

PRELIMINARY STUDIES OF THE HEAT RESISTANCE OF BACTERIAL SPORES ON PAPER CARRIERS

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RECENTLY there has been a reappraisal of the methods of testing sterilisers and sterilising techniques¹⁻⁴ and spore impregnated papers have been introduced for this purpose. The spore papers* are available as paper strips, impregnated with spores of *Bacillus stearothermophilus*, sealed in glassine envelopes. Spores on these strips are stated to survive 5 minutes at 121° moist heat, but to be killed after 12 minutes.

The resistance to moist heat at 121° was tested in an autoclave, the lid of which contained a plunger-type device to facilitate the rapid movement of the spore papers in and out of the autoclave. The temperature in the autoclave was recorded by a thermometer and a pressure gauge.

TABLE I
EFFECT OF RECOVERY IN MEDIUM, A, B OR C ON THE APPARENT SURVIVAL TIME OF SPORE STRIPS EXPOSED TO STEAM AT 121°

Time in minutes at 121°	Recovery broth								
	A			B			C		
3	+	+	+	+	+	+	+	+	-
3½	+	+	+	+	+	+	+	+	+
4	-	-	-	+	+	+	+	+	+
4½	-	-	-	+	+	+	+	+	-
5	-	-	-	+	+	+	+	+	+
5½	-	-	-	+	+	+	+	+	+
6	-	-	-	+	+	+	+	+	+
6½	-	-	-	+	+	+	+	+	+
7	-	-	-	-	-	-	+	+	+
7½	-	-	-	-	-	-	+	+	+
8	-	-	-	-	-	-	+	-	-

Each symbol indicates the response of one spore strip.
+ = growth.
- = no growth.

The spore strips were cut into halves and several halves fitted separately on to the plunger barrel which was then brought into contact with steam at 121°. The time of contact was measured by a stop watch, and at the end of this the plunger was withdrawn from the steam. Thermocouple readings indicated that the spore strips reached the temperature of the steam in less than 8 seconds. Immediately after removal from the steam, the strips were transferred to the recovery medium, incubated at 55° and examined at intervals for up to 2 weeks.

Three kinds of recovery medium were used in 10 ml. quantities.

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

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(A) Oxoid Dextrose Tryptone Broth, Code number CM73 containing 0.5 per cent dextrose, 1 per cent tryptone (Oxoid), 0.004 per cent brom-cresol purple. This is recommended as a recovery medium by the makers of the spore strips.

(B) Broth containing 0.5 per cent dextrose, 1 per cent tryptone (Difco).

(C) Broth containing 0.5 per cent dextrose, 1 per cent tryptone (Oxoid).

The results of over 300 observations of the effect of recovery in medium A or B on the apparent survival time of spore strips after exposure are given in Figure 1. Experiments using medium C are as yet incomplete and the data have not been included in the Figure. However, sufficient

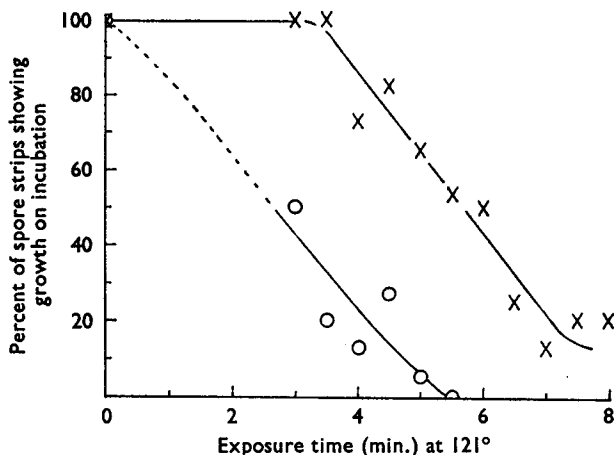


FIG. 1. Effect of recovery medium on the apparent survival time of spore strips exposed to steam at 121°.

○, recovery medium A; X, recovery medium B.

results have been obtained to justify preliminary comparison with results from A and B. Table I gives the combined results of two separate experiments using A, B and C as recovery media.

The claim made for the survival of these spores has not been substantiated by our results (Fig. 1). When using the recovery medium A, 50 per cent of the spore strips failed to show growth after 3 minutes' exposure.

These results also show that all the spore strips after 3 minutes' exposure were capable of producing growth when recovered in medium B but the percentage dropped rapidly with increased heating time.

Table I indicates that there is little, if any, difference between broths B and C in their ability to support growth from heated spore strips.

Since medium A is medium C with added brom-cresol-purple, it is likely that the absence of the dye from the recovery medium accounts for the increase in the apparent survival time of heated spore strips which occurs with medium C.

Most of the spore strips which showed growth on incubation in the recovery media did so within 24 hours. Growth was not observed after 14 days with any spore strip which had failed to show growth after 36 hours.

REFERENCES

1. Kelsey, *Lancet*, 1958, **1**, 306.
2. *Present Sterilising Practise in Six Hospitals*. Nuffield Provincial Hospitals Trust Report (1958).
3. M.R.C. Report, *Lancet*, 1959, **2**, 425.
4. Brown and Ridout, *Pharm. J.*, 1960, **184**, 5.

After Mr. Brown presented the paper there was a DISCUSSION. The following points were made.

The introduction of the plunger did not influence the temperature: pressure relationship in the autoclave. The work had not set out to prove or disprove the manufacturer's claims for spore papers, but was part of a study on the heat resistance of spores. The organisms used were unlikely to withstand the conditions in dressing sterilisers working at 30 p.s.i. for 10 minutes.