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# Effect of storage on the heat resistance of bacterial spore papers

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Papers impregnated with $10^4$ *Bacillus stearothermophilus* spores in water and subsequently stored on the bench can lose heat resistance and viability. This loss of viability was accelerated with storage over silica gel. Papers containing more than about $10^6$ spores retained significant heat resistance over long periods. The pH of the broth significantly affected recovery from heated spore papers.

THIS paper states results of tests on the viability and heat resistance after storage of papers impregnated with *Bacillus stearothermophilus* spores described previously (Cook & Brown, 1965).

## Experimental

### METHODS AND MATERIALS

Spore papers were prepared and tested as described by Cook & Brown (1965). In addition, papers were prepared using spores suspended in *Mist. Dessicans* (Fry & Greaves, 1951) which contained 7·5 g glucose, 1 g peptone, 0·5 g sodium chloride, 75 ml serum and water to 100 ml. Papers were either impregnated with spore suspension individually using a dropping pipette (Method A) or in bulk (Method B) (Cook & Brown, 1965). Spore papers were stored at room temperature in screw capped dark coloured jars either on the bench or over silica gel in a vacuum desiccator. Details of the spore papers are given in Table 1.

*Effect of storage on viability.* Unheated spore papers were incubated in broth after storage and the results for papers prepared as described

TABLE 1. DETAILS OF SPORE PAPERS

| Code | Storage conditions | Sporulation time of spores (days) | Times washed | Suspending medium | Method of preparation | Number of spores per paper |
|---|---|---|---|---|---|---|
| E | Over silica gel | 5 | 6 | Water | A | $10^4$ |
| | Bench | | | | | |
| G | Over silica gel | 5 | 0 | Broth culture | A | $10^4$ |
| | Bench | | | | | |
| A | Bench | 2 | 5 | Water | A | $3 \times 10^4$ |
| D | Bench | 8 | 5 | Water | B | $10^6$ |
| C | Over silica gel | 8 | 5 | Water | B | $10^7$ |
| | Bench | | | | | |
| F | Over silica gel | 8 | 5 | *Mist. Dessicans* | B | $10^7$ |
| | Bench | | | | | |

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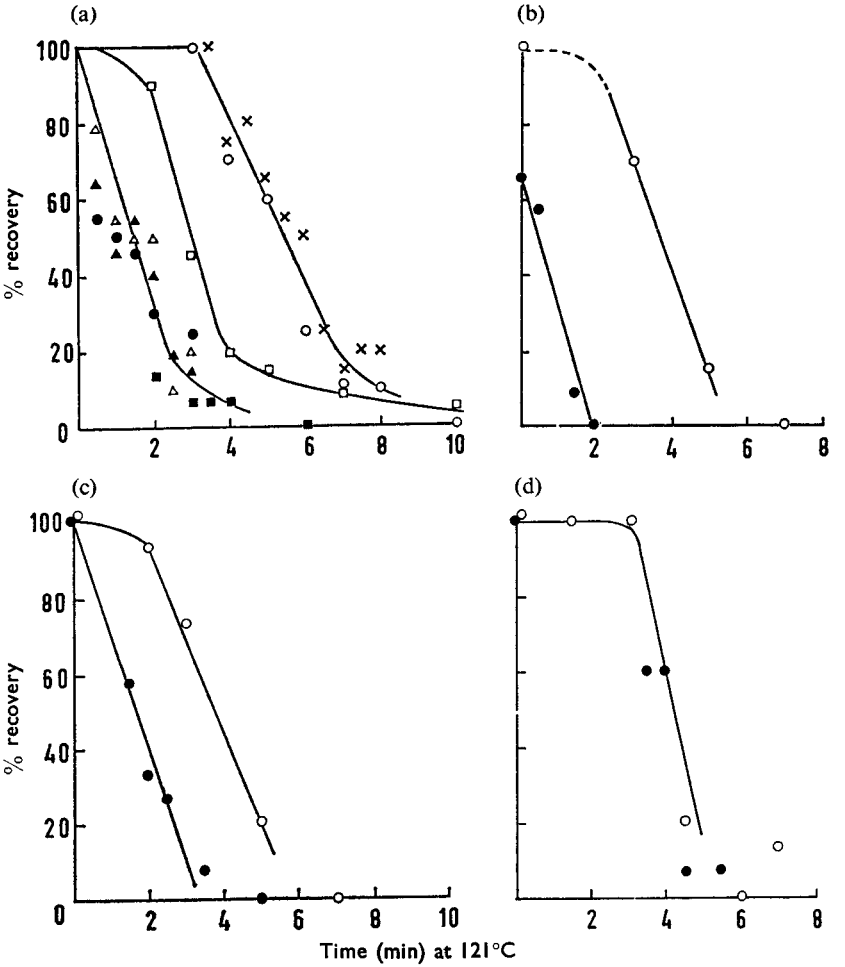


FIG. 1. Effect of bench storage upon heat resistance of spore papers. (a) Commercial papers. ○, ×, Zero storage time. □, Stored 5 weeks. ▲, △, ●, ■, Stored 16 weeks. (b) Papers coded A. ○, Zero time. ●, Stored 16 weeks. (c) Papers coded G. ○, Zero time. ●, Stored 16 weeks. (d) Papers coded D. ○, Zero time. ●, Stored 22 weeks.

above and for papers from a commercial source (Ox.S)* are recorded in Table 2.

*Effect of storage on heat resistance.* Spore papers prepared in different ways were tested for resistance to wet heat before and after storage. Results of such experiments for the papers coded Ox.S, A, G and D, stored on the bench are illustrated in Fig. 1. Results of experiments made to compare the effect of bench and desiccator storage conditions of C and F papers are given in Figs 2 and 3.

* Oxoid Spore Strips, Code number BR23, Oxoid Division, Oxo Ltd., London, E.C.4.

TABLE 2. EFFECT OF STORAGE UPON VIABILITY OF UNHEATED SPORE PAPERS

| Paper | Weeks stored | Storage conditions | Number of papers tested | % showing viability |
|---|---|---|---|---|
| G .. .. .. | 37 | Bench | 15 | 100 |
| | 20 | Over silica gel | 140 | 0 |
| E .. .. .. | 37 | Bench | 5 | 80 |
| | | Over silica gel | 5 | 0 |
| Ox.S .. .. | 26 | Bench | 30 | 90 |

*Effect of recovery medium pH.* Unheated spore papers were incubated in broths of pH below 5·1 and above 6·3. It was observed that broths of pH 5·1 or less did not support the growth of the spores on the paper D but growth did occur at pH 6·3 or above. Spore papers coded D were also tested for resistance to wet heat and recovered in broths of differing pH (Fig. 4).

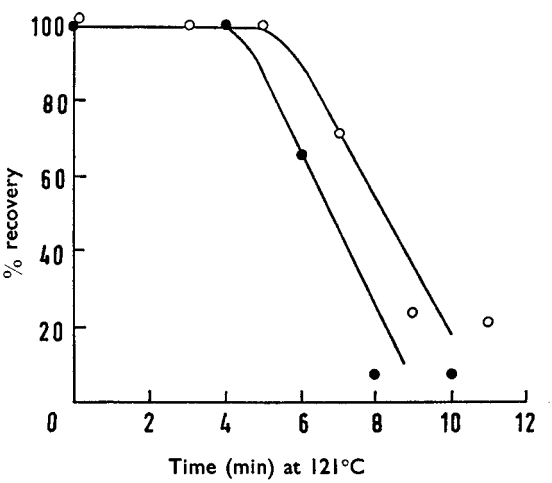


FIG. 2. Effect of storage over silica gel on heat resistance of papers coded C. ○, Zero time. ●, Stored 16 weeks.

## Discussion

Spores can lose viability during storage on paper. Papers Ox.S and E ($10^4$ spores per paper) began to lose viability after 26 and 37 weeks bench storage respectively (Table 2). Papers coded G ($10^4$ spores per paper, unwashed) showed no loss in viability when stored on the bench for 37 weeks. Storage over silica gel resulted in complete loss of viability with E and G papers after 37 and 20 weeks respectively.

The papers with the smaller inocula ($10^4$ spores per paper) and Ox.S papers, lost heat resistance after less than 16 weeks bench storage (Fig. 1). Unlike G and Ox.S papers, papers coded A not only lost resistance

but also viability after 16 weeks bench storage (Fig. 1). G papers were inoculated with an unwashed suspension, and the spores in the finished papers may have been protected from harmful effects of storage by the presence of dried nutrients.

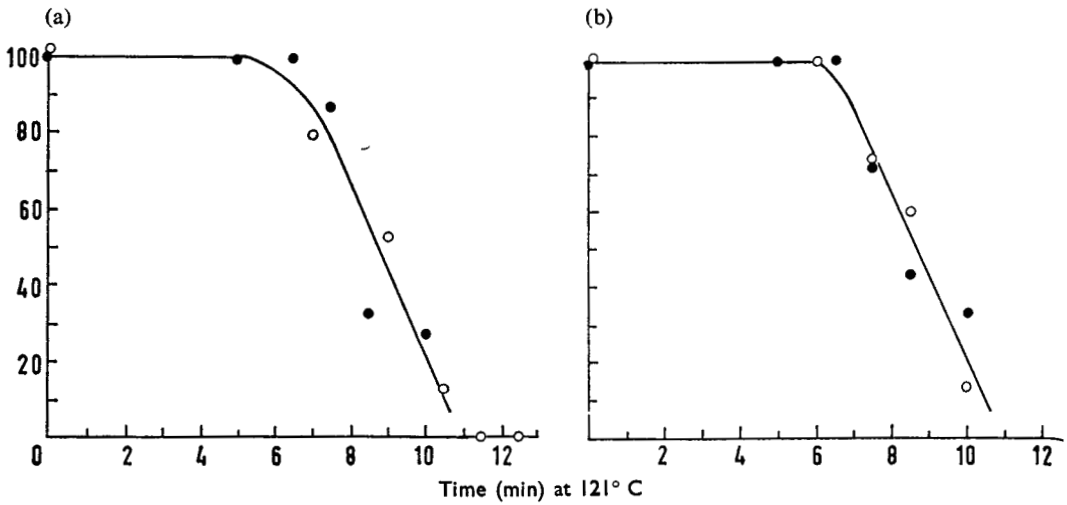


FIG. 3. Effect of storage on bench and over silica gel on heat resistance of papers coded F. Ordinate: % recovery. (a) Bench. ○, Zero time. ●, Stored 34 weeks. (b) Silica gel. ○, Zero time. ●, Stored 34 weeks.

With one exception, papers with more than about $10^6$ spores showed no significant loss of heat resistance over the storage periods stated [Figs 1 (d), 2 and 3]. C coded papers appear to have lost some heat resistance after storage over silica gel for 16 weeks (Fig. 2). This finding accords with

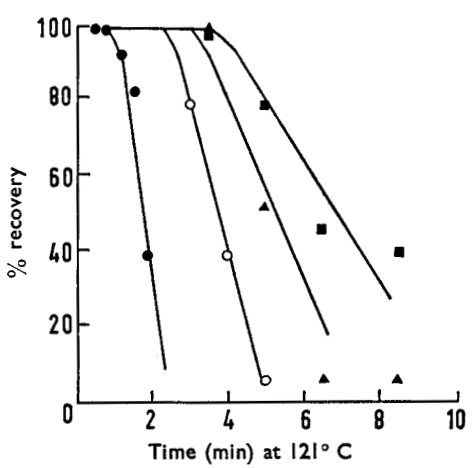


FIG. 4. Effect of pH of recovery broth on apparent heat resistance of papers coded D. ■, pH 7·4. ○, pH 6·8. ●, pH 6·3. ▲, pH 7·9.

loss of viability obtained after similar storage conditions for papers G and E (Table 2). No comparable experiment was made after 16 weeks bench storage with papers coded C, but no loss in resistance was observed after 5 weeks bench storage. F papers did not lose heat resistance when stored over silica gel or on the bench for 34 weeks (Fig. 3). The harmful effects of drying over silica gel were perhaps minimised in this instance by the presence of glucose and other ingredients in the *Mist. Dessicans* used to suspend the spores with which F papers were impregnated.

The pH of the broth used to recover heated spore papers is important; maximum recovery of heated spores occurred at about pH 7·4 (Fig. 4). These results agree with those for the recovery of heated spores in aqueous suspension (Brown, 1962) where increased pH of the medium, up to a maximum correlated with increased recovery.

## References

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