EFFECT OF SUSPENDING MEDIA ON THE FREEZE-DRYING AND SUBSEQUENT STORAGE OF ECBO VIRUS SEROTYPE VG(5) 27

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Animal enteroviruses comprise approximately 20% of more than 500 animal viruses maintained by the American Type Culture Collection. From an economic viewpoint preservation of viruses by freeze-drying, where it can be done successfully, is preferred to maintenance in the frozen state because of the greater convenience of handling and storage of dried cultures. The virus used in the present study was an animal picornavirus, ECBO virus serotype VG(5)27 (McFerran, 1958). In the past enteroviruses have been found to be extremely difficult to freeze-dry successfully and hence it was considered that an appraisal of a wide variety of suspending media, which have been used effectively in the freeze-drying of other viruses, would give an indication of media worth examining in more detail.

It was found that an aqueous solution of 1M tris pH8.5 (Berge, Jewett and Blair, 1971) provided excellent protection for VG(5)27 virus during the freeze-drying cycle although subsequent stabilisation during storage at 4°, 20° and 37°C was poor. In order to overcome this problem low molecular weight species were removed from VG(5)27 virus infected tissue culture fluid by either ultracentrifugation or ultrafiltration prior to freeze-drying the virus in 1M tris pH8.5. Although both techniques lowered the sodium ion concentration by approximately 1000 fold there was no marked improvement in VG(5)27 virus stability during storage (Fig. 1).

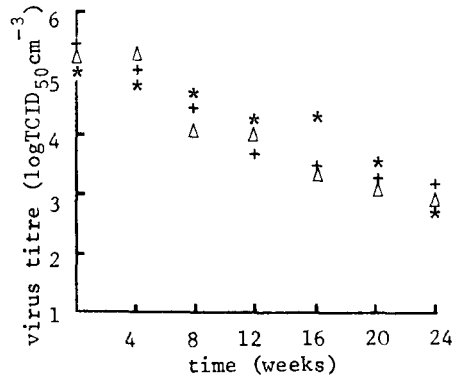

Fig. 1. Showing the stability of VG(5)27 virus freeze-dried in 1M tris pH8.5 and stored at 4°C.

virus infected tissue culture fluid (control) Δ

ultracentrifuged virus *

ultrafiltered virus +

The combination of 1M tris pH8.5 with other additives (5% w/v magnesium chloride, 1% w/v cysteine, 7.5% w/v sucrose) resulted in an increased retention of VG(5)27 virus infectivity during storage. Chemical modification of tris buffer, prior to its use as a suspending medium, led to the complete loss of its protective action. The use of various other buffers (1M boric acid pH8.5, 1M glycine pH8.5) was found to be completely ineffective in protecting VG(5)27 virus during the freeze-drying cycle. Therefore, it is possible that the stabilising action of 1M tris pH8.5 is due to a specific interaction with the virus particle rather than the general protective effect which is attributed to most suspending media (Orndorff and MacKenzie, 1973).

Berge, T.O., Jewett, R.L. and Blair, W.O. (1971). Appl. Microbiol., 22, 850-853.
McFerran, J.B. (1958). Vet. Rec., 70, 49.
Orndorff, G.R. and MacKenzie, A.P. (1973). Cryobiology, 10, 475-487.