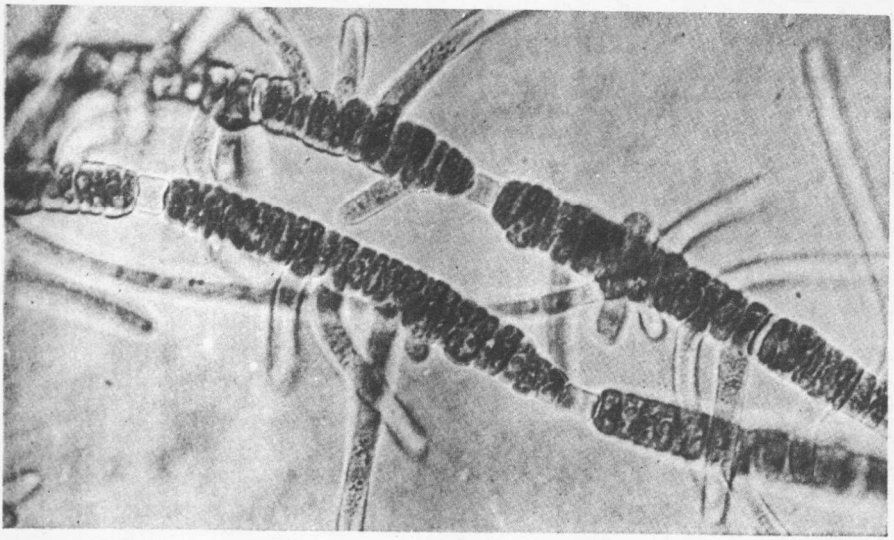

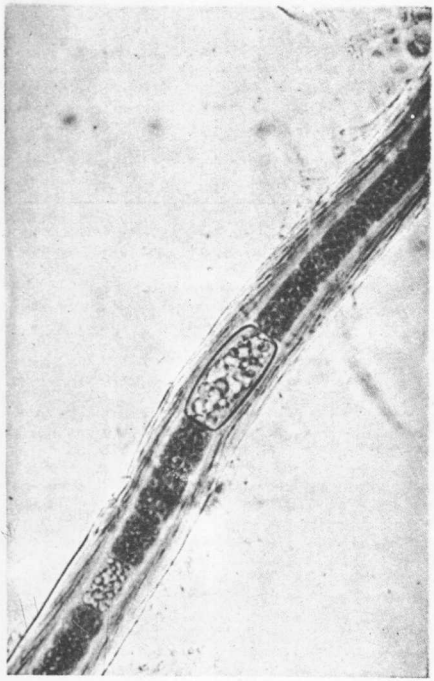

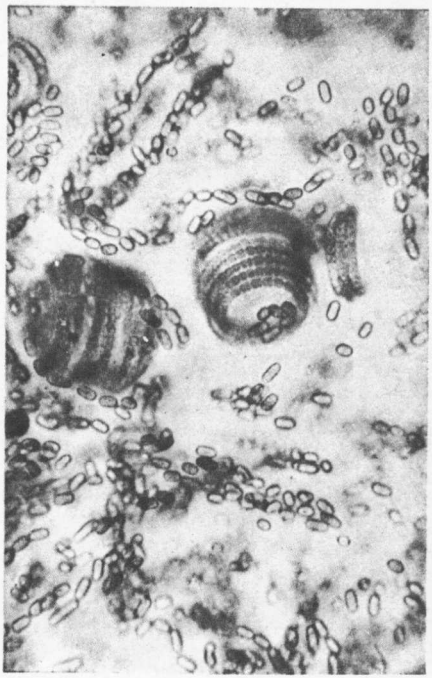

Frontispiece

TOP. *Fischerella*, showing primary trichomes and branches. Neofluar 40/0.75

BOTTOM (*left*). *Scytonema myochrous*, showing recently formed heterocyst and lamellate sheath. Neofluar 40/0.75.

BOTTOM (*right*). *Anabaena*, showing whorled trichomes, and also profuse spore formation. Planachromat 25/0.45.

Courtesy of *Zeiss Information* and Dr G. H. Schwabe, Hydrobiologische Anstalt der Max-Planck-Gesellschaft, Plön.

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CHAPTER 1

SYNTHESIS OF METABOLIC INTERMEDIATES

ARNOLD J. SMITH

1. INTRODUCTION

Blue-green algae are capable of adopting several different modes of metabolism with respect to their sources of carbon and energy. All of the known species have the ability to grow in the light with carbon dioxide as the principal, if not sole, carbon source. When provided with organic compounds in addition to carbon dioxide, many cyanophytes utilize organic carbon in cell synthesis. The contribution of the organic substrate to cell synthesis varies widely from negligible amounts to the situation where enough of the organic compound is utilized to account for all of the carbon in newly synthesized cell material. Finally, a few species are reputed to be capable of growth in the dark on organic media; under these conditions, the organic compounds presumably act as a source of energy as well as a major source of carbon. For simplicity I have divided this account of the biosynthesis of metabolic intermediates in blue-green algae into three main sections, one for each of the modes of metabolism displayed by this group of organisms.

2. PHOTOASSIMILATION OF CARBON DIOXIDE BY BLUE-GREEN ALGAE

Most blue-green algae have the ability to use carbon dioxide as sole source of carbon. In this respect they are similar to green plants and to many eukaryotic algae, photosynthetic bacteria and chemolithotrophic bacteria. Although many cyanophytes flourish in environments rich in organic compounds, none of those isolated from a natural environment has yet been shown to have an absolute requirement for a pre-formed organic compound apart from a few marine strains which require vitamin B_{12} (Pinter & Provasoli 1958, Van Baalen 1962). In this connection, the report of an endophytic species of *Nostoc punctiforme* scarcely capable of autotrophic growth (Winter 1935) deserves re-examination.

Autotrophic carbon dioxide fixation

The studies of Calvin and his co-workers (see Bassham & Calvin 1957, Calvin & Bassham 1962) together with the studies of others (see Vishniac *et al.* 1957) have established the route for the net fixation of carbon dioxide in green plants and green algae (Fig. 1.1). These investigations have defined the experimental approach which should be adopted to establish the ribulose diphosphate cycle as the pathway for the net assimilation of carbon dioxide in an autotrophic organism. This involves short-term and steady-state labelling experiments with intact cells or tissues as well as an investigation of the catalytic activities of cell-free preparations. Brief exposure of organisms assimilating carbon dioxide, via the reactions of the cycle, to radioactive carbon dioxide, labels the ethanol soluble fraction of cell material in a highly specific manner. After exposure to ^{14}C-carbon dioxide for a few seconds, 3-phosphoglyceric acid is the most heavily labelled compound in the ethanol soluble fraction and the bulk of the isotope in this compound is located in the carboxyl carbon atom. An increase in the period of exposure to labelled carbon dioxide results in a decrease in the percentage of total radioactivity fixed in 3-phosphoglyceric acid and the appearance of isotope in a highly specific manner in C_3, C_4, C_5, C_6 and C_7 carbohydrates. The removal of the energy source for carbon dioxide fixation or a reduction in the availability of carbon dioxide both induce reciprocal but opposite changes in the size of the intracellular pools of ribulose diphosphate and 3-phosphoglyceric acid in organisms assimilating carbon dioxide under steady-state conditions. Finally, cell-free preparations should contain all of the enzymes of the cycle in amounts consistent with the rate of carbon dioxide fixation by intact cells or tissues.

The rigorous application of this experimental approach in studies of

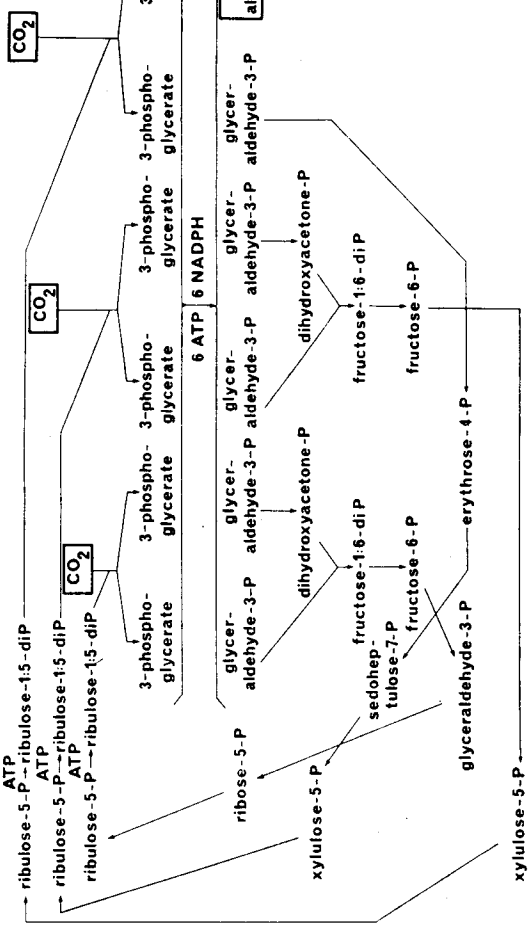

FIG. 1.1. Ribulose diphosphate cycle of CO_2 assimilation (after Quayle 1969). For a variant of the cycle involving sedoheptulose diphosphate, see Krebs & Kornberg (1957).

autotrophic carbon dioxide fixation has been the exception rather than the rule. The ribulose diphosphate cycle has frequently been cited as the route for the net fixation of carbon dioxide by particular autotrophic organisms without adequate experimental evidence. Although one of the key enzymes of the cycle, ribulosediphosphate carboxylase, has been found in all autotrophic organisms, recent work has revealed the existence of supplementary mechanisms for the fixation of carbon dioxide in photosynthetic bacteria (Evans *et al.* 1966) as well as adjuncts to the ribulosediphosphate cycle which serve to increase the intracellular concentration of carbon dioxide in algae (Graham & Reed 1971) and tropical grasses (Andrews *et al.* 1971, Hatch 1971). These findings emphasize the need for adequate experimental investigation before accepting the ribulosediphosphate cycle as the sole route for carbon dioxide assimilation by an autotrophic organism.

A. *Assimilation of* ^{14}*C-carbon dioxide*

The mechanism of carbon dioxide fixation in blue-green algae has been investigated in a number of different species but, without exception, the experimental approach outlined in the previous section has never been applied in its entirety to a particular species of blue-green alga. Kandler (1961) has determined the distribution of radioactivity amongst the compounds of the ethanol soluble fraction of *Anacystis nidulans* after exposure to ^{14}C-labelled carbon dioxide for various periods of time ranging from 5 sec to 2 min (Fig. 1.2a). 3-Phosphoglyceric acid was the most heavily labelled compound after exposure for 5 sec and the percentage of the total radioactivity fixed in this compound decreased as the period of exposure to labelled carbon dioxide increased. In contrast, there was an increase in the amount of radioactivity in all of the other compounds. Throughout the experiment, 20 to 30% of the incorporated radioactivity was present in organic phosphates whereas the amount of isotope in aspartate was always less than 5%. The pattern of labelling is similar to that obtained with *Scenedesmus obliquus* (Fig. 1.2b).

Richter (1961) has briefly reported the results of similar studies with the same organism. Unlike Kandler, he found large amounts of isotope in glutamate, aspartate, alanine and phosphoenol-pyruvate as well as 3-phosphoglyceric acid after exposure to ^{14}C-carbon dioxide for periods ranging from 5 to 30 sec; over the same period hexose monophosphates were only weakly labelled. Norris *et al.* (1955) determined the amount of isotope in the compounds extracted by hot ethanol from four species of blue-green algae after exposure to labelled carbon dioxide for 5 min. With three of these organisms, species of *Phormidium*, *Nostoc* and *Synechococcus*, the patterns of labelling were similar to those for *Anacystis nidulans* (Kandler 1961),

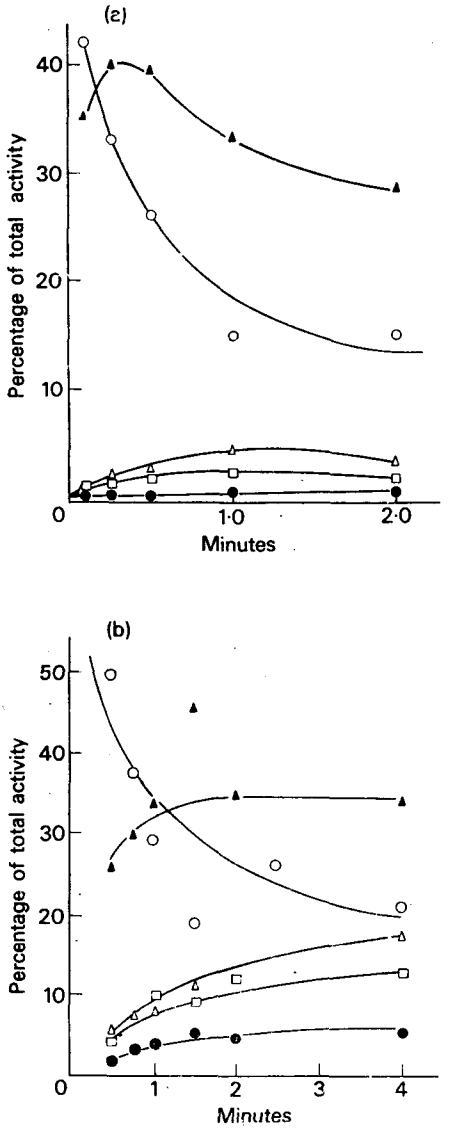

FIG. 1.2. Labelling of ethanol soluble compounds in (a) *Anacystis nidulans* (Kandler 1961) and (b) *Scenedesmus obliquus* (Calvin *et al.* 1951) during brief exposure to ^{14}C carbon dioxide [○, 3 phosphoglyceric acid; ● alanine; △ aspartic acid; ▲ organic phosphates; □ triose phosphates].

Chlorella sp. and *Scenedesmus* sp. (Norris *et al.* 1955) (Table 1.1) except for small amounts of label (0·9 to 6·7% of the total in the ethanol extract) which were present in an unidentified compound running near to alanine. The pattern of labelling in *Nostoc muscorum*, the other organism used by Norris *et al.*, was markedly different from the others (Table 1.1); 20·9% of the radioactivity in the ethanol extract (equivalent to 17% of total isotope incorporation), the largest amount found in any single compound, was present in the same unknown compound running close to alanine. Linko *et al.* (1957) identified this compound as citrulline. They showed that the specific activity of this compound was about half that of the ^{14}C-bicarbonate assimilated by the organism; the bulk of the radioactivity was located in the carbamyl moiety. The same group examined the kinetics of citrulline labelling in *N. muscorum* in the light and dark. The amount of radioactivity in citrulline after 8 min exposure to ^{14}C-carbon dioxide in the light accounted for 1% of the total incorporation This level of labelling is considerably lower than that reported by Norris *et al.* (1955). Citrulline was also labelled in comparable experiments in the dark but the rate of labelling, on a percentage basis, was significantly lower. The situation is further complicated by the results of an earlier investigation of the products of carbon dioxide assimilation in a strain of the same species; after exposure to ^{14}C-carbon dioxide for 30 min. Allison *et al.* (1953) found radioactivity in aspartate, glutamate, sucrose and phosphate esters but not in the area of the chromatogram where citrulline would be expected to occur.

Kindel & Gibbs (1963) have determined the distribution of radioactivity amongst the carbon atoms of hexose residues from the polysaccharide fraction of *Anacystis nidulans* after exposure to ^{14}C-carbon dioxide for periods of time ranging from 5 to 30 sec. Most of the label was present in carbon atoms 3 and 4. A similar pattern of isotope distribution was found by the same workers in *Chlorella pyrenoidosa* which had been exposed to ^{14}C-carbon dioxide under identical conditions. This is the only investigation that has been made of the distribution of label in a product of carbon dioxide assimilation by a blue-green alga.

B. *Catalytic activities of cell-free preparations*

Information at the level of the enzymes of the ribulosediphosphate cycle in blue-green algae is sparse (Table 1.2, section A). Only one organism, *Tolypothrix tenuis*, has been shown to contain all of the enzymes of the cycle (Latzko & Gibbs 1969). Whilst the levels of most of the enzymes in extracts were sufficient to account for the rate of carbon dioxide assimilation by intact cells, the amount of ribulosediphosphate carboxylase was only enough to account for assimilation at one-tenth of the observed rate. Although this

TABLE 1.1. Distribution of ^{14}C amongst the ethanol soluble compounds from algae exposed to ^{14}C-carbon dioxide

	*Anacystis nidulans**	*Nostoc muscorum*†	*Synechococcus* sp.†	*Chlorella* sp.†	*Scenedesmus* sp.†
Time of exposure to ^{14}C-carbon dioxide (min)	2·0	5·0	5·0	5·0	5·0
^{14}C fixed in the 80% ethanol soluble fraction (% of total ^{14}C fixed)	60·0	83·3	28·8	—	64·1
Compounds present in the ethanol soluble fraction		*% of ^{14}C in the ethanol soluble fraction*			
Phosphoglyceric acid	25·0	2·5	16·8	25·0	4·5
Pentose and triose phosphate	3·3	14·6	3·1	t	1·1
Diphosphates	11·0	5·5	20·8	2·8	2·5
Hexose and heptose phosphate	34·0	6·7	20·8	12·1	17·4
UDP hexoses	2·5	7·5	8·3	7·6	
Phosphoenol-pyruvate	5·5	t	2·8	1·9	0·6
Aspartic acid	6·0	11·7	4·9	5·5	19·2
Glutamic acid	1·0	0·4	0·3	0·6	2·1
Alanine	1·7	1·9	1·2	4·5	4·0
Serine and glycine	2·0	1·8	1·5	10·6	8·8
Unknown near alanine	2·5	20·9	6·7	—	0·2

* Kandler (1961)
† Norris *et al.* (1955)
t = trace