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Stabilization of Enveloped Viruses by Dimethyl Sulfoxide

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In a recent review on the storage of animal viruses at subzero temperatures (8), it was pointed out that enveloped viruses present special problems. For example, herpesvirus even at −90 C loses infectivity after prolonged storage and at −20 C is even more labile than at 4 C. Repeated freezings and thawings of this virus result in marked loss in activity. Members of the herpesvirus group can be stabilized to some degree at freezing temperatures by suspending the viruses in serum, glycerol, sorbitol, sucrose, skim milk, or other proteins (1, 3, 11, 13, 14). These stabilizers are believed to act by preventing the formation of destructive ice crystals. The stabilizers of herpesviruses are also recognized as preservatives for cultured cells in the frozen state. The protective effects of sugars and proteins on cells have been attributed to the action of these compounds in lowering the effective concentrations of electrolytes, and thus preventing the denaturation of the cells by the toxic effects of concentrated salts and solutes, or by preventing the formation of intracellular ice crystals (4–7, 9). Since the infectivity of enveloped viruses may depend on the integrity of the envelope derived from the host cell (2, 10, 12), their preservation during freezing may require treating the viruses as if they were cultured cells. This led us to investigate dimethyl sulfoxide (DMSO), as a preservative for enveloped viruses, because this compound is the current preservative of choice for cultured cells.

All viruses used in this study were grown in green monkey kidney (GMK) cells maintained with protein-free medium B (0.5% lactalbumin hydrolysate in Earle's salt solution). Viruses were assayed in GMK cultures using the plaque-forming unit (PFU) method. Herpesvirus (JES strain) was used as a model agent. Other enveloped viruses used were measles (Edmonston strain), Sindbis (ar 334 strain), and vesicular stomatitis virus (Indiana strain). Nonenveloped viruses studied were vaccinia (WR strain), adenovirus (SV15 strain), and poliovirus (type 1 Mahoney strain).

Since repeated freezing and thawing are deleterious to enveloped viruses, DMSO was compared with fetal calf serum, which has well known preservative properties. Figure 1 shows the results of repeated freezing and thawing on undiluted herpesvirus (control) and on virus in the presence of DMSO and of serum. Herpesvirus was inactivated twofold after a single freezing and thawing; after four cycles of freezing and thawing, a 10-fold decrease in titer was manifested. Fetal serum conferred protection, but not as well as DMSO.

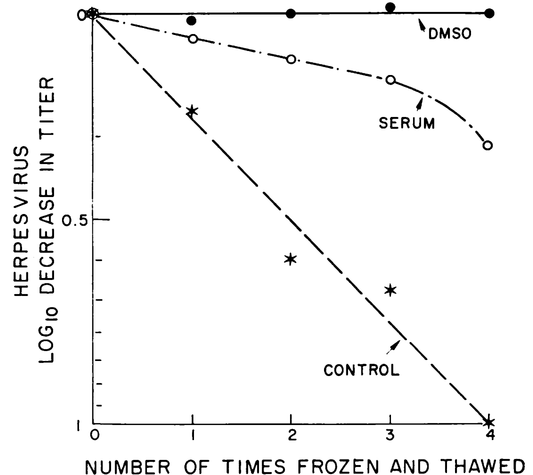

FIG. 1. *Nine parts of undiluted herpesvirus were mixed with one part of (i) distilled water (control), (ii) undiluted fetal serum, and (iii) undiluted dimethyl sulfoxide. Representative samples were frozen by reducing the temperature from 25 to −40 C in about 20 min. The virus was thawed rapidly at 37 C (10 to 20 sec). Control samples were held at 4 C and shifted to 37 C with the frozen and thawed samples; they showed no loss in titer and are not shown in the figure.*

A second series of experiments was conducted with enveloped viruses diluted at least 1,000-fold beyond the protective effects of materials in the culture harvests. Nonenveloped viruses were also tested (Table 1). The enveloped viruses were completely stabilized by DMSO against repeated freezing and thawing, whereas control samples free of protective agents were completely inactivated. Serum partially spared the enveloped

TABLE 1. *Effects of repeated freezings on enveloped and nonenveloped viruses*

Virus[a]	Concn of virus harvest	Avg no. of PFU/0.1 ml of virus			
		Controls	Frozen and thawed four times in		
			Distilled water	10% Serum	10% DMSO
Enveloped					
Herpes	10^{-3}	1,200	0	320	1,150
Measles	$10^{-2.5}$	940	0	100	890
Vesicular stomatitis	10^{-4}	1,880	0	700	1,800
Sindbis	10^{-4}	750	0	50	700
Nonenveloped					
Type 1 polio	10^{-4}	2,000	1,800	2,100	1,900
Adeno	$10^{-4.5}$	1,950	1,900	1,700	1,750
Vaccinia	10^{-4}	800	750	840	810

[a] Viruses were diluted in distilled water to contain about 2,000 PFU/0.1 ml and were then mixed with an equal volume of (i) distilled water, (ii) 20% fetal serum, and (iii) 20% DMSO. Representatives samples were frozen and thawed with the methods described in Fig. 1.

viruses but not nearly as well as DMSO. Concentrations of DMSO as low as 5% effectively protected the enveloped viruses against the trauma of freezing, but lower concentrations were less efficient. The nonenveloped viruses were not inactivated by freezing and thawing even in the absence of protective agents. The stable characteristics of these viruses are well known (8), and they afford no problems in storage.

The inactivation of herpesvirus and other enveloped viruses by freezing apparently is caused by denaturation of the host-cell component surrounding the viruses. Lovelock (6) proposed that the electrolyte disturbances associated with freezing of cell suspensions can result in the dispersion of lipids and lipoproteins from cell membranes, leading to permeability changes and cellular dissolution. He has further shown that lipoproteins are highly sensitive to freezing and that DMSO stabilizes lipoprotein complexes. Thus, if similar changes take place in virus envelopes which also contain essential lipoproteins (presumably derived from the host-cell membrane), one should expect enveloped viruses to be inactivated unless a membrane preservative is present.

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