2,3-diPGA there is, in effect, an intermediate glyceric acid moiety and two intermediate phosphate groups; whereas in the phosphoenzyme mechanism the glyceric acid moiety is transferred directly and there is only one intermediate phosphate.

[^{32}P]3-PGA was incubated with rabbit muscle phosphoglycerate mutase in a tris buffer, pH 7.4, at 30° until equilibrium was reached, and samples for assay of 2-PGA concentration and radioactivity were taken. Then unlabelled 2-PGA was added in small volume and further samples were taken. The concentration of 2-PGA was determined by the formation of NADH in the enzymic conversion of 2-PGA into lactate; the radioactivity was determined as phosphate after hydrolysis of the ATP formed. The initial concentrations after addition of unlabelled 2-PGA were: 2-PGA, 1.47 mM; 3-PGA, 0.913 mM; 2,3-diPGA, 9.64 μM . The radioactivity in the 2-PGA fell during the reaction by about 38% and then returned to its original value; and the results corresponded well with the computed co-transport curve for a single intermediate phosphate. Experiments at one-half the substrate concentrations gave similar results.

The above experiments were repeated with ^{14}C -labelled substrate and the radioactivity in the 2-PGA was determined as lactate after removal of phosphates with ZnSO_4 and $\text{Ba}(\text{OH})_2$. At both concentrations the radioactivity in the 2-PGA remained substantially constant throughout the reaction, indicating that the fluxes closely obeyed the Independence relationship (Britton, 1965). This result indicates that the glyceric acid moiety is transferred directly and also shows that any isomerization of the enzyme cannot be rate-limiting.

The results thus provide strong evidence that the mechanism involves a phosphoenzyme, and exclude both the intermolecular and the direct transfer of phosphate.

We thank the Wellcome Trust for financial support. J. B. C. is a Wellcome Trust Research Assistant.

Britton, H. G. (1965). *Nature, Lond.*, **205**, 1323.

Britton, H. G. & Clarke, J. B. (1968). *Biochem. J.* **110**, 161.

Grisolia, S. & Cleland, W. W. (1968). *Biochemistry*, **7**, 1115.

Spatial Recognition of Methionine in the Tropocollagen Macromolecule

By JACQUELINE B. WEISS and JOHN BOWDEN.
(*Rheumatism Research Centre, University of Manchester, M13 0JJ*)

An electron-microscopic method of ordering the cyanogen bromide peptides of the α_1 chain of acid soluble calf skin collagen has recently been described by Rauterberg & Kuhn (1968). The method

employed involved the formation of long spacing segments (SLS) from the large (non-dialysable) peptides, and comparison of the electron-microscopic appearance of these segments with untreated α_1 chain SLS. By this means four of the seven methionine residues could be assigned positions in the α_1 chain of tropocollagen.

This method is technically difficult, and has the obvious disadvantage that some cyanogen bromide peptides are too small for SLS formation and the location of the methionine residues in these is therefore not possible.

We are currently devising a system for electron-microscopic location of specific amino acid residues in tropocollagen which depends on treating specific amino acid side chains with a linkage molecule capable of metal-ligand formation. We have recently reported the specific spatial location of arginine in tropocollagen by such a method (Weiss & Bowden, 1968; Weiss & Bowden, 1969) and we are now able, by the same principle, to locate methionine residues.

SLS collagen is precipitated on grids and treated with 4-bromoacetamido-2-nitrophenol, a reagent reported by Conway & Koshland (1967) to react specifically with methionine and cystine. As there is no cystine in collagen the reaction is in this case specific for methionine. Subsequent treatment with a metallic salt, under conditions in which ionization of carboxylic groups is minimal, allows metal-ligand formation with the free phenolic group of 4-bromoacetamido-2-nitrophenol and reveals the location of the methionine residues.

We are grateful to Dr A. Conway who kindly supplied us with the 4-bromoacetamido-2-nitrophenol used in this work.

Conway, A. & Koshland, D. E. (1967). *Biochim. biophys. Acta*, **133**, 593.

Rauterberg, J. & Kuhn, K. (1968). *FEBS Lett.* **1**, 230.

Weiss, J. B. & Bowden, J. (1968). *Nature, Lond.*, **220**, 16.

Weiss, J. B. & Bowden, J. (1969). (in preparation).

Ion Gradients and the Accumulation of Various Amino Acids by Mouse Ascites-Tumour Cells Depleted of Adenosine Triphosphate

By A. A. EDDY, C. HOGG and M. REID. (*Department of Biochemistry, University of Manchester Institute of Science and Technology*)

The accumulation of glycine by preparations of mouse ascites-tumour cells suspended in the presence of sodium cyanide in a Ringer solution lacking glucose appears to be controlled by the concentrations of Na^+ ions inside and outside the cells. The distribution of K^+ ions also affects the accumulation process. Substantial gradients of

glycine concentration may develop across the cell membrane when $[Na^+]$ and $[K^+]$ in the two phases are appropriately manipulated in circumstances where the spontaneous movements of the ions across the cell membrane appear to provide the energy needed to concentrate the amino acid (Eddy, 1968a,b). Analogous observations have now been made in a modified system containing 2mM-sodium cyanide to inhibit respiration and 10mM-2-deoxyglucose to trap ATP. The accumulation of L-alanine, 2-aminoisobutyric acid, L-leucine, L-phenylalanine and L-methionine has been studied in the modified system at both large and small values of cellular and extracellular $[Na^+]$. The results, though less detailed than those previously obtained with glycine, encourage the view that similar mechanisms are involved in the transport of all these amino acids.

Eddy, A. A. (1968a). *Biochem. J.* **108**, 195.

Eddy, A. A. (1968b). *Biochem. J.* **108**, 489.

The 'Active' Accumulation of Tetracycline by *Escherichia coli*

By T. J. FRANKLIN and B. HIGGINSON. (*Imperial Chemical Industries Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire*)

The tetracycline antibiotics inhibit protein biosynthesis in cell-free preparations from both mammalian and bacterial cells (Franklin, 1963). The selective antimicrobial activity of these drugs *in vivo* may therefore depend in part at least on the curious ability of bacterial cells to accumulate them to high intracellular concentrations by an energy-dependent 'active' process (Izaki & Arima, 1963; Franklin & Godfrey, 1965; Franklin, 1967), which apparently does not occur in mammalian cells (Franklin, 1966).

The 'active' accumulation of a toxic substance not obviously structurally related to any known nutrient is a remarkable phenomenon and it has been found to have a number of unusual features. The initial rate of uptake of $[^3H]$ tetracycline by a tetracycline-sensitive strain of *Escherichia coli* is linearly dependent on the concentration of the drug in the medium, and no trend towards a saturation effect has been found at tetracycline concentrations up to approximately 1mM. Wilbrandt & Rosenberg (1961) pointed out that such linear kinetics do not preclude the possibility of a carrier-mediated transport if it is assumed that the affinity of the postulated carrier for the transported solute is very low.

When *E. coli* cells are incubated with low concentrations of $[^3H]$ tetracycline, e.g. 10 $\mu g./ml.$, the

accumulation of drug reaches a plateau in 7–10min. If the cells are then transferred to drug-free medium the label is rapidly lost from the cells. This exit reaction is markedly temperature-dependent, and is accelerated by the addition of 1mM-2,4-dinitrophenol. In contrast, when cells are preincubated with $[^3H]$ tetracycline at concentrations greater than 50 $\mu g./ml.$, uptake of the drug remains approximately linear for about 1hr. When these cells are transferred to drug-free medium, the rate of the exit reaction is found to be strikingly decreased compared with that in cells preincubated with lower concentrations of drug. Indeed, when the cells are preincubated with 200 $\mu g.$ of $[^3H]$ tetracycline/ml. the exit reaction is negligible, although loss of label from these cells can be readily provoked by dinitrophenol at 37°. It seems probable therefore that the intracellular accumulation of tetracycline above a certain concentration results in a progressive inhibition of the exit reaction. This in turn accounts for the continued uptake of the drug by *E. coli* when the extracellular drug concentration is greater than 50 $\mu g./l.$

Franklin, T. J. (1963). *Biochem. J.* **87**, 449.

Franklin, T. J. (1966). *Symp. Soc. gen. Microbiol.* **16**, 192.

Franklin, T. J. (1967). *Biochem. J.* **105**, 371.

Franklin, T. J. & Godfrey, A. (1965). *Biochem. J.* **95**, 54.

Izaki, K. & Arima, K. (1963). *Nature, Lond.*, **200**, 192.

Wilbrandt, W. & Rosenberg, T. (1961). *Pharmacol. Rev.* **13**, 109.

The Degradation of 2-Thiophenecarboxylic Acid by a *Flavobacterium* Species

By M. J. AMPHLETT and A. G. CALLELY. (*Department of Microbiology, University College of South Wales and Monmouthshire, Cardiff*)

The thiophen ring is of restricted distribution in Nature, though a number of polythienyls have been isolated from plants in recent years (Gronowitz, 1963). However, little attention has been devoted to the microbiological dissimilation of thiophen (Knecht, 1961; Isenberg, 1961).

Attempts were made to isolate by enrichment culture micro-organisms able to degrade simple thiophens, but such attempts were unsuccessful. However, by using low (<0.5mM) concentrations of the enrichment substrate, a micro-organism was isolated that was able to grow in a simple mineral-salts medium with thiophen-2-carboxylate as sole source of organic carbon, and was provisionally identified as a *Flavobacterium* sp.

When the organism was grown in succinate medium, washed cells were able to oxidize thiophen-2-carboxylate at 0.1 $\mu mole/mg.$ dry wt. of cells/hr. in the presence of chloramphenicol at a concentra-