STUDIES OF THE HEMOLYSIS OF RED BLOOD CELLS BY MUMPS VIRUS*

II. The Relationships of Hemagglutination, Virus Elution, and Hemolysis

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It has been shown in previous studies (1) that in addition to agglutinating erythrocytes, mumps virus, under appropriate conditions, will hemolyze red blood cells. This hemolytic activity was destroyed by a variety of physical and chemical agents which left intact the capacity of the virus to agglutinate erythrocytes and to elute therefrom (2). These findings appeared to indicate that the hemolytic reaction involves some labile component of the virus that is not essential for hemagglutination or elution from erythrocytes. However, since both hemolysis and hemagglutination appeared to follow union between virus and red blood cell, the discovery of certain factors of mutual importance for these two reactions could be anticipated. In order to study the relationships between hemagglutination, virus elution, and hemolysis, the agglutination and lysis of red blood cells by mumps virus were observed under conditions affecting (*a*) the red cell receptors, (*b*) adsorption of virus on unmodified erythrocytes, and (*c*) elution of virus after its adsorption on the red blood cell. The results of these experiments are presented in this report.

Materials and Methods

Viruses.—The egg-adapted strain of mumps virus described in previous studies was employed (1, 2). The virus was cultivated in the amniotic sac of 7-to-8-day-old chick embryos. After incubation at 35°C., for 5 days, the amniotic fluid was harvested, placed in glass ampoules, sealed, and stored at −70°C. until required.

The PR8 strain of influenza A virus and the Lee strain of influenza B virus were employed. These viruses were cultivated in the allantoic sac of 10-to-12-day-old embryos. The allantoic fluid was collected after 48 hours' incubation at 35°C. and handled in the same manner as the fluids infected with mumps virus.

Red Blood Cells.—Blood specimens from the various animals were mixed with an equal volume of Alsever's solution and stored at 4°C. for use during periods up to 10 days. Unless otherwise noted, chicken erythrocytes were used in all tests. The cells were washed 3 times in 0.85 per cent NaCl solution buffered at pH 7.2 (0.025 M phosphate) and made up to the desired concentration in this buffered saline.

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Virus Hemagglutination Titrations.—The technique of the previous report (2) was used.

Virus Hemolysin Titrations.—The method already described (2) was employed, using chicken red blood cells.

Normal Allantoic and Amniotic Fluids.—Allantoic and amniotic fluids were harvested separately from 12-to-14-day-old embryos and stored at 4°C. until needed.

Egg White Inhibitors.—Egg white inhibitors were prepared by Dr. K. Penttinen by a method similar to that used by Lanni and associates (3). These are designated E17 and EI, and both showed inhibition at a dilution of 1:25,000 when tested against 2 hemagglutinating units of heated influenza B virus (Lee).

Extract of Human Erythrocytes.—Dr. C. Howe prepared this red cell extract by the method described previously (4).

Vibrio Culture Filtrate.—This filtrate was obtained by Dr. F. S. Stewart from a culture of vibrio No. 4711 (British National Type Culture Collection). Such material had been shown to have a strong red cell receptor-destroying action in experiments (5) with influenza A virus (PR8).

Sodium Periodate Solution.—A stock solution of M/100 sodium periodate solution was prepared in phosphate buffer saline from which further dilutions were made in this buffer for use in the experiments. Five per cent glucose in neutral phosphate buffer was used to reduce the periodate where indicated.

EXPERIMENTAL

A. Hemagglutination and Hemolysis under Conditions Which Affect the Cell Receptors

The relationship of the cell receptors involved in hemagglutination and in hemolysis was studied by determining whether hemolysis would occur with red cells of sorts which do not possess the receptors for the hemagglutinin and with cells in which these receptors had been destroyed or removed. In the first instance, a study was made of the susceptibility of the erythrocytes of various animal species to hemagglutination and hemolysis by mumps virus, the presence or absence of agglutination being interpreted as indicating the presence or absence of the specific receptors for hemagglutination. In addition, experiments were carried out with chicken red cells before and after they were deprived of receptors by the action of mumps or influenza virus, periodate or vibrio filtrate to determine the effect of destruction of receptors on susceptibility to hemolysis by mumps virus.

1. Susceptibility of Erythrocytes of Various Animal Species.—

Observations were made on the hemagglutination and hemolysis by mumps virus of the red cells of eight different species in addition to those of chicken, man, and sheep reported previously (1). All red cells were treated in exactly the same manner and the results were evaluated by comparison with the results obtained in simultaneous titrations using chicken red cells. Table I summarizes the results. These data show that erythrocytes agglutinated by mumps virus were also hemolyzed by it.

2. Destruction of the Cell Receptors.—

Mumps and Influenza Viruses.—Chicken red cells were saturated with mumps virus or influenza viruses Type A or B at 4°C. The red cell–virus suspensions were then incubated at 37°C. with shaking for 3 or more hours. To insure complete elution of the adsorbed virus, the cells were washed and reincubated for 3 additional hours. In order to avoid hemolysis during elution of the mumps virus, an infected fluid with little hemolytic activity was used.

Erythrocytes treated in this manner with mumps or influenza viruses were found to be neither agglutinated nor hemolyzed by an amniotic fluid infected with mumps virus which would hemolyze untreated red cells.

TABLE I

Hemagglutination and Hemolysis of Red Blood Cells of Various Animal Species by the Amniotic Fluid Infected with Mumps Virus

Species	Hemagglutination	Hemolysis
Chicken	+++	+++
Man	+++	+++
Sheep	+++	+++
Monkey	+++	+++
Guinea pig	+++	+++
Rabbit	−	−
Mouse	±	+
Horse	++	+++
Cow	+++	+++
Hog	++	++
Bear	−	±

+++, ++, + indicate degrees of hemagglutination or hemolysis as compared with results obtained using chicken erythrocytes. +++ indicates that at least 50 per cent of the cells were hemolyzed. − indicates absence of hemagglutination or hemolysis and ± indicates inconsistent or doubtfully positive results.

Mumps virus heated to 50°C. for 30 minutes, or exposed to 0.2 per cent formaldehyde, which readily adsorbed on and eluted from erythrocytes but which had lost all its hemolytic activity, was used to treat chicken erythrocytes. Such red cells proved insusceptible to hemagglutination or hemolysis by mumps virus.

Periodate.—Although periodate in adequate concentrations can hemolyze chicken red cells, lower concentrations inactivate most of the cell receptors without causing hemolysis.

One volume of 10 per cent chicken red cells was mixed with 1 volume of serial dilutions of periodate solution. The mixture stood at room temperature for 30 minutes. At this time an equal volume of 5 per cent glucose solution was added to reduce the periodate. Red cells were then washed with the glucose so-

lution and with buffered saline before preparing suspensions for hemagglutination and hemolysis tests with mumps virus. Red cells treated simultaneously with M/500 periodate solution previously reduced with glucose were used as controls.

The results, which are presented in Table II, show that concentrations of sodium periodate of M/1000 or higher hemolyze the red cells. At a concentration of M/2000, the cells show a definite decrease in susceptibility to hemagglutination and hemolysis by mumps virus. Less effect is observed with sodium periodate at concentrations of M/4000 and lower. Periodate affects similarly the cell receptors important for hemagglutination and hemolysis, a fact which suggests their close relationship.

TABLE II

Hemagglutination and Hemolysis of Periodate-Treated Red Cells by Mumps Virus

Sodium periodate	Hemagglutination titers*	Hemolysis						
		Dilutions of virus						Buffer control
		8	16	32	64	128	256	
conc.								
M/500	—	Completely hemolyzed						
M/1000	<32	43‡	43	48	47	46	43	40
M/2000	64	7	6	4	2	0	0	0
M/4000	256	18	17	14	9	4	0	0
M/10000	1024	27	27	26	17	12	4	0
M/500 + glucose	1024	30	29	28	21	13	7	0

* Expressed as the reciprocal.

‡ Per cent hemolysis.

Vibrio Filtrate.—

The filtrate produced some hemolysis of the red cells when incubated at 37°C. but this could be avoided if they were kept at 9°C. Chicken red cells were suspended in vibrio filtrate and placed at 9°C. for 1 hour. Following this they were washed in buffered saline and incubated at 37°C. for 3 hours.

Cells treated in this way showed a reduction in susceptibility to hemolysis by mumps virus which was accompanied by a decreased activity for virus adsorption and hemagglutination. These findings suggest that adsorption of the virus to intact cell receptors is essential for the hemolytic action.

B. Hemagglutination and Hemolysis under Conditions Which Affect Virus Adsorption

In a mixture of virus and red cells, adsorption of the virus to the cell receptors can be interfered with by the presence of the so called hemagglutination

inhibitors which combine with the virus or by the presence of other viruses which act on the same receptors. The effects of these two types of interfering agents on hemolysis by the mumps virus have been studied in the following experiments.

1. Effect of Inhibitors of Hemagglutination.—

Normal Allantoic and Amniotic Fluids.—The results of experiments using normal allantoic or amniotic fluids as diluents for the virus are presented in Table III. Both of these materials had inhibitory effects on hemagglutination and hemolysis. Normal allantoic fluid appeared to be more active than amniotic fluid in the inhibition of both hemagglutination and hemolysis by the mumps virus and had a greater effect on hemolysis. The inhibitory effect of

TABLE III

Inhibition of Mumps Virus Hemagglutination and Hemolysis of Chicken Erythrocytes by Normal Allantoic and Amniotic Fluids

Diluent	Test	Dilutions of virus									Control
		8	16	32	64	128	256	512	1024	2048	
Phosphate buffer	Hemagglutination	+	+	+	+	+	+	+	+	±	0
	Hemolysis	58*	54	44	24	15	10	5	0	0	0
Normal allantoic fluid	Hemagglutination	+	+	+	+	+	0	0	0	0	0
	Hemolysis	16	8	4	2	0	0	0	0	0	0
Normal amniotic fluid	Hemagglutination	+	+	+	+	+	+	+	0	0	0
	Hemolysis	37	26	11	7	4	0	0	0	0	0

* Per cent hemolysis.

allantoic fluid was not destroyed by incubation with fresh mumps virus for 3 hours at 37°C.

Egg White Inhibitor and Red Cell Extract.—

Inhibitors prepared from egg white were diluted 1:10 in phosphate-buffered saline. The red cell extract was dissolved in distilled water by heating at 56°C. for 30 minutes and further diluted in buffered saline for use. Serial dilutions of virus were made in solutions containing the inhibitors and the tests for hemagglutination and hemolysis were performed in the usual manner. The hemagglutination test with red cell extract contained 250 micrograms per tube and the hemolytic test 500 micrograms per tube. A series in which the virus was diluted in normal allantoic fluid from 12-day embryos was included for comparison and another series using phosphate-buffered saline as diluent served as control.

It will be noted from the data in Table IV that the egg white inhibitors have a greater inhibitory effect on hemagglutination than on

hemolysis. Since a period of incubation at 37°C. is used in the hemolytic test, the difference observed might have been due to the fact that mumps virus destroyed the egg white inhibitor during this period of incubation. The inhibition titer of the two substances was reduced eightfold or more when they were incubated with virus at 37°C. for 3 hours before adding the red cells. This reduction in titer indicates that the inhibitors were destroyed by the virus at 37°C. The inhibiting action of the red blood cell extract was also destroyed by previous incubation with virus at 37°C.

TABLE IV

Inhibition of Mumps Virus Hemagglutination and Hemolysis of Chicken Red Cells by Egg White Inhibitors and Human Red Cell Extract

Diluent	Test	Dilution of virus									Control
		8	16	32	64	128	256	512	1024	2048	
Phosphate buffer	Hemagglutination	+	+	+	+	+	+	+	+	0	0
	Hemolysis	76*	62	52	35	24	13	8			0
Egg white inhibitor E17 (1:10)	Hemagglutination	+	+	+	0	0	0	0	0	0	0
	Hemolysis	54	51	45	27	16	7	4			0
Egg white inhibitor EI (1:10)	Hemagglutination	+	+	+	0	0	0	0	0	0	0
	Hemolysis	52	49	39	26	16	4	2			0
Normal allantoic fluid	Hemagglutination	+	+	+	+	0	0	0	0	0	0
	Hemolysis	28	11	4	3	0	0	0			
Phosphate buffer	Hemagglutination	+	+	+	+	+	+	+	+	+	0
	Hemolysis	54*	46	34	26	14	11				0
Red cell extract solution	Hemagglutination	+	+	+	+	+	0	0	0	0	0
	Hemolysis	23	21	11	6	3	0				0

* Per cent hemolysis.

2. Interfering Action of Homologous and Heterologous Viruses.—

Previous experiments have shown that proper treatment with heat can destroy the hemolytic property of mumps virus while leaving the hemagglutinating activity relatively unaltered. Experiments were performed using heat-inactivated virus as an interfering agent for hemolysis with active mumps virus. Since influenza virus acts on the same cell receptors as mumps virus in hemagglutination reactions, but shows no hemolytic activity under the conditions of this test (1), it was likewise used. It was thought that these viruses would interfere with hemolysis by a blocking action on the cell receptors, so the effect of changing the order of adding the hemolytically active and inactive viruses was also studied with mumps virus.

Effect of Inactivated Mumps Virus.—Amniotic fluid infected with virus (hemagglutinin titer of 1:1024) was heated to 50°C. for 30 minutes. The technique used consisted of mixing 1 ml. heated virus, 0.5 ml. of serial dilutions of active virus, and 0.5 ml. of 4 per cent chicken cells in the manner indicated in Table V. Two controls were included: a series using only buffered saline without heated virus and another in which virus whose hemagglutinating capacity had been destroyed by heating to 56° for 30 minutes was used. The hemolytic tests were then carried out as in previous experiments.

Mumps virus heated at 50°C. interferes with the hemolysis of erythrocytes by unheated mumps virus and this interfering action is more marked when the heated virus is added 30 minutes before the unheated virus. When the heat treatment is sufficient to destroy the hemagglutinating capacity of the virus (*i.e.* 30 minutes at 56°C.), the virus has little interfering action, a fact which

TABLE V

Interference with Hemolysis by Inactivated Mumps Virus

Hemolysis tests	Hemolysis							
	Dilutions of active virus							Control
	8	16	32	64	128	256	512	
1. Active virus in phosphate buffer	45*	49	43	26	15	9	4	0
2. Active virus added before inactive virus‡	25	15	8	3	0	0	0	0
3. Active and inactive viruses added at same time	14	8	4	2	1	0	0	0
4. Inactive virus added before active virus‡	4	2	1	0	0	0	0	0
5. Active virus with virus heated at 56°C.	45	32	21	11	5	4	1	0

* Per cent hemolysis.

‡ Time interval was 30 minutes.

suggests that combination with the cell receptors is essential for this effect. Mumps virus eluted into buffered saline was employed to eliminate the components present in amniotic fluid, and comparable results were obtained, indicating that the inhibitory substances present in normal amniotic fluids are not responsible for the interfering action observed.

Effect of Influenza Virus.—

Influenza A and B viruses were used. One-half ml. amounts of various dilutions of mumps virus were mixed with 0.5 ml. allantoic fluid infected with influenza virus and 1 ml. of 2 per cent chicken red cells was then added. Both influenza viruses had a hemagglutination titer of 1:1280.

When either influenza A or B virus was used in equal amounts as indicated by the hemagglutination titer, they exerted the same marked inhibitory effect on hemolysis as shown in Table VI. This interfering action was greater than that observed with uninfected allantoic fluid (Table III).

C. Relationship of Hemolysis and Virus Elution

All the evidence so far presented suggests that lysis of the red cells by the mumps virus occurs only under conditions which allow for elution of the adsorbed mumps virus. It was of interest to determine whether quantitative relationships exist between the degree of hemolysis and the amount of virus eluted, and whether such relationships can be altered by varying the conditions under which these reactions occur.

1. Correlation between Hemolysis and Virus Elution.—

The method used was adapted from the "step-wise elution" technique of Björkman and Horsfall (6).

One-half ml. of infected amniotic fluid was mixed with 1 ml. of 5 per cent chicken red cells and 3.5 ml. of buffered saline to make the final volume 5 ml. Adsorption of mumps virus was allowed to take place at 4°C. for 1 hour. The supernatant fluid was discarded and cells were then resuspended in 5 ml. buffered saline which had been warmed to 37°C. The tube was immediately placed in a water bath at the same temperature. At certain time intervals, the mixture was sedimented in the centrifuge and the supernatant fluids removed for determination of the amount of hemolysis that had occurred during the interval and of their hemagglutinin titer. Five ml. of warmed phosphate saline was then added and the cells gently shaken and reincubated. The results are presented in Fig. 1.

TABLE VI

Interfering Action of Influenza Viruses on Hemolysis of Red Cells by Mumps Virus

Interfering virus	Hemolysis							
	Dilutions of mumps virus							Control
	8	16	32	64	128	256	512	
None	49*	45	44	35	28	19	11	0
Influenza A	13	4	1	0	0	0	0	0
Influenza B	13	5	1	0	0	0	0	0

* Per cent hemolysis.

The results show a close relationship between the degree of hemolysis and the amount of virus liberated at 37°C. Lysis of the red cell occurs during elution of the active virus. The rate of elution appears to be related to the degree of hemolysis produced.

2. Occurrence of Virus Elution during Hemolysis.—

In the case of influenza virus, failure of elution with retention of hemagglutinating activity has been reported, either by modification of the virus by heat (7, 8) or by periodate (9). Partial failure of elution was demonstrable when the mumps virus was heated to 50–56°C. for various periods of time, but the he-

magglutinating capacity was also greatly impaired by such treatment. Complete failure of elution without reduction in hemagglutinin titer was accomplished by periodate treatment in the following experiment.

One ml. of amniotic fluid infected with mumps virus was mixed with 1 ml of different periodate solutions. After standing at room temperature for 30 minutes, 2 ml. of 5 per cent glucose solution was added to reduce the excess reagent. Virus treated with periodate solution previously reduced by glucose served as control. Tests for hemolytic activity and ability to adsorb to and elute from red cells were made on each virus preparation. For the hemolytic

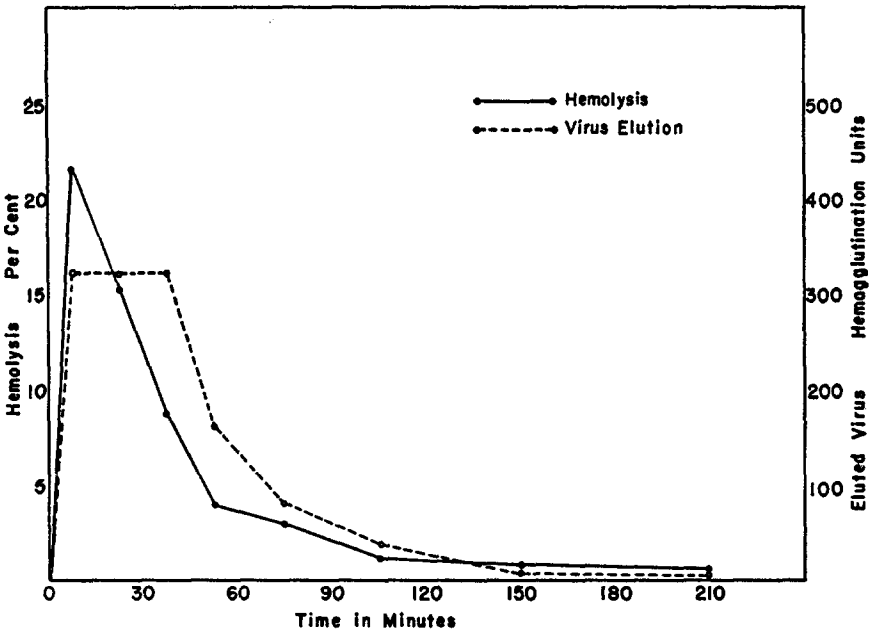

FIG. 1. Relationship of virus elution and hemolysis.

test, the usual technique was used. For study of the adsorption-elution mechanism, 1 ml. of treated or control virus (diluted 1:4) was added to 1 ml. of 2 per cent chicken red cells. The mixture was placed at 4°C. for 1 hour, centrifuged, and the hemagglutinin content of the supernatant fluid determined. After adding 2 ml. of buffered saline to resuspend the cells, the virus was allowed to elute at 37°C. for 3 hours. The suspension was again sedimented in the centrifuge and the hemagglutinin content of the eluate determined.

The data in Table VII indicate that the virus treated with periodate failed to hemolyze red cells when it had lost the capacity to elute following adsorption on these cells.

3. Comparison of Hemolysis and Virus Elution under Different Conditions.—

In previous studies (1) it has been shown that the degree of hemolysis produced by the same virus preparation depends on the temperature and hydro-

gen ion concentration of the medium in which the reaction takes place. It was of interest to determine whether reduction in hemolysis with suboptimal conditions of temperature and pH would be accompanied by a corresponding reduction in the amount of virus liberated from the red cells.

Hemolysis and Virus Elution at Different Temperatures.—

One-half ml. amounts of infected amniotic fluid were mixed with 1 ml. of 10 per cent chicken red cells, to which 3.5 ml. of buffered saline was added to make the final volume 5 ml. Two tubes were prepared in the same manner and placed at 4°C. for 1 hour to allow the virus to adsorb to the cells. After centrifuging, the supernatant fluid was poured off and replaced by 5 ml. of buffered saline. The cells were resuspended by gentle shaking and elution of the adsorbed virus was allowed to take place at 37°C. and 27°C. respectively. After 4 hours

TABLE VII

Effect of Sodium Periodate on Hemolysis and Elution of the Mumps Virus

Mumps virus treated with periodate	Virus adsorption and elution			Hemolytic activity of virus						Buffer control
	Hemagglutination titer			Dilutions of virus						
	Original fluid	Supernatant fluid	Eluate	16	32	64	128	256	512	
conc.										
M/100 + glucose	2048*	<16	1024	51‡	45	43	28	18	12	0
M/100	2048	<16	<16	0	0	0	0	0	0	0
M/1000	2048	<16	1024	41	40	38	27	16	12	0
M/10000	2048	<16	1024	43	42	38	30	20	14	0

* Expressed as the reciprocal.

‡ Per cent hemolysis.

at 37°C. and 7 hours at 27°C., the suspensions were again centrifuged and the degree of hemolysis in the eluates determined. The hemagglutinating and hemolytic activities of the virus in the eluate were determined with fresh red cells. In order to obviate the interference of hemoglobin already present in the eluate in the determination of its hemolytic activity, the test was modified in the following manner. After adding serial dilutions of the eluate to the red cells, the tubes were placed at 4°C. for 1 hour to allow maximal adsorption of virus, the cells were spun down, and the supernatant fluids were replaced by buffered saline before incubation at 37°C. To make the results comparable, all the hemolysis tests in this experiment were performed in a similar manner. The results are presented in Table VIII.

The results show that elution of the virus at 37°C. in 4 hours produces more hemolysis than its release at 27°C. in 7 hours. Thus, the same amount of virus (as indicated by equal hemagglutinin and hemolysin titers in the eluates) can elute at different temperatures with the production of different degrees of hemolysis. These processes do not appear, therefore, to be correlated.

Hemolysis and Virus Elution at Different pH Levels.—

One ml. amounts of a 10 per cent chicken red cell suspension in isotonic phosphate buffers of various pH values (4.5–9.0) were allowed to stand for 3 hours. After washing with 2 changes of phosphate buffer at the respective pH levels, the cells were resuspended in 4.5 ml. of the

TABLE VIII

Comparison of Hemolysis and Elution of Mumps Virus at Different Temperatures

Fluid tested	Hemolysis in fluid	Hemagglutination titer	Titration of hemolytic activity						
			Dilutions of fluid						Control
			20	40	80	160	320	640	
	per cent								
Original amniotic fluid	0	1280*	28‡	23	17	13	6	4	0
Supernatant at 4°C.	0	40	0	0	0	0	0	0	0
Eluate at 37°C. for 4 hrs.	19	1280	22	15	9	5	2	0	0
Eluate at 27°C. for 7 hrs.	8	1280	26	18	12	8	3	2	0

* Expressed as the reciprocal.

‡ Per cent hemolysis.

TABLE IX

Effect of pH of the Diluent on Adsorption and Elution of Mumps Virus and the Degree of Hemolysis Produced

pH of diluent	Supernatant fluid		Eluate	
	Hemagglutination titer	Hemolysis	Hemagglutination titer	Hemolysis
		per cent		*per cent*
4.5	<32*	2	64*	100
5.0	<32	1	1024	1
5.5	<32	0	1024	1
6.0	<32	0	1024	1
6.5	<32	0	1024	14
7.2	<32	0	1024	25
8.0	<32	0	1024	19
8.5	<32	0	1024	10
9.0	<32	0	512	7

* Expressed as the reciprocal.

corresponding buffer solutions. To each of these cell suspensions, 0.3 ml. of infected amniotic fluid was added to make the final dilution of virus 1:16. Adsorption was allowed to occur at 4°C. for 1 hour and the hemagglutination titer of the supernatant fluid as well as the amount of hemolysis, if present, determined. After replacing the supernatant fluid with an equal amount of fresh buffer solution at the same pH, the tubes were placed in a water bath at 37°C. for 3 hours. The degree of hemolysis in the eluate was determined and the hemagglutinin content titered as noted in Table IX.

Adsorption and elution of the mumps virus appeared to occur about equally well within pH range 5.0–8.5, but marked differences were observed in the proportion of red cells hemolyzed during elution of the virus. Maximal hemolysis occurred when the pH of the medium was near 7.0. However, the hemolytic property of the virus eluted at high or low pH values was not destroyed. This was indicated by the fact that when the reaction of these eluates was adjusted to neutrality and they were then tested with fresh cells in neutral phosphate-buffered saline approximately the same degree of hemolysis was obtained as that produced by virus eluted at pH 7. The data prove that with certain hydrogen ion concentrations, mumps virus can elute from chicken erythrocytes with little hemolytic action and yet show no permanent loss of hemolytic activity when subsequently tested at pH 7. This demonstrates that hemolytic action is not identical with the process of virus elution and that it is much more the responsive to changes in hydrogen ion concentration.

DISCUSSION

The results of comparison of the susceptibility of red blood cells to agglutination and hemolysis, respectively, by mumps virus suggest that the same receptor areas of the cell are important in both reactions. Cells which were not agglutinable by mumps virus were also not hemolyzed by the virus, a fact holding true whether the receptors were absent naturally or were destroyed by various means. The same locus on the red cell surface appeared to be involved in both hemagglutination and hemolysis.

When adsorption of hemolytically active mumps virus on red cells was interfered with by the presence of inhibitors of various types or by the addition of viruses lacking hemolytic activity, the hemolysis produced by active mumps virus was reduced. The inhibitors presumably combine with the virus to prevent its attachment to the red cell whereas the addition of interfering viruses saturates the receptor areas of the cell and prevents subsequent adsorption of mumps virus. The data provide further evidence that adsorption of mumps virus on the erythrocyte is an essential stage in the hemolytic action. The relative potency of inhibitors in the prevention of virus hemagglutination and in the suppression of hemolysis seemed to depend upon the ease with which the inhibitor was destroyed by virus at 37°C. Egg white inhibitors, which were readily inactivated by the virus, had much less effect on hemolysis than on hemagglutination. On the other hand, the component in normal allantoic fluid, whose inhibitory effect on hemagglutination and hemolysis did not appear to be readily destroyed by mumps virus, had a somewhat greater activity in preventing hemolysis than in suppressing hemagglutination.

The results of certain experiments indicated that hemolysis was related to elution of the virus from erythrocytes. For example, hemolysis did not occur

when adsorbed virus failed to elute from erythrocytes as a result of physical (2) or chemical treatment; *e.g.*, periodate. However, it is not evident from these experiments whether or not the loss of these two properties by the virus was mutually interdependent. Since loss of hemolytic activity occurred without loss of power to elute as a result of mild treatment of virus with heat or formaldehyde (2), it appears possible that periodate-treated virus did not hemolyze erythrocytes because its hemolytic property had been destroyed and not because it failed to elute from the cell. It is not unlikely that mumps virus is denatured by periodate, since loss of infectivity of other viruses following treatment with periodate has been observed (9, 10), and loss of hemolytic activity might accompany this denaturation.

Though hemolysis did not occur when there was no elution of mumps virus, hemolytically active virus can elute under certain conditions, after adsorption to the red cell, with production of little or no hemolysis. Experiments involving elution of virus under various conditions of temperature or pH showed that virus could elute from red cells, with consequent destruction of cell receptors, without hemolysis. Such findings indicated that virus elution and hemolysis were not identical processes. Furthermore, the previously mentioned observations (2) that hemolytic capacity could be destroyed by exposure to heat and formaldehyde, without seriously affecting the ability of the virus to elute, provided additional evidence of the dissimilar nature of these activities.

In the consideration of these experiments, it is important to point out that the hemolytic property of virus, which eluted from red cells at certain hydrogen ion concentrations without producing hemolysis, was not destroyed; for such eluates hemolyzed erythrocytes when tested with fresh cells at pH 7.2. This reversible inactivation of the hemolysin by changes in hydrogen ion concentration is additional evidence of its enzymatic nature (1).

At the present stage of these studies, it can be stated that adsorption of the virus to the red cell is an indispensable step for hemolysis and that disruption of the cell appears to occur during elution of the virus. The hemolytic process seems to involve two enzyme-like reactions, namely, destruction of red cell receptors and lysis of the cell. The differences discussed above suggest that these are distinct reactions. Furthermore, these two types of activity differ in their occurrence among viruses that agglutinate chicken red cells. Influenza and Newcastle disease viruses possess the capacity to destroy the same kind of red cell receptors affected by mumps virus, whereas Newcastle disease virus has been found to have a definite hemolytic action on erythrocytes (11) while influenza virus has not (1).

The phenomenon of hemolysis appears to involve some further action of the virus in addition to its destruction of red cell receptors during its release after adsorption to the cell. This additional activity has many of the characteristics associated with relatively labile enzymes.

SUMMARY

The relationship between hemagglutination and hemolysis by the mumps virus has been studied under conditions which affect (*a*) the receptors of chicken red cells and (*b*) the adsorption and subsequent elution of the virus from these cells. The results show that the hemolytic action of the virus appears to involve some of the same receptor areas of erythrocytes that are implicated in hemagglutination. Materials such as allantoic fluid, egg white, and red cell extract, which inhibit the agglutination of chicken red cells by mumps virus, also interfere with its hemolytic activity. Of these inhibitors, egg white and red cell extract, which are readily destroyed by the virus during incubation at 37°C., exert a greater antagonistic effect on hemagglutination than on hemolysis. Heated mumps virus or unheated influenza virus interferes with the hemolysis of red cells by untreated mumps virus.

Though hemolysis takes place during elution of the virus after its adsorption on the red cell, the processes are apparently distinct. The hemolytic activity is easily affected by certain conditions of pH and temperature which have no effect on the ability of mumps virus to adsorb on and elute from red cells.

BIBLIOGRAPHY

1. Morgan, H. R., Enders, J. F., and Wagley, P. F., *J. Exp. Med.*, 1948, **88,** 503.
2. Chu, L. W., and Morgan, H. R., *J. Exp. Med.*, 1950, **91,** 393.
3. Lanni, F., Sharp, D. G., Eckert, E. A., Dillon, E. S., Beard, D., and Beard, J. W., *J. Biol. Chem.*, 1949, **179,** 1275.
4. de Burgh, P. M., Yu, P. C., Howe, C., and Bovarnick, M. J., *J. Exp. Med.*, 1948, **87,** 1.
5. Stewart, F. S., *J. Path. and Bact.*, in press.
6. Björkman, S. E., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1948, **88,** 445.
7. Hirst, G. K., *J. Exp. Med.*, 1948, **87,** 315.
8. Chu, C. M., *J. Hyg.*, 1948, **46,** 247.
9. Hirst, G. K., *J. Exp. Med.*, 1949, **89,** 233.
10. Goebel, W. F., Olitsky, P. K., and Saenz, A. C., *J. Exp. Med.*, 1948, **87,** 445.
11. Kilham, L., *Proc. Soc. Exp. Biol. and Med.*, 1949, **71,** 63.