It was also found by means of the ^{14}C -labelled yeast that glucose, like 3-FG, stimulated endogenous metabolism so that about one-third of the carbon dioxide evolved was of cellular origin.

The observed increase in oxygen uptake and carbon dioxide production after the addition of 3-FG to resting cells of *Saccharomyces cerevisiae* must be accounted for by an increase in endogenous metabolism.

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Photosynthetic-Carbon-Cycle Enzyme Activities in Leaves of a Chlorophyll-less *Phaseolus vulgaris* Plant

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Chlorophyll-less plants of *Phaseolus vulgaris* cultivar Alabaster seem to occur quite rarely. When such a plant was found in a batch of seedlings that had been allowed to green in natural daylight in July, the primary leaves were taken for electron microscopy and the assay of enzyme activities (Bradbeer, 1969) at the stage at which the leaves appeared to have reached their maximum size, fresh wt. 133 mg./leaf. No indication of the onset of senescence in these leaves was obtained from their protein content, 7.02 mg./leaf, or from electron microscopy. The electron micrographs revealed the presence of plastid-like organelles that did not show any internal lamellar structure.

The chlorophyll-less leaves have been compared with the etiolated leaves of plants grown for 14 days in the dark (fresh wt. 35 mg., protein content 1.93 mg.) and also with the fully greened leaves of plants grown for 14 days in darkness followed by 5 days of continuous illumination at $500 \mu\text{w}/\text{cm}^2$ (fresh wt. 133 mg., protein 4.72 mg.). The etiolated leaves contained all of the investigated enzymes of the photosynthetic carbon cycle but the activities were very much lower than those of fully greened leaves.

The three photosynthetic-carbon-cycle enzymes that are confined to the chloroplasts, namely ribulose diphosphate carboxylase, phosphoribulokinase and NADP-linked triose phosphate dehydrogenase, were present in the chlorophyll-less leaves at about the same activities as in etiolated leaves.

Four enzymes, ribose phosphate isomerase, phosphoglycerate kinase, fructose diphosphate aldolase and transketolase, showed activities almost

midway between those of etiolated and fully greened leaves. These enzymes are all implicated in the photosynthetic carbon cycle but they are not confined to the chloroplasts.

Triose phosphate isomerase, NAD-linked triose phosphate dehydrogenase and phosphopyruvate carboxylase occurred in chlorophyll-less leaves at about twice the activity found in fully greened leaves. Of these three enzymes only triose phosphate isomerase appears to be a component of the photosynthetic carbon cycle in beans, and this enzyme and NAD-linked triose phosphate dehydrogenase occur both inside and outside of the chloroplasts.

Chlorophyll-less plants of *Vicia faba* have been reported to lack chloroplast ribosomes (T. A. Dyer, R. Miller & A. D. Greenwood, unpublished work), but no information on this point is available for *Phaseolus*.

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The Purification and some Properties of a Monohaloacetate Dehalogenase from *Pseudomonas dehalogenans* N.C.I.B. 9061

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Several micro-organisms can eliminate halogen atoms from halogenated aliphatic or aromatic compounds; such reactions play an important part in the breakdown of halogenated herbicides in the soil. The enzymic release of the halogen atom of monohaloacetate as inorganic halide has been studied in cell-free extracts (Davies & Evans, 1962) and with partially purified enzymes (Goldman, 1965; Goldman & Milne, 1966; Goldman, Milne & Keister, 1968).

Pseudomonas dehalogenans N.C.I.B. 9061 was grown aerobically at 28–30° on a mineral salts medium containing monochloroacetate (0.37%) as sole carbon source. Cell-free extracts, prepared by ultrasonic disintegration of the harvested cells, contained an enzyme that catalysed the release of inorganic chloride from monochloroacetate. This enzyme was assayed by conductimetric titration with 1 mM-AgNO₃ of the chloride released after 10 min. reaction at pH 9.0 and 30°.

In agreement with the other haloacetate dehalogenases the enzyme had a pH optimum of 9.5; below pH 5.0 activity was irreversibly lost. Dialysis for 10 hr. at pH values between 7.0 and 10.0 produced no significant loss of activity. At pH 7.0 the enzyme was stable for at least 48 hr. at ambient temperatures, and indefinitely at 4°; the activity

and stability were not affected by GSH, cysteine or mercaptoethanol (all at 1 mM). However, virtually complete loss of activity was effected by 0.1 mM *p*-chloromercuribenzenesulphonate (98% inhibition) and 1.0 mM *N*-ethylmaleimide (95% inhibition).

At pH 7.0 the rates of hydrolysis of the monobromo and moniodo homologues were 140% and 90% respectively of the rate with monochloroacetate.

The approximate molecular weight as determined by chromatography on Sephadex G-100 was 14500.

The enzyme was isolated from the cell-free extract by a six-stage purification. The final specific activity was 120 μ moles/min./mg. of protein. The purified enzyme ran as a single band on disc gel electrophoresis in two buffer systems a pH 9.5 and pH 8.5.

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Extracellular Nitrogen and the Control of Arginine Biosynthesis in *Anabaena variabilis*

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A general feature of the growth of blue-green algae is the liberation into the culture medium of significant amounts of peptides and amino acids (Fogg, 1962). In *Anabaena cylindrica* 30% of the total combined nitrogen was present as extracellular material and the rate of excretion was greatest in rapidly growing cultures (Fogg, 1952). The function of this extracellular material, representing as it does a considerable proportion of the biosynthetic activity of the micro-organisms, is uncertain, although a protective action against polymyxin B has been suggested (Whitton, 1965). We suggest that the widespread presence of extracellular peptides and amino acids may be a consequence of failure to exert control over amino acid biosynthesis. It has been shown that in *Anabaena variabilis* several examples of metabolic control by repression and de-repression of enzyme formation are absent (Pearce & Carr, 1967, 1969). There were a total of 18 amino acids in the supernatant after growth of *A. variabilis* with glutamate as the major constituent (23%); ornithine (3.6%) and arginine

(2.3%) were both present, the latter being entirely peptide-bound. When [U-¹⁴C]arginine (5 μ C; 1 mM) was included in the otherwise autotrophic growth medium, radioisotope incorporation was linear with growth and accounted for 5% of the total cell mass synthesized. Feedback inhibition of *N*-acetylglutamate phosphokinase by its end product arginine has been demonstrated in blue-green algae by Hoare & Hoare (1966), who also observed that *N*-acetylornithine was converted into ornithine by a transacetylase rather than by a deacetylase. Several of the enzymes necessary for the biosynthesis of arginine from glutamate, including *N*-acetylglutamate phosphokinase, *N*-acetylornithine transaminase, *N*-acetylornithine transacetylase, argininosuccinate and ornithine transcarbamoylase, have been measured in extracts of *A. variabilis*. Cell-free extracts prepared from *A. variabilis* grown in the presence and absence of arginine (1 mM) showed no significant differences in the enzymic activities listed above. The catabolism of arginine probably proceeds via citrulline in this organism, using arginine desiminase which is present equally in organisms from autotrophic and arginine-enriched cultures. No urea was detected when arginine was incubated with either type of extract, indicating the absence of arginase although this may be a result of the active urease present. The results presented are comparable with the results on the biosynthesis of branched-chain amino acids, in which end product inhibition but not control over enzyme synthesis was found in this photosynthetic micro-organism. The extent to which lack of control by enzymic repression occurs in other amino acid-biosynthetic routes is under examination.

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A Tyrocidine-Synthesizing Cell-Free Extract from *Bacillus brevis* A.T.C.C. 10068

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The composition of tyrocidine synthesized *in vivo* by *Bacillus brevis* A.T.C.C. 10068 (Mach & Tatum, 1964) or by extracts from *B. brevis* A.T.C.C. 8185 (Fujikawa, Sakamoto, Suzuki & Kurahashi, 1968) is altered by changing the amino acid content of the medium. We have obtained a cell-free extract from *B. brevis* A.T.C.C. 10068 that synthesizes tyrocidine.