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.

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Spatial Recognition of Methionine in the Tropocollagen Macromolecule

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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.

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

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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