

## STABILIZING EFFECT OF MAGNESIUM CHLORIDE AND SUCROSE ON SABIN LIVE POLIO VACCINE(\*)

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### ABSTRACT

A trivalent vaccine was stabilized with (a) 70% sucrose, (b) MgCl<sub>2</sub> 1M, and (c) 18% sucrose plus MgCl<sub>2</sub> 1/2 M. A portion of each batch was kept at + 4°C and 22-25°C.

No change in titre for all 3 preparations was recorded after 9 weeks storage at +4°C. While the potency of vaccines containing MgCl<sub>2</sub> alone or mixed with sucrose and kept at + 22-25° C for 5 weeks was not altered, the vaccine containing only sucrose was less stable, and a drop of titre was noticed after 2 weeks of storage at + 22-25°C.

Monovalent polio vaccines were also stabilized as above and kept at + 4°C or at -20°C. It was found that regardless of the type of stabilizer used, 82 to 97 per cent of potency was retained after 9 months of storage at + 4°C or at -20°C.

Sucrose and magnesium chloride (MgCl<sub>2</sub>) are two stabilizers currently used with oral polio vaccine. According to Wallis and Melnick (1961), MgCl<sub>2</sub> protects this virus without loss of titre for 3 months at + 4°C or 25 days at 22-25° C. Perkins and Magrath (1963) suggest a life limited to 6 months at + 4°C or 14 days at 25°C for both stabilizers.

This report is concerned with protection of sucrose and MgCl<sub>2</sub> alone or mixed, when virus preparations are kept at cold or at room temperature.

### MATERIALS AND METHODS

#### *Stabilization and storage of vaccine*

One trivalent and 3 monovalent polio vaccines were divided into three equal parts. One part was equally mixed with sucrose 70%, another part was blended with MgCl<sub>2</sub> 1M and the last part was stabilized with 35% sucrose together with MgCl<sub>2</sub> 1/2M.

These preparations were distributed into a sterile American type 1 glass of 5 ml. Each vial holds 23 human doses or 3.4 ml of vaccine (the volume of a human dose was 0.15 ml or 3 drops). The vials were tightly sealed in order to avoid rise of pH by loss of CO<sub>2</sub>.

Equal numbers of each preparation were held at -20°C, + 4°C or at + 22-25°C. All samples selected at random for titration were stored frozen at -70°C.

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Each time, 10 samples were collected and were mixed at the time of titration.

### Virus assay

Monolayers of "Vero" cells in tubes were washed with 3 ml of serum free maintenance medium and were fed with 1 ml of fresh maintenance medium 0.5% lactalbumin hydrolysate, 0.22% sodium bicarbonate, 0.2% bovine albumin as well as with 100 units of penicillin and 100 of streptomycin per ml. Tenfold dilutions were made of the samples and 0.2 ml of virus dilution were added to all 10 tubes. The tubes were incubated at 36°C in stationary position and the final reading was made on day seven. The titres were expressed as log 10 TCID50 per ml of virus suspensions.

## RESULTS

The results of the assay of the samples held 9 months in cold storage are reflected in Table 1. The following conclusions may be drawn from the presented figures.

Table 1. Loss of potency of stabilized polioviruses stored 9 months at 4°C or 20°C

Stabilizer of Virus	Type of Virus	Temperature of Storage	Loss of Potency		
			Log	Percentage	
Sucrose 35%	I	+ 4°C	0.12	15	
	II		0.10	16	
	III		0.08	14	
MgCl <sub>2</sub> 1M	I		0.08	11	
	II		0.06	10	
	III		0.1	16	
Sucrose 18% + MgCl <sub>2</sub> 1/2M	I		0.02	3	
	II		0.04	7	
	III		0.04	7	
Sucrose 35%	I		-20°C	0.14	18
	II			0.06	9
	III			0.12	18
MgCl <sub>2</sub> 1M	I	0.04		6	
	II	0.02		4	
	III	0.08		12	
Sucrose 18% + MgCl <sub>2</sub> 1/2M	I	0.06		8	
	II	0.07		7	
	III	0.04		7	

(a) There was not a significant difference between the loss of titre of viruses blended with sucrose or MgCl<sub>2</sub> and kept 9 months at + 4°C or at -20°C; this drop of titre was of 10 to 18%. The losses were less, not exceeding 6% in the case of polio viruses types I and II, containing MgCl<sub>2</sub> and held 9 months at -20°C.

(b) The viruses blended with a mixture of sucrose and MgCl<sub>2</sub> and stored at + 4°C or at -20°C had lost 3 to 8% of the original titre after 9 months.

(c) Trivalent vaccine added with MgCl<sub>2</sub> alone or mixed with sucrose and held at +22-25°C maintained its original titre after 4 weeks; the loss was however 0.25 to 0.5 log at the end of the 5th week of conservation at this temperature

(Table II). The titre of the sample containing sucrose dropped gradually and slowly. A drop of one log was recorded after 5 weeks of storage at + 22°-25°C.

Table II. Loss of titre of stabilized trivalent polio vaccine stored at + 22-25°C.

Stabilizer	Loss of Titre in Log 10 after				
	1Week	2Weeks	3Weeks	4Weeks	5Weeks
Sucrose 35%	0.5	0.5	0.65	0.9	1
MgCl <sub>2</sub> 1M	-	-	-	-	0.5
Sucrose 18% + MgCl <sub>2</sub> 1/2 M	-	-	-	-	0.25

## DISCUSSION

Bezzulov et al. (1961) have observed that in the presence of 50% sucrose at 22°C, live polio vaccine showed 1 log decline after 14 days and 3 logs after 20 days in comparison with initial titre. Lashkevich et al. (1961), on the other hand, have found that when live attenuated poliovirus was used in dragée-candy and kept at room temperature, there was a drop in titre of about 25% after 3 days, 50% after 7 days and 75% after 15 days. These authors have also reported that storage of candied vaccine at + 4°C or at -20°C did not cause any loss in titre.

The cation stabilizers of live polio vaccine were first introduced by Wallis and Melnick (1961) According to these authors the addition of 1 molar of MgCl<sub>2</sub> to attenuated polio strains maintained them without loss of titre for 3 months at + 4°C or 25 days at + 22-25°C. This finding was confirmed by Perkins and Magrath (1963, 1976) who emphasized the necessity of preventing the rise of pH that occurs with the loss of CO<sub>2</sub> from the container. These investigators suggest a life limited to 6 months at + 4°C or 14 days at + 25°C for the above-mentioned stabilizers.

In the present experiments, we attempted to use both stabilizers, alone or mixed, in order to find optimum conditions for stabilizing Sabin vaccine produced in human diploid cells. Our data indicated that if vaccines are stabilized with sucrose or MgCl<sub>2</sub> and stored tightly closed, the loss of titre after 9 months of storage at + 4°C or at -20°C will be negligible. Under similar conditions, a mixture of both stabilizers better protects polio viruses. We do not recommend however the use of this bitter sweet mixture which tastes bitter and, if used, the vaccine will lose its advantage of being tasty.

At room temperature, MgCl<sub>2</sub> protects the poliovirus better than sucrose. In our experiment, a trivalent vaccine containing MgCl<sub>2</sub> 1M maintains its titre after 1 month of storage at room temperature.

Other factors outlined by Melnick and Wallis (1963) or by Perkins and Magrath (1963, 1976) play an important role in the protection of live poliovaccine. The type of container, the volume and pH of the fluid and the way of sealing containers are among the most important factors to be taken into account.

In accordance with the findings of the above-mentioned investigators, we have noted that if the top of a vial is not sealed tightly regardless of the type of stabilizer present in the vaccine, the pH will change and a significant loss in titre will occur.

#### REFERENCES

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