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A critical evaluation of clinical trials in reactions to sulfites

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Sulfiting agents are defined as sulfur dioxide and several forms of inorganic sulfites that liberate SO_2 under appropriate conditions. Sulfites are usually added to foods, although they can occur naturally as a consequence of fermentation. For example, *Saccharomyces cerevisiae* generates between 1 and 30 ppm of SO_2 during the fermentation of wine; some strains may produce in excess of 100 ppm.[1] Thus naturally occurring sulfites constitute a substantial portion of the total sulfite found in wine and beer.

Historically the ancient Greeks used SO_2 to fumigate their homes. Later the Romans and Egyptians used SO_2 to cleanse their wine vessels. The first recorded use of sulfites as food preservatives occurred in 1664, when cider was added to flasks of SO_2 to retard spoilage.[2] SO_2 has enjoyed widespread use in the United States since the late 1800s, and the sulfite salts have been used since the 1920s. They were first used in the manufacture of wine and beer and subsequently have been used in many other products.

Heretofore, SO_2, potassium metabisulfite, sodium metabisulfite, potassium bisulfite, sodium bisulfite, and sodium sulfite have been classified GRAS (gen-

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TABLE I. Some common uses of sulfiting agents

Use	Example
Control of enzymatic browning	Lettuce, guacamole, cut fruits, fresh mushrooms, shrimp
Control of nonenzymatic browning	Dehydrated potatoes, white grape juice, dried fruit, wine
Antimicrobial action	Occurs naturally in fermentation of wine and beer; agents added to control microbial growth in production of corn syrup, beer, and wine
Reducing agent	Corn wet-milling, dough conditioner
Bleaching	Maraschino cherries
Processing aid	Beet sugar

ulations permit their use in a variety of foods except for meats and other foods recognized as sources of thiamine. However, these regulations are under review and are subject to change. The report by the ad hoc panel of the Life Sciences Research Office, Federation of American Societies for Experimental Biology, indicates that for the majority of the population sulfites do not constitute a health hazard at current levels of use; however, for sulfite-sensitive persons use of these agents in fresh food should be discontinued.[3]

Sulfiting agents serve many important technical purposes in the food industry (Table I). In many products, sulfites serve more than one purpose. Alternatives to sulfiting agents are currently being investigated. However, possible alternatives may be less effective or more expensive and may impose their own health risks.

CHEMISTRY

Forms of sulfite exist in dynamic equilibrium in foods. In solution a variety of sulfite species can exist (Fig. 1). SO_2 readily dissolves in water to produce sulfurous acid. Bisulfites or metabisulfite salts react with water producing bisulfite ion, HSO_3^-. At low pH, equilibrium favors H_2SO_3; at intermediate pH (4.0), HSO_3^- prevails; at high pH, sulfite ion, SO_3^{2-}, predominates (as in the small bowel). SO_2 can be generated from H_2SO_3 at acidic pH (as in the stomach). At pH 2.5, approximately 16% of the SO_2 can be liberated from H_2SO_3; at pH 2.0, 37%; and at pH 1.0, 86%.[1] Thus the effect of pH on these sulfite reactions must be considered in designing challenge studies in nents to form combined sulfites. Some of these reactions are reversible, while others are virtually irreversible. The dissociable combined forms can serve as reservoirs for "free" sulfites, but irreversible reactions remove sulfite permanently from the pool of available free SO_2. Since free SO_2 is the most likely cause of adverse responses to sulfiting agents, these chemical reactions have significant implications regarding which foods may cause difficulty in sensitive patients.

When ingested, free sulfite is oxidized to sulfate (which is excreted in the urine) by the enzyme sulfite oxidase. This enzyme is widely distributed in the body, with the highest activity found in the liver and kidney.[1] Defects in sulfite oxidase activity may potentially be of importance in the pathogenesis of adverse reactions to sulfites.[4]

MEASUREMENT OF SULFITES

Several methods for measuring sulfite residue levels in foods are available. Measurement of sulfite levels is important in determining consumption and assessing risk for patients suffering adverse reactions to sulfiting agents.

Sulfites exist in foods as H_2SO_3, inorganic sulfites, and a variety of combined forms. Since sulfite salts can release SO_2 under some assay conditions, levels of sulfiting agents in foods are usually expressed as SO_2 equivalents. Variations of the Ripper method[5] are used to detect "free" SO_2 (undissociated H_2SO_3, HSO_3^-, and SO_3^{2-}). The Monier-Williams method[6] measures "total" SO_2, which includes the same substances detected by the Ripper method plus some combined forms of sulfites. Neither method is entirely satisfactory since nonsulfite substances may interfere in the analyses, some hazardous combined forms of sulfites may not be measured under the assay conditions, or some combined forms that are not hazardous may be detected. Ideally, methods can be developed that will enable us to specifically identify and measure those forms of sulfite that pose health risks.

LEVELS OF CONSUMPTION/EXPOSURE

Adequate assessment of consumer exposure to sulfites in foods is wanting. This is partially because of difficulties with methods of measuring sulfites in foods. Furthermore, the amounts of sulfites used initially to treat food do not reflect residue levels after processing. Storage and preparation of food will also affect the final amount of sulfite available for consumption. Caution should be exercised when evaluating reports from other countries incriminating foods

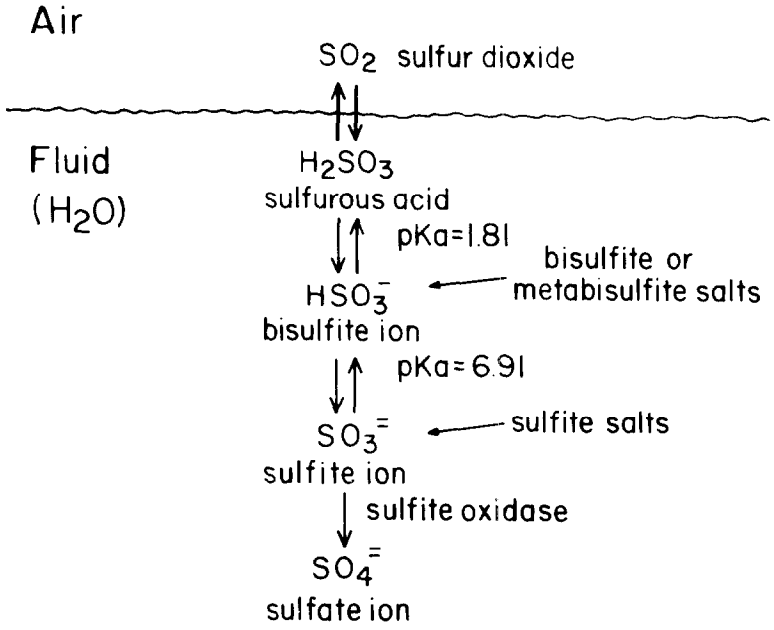

FIG. 1. Chemical reactions of sulfites in solution.

Estimated consumption levels for U.S. citizens have recently been reviewed.[3] The total daily per capita intake of sulfites for foods, expressed as SO_2 equivalents, is approximately 6 mg. Beer and wine contribute additional amounts, with another 30 mg for each 200 ml of wine and 10 mg for each liter of beer consumed. During the course of a restaurant meal, however, higher amounts may be encountered, the most widely cited example being that of lettuce from salad bars, which may be treated with sulfites. Taylor et al.[8] treated lettuce with a commercial salad freshener and detected 450 to 950 ppm of SO_2. The levels were virtually unchanged after 24 hours of refrigeration. Thus if an individual consumes a serving of 100 gm of treated lettuce, he would consume 95 mg of sulfite as SO_2. Consumption of 200 ml of wine containing 150 ppm of SO_2 would provide an additional 30 mg; lesser amounts may be contributed by dehydrated potatoes and shrimp, for a total of 175 to 180 mg for a restaurant meal.

In addition to exposure through ingestion, individuals are also exposed to SO_2 by inhalation. Using estimates of air quality standards, the Federation of American Societies for Experimental Biology panel[3] predicted that an individual may be exposed to 0.85 mg of SO_2 per 24 hours, assuming a 0.3 ppm level in the air during the course of 1 year. In industrial environments, higher intermittent levels of up to 5 ppm per 8 hours are permitted. Such exposure could diseases, contain sulfiting agents.[9, 10] Ordinarily, exposure levels through these agents are quite small. However, infusion of 500 ml of a solution containing 0.1% $NaHSO_3$ would result in an intake of 300 mg of SO_2 equivalents.[3]

ADVERSE RESPONSES TO SULFITES IN NONASTHMATIC SUBJECTS

Exposure to sulfites can occur through a variety of routes (Table II). Toxicity studies in normal individuals have been conducted, primarily through oral challenges and inhalation studies. Small numbers of normal individuals have ingested doses of as much as 400 mg of SO_2 equivalents per day without adverse effect.[1] However, doses of 4 to 6 gm per day predictably caused nausea, vomiting, gastric irritation, and occasional gastrointestinal hemorrhaging.[11] Inhalation studies indicate that SO_2 at 6 to 12 ppm causes immediate irritation of the nose and throat and bronchoconstriction, and excessive concentrations of SO_2 cause laryngotracheal and pulmonary edema and death.[12] Härkönen et al.[12] observed long-term obstructive airway changes and airway hyperreactivity to inhaled histamine in four individuals acutely exposed to high concentrations of SO_2.

In spite of a great deal of attention in the popular media and anecdotal reports, adverse reactions to sulfites in nonasthmatics are extremely rare. Fisher[13] described dermatitis of the hand in a food handler ex-

TABLE II. Routes of sulfite exposure

Route	Example
Ingestion as solid	Oral medication
Ingestion as solution	Beer, wine, sprays on vegetables and fruits at salad bar
Inhalation	Smog, opening package of dried fruit, air above salad bar, bronchodilator solution aerosols
Subcutaneous injection	Epinephrine, lidocaine
Intravenous injection	Corticosteroid preparations, metoclopramide

small number of patients. No direct evidence for this association was presented. However, injection of 1.2 to 2.4 mg of $NaHSO_3$ into the subarachnoid space of rabbits produced a chronic hindlimb paralysis similar to the clinical picture in humans. Administration of the anesthetic without $NaHSO_3$ had no effect.

Prenner and Stevens[15] were the first to report allergic-like symptoms. These occurred in a 50-year-old man who had generalized urticaria and angioedema of the tongue with swallowing difficulty and chest tightness after ingesting restaurant salads. When the patient underwent oral challenge with $NaHSO_3$ solutions to a total dose of 10 mg, he experienced itching, nausea, warmness, cough, tightness in the throat, and erythema of the shoulder but not urticaria or angioedema. Both scratch and intradermal testing with $NaHSO_3$ solution (10 mg/ml) produced a wheal-and-flare response. Control individuals were negative to scratch and intradermal testing with the same solution. When the patient's serum was passively transferred to a nonatopic individual and the site challenged with $NaHSO_3$ solution, a wheal-and-flare reaction appeared. While the passive transfer test suggests an IgE mechanism, a specific antibody to sulfite was not identified.

Habenicht et al.[16] described two women who reported urticaria and angioedema after ingestion of sulfited foods. One patient underwent an oral challenge with capsules of $K_2S_2O_5$ in increasing doses, without a placebo control. Fifteen minutes after the 25 mg dose she developed burning of the scalp and urticaria. No changes in pulmonary function were noted.

Schwartz[17] performed challenge studies in two individuals. The first, a 24-year-old man, had symptoms of angioedema involving the face and periorbital area, abdominal cramps, and diarrhea 10 minutes after ingesting a lettuce salad in a restaurant. A placebo-

developed tightness in the stomach and light-headedness; with 25 mg he developed abdominal distress, light-headedness, dizziness, and hypotension. Pulmonary functions were unchanged. The second individual, a 34-year-old woman with asthma, experienced dizziness, nausea, dysphasia, and chest tightness after a restaurant meal. A similar challenge produced weakness, dizziness, and nausea, with mild hypotension but no change in pulmonary function after a 30 mg dose of $K_2S_2O_5$.

Huang and Fraser[18] suggested that subcutaneous administration of sulfites could also provoke urticaria, angioedema, and laryngeal edema. Subcutaneous injection of 1.8 ml of lidocaine, which contains 0.5 mg of $NaHSO_3$, produced palmar pruritus in the patient. No control challenge was administered.

Flaherty et al.[19] identified a patient with sclerosing cholangitis whose liver condition worsened after sulfited foods were ingested. Liver functions improved on a sulfite-free diet. Double-blind oral challenge with 500 mg of metabisulfite and lactose resulted in an increase in liver enzymes after sulfite challenge but not with placebo. The response could be inhibited by prior oral administration of 3 mg of vitamin B_{12}.

Challenge studies in larger numbers of patients with a risk for adverse reactions to sulfites have failed to identify a significant number of reactors. Sonin and Patterson[20] challenged 10 control subjects, one patient with chronic urticaria, and 12 patients with idiopathic anaphylaxis, nine of whom had a history of reactions associated with restaurant meals, with increasing oral doses of $Na_2S_2O_5$ dissolved in lemonade. None of the patients reacted to doses totaling 391 mg. Challenge studies from the National Institutes of Health[21] conducted on 25 patients with unexplained anaphylaxis and eight patients with systemic mastocytosis produced anaphylactic episodes in only two patients, both of whom had unexplained anaphylaxis. However, the same two individuals also reacted to placebo challenge. Therefore it appears that anaphylactoid reactions may occur in otherwise normal individuals, but such reactions are rare.

ASTHMATIC RESPONSES TO SULFITING AGENTS

Sufficient data have accumulated to implicate ingestion of sulfites in the production of attacks of asthma. In the earliest report, Kochen[22] described a young child with repeated episodes of asthma after ingestion of dried fruits contained in hermetically sealed bags. However, confirmatory provocative challenges were not conducted. Subsequent studies by Stevenson and

after ingestion of sulfiting agents. They reported four steroid-dependent women, aged 27 to 65 years, who experienced episodes of asthma associated with anaphylactoid symptoms such as flushing, weakness, dizziness, and cyanosis with loss of consciousness. In a single-blind, placebo-controlled $K_2S_2O_5$ oral capsule challenge, all four subjects experienced significant decreases in FEV_1 (23% to 49%). The challenge was repeated in one individual, with similar results. Results of skin tests with sulfiting agents were uniformly negative, as were basophil histamine release assays performed on the patients' peripheral blood. These results suggested that an IgE-mediated reaction was not involved. Simultaneously, Australian investigators[24] described two steroid-dependent asthmatic women who related attacks of asthma associated with ingestion of sulfited foods and beverages. One also had experienced a respiratory arrest after intravenous infusion of a sulfited dexamethasone preparation. Both individuals had severe bronchospasm on double-blind challenge with a 500 mg dose of $Na_2S_2O_5$. These studies provide convincing evidence that capsules containing sulfiting agents can provoke episodes of wheezing in certain asthmatics.

Ingestion of sulfited solutions is more likely to precipitate asthma attacks than is ingestion of encapsulated sulfites. Towns and Mellis[25] found that 19 of 29 (66%) steroid-dependent asthmatic children responded to an acidic metabisulfite solution challenge, whereas none of the children reacted to metabisulfite in capsule form. Freedman[7] challenged 14 asthmatics with a history of exacerbation of asthma after drinking a sulfited orange drink preparation. The challenge solution contained $Na_2S_2O_5$ in citric acid and was calculated to contain 100 ppm of SO_2. The study was not placebo controlled or double blinded. Eight of the 14 individuals reacted with a 12% or greater drop in FEV_1 (range, 12% to 57%). One individual who experienced only a 12% drop was rechallenged with an additional 75 mg of $Na_2S_2O_5$ and responded with a 37% decrease in FEV_1. We have prepared a solution similar to that described by Freedman and found the pH to be 2.9. At this pH, most of the free SO_2 would exist as HSO_3^- and about 10% as H_2SO_3. About 6% of the sulfite would be evolved as gaseous SO_2 at this pH. Therefore it is highly likely that volatilized SO_2 was being inhaled by the individuals reported by Freedman and Towns and Mellis.

Asthmatic subjects have significant bronchoconstriction on inhalation of 1 ppm of SO_2.[26] The bronchoconstricting effects of SO_2 inhalation are further potentiated by mild exercise. SO_2 is highly soluble in 50 ppm, approximately 99% of SO_2 is absorbed by the upper airways.[12] Animal studies indicate that insufflation of SO_2 into the isolated upper airway caused constriction of both upper and lower airways. These responses were further shown to be dependent on innervation of the larynx and tracheobronchial tree.[26] The effects can be partly blocked by administration of aerosolized atropine, suggesting that the bronchospasm caused by SO_2 is mediated through the parasympathetic pathway and is probably initiated by stimulation of afferent nerve endings lying superficially in the larynx or tracheobronchial tree.[26] To date, no direct proof for this exists in human beings.

Not unexpectedly, acute bronchoconstriction can also be provoked when asthmatic subjects are exposed to sulfiting agents by inhalation. Twarog and Leung[27] described a steroid-dependent asthmatic who on two occasions developed severe airway obstruction requiring intubation and mechanical ventilation shortly after receiving nebulized isoetharine (a sulfited bronchodilator). Intradermal skin testing of the patient to an aqueous $NaHSO_3$ solution (0.1 mg/ml) produced a wheal-and-flare reaction. Three control subjects injected intradermally with 1 mg/ml did not react. Leukocytes isolated from the patient's peripheral blood released 20% of their total histamine content when they were exposed to $Na_2S_2O_5$ at 10^{-3} to 10^{-7} mol/L. Leukocytes from nonsensitive individuals showed no significant histamine release when treated in a similar fashion. Attempts to passively sensitize a monkey with the patient's serum were unsuccessful. The patient's sensitivity to sulfites was confirmed by a placebo-controlled, double-blind oral challenge, with graded doses of $Na_2S_2O_5$ in water as the challenge material. Ten minutes after ingesting 5 mg of $Na_2S_2O_5$ the patient developed acute wheezing and generalized flushing without urticaria, and the FEV_1 dropped 52% from baseline. There was no evidence of an increased level of histamine in the patient's plasma, and there was no change in total hemolytic complement values.

Koepke et al.[28] detected varying concentrations of SO_2 produced during nebulization of several bronchodilator solutions. These ranged from 6 ppm for isoproterenol (Isuprel), to 1.4 ppm for isoetharine (Bronkosol), to 0.4 ppm for metaproterenol (Alupent). The authors performed aerosolized sulfite challenges on two individuals previously shown to be sensitive to sulfiting agents by oral challenge. The subjects received control inhalations of normal saline solution. After inhalation of varying amounts of the 0.6 mg/ml $Na_2S_2O_5$ solution, the patients had a marked fall in pulmonary function. Interestingly, they exhib-

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