Influence of Salts on Foot-and-Mouth Disease Virus

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ABSTRACT

FELLOWES, O. N. (Plum Island Animal Disease Laboratory, Greenport, N.Y.). Influence of salts on foot-and-mouth disease virus. Appl. Microbiol. 14:206–211. 1966.—The effect of sodium and magnesium chloride in 1 and 2 M concentration at temperatures of 37 and 50 °C on type C, strain 149, foot-and-mouth disease virus during storage for 6 days was studied. The exclusively passaged cattle strain and its tissue culture-adapted line were compared. Preparations of the various chemicals and their concentrations were made directly in suspensions of the virus, which, together with untreated control virus suspensions, were stored at indicated temperatures and tested daily for concentration of virus present. Both 1 and 2 M concentrations of Mg markedly slowed the degradation of the bovine-passaged virus, as compared with untreated virus stored at 37 or 50 °C. Such was not the case with 1 and 2 M concentrations of Na at 37 and 50 °C, in which instance the treated virus was degraded faster than the untreated controls at 37 °C, and but slightly influenced at 50 °C. The tissue culture-adapted virus at the 25th passage was not stabilized by any concentration of chemical additive either at 37 or 50 °C, except for 1 and 2 M concentrations of Na at 37 °C, which partially retarded degradation of the virus. After 91 passages of the virus in tissue culture, only a suggestion of the influence of 1 and 2 M concentrations of Na at 37 °C remained to show a stabilizing effect. These responses tend to separate the bovine-passaged virus from the tissue culture-adapted virus under the conditions of this study.

It becomes more and more important to define the environment in which a line or type of virus has been maintained since its initial isolation. The hosts in which the virus has been propagated or the artificial media in which the virus has been cultivated may modify attributes or properties of an infectious agent so treated. It has been reported that the presence of monovalent or divalent cations, at various concentrations, will stabilize enteroviruses for varying periods of time when heated at 37 or 50 °C (9). With other viruses, the effect of certain cations and heat will degrade the virus (11). With polioviruses, the cation effect with heat has served to distinguish between the attenuated and virulent strains of the same virus (10). The objective of this study was to determine the influence of an environment containing sodium or magnesium chloride at 1 and 2 M concentration on cattle-propagated and tissue culture-adapted line of the same virus stored at 37 and 50 °C.

MATERIALS AND METHODS

Virus. The model virus was type C, strain 149, maintained at Pirbright Laboratories in England for some 30 years by passage in cattle. At Plum Island, the virus was used in its 5th cattle passage and 25th and 91st tissue culture passage.

A 10% suspension of infected tongue epithelium was prepared by grinding tissue in a mortar and diluting with tissue culture maintenance fluid containing Hanks’s salt solution, lactalbumin hydrolysate, normal bovine serum, phenol red, and small amounts of penicillin and streptomycin. The supernatant fraction of the suspension, after centrifugation at 900 \( \times \) g for 20 min, was recovered. Additional antibiotics to the amount of 250 units of penicillin and 250 \( \mu \)g of streptomycin per ml were added, and the pH was adjusted to 7.5 with 1 N NaOH. The pool of virus in 100-ml amounts was stored at −50 °C. The virus concentration varied from \( 10^{4.4} \) to \( 10^{6.4} \) mouse LD\(_{50}\) per milliliter.

Tissue culture virus was prepared from cultures of calf kidney cells growing as monolayers in Povitsky bottles. The technique of Patty and May (6) was used. The pool of virus in 100-ml amounts was stored at −50 °C. The virus concentration varied from \( 10^{5.8} \) to \( 10^{6.4} \) plaque-forming units (PFU) per ml.

Treatment of virus. Working volumes of 80 to 100 ml of the thawed virus suspensions were used. Sufficient MgCl\(_2\) or NaCl was added to individual volumes of virus to make a 1 or 2 M concentration. The solution of the salt in the medium was effected readily without the use of heat and with regard for volume expansion. Controls without additives were included.
and all preparations were dispensed in 4-ml amounts into Pyrex ampoules which were flame-sealed. Samples of each combination of reactants were placed at 37 and 50 °C for storage and were assayed daily for a period of 6 days for the concentration of surviving virus.

**Virus assay.** The virus concentration in tissue culture samples was determined by plaque assay on monolayers of calf kidney cells according to the general method of Bachrach et al. (1). The virus in tissue suspension was assayed for lethal effect by inoculation of serial 10-fold dilutions intraperitoneally into suckling mice, with the use of 10 mice per dilution.

**Procedure.** Each experiment was repeated three times at different intervals of time to get replicate sets of data for each cation, cation concentration, and storage temperature. Considering the method of assay and the use of 10-fold dilutions of samples, values differing by no more than 1 log in range for the same experiment were included in the mean PFU per milliliter. The mean PFU per milliliter concentration was plotted on graph paper to depict the survival of the virus in time and concentration while under the influence of the environment being tested.

**Results**

In Fig. 1 and 2, the 1 and 2 M concentrations of MgCl₂ slowed the rate of degradation of the cattle-passaged type C, strain 149 virus at both 37 and 50 °C storage temperatures for a period of 6 days, as compared with untreated control virus. The 1 and 2 M concentration of NaCl did not exhibit this same influence on virus at 37 °C, but did show a diminished slowing effect on the virus loss at 50 °C storage, as depicted in Fig. 3 and 4.

The 1 and 2 M concentrations of MgCl₂ did not slow the inactivation of virus in its 25th tissue...
tissue culture passage at 37 C storage, but had no such effect at 50 C storage, as indicated in Fig. 6 and 7. The effect of 1 M NaCl was greater than 2 M on the virus at 37 C.

![Figure 4](image1.png)  
**Fig. 4.** Monovalent cation effect on virus from fifth bovine passage. Symbols: $\triangle = $ virus control, 37 C; $\Delta = $ virus control, 50 C; $\times = 1$ M Na, 37 C; $\bigcirc = 1$ M Na, 50 C.

![Figure 5](image2.png)  
**Fig. 5.** Divalent cation effect on virus from 25th tissue culture passage. Symbols: $\triangle = $ virus control, 37 C; $\Delta = $ virus control, 50 C; $\times = 2$ M and 1 M Mg, 37 C; $\bigcirc = 2$ M and 1 M Mg, 50 C.

![Figure 6](image3.png)  
**Fig. 6.** Monovalent cation effect on virus from 25th tissue culture passage. Symbols: $\triangle = $ virus control, 37 C; $\Delta = $ virus control, 50 C; $\times = 2$ M Na, 37 C; $\bigcirc = 2$ M Na, 50 C.

![Figure 7](image4.png)  
**Fig. 7.** Monovalent cation effect on virus from 25th tissue culture passage. Symbols: $\triangle = $ virus control, 37 C; $\Delta = $ virus control, 50 C; $\times = 1$ M Na, 37 C; and 1 M Na, 50 C.
Figures 8 and 9 show that the untreated virus control at 37 C from the 91st tissue culture passage contained resistant virus exceeding in titer and persistence that of the treated virus at either 1 or 2 M concentrations of MgCl₂ at 37 or 50 C. The 1 and 2 M concentrations of NaCl still exhibited a slight slowing effect on loss of 91st tissue culture passage virus at 37 C storage, compared with the same treatment of 25th passage tissue culture virus and with untreated controls of 91st tissue culture passage virus. The 1 M concentration of NaCl had the greater effect, as indicated in Fig. 10 and 11.

For purposes of comparison, some infective levels of various passages in both tissue culture and cattle, as assayed in both tissue culture and suckling mice, are shown in Table 1. There is little difference shown between PFU and LD₅₀ values obtained in tissue culture and mice when the same sample is tested for infectious activity in both media.

**DISCUSSION**

Gradually, the means of identifying certain viruses by characteristics or markers has emerged. Foot-and-mouth disease virus (FMDV) has been found to possess infectious ribonucleic acid (2, 5). Like other small ribonucleic acid viruses, it is resistant to ether. Virus exclusively maintained in cattle can possibly be distinguished from the...
same strain adapted and grown in tissue culture by other than serological means. The virus adapted to growth in tissue culture had lost its ability to survive treatment by 1 and 2 mM MgCl₂ at 37 and 50 °C over a period of 6 days, as compared with the same strain maintained in bovine passage. This effect seems to be due to the cation rather than the anion of the chemical additives, since the chloride anion was common to all. The rapid inactivation of the tissue culture virus under these conditions might lend itself to the production of an antigen to be used in immunizing or diagnostic procedures.

The preservation of bovine FMDV by a concentration of salt as high as 25% has been known for many years (7). This property is also possessed by mengo, encephalitis, and polio viruses heated in hypertonic salt solution (8). In studies performed in this laboratory, suspensions of bovine tissue and tissue culture containing type A, strain 119 FMDV were stored for 6 days at 37 °C without additives. The tissue culture virus survived better than did the tongue tissue virus (4). Exposure of virus in tissue suspension to higher temperatures has revealed infectious virus present after 4 hr at 84 °C but not at 6 hr for 85 °C (3). In Fig. 8 and 9, the untreated virus control preparations (both tissue culture lines) seem to have heat-resistant members present in the FMDV population. It is possible, therefore, to have heat-resistant virus in the population of virus being tested on a random basis.

From these data, it is now reasonable to theorize that survival of bovine FMDV at higher temperatures (50 °C) is conditioned by at least two factors: the random presence of heat-stable virus in the population or the presence of 1 or 2 mM MgCl₂ in the medium. The first factor can, of course, be detected by the virus control when exposed to higher temperatures.

The choice of type C, strain 149, FMDV as a model was highly influenced by the knowledge that it had been maintained exclusively for 30 years in cattle. The cattle strain of FMDV, when well adapted to growth in tissue culture, will exhibit a PFU value approximating very closely the LD₅₀ value in suckling mice. However, in this instance, so as not to modify any attributes of the cattle-passage type C, strain 149 by testing in an artificial medium, the assays of such tissue suspensions were performed in mice. It is noted that at the 25th tissue culture passage level the virus had already lost its ability to be stabilized against heat treatment by magnesium chloride. It may be that this characteristic is a desirable one or is a marker for some other wanted attribute, in which case it would be necessary to stop serial passage of the virus in tissue culture at some earlier level. It would be interesting to determine if the virus from the 91st tissue culture passage when passed one or more times in cattle would regain its lost characteristics with regard to magnesium chloride. This is being done and the results will be reported at a later time.

**Addendum in Proof**

The return of the 91st tissue culture passage virus to two cattle by the lingual route did not change the
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characteristics of the virus, with regard to Mg and Na ions, to those described for exclusively passaged bovine virus.

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LITERATURE CITED


