THE EFFECTS OF CATIONS (Mg^{++} , Ca^{++}) ON ENTERO VIRUSES

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INTRODUCTION

Since it is known that attachment of phage on the surface of bacterial cells requires cation¹⁾²⁾, the effect of cations on various viruses has been widely studied. In poliovirus, Bachtold et al.³⁾ found that either Ca^{++} or Mg^{++} was necessary for a maximum rate of attachment of virus to MK cell. Recently, Wallis and Melnick⁴⁾ found that molar $MgCl_2$ stabilized poliovirus very effectively.

Taking this information into consideration, the present study was designed to clarify the different effects of cations on entero viruses in respect to stabilization, attachment on cells and growth, especially of plaque formation by different types of poliovirus.

MATERIALS AND METHODS

Viruses

Coxsackie B group (B1 - B5) was used for experiment on thermal stabilization by magnesium chloride solution, and poliovirus on the effect of cations on plaque formation.

The poliovirus contained the strains of Sabin, Mahoney, Y-1 (Type 1), MEF₁ (Type 2) and Saukett (Type 3). The strain designated with Y-1 was a wild strain isolated from a paralytic poliomyelitis patient. Stock viruses were obtained by propagating in MK cell monolayer in the cysteine medium.

Cell culture

HeLa cells were used throughout this experiment. Cells were grown with Melnick-Hanks' solution containing 10 % bovine serum, at 37°C in bottles or tubes, and maintained in Melnick-Earle's solution to which was added 10 % skim milk solution (10 %). Puck's saline was used for washing the culture and diluting viruses and salt solutions.

Titration of viruses

The tubes of HeLa cell were washed once with Puck's saline and the maintenance medium added. After 24 hours they were inoculated with 0.1 ml of 10-fold dilutions of viruses. The cytopathogenic effect by viruses was observed for 9 days, and TCD₅₀ was calculated. For the experiment on the stabilization by Mg^{++} , 0.1 ml of virus was added to 0.9 ml 1 M $MgCl_2$, and the mixture was heated at 50°C for 10, 20 and 30 minutes. This was followed by diluting with Puck's

saline and titration. A control experiment was carried out on the mixture of 0.1 ml of virus and 0.9 ml Puck's saline and treated according to the same procedure as above.

Plaque formation

The cell monolayer grown in the bottle was washed once with Puck's saline, maintenance medium added and virus inoculation done after 24 hours. After removal of the maintenance medium, the monolayer was washed with Puck's saline, and 0.1 ml of 10^{-3} and 10^{-4} dilution of viruses added with 0.1 ml of the appropriate concentration of salt (with 0.1 ml of Puck's saline in the control experiment). After adsorption for 1 hour, agar overlay was added. The overlay consisted of 1.5 % agar (Difco Bacto) in Hank's BSS containing 2% calf serum, 0.22 % sodium bicarbonate and neutral red at a concentration of 1 : 60,000. In one experiment, 1 % calf serum and 1 % bovine albumin V fraction (5.5 % solution) were used instead of 2 % calf serum. After the agar had solidified, the bottles were kept at 37°C and the number and the size of plaque observed on the third and fifth day from inoculation.

RESULTS

The effect of different cations on the plaque size of polioviruses

The effects of K^+ , Na^+ , Ca^{++} and Mg^{++} were tested on the plaque formation of Mahoney and Sabin strain of type 1 poliovirus. Table 1 shows the result of

TABLE 1
Plaque formation of type 1 poliovirus in the presence of mon- or divalent cation

salt	M ($\times 1/2$)	Mahoney		Sabin 1	
		logPFU	size, mm	logPFU	size, mm
KCl	1	6.8	5	6.2	4
	10^{-1}	7.0	6	6.0	4
	10^{-2}	7.0	6	6.0	4
	10^{-3}	6.9	6	6.0	6
NaCl	1	6.7	6	6.0	5
	10^{-1}	—	—	6.2	4
	10^{-2}	7.2	5	6.0	6
	10^{-3}	7.0	5	5.6	4
CaCl ₂	1	6.8	10	5.3	4
	10^{-1}	—	—	6.2	6
	10^{-2}	7.0	7	5.8	5
	10^{-3}	6.8	6	6.0	5
MgCl ₂	1	7.2	10	6.2	8
	10^{-1}	7.2	10	6.0	7
	10^{-2}	7.2	6	6.0	4
	10^{-3}	7.0	4	6.0	4

fifth day observation. It is ascertained that K^+ , Na^+ , Ca^{++} had no effect on the plaque formation, except in the case of Mahoney strain in the presence of $1/2$ M $CaCl_2$. Remarkable effects were shown in the case of Mg^{++} . Both strains formed large plaques in the presence of $1/2$ M $MgCl_2$, being twice in diameter as compared with the case of more diluted concentrations of $MgCl_2$.

The effect of Mg^{++} on the plaque formations of poliovirus

An attempt was made to determine the effect of Mg^{++} in detail, and also to inspect how plaque formation is changed by Mg^{++} in each of attenuated and virulent strains, or in each of type 1, 2 polioviruses. The results are shown in Table 2. In experiment 1, overlay contained 2% calf serum, while in experiment 2, it contained 1% calf serum and 1% bovine albumin V fraction (5.5% solution).

TABLE 2
Plaque formation of polioviruses in the presence of Mg^{++}

		$MgCl_2$	from inoculation			
			3rd day		5th day	
Expt. 1*	Sabin 1	M ($\times 1/2$)	logPFU	size, mm	logPFU	size, mm
		0	5.4	1.0	6.2	3.5
		10^{-4}	5.5	1.0	6.3	3.0
		10^{-3}	5.8	1.0	6.3	4.0
		10^{-2}	6.0	1.0	6.4	4.0
		10^{-1}	6.2	3.0	6.3	7.0
	Mahoney	0	6.6	1.5	6.8	4.0
		10^{-1}	6.8	4.0	6.8	9.0
Expt. 2*	Y-1	0	6.7	1.5	7.4	3.5
		10^{-1}	7.4	3.0	7.5	7.5
	Sabin 1	0	6.2	2.0	6.3	5.0
		10^{-1}	6.6	4.0	6.6	8.5
	MEF ₁	0	6.6	3.0	6.6	7.0
		10^{-1}	6.5	6.0	6.5	11.0
	Saukett	0	5.7	1.5	5.8	5.0
		10^{-1}	5.8	7.0	5.9	11.0

* Agar overlay contains 2% calf serum in Expt. 1, and 1% calf serum plus 1% bovine albumin V fraction in Expt. 2.

It is evident that the plaque size depends on the concentration of Mg^{++} . As shown in the table, experiment 1, Sabin, the size of plaque in the presence of $1/2 \times 10^{-2}$, $1/2 \times 10^{-3}$ and $1/2 \times 10^{-4}$ M $MgCl_2$ was identical to that of control. The concentration of $1/2 \times 10^{-1}$ M led to the formation of large plaque. As the other

virus strains showed the same tendencies, the data of low concentrations of MgCl_2 were omitted from the table. The difference in the size on the third day was more distinct than on the fifth day from inoculation between the presence and the absence of Mg^{++} . When the overlay contained bovine albumin, the plaque tended to be large but the boundary rather irregular.

PFU on the third and fifth days from inoculation was almost identical in the virulent strains (Mahoney, MEF_1 and Saukett) in either presence or the absence of Mg^{++} . But Sabin 1 showed low PFU on the third day in the absence of Mg^{++} , and reached a constant value on the fifth day, while it showed that PFU values were constant both the third and fifth days in the case of the presence of Mg^{++} .

Stabilizing effect of Mg^{++} to Coxsackie B group

Coxsackie B1, B2, B3, B4 and B5 viruses were mixed with 1 M MgCl_2 (final concentration, 0.9 M) and heated at 50°C , and the stabilizing effect of Mg^{++} was tested by titrating the treated mixture using HeLa cell. The control virus was mixed with Puck's saline for MgCl_2 . Fig. 1 illustrates graphically the titer decrease in $\log\text{TCD}_{50}$ against the time of heating. Though viruses tested were inactivated rapidly by 50°C , heating with Mg^{++} brought about virtually no inactivation for 30 minutes, except a slight inactivation of Coxsackie B5.

Fig. 1. Stabilizing effect of MgCl_2 to Coxsackie B group.

- Coxsackie B1 × Coxsackie B2
- Coxsackie B3 △ Coxsackie B4
- Coxsackie B5

DISCUSSION

While the monovalent cations, K^+ and Na^+ , do not affect the size of plaque, the divalent cations do at high concentration (Table 1). In the presence of 1/2 M CaCl_2 , Mahoney strain formed large plaque while Sabin 1 strain did not. It remains to be studied whether the difference in the plaque size is commonly obser-

ved between virulent and attenuated strains of polioviruses in general.

It was ascertained that Mg^{++} at the concentration of $1/20$ or $1/2$ M increased the size of plaque formed by poliovirus on HeLa cell. This effect is common to different strains of poliovirus, attenuated or virulent. In the course of the preparation of this report, Wallis and Melnick⁵⁾ reported the same phenomena, and they concluded from their detailed data that the increase in the size of plaque was due to the shortening of the maturation time in the cell, resulting in an increase in growth rate.

In the present experiment, it was shown that PFU of Sabin type 1 strain on the third day increased with increasing Mg^{++} concentration, but reached a constant value on the fifth day independent of the concentration of Mg^{++} (Table 2). It is quite possible, therefore, that Mg^{++} promotes the growth of poliovirus.

The difference in PFU with different concentrations of Mg^{++} on the third day was observed in the attenuated strain of poliovirus but not in the virulent strain, suggesting that the former is more sensitive to Mg^{++} than the later.

As the monolayer culture was washed with Puck's saline before virus inoculation, Mg^{++} and Ca^{++} contained very little if any. Thus, Mg^{++} was added to the monolayer with virus at the time of adsorption for 1 hour in contrast to adding agar overlay as in the experiment of Wallis and Melnick⁵⁾. It is therefore evident with a result of constant PFU values in either absence or presence of Mg^{++} at fifth day that adsorption of virus on HeLa cell requires no appreciable amount of Mg^{++} , and it seems that about 0.13 M Na^+ and K^+ contained in Puck's saline is enough to adsorb on the cell.

As mentioned above, the constant PFU value and large plaque formation were obtained in the presence of Mg^{++} for only 3 days after inoculation, so for the virus titration using plaque method it is possible to shorten the observation.

The results of the remarkable stabilizing effect of Mg^{++} against heat to Cocksackie viruses are essentially the same as those obtained by Wallis and Melnick⁶⁾.

SUMMURY

The effect of cations on the plaque formation of polioviruses, and stabilization of Cocksackie B group by Mg^{++} were studied.

The results obtained are as follows:

1. $1/2 \times 10^{-1}$ M $MgCl_2$ caused the large plaque formation of poliovirus, being twice or larger in diameter.
2. Mg^{++} did not affect the PFU Value, suggesting no requirement of Mg^{++} for adsorbing on the surface of HeLa cell.
3. In the presence of $MgCl_2$, the plaque counts can be carried out at only third day from inoculation.
4. Monovalent cations, Na^+ , K^+ , showed no effect on the plaque formation of poliovirus.
5. Mg^{++} stabilized Cocksackie virus against heat effectively.

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