Differential Toxicity of Manganese for Virulent and Avirulent Group A Streptococci

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SUMMARY

Mn²⁺ depressed the rate and extent of multiplication of several virulent strains of group A beta-haemolytic streptococci (*Streptococcus pyogenes*), and caused a 6–12 hr increase in the lag phases of related avirulent strains. Several other cations did not exert such differential inhibitory effects. Ca²⁺ overcame the inhibitory effects of Mn²⁺ in both kinds of organism. In suspensions of virulent strains, Mn²⁺ depressed both nucleic acid and protein syntheses below the control values, but only nucleic acid synthesis was depressed in avirulent-organism suspensions; protein synthesis was largely unaffected. The oxidation of glucose was depressed below control values by Mn²⁺ to a much greater extent in virulent strains than in avirulent strains.

INTRODUCTION

In previous experiments with pneumococci (*Streptococcus pneumoniae*, Firshein, 1960, 1962), Mn²⁺ was shown to stimulate the multiplication of virulent strains and to produce either inhibitory or no effects in the case of avirulent strains. In this respect, Mn²⁺ acted in a manner similar to that of certain deoxyribonucleic acid (DNA) degradation products, which also enhanced multiplication of virulent strains of pneumococci. However, Mn²⁺ inhibited the multiplication of several avirulent strains of pneumococci, whereas DNA degradation products did not depress the multiplication of any avirulent strains tested. It was ascertained (Firshein, 1962) that the stimulatory effects of Mn²⁺ on multiplication of virulent pneumococci were related to an enhancement of respiration, whereas the toxic effects of Mn²⁺ on multiplication of avirulent pneumococci involved, in part, a depressing effect on respiration. Recent results (Firshein & Zimmerman, 1964) have shown that DNA degradation products also stimulate multiplication of virulent strains of group A streptococci. However, in contrast to pneumococci, these virulent group A streptococci showed a depressed rate and extent of multiplication in the presence of Mn²⁺. The present report is concerned with an analysis of this Mn²⁺-effect on virulent streptococci as well as with a study of the effects of Mn²⁺ on multiplication of avirulent streptococci. The effects of Mn²⁺ on respiration, nucleic acid synthesis, and protein synthesis of both cell types will also be described. In part, the interest of the results with Mn²⁺ lies in their contrast to the results described in the accompanying paper (Firshein & Zimmerman, 1964) with DNA degradation products. The results of both papers are discussed together in the next paper (Firshein & Zimmerman, 1964).

METHODS

General. Three pairs of virulent and avirulent strains of group A streptococci were used. They were identical to those used by Firshein & Zimmerman (1964). Maintenance of these strains, medium, viable counts, techniques of preparing and performing experiments with suspensions of organisms, were as reported by Firshein & Braun (1960), Firshein (1961) and Firshein & Zimmerman (1964). Mn^{2+} (as $MnSO_4$) and Ca^{2+} (as $CaCl_2 \cdot 2H_2O$) were dissolved in distilled water before use. Other cations

Fig. 1. Viable counts of virulent and avirulent group A streptococci in the presence and absence of Mn^{2+} . Concentration of Mn^{2+} was the same for all strains tested ($4.0 \mu M$). Medium was brain heart infusion neopeptone blood broth (Firshein & Zimmerman, 1964). Virulent, solid lines; avirulent, broken lines. ($\circ$) ($\bullet$), with Mn^{2+} ; ($\square$) ($\blacksquare$), control. 1. Strain M-18 (virulent) and 18AM⁻ (avirulent). 2. Strain M-6 (virulent) and 6AM⁻ (avirulent). 3. Strain Q-43x (virulent) and Q-496 (avirulent).

that were tested included Mg^{2+} (as $MgSO_4 \cdot 7H_2O$), Zn^{2+} (as $ZnSO_4$), and Fe^{2+} (as $FeSO_4$). Manometric experiments were made as described in Firshein & Zimmerman (1964). As in this latter report, it was necessary to determine the effects of Mn^{2+} on the length and percentage of chains of cocci in liquid cultures. An effect of Mn^{2+} on either variable could produce an error in counting the actual number of viable cocci (when this number is determined by plating serial dilutions of the cultures). It was found that Mn^{2+} had no effects on these variables.

RESULTS

Effects of Mn^{2+} on multiplication

Figure 1 shows the effects of Mn^{2+} on the multiplication of virulent and avirulent strains of group A streptococci in broth cultures. In all cases, the same concentration of Mn^{2+} ($4.0 \mu M$) depressed the rate and extent of multiplication of virulent strains and caused a 6–12 hr increase in the lag phases of avirulent strains. Table 1

Fig. 2. Effects of concentration of Mn^{2+} on the number of viable cocci of a virulent and avirulent strain of group A streptococci after incubation for 18 hr. Medium was brain heart infusion neopeptone blood broth (Firshein & Zimmerman, 1964). Inocula ($\times 10^5$); strain Q-43x (virulent), 50; strain Q-496 (avirulent), 35. Virulent, solid line; avirulent, broken line.

Table 1. *Analysis of total yields of organisms and generation times for virulent and avirulent Group A streptococci in the presence and absence of Mn^{2+}*

Data are based on results of Fig. 1. Generation times are determined in the log phase of growth, which varied from strain to strain.

Organism	Yield of cocci after 18 hr (Count $\times 10^5$ /ml.)		Relative decrease from control value *	Generation time (min.)		Relative increase from control value*
	Mn^{2+}	Control		Mn^{2+}	Control	
Q-43x (virulent)	80	300	3.75	52	48	1.1
Q-496 (avirulent)	6	200	33.3	144	23	6.4
M-18 (virulent)	50	170	3.1	190	60	3.1
18AM ⁻ (avirulent)	20	500	25	276	45	6.1
M-6 (virulent)	65	130	2.0	180	70	2.6
6AM ⁻ (avirulent)	15	150	10.3	90	18	5.0

* These values connote the number of times greater or less, the Mn^{2+} -supplemented values differ from the control values.

compares the generation times and total yields of avirulent and virulent organisms in the presence and absence of Mn^{2+} . After incubation for 18 hr, viable counts in the presence of Mn^{2+} were much smaller for avirulent cultures than for virulent cultures. Similarly, the rates of multiplication of avirulent strains were lower than those of virulent strains, as evidenced by changes in the generation times. The actual duration of the lag and log phases remained essentially unaltered in Mn^{2+} -supplemented and unsupplemented cultures of virulent strains. However, with avirulent strains, the length of these two phases was drastically altered in the presence of Mn^{2+} . Thus, by the time avirulent cocci from unsupplemented cultures had been in the stationary phase for 6–12 hr, avirulent cocci from cultures containing Mn^{2+} were just beginning to enter the log phase.

Table 2. *Effects of Mn^{2+} on multiplication of group A streptococci in different media*

Concentration of Mn^{2+} was $4.0 \mu M$. Inocula ($\times 10^5$): strain Q-43x, 10; strain Q-496, 14; strain M-18, 6.8; strain 18 AM⁻, 12.

Medium	Organism	Additions		Viable cell count after 12 hr ($\times 10^{-5}$)
		Mn^{2+}	Control	
Difco Todd-Hewitt broth + 0.5 % (v/v) defibrinated sheep blood	Q-43x (virulent)	+	—	50
		—	+	310
	Q-496 (avirulent)	+	—	15
		—	+	240
CAT medium (Marmur & Hotchkiss, 1955)	Q-43x	+	—	24
		—	+	105
	Q-496	+	—	4
		—	+	125
Difco Todd-Hewitt broth + 0.5 % (v/v) defibrinated sheep blood	M-18 (virulent)	+	—	43
		—	+	305
	18 AM ⁻ (avirulent)	+	—	7
		—	+	850
CAT medium	M-18	+	—	56
		—	+	110
	18 AM ⁻	+	—	4
		—	+	710

By using a related pair of virulent and avirulent strains (Q-43x, Q-496), it was determined that maximum inhibition of multiplication for both strains occurred at a concentration of $4.0 \mu M$ Mn^{2+} , although, as suspected, the inhibition was much greater in avirulent cultures (Fig. 2).

Table 2 shows that the inhibitory effects of Mn^{2+} on multiplication of virulent and avirulent streptococci were not restricted to one medium, but occurred in other media that supported the multiplication of streptococci.

Effects of other cations on multiplication

It was found (Firshein, 1962) that the stimulatory effects of Mn^{2+} on multiplication of pneumococci were annulled by the addition of Ca^{2+} . Similarly, in studies with streptococci strains Q-43x and Q-496 (Table 3), a substantial portion of the inhibition of multiplication of both organisms by Mn^{2+} could be prevented by the addition of Ca^{2+} to a level of $4.0 \mu M$.

Fig. 3. Effects of Mn^{2+} on oxygen uptake of virulent and avirulent strains of group A streptococci in suspensions containing glucose. For concentration of Mn^{2+} , see Table 1. Concentration of glucose was the same as that in washed suspensions ($50 \mu M$). Endogenous rates are not shown on the graph because they were negligible for all strains tested. 1. Strain Q-43x (virulent) and Q-496 (avirulent). 2. M-18 (virulent) and 18 AM- (avirulent). Solid lines, glucose alone; dashed lines, glucose + Mn^{2+} . (○), virulent; (■), avirulent. Inocula for all strains were adjusted to the same number, 8.5×10^8 viable cocci/ml.

Table 3. *Reversal of the inhibitory effects of Mn^{2+} on multiplication of streptococci by Ca^{2+}*

Medium was brain heart infusion neopeptone blood broth (Firshein & Zimmerman, 1964). Inocula: ($\times 10^5$), strain Q-43x, 24; Q-496, 15.

Organism	Additions (to μM)		Viable count after 12 hr ($\times 10^{-5}$)
	Ca^{2+}	Mn^{2+}	
Q-43x (virulent)	.	.	340
	.	4.0	45
	0.4	4.0	80
	4.0	4.0	290
	8.0	4.0	200
	0.4	.	300
	4.0	.	245
	8.0	.	190
Q-496 (avirulent)	.	.	200
	.	4.0	10
	0.4	4.0	30
	4.0	4.0	250
	8.0	4.0	150
	0.4	.	200
	4.0	.	410
	8.0	.	300

Several other divalent cations did not exert inhibitory effects on multiplication of group A streptococci (Table 4). One cation, Mg^{2+} , enhanced the multiplication of virulent and avirulent strains slightly.

Effects of Mn^{2+} on respiration, nucleic acid synthesis, and protein synthesis

The ability of Mn^{2+} to affect respiration of pneumococci (Firshein, 1962) suggested a study of the effects of Mn^{2+} on respiration of group A streptococci. Using oxidation of glucose as a parameter of respiration, it was found (Fig. 3) with two related pairs of virulent and avirulent strains that Mn^{2+} depressed oxygen uptake in both virulent

Table 4. *Effects of Mg^{2+} , Zn^{2+} , and Fe^{2+} on multiplication of group A streptococci*

Since no inhibitory effects on multiplication were exerted by the cations tested, only the results with one concentration (indicated in parentheses) are shown. Medium was brain heart infusion neopeptone blood broth (Firshein & Zimmerman, 1964). Inocula ($\times 10^6$); strain Q-43x, 8; strain Q-496, 5; strain M-18, 15; strain 18AM⁻, 15; strain M-6, 20; strain 6AM⁻, 12.

Organism	Cation tested	Viable count after 12 hr ($\times 10^5$)
Q-43x (virulent)	Mg^{2+} (2.0 μM)	520
	Zn^{2+} (4.0 μM)	210
	Fe^{2+} (4.0 μM)	220
	None	300
Q-496 (avirulent)	Mg^{2+}	250
	Zn^{2+}	100
	Fe^{2+}	150
	None	105
M-18 (virulent)	Mg^{2+}	950
	Zn^{2+}	550
	Fe^{2+}	440
	None	550
18AM ⁻ (avirulent)	Mg^{2+}	1000
	Zn^{2+}	730
	Fe^{2+}	680
	None	700
M-6 (virulent)	Mg^{2+}	210
	Zn^{2+}	95
	Fe^{2+}	90
	None	100
6AM ⁻ (avirulent)	Mg^{2+}	450
	Zn^{2+}	220
	Fe^{2+}	160
	None	200

and avirulent-cell suspensions containing glucose. However, the inhibitory effects were more pronounced in virulent than in avirulent-cell suspensions, particularly for one pair (Q-43x and Q-496). These results were surprising in view of the fact that multiplication was depressed to a greater extent in avirulent strains than virulent strains. To test whether other metabolic systems in avirulent strains may have been affected by Mn^{2+} , measurements of nucleic acid and protein synthesis

were made in washed-cell suspensions in the presence and absence of the cation. In addition, effects of Mn^{2+} on nucleic acid and protein syntheses of virulent strains were determined. The results of these experiments, replicated several times (Table 5), demonstrated that: (1) nucleic acid synthesis and protein synthesis were depressed equally from control levels in suspensions of virulent strains; (2) only nucleic acid synthesis was depressed in suspensions of avirulent strains, protein synthesis was largely unaffected; and (3) nucleic acid synthesis was depressed to a much greater extent from control levels in suspensions of avirulent than virulent strains.

Table 5. *Effects of Mn^{2+} on nucleic acid and protein synthesis of Group A streptococci*

Concentration of Mn^{2+} was $4.0 \mu M$. Preparation of washed cocci, suspending medium, extraction and measurement of nucleic acids and protein were as described previously (Firshein, 1961).

Organism	Additions		% increase over zero time after 70 min.		
	Mn^{2+}	Control	DNA	RNA	Protein
Q-43x (virulent)	+	—	50	45	42
	—	+	69	54	64
Q-496 (avirulent)	+	—	25	23	65
	—	+	68	71	74
M-18 (virulent)	+	—	14	10	15
	—	+	21	18	23
18 AM ⁻ (avirulent)	+	—	1	—	14
	—	+	13	14	16
M-6 (virulent)	+	—	21	20	18
	—	+	34	40	31
6 AM ⁻ (avirulent)	+	—	—	1	21
	—	+	24	25	19

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