Greenberg (1957) did not alter the ratio of protein to radioactivity.

When the two isomers of the ^{14}C -labelled anilino-propionamide were supplied separately to the tissue, a similar amount of binding was observed in each case.

If this binding to proteins is by a normal peptide bond this would mean that with the herbicidal isomer of the anilinopropionamide a derivative of D-alanine is incorporated into protein. This could cause inhibition of subsequent protein synthesis. With the non-herbicidal isomer a derivative of the naturally occurring L-isomer of alanine would be incorporated. This may not interfere with the normal mechanism of protein synthesis.

Further work is being carried out to elucidate the precise effect of 2-(4-methyl-2,6-dinitroanilino)-N-methylpropionamide on the mechanism of protein synthesis in plants (Baxter & Weeks, 1971).

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Studies on the L-Serine O-Sulphate-Degrading Activity of Pig Liver

By N. TUDBALL and P. THOMAS (*Department of Biochemistry, University College, Cathays Park, Cardiff CF1 1XL, U.K.*)

An enzyme system that catalyses the $\alpha\beta$ -elimination of L-serine O-sulphate has now been obtained from pig liver in a homogeneous form (Tudball *et al.*, 1971). The system is unique in that it does not require the obligatory participation of pyridoxal phosphate as a cofactor (Tudball *et al.*, 1969). It has a limited specificity, acting only on N-terminal serine O-sulphate residues and on β -chloro-L-alanine (Thomas & Tudball, 1967). The substrates normally employed for enzyme assay have not yet been shown to occur in Nature, and it seems unlikely that the normal function of the system is associated with these compounds.

To obtain more information on the true function of the enzyme and its mode of action, the features of the L-serine O-sulphate molecule that are necessary for binding and catalytic activity were investigated. To this end an initial assumption was made that the conformation adopted by the substrate in the crystal form (N. Tudball, J. P. G. Richards & H. Davies, unpublished work) resembled to a high degree that form of the substrate in solution normally interacting with the enzyme.

By using the extended-chain crystal form of L-

serine O-sulphate as a model, it was predicted from stereochemical considerations that cyclic compounds related to isophthalic acid would act as reversible competitive inhibitors of the enzyme. A large number of compounds possessing the desired properties were tested and found to be good competitive inhibitors, the degree of inhibition varying with the nature of substituent groupings.

Attempts at varying the basic L-serine O-sulphate structure with a view to promoting substrate-like properties generally proved unsuccessful, though it is now possible to define those features of the molecule necessary for activity. Any potential substrate must possess an electronegative β -substituent, either a $-\text{Cl}$ grouping or an $-\text{OSO}_3^-$ grouping. The hydrogen atom attached to the α -carbon cannot be replaced and the α -amino grouping must be present in an unmodified form.

The implications of the above results will be discussed.

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Separation of the Adenosine 3'-Phosphate 5'-Sulphatophosphate-Degrading Enzymes of Bovine Liver

By W. H. B. DENNER, A. M. STOKES and K. S. DODGSON (*Department of Biochemistry, University College, P.O. Box 78, Cardiff CF1 1XL, U.K.*)

The tissues of a variety of species have been shown to possess enzymes which degrade adenosine 5'-sulphatophosphate and adenosine 3'-phosphate 5'-sulphatophosphate (Brunngraber, 1958; Balasubramanian & Bachhawat, 1962; Adams, 1964; Austin *et al.*, 1969; Armstrong *et al.*, 1970; Koizumi *et al.*, 1969; Farooqui & Balasubramanian, 1970; Bailey-Wood *et al.*, 1969, 1970). Some of the observations on adenosine 3'-phosphate 5'-sulphatophosphate degradation have been made with relatively crude enzyme systems and long incubation periods. Under these conditions it is not easy to distinguish between the production of inorganic sulphate by (a) direct desulphation of adenosine 3'-phosphate 5'-sulphatophosphate by an adenosine 3'-phosphate 5'-sulphatophosphate sulphohydrolase and the sequential action of (b) a 3'-nucleotidase, forming adenosine

5'-sulphatophosphate, and (c) an adenosine 5'-sulphatophosphate sulphonyhydrolase:

Bailey-Wood *et al.* (1970) purified an adenosine 5'-sulphatophosphate sulphonyhydrolase from rat liver high-speed supernatant with an optimum pH of 5.2. This enzyme, together with a 3'-nucleotidase, could account for the degradation of adenosine 3'-phosphate 5'-sulphatophosphate at acid pH. However, preliminary experiments suggested that, at alkaline pH, direct desulphation of adenosine 3'-phosphate 5'-sulphatophosphate occurred by the action of an adenosine 3'-phosphate 5'-sulphatophosphate sulphonyhydrolase. The present study was undertaken to clarify the situation and reports the separation of the three enzyme activities, adenosine 3'-phosphate 5'-sulphatophosphate sulphonyhydrolase, adenosine 5'-sulphatophosphate sulphonyhydrolase and 3'-nucleotidase, from the high-speed supernatant fraction of bovine liver.

The subcellular distribution of adenosine 3'-phosphate 5'-sulphatophosphate-degrading enzymes in bovine liver was determined and activity was found in all five of the main subcellular fractions. However, most of the activity was present in the high-speed supernatant fraction. $(\text{NH}_4)_2\text{SO}_4$ fractionation of the high-speed supernatant gave a fraction (0–40% saturation) free from significant amounts of adenosine 5'-sulphatophosphate sulphonyhydrolase activity. This fraction was still capable of degrading adenosine 3'-phosphate 5'-sulphatophosphate with the production of both inorganic sulphate and adenosine 5'-sulphatophosphate and hence contained both adenosine 3'-phosphate 5'-sulphatophosphate sulphonyhydrolase and 3'-nucleotidase activity. These activities were subsequently separated by column chromatography on ECTEOLA-cellulose. Most of the adenosine 5'-sulphatophosphate sulphonyhydrolase activity was present in the 40–55%-saturated- $(\text{NH}_4)_2\text{SO}_4$ fraction. This was separated from residual adenosine 3'-phosphate 5'-sulphatophosphate sulphonyhydrolase and 3'-nucleotidase activity by column chromatography on DEAE-cellulose. Experiments on the properties of these enzymes have shown that the 3'-nucleotidase has optimum pH 6.0 and is markedly stimulated by Co^{2+} , whereas the adenosine 3'-phosphate 5'-sulphatophosphate sulphonyhydrolase has optimum pH 8.8 and the presence of Co^{2+} has no significant effect on the activity.

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The Purification and Properties of Adenosine 5'-Sulphatophosphate Sulphonyhydrolase from Bovine Liver

By A. M. STOKES, W. H. B. DENNER and K. S. DODGSON (*Department of Biochemistry, University College, P.O. Box 78, Cardiff CF1 1XL, U.K.*)

Previous studies from these laboratories have described the partial purification and properties of adenosine 5'-sulphatophosphate sulphonyhydrolase from rat liver (Bailey-Wood *et al.*, 1970). Studies on the enzymic degradation of adenosine 3'-phosphate 5'-sulphatophosphate and adenosine 5'-sulphatophosphate by bovine liver have revealed the existence of a similar adenosine 5'-sulphatophosphate sulphonyhydrolase in this tissue, and its partial purification has been reported (Denner *et al.*, 1972). The enzyme has now been purified 1200-fold from this tissue and its properties have been compared with those of the rat liver enzyme.

A crude active enzyme preparation was obtained from fresh bovine liver high-speed supernatant by fractionation with $(\text{NH}_4)_2\text{SO}_4$ (40–55% saturation). Purification was achieved by chromatography first on fibrous DEAE-cellulose (Whatman DE11) and then on microgranular DEAE-cellulose (Whatman