J. Hyg., Camb. (1970), **68**, 457
With 1 *plate*
Printed in Great Britain

Recoveries of bacteria after drying and heating in glutamate foams

By D. I. ANNEAR
Department of Microbiology, Royal Perth Hospital, Perth, Western Australia

(*Received* 24 *March* 1970)

SUMMARY

A method of drying bacteria is described, in which the bacterial suspension was made in 40% sodium glutamate, and 0·1 ml. volumes of this in 8 ml. ampoules were dried *in vacuo* while being held in a water bath at 25° C. After 1 hr. with the pump still running, the ampoules were immersed in water at 100° C. The partly dried suspension expanded rapidly into a homogeneous white foam. After 30 min. the ampoules were taken off the manifold; small tubes containing dry P_2O_5 were inserted in the ampoules which were then sealed in air. Preliminary results with three organisms, *Salmonella ndolo*, *Staphylococcus aureus* and *Serratia marcescens* showed high survivals immediately after the 'foaming' period, and good stability after 1 or 2 days at 100° C.

INTRODUCTION

In a previous study (Annear, 1966) it was shown that bacteria suspended in sodium glutamate could be recovered in appreciable numbers after the suspensions were dried *in vacuo* while immersed in a bath at 100° C. Under these conditions vigorous spluttering occurred and the suspensions dried rapidly in foamy flakes patchily distributed on the walls of the ampoules.

Subsequent studies have shown that a more controlled foaming resulted if the suspension was initially dried from the liquid state at a lower temperature (*ca.* 25° C.) before being subjected to a higher one. Under these conditions the suspension dried initially as a scaly bubbly residue in the bottom of the ampoule (Plate 1 A). When subjected to a high temperature (100° C.), and while still being evacuated, the desiccate expanded and set within a second or two into a fairly homogeneous white foam. The density and volume of the foam was modified by the volume and/or glutamate concentration of the solution dried (Plate 1 B, C). The appearance of the foam did not seem to be affected by the interval between the initial drying and the heating, desiccates left on the pump for several days responding in a similar manner to those left for 1 hr. Water loss from the desiccates during foaming is undoubtedly accelerated both because of the increased temperature and because of the enormous increase in surface area which occurs in the dried material.

The results of several experiments are published here to illustrate the method of drying micro-organisms in glutamate foams and the relative stability of the

desiccates to high temperatures when held in air and in the presence of dry P_2O_5. Previous studies by Annear & Bottomley (1965) have shown that bacteria sealed under such conditions but dried by means other than those described here were remarkably stable to heat.

METHODS

The organisms chosen are shown in Table 1. They were grown on nutrient agar plates for 18 hr. at 30° C. and heavy suspensions made in 40 % glutamate. Ampoules of 8 ml. capacity were inoculated with 0·1 ml. volumes of these suspensions which were then dried *in vacuo* on a horizontal manifold using P_2O_5 as a desiccant and while held in a water bath at 25° C. (Annear, 1961). Drying was carried out under these conditions for 1 hr., most of the water being removed within the first few minutes to leave behind scaly residues. While still being pumped, the ampoules were then immersed in a boiling water bath to produce foaming and were left at this temperature for a period of 30 min. to effect further dehydration of the foams.

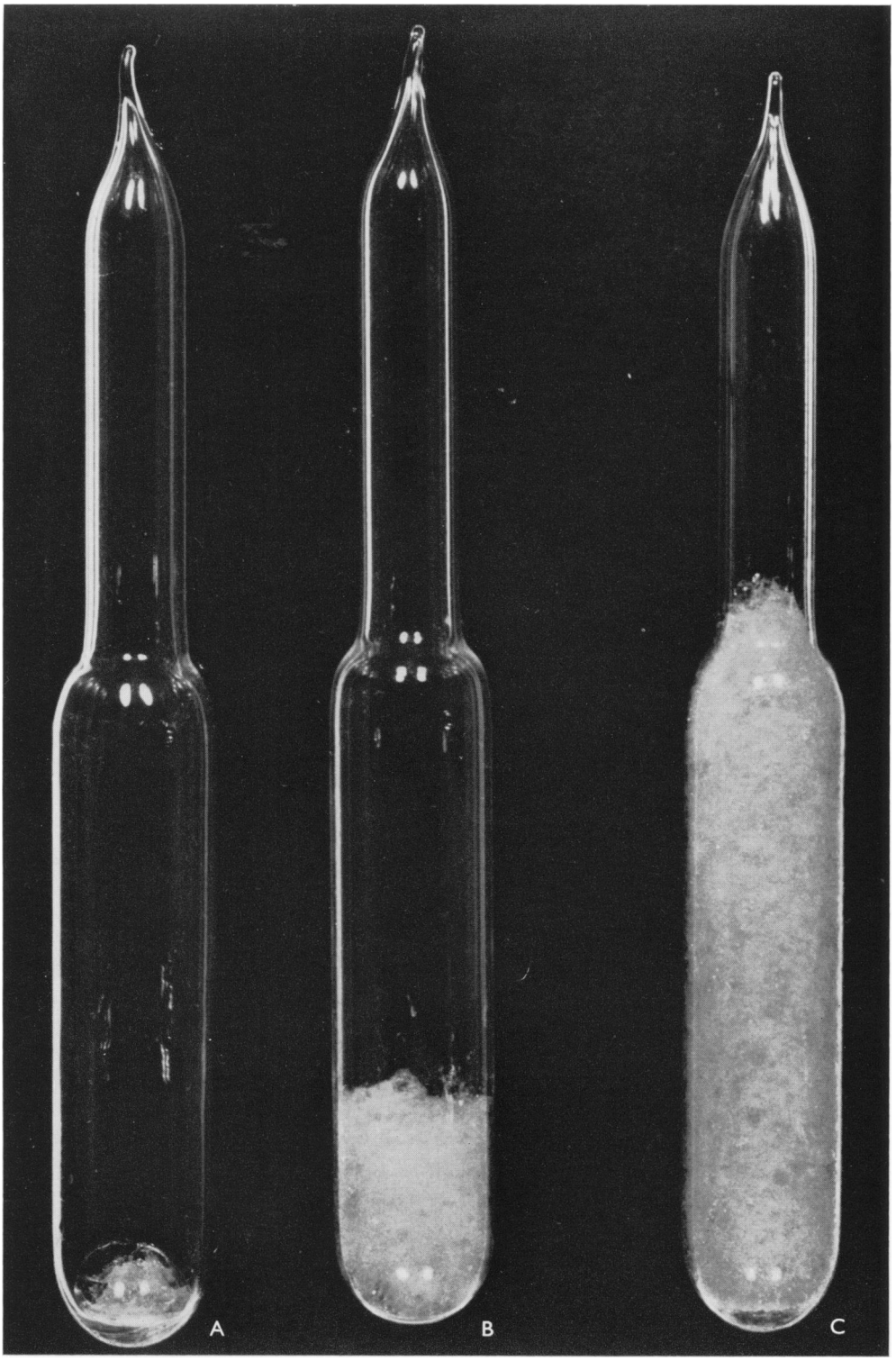
Table 1. *Recoveries (log_{10}) of bacteria after drying, foaming and heating in sodium glutamate*

		Recovery				
		Immediately after	After	After exposure to 100° C. for		
Organism	Inoculum	drying	foaming	1 day	2 days	3 days
Salmonella ndolo (NCTC 8700)	9·0	8·9	8·8	7·2	5·1	2·1
Staphylococcus aureus (NCTC 6571)	8·9	8·9	8·7	6·1	3·4	< 1
Serratia marcescens (NCTC 1377)	8·8	8·8	8·7	7·1	5·3	3·0

After removal from the manifold, small tubes of dry P_2O_5 were introduced into the ampoules which were then sealed in air. The ampoules were transferred to a boiling water bath and viable counts made on the desiccates at daily intervals. Counts were also performed immediately after the first stage of drying at 25° C. and after the initial exposure to 100° C. used to produce the foams. All counts were made on blood agar plates after slow rehydration of the desiccates (Annear, 1965).

RESULTS AND DISCUSSION

The results in Table 1 show that very little loss occurred with any of the three organisms tested during either the initial drying from the liquid state at 25° C. or during the high temperature treatment used to produce the foams. The survival of the organisms during subsequent exposure to 100° C. indicates reasonable stability and permits reliable prediction of high survival in such desiccates during storage at ambient temperatures. However, a more systematic study of the various factors involved would be necessary for the understanding of survival in this type of desiccate.



D. I. ANNEAR

(*Facing p.* 459)

It is not known at present what application if any this particular method of drying has. It does offer a means of drying a small volume of suspension from the liquid state efficiently because of the enormously increased surface area the desiccates present when expanded into foams. In this respect the desiccates resemble the peptone foams made from freeze-dried peptone plugs (Annear, 1956). Foaming in those preparations was shown to be dependent upon entrapped air in the matrix of the freeze-dried peptone.

While entrapped air would seem also to be involved in the production of the glutamate foams, it is of interest that failure has resulted in attempts to produce foams with a number of other compounds commonly used in preservation of micro-organisms. Compounds tested included glucose, sucrose, sorbitol, mannitol and higher molecular weight compounds such as dextrins, starch, dextrans and polyethylene glycols. None foamed as glutamate did and it would be interesting to find an explanation for this behaviour which at present seems fortuitously associated with a compound so useful in the preservation of micro-organisms.

REFERENCES

ANNEAR, D. I. (1956). The preservation of bacteria by drying in peptone plugs. *Journal of Hygiene* **54**, 487.

ANNEAR, D. I. (1961). Recoveries of *Strigomonas oncopelti* after drying from the liquid state. *Australian Journal of Experimental Biology and Medical Science* **39**, 295.

ANNEAR, D. I. (1965). Effect of heat during drying on survival of bacteria in desiccates. *Australian Journal of Experimental Biology and Medical Science* **43**, 665.

ANNEAR, D. I. (1966). Recoveries of bacteria after drying *in vacuo* at a bath temperature of 100° C. *Nature, London* **211**, 761.

ANNEAR, D. I. & BOTTOMLEY, G. A. (1965). Survival of bacteria in desiccates at 100° C in dry atmospheres. *Nature, London* **206**, 1373.

EXPLANATION OF PLATE

Glutamate desiccates before and after foaming. Organisms dried in 40 % sodium glutamate.

(A) Desiccate resulting from drying 0·1 ml. of suspension *in vacuo* at a bath temperature of 25 °C.

(B) Appearance of (A) after subjecting it to 100 °C. *in vacuo*.

(C) As for (B) but resulting from an initial suspension of 0·5 ml. volume.