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An investigation of the inhibitory properties of sodium thioglycollate in media for the recovery of clostridial spores

By H. R. HIBBERT and R. SPENCER
British Food Manufacturing Industries Research Association, Leatherhead, Surrey

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SUMMARY

The effect of various concentrations of sodium thioglycollate (0–0·05 %) on the recovery of spores of four strains of clostridia was investigated. The results showed that thioglycollate at a concentration of 0·01 % can be inhibitory to some clostridia. However, the results also indicated that the inhibitory effect is dependent upon the composition of the growth medium. The presence of glucose appeared to be particularly important in reducing the inhibitory effect. These findings support the view that the use of sodium thioglycollate in sterility test media should be discontinued.

INTRODUCTION

Mossel & Beerens (1968) drew attention to the continued use of media containing sodium thioglycollate for the recovery of clostridial spores in the testing of the sterility of surgical apparatus, despite the demonstration by Hirsch & Grinsted (1954) and by Galesloot (1961) that under some circumstances sodium thioglycollate may inhibit the germination of the spores of some clostridia.

Mossel & Beerens (1968) studied the recovery of 14 strains of clostridia in five different media, including media containing sodium thioglycollate, and interpreted the reduced recovery in the presence of sodium thioglycollate as confirmation of the inhibitory effect of this compound on the germination of some clostridial spores. Their results do not, however, unequivocally support their conclusions. The recovery in thioglycollate agar USP (U.S. Pharmacopoeia, 1965) was not greatly different from the recovery in this medium when sodium thioglycollate was omitted. Recovery in Difco thioglycollate agar (Difco Manual, 1953) was better than that obtained in USP-thioglycollate agar and was further improved by the addition of glucose to this medium, although recovery was not as good as in a fifth medium of different basic composition which contained cysteine to reduce the redox potential. The effect of omitting sodium thioglycollate from the Difco medium or of adding it to the fifth medium was not investigated. Thus the results obtained by Mossel & Beerens (1968) supported the interpretation that the recovery of clostridia was dependent upon the nutritional state of the medium at least as well as the interpretation that the presence of sodium thioglycollate inhibited the recovery of clostridial spores.

In addition to their well established use in the sterility testing of surgical apparatus and pharmaceutical preparations (Pittman, 1946; Sykes, 1956; Report, 1960), media containing sodium thioglycollate are still used for the sterility testing of canned foods and for counting clostridial spores in foods (Sharf, 1966; Thatcher & Clark, 1968; Hersom & Hulland, 1969). In view of the relevance to the examination of foods of the comments of Mossel & Beerens (1968) in relation to surgical apparatus, and the nature of these workers' results, the possible adverse effect of sodium thioglycollate on the recovery of clostridia from spores was investigated further. This investigation was undertaken by determining the extent of recovery of several species of clostridia in several media in the presence of various concentrations of sodium thioglycollate. In addition, recourse was made to the results of a large series of sterility tests of canned meats in which liver broth and USP-thioglycollate medium were used in parallel.

MATERIALS AND METHODS

The clostridia examined were from the culture collection of the laboratories of the British Food Manufacturing Industries Research Association, Leatherhead, England, and consisted of one strain each of *Clostridium welchii*, *Cl. histolyticum*, *Cl. tertium* and *Cl. paraputrefaciens*.

Spore-bearing cultures of these organisms were obtained by culturing them in reinforced clostridial medium (RCM, Oxoid Manual, 1967) for 48 hr. at 37° C. and then storing the cultures at room temperature for 6 weeks. The cells were separated from the medium by centrifugation, washed three times in distilled water, and finally resuspended in distilled water. Immediately before use the suspensions were heated at 80° C. for 10 min. to kill vegetative organisms as in the investigation of Mossel & Beerens (1968).

The following media were used with varying concentrations of sodium thioglycollate up to 0·05 %.

Medium A. Reinforced clostridial agar (Oxoid).

Medium B. USP-thioglycollate agar with the omission of thioglycollate (U.S. Pharmacopoeia, 1965).

Medium C. Soya–peptone agar; Phytone, Baltimore Biological laboratories, 15 g./l.; yeast extract, 5 g./l.; cysteine-HCl, 0·5 g./l.; disodium phosphate, 2·5 g./l.; sodium chloride, 2·5 g./l.; agar 15 g./l.; distilled water to 1 l.: pH $7{\cdot}2 \pm 0{\cdot}1$. This medium was recommended for glycolytic clostridia (Mossel *et al.* 1965), in which category *Cl. welchii* comes, and was used as a reference medium by Mossel & Beerens (1968).

The recovery of the various strains of clostridia on these media was determined in duplicate using a surface-plate drop-count technique similar to that of Miles & Misra (1938) but with anaerobic incubation of the plates for 3 days at 37° C. after which the colonies were counted. This procedure has been shown to be satisfactory for colony counts of several species of clostridia (R. Spencer, unpublished results).

In the sterility testing of canned meats, a sample of meat was taken with aseptic precautions from a can and broken up in quarter strength Ringer solution con-

taining 0·1 % peptone and 0·1 % yeast extract. Duplicate portions of the suspension were inoculated into tubes of both liver broth and USP-thioglycollate broth. One tube of each medium was incubated at 55° C. and one tube of each medium at 37° C. After 6 days incubation the cultures were examined for growth by aerobic and anaerobic subculture.

RESULTS

Table 1 shows the extent to which the four clostridia were recovered on the three media at various concentrations of sodium thioglycollate.

Three of the clostridial cultures were inhibited by 0·01 % sodium thioglycollate in the soya-peptone medium; the fourth culture did not grow in this medium. It

Table 1. *The effect of increasing concentrations of sodium thioglycollate in different media on the recovery (as log_{10} counts) of four species of clostridia*

(The standard deviation of the differences between replicate log counts is 0·02, and the maximum deviation at the 95 % probability level is ± 0·04. RCM = reinforced clostridial medium; USP-thio = USP-thioglycollate; soya-pep. agar = soya-peptone agar.)

		Recovery counts (log_{10}) in media containing sodium thioglycollate (%)			
	Medium	0	0·01	0·03	0·05
Cl. welchii	RCM	6·06	6·24	6·25	6·17
	USP-thio.	4·96	4·98	5·05	4·97
	soya-pep. agar	5·12	< 2·44	< 2·44	< 2·44
Cl. paraputrefaciens	RCM	6·80	6·75	6·32	6·78
	USP-thio.	5·37	5·48	5·95	6·18
	soya-pep. agar	5·01	4·90	4·93	4·99
Cl. histolyticum	RCM	5·91	5·75	5·83	5·74
	USP-thio.	5·07	< 2·44	< 2·44	< 2·44
	soya-pep. agar	< 2·44	< 2·44	< 2·44	< 2·44
Cl. tertium	RCM	5·04	5·05	5·06	5·14
	USP-thio.	5·46	5·51	5·51	5·56
	soya-pep. agar	3·47	< 2·44	< 2·44	< 2·44

Table 2. *Influence of medium on isolation of clostridia from canned meat*

Number of samples positive in:		
Liver broth	USP-thioglycollate	Both liver broth and USP-thioglycollate
3	7	0

$\chi^2 = 1{\cdot}6$; d.f. = 1; $P > 0{\cdot}05$

should be noted that this medium was developed for use with glycolytic clostridia and that the culture which did not grow on it even in the absence of sodium thioglycollate, *Cl. histolyticum*, is a proteolytic clostridium. Mossel *et al.* (1965) showed that *Cl. histolyticum* is particularly sensitive to the composition of the medium used to recover it from spores.

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One culture, *Cl. histolyticum*, was inhibited by 0·01 % sodium thioglycollate in the USP-thioglycollate agar while the recovery of the culture of *Cl. paraputrefaciens* was apparently increased by increasing concentrations of sodium thioglycollate in USP-thioglycollate agar.

No culture was inhibited by 0·05 % sodium thioglycollate in RCM.

Table 2 shows the extent to which clostridia were recovered from canned meats cultured in liver broth and in USP-thioglycollate medium. No attempt was made to identify the clostridia isolated. The recoveries in the two media were not significantly different ($P > 0·05$) and thus no evidence was forthcoming that the thioglycollate medium was inferior to liver broth in the recovery of clostridia from canned meats.

DISCUSSION

It seems that Clark (1943) first commented on the possible toxicity to thermophilic anaerobes of sodium thioglycollate, a substance recommended by Brewer (1940) for reducing the redox potential in anaerobic media. Hirsch & Grinsted (1954) observed that mesophilic clostridia were sensitive to sodium thioglycollate when it was incorporated in a medium at a concentration of 0·1 %, but not when it was present in the medium at 0·01 %. The effect of sodium thioglycollate at 0·03–0·05 %, as in USP-thioglycollate medium, was not determined. The results of Mossel & Beerens (1968), as pointed out above, whilst certainly providing evidence of inadequacies in the USP-thioglycollate medium so far as the recovery of clostridia from spores is concerned, do not demonstrate that sodium thioglycollate at a concentration of 0·03–0·05 % is inhibitory to clostridia.

The results of the present investigation demonstrate clearly that sodium thioglycollate at as low a concentration as 0·01 % can be inhibitory to some clostridia. Equally clearly the inhibitory properties of sodium thioglycollate depend very much upon the medium and were not apparent in RCM. Sodium thioglycollate at a concentration of 0·03–0·05 % in a nutritionally rich medium, and in particular in one containing glucose, may well be a satisfactory substance for reducing the redox potential of media for clostridia.

In contrast to these findings, there was no evidence that USP-thioglycollate medium was inhibitory when used in the sterility testing of canned meats. It is, however, known that thioglycollate can be neutralized by meat particles (Clark, 1943) which may account for the results obtained.

Although this investigation has been restricted to only four strains of clostridia, the results obtained substantiate previous reports that thioglycollate can exert an inhibitory effect against some clostridia. On the basis of these findings, and those of Mossel & Beerens (1968), USP-thioglycollate medium would appear to be inadequate as a sterility test medium.

The data on the recovery of clostridial spores from canned meats were provided by Brooke Bond Liebig Research Centre to whom we are obliged for permission to include these data in this report.

Much of the experimental work was carried out by Mr S. A. H. Razvi.

REFERENCES

BREWER, J. H. (1940). Clear liquid mediums for the 'aerobic' cultivation of anaerobes. *Journal of the American Medical Association* **115**, 598.

CLARK, F. M. (1943). Observations on growth of thermophilic anaerobes in Brewer's Medium. *Food Research* **8**, 327.

Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Chemical Laboratory Procedures (1953). 9th ed., p. 195. Detroit: Michigan.

GALESLOOT, TH. E. (1961). De anaerobe sporevormers die bedorf von smeltkass kunnen veroorzoken. *Nederlandsch Melk-en Zuiveltijdschrift* **15**, 263.

HERSOM, A. C. & HULLAND, E. C. (1969). *Canned foods: an introduction to their microbiology* (*Baumgartner*). 6th ed. London: Churchill.

HIRSCH, A. & GRINSTED, E. (1954). Methods for the growth and enumeration of anaerobic sporeformers from cheese, with observations on the effect of nisin. *Journal of Dairy Research* **21**, 101.

MILES, A. A. & MISRA, S. S. (1938). The estimation of the bactericidal power of the blood. *Journal of Hygiene* **38**, 732.

MOSSEL, D. A. A. & BEERENS, H. (1968). Studies on the inhibitory properties of sodium thioglycollate on the germination of wet spores of clostridia. *Journal of Hygiene* **66**, 269.

MOSSEL, D. A. A., BEERENS, H., TAHON-CASTEL, M., BARON, G., POLSPOEL, B. (1965). Etude des milieux utilizés pour le dénombrement des spores des bactéries anaérobies en microbiologie alimentaire. *Annales de l'Institut Pasteur de Lille* **16**, 147.

Oxoid Manual (1967). 3rd ed. London: Oxoid, Ltd.

Pharmacopoeia of the United States of America (1965). XVIII revision, p. 829.

PITTMAN, M. (1946). A study of fluid thioglycollate medium for the sterility test. *Journal of Bacteriology* **51**, 19.

SHARF, J. M. (1966). *Recommended methods for the microbiological examination of foods.* 2nd ed., p. 147. New York: American Public Health Assoc., Inc.

SYKES, G. (1956). The technique of sterility testing. *Journal of Pharmacy and Pharmacology* **8**, 573.

THATCHER, F. S. & CLARK, D. S. (1968). *Micro-organisms in foods, part III*, p. 169. Toronto: University of Toronto Press.

Report (1960). General requirements for the sterility of biological substances. *World Health Organization Technical Report Series no.* 200.