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Biological and Toxicological Consequences of Reactions Between Sulphur (IV) Oxoanions and Food Components

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ABSTRACT

The studies of the toxicological effects of sulphur dioxide and sulphur (IV) oxoanions are briefly summarised from 1946 to the establishment of an ADI by WHO in 1974. Sulphate oxidase is dependent on molybdenum, located in some tissues, but absent in others. The reactions of sulphur (IV) oxoanions with nucleic acids, vitamins, essential polyunsaturated fatty acids, carbonyl compounds, Maillard intermediates, proteins and carbohydrates are discussed, resulting in the conclusions that there is less known about the biological consequences of the chemical reactions than about these reactions themselves, and that, after investigations have progressed further, regulatory limits for SO_2 in foods may be able to be relaxed.

INTRODUCTION

From a regulatory viewpoint, sulphur dioxide and sulphur (IV) oxoanions are usually classified as preservatives but, of course, they have many other functions in foods, for example, in controlling enzymic and non-enzymic browning reactions, as antioxidants, in modifying protein texture and in the preparation of protein isolates. Prior to 1972, there was a voluminous literature on the toxicological aspects of sulphur dioxide *per se* (see review by Gunnison, 1981) but much of the research was directed at exposure by inhalation in industrial or urban atmospheres; little work has been done on exposure in the diet. Early studies by Fitzhugh *et al.* (1946) led to the conclusion that the highest dietary level of sodium bisulphite which

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caused no obvious adverse effects in rats was 0·05 %, equivalent to 15 mg SO_2/kg body weight per day, but later workers found conflicting results and concluded that higher daily intakes were tolerated when sulphite was administered in drinking water or in wine (Lockett & Natoff, 1960; Causeret & Hugot, 1966; Causeret *et al.*, 1965; Cluzan *et al.*, 1965; Lanteaume *et al.*, 1965). It was suggested that the conflicting results arose from the fact that sulphite in the diet was leading to destruction of thiamine (Joslyn & Leichter, 1968; Hermus, 1969) and that at least some of the adverse effects arose from nutritional deficiencies. Subsequently, Til *et al.* (1972*a*, *b*) performed long-term three-generation studies on rats in which sodium metabisulphite was administered in thiamine-enriched diets, stored under conditions (−18 °C) which minimised destruction of thiamine; they also performed a 48-week study on pigs similarly treated. On the basis of this work, a no adverse effect level was established in the rat of 0·25 % $Na_2S_2O_5$, equivalent to 70 mg SO_2/kg body weight. At higher dose levels, hyperplastic changes were seen in the forestomach and glandular stomach of the rat, with ulceration and inflammatory changes also evident. These changes probably resulted from chronic irritation at high dose levels, but there may have been effects on the synthesis of prostaglandins from arachidonic acid; prostanoids are known to have a cytoprotective rôle and to modulate mucus secretion in the stomach (Robert, 1979; Miller & Jacobson, 1979) and sulphite is known to cause free-radical destruction of essential fatty acids. Consequent on these long-term studies, in 1974 the Joint FAO/WHO Expert Committee on Food Additives reviewed the toxicological data on sulphur dioxide and established an ADI of 0–0·7 mg SO_2/kg body weight (WHO, 1974).

The systemic toxicity of sulphite ion *per se* appears to be fairly low, the intra-peritoneal LD_{50} being around 500 mg/kg body weight in rats (Cohen *et al.*, 1973). Metabolic studies in rats, mice and primates have indicated that the greater part of ingested sulphite is absorbed and excreted in urine as sulphate within 24 h (Gibson & Strong, 1973). The enzymic conversion of sulphite to sulphate is readily effected in mammals and the capacity of endogenous sulphite oxidase (sulphite: O_2 oxido-reductase: EC1.8.3.1) is substantial—it has been estimated that in the rat the tissues can oxidise sulphite at a rate of approximately 750 mmol per kg tissue per day (Cohen *et al.*, 1973). Sulphite oxidase is a molybdenum-dependent enzyme located in the mitochondria not only of liver but also of other tissues including kidney, lung, heart, intestine and adrenal, but is absent from brain, muscle, blood, adipocytes and thymus.

TABLE 1
Known Cellular/Dietary Components which Interact with Sulphur (IV) Oxoanions

1. Nucleic acids	6. Polyunsaturated fatty acids
2. Thiamine	7. Carboxyl compounds
3. Flavins	8. Maillard intermediates
4. Folic acid	9. Proteins
5. Vitamin A (precursors)	

INTERACTIONS OF SULPHUR (IV) OXOANIONS

Many of the more important interactions of sulphite with cellular or dietary components have been discussed by other authors in this issue and are summarised in Table 1.

REACTIONS WITH NUCLEIC ACIDS

Uracil and cytosine and its nucleosides can react reversibly with sulphite ion under mild physiological conditions to yield dihydro base sulphonates (Hayatsu, 1976). Furthermore, 5,6-dihydrocytosine-6-sulphonate can be deaminated to the corresponding uracil adduct (Shapiro *et al.*, 1974). This raises a toxicological question concerning the possible mutagenicity of SO_2 and its anions. At high concentrations, bisulphite is indeed mutagenic in a number of bacteria and phage and a base pair transition mutation has been proved in an *E. coli* revertant system and a T4 phage rII system (Hayatsu & Muira, 1970; Mukai *et al.*, 1970; Summers & Drake, 1971). In human lymphocyte cultures exposed to 5·7 ppm SO_2 in air bubbled through the culture medium there was reduced cell growth, DNA synthesis and mitotic indices compared with controls; chromosome abnormalities were also seen (Schneider & Calkins, 1970). Chromosomal abnormalities have also been induced in oocytes of mouse, ewe and cow undergoing meiosis (Jagiello *et al.*, 1975).

However, there is no convincing evidence that SO_2 or sulphites are mutagenic to mammals *in vivo* and this is probably for two major reasons: (a) the concentrations of HSO_3^- required for reaction with cytosine or uracil are higher than would be encountered at tolerable exposure levels

and (b) HSO_3^- is very rapidly metabolised in intact tissues. Assays in mammalian systems, i.e. host-mediated assays, cytogenetic assays and dominant lethal mutation assays, have proved negative (Maxwell & Newell, 1972) and long-term carcinogenicity studies on dietary sulphites have similarly proved negative. At the present time, it appears that the interaction of sulphites with nucleic acids does not present a major genotoxic hazard to man. However, in passing, it should be noted that rats rendered sulphite oxidase-deficient by treatment with tungsten had a low incidence of mammary adenocarcinomas (Gunnison, 1981).

REACTION WITH VITAMINS

Thiamine

The interaction of sulphur dioxide with thiamine in stored diets was among the first of such interactions to be recognised as being of nutritional and toxicological significance. As indicated in the Introduction, early toxicological studies on sulphite were complicated by thiamine deficiency so the significance is proven, at least at high dietary levels of SO_2. The thiamine enrichment and conservative storage procedures adopted by Til *et al.* (1972*a*, *b*) led only to minor losses (*ca.* 2%) of thiamine at dietary sodium metabisulphite concentrations up to 0·25%; the losses rose to no more than 15% at dietary metabisulphite concentrations of 2%. Thus, complications of thiamine deficiency were avoided in these experimental studies. Whether sulphite-induced thiamine deficiency may represent a serious hazard to man is also less easily established. In general, the applications of SO_2 in foods are controlled both by regulations and technological practice so that total dietary intake of thiamine is maintained at an adequate level. Since beri beri is *not* endemic in developed countries, it would appear that thiamine intakes are adequate, in the main, but changes in eating habits may require close monitoring and thiamine figures in dietary Tables may be unreliable as more and more convenience foods are consumed. However, it is known that the ADI for SO_2 and its salts may easily be exceeded by heavy drinkers of wines and beer, and nutritional deficiencies do appear in these sub-populations, but this probably relates as much to the poor nutritional habits of many heavy drinkers as to the effect of SO_2.

Other water-soluble vitamins

While it is known that some other water-soluble vitamins can react with sulphite, including flavins (Müller & Massey, 1969; Hevesi & Bruice, 1973) and folic acid (Vonderschmidtt *et al.*, 1967), the nutritional or toxicological significance remains to be assessed. Destruction of these vitamins in addition to thiamine may have contributed to some of the differences and conflicting results obtained in studies on stored, sulphited diets in experimental animals, but no clear evidence has yet emerged of specific deficiencies induced by sulphites either in experimental animals or in man. It has been suggested, however, that the severe anaemia seen in young male rats given high dietary concentrations of sulphite was due to the interaction with dietary components, possible cyanocobalamine (Gunnison *et al.*, 1981).

Vitamin A (precursors)

Similarly, with the fat-soluble vitamin A and its precursors (Peiser & Yang, 1979) the chemical observations have outstripped the biological evaluation. It would seem likely that significant losses in carotenoids in sulphited foods would have deleterious effects on the visual appeal of foods rich in these vitamin A precursors and hence that technological and commercial factors would regulate the magnitude of the losses. However, within limits, vitamin A intakes over and above the levels required to prevent a frank deficiency have been claimed to be protective against some tumours of epithelial tissues (Hicks, 1983) and, in this regard, the intake of many people may be sub-optimal—further losses due to processing of foods may therefore need to be watched. Furthermore, as yet we know virtually nothing of the toxicological or anti-nutritional properties of the oxidation products of caretenoids produced by sulphite-catalysed free radical processes.

Essential polyunsaturated fatty acids

To add to the catalogue of ignorance, as with vitamin A, we know very little of the biological consequences of the sulphite-induced free radical oxidation of essential fatty acids which are required for the synthesis of the physiologically active prostanoids (prostaglandins, prostacyclins, etc.). Nor do we know much about the toxicology of the oxidation

products. Since the major dietary sources of polyunsaturated fatty acids are rarely treated with sulphite, it is unlikely to present a dietary hazard but *in vivo* interactions may complicate studies in experimental animals. As mentioned earlier, prostaglandins have a cytoprotective rôle in the gastric mucosa and also modulate mucus synthesis and secretion. The gastric erosions seen in the toxicological studies on metabisulphite in rats may thus have a rational basis—other ulcerogenic compounds, such as aspirin and indomethacin, are inhibitors of prostaglandin synthesis *in vivo*.

REACTIONS WITH CARBONYL COMPOUNDS

Work on the fate of sulphite used in controlling non-enzymic browning reactions and in fermented beverages has indicated that a large proportion of the SO_2 which is bound occurs in the form of hydroxy sulphonates of simple aldehydes, ketones and reducing sugars, involving reaction with glucose, acetaldehyde, galacturonic acid, pyruvate, 2,5-diketogulonic acid, etc.

Several studies have been directed at evaluating the oral toxicity of acetaldehyde hydroxysulphonate (α-hydroxyethane sulphonate) and glucose hydroxysulphonate in experimental animals (Bhagat & Lockett, 1964; Hötzel *et al.*, 1966) and in man (Hötzel *et al.*, 1969; Bitsch *et al.*, 1969). Since the hydroxysulphonates are labile *in vitro* (Burroughs & Sparks, 1973*a*, *b*) it is perhaps not surprising that they produced gastrointestinal effects similar to those produced by sulphite alone. Hötzel *et al.* (1966) considered that, because acetaldehyde hydroxysulphonate was more stable than other SO_2 adducts in wine, it might be considered that the toxicity of all the other bound SO_2 products would be less than the acetaldehyde derivative. Results of metabolism studies on sulphite/glucose mixtures in rats, mice and monkeys and on acetaldehyde hydroxysulphonate in rats were indistinguishable from sulphite alone, confirming the lability of these compounds *in vivo* (Gibson & Strong, 1973; Gibson & Strong, 1976).

Incidentally, some hydroxysulphonates have also been evaluated as drugs used in the treatment of 'molimina crurum nocturna' (restless leg syndrome) but daily administration of sodium 1-hydroxyheptyl sulphonate or sodium α-hydroxyfurfuryl sulphonate to man for up to 45 months was without effect on pulse rate, blood pressure, haematological parameters, urinalysis or transaminase activity (Brenning, 1971).

In general, then, the interaction of sulphites with carbonyl compounds to yield simple hydroxysylphonates does not appear to have significant toxicological consequences, for better or worse.

INTERACTIONS WITH MAILLARD INTERMEDIATES

One of the products of interaction of SO_2 with unsaturated osuloses produced in the Maillard reaction is 3-deoxy-4-sulphohexosulose and the corresponding pentosulose. These interaction products have been shown to be a major end-product of SO_2 in dehydrated vegetables and possibly also in jams made from sulphited fruit pulp (Wedzicha & McWeeny, 1974; Wedzicha & McWeeny, 1975; McWeeny *et al.*, 1980). The metabolism, mutagenicity and acute and short-term toxicity of 3-deoxy-4-sulphohexosulose (DSH) have been studied recently (Walker *et al.*, 1983*a*, *b*). In both rats and mice, the acute intragastric LD_{50} was in excess of 5 g/kg body weight which is indicative of a low toxicity: most regulatory authorities would not require the LD_{50} to be determined if it exceeds 5 g/kg body weight. In fact, even higher single doses, up to 6·5 g/kg in rats and 10·7 g/kg in mice, were without toxic effect other than a transient diarrhoea which probably resulted from osmotic effects of the high load in the GI tract. These figures compare with an oral LD_{50} for SO_2 in the rabbit of 600–700 mg/kg body weight (WHO, 1974), so the interaction to form 3-sulpho-4-deoxyosuloses appears to reduce the acute toxicity. In addition, DSH was not mutagenic nor cytotoxic in the Ames test using four strains of *Salmonella typhimurium*, which contrasts with SO_2 *per se*, and so the interaction product appears to carry less of a mutagenic hazard than SO_2 itself.

Metabolic studies in rats and mice over a wide range of dose levels from 100 mg/kg body weight to over 10 g/kg body weight indicated that the material was poorly absorbed from the GI tract, faecal excretion varying between 58% and 73% in the rat, and was metabolically inert, i.e. only unchanged material was detected in urine, which accounted for 16% to 31% of the administered dose. Similar results were obtained with ^{35}S- or ^{14}C-DSH, confirming metabolic inertness. There was no evidence of accumulation in the tissues, activity being rapidly cleared after dosing.

Short-term (90-day) studies in mice were carried out at doses of 0·5, 1·0 and 2% of the diet and again at the 0·5 and 1% levels, no gross adverse effects being seen (R. Walker, M. A. Mendoza-Garcia and E. Quattrucci;

unpublished). However, at the 2% level, about 10% of the animals developed bladder stones composed mainly of calcium oxalate. Since metabolic studies have demonstrated that the oxalate did not arise from the parent compound, it is likely that the stones arise from secondary disturbances to mineral absorption in the gastrointestinal tract. Similar effects are seen with high dose levels of other osmotically active and poorly absorbed materials such as xylitol (WHO, 1978) and are unlikely to be of any significance in relation to levels of human exposure.

INTERACTIONS WITH MAJOR DIETARY COMPONENTS

Proteins

In studies using ^{35}S-sulphite, Thewlis & Wade (1974) found that 63% of the sulphite added to hard, sweet biscuit doughs was combined in organic form, believed to arise from interaction with disulphide bonds, producing S-sulphocysteine. This sort of interaction is known to occur *in vivo*, inhaled $^{35}SO_2$ combining principally with plasma α-globulins but also with albumin, γ-globulin and β-globulin (Yokoyama *et al.*, 1971). The clearance of these sulphonated proteins is relatively slow and, after injection of radiolabelled sulphited rat serum proteins in the rat, Gibson & Strong (1974) found that 5–7% of the activity remained in the carcass after 2 weeks, principally associated with liver and spleen. However, this contrasts with the fate of similar sulphited proteins given orally, when 42–62% of the dose appeared in urine as sulphate, with a further 27–50% in faeces, and there was very little retention in the carcass (Cole, 1967; Gibson & Strong, 1974). Sulphited proteins in the diet might then be expected to present little hazard. However, Bhagat & Lockett (1964) investigated the effects of sulphited casein in semi-synthetic rat diets and claimed to have encountered adverse effects. With diets formulated from casein which had been stored for a short period with metabisulphite, the toxicity appeared to be a thiamine deficiency complicated by the presence of weak anti-thiamine activity. In diets using casein stored for longer periods of 3–4 months, the effects were only partially ameliorated by large amounts of supplementary thiamine, and diarrhoea was observed. These workers also reported that autoclaving the sulphited protein diet resulted in a 50% deficit in weight gain in female rats and this could not be accounted for solely by the heat treatment.

Attempts to repeat this work, both at BIBRA and in my own laboratory, have failed to reproduce the toxic effects and, since Bhagat and Lockett did not identify the toxic interaction product, it is not possible to explain the discrepancy. The possibility that toxic interaction products of sulphites with proteins or that selective destruction of sulphur amino acids was at the root of the problem cannot be discounted.

Carbohydrates

Again, attempting to repeat some of the work of Bhagat & Lockett (1964), we have used lactose stored with sulphite as part of the carbohydrate content of laboratory rat diets. In this case biological effects were observed in the form of a transient alopecia in weanling animals from which they recovered as they matured and were growing more slowly. We would not have expected such an effect from a simple hydroxysulphonate adduct on the basis of experience with, for example, glucose hydroxysulphonates (*vide supra*) but the causal factor remains to be identified. It may be that the relatively loosely bound sulphite was interacting with dietary riboflavin, since a similar alopecia is seen in vitamin B_2 deficiency; alternatively, interference with sulphur amino acid metabolism may have limited the utilisation in keratin synthesis (keratin being rich in S-amino acids), but these are speculations which require further investigation.

CONCLUSIONS

Summing up, much more is known about the chemical reactions of sulphur (IV) oxoanions in food systems than about the biological consequences—the chemistry has outstripped the biological investigations. But, clearly, closer collaboration between the chemist and toxicologist is necessary if the significance of the interactions for man is to be appreciated fully.

It is evident that reactions with dietary components have complicated earlier toxicological/nutritional studies and that many known interactions/products have not been evaluated biologically. As we have seen, interactions may lead to enhanced or reduced toxicity relative to SO_2 *per se*; a more complete understanding may make it possible to re-evaluate,

and possibly even relax, the regulatory limits for SO_2 in foods—but we are not yet in such a position.

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