Technical Papers

Lack of Influence of Thiosulfate and Metabisulfite on the Antibiotic Activity of Penicillin

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Geiger and Conn (*5*) have reported that clavacin, an antibiotic containing an unsaturated ketone group, is inactivated 97 per cent or more by sodium thiosulfate as well as by cysteine and thioglycollate. Penicillic acid, also an unsaturated ketone, was not affected by thiosulfate but was inactivated by the thiols. Reiner and Leonard (*9*) found that p-benzoquinone, which is highly toxic to trypanosomes, was detoxified by sodium thiosulfate and by sodium metabisulfite. Arsenoxides were not detoxified by sodium thiosulfate.

Penicillin does not affect the course of *Trypanosoma equiperdum* infection in the rat,[1] yet oxophenarsine dramatically arrests such infection (*10*).

It seemed of interest to observe whether penicillin, whose structure may contain two ketone groups, or a keto and an incipient azlactone structure (*4*), would be inactivated by sodium thiosulfate and by sodium metabisulfite.[2] It has been shown by Cavallito and co-workers (*1, 2*) and by Chow and McKee (*3*) that penicillin is inactivated by cysteine and certain other thiols, and the view has been advanced that penicillin attacks essential SH enzymes of microorganisms affected by it. If this action thus resembles the action of arsenicals postulated by Voegtlin, Dyer, and Leonard (*10*) and strongly supported by the work of Peters and his co-workers (*7*), it is difficult to see why a host toxicity similar to that of the arsenicals is not displayed by penicillin even in massive doses. Penicillin is, however, reported by Walker, *et al.* (*11*) to exert a toxic action when applied to the cerebral cortex of man and mammals. Early reports on penicillin toxicity are difficult to evaluate because of the impurity of the preparations employed.

EXPERIMENTAL PROCEDURE

Cup tests were made using a standard strain of *Staph. aureus* #209 as the test organism on nutrient agar at pH 7.[3] Pure sodium penicillin (Merck) was dissolved in sterile normal saline to make a dilution of 3 units/cc. and mixed 1:1 with various dilutions of sodium thiosulfate (Merck reagent) and of sodium metabisulfite (C.P., Baker). After standing at room temperature for one hour the mixtures were filled into the cups, 0.3 cc. each, hence .5 unit or 0.3 μg. of penicillin per cup. Control plates consisted of (1) a plate with various dilutions of penicillin and normal saline, (2) a plate of the dilutions of sodium thiosulfate alone in saline, and (3) a plate of the various dilutions of sodium metabisulfite alone in saline. The dilutions of the tested substances were 1:200, 1:2,000, and 1:20,000; hence the cups contained 3 mg., 0.3 mg., and 0.03 mg. of these sulfur compounds, respectively. After 18 hours incubation at 37° C. the zones of inhibition were measured (Table 1).

TABLE 1
DIAMETER OF ZONES OF INHIBITION

	Penicillin Controls			
Units per cup	1	.5	0.1	0.01
Diameter (mm.)	20	15	10	8

	Dilutions of the Tested Sulfur Compound		
	1:200	1:2,000	1:20,000
Sodium thiosulfate alone (A).	..	..	..
Sodium metabisulfite alone (B)	..	..	..
Penicillin + A .5 unit	15 mm.	15 mm.	15 mm.
Penicillin + B .5 unit	15 mm.	15 mm.	15 mm.

RESULTS

At all concentrations tested sodium thiosulfate exerted no inhibitory action on *Staph. aureus*. Sodium metabisulfite likewise displayed no inhibitory action. Penicillin activity was not decreased by either compound.

DISCUSSION

Unlike clavacin and p-benzoquinone, penicillin is not inhibited in antibiotic activity by thiosulfate. Unlike p-benzoquinone, it is not inhibited by metabisulfite. In the inhibition of quinone by these reducing agents the present author held the view that this was simply an *in vitro* reduction of the quinone to the less toxic hydroquinone. Cavallito and co-workers (*2*) admit, in the case of the action of thiols on pyocyanine, that this is an *in vitro* reduction of the antibiotic, for here the colored pyocyanine is decolorized by the thiol.

[1] Unpublished experiments of the author.

[2] Penicillin contains a thiazolidine ring. The antibiotic activity is not inherent in this structure, for other thiazolidines do not display antibiotic activity. We have tested by cup test, diethylrhodanine (5-diethyl-2-thioketo-4-thiazolidine) at various concentrations against *Staphylococcus aureus* and *Escherichia coli*. There was no zone of inhibition of either organism. The diethylrhodanine was prepared by the method of Leonard (*Meddelanden fran K. Vetensk. Nobelinst.*, 1921, 4, No. 14).

[3] I desire to thank C. Grubaugh, of our Bacteriology Department, for aid in these cup tests.

Is the action of the thiols on ketonic antibiotics a simple *in vitro* reduction of the active keto- and lactone groups, thus inactivating the antibiotic? *In vivo* thiols of the host do not appear to inactivate penicillin. Does this necessarily mean that the attack upon the microorganisms is an attack upon specific sulfhydryl enzymes of the parasite affected, while there is little or no attack by some of the antibiotics, such as penicillin, on the essential SH enzymes of the mammalian host or of trypanosomes? Low concentrations of sodium thioglycollate accelerate the action of penicillinase to inhibit penicillin action (*12*). The rapid urinary and biliary excretion of unaltered penicillin would indicate little or no cumulative binding of it by host cells (*8*), such as occurs with arsenicals. The inactivation of penicillin by cysteine has been shown by Hirsh and O'Neil (*6*) to take place only in saline or on agar and not in the presence of broth or serum.

The writer suggests that until it can be proven that penicillin binds to the SH groups of reduced bacterial protein, in proportion to the number of SH groups in such protein, after the manner in which Peters' colleagues showed that As does so bind to kerateine (the reduction product of keratin), the actual point of attack of penicillin on microorganisms cannot definitely be concluded to be upon the thiols of the organism. The inactivating, reducing agents may be simply reducing the antibiotics to inactive substances *in vitro* rather than removing them from combination with thiols of the microorganisms. The lack of host toxicity of penicillin (except at high concentration on brain cells) leads one to consider it possible that its attack may be upon an essential enzymatic process of bacteria and spirochetes, which is not essential to the mammal or the trypanosome. Is this necessarily an "SH" enzyme?

It would be interesting to know the oxidation-reduction potentials of penicillin and clavacin and their position with respect to the oxidation-reduction potentials of the cystine-cysteine system and other oxidative enzyme systems of the organism. Perhaps some of the ketonic antibiotics are at an E_h level such that they can be reduced by both thiosulfate and thiols, while others are at a level requiring the reduction potentials of certain thiols to cause their reduction.

Apparently the ketonic antibiotics can be classified in two groups: (1) those which are inactivated by thiosulfate as well as by thiols, as are clavacin and p-benzoquinone, and (2) those unaffected by thiosulfate but inactivated by certain thiols, as are penicillin and penicillic acid.

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Protective Action of Desoxycorticosterone Acetate Against X-Ray-induced Liver Changes[1]

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X-ray and radium radiations, besides producing local effects, cause a general intoxication clinically known as "radiation sickness." This intoxication is one of the limiting factors in the successful radiological treatment of cancers, especially if the irradiation of larger volumina of the body is required. Many efforts have been made to control this condition, but "the list of remedies recommended for X-ray sickness is noteworthy more for its length than for any benefit that it has provided for sufferers from this distressing complaint" (*7*). This is largely due to the fact that no objective method for the evaluation of these therapeuticals has so far been available.

Data obtained during our previous "lethal dose studies with X-rays" (*2*) and especially some observations concerning fatty changes in the livers made during these investigations (*3*) seem to offer possibilities of a more objective approach to this problem.

Furthermore, the accumulated evidence indicates that histamine-like substances, if not histamine itself, cause the symptoms of radiation sickness (*1*) and also the above-mentioned liver changes (*3*).

On the basis of the histamine theory of radiation effects (*1*), desoxycorticosterone acetate has been chosen in the present study for the evaluation of its usefulness as a remedy for radiation sickness. This sterone is known to counteract histamine effects (*4–6*) and has also been recommended for the treatment of radiation sickness (*8*).

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