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

homogenate was prepared and incubated at pH 8.0 and 37°C with GSH and 1-menaphthyl sulphate. Only when the liver preparation was included in the incubation mixture was it possible to demonstrate chromatographically the formation of significant amounts of *S*-(1-menaphthyl)glutathione.

A spectrophotometric assay of the interaction of 1-menaphthyl sulphate and GSH was developed after it had been observed that ϵ for the ester was 1400 at 298nm whereas for *S*-(1-menaphthyl)glutathione it was 3900 at this wavelength. The enzyme responsible for catalysing the reaction of the ester with GSH was purified 15-fold in a manner similar to that described by Johnson (1966) for glutathione *S*-alkyltransferase. The present enzyme, however, differed from that described by Johnson (1966) in that it showed much greater stability to dialysis against water.

In the presence of the purified enzyme 1-menaphthyl alcohol served neither as a substrate for the aralkylation of GSH nor as an inhibitor of the reaction between 1-menaphthyl sulphate and GSH. When sodium 1-menaphthyl sulphate and GSH were incubated at 37°C and pH 8.0 in the presence of the purified enzyme the amount of *S*-(1-menaphthyl)glutathione formed corresponded to the amount of SO_4^{2-} released, whereas no significant liberation of SO_4^{2-} occurred when GSH was omitted from the incubation mixture. In view of these various observations it appears that it is the ester and not the alcohol that acts as the substrate for this enzyme.

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Acid Glycosaminoglycans of Elastic Cartilage

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The acid glycosaminoglycan composition of ligamentum nuchae, a connective tissue composed almost entirely of elastic fibres, differs from that of tendon, where the fibres are exclusively collagenous (Meyer, Davidson, Linker & Hoffman, 1956). In this communication two approaches are described by which it was hoped to establish whether the presence of elastic fibres in cartilage is associated with differences in acid glycosaminoglycan composition or in the pattern of their age-related changes already established for hyaline cartilages (Mathews, 1965).

Six cartilages, two elastic (external ear and

epiglottis) and four hyaline (nasal septum, trachea, thyroid and cricoid), from a 2-year-old steer and the external ear of New Zealand White rabbits (of ages ranging up to 2 years) were dissected free of surrounding tissue. Dry cartilage was subjected to proteolytic digestion (Scott, 1960) and, after preliminary purification (Wusteman, Johnson, Dodgson & Bell, 1968), the glycosaminoglycan mixture was fractionated on Dowex 1 (X2; 200–400 mesh; Cl^- form) with a stepwise gradient of 0.8M-, 1.7M- and 5.0M-NaCl. Collagen and elastin content of the dried cartilage was deduced from the hydroxyproline content (Stegemann, 1958) before and after alkaline extraction (Lansing, Rosenthal, Alex & Dempsey, 1952).

Though the elastic cartilages contain less acid glycosaminoglycan per unit dry weight than the hyaline cartilages, no significant differences between the two types of cartilage were seen when results were expressed relative to the collagen content. Like the hyaline cartilages, elastic cartilage contains small quantities of keratan sulphate, whereas the bulk (over 90%) of the acid glycosaminoglycans are chondroitin 4-sulphate and chondroitin 6-sulphate. Neither the ratio of chondroitin 4-sulphate to chondroitin 6-sulphate nor the degree of sulphation of the polysaccharides could be correlated with the presence or absence of elastic fibres. The only respect in which elastic and non-elastic cartilages differed slightly lay in the unsulphated glycosaminoglycan fraction eluted by 0.8M-NaCl. This accounted for 3.2 and 4.3% of the total uronic acid of ear and epiglottis cartilage respectively, but was less than 2.4% in the hyaline cartilages.

Changes noted during the aging of rabbit ear cartilage were similar to those already reported for the hyaline cartilage of human bronchus (Mason, Dodgson & Wusteman, 1969).

The implication of these results will be discussed.

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