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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Davies, J. I. & Evans, W. C. (1962). *Biochem. J.* **82**, 50P.

Goldman, P. (1965). *J. biol. Chem.* **240**, 3434.

Goldman, P. & Milne, G. W. A. (1966). *J. biol. Chem.* **241**, 5557.

Goldman, P., Milne, G. W. A. & Keister, D. B. (1968). *J. biol. Chem.* **243**, 428.

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.

Fogg, G. E. (1952). *Proc. Roy. Soc. B*, **139**, 372.

Fogg, G. E. (1962). In *Physiology and Biochemistry of Algae*, p. 475. Ed. by Lewin, L. A. New York: Academic Press Inc.

Whitton, B. A. (1965). *J. gen. Microbiol.* **40**, 1.

Pearce, J. & Carr, N. G. (1967). *J. gen. Microbiol.* **49**, 301.

Pearce, J. & Carr, N. G. (1969). *J. gen. Microbiol.* **54**, 451.

Hoare, D. S. & Hoare, S. L. (1966). *J. Bact.* **92**, 375.

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.

Cells grown to a density of 1.3 mg. dry wt./ml. of medium in shallow stationary culture with peptone-yeast extract were washed once in 5 mM-MgCl₂, crushed with a modified Hughes press (Sutton & King, 1966), and then ground with alumina in 50 mM-tris-HCl buffer, pH 7.5, containing 5 mM-MgCl₂ and 13 mM-2-mercaptoethanol and centrifuged at 30 000g. Supernatant containing 16 mg. of protein was added to buffer, MgCl₂, ATP, 1 μ mole each of L-asparagine, L-glutamine, L-ornithine, L-proline, L-tyrosine and L-valine, and 0.2 μ mole of DL-[1-¹⁴C]phenylalanine in a final volume of 3 ml. After 1 hr., trichloroacetic acid to a final concentration of 5% (w/v) and 0.5 mg. of tyrocidine were added, the mixture was centrifuged and the sediment washed successively with 5% (w/v) trichloroacetic acid, 2% (w/v) NaCl and ethanol-0.2 M-HCl (9:1, v/v). Tyrocidine was separated from the ethanol-soluble fraction on acid alumina (Fujikawa, Suzuki & Kurahashi, 1968) and its purity checked by chromatography on Sephadex LH-20 with the solvent *NN*-dimethylformamide-water (9:1, v/v) (I. Bartley & B. Hodgson, unpublished work).

With 0.1 M-tris-HCl buffer, pH 7.5, 2 mM-ATP and 10 mM-MgCl₂, 1.29 and 0.86 nmole of [¹⁴C]-phenylalanine were incorporated into ethanol-soluble material and tyrocidine respectively. Freezing and thawing the extract decreased the incorporation into the respective fractions to 0.41 and 0.14 nmole after 24 hr. at -15°, and to 0.24 and 0.03 nmole after a second freezing. Extracts from cells harvested at either 0.9 and 1.6 mg. dry wt./ml. of medium gave less incorporation.

With 2 mM-ATP and 10 mM-MgCl₂ and either 0.1 M-tris-HCl buffers in the pH range 7.2-8.5 or 0.1 M-KH₂PO₄-KOH buffers in the pH range 6.2-7.8, the extract catalysed a maximum incorporation of [¹⁴C]phenylalanine into tyrocidine at pH 7.5 of 0.91 and 1.32 nmole respectively; 1 mM-ATP with the KH₂PO₄-KOH buffer, pH 7.5, allowed an incorporation of 0.60 nmole of [¹⁴C]-phenylalanine into tyrocidine, and either 10 mM-ATP or 1 mM-ATP + 10 mM-phosphoenolpyruvate + pyruvate kinase allowed a maximum incorporation of 2.04 nmole. Added MgCl₂ was not required. Under optimum conditions more than 90% of the counts in the ethanol-soluble material were recovered as tyrocidine.

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Fujikawa, K., Sakamoto, Y., Suzuki, T. & Kurahashi, K. (1968). *Biochim. biophys. Acta*, **169**, 520.

Fujikawa, K., Suzuki, T. & Kurahashi, K. (1968). *Biochim. biophys. Acta*, **161**, 232.

Mach, B. & Tatum, E. L. (1964). *Proc. nat. Acad. Sci., Wash.*, **52**, 876.

Sutton, C. R. & King, H. K. (1966). *Biochem. J.* **101**, 20P.

A Comparison of Dehydrogenase Activities in Tissue Homogenates and Tissue Sections

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Pentose-shunt dehydrogenation can be demonstrated in the supernatant fraction of tissue homogenates by following the production of NADPH at 340 nm. (Glock & McLean, 1953). It can also be demonstrated in intact tissue sections by following the reduction of certain tetrazolium salts to insoluble coloured formazans (Altmann & Chayen, 1965). These formazans can be eluted from the sections and measured quantitatively (Altmann, 1969; F. P. Altmann, unpublished work). 6-Phosphogluconate dehydrogenase has been tested, under optimum conditions (Altmann, 1968), in homogenates and intact sections of rat liver taken from the same animal. Activities from the homogenate and section experiments were converted into the common unit of μ moles of hydrogen formed/cm.³ of tissue/30 min. at 37°.

The homogenate gave 212 units of activity for 6-phosphogluconate dehydrogenase. The sections were tested with seven different tetrazolium salts: MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; INT, 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride; TNBT, 2,2',5,5'-tetra-*p*-nitrophenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene)ditetrazolium chloride; NBT, 2,2'-di-*p*-nitrophenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene)ditetrazolium chloride; BT, 2,2',5,5'-tetraphenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene)ditetrazolium chloride; NT, 2,2'-(*p*-diphenylene)-bis-(3,5-diphenyl)ditetrazolium chloride; TT, 2,3,5-triphenyltetrazolium chloride. The most active of these (MTT), in the presence of phenazine methosulphate (PMS), gave 250 units of activity. Within experimental error, therefore, the activity recorded was the same in both systems. The homogenate system measures the total amount of NADPH produced by the dehydrogenation (NADPH-oxidizing systems were absent). With MTT and PMS the sections gave the same activity. In the absence of PMS, the activity was decreased to 77 units. This fall may represent NADPH hydrogen that is being used for fat biosynthesis; adipose tissue shows a far greater increase in activity with PMS (about ten times). The activities recorded with some of the other tetrazolium salts were lower; for example, NT gave 220 and 87 units with and without PMS respectively, and TT gave 200 and 60 units respectively. These lower activities obtained with tetrazolium salts that accept the NADPH hydrogen at points further along the