The Preservation of Leptospires by Drying from the Liquid State

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SUMMARY

Four strains of *Leptospira* were preserved by drying, *in vacuo*, small volumes (0.04 ml.) of suspension of the organism from the liquid state. The leptospires were suspended and dried in a mixture of equal parts of Korthof's medium and 20% (w/v) glucose. Recoveries of the organisms after storage for one year were appreciably higher from desiccates stored at 4° than from those stored at about 20°.

INTRODUCTION

A wide range of micro-organisms is now routinely preserved in the dried state. This is not so however for leptospires, which are maintained by animal passage, in culture, or in frozen suspensions. The recovery of leptospires from desiccates after storage for 2 years has been reported previously (Annear, 1958*a*), but the results revealed the method to be of doubtful value for long-term preservation of the organisms. Moreover, the drying method was a relatively complex one. The present paper describes the drying of leptospires by a simpler method which the results indicate to be more efficient. Briefly the procedure consists of drying *in vacuo* and over P₂O₅ small volumes of microbial suspension in ampoules which are immersed in a water bath at room temperature to prevent freezing during evaporation. The method has been previously applied to the preservation of other bacteria (Annear, 1958*b*) and the protozoan *Strigomonas oncopelti* (Annear, 1961).

METHODS

The four strains of *Leptospira* investigated are shown in Table 1. The organisms were grown in 200 ml. volumes of Korthof's medium at 28°, and 3-6 days after the first appearance of turbidity were harvested by centrifuging the culture in 1 oz. bottles. The deposited organisms were resuspended and pooled in about 2 ml. of the supernatant fluid, and with a syringe-pipette (Annear, 1956) single drops were delivered into ampoules (Edwards High Vacuum Ltd., E30/C17) which had been previously charged with one drop of 20% (w/v) glucose solution; the drop volume in each case was about 0.02 ml. Cotton-wool plugs were pushed down the necks of the ampoules, which were then connected to a manifold containing P₂O₅ (Annear, 1958*b*). The bottoms of the ampoules were immersed in a water bath at room temperature (about 20°) and the suspensions dried from the liquid state. After pumping overnight, the ampoules were constricted, placed on a vertical manifold

and sealed *in vacuo* after a further 6 hr. of pumping. The sealed ampoules were stored at 4° or at room temperature (usually about 20°, but within a range of 17–30°).

Rehydration of the desiccates was effected by resuspending the dried organisms in about 0.5 ml. of Korthof's medium in the ampoule and then pipetting this volume into a further 9.5 ml. volume of the same medium. A drop of the smaller volume was examined microscopically. The 10 ml. volume of suspension was divided into four samples which were incubated at 31°. Daily examinations of the tubes were made and those showing turbidity were examined microscopically.

Table 1. *Recoveries of leptospire after drying and storage for 12 months*

Organism	Expt.	Storage temperature	No. of desiccates examined	No. of days* for cultures to show visible growth
<i>L. icterohaemorrhagiae</i> (Jackson)	1a	4°	1	6
	b	Room	1	8
	2a	4°	1	6
	b	Room	1	8
	3	4°	3	5, 5, 5
	4a	4°	2	5, 5
	b	Room	2	7, 8
	5a	4°	1	8
	b	Room	3	8, 10, 11
<i>L. icterohaemorrhagiae</i> (W)	6	4°	1	6
<i>L. canicola</i> (Berlin)	7	4°	1	6
<i>L. pomona</i> (Staines)	8	4°	1	9
	9	4°	2	7, 7

* The values in the column are means of the sets of four samples.

RESULTS

Numerous desiccates have been examined during 12 months of storage, all of them yielding living leptospire. The results shown in Table 1 are those from ampoules examined at the end of this period. Microscopically all of the rehydrated desiccates showed large numbers of inert leptospire. However, actively motile leptospire were seen on rehydrating many of the desiccates which had been stored at 4° and in some of those stored at room temperature. No attempt was made to assess the fraction of active leptospire but in some of the preparations there were hundreds of them in each low-power field ($\times 200$). When first rehydrated the motile organisms did not have the morphology of actively growing leptospire but appeared somewhat distorted and compressed. Within a few hours, however, many normal forms were visible. On culture, all desiccates, including those in which no active forms were seen, yielded growth. An occasional tube among the sets of four samples became contaminated. Except for Expt. 5b (Table 1), there was no more than a difference of one day among the recovery periods of each set of samples or between the means of sets of samples from replicate desiccates. In Expt. 5b there were differences of as many as three days in both cases. As in the previous study (Annenar, 1958a)

there were considerable differences between recovery periods from ampoules stored at room temperature and those stored at 4°, and these differences will undoubtedly increase with further storage.

DISCUSSION

The results from storage at 4° are encouraging and suggest that the method of drying is worth further study for long-term preservation of leptospire. The recovery periods shown in Table 1 are little or no longer than those from desiccates rehydrated at the beginning of the storage period, indicating that there has been no appreciable deterioration during storage. The conditions of drying so far have been quite arbitrary and the critical investigation of such factors as the age of the cultures and the composition of the suspending medium may well lead to more efficient drying of the organisms and to higher storage survival, particularly at room temperature.

In previous work (Annear, 1958*b*) considerable variation occurred among recoveries from replicate desiccates of salmonellas dried from the liquid state on glass surfaces of ampoules, whereas the results were more uniform when the same suspensions were dried on tufts of cotton wool. It should be profitable to make such comparisons with leptospire. Also the concentration of organisms in the suspension to be dried might be increased by decreasing the volume of the supernatant fluid in which the organisms are suspended after centrifugation, and this might result in more rapid recoveries after drying. Similar results to those shown here were obtained when glucose was added to the suspension of the organism before it was delivered into the ampoules. Such a procedure is simpler, in that it involves only a single charging of the ampoules, but the technique as described here does constitute a convenient method for testing a range of preserving media in the one experiment.

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