obtained from the straight line and those calculated from data published by Hine and Brownell[1] lie within the experimental errors.

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BIOCHEMISTRY

Oxidative Phosphorylation: Synthesis of Adenosine Diphosphate by the Oxidation of Quinol Phosphates

THE importance of oxidative phosphorylation in the respiratory chain of living organisms has led to intensive research into the possible *in vivo* and *in vitro* mechanisms of phosphorylation[1], and several theories have been advanced which postulate quinol phosphates[2–4] (or analogous nitrogenous systems derived from riboflavin[5,6]) as the active intermediates in phosphate transfer.

An earlier communication from this Laboratory[4] reported the formation of inorganic pyrophosphate during the oxidation of 2-methyl naphtha-1 : 4-quinol diphosphate in aqueous solution in the presence of orthophosphate, and this work has now been extended to the synthesis of adenosine diphosphate. Two routes have been successfully followed, in one of which phosphate has been transferred to adenylic acid as substrate, and in the other the nucleotide has been transferred to inorganic phosphate as substrate.

In the first of these two syntheses of adenosine diphosphate, 2 : 3-dimethyl naphtha-1 : 4-quinol monophosphate was oxidized by bromine in the presence of the tetra *n*-butyl ammonium salt of adenosine-5′-phosphate under rigorously anhydrous conditions in N,N-dimethyl formamide. After removal of excess bromine by *cyclo*hexene and evaporation of the solvent, the residue was dissolved in water and passed down a cation-exchange column ('Amberlite *IR*-120', H^+ form). The eluate and washings were freeze-dried, the residual gum dissolved in ethanol, and the nucleotide fraction precipitated with ether[7]. Repetition of this procedure removed ortho- and pyrophosphate. The nucleotide fraction was chromatographed on an anion-exchange column ('Dowex-1', Cl^- form) and the adenosine diphosphate isolated as the free acid (yield estimated spectrophotometrically, 25 per cent). The product had the correct analysis, a phosphorus to nitrogen ratio of 1 : 2·51, and ran as a single spot during paper chromatography and paper electrophoresis. An enzymic estimation (on a sample which had been kept for a week) using phospho-enol pyruvate-pyruvate kinase and myokinase showed the sample to contain 72 per cent adenosine diphosphate and 28 per cent adenylic acid. A parallel assay on the sodium salt of adenosine diphosphate supplied by the Sigma Chemical Co., St. Louis, Missouri, gave values of 87 per cent adenosine diphosphate and 13 per cent adenylic acid.

In the second synthesis, a naphtha-1 : 4 quinol ester of adenylic acid was oxidized in the presence of inorganic phosphate. Adenosine diphosphate was again isolated as the free acid and assayed as before. The results obtained in the two syntheses were virtually identical.

Further details of this work will appear elsewhere. We are indebted to Dr. K. J. M. Andrews (Roche Products, Ltd.) for helpful discussion and the provision of certain materials, and to Dr. J. B. Chappell (Department of Biochemistry, Cambridge) for the enzymic assays.

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N-Terminal Amino-Acids of Bovine Antibody

BOVINE γ-globulin has been shown to be a mixture of molecules having N-terminal amino-acids, aspartic acid, glutamic acid, serine, alanine and valine with a total of less than 1 mole/mole of γ-globulin[1]. A partial fractionation was achieved by partition chromatography of γ-globulin; the molecules with N-terminal aspartic acid, serine and alanine were concentrated in the faster running fractions, but valine, the principal N-terminal acid, was present in all the fractions[2]. Glutamic acid was present in only trace amounts in all the fractions.

In view of these results, we suggested that there were two types of γ-globulin: (1) with N-terminal aspartic acid, serine and alanine; (2) with N-terminal valine, and that a partial fractionation of these two types had been obtained. McFadden and Smith[1] analysed γ-globulin from a cow which had been strongly immunized with mixed antigens and found a much higher content of N-terminal valine and only traces of other amino-acids.

This led us to suggest that antibody γ-globulin had only N-terminal valine, and that the molecules with the other N-terminal amino-acids were really β-type globulins which have an electrophoretic mobility similar to that of antibody. We also found that true β-globulin had N-terminal aspartic acid, glutamic acid, serine, alanine, threonine, but no N-terminal valine.

The gift of bovine antibody to a well-characterized antigen (rabbit serum albumin) by Drs. F. J. Dixon and W. O. Weigle enabled us to test our suggestion. The bovine antibody was received as a freeze-dried specific precipitate prepared from the colostrum of an immune cow. It contained 19 per cent of rabbit serum albumin. Rabbit albumin was found to have glutamic acid as N-terminal amino-acid, present in 0·6 mole/mole albumin. The N-terminal amino-acids of the specific precipitate, after allowance for