units, with closely similar amino acid compositions and peptide 'maps'. The reactive thiol groups have a similar reaction rate with iodoacetamide that is characteristically independent of pH. However, although the effect of single substrates is slight, combinations of substrates alter the inhibition reaction in quite a different way. The most striking difference is with creatine plus Mg-ADP. This combination, shown by O'Sullivan, Diefenbach & Cohn (1966) to give almost complete protection to the rabbit enzyme against inhibition by iodoacetamide, strongly enhances the rate of inhibition of human enzyme. Varying the pH of the reaction shows that the reactive thiol groups have become freely ionizing with a pK near 8.6. Concentrations of this substrate combination that less than half-saturate the enzyme have a complex effect in which, as compared with the control, the reactive thiol groups appear first activated and then protected as the reaction proceeds. Inhibition rarely exceeds 60% under the conditions used, whereas with the rabbit enzyme 90% inhibition is achieved. An equilibrium mixture of substrates shows the same phenomenon, but the active combination is probably creatine plus Mg-ATP.

These results seem to be compatible only with creatine plus Mg-ADP enhancing the activity of the thiol in the subunit to which they bind but causing protection of the reactive thiol group in the subunit without substrate. Some form of co-operatively induced conformational change between the two subunits is indicated, and if it were stabilized by the alkylation of the more reactive thiol group then this would explain why the reaction does not go to completion. The reaction goes much nearer to completion in the presence of high concentrations of creatine plus Mg-ADP, which would simultaneously activate the thiol groups on both subunits.

Unlike the rabbit enzyme no protective effects could be detected that might be interpreted as an interaction of the reactive thiol group with creatine in an intermediate complex of the enzyme reaction. Hence the mechanism of action suggested by Watts & Rabin (1962) is not supported and a more conformational role for the thiol group is indicated (Watts, 1966).

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The Significance of Anions in the Protection by Substrates of Adenosine Triphosphate-Creatine Phosphotransferase Against Inhibition by Iodoacetamide

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Rabbit muscle creatine kinase was first observed to be protected against inhibition by iodoacetamide by an equilibrium mixture of substrates (Watts & Rabin, 1962). In these experiments the ionic strength was controlled with NO_3^- ions. The enzyme was also found to be strongly protected by creatine plus Mg-ADP in the presence of 50mm- Cl^- ion (O'Sullivan, Diefenbach & Cohn, 1966). These univalent anions inhibit normal enzyme activity to varying degrees, but acetate does not. This indicated the desirability of determining the precise effect of anions in iodoacetamide experiments.

By using 50mm-bicine-NaOH buffer throughout it was found that creatine (40mm) plus ADP (1mm) and magnesium acetate (10mm) were completely without effect on the inhibition of creatine kinase by iodoacetamide at various pH values. However, the addition of 0.1M-sodium chloride resulted in the previously observed protection. Nitrate was an even better protecting agent and the order of efficiency of a series of anions in facilitating protection was $\text{NO}_3^- > \text{Cl}^- > \text{HCO}_3^- > \text{Br}^-$; acetate ions were completely without effect. The anions without substrates did not affect the rate of inhibition and the type of univalent cation present was unimportant.

Lineweaver-Burk plots with Mg-ATP as the variable substrate and fixed concentrations of Mg-ADP as the competitive inhibitor gave K_i values for the Mg-ADP complex of 0.24mm in 0.1M-acetate and of 0.055mm in 0.1M-chloride. With phosphocreatine as variable substrate K_i for creatine was 40mm in acetate but 6mm in chloride. However, the K_m values for creatine and Mg-ADP acting as substrates were not decreased in chloride compared with acetate. It would appear that the inhibitory anions selectively mediate the binding of creatine and Mg-ADP to the enzyme to form a dead-end complex that protects the essential thiol group. The consequence of this complex is also seen in normal enzyme progress curves in which the initial part becomes more curved as a more inhibitory anion is used, rendering kinetic data unreliable.

With acetate as added anion an equilibrium mixture of substrates gave a small but reproducible protection against iodoacetamide. This could represent a short-lived intermediate complex forming part of the mechanism suggested by Watts & Rabin (1962). Sterically suitable anions may

mimic the phosphoryl group by permitting the formation of a stable, quasi-transition-state complex.

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Studies on the Effect of Sulphate Nutrition on the Flavour Components of Onion (*Allium cepa*)

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The occurrence of alkyl mono-, di- and tri-sulphides in the volatile components of raw and cooked *Allium* species is well established (e.g. Carson & Wong, 1961; Oaks, Hartmann & Dimick, 1964; Brodnitz, Pollock & Vallon, 1969). The characteristic flavours of certain species, e.g. *A. cepa* (onion), *A. sativum* (garlic) and *A. tuberosum* (Chinese chive), are determined by the proportions of *n*-propyl, allyl and methyl radicals found in the sulphides (Saghir, Mann, Bernhard & Jacobsen, 1964). Varietal differences in onion flavour strength are known (Schwimmer & Guadagni, 1962), but the effects of environmental factors on the volatile flavour components of vegetables have not been reported in any detail. Schwimmer (1968) has described how the flavour components of raw onions manifest themselves by four effects on the sensory organs: a lachrymatory effect, a burning astringency and later a bitter taste on the tongue accompanied by the typical aroma of onion.

We have investigated the relationship between the sulphur nutrition of *A. cepa* (var. White Lisbon) and its flavour components by sensory and g.l.c. methods. The plants were grown in washed sand in a glasshouse and the nutrient solutions contained five concentrations of sulphate (0, 0.05, 0.1, 1 and 3 mequiv/l respectively), the remaining nutrients were at the optimum concentrations (Hewitt, 1966).

The plants were harvested as salad onions after 80 days' growth. Maximum growth was obtained with sulphate concentrations greater than 0.2 mequiv/l. Below this concentration there were symptoms of sulphur deficiency (Wallace, 1961) and the growth response was directly related to sulphate concentration in the nutrient media. Flavour strength as determined by triangular tasting tests, sensory (taste) threshold concentration and pyruvic acid concentration in the expressed juice (Schwimmer & Guadagni, 1962), and peak area in gas-liquid chromatograms of head space vapours, were positively correlated with growth response. Lachrymatory potency increased with the increase in area of the propionaldehyde g.l.c.

peak. As the aldehyde is known to be formed by decomposition of 1-propenylsulphenic acid (Virtanen, 1965), this suggests the basis of an instrumental method of determination of this component.

The conclusions arising from these observations will be discussed.

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The Interaction of Sulphate Esters and Glutathione *in vivo*

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The finding that the administration of certain carboxylic acid esters of 1-menaphthyl alcohol to rats is followed by the excretion of 1-menaphthylmercapturic acid (Hyde & Young, 1968) led us to investigate the possibility that sulphate esters also give rise to the formation of mercapturic acids *in vivo*. The acid sulphate esters of benzyl alcohol and 1-menaphthyl alcohol were prepared by the action of chlorosulphonic acid on the alcohols in pyridine solution and were separated as their sodium salts. After the injection of sodium benzyl sulphate and sodium 1-menaphthylsulphate into rats, benzylmercapturic acid and 1-menaphthylmercapturic acid were isolated from the urine of the dosed animals in amounts corresponding to the conversion of 8.3 and 5.0% respectively of the administered sulphate esters.

Rat liver contains enzymes that catalyse the conjugation of certain foreign compounds with GSH to form *S*-substituted derivatives (Boyland & Chasseaud, 1969) and it is now generally accepted that this reaction is an early step in the formation of mercapturic acids. This prompted us to determine whether an aralkyl sulphate may participate in an enzyme-catalysed *S*-aralkylation of glutathione. A 100000g supernatant fraction from a rat liver