and was shown not to correspond to any of the radioactive spots. Short chain acyl carnitines would not be expected to contaminate washed lipid extracts.

The observation that TMAA is incorporated into a phospholipid may be of importance in elucidation of the mechanism of its inhibitory action. Thin-layer chromatography on silica gel *G* using chloroform–methanol–acetic acid–water (25 : 15 : 4 : 2) indicates that several of the unusual phospholipids formed when dietary choline is replaced by other compounds can be separated from each other. Phosphatidylcholine, phosphatidyl β-methylcholine and phosphatidyldimethylaminoethanol have R_F values of 0·33, 0·47 and 0·67, respectively. The lipid formed from TMAA has an R_F of 0·19. Furthermore, all these compounds can be clearly separated from each other after being mixed together. In addition to demonstrating that TMAA is incorporated into a lipid soluble derivative, this supports the observation of Bieber and Newburgh[13] that dimethylaminoethanol is incorporated into the phospholipid fraction and is not methylated to form phosphatidylcholine after incorporation.

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H. M. MEHENDALE
W. C. DAUTERMAN
ERNEST HODGSON

Department of Entomology,
North Carolina State University,
Raleigh.

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MICROBIOLOGY

Recoveries of Bacteria after Drying *in vacuo* at a Bath Temperature of 100° C

FOR the purposes of preservation, micro-organisms may be dried either from frozen or liquid suspensions and the recoveries are governed by a number of factors, in particular by the nature of the suspending medium[1]. So far, bath temperatures of no higher than about 30° C appear to have been used to maintain suspensions in the liquid state during drying *in vacuo*.

Recent investigations of the resistance of bacteria to heat in the dried state[2,3] prompted an attempt to determine what fraction, if any, of bacteria would survive the actual drying process when it was carried out *in vacuo* and when the ampoules were held at a bath temperature of 100° C. Under these conditions, drying could be expected to proceed very rapidly.

Suspensions of the test organism, *Salmonella ndolo* (N.C.T.C. 8700), were prepared in 20 per cent sodium glutamate[2] and 0·1 ml. volumes of it were dispensed into ampoules (Edwards, 36/*H*/0800). The ampoules were connected to a horizontal manifold[4] charged with phosphorus pentoxide and the assembly was connected to a 'Speedivac *SSC*20' vacuum pump. Three drying treatments were studied; the pump was switched on (*A*) 5 sec before the ampoules were immersed in boiling water, (*B*) at the same time as the immersion, or (*C*) 5 sec after immersion. As the suspensions were subjected to reduced pressure and heat, vigorous spluttering occurred and the suspensions dried as foamy flakes on the walls of the ampoules. After 30 min, the bath was removed, the ampoules were constricted, replaced on the manifold and exposed for a further 30 min to 100° C at high vacuum, after which they were sealed *in vacuo*. The heat resistance of the sealed desiccates was tested by total immersion of the ampoules in a boiling water bath. Using "slow" rehydration procedures[3], viable counts on the desiccates were made immediately after the ampoules were sealed and at intervals during the secondary heating.

The only treatment of the three studied (Table 1) which resulted in heavy loss was treatment *C*, in which the ampoules were heated for 5 sec before evaporative cooling *in vacuo* began. With the other two treatments, where evaporative cooling began at the same time or 5 sec before the suspensions were exposed to high temperature, appreciable recoveries (5–16 per cent) of the organism were obtained. The recoveries from treatments *A* and *B* after heating the sealed ampoules at 100° C also indicate that they would yield desiccates with long storage life at ambient temperatures[3].

Table 1. RECOVERIES (LOG_{10}) OF *Salmonella ndolo* AFTER DRYING *in vacuo* AT A BATH TEMPERATURE OF 100° C AND AFTER FURTHER EXPOSURE OF THE SEALED DESICCATES TO 100° C

Drying treatment (see text)	Recoveries (log_{10}) Immediately after ampoules were sealed	After exposure to 100° C for: 24 h	48 h
A	8·9 (16 per cent)	5·6	2·9
B	8·4 (5 per cent)	4·8	1·0
C	5·8	< 1	< 1

Number of organisms dried (log_{10}) = 9·7.

No attempt has been made to measure temperature changes during the brief drying periods in these experiments. The cooling during drying *in vacuo* will be markedly modified by the heat input from the bath and the resulting temperatures could be expected to fluctuate violently.

Working at much lower temperatures, and in more slowly evaporating systems, other workers[1] found specimen temperatures within evacuated ampoules to be up to 60° C below the temperature of the bath surrounding the ampoules.

Whereas it is firmly established that glutamate protects organisms during heating in the dried state, there is no evidence from this or previous work that it does so during the early stages of drying. It is, of course, difficult to examine the two stages separately. No suggestion is made here that any of the treatments described should be seriously considered for preserving organisms, but the results do indicate that higher bath temperatures than have hitherto been contemplated could be used for drying some micro-organisms with a reasonable expectation of survival.

D. I. ANNEAR

Cell Preservation Section,
Royal Perth Hospital,
Perth, Western Australia.

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Fragile Cultures of *Escherichia coli* produced by Growth in High Concentrations of Various Salts

SUCROSE-DEPENDENT mutants have been reported which permit total isolation under mild conditions of cellular ribonucleic acid and proteins from actively growing cultures[1]. The considerable time and effort required to isolate equivalent mutants in strains of other genetic provenance had led us to search for a general method to