nictitating membrane were partially re-established in the absence and presence of DCI. These results should make it obvious that we cannot expect all parameters made tachyphylactic by ephedrine to be simultaneously reversed (partially or completely) by an infusion of norepinephrine.

The observations made on blood pressure responses to single norepinephrine injections preceding and following ephedrine tachyphylaxis development exclude adrenergic contractile receptor saturation as an explanation for ephedrine tachyphylaxis. This is further corroborated by the fact that norepinephrine can be infused in amounts too large to prevent reversal which represents true "receptor saturation." This is not in complete agreement with the suggestions of Winder (15) and of Horita, West, and Dille (16) that "receptor saturation" is the true explanation for tachyphylaxis to sympathomimetic amines.

In view of the above considerations, full reversal of both phases of ephedrine tachyphylaxis with norepinephrine could not be expected. Nevertheless, our original postulate (that the loss of norepinephrine from critical sites as an important etiological factor in the development of sympathomimetic amine tachyphylaxis) was fully substantiated.

Norepinephrine shows a unique specificity since epinephrine cannot be substituted in its place as a reversing agent. The precursors of norepinephrine may be effective in reversing ephedrine tachyphylaxis if the synthesis of norepinephrine is not influenced by the presence of large concentrations of ephedrine. To evaluate fully the nature of ephedrine tachyphylaxis on smooth muscle, the nictitating membrane might constitute a very reliable test organ *in situ* if studied under more favorable conditions, such as by close-arterial injections. We agree with the findings of Trendelenburg, *et al.* (17), that ephedrine is neither a purely directly acting nor purely indirectly acting sympathomimetic amine, and this fact may be responsible for only the partial reversal of ephedrine tachyphylaxis by norepinephrine infusions.

In view of the dual nature of ephedrine, which is further substantiated by our observations, it is realized that this amine is not the best drug to demonstrate fully the phenomenon of tachyphylaxis reversal. We are investigating, therefore, tachyphylaxis reversal by norepinephrine of sympathomimetic amines reported to have a greater indirect effect than ephedrine (5–7, 17), *e.g.*, tyramine and amphetamine. These results have been reported in part and will be published in the near future.

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Studies of Preservatives of Poliomyelitis (Salk) Vaccine I

Benzethonium Chloride

By HILLIARD PIVNICK, J. M. TRACY, and D. G. GLASS

Several antimicrobial materials may be present in killed Salk poliomyelitis vaccine. They are (*a*) antibiotics which are added to the tissue culture to inhibit growth of bacterial contaminants without inhibiting growth of the poliomyelitis virus, (*b*) formaldehyde added to kill the virus, and (*c*) preservatives such as benzethonium chloride added to the finished vaccine to prevent the growth of bacterial and fungal contaminants. The stable antibiotics contribute considerable antibacterial activity; the formaldehyde, if not neutralized by bisulfite, is an effective, stable antibacterial agent with some antifungal activity. Benzethonium chloride adds little, if any, antibacterial activity to that obtained either by antibiotics or formaldehyde but does furnish some antifungal activity.

PRESERVATIVES for killed poliomyelitis (Salk) vaccine must be chosen with special care because the antigenic potency of the vaccine is easily destroyed. Benzethonium chloride (BEC) has been used as a preservative for poliomyelitis vaccine and has been found to cause no destruction of the virus antigen when used in concentrations of about 25 p.p.m. (1). This concentration

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of BEC is a good preservative against fungi and some G-positive bacteria but has little or no effect against many G-negative species, especially members of the genus *Pseudomonas* (2–4).

Besides the preservatives which are added to poliomyelitis vaccine at the end of the manufacturing process, other antimicrobial materials are added during the manufacture of the vaccine. Antibiotics such as streptomycin, neomycin, and polymyxin B are added to the tissue culture fluid to control bacterial contaminants. Some of these antibiotics, depending on their stability, will be present in the finished vaccine. In addition, formaldehyde is added to kill the virus and, unless neutralized, about three-quarters of the original amount of this compound will still be present in the finished vaccine. Thus poliomyelitis vaccine may possess considerable preservative activity without the addition of extra preservatives.

Unfortunately, despite the numerous antimicrobial compounds, contaminants do grow in poliomyelitis vaccine.

During a 3-year period, 13 partially used multiple-dose vials of poliomyelitis vaccine or poliomyelitis vaccine combined with other antigens were returned to this laboratory because of apparent contamination. All of these vials had been preserved by the addition of 25 p.p.m. of BEC; all contained some residue of the antibiotics added to the tissue culture medium. None of them, however, contained residual formaldehyde as it had been neutralized by sodium bisulfite. Two of the vials were contaminated with molds. The remainder yielded species of the genus *Pseudomonas* and two of these contained *Pseudomonas aeruginosa*. This species may cause fatal septicemia (5, 6).

All the vials had been entered and part of the contents withdrawn. Three of them, each from a different lot of poliomyelitis vaccine, were returned by a single physician, and each yielded the same species of *Pseudomonas*, but this species was not encountered in the other contaminated vials. No unused vials have shown contamination. Obviously, the entry of nonsterile needles had introduced microorganisms which had grown despite the presence of BEC. Apparently BEC was inadequate as a preservative; this report attempts to explain the reasons for the inadequacy.

Table I.—Cultures Used

Code	Culture	Code	Culture
C-1	*Pseudomonas sp.*[a]	C-12	*Salmonella paratyphi* C[c]
C-2	*Pseudomonas sp.*[a]	C-13	*Pseudomonas aeruginosa*[b]
C-3	*Pseudomonas sp.*[a]	C-16	*Pseudomonas aeruginosa*[b]
C-4	*Pseudomonas aeruginosa*[a]	C-27	*Saccharomyces ellipsoideus*[e]
C-5	*Pseudomonas sp.*[a]	C-28	*Debaromyces kloeckeri*[e]
C-6	*Pseudomonas aeruginosa*[b]	C-29	*Saccharomyces rouxii*[e]
C-8	*Shigella flexneri*[c]	C-30	*Rhodotorula glutinis*[e]
C-9	*Proteus sp.*[b]	C-32	*Rhizopus oryzae*[e]
C-10	*Staphylococcus aureus*[d]	C-33	*Fusarium lini*[e]
C-11	*Staphylococcus aureus*[d]	C-35	*Circinella spinosa*[e]

[a] Contaminated poliomyelitis vaccine. [b] Contaminated tissue culture. [c] Sick monkey. [d] Clinical laboratory. [e] Stock cultures from Ontario Agricultural College, Guelph, Ontario, Canada.

EXPERIMENTAL

Vaccines and Other Preserved Media

During this investigation many preparations, described below, were preserved and challenged.

HB597 Medium.—This is a nutrient solution used in tissue culture for the growth of poliomyelitis virus. It differs from medium 199 of Morgan, *et al.* (7), in that it contains no purines, pyrimidines, ribose, deoxyribose, or adenosinetriphosphate. In addition, Hanks' balanced salt solution is used instead of Earle's solution.

Killed Poliomyelitis (Salk) Vaccine.—This consists of HB597 medium with antibiotics, virus of the three types of poliomyelitis, soluble residue from the monkey kidney cell tissue culture, formaldehyde which may or may not be neutralized by sodium bisulfite, and a preservative. During these experiments, as discussed later, the antibiotics, preservatives, and formaldehyde have been varied.

Diphtheria - Pertussis - Tetanus - Poliomyelitis (DPT Polio) Vaccine.—This is a mixture of diphtheria and tetanus toxoids, pertussis vaccine, and Salk vaccine. The poliomyelitis component of this mixture is 96% of the total volume.

Preservatives

BEC, at a concentration of 0.5% in Hanks'

Table II.—BEC in Commercially Produced Vaccines

Manufacturer	Vaccine Type	BEC, p.p.m.
A	Poliomyelitis[a]	18
A	DPT Polio[b]	16
B	DPT Polio[b]	12
C	Poliomyelitis (5 lots)[a]	25 ± 1
C	DPT Polio (10 lots)[b]	6 ± .05

[a] Trivalent poliomyelitis vaccine (Salk vaccine). [b] Salk vaccine with diphtheria and tetanus toxoids and pertussis vaccine.

Table III.—Recovery of BEC from Poliomyelitis Vaccine Dispensed in Different Sized Containers

BEC Added, p.p.m.	BEC Found by Analysis, p.p.m. In 10-ml. vials	In 3-ml. vials	In 1-ml. ampuls
31	24.6 (4)[a]		
28	22.4 (12)	22.1 (5)	20.5 (9)

[a] Number of lots examined indicated by parentheses.

TABLE IV.—BEC CONTENT OF FOUR LOTS OF DPT POLIO VACCINE COMPARED WITH THAT OF THE POLIOMYELITIS VACCINE USED IN MAKING THEM

Lot	Polio Vaccine, BEC Content	DPT Polio Vaccine, BEC Content
1	25	7
2	28	8
3	26	7
4	23	9

balanced salt solution, was added with constant mixing to obtain the desired concentration in the medium or vaccine. Analysis of BEC was by a modification (8) of the method of Auerbach (9). Antibiotics were determined quantitatively in the vaccine by usual methods (10). When more than one antibiotic was present, assay organisms were used which were resistant to all antibiotics except the one being measured. Formaldehyde was determined by the method of Nash (11) and by a modification of the Nash method (12).

Bacteria

All bacterial cultures (see Table I) were stored in the lyophilized state or on nutrient agar slopes at −20°. To obtain cells for challenging preserved vaccines, Difco[1] nutrient broth was inoculated and incubated 2 days at 30°. During the second day of incubation the culture was aerated on a reciprocating shaker. The broth culture was then sedimented by centrifugation and resuspended to one-fifth of the original volume in Difco tryptose (0.1%)-saline (0.5%). Counts of viable cells in these suspensions were made on numerous occasions and found to vary from 0.5 to 5.0 × 10^9 cells per ml.

Yeasts and Molds

These cultures (see Table I) were stored at 4° on Difco Sabouraud agar. To obtain material for challenging samples they were grown for 5 days at 30° on Sabouraud agar slopes, washed off with tryptose-saline, and shaken vigorously to break up clumps. Several determinations indicated viable populations of 10^7 and 10^8 cells per ml.

Challenge of Preserved Systems

Medium or vaccines were dispensed, 4 ml. per screw-capped test tube, and challenged with 0.1 ml. of the suspended culture undiluted, diluted 1 × 10^{-3} and 1 × 10^{-6}.

All tubes were incubated at room temperature (20 ± 4°) and examined after 4, 11, and 30 or 60 days for macroscopic growth and/or acid production. Tubes in some experiments were incubated at 4°. When there was doubt concerning growth, media challenged with bacteria were subcultured to Difco nutrient broth or a neutralizing broth (13), and fungi were subcultured to Sabouraud agar. If positive, the medium was considered to have macroscopic evidence of growth. Tubes which showed no obvious signs of bacterial growth (no color change of phenol red indicator or no increase in turbidity above that contributed by the inoculum) were recorded as negative. In many experiments, all tubes which showed no macroscopic evidence of growth were subcultured 11 or 30 days after challenge. The objectives of this investigation were, however, concerned mainly with inhibition of growth, regardless of whether inhibition was due to bacteriostasis or to death of the challenge cultures.

Most results were obvious after 11 days of incubation, but occasionally some tubes which appeared negative at 11 days contained visible growth after 30 days at room temperature.

[1] Difco Laboratories, Detroit, Mich.

TABLE V.—ADSORPTION OF BEC BY PERTUSSIS VACCINE

Vaccine	BEC, p.p.m. Added	BEC, p.p.m. Found
Tetanus toxoid	25	18
Tetanus toxoid with polio vaccine	25	19
Diphtheria toxoid with polio vaccine	25	19.5
Tetanus toxoid, diphtheria toxoid, and pertussis vaccine with polio vaccine (DPT Polio)	25	7
Pertussis vaccine	50	15

RESULTS

Benzethonium Chloride Content of Vaccines.—Table II shows the BEC content found in several commercially produced vaccines.

Table III shows that if BEC is added to poliomyelitis vaccine only about 80% can be found upon analysis when the vaccine is dispensed in 10-ml. amounts in multiple-dose vials and even less is found in 1-ml. ampuls. The reason for less than complete recovery of the BEC is probably adsorption on the glass as found by Fogh, *et al.*, with cetylpyridinium chloride (14). The smaller containers would have a larger *surface of glass/volume of liquid* ratio than the 10-ml. vials.

Table IV shows that BEC also disappears when poliomyelitis vaccine is converted to a multivalent vaccine (DPT Polio) by the addition of four volumes of combined diphtheria toxoid, tetanus toxoid, and pertussis vaccine to 100 volumes of poliomyelitis vaccine. The three additives had been preserved by the addition of at least 25 p.p.m. of BEC, so dilution of the BEC in the poliomyelitis vaccine was not a factor in producing the low levels found.

Apparently either the toxoids or pertussis cells adsorb or otherwise change benzethonium chloride. Table V shows that the presence of pertussis cells is a cause of failure to recover all of the added

TABLE VI.—PRESERVATIVE ACTIVITY OF BEC IN HB597 MEDIUM

BEC, p.p.m.	Challenge Culture, Dilution	No. tubes with growth / No. tubes challenged: Bacteria	Yeasts and Molds
0	None	13/13	7/7
	10^{-3}	13/13	6/7
	10^{-6}	13/13	2/7
24	None	11/13	6/7
	10^{-3}	11/13	0/7
	10^{-6}	11/13	0/7

TABLE VII.—ANTIBIOTIC CONCENTRATION IN POLIOMYELITIS FLUIDS DURING VARIOUS STAGES OF PRODUCTION

Tissue Culture Fluid	Antibiotic Concn. (mcg. per ml.)		
	Polymyxin	Neomycin	Streptomycin
Live virus fluid	<2	10	180
Inactivated 7 days	<2	14	170
Inactivated 12 days	0	10	135

benzethonium chloride. The toxoids do not remove appreciable amounts of BEC.

Preservative Activity of BEC.—To test the antimicrobial activity of BEC in systems uncomplicated by the presence of antibiotics normally found in poliomyelitis vaccine, BEC was added to antibiotic-free HB597 medium and the 4 ml. of medium was challenged with 0.1 ml. of the challenge cultures previously described. Table VI shows that 24 p.p.m. of BEC has little antibacterial activity but has some antifungal activity against the lower concentrations of fungal cells. The two bacterial cultures inhibited by the BEC were *Staphylococcus aureus*.

As stated previously, BEC is not the only preservative in poliomyelitis vaccine. In production of vaccine the HB597 medium used in the tissue culture had added to it 200 units of streptomycin, 20 units of polymyxin, and 10 units of neomycin per ml. Table VII shows that the polymyxin is completely destroyed during the production of vaccine but that the neomycin and streptomycin are stable.

As a check on the preservative power of the residual antibiotics in poliomyelitis vaccine, 50 strains of *Staphylococcus aureus* were obtained from a clinical laboratory. It was assumed (but not checked) that from such a source many of the organisms would be resistant to antibiotics. Fifty test tubes were charged with poliomyelitis vaccine in which the formaldehyde had been neutralized and to which no preservative had been added. Each tube was inoculated with about 10^8 cells of a different strain of *S. aureus*. No growth was observed in any of the tubes. The residual antibiotics were evidently effective preservatives against most strains of *S. aureus*.

Table VIII shows that the antibiotics remaining in finished poliomyelitis vaccine possess some activity against the test organisms and that this activity is not increased appreciably by BEC.

Experiments similar to those depicted in Table VIII but with tubes incubated at 4° showed that many of the G-negative bacteria were able to grow at refrigerator temperature if the incubation period was extended to 2 months.

To test the effect of the addition of polymyxin to finished poliomyelitis vaccine which contained BEC, a vaccine which contained only streptomycin (200 mcg. per ml.) was used. Three separate experiments were run. The aggregated results (Table IX) show that polymyxin and neomycin do not increase the preservative activity obtained by streptomycin alone.

Table IX, like Table VIII, shows that the addition of BEC to vaccine which contains only streptomycin gives a slight increase in preservative activity. However, when polymyxin and neomycin as well as BEC were added to the vaccine containing streptomycin there was a definite increase in preservative activity. Apparently there is some synergistic or additive effect between polymyxin and BEC. (Compare with Table VIII.)

TABLE VIII.—EFFECT OF BEC ON THE PRESERVATIVE ACTIVITY OF POLIOMYELITIS VACCINE[a] CONTAINING RESIDUAL STREPTOMYCIN AND NEOMYCIN

Challenge Culture (bacteria), Dilution	No. tubes with growth / No. tubes challenged, BEC concn., p.p.m. 0	8	22
None	13/13	13/13	9/13
10^{-3}	7/13	7/13	5/13
10^{-6}	3/13	2/13	4/13

[a] For maldehyde neutralized with sodium bisulfite.

TABLE X.—DISAPPEARANCE OF POLYMYXIN B FROM POLIOMYELITIS VACCINE DURING STORAGE

Time of Storage, wks.	Concn. Polymyxin, p.p.m. 4°C.	25°C.
0	20	20
2		11.2
5	21	
6		4
9	12.5	

TABLE IX.—ANTIBIOTICS AND BEC AS ANTIBACTERIAL AGENTS IN POLIOMYELITIS VACCINE[a]

Antibiotic Mixture, mcg. per ml.		BEC, p.p.m.	Challenge Cultures (bacteria), Dilution	Growth[b] Challenged	Positive, %
Streptomycin	200		None	36/37[c]	97
Polymyxin	0	0	10^{-3}	26/38	68
Neomycin	0		10^{-6}	16/38	42
Streptomycin	200		None	36/39	92
Polymyxin	0	26	10^{-3}	18/39	46
Neomycin	0	(range 24–29)	10^{-6}	12/39	31
Streptomycin	200		None	37/39	95
Polymyxin	20	0	10^{-3}	26/39	70
Neomycin	8		10^{-6}	15/39	39
Streptomycin	200		None	30/36	83
Polymyxin	20	26	10^{-3}	11/36	31
Neomycin	8	(range 24–29)	10^{-6}	4/36	11

[a] Formaldehyde neutralized with sodium bisulfite. [b] Number of tubes with growth/number of tubes challenged. [c] Aggregate of three separate experiments using cultures C-1 to C-16.

TABLE XI.—LOSS OF PRESERVATIVE EFFECT RESULTING FROM THE NEUTRALIZATION OF FORMALDEHYDE IN SALK VACCINE[a] WITH SODIUM BISULFITE

Medium	Free Formaldehyde, p.p.m. (modified Nash test)	Challenge Culture, Dilution	No. tubes with growth / No. tubes challenged: Bacteria	Yeasts and Molds
HB597	4.0	None	13/13	7/7
		10^{-3}	13/13	6/7
		10^{-6}	13/13	5/7
Lot 155, polio vaccine	72.8	None	2/13	6/7
		10^{-3}	0/13	2/7
		10^{-6}	0/13	1/7
Lot 155, polio vaccine with bisulfite	7.5	None	11/13	7/7
		10^{-3}	3/13	5/7
		10^{-6}	0/13	6/7

[a] BEC not added.

TABLE XII.—ANTIFUNGAL ACTIVITY OF BEC IN POLIOMYELITIS VACCINE CONTAINING ANTIBIOTICS AND NON-NEUTRALIZED FORMALDEHYDE

Medium	Challenge Culture, Dilution	No. tubes with growth / No. tubes challenged: Bacteria	Yeasts and Molds
HB597	None	13/13	7/7
	10^{-3}	13/13	6/7
	10^{-6}	13/13	2/7
Polio vaccine	None	1/13	6/7
	10^{-3}	0/13	2/7
	10^{-6}	0/13	1/7
Polio vaccine with 20 p.p.m. benzethonium chloride	None	1/13	3/7
	10^{-3}	0/13	0/7
	10^{-6}	0/13	0/7

It appeared desirable to increase the preservative activity in poliomyelitis vaccine, either by increasing the concentration of BEC or by adding polymyxin to the inactivated vaccine just before packaging. Unfortunately, increasing the concentration of BEC to 33 p.p.m. caused a precipitate in the vaccine and as shown in Table X, polymyxin is unstable even at 4° and would be valueless in vaccines stored for several months.

The above results indicated that BEC added little antibacterial activity to that obtained from the residual antibiotics in Salk vaccine. It did, however, confer some antimycotic activity (Table VI).

In contrast to the inadequate antibacterial activity of BEC it was found that formaldehyde was an excellent preservative in poliomyelitis vaccine. Table XI shows that the neutralization of formaldehyde by bisulfite decreases markedly both the antibacterial and antimycotic activity in poliomyelitis vaccine.

The addition of BEC to poliomyelitis vaccine containing residual formaldehyde does not materially increase the antibacterial effect on the test organisms used but, as Table XII shows, the BEC increases the antifungal activity of the preservatives in the poliomyelitis vaccine.

DISCUSSION

The objective of this research was to determine the reason for growth of bacterial and fungal contaminants in poliomyelitis vaccine preserved by the addition of 25 p.p.m. of BEC. McLean (1), Schuchardt, *et al.* (15), and Schuchardt (16) have shown that this compound, added as a preservative to poliomyelitis vaccine, did not significantly damage the antigenicity of the vaccine. They stated that it should be used preferably in vaccines to which bisulfite had not been added, but they failed to present data concerning its efficacy as a preservative with or without neutralization of formaldehyde.

The work described in this report furnishes two reasons for the failure of BEC to give adequate preservation to poliomyelitis vaccine and DPT Polio vaccine. The most important reason is that BEC at 25 p.p.m. or less is not a very effective inhibitor of G-negative bacteria. Other workers have reported this deficiency (2, 3). The other apparent reason is the loss of BEC, probably because of adsorption on glass or bacterial cells. The phenomenon of adsorption on glass has been reported for other quaternary ammonium compounds by Fogh, *et al.* (14).

The challenge dose used in these experiments was, in our opinion, severe. Not only were large numbers of organisms used (up to 10^8 viable cells), but also many of the cultures were isolated from contaminated vaccines. The necessity of using a severe challenge is evident; the desirability of using contaminants from biologicals has been emphasized by Pittman and Feeley (17). The use of less severe challenge doses (Table VI) shows clearly the lack of ability of BEC to inhibit even small numbers (10^2 to 10^5 cells) of G-negative bacteria.

There is little or no need for BEC as an inhibitor of G-positive bacteria in poliomyelitis vaccine and (as stated above) its ability to inhibit many G-negative bacteria is questionable. As an antifungal agent it does serve some purpose. Therefore, a preservative which has the antifungal activity of BEC and the antibacterial activity of formaldehyde is still needed in poliomyelitis vaccine, especially if the formaldehyde has been inactivated by bisulfite. Such a compound has been found and its effectiveness will be the subject of a later report.

SUMMARY

Benzethonium chloride (BEC) is adsorbed on glass and on bacterial cells.

BEC alone does not inhibit the growth of sev-

eral G-negative bacteria but does inhibit the growth of G-positive bacteria, yeasts, and molds.

BEC adds little antibacterial effect to the residual antibiotics present in poliomyelitis vaccine but does contribute antimycotic activity.

Formaldehyde, if not neutralized by bisulfite, is highly antibacterial and exhibits some antimycotic activity.

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Oxidation of Sulfurous Acid Salts in Pharmaceutical Systems

By LOUIS C. SCHROETER

Many aqueous, oxygen-labile pharmaceutical systems may be effectively protected by the antioxidant activity of sulfurous acid salts. The sulfite system is inordinately sensitive to trace amounts of heavy metal catalysts and a wide variety of organic compounds which act as oxidation inhibitors. Mechanism of sulfite oxidation in the presence of known inhibitors at levels normally encountered in pharmaceutical formulations appears to involve heavy metal catalysts. At the concentration (p.p.m.) of heavy metals normally present in formulations, the rate of oxidation and antioxidant activity is directly dependent upon the heavy metal concentration. However, a slow but measurable rate of sulfite oxidation occurs in systems especially purified to remove metal ions. The copper-catalyzed oxidation of sulfurous acid salts in buffered systems containing ethanol as an inhibitor may be described by first-order kinetics. The pH profile of the absolute initial rate of the copper-catalyzed oxidation has been experimentally determined and found to be in reasonable agreement with a theoretically derived curve.

ORGANIC COMPOUNDS in very low concentrations (10^{-6} M) appear to inhibit the rate of oxidation of aqueous solutions of sulfurous acid salts (1). This inhibitory action has been demonstrated by many compounds representing diverse structural features and functional groupings—typical drug molecules may be expected to retard the rate of sulfite oxidation (2). This fact may at first seem paradoxical since it is well known that sulfurous acid salts are employed as pharmaceutical antioxidants (3). Pharmaceutical systems generally contain much more drug than antioxidant: the molar concentration of drug may be as much as two orders of magnitude greater than that of the antioxidant.

Effectiveness of sulfurous acid salts as pharmaceutical antioxidants in most aqueous systems appears to depend on their avidity for free radicals such as OH or simply on the ease with which they are oxidized in comparison with most autoxidizable drugs (3). However, this reveals little about the mechanism of sulfite oxidation in the presence of known inhibitors.

Inhibition of the rate of sulfite oxidation may be described by an equation (1–3)

$$-\frac{d(S_t)}{dt} = \frac{k_1(S_t)A}{B + M} \qquad \text{(Eq. 1)}$$

where S_t is the concentration of total sulfurous acid species, k_1 is the specific rate constant for the uninhibited reaction, M is the molar concentration of additive, and A and B are constants generally of the magnitude 10^{-5}. The specific rate constant, k_1, for the air oxidation (atmospheric pressure, 25°) of pure aqueous sulfite is 2.9×10^{-3} seconds^{-1} under conditions such that the diffusion or dissolution rate of oxygen is not rate limiting; the copper-catalyzed (10^{-6} M Cu^{2+}) reaction yields an apparent rate constant of 5.1×10^{-3} seconds^{-1}. Inhibition of sulfite oxidation by a variety of compounds over

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