

Short Communication

Heat Stability of the Lyophilized Sabin Poliovaccine

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SUMMARY: In this study we lyophilized three types of live attenuated polioviruses (Sabin vaccine strains) and evaluated the lyophilized vaccine viruses' heat stability. The virus titers were measured after heating at 37°C and 45°C and then compared with the titers of conventional liquid vaccine viruses similarly treated. The results showed that lyophilization, while slightly reducing vaccine virus titers, had a far greater sparing effect on subsequent heat inactivation of lyophilized vaccine viruses, thus demonstrating its validity for the improvement of the vaccine.

Live attenuated poliovaccine is known as one of the most heat-labile vaccines currently in use (1).

Presently, in order to increase its heat stability, a high concentration of MgCl₂ (1 molar) or sucrose or sorbitol (35-70%) is used for the preparation of the vaccine by manufacturers, and a reduction in vaccine potency of ~0.5 log₁₀ after 1 week of incubation at 37°C has been adopted by many manufacturers as a customary standard. These stabilizers, while effective for long storage of the vaccine virus at relatively low temperatures (2-8°C), are not effective for lengthy exposure to higher temperatures (>37°C). Therefore, full provision of "the cold chain" is indispensable in tropical countries, in order to prevent the loss of potency of the vaccine by exposure to higher ambient temperatures during transportation and storage until use in vaccination programs in such countries (2).

In addition to heat sensitivity, enteroviruses, including poliovirus, are known to be very labile to lyophilization. In fact, lyophilization of these viruses has been unsuccessful so far in almost all trials conducted (3-7). Until recently, we have investigated the possibility of lyophilization of live attenuated polioviruses (Sabin strains of types 1, 2, and 3) in order to develop a more heat-stable poliovaccine (8,9). The results showed that lyophilization of vaccine viruses is feasible and that lyophilizing medium consisting of 2.5% (wt/vol) sorbitol, 0.06-0.1% glutamine, and 0.01% BSA and a reconstituting medium containing 5-10% polypeptone S and 10-15% sucrose greatly increased the heat stability of the vaccine viruses. In the present paper, the heat stability of the aforementioned lyophilized vaccine was compared with that of the liquid vaccine suspended in the same solution as is used for the current vaccine preparation by measuring titers of vaccine viruses after long time exposure to higher temperatures (at 37°C for 1 and 3 weeks and at 45°C for 1 week).

The types 1, 2, and 3 Sabin vaccine viruses (strains LS-c, 2ab; P712, Ch, 2ab; and Leon, 12a,b; respectively) used in this study had been thrice passaged in primary monkey kidney cell cultures from Sabin's original viruses and were kindly

provided by the Japan Poliomyelitis Research Institute, Tokyo. Based on our previous study (9), lyophilizing medium consisting of 2.5% sorbitol, 0.06% glutamine, and 0.01% BSA was used to suspend the viruses before lyophilization. Eagle's basal medium supplemented with 35% sucrose (kindly provided by the Japan Poliomyelitis Research Institute), which is employed in the currently used vaccine preparation (current vaccine medium), was used as control medium. One volume of each vaccine virus was suspended in 100 volumes of either of the mediums mentioned above.

The viruses (0.5 ml in volume) were lyophilized in a small vial using a freeze-drier (Modulio EF-4, Edwards, Crawley, Sussex, England) equipped with a rotary vacuum pump (E2M5, Edwards). Lyophilization consisted of two stages: first, 3 days at -45°C, and second, 2 days at -5°C. During lyophilization, the vacuum level was kept below 6.0×10^{-2} hect pascals (hPa) and the temperature of the trap chamber was maintained at -65°C. After lyophilization, small vials containing viruses were rubber-stoppered in a vacuum, then tightly sealed with an aluminum cap under normal air pressure. Small vials containing the lyophilized viruses and others containing viruses suspended in the current vaccine medium (0.5 ml) were put into larger dark brown-colored bottles and submerged and incubated for 1 or 3 weeks in a 37°C water bath or for 1 week in a 45°C water bath. After incubation, the vials were stored at below -30°C until tested. The lyophilized viruses in the small vials were dissolved with a 0.5-ml solution containing 10% polypeptone S and 15% sucrose (reconstituting medium) just before virus titration(9).

After heating for a definite period of time, virus titers were measured by means of plaque assay using monolayers of GMK2 (established green monkey kidney) cells formed in a 6 well-tissue culture plate (BD Biosciences, Billerica, Mass., USA, well diameter: 3.5 cm) as described previously (10). Dilutions of viruses from a vial were inoculated onto more than 6 wells per dilution (inoculum size: 0.05 ml). Virus titer was expressed as a logarithm (log₁₀) of plaque forming unit (PFU)/0.05 ml. In order to obtain accurate virus titers under different treatments, virus titers from more than 10 vials were usually measured for each treatment.

After each lyophilization treatment and heating of types 1, 2, and 3 vaccine viruses, more than 10 treated samples (vials) were subjected to infectivity assay. Based on the infectivity titers (expressed in log₁₀ PFU/0.05 ml) of samples after treatment, geometric mean titer and geometric standard deviation

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were computed. In order to make easily comparable the log reduction of virus titers by heat treatments between lyophilized viruses and viruses suspended in the current vaccine medium, all of the means of virus titers (geometric mean titer) before heat treatment were adjusted to 0 log₁₀. On this basis, the log reduction in titer after each treatment was calculated and expressed by the mean log reduction ± geometric standard variation.

As shown in Figure 1A, in the conventional medium, type 1 vaccine virus showed a log titer reduction of 1.09 and 3.54 after heating at 37°C for 7 days and 21 days, respectively, while the inactivation of virus by heating at 45°C for 7 days was the highest, showing a 4.37 log₁₀ reduction. In contrast, lyophilization treatment, while lessening vaccine virus titer by 0.52 log₁₀ by itself, had a markedly sparing effect on the subsequent heat inactivation of lyophilized vaccine virus: the reduction in titer by heating at 37°C for 7 days and 21 days remained 0.48 log₁₀ and 1.0 log₁₀, respectively, and that at 45°C for 7 days remained 1.29 log₁₀. Geometric standard deviation of the virus titers after heat treatment showed a wider distribution of the titers for lyophilized vaccine virus than for virus suspended in conventional medium, with the exception of those after 7 days at 45°C.

As for type 2 vaccine virus (Fig. 1B), the reduction of virus titer in the conventional medium after heating at 37°C for 7 days and 21 days and heating at 45°C for 7 days was 0.97 log₁₀, 3.96 log₁₀, and 4.09 log₁₀, respectively. While lyophilization reduced the virus titer by 0.68 log₁₀, type 2 virus after lyophilization was very stable in heat, as was type 1 virus: the log titer reduction of lyophilized type 2 virus after 7 days' and 21 days' incubation at 37°C and 7 days' incubation at 45°C was 0.57 log₁₀, 0.77 log₁₀, and 1.44 log₁₀, respectively.

As for type 3 (Fig. 1C), both the virus suspended in liquid medium and lyophilized virus were more heat-labile than their counterpart type 1 and type 2 viruses. In conventional medium, the reduction in titer after 7 days and 21 days at 37°C and 7 days at 45°C was 1.75 log₁₀, 4.52 log₁₀ and 5.13 log₁₀, respectively. Reduction of infectivity by lyophilization itself (1.29 log₁₀) was also the greatest among the three types of viruses. Loss of infectivity after 7 days and 21 days at 37°C and 7 days at 45°C was 0.38 log₁₀, 1.37 log₁₀, and 1.88 log₁₀, respectively. A tendency for titers of lyophilized virus to be more widely dispersed than titers of virus in liquid medium was also observed for type 2 and type 3 viruses.

Lyophilized live virus vaccines with various stabilizers have been successfully employed for the control of measles, mumps, and rubella (11). However, lyophilization of poliovaccine has not been put to practical use because of the generally known lability of poliovirus to this treatment (3, 5, 6). Portocala et al. (6) showed that following lyophilization, infectivity of cell culture-grown poliovirus (Sabin type 1) decreased by approximately 6 log₁₀. Kraft and Pollard (3) reported that lyophilization of virulent polioviruses (mouse-brain grown type 2 MEF1 and type 3 Leon strains) by the use of either 5-30% peptone or 5% thioglycolate as the lyophilizing medium or by reconstituting lyophilized virus with 30% peptone, minimized the reduction of infectivity by the lyophilization process and that the lyophilized virus withstood heating at 40°C for 8 h with a relatively small, ~1.4 log₁₀, loss of infectivity. Previously, minimal loss of infectivity of poliovirus by lyophilization was reported by Berge et al. (7) using type 1 (Chat), 2 (MEF 1), and 3 (Saukett) viruses that were desalted by ultrafiltration through a Diaflo membrane, and resuspended in Tris buffer; loss of infectivity by lyophiliza-

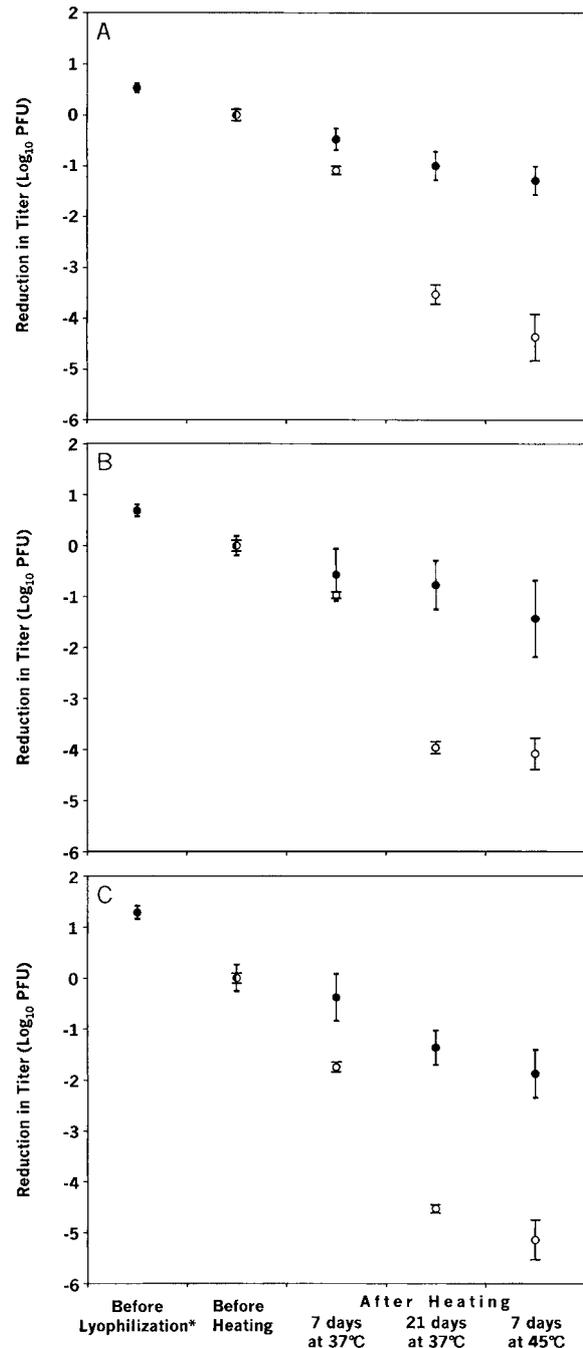


Fig. 1. Heat sensitivity of conventional (O) and lyophilized (●) Sabin vaccine viruses. Fig. 1A: Heat sensitivity of Sabin type 1 viruses. Fig. 1B: Heat sensitivity of Sabin type 2 viruses. Fig. 1C: Heat sensitivity of Sabin type 3 viruses. Titers of virus samples are expressed by the geometric mean titer ± geometric standard deviation. *Virus suspended in lyophilizing medium.

tion was 0.3-1.2 log₁₀ and further loss by incubation at 37°C for 5 days was 2.1-2.3 log₁₀.

In the present study, while the lyophilization of three types of vaccine virus showed a loss of infectivity of 0.52-1.29 log₁₀ comparable to that reported by Berge et al., the subsequent loss by incubation at 37°C for 7 days was very small, 0.38-0.57 log₁₀. Furthermore, lyophilized vaccine viruses were found to be fairly stable following lengthy exposure to higher temperatures, compared with the current liquid vaccine preparations, as seen in Figures 1A, B, and C. Taking the loss of

infectivity caused by lyophilization into consideration, the reduction in titer of lyophilized vaccine viruses from the original virus titers was roughly the same as that of conventional vaccine viruses at a period of 1 week incubation at 37°C. Further, it was found that the longer the incubation time and the higher the incubation temperature, the more heat-stable was the lyophilized vaccine.

Lyophilization of poliovaccine may facilitate handling the vaccine products at ordinary ambient temperatures and shipping to remote vaccination sites in various geographic areas, including tropical countries. The result may also encourage further studies on lyophilization of enterovirus as a whole, which to date has generally been considered to be impractical.

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