A NOTE ON THE PRESERVATION OF INFLUENZA VIRUS

By D. I. ANNEAR and T. S. L. BESWICK*
Department of Pathology, University of Cambridge

(With 1 Figure in the Text)

The main object of the experiment which this note describes was to determine whether influenza virus could be preserved by the 'peptone-plug' method of drying which has recently been found to be very effective for bacteria (Annear, 1956). Two other methods of preservation were also compared in parallel with it: (*a*) the centrifugal freeze-drying technique (Fry & Greaves, 1951) and (*b*) deep-freezing.

In the peptone-plug method of preservation, very small volumes (single drops) of suspension are dried in individual ampoules. Similar aliquots were therefore preserved by the other two methods, although the volume of the preserving medium to which these single drops were added was different in each case. The same preserving medium was used for each drying method.

METHODS

The virus dried was the PR8 strain of Influenza A and the material used was freshly harvested allantoic fluid from eggs infected 48 hr. previously. The single-drop inocula (0·02 ml.) were dispensed in the following manner:

(*a*) Into 5 ml. volumes of serum broth (10% horse serum in nutrient broth); these were used for the titration of the virus which was done immediately and in duplicate.

(*b*) Into 5 ml. volumes of serum broth contained in ampoules; these were immersed immediately in an alcohol bath at $-20°$ C. Later the ampoules were sealed and stored in a dry-ice cabinet.

(*c*) Into 0·25 ml. volumes of 6% peptone (Evans) + 0·5% starch contained in freeze-drying tubes. These tubes were subjected immediately to centrifugal freeze-drying in a small L5 unit (Edwards High Vacuum Ltd.).

(*d*) Into peptone-starch plugs (1·5 ml.) made from the same solution as used in (*c*) above. Each plug was exposed to high vacuum as soon as it had been inoculated (drying method 2; Annear, 1956).

After about 4 hr. primary drying in the centrifugal freeze-drying machine the desiccates were transferred to the manifold on which the peptone plugs were being dried. Then all tubes were constricted one at a time, returned to the manifold and finally sealed *in vacuo* after a further 20 hr. drying. The desiccates were stored in the dark at room temperature.

* Present address: Biological Standards Control Laboratory, Medical Research Council Laboratories, Holly Hill, Hampstead, London, N.W. 3.

https://doi.org/10.1017/S0022172400044788 Published online by Cambridge University Press

The titration of the virus for infectivity was carried out along conventional lines. After reconstituting the dried material and making the volumes up to 5·0 ml., which gave an initial dilution of 1/250, tenfold dilutions of the suspensions were made in serum broth. Ten-day-old fertile eggs were inoculated with 0·2 ml. volumes of the dilutions (six eggs at each dilution). The eggs were incubated for 3 days, chilled overnight at 4° C. and the allantoic fluids harvested. The haemagglutination tests were carried out by adding one drop (0·02 ml.) of each allantoic fluid to 0·5 ml. of a 0·5 % suspension of human erythrocytes (Group O) in small tubes, and the tubes were read after 1 hr. at room temperature.

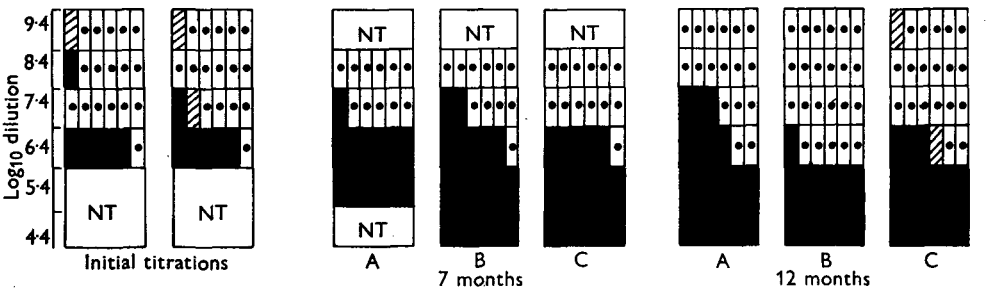

Fig. 1. Titrations of Influenza A virus before and during preservation. A, preserved by deep-freezing; B, preserved by drying in peptone plugs; C, preserved by centrifugal freeze-drying. NT = not tested. ■ Positive. ⊡ Negative. ▨ Dead.

RESULTS AND DISCUSSION

The results of the haemagglutination tests are shown in Fig. 1. The calculation of titres for such a small number of observations would be of doubtful value; useful information may, nevertheless, be gained from a consideration of the results shown in the figure. The actual infectivity titre of the original allantoic fluid was about $4{\cdot}3 \times 10^7$ ID_{50} per ml.

The titre of the original material was rather lower than was expected, hence the lack of information for the lower dilutions of these titrations. However, the results obtained at the dilutions tested make it clear that the titre cannot have been significantly higher than that of the frozen material tested 7 months later.

After 7 months' storage there was little if any loss of infectivity in any of the stored preparations. The results at 12 months indicate an undiminished activity for the frozen virus and a questionable fall in titre for the two dried preparations with little, if any, difference between them.

Peptone appears to be a very satisfactory medium in which to dry influenza virus. A similar observation was made for vaccinia virus by Collier (1955) in his extensive studies on the development of a stable smallpox vaccine.

There is very little quantitative information to date on the survival of influenza virus in the desiccated state. The investigations carried out by Greiff, Blumenthal, Chiga & Pinkerton (1954) have furnished a great deal of data on the physical factors which influence the survival of the virus during freezing and thawing and during drying from the frozen state, but their data include no results from stored desiccates. It would appear both from the work of Greiff *et al.* and from the results

published here that the virus may be dried under a variety of conditions with little or no loss in infectivity, and that the storage of the desiccated material at room temperature is a satisfactory and useful means of preserving it.

One of us (D.I.A.) is indebted to the Medical Research Council for a personal research grant.

REFERENCES

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

COLLIER, L. H. (1955). The development of a stable smallpox vaccine. *J. Hyg., Camb.*, **53**, 76.

FRY, R. M. & GREAVES, R. I. N. (1951). The survival of bacteria during and after drying. *J. Hyg., Camb.*, **49**, 220.

GREIFF, D., BLUMENTHAL, H., CHIGA, M. & PINKERTON, H. (1954). The effects on biological materials of freezing and drying by vacuum sublimation. II. Effect on influenza virus. *J. exp. Med.* **100**, 89.

(*MS. received for publication* 8. v. 56)