ADDENDUM

The Survival of Bacterial Spores in Arsphenamines

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Sterility tests carried out by us over many years have indicated that bacteria can be found in arsphenamines prepared without aseptic precautions and that they may remain viable for years in contact with the drug and under nitrogen. It seemed desirable to make some study of the virulence of such organisms and the following work was initiated for this purpose. It was obviously desirable to use an organism which would survive a drying process and which was pathogenic to small animals, and since members of the *Clostridium* group have frequently been found responsible for deaths after infection (see Coulthard and Sykes[1]) it was decided to use one of this group if possible. One difficulty with the clostridia is that some do not regularly produce spores, but after a prolonged search, we found that a *Clostridium septicum* isolated from a sheep by Mr. J. E. K. Lineham, at that time of our Veterinary Research Division, appeared to be suitable. It spored fairly readily on coagulated blood-serum and remained virulent on drying.

Experimental

The *Cl. septicum* was grown anærobically on coagulated blood-serum slopes at 37° C. for 24 hours. The growth, containing many spores, was suspended in Ringer solution and distributed in 0·2 ml. amounts into 5-ml. ampoules, which were immediately dried under vacuum at 50° C. Neoarsphenamine B.P. (Boots) 0·6 g. was then dispensed into each ampoule and these, after being filled with nitrogen, were sealed. These ampoules, half of which were stored at room temperature and half at 4° C., were tested for surviving bacteria at intervals by animal inoculation.

TABLE I

Survival of virulent bacterial spores in ampoules of neoarsphenamine under nitrogen

Test	Storage time of ampoules (weeks)	Dose of neoarsphenamine per mouse mg.	Method of sampling	Mice killed by injection from infected ampoule stored at:	
				Room temperature	4° C.
1	0	5·4	A	+	
2	0	5·0	B	+	
3	1	3·1	A	+	
4	1	4·0	A		+
5	2	5·0	B	+	
6	2	5·0	B		+
7	2	3·25	A	+	
8	2	2·26	A		+
9	14	5·0	B	+	
10	14	5·0	B		+
11	26	5·0	B	+	
12	26	5·0	B		+
13	48	?	A	+	+

A = Contents decanted and ampoule tested. B = Aliquot of entire contents tested.
Of the groups of 5 mice injected in each test, all died except in tests 1, 4, 9 and 10, where 4 out of 5 died. The presence of *Cl. septicum* was confirmed by autopsy and culture of at least one mouse from each test.

At first, to reduce the dose of neoarsphenamine, the major portion of the contents of the ampoule was decanted. The ampoule was then rinsed with 1·0 ml. of distilled water and 0·2 ml. of this injected intramuscularly into each of 5 mice. It was subsequently found possible to modify this procedure by dissolving the entire contents of the ampoule in 24 ml. of distilled water and injecting 0·2 ml. aliquots into each of 5 mice. To ensure that the deaths were not due to the toxicity of the drug a corresponding dose of sterile neoarsphenamine was injected into a control group of animals. When the first procedure was adopted this dose was calculated from the difference in the weight of the ampoule.

As a further control to make certain that the deaths were due to the multiplication of organisms and not to toxins carried over, autopsies were performed on representative mice and the presence of typical *Cl. septicum* confirmed by culture and animal inoculation.

Results

The results which are given in Table I show clearly that the dried *Cl. septicum* spores retained their virulence in contact with neoarsphenamine and under nitrogen for at least 48 weeks.

Summary

1. Spores of *Cl. septicum* in sealed ampoules of neoarsphenamine B.P. have been shown to survive and retain their virulence for at least 48 weeks.

Reference

1. Coulthard and Sykes, *Pharm. J.*, 1936, **137**, 39.