16696

Influence of Saliva upon Hemagglutination by Influenza Virus.*

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Study of the mechanisms involved in infection and resistance to influenza virus led to the demonstration of a virus-inactivating property in nasal secretions and in sputum.[1,2] The bulk of evidence points to the probability that the essential agency is specific antibody.[3] Rose[4] has continued further studies of human sputum, demonstrating wide variation in the capacity of different samples to neutralize influenza virus.

Recently several authors[5-8] have described the inhibition of the hemagglutinating action of influenza virus by carbohydrates and viscous materials. Although in an earlier study[2] saliva was not found to neutralize influenza virus in mice, the present study was undertaken to determine the effect of saliva upon hemagglutination since that fluid commonly contains a variety of substances similar in character to those shown to influence the action of virus upon erythrocytes *in vitro*.

Materials and Methods. Collection and Treatment of Specimens. Twenty to 25 cc of saliva were collected without chewing, in 50 cc graduated centrifuge tubes. Specimens were centrifuged at 2000-2500 r.p.m. for half an hour to remove solid particles and mucus because these substances were found to cause the chicken red cell suspensions to settle out on the bottom of the tubes in the same pattern as that seen with influenza virus and red cells. If supernatants were clear after centrifuging they were removed and used with no further treatment. However, when the specimens contained a great deal of mucus, further treatment was necessary. They were ground in mortars with alundum and centrifuged again. If still not clear and free of mucus, they were filtered through cotton and gauze. Even then small amounts of mucus remained in some specimens. It most cases the saliva was used the same day as collected.

Virus Strain. Allantoic fluid of the PR8 strain of influenza virus Type A which had 593 mouse passages and 71 egg passages was used in the majority of the experiments. In a few of the early experiments an eluate prepared from the 65th egg passage and allantoic fluid from the 70th egg passage of the same strain were used.

Red Cell Suspensions. From a stock 10% suspension of washed chicken erythrocytes, 0.25% suspensions were prepared daily. No cells more than 5 days old were used.

Experimental. 1. *Effect of saliva on PR8 strain of influenza virus.* Two-fold dilutions of virus were made in 0.25 cc volumes of physiological salt solution. To each dilution 0.25 cc of undiluted centrifuged saliva was added and mixed. This mixture stood at room temperature for 30-60 minutes before 0.5 cc of 0.25% chicken red cell suspension was added. Control titrations of virus and saliva alone were carried out under the same conditions.

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The effect of saliva on the hemagglutinating titer of the PR8 strain of influenza virus was to decrease it from 8 to 512 times. The amount of inhibitor in saliva varied from person to person as well as from day to day in the same individual. Results of experiments with saliva from one individual on 6 occasions are shown in Table I. The results suggest that some deterioration of the inhibiting substance occurs on standing, even at 4°C.

2. *Inhibition tests with dilutions of saliva.* An inhibition test was performed employing a procedure similar to that described by Salk.[9] Two-fold dilutions of saliva were made directly in 0.5 cc of 0.25% red cell suspension. To the dilutions of saliva, 0.5 cc of a 1/1000 dilution of PR8 virus was added. This amount of virus constituted a final dilution of two agglutinating units in each tube.

This method of measuring inhibition by saliva, at first glance, did not seem to be as effective as that of the first experiments, owing perhaps to the fact that in the second type of test the saliva and virus do not stand together before the indicator (cell suspension) is added. Nevertheless, in terms of the hemagglutinating units inhibited in the two types of test the differences are not great. The results with several specimens are shown in Table II.

3. *Effect of saliva on chicken red cells.* From 3.5 to 4 cc of saliva were mixed with 8 cc of 0.25% chicken red cells and allowed to stand at room temperature for 30-60 minutes. The specimens were thoroughly shaken several times during that period. The cells were centrifuged and resuspended in 8 cc of fresh salt solution without washing. These cells were then added to a series of virus dilutions. A cell control of 0.5 cc salt solution and 0.5 cc of cells was included.

It was found that when a specimen of saliva caused spontaneous agglutination of red cells, the cells continued to agglutinate even though removed from the mixture and resuspended in fresh salt solution. In most cases treating cells with saliva had very little effect on the virus titrations. Thus the influence of the inhibitor appears to be upon the

[9] Salk, J. E., *J. Immunol.*, 1944, **49**, 87.

TABLE I.

Effect of Saliva from One Individual Upon Hemagglutination by the PR8 Strain of Influenza Virus.

Specimen	Experiment	Date of test	Age of saliva, days	Final dilution of virus: 4	8	16	32	64	128	256	512	1024	2048	4096	8192	16,384	Titer of virus	Agg. units of virus inhibited
Control	0.5 cc virus dil.	With all tests		+	+	+	+	+	+	+	+	+	+	+	0	0	4096	
	+																	
	0.5 cc cell suspension																	
E.M.	0.25 cc virus dil.	1-22-48	0	+	+	0	0	0	0	0	0	0	0	0	0	0	8	256
	+		0	+	+	+	+	0	0	0	0	0	0	0	0	0	32	64
	0.25 cc saliva	27																
		2-23	6	+	+	+	+	+	+	+	+	0	0	0	0	0	512	4
	+	3-2	1	+	+	+	+	+	+	0	0	0	0	0	0	0	128	16
	0.5 cc cell suspension	3																
		5	0	+	0	0	0	0	0	+	0	0	0	0	0	0	256	8
																	4	512

+ = Agglutination.
0 = No agglutination.

TABLE II.
Effect of Saliva upon Hemagglutination of PR8 Strain of Influenza Virus as Indicated by the Inhibition Test.

Specimen	Date of test	Age of saliva in days	Inhibition titer of saliva	Aggl. units of virus inhibited
B.B.	2–23–48	6	< 4	0
	26	0	32	64
	3– 2	1	64	128
	3	1	32	64
	29	0	32	64
A.Z.	17	0	8	16
J.Q.	17	0	8	16
E.M.	2–23	6	< 4	0
	3– 2	1	< 4	0
	3	1	4	8
	5	0	32	64

TABLE III.
Lack of Effect of Saliva Upon the Agglutinability of Chicken Red Cells.

Specimen	Experiment	Date of test	Age of saliva in days	Titer of virus	Aggl. units of virus inhibited
Control	0.5 cc virus dil. + 0.5 cc normal cells	With all tests		4096	
B.B.	0.5 cc virus dil. + 0.5 cc cells saliva-treated	1-28-48	0	1024	2
		2-23	6	2048	1
		26	0	2048	1
A.Z.	”	26	0	4096	0
J.Q.	”	28	0	4096	0
E.M.	”	22	0	256	8
		27	0	512	4
		2-23	6	2048	1

virus rather than upon the erythrocytes. The results shown in Table III illustrate the general lack of effect as well as the exceptional experience with saliva of E.M.

4. *Effect of heating saliva and virus.* Samples of saliva or virus were heated at 56°C for 30 minutes. All various combinations of the above tests were tried:

(a) Constant amount of heated saliva added to serial dilutions of unheated virus.

(b) Inhibition test—serial dilutions of heated saliva with constant amount of unheated virus.

(c) Unheated saliva added to heated virus.

(d) Inhibition test—unheated saliva with heated virus.

(e) Heated saliva added to heated virus.

(f) Inhibition test—heated saliva with heated virus.

One effect of heating the saliva was the elimination of spontaneous agglutination of red cells by certain specimens. In only one case did heating the saliva fail to eliminate this influence. Otherwise the heating of virus or saliva exerted no significant effect upon the action of saliva, illustrating that in this

respect the inhibitory influence of saliva differs from that of the normal serum inhibitor.

Summary. Saliva of human adults possesses the capacity to inhibit agglutination of erythrocytes by influenza virus. Under the conditions of study the effectiveness varies among individuals and at different times. Its action is not significantly affected by heating at 56° C for 30 minutes; nor is its behavior influenced by heating of the virus. Nevertheless, the action of the inhibitor appears to be upon the virus rather than upon the erythrocytes. The nature of the inhibitor has not been determined but it is suggested that saliva represents a physiologic source of materials such as have been shown to interfere with hemagglutination in *in vitro* systems.

16697

Alteration of *Pasteurella pestis* Bacteriophage Following Successive Transfer on *Pasteurella pseudotuberculosis* and on Shigellae.

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A previous report[1] has described the activity of a strain of *Pasteurella pestis* bacteriophage which was able to lyse 19 of 27 strains of *Pasteurella pseudotuberculosis* and 6 of 37 strains of shigellae. By repeated transfer on *P. pseudotuberculosis* (Spokane strain) this phage became adapted so that it lysed all 27 strains of this species in high dilution. After transfer on shigellae, the phage increased its potency for sensitive strains of this genus. The present report describes the effect of successive transfer of the same *P. pestis* phage on *P. pseudotuberculosis* and on certain species of shigellae in various combinations.

The methods used in this study have been recorded.[1] All tests were incubated at 37°C. The transfers of phage were made by adding 0.5 ml of the previous passage to 10 ml of a broth culture of bacteria about two hours old and incubating until clear. After about 5 transfers no secondary resistant growth appeared and filtration of the lysate was unnecessary. The "purified" *P. pestis* phage previously described was used as a starting point in this study. The bacterial cultures were the same as those studied in the first report. Thirteen additional strains of *P. pseudotuberculosis* were investigated, of which all but one were lysed by the original phage.

In the first experiment, the *P. pestis* phage was transferred 22 times on *P. pseudotuberculosis* (Spokane strain) and then five times on each of the following in succession: *S. dysenteriae* (No. 44), *S. paradysenteriae* (type 103 No. 12), *S. sonnei* (No. 6), and *S. ambigua* (No. 33). It was found that any significant change occurred within 5 transfers on a given culture and that further transfers up to 25 made no difference. After each series of transfers, the adapted phages were tested on agar plates against each of the organisms listed and against *P. pestis*. Representative results are shown in Table I.

The results after transfer on 2 or 3 of the species of shigellae were essentially the same as those after transfer on all 4 species. The right hand column in Table I represents the effect of successive transfer on all 4 shigellae. It is noteworthy that after passage first on *P. pseudotuberculosis* and then on *S. dysenteriae*, the phage no longer lysed *P. pseudotuberculosis*.

A second series of experiments was planned to show the effect of successive transfer on

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