Chemical Sterilization of Bacteriological Media by Means of Mercuric Oxycyanide and Subsequent Inactivation of the Mercurial by Thioglycolate

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Sterilization of bacteriological media is accomplished by heat or filtration. Media containing blood, ascites, etc. cannot always be treated along these lines, and there remains only aseptic handling of these compounds, which is, however, not always feasible.

We therefore tried to achieve a chemical sterilization of bacteriological media. The principle of the method is, in brief, addition of a chemical disinfectant, the effect of which is afterwards cancelled out. An effect of this type was demonstrated by Brewer (*2*) and Fildes (*3*), who were able to neutralize the bactericidal action of various mercurials by addition of substances containing —SH groups. Similarly, Woods (*5*) showed that *p*-aminobenzoic acid antagonizes the action of sulfanilamide. Only bactericidal agents are suitable for sterilization by chemical means; materials which act mainly bacteriostatically do not fit the purpose. Also, not every antiseptic whose action can be neutralized is suitable; for example, sublimate in bactericidal concentration is a strong coagulant of proteins, thus changing the chemical as well as the physical properties of the medium.

TABLE 1

THIOGLYCOLATE CONCENTRATION NEEDED FOR REACTIVATION OF MERCURIC OXYCYANIDE (MOC) STERILIZED MEDIUM

Organism tested	Medium	Bactericidal concentration of MOC (no growth)	Neutralizing concentration of thioglycolate (full growth)	MOC: thioglycolate ratio
Staphylococcus aureus	Broth	1:100,000	1:50,000	1:2
	Broth + 5% blood	1:10,000	1:5,000	1:2
	Broth + 10% blood	1:10,000	1:5,000	1:2
Streptococcus pyogenes	Broth + 10% serum	1:25,000	1:10,000	1:2,5
Escherichia coli	Broth	1:100,000	1:50,000	1:2
Eberthella typhosa	Broth	1:100,000	1:50,000	1:2

The proposed method consists in treating the medium to be sterilized with a bactericidal concentration of mercuric oxycyanide (MOC). MOC, though a stronger bactericide than sublimate, has practically no coagulating action on albuminoid material and does not attack metals (*4*).

The procedure is as follows: The bactericidal concentration of the mercurial is allowed to act on the medium for at least 24 hours at 37° C. or any other temperature desired. This concentration varies for different media and ranges between 1:50,000 and 1:1,000. If the container for the medium has not previously been sterilized, it should be stoppered with rubber and shaken several times during the procedure to insure complete wetting of the inner surface. Reactivation of a portion of the medium is then performed by adding sterile sodium thioglycolate. The ratio of neutralizing MOC to sodium thioglycolate is 1:4 according to weight, *i.e.* 20 molecules of thioglycolate neutralize 1 molecule of mercuric oxycyanide (see Table 1). After addition of the proper amount of thioglycolate, the reactivated medium is divided into two parts: one is left uninoculated and serves as control, while the other is inoculated with any organism desired. If sterilization and reactivation are both complete, the control tube remains sterile, while full growth is obtained in the inoculated tube.

There are two shortcomings in the method proposed: (1) MOC, like other inorganic mercurials, sterilizes spores rather slowly (*1–3*). Media, even if heavily contaminated by vegetative forms, become sterile at 37° C. within 6–9 hours, whereas the destruction of spores takes several weeks. This method is, therefore, limited to sterilization of materials and media containing only small numbers of spores. (2) When media containing blood are exposed to the action of MOC for more than 12 hours, haemolysis occurs. This can, however, be stopped by neutralization with thioglycolate.

The advantages of the method are: (1) MOC does not deprive the medium of any essential —SH substances which could not be replaced by thioglycolate. In this respect the medium remains unchanged. The small excess of thioglycolic acid present after inactivation of the mercurial does not affect aerobic organisms, but definitely stimulates the propagation of facultative anaerobes. (2) The inactive complex, MOC-thioglycolate, thus obtained remains stable for at least a month. On the other hand, merthiolate, for example, when similarly treated, gives an unstable compound which releases the free antiseptic after 24 hours. (3) Variations as to the concentration, temperature, and time of exposure to the mercurial are easily produced according to need.

Investigations into the mechanism of the action of mercurials and quantitative relationship for various —SH substances and different bacteria are under way and will be published elsewhere.

References

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