Stabilization of Hog Cholera Virus by Dimethyl Sulfoxide

J. Tessler, W. C. Stewart and J. I. Kresse*

ABSTRACT

The stability of hog cholera virus through five freeze-thaw cycles in the presence and absence of dimethyl sulfoxide was studied. In the absence of dimethyl sulfoxide the hog cholera virus titer was reduced 52% to 91% following successive freezing and thawing cycles. However, when dimethyl sulfoxide was added to the viral suspension the virus titer appeared to remain the same after the same number of freezing and thawing cycles.

RÉSUMÉ

Cette expérience visait à étudier la stabilité d'une souche du virus de la peste porcine, soumise à cinq gels et dégels successifs, tant en présence qu'en l'absence de sulfoxyde d'éthane. En l'absence de ce produit chimique, la suspension virale subit une perte de vitalité de l'ordre de 52 à 91%. Par ailleurs, la présence de sulfoxyde d'éthane dans la suspension virale lui pemit de conserver toute sa vitalité.

*National Animal Disease Center, North Central Region, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010 (Tessler) and National Animal Disease Center, Veterinary Services Laboratories, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, Ames, Iowa 50010 (Stewart and Kresse).

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Melnick (4) reported earlier that enveloped viruses stored at –90°C or –20°C lost infectivity when they were subjected to successive freezing and thawing cycles. Wallis and Melnick (6) solved this problem in the herpes virus system by the addition of dimethyl sulfoxide (DMSO). The purpose of the present study was to determine a method of maintaining hog cholera virus (HCV) at –20°C without consequent loss in titer.

The HCV used was the noncytopathogenic strain A (5). Stock virus was propagated in 75 cm^2 plastic flask[a] cultures of confluent PK-15 cells containing Earle's balanced salt solution and 5% specific pathogen free (SPF) calf serum. The cultures were incubated at 37°C for three days, then frozen and thawed. Virus was centrifuged at 2,000 x G for 15 minutes at 4°C and divided into two aliquots. Dimethyl sulfoxide was added to one aliquot to make a 10% concentration. The other aliquot remained unchanged. Both aliquots were stored at –20°C. Then each aliquot was thawed after two days and viable virus content was assayed in PK-15 cell cultures in Leighton tubes as described by Carbrey *et al* (1, 2). The samples were frozen promptly and stored again at –20°C. Subsequently, aliquots were thawed and titrations were performed on days 4, 11, 16 and 18. In the absence of DMSO, the HCV virus titer was reduced 52% to 91% (Table I)

[a]Falcon Plastics, Oxnard, California 93030.

TABLE I. Stability of Hog Cholera Virus (Strain A) in 5% SPF Calf Serum and 10% Dimethyl Sulfoxide Stored at −20°C

Freeze and Thaw Cycle	Virus Titer[a] in 10% Dimethyl Sulfoxide (DMSO) x 10^6	Virus Titer in 5% SPF Calf Serum x 10^6	Viral Loss as Shown by Difference Between DMSO and Calf Serum
Cycle 1 Day 2	6.20	3.00	52%
Cycle 2 Day 4	9.50	4.10	57%
Cycle 3 Day 11	2.00	0.45	78%
Cycle 4 Day 16	3.90	0.40	90%
Cycle 5 Day 18	4.70	0.42	91%

[a]Immunofluorescence plaque forming units/ml

through five successive freeze-thaw cycles. However, in the presence of 10% DMSO there was no loss in titer through the five cycles.

These findings are similar to those of Wallis and Melnick (6) with herpes viruses. The study indicates also that an ultra low temperature freezer may not be required for storage of HCV. Other laboratories may wish to study their enveloped viruses in a similar way. The mechanism of action of DMSO is still not clear. Lovelock (3) hypothesized that DMSO stabilized the lipoprotein complexes. Eventually, biophysicists may derive an explanation so that virologists can apply DMSO to other problems with enveloped viruses.

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BOOK REVIEW

AN ATLAS OF MAMMALIAN CHROMOSOMES. VOLUME 8. *T. C. Hsu and K. Benirschke. Published by Springer-Verlag, New York. 1974. Price $19.80.*

This folio contains karyotypes of animals, the majority of them being of the order Rodentia. In addition, however, there are karyotypes of some of the less common species of Marsupials, Insectivores and Cetaceans. As in previous volumes, the karyotypes are of good quality and credit for their preparation is included.

The karyotype of *Peromyscus maniculatus* is included, but readers should heed the authors' caution in that *P. maniculatus* contains many subspecies in which there is a great deal of variation in the morphology of the chromosomes. Incidentally, the various subspecies of *P. maniculatus* could possibly be used in a study of the changes in chromosome morphology within a species. — *Y.S. Moon.*