to be transferred to the shoots. The extent of this microbial participation in the apparent absorption of phosphate by the plants is influenced by several factors: first, it is of importance only at low concentrations; secondly, because of the known sensitivity of bacteria to hydrogen ion concentra-

tion, it increases with increasing pH; and thirdly, it is affected by the exudation of organic substances from the root.

These findings will be discussed in relation to the possible influence of the rhizosphere flora on the absorption of inorganic nutrients by plants.

COMMUNICATIONS

Threonine Deaminase and Acetolactate Synthetase in *Anabaena variabilis*

By W. HOOD and N. G. CARR (*Department of Biochemistry, University of Liverpool*)

Recent work has indicated that species of blue-green algae hitherto considered to be strict autotrophs will incorporate and metabolize carbon compounds other than carbon dioxide; ^{14}C -labelled sugars, carboxylic acids and amino acids were assimilated into *Anabaena variabilis* and *Anacystis nidulans*. However, Pearce & Carr (1967) have found no evidence of repression or de-repression of enzymes concerned in acetate metabolism after growth in the presence of this substrate. This communication describes the initial attempts to estimate the extent of metabolic control by either repression or feed-back inhibition in amino acid biosynthesis. This has been only briefly examined in the blue-green algae: Hoare & Hoare (1966) demonstrated feed-back control in arginine formation.

Anabaena variabilis was grown (Pearce & Carr, 1967) autotrophically and in the presence (100 mg./l.) of L-valine or L-valine, L-leucine and L-isoleucine; L- ^{14}C valine was shown to be assimilated by the significant uptake of ^{14}C into cell material during growth. Cell-free extracts from each type of culture were prepared by ultrasonic treatment and the activities of enzymes concerned in branched-chain amino acid biosynthesis were examined. Acetolactate formation from pyruvate was measured by conversion into acetoin and spectrophotometric determination of this product (Westerfield, 1945). Acetolactate synthetase activity had a broad pH range with two optima, indicative of two isoenzymes, as found in *Escherichia coli* (Radhakrishnan & Snell, 1960). Variations in the growth conditions quoted above did not alter the specific activities of this enzyme in extracts at any pH value, but valine exerted 50% inhibition on activity in extracts assayed at pH 8.0. A lack of repression was also observed when threonine deaminase was examined in extracts from organisms grown autotrophically (0.17 $\mu\text{mole/mg. of protein/hr.}$) and in the presence of isoleucine (0.16 $\mu\text{mole/mg./hr.}$) or valine, leucine and iso-

leucine (0.19 $\mu\text{mole/mg./hr.}$). In contrast with the failure to exhibit enzyme repression, threonine deaminase was competitively inhibited by its end product, isoleucine, and this action was relieved by valine. In this respect threonine deaminase in blue-green algae is similar to that in other organisms.

The lack of enzymic repression in *Anabaena variabilis* contrasts with results obtained with other procaryotic organisms and indeed with *Anacystis nidulans*, preliminary results with which indicate slight repression of acetolactate synthetase by growth in the presence of valine.

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Reduced Nicotinamide-Adenine Dinucleotide Phosphate Oxidase in the Autotrophic Blue-Green Alga *Anabaena variabilis*

By C. K. LEACH and N. G. CARR (*Department of Biochemistry, University of Liverpool*)

The observation that there is significant assimilation of reduced ^{14}C -labelled carbon compounds by strict autotrophs has renewed interest in the causative reasons of obligate autotrophic metabolism. The earlier view that such organisms were generally impermeable to organic compounds is now unlikely to be correct. An incomplete tricarboxylic acid cycle has been observed by several groups and its role in autotrophy discussed. Smith, London & Stanier (1967) suggest that the absence of α -oxoglutarate dehydrogenase and, more crucially, NADH oxidase, are the essential features of obligate autotrophy in both blue-green algae and the chemosynthetic bacteria, and have suggested certain evolutionary implications of these deletions.

This communication reports that in extracts of several blue-green algae an NADPH oxidase is

present. *Anabaena variabilis* was grown autotrophically, harvested at 1000g for 5min. and disintegrated by 3×45 sec. ultrasonic treatment at 0°, and a crude extract was prepared as previously described (Pearce & Carr, 1967). Incubation of NADPH (0.1 μ mole) in potassium phosphate buffer, pH 7.6 (200 μ moles), in the presence of algal extract (3–6mg. of protein), led to a steady but slow decrease in E_{340} when compared with a non-enzyme or boiled-enzyme control. This rate, which was measured in an Optica CF4R recording spectrophotometer with a scale expanded to 0.2, corresponded to 0.38 ± 0.04 μ mole of NADPH oxidized/min./mg. of protein. Under anaerobic conditions, no oxidation was obtained. When NADPH (0.2 μ mole) was added to a pre-equilibrated extract in a Clark oxygen electrode assembly, an increase in rate of O₂ uptake was recorded; this rate was equivalent to 0.21 μ mole of O₂/min./mg. of protein or 0.42 μ mole of NADPH oxidized/min./mg. of protein.

A smaller rate (0.18 ± 0.03 μ mole/min./mg. of protein) of oxidation was found when NADH was incubated and E_{340} measured. Transhydrogenase activity was not detected under the conditions employed.

Some attention has been directed to proving that these activities were obtained with extracts from pure blue-green algae cultures. For example, before harvesting, samples of grown cultures were inoculated into various yeast-extract solutions as well as on to nutrient agar made up with tap water or with spent autotrophic media. No growth was obtained after incubation in the dark at 30° for 4 weeks. Extracts containing NADPH oxidase, markedly more active than NADH oxidase, have been obtained from *Nostoc muscorum*, *Mastigocladus laminosus*, *Anabaena cylindrica* and *Framyella diplosiphon*. The data present will be discussed in relation to possible mechanisms of autotrophic metabolism.

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The Photometabolism of Benzoic Acid by *Rhodopseudomonas palustris*: A New Pathway of Aromatic Ring Metabolism.

By P. L. DUTTON and W. C. EVANS (*Department of Biochemistry and Soil Science, University College of North Wales, Bangor*)

The aerobic pathways of oxidative breakdown of aromatic compounds by micro-organisms have been well described. The absence of enzymes of these oxidative pathways in *Rhodopseudomonas palustris* grown anaerobically in the light (i.e. photosynthetically) on aromatic substrates has been demonstrated (Hegeman, 1967; Dutton & Evans, 1967). Furthermore, extensive cultural investigations of the organism grown photosynthetically on benzoate failed to detect any of the intermediates of the known oxidative metabolic pathways of benzoate. Coupled with the facts that both light and an absence of molecular oxygen are necessary for the photosynthetic breakdown of benzoate, this indicates that another mode of aromatic breakdown may be employed by *Rhodopseudomonas palustris*.

Isotope-dilution experiments have been conducted on cells grown photosynthetically on benzoate, to identify the intermediates of this pathway. Buffered cell suspensions [3% (wet wt.) in 0.05M-tris-HCl buffer, pH 7.2 (15ml.)] were illuminated under argon with [*carboxy*-¹⁴C]benzoate (22 μ moles, 5 μ C) in the presence of possible intermediates (0.5mg.) for 90min., after which the cells were centrifuged and the acidified supernatant was extracted with ether. This extract was resolved by thin-layer chromatography on silica gel G developed in two dimensions with benzene-dioxan-acetic acid (45:5:2, by vol.). Radioautograms were prepared from the plates. It was found that the cyclohexanol-2-carboxylic acid and pimelic acid spots contained labelled material. Further chromatography on thin-layer plates of the cyclohexanol-2-carboxylic acid spot (silica gel G, six different solvents) and the pimelic acid spot (silica gel G, five solvents; cellulose powder, four solvents) failed to resolve the radioactivity from the respective carrier. After addition of more carrier (200mg.) to the two extracted spots, both were recrystallized five times without loss of specific radioactivity.

Both cyclohexanecarboxylic acid and Δ^1 -cyclohexenecarboxylic acid were also labelled when added to the system. Experiments are in progress to confirm that the radioactivity is associated with these compounds; to date, cyclohexanecarboxylic acid has been run in five solvents on silica gel G plates without separation of the radioactivity. G. D. Hegeman (personal communication) has noted that *Rhodopseudomonas palustris* grown photosynthetically on benzoate are induced to the assimilation of cyclohexanecarboxylate.