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Effect of Sodium Thiosulfate Upon Mustard-Virus Interaction.* (21132)

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It was previously reported that exposure of influenza virus to appropriate concentrations of mustard resulted in selective inactivation of certain viral attributes such as infectivity and toxicity(1). In analyzing the early stages of destruction of viral toxins by mustard, it was necessary to circumvent the inherent toxicity of mustard itself. The use of sodium thiosulfate in this connection resulted in a rapid detoxification of mustard by thiosulfate(2). This observation along with previous reports of mustard-thiosulfate interaction(3) suggested a possible influence of thiosulfate upon inactivation of virus by mustard. This paper reports the results of such studies.

Materials and methods. Virus. Influenza A (PR8) virus was prepared as described(2). Harvested virus was stored in ampoules in a dry-ice cabinet. When needed, an ampoule of virus was placed in a 37°C water bath for 30 minutes. Following this, the viral suspension was centrifuged 15 minutes at 3500 rpm; the supernatant fluid was removed, allowed to attain room temperature and then used as the source of virus. *Mustard.* The chloroethylamine derivative used in these investigations was cyclohexyl [bis(β-chloroethyl)] amine hydrochloride, kindly supplied by Dr. H. B. Woodruff of Merck and Co., Inc. The chemical solution was prepared in triple-distilled water to yield for the 0.01 M solution of mustard a final pH of 2.6. *Thiosulfate.* Freshly prepared solutions of sodium thiosulfate in distilled water were used.

Exposure of virus to mustard. This was carried out as previously described(2) except that the reaction temperature in these experiments was 25°C. In testing for the effect of thiosulfate upon these mustard-virus systems, sufficient 3 M sodium thiosulfate was added to the samples (before addition of mustard) to yield a final thiosulfate concentration of 0.075 M. *Test of viral toxicity.* Serial 2-fold dilutions of mustard-treated virus were made in normal allantoic fluid; these were tested for residual viral toxicity by injecting 1.0 ml of dilution intravenously into a mouse, 6 mice being used for each dilution. The animals employed in the tests were Swiss mice (Webster strain) 12 to 15 g weight and of random sex. All animals were observed daily for a period of 7 days, and deaths occurring 24 hours or later after intravenous injection were considered to be of viral origin. *Infectivity test.* This was carried out as described previously(2).

Results. Effect of thiosulfate upon destruction of viral toxicity and infectivity by mustard. The effect of sodium thiosulfate upon mustard-virus interaction is shown in Table I. The results presented in this table are representative of a series of similar experiments.

In terms of mustard inactivation of viral toxicity, it is apparent that there was less destruction of viral toxin in the presence of sodium thiosulfate. The greater residual toxicity of virus exposed to mustard in the pres-

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TABLE I. Effect of Sodium Thiosulfate in Mustard Inactivation of Virus.

Sample	(M) Thios.	(M) Mustard	Toxicity of sample* u	1:2	1:4	Neg log ID_{50} remaining
Virus	.075	5.0×10^{-4}	1/6			4.7
"	"	2.5 "	4/6			6.3
"	"	1.25 "	6/6	4/6	0/6	7.3
"	"	6.25×10^{-5}	6/6	4/6	0/6	7.3
"	0	5.0×10^{-4}	0/6			<1.0
"	"	2.5 "	0/6			4.7
"	"	1.25 "	1/6	0/6		5.7
"	"	6.25×10^{-5}	5/6	1/5	0/6	7.3
"	"	0	6/6	6/6	1/6	8.3
"	.075	0	5/5	6/6	1/6	8.5
NAF	0	5.0×10^{-4}	0/6			
"	.075	"	0/6			

NAF = Normal allantoic fluid.
(M) = Final molar concentration.
Blank spaces indicate samples not tested.

* Samples were left 3 hr at room temp. and 18-20 hr in refrigerator before tests for residual viral toxicity and infectivity. The 1:2 and 1:4 dilutions of samples were made in NAF. u represents undiluted sample.

ence of thiosulfate was most evident when 1.25×10^{-4} M mustard was used. These deaths are properly attributable to viral toxicity, for normal allantoic fluid treated with mustard alone or in the presence of thiosulfate proved innocuous upon intravenous injection of mice. The "sparing" action of 0.075 M sodium thiosulfate upon mustard inactivation of virus extended also to viral infectivity. Thus, for the 3 highest concentrations of mustard, destruction of viral infectivity, as measured by the log number of egg-infective doses remaining, was approximately 1.6 to over 3.7 log units greater in the absence of thiosulfate.

Effect of thiosulfate upon rate of destruction of viral infectivity by mustard. The conclusions derived from Table I represent the end results of mustard action.. An earlier report(2) had indicated the extreme rapidity of mustard-virus interaction. To determine the effect of thiosulfate upon the rate of reaction between mustard and virus, the experiments shown in Table II were carried out.

It is evident that thiosulfate had little influence upon the course of events occurring during the first minute of reaction. Addition of mustard to virus resulted in an approximate drop of 3 log units irrespective of the presence or absence of thiosulfate. The effect of thiosulfate upon this system became apparent only after the first minute of reaction; thus, while a further drop in viral infectivity (approximately 2 log units) occurred in the next 4 minutes in the mixture without thiosulfate, no additional loss was detected in the system containing 0.075 M thiosulfate. In other experiments (results not shown) using 5×10^{-4} M mustard, it was found that an increase in the concentration of thiosulfate from 0.075 M to 0.5 M still failed to prevent a marked initial drop (*i.e.* in the first minute) in viral infectivity upon addition of mustard to virus.

Discussion and summary. The present studies would appear to indicate that the rate of reaction of mustard with virus was extremely rapid and that there were chemical groups in influenza virus which rendered them highly susceptible to mustard action. The rapidity of reaction at room temperature was attested by the observation that the greatest portion of the viral population was inactivated during the first minute of mustard-virus interaction. The inability of sodium thiosulfate, a substance reputedly having marked affinity for mustard molecules, to influence the course of events during the first minute of reaction seemingly supported the second of the above premises; presumably thiosulfate failed to compete successfully with virus for the molecules of mustard, thus accounting for the rapid initial destruction of virus. The partially "protective" action of thiosulfate upon destruction of virus by mustard, as reported herein, was confined to events after the first minute of reaction, and was probably related to a tardy combination of thiosulfate and mustard molecules.

TABLE II. Effect of Thiosulfate upon Initial Stages of Mustard-Virus Interaction.

Viral sample	(M) Mustard	(M) Thios.	Time of sampling (min.)	Log ID_{50} of sample
1	5×10^{-4}	.075	1	5.7
2	"	"	5	5.5
3	"	0	1	5.5
4	"	"	5	3.7
5	0	"		8.7
6	"	.075		8.5

Reaction was at room temp.

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Effect of Hyaluronic Acid on Skin Lesions Produced by Staphylococci.* (21133)

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The increasing interest aroused by recent biochemical and enzymological researches on the mucopolysaccharides of the connective tissue ground substance has led us to carry out a series of investigations on the significance of such substances in inflammation processes and experimental carcinogenesis. In this first paper some observations are reported on the action of hyaluronic acid and its enzymatic split products on an inflammation experimentally produced in the rabbit skin by inoculation with pathogenic staphylococci. These investigations follow the observations of Scott *et al.*(1) who reported a slight shortening of incubation period in experimental syphilis, if spirochetes are inoculated in the rabbit testis together with hyaluronidase. According to these authors, this is due either to direct action of hyaluronidase on spirochetes or on the ground substance. On the other hand, evidence has been repeatedly given (Meyer and Chaffee(2), Campani(3), Caputo(4), Cutinelli(5), McClean *et al.*(6)) of the participation of mucopolysaccharides of the connective tissue ground substance in inflammation processes and in the formation of granulation tissue (Caselli(7), Baggi (8-12)).

Methods. The following materials have been used: A) 24-hr agar cultures of *Staphylococcus aureus*.† Immediately before the experiment the bacterial growth of each culture was suspended in 10 ml of saline. B) Potassium hyaluronate solution (7.5 mg/ml) in saline, solution pH 7.3.‡ C) Split products of potassium hyaluronate obtained by incubating for 2 hours at 37°C the solution listed as B with hyaluronidase§ (10 Schering units/1 ml of hyaluronidase solution), then inactivating the enzyme by keeping mixture in water bath at 80°C, 30 minutes. D) 24-hr agar cultures of the same strain of *S. aureus* A, seeding from a broth culture after 5 successive cultures in broth (4 ml) to which one ml potassium hyaluronate had been added at concentration of 2 mg/ml. The fifth broth culture was then seeded in agar and the growth suspended in 10 ml saline. Experiments were made on 9 albino rabbits from the same breed, weighing approximately 2.5 kg. The back was carefully shaved, not to produce abrasions. Each animal was given 2 intradermal injections on both sides at 8 cm distance from each other. The animals were divided into 3 groups: *1st group.* 4 rabbits injected in left side skin with 0.4 ml (2 animals) and 0.2 ml (2 animals) of a mixture made with equal volumes of bacterial suspension A and potassium hyaluronate solution B. Controls were injected in the right side with the same amount of a mixture containing the bacterial suspension and saline in equal volumes. *2nd group.* 3 rabbits were injected in the left

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† A strain of staphylococcus has been used recently isolated from an abscess.

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§ Throughout our experiments hyaluronidase (Kinaden) has been used prepared by the Schering Laboratory, Berlin.