ADSORPTION OF INFLUENZA HEMAGGLUTININS AND VIRUS BY RED BLOOD CELLS

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In a recent paper on the agglutination of chicken red blood cells by suspensions of influenza virus (1), the majority of the experiments dealt with the agglutination-inhibiting properties of immune sera, and correlations were drawn between measurements of influenza antibodies done *in vitro* and those obtained by protection tests done in mice. In the present paper are reported the results of experimentation on the agglutination reaction itself.

It has previously been shown that a high percentage of the infective agent was removed from an influenza virus suspension when washed chicken cells were added and centrifuged out at low speed (2). The adsorption of influenza virus on chicken red cells was confirmed by the work of McClelland and Hare (3) who showed in addition that the adsorbed virus could be detected on the sedimented cells. Further work on the hemagglutinins present in influenza virus suspensions and on their adsorption on red cells is reported in the present communication.

Methods

Preparation of Virus Suspensions.—The two viruses used in this work were the mouse virulent PR8 strain of influenza A virus (4) and the Lee strain of influenza B virus (5). Suspensions of both strains were prepared from allantoic fluid by a technique which has been previously described (6). Briefly the method consisted of inoculating the allantoic sac of 11-day old white Leghorn embryos with 0.1 cc. of a 10^{-3} dilution of infected allantoic fluid. The eggs were incubated at 37°C. for 48 hours, and the allantoic fluid was removed without contamination with embryonic red cells. The cellular debris was removed from the fluid by low speed centrifugation. Large pools of allantoic fluid containing each virus were made at one time. The pools were distributed in small amounts into lusteroid tubes, which were stored at −72°C. until used. All of the work reported here was done with fluid from these pools.

Agglutination Titrations.—All estimations of agglutination titer were done in the same manner. Twofold dilutions of the virus suspension were made in saline. The test tubes used had an internal diameter of 0.8 cm. and a length of 7 cm. To 1 cc. of each dilution was added 1 cc. of a 1.5 per cent suspension of chicken red cells. The cells were added with an automatic pipetting machine which delivered them with such force that sufficient mixing took place at once. The degree of agglutination was read after the suspensions stood 75 minutes at room temperature.

The method of estimating the degree of agglutination and of calculating the agglutination titer was changed from that formerly used (6). Previously we measured the amount of agglutination in a given tube by comparing the density of the suspended cells in the supernatant portion of the fluid column with the density of standard red cell suspensions. In the present work the densities of the supernatant red cell suspensions were estimated by measuring the amount of light transmitted through a portion of the supernatant by means of a photoelectric densitometer. The details of construction of this optical densitometer will be given in a later report (7). The galvanometer readings were converted into values expressing the percentage of cells added to a tube, which remained in suspension after 75 minutes. The end point of a titration was taken to be the dilution of virus suspension where 50 per cent of the original cells added had sedimented to the bottom during the incubation period. Thus if a 1.5 per cent suspension of cells was added to a titration (1 cc. of virus dilution plus 1 cc. of cell suspension), the end point was in a tube with a supernatant cell density equal to that of a 0.37 per cent suspension of red cells. Usually, however, the 50 per cent sedimentation point fell between two dilutions, and it was necessary to calculate the titer by calculation from the two values obtained from dilutions on either side of the end point. This calculation was based on the fact that in an agglutination titration when the percentage of cells sedimented was compared with the dilution of virus suspension causing the agglutination, the curve for sedimentation was linear over a wide range when the dilution of virus was plotted on a logarithmic scale (7).

For example, in an agglutination titration 42 per cent of the cells in the 1:128 virus dilution remained suspended at 75 minutes, while in the 1:256 dilution 56 per cent of the cells did not completely sediment. The 50 per cent sedimentation point was calculated as $\frac{50 - 42}{56 - 42}$ or $^4/_7$ of the distance between the two dilutions. Since the scale of dilution was logarithmic, this distance was $^4/_7 \times \log 2$ or $0.57 \times 0.301 = 0.172$. Log 128 = 2.107 + 0.172 = 2.279, and antilog 2.279 = 190, which was the final titer. All titers are expressed as the reciprocal of the final dilution of the original virus suspension.

The use of a photoelectric densitometer for these readings has increased the accuracy of determination of end points over the visual method formerly employed. In general, duplicate determinations of agglutination titer checked within less than ± 10 per cent of the estimated value, although occasional duplicate titrations showed greater disparity than this.

Chicken Red Cells.—All of the red cells used were obtained from chickens in a local slaughterhouse. After the cervical vessels were severed the chicken blood was collected in one volume of 2 per cent sodium citrate. The cells were washed three times in physiological saline and were centrifuged at 1000 R.P.M. for 4 minutes after the final washing. The packed cells were stored at 4°C. and were diluted before use in saline. 1.5 per cent suspensions (by packed volume) were used for agglutination titrations. The suspensions, first made up by volume, were changed to a standard density (as measured on the optical densitometer) by the addition of small amounts of cells or saline.

Virus Titrations in Mice.—Titrations of virus suspensions in mice were done in

the usual way except that the virus was diluted in twofold steps. The 50 per cent mortality titer was calculated from the deaths which occurred within the first 10 days after inoculation.

EXPERIMENTAL

Adsorption and Elution of Influenza Hemagglutinins by Chicken Red Blood Cells.—50 cc. of a suspension of Lee virus and 50 cc. of a 3 per cent suspension

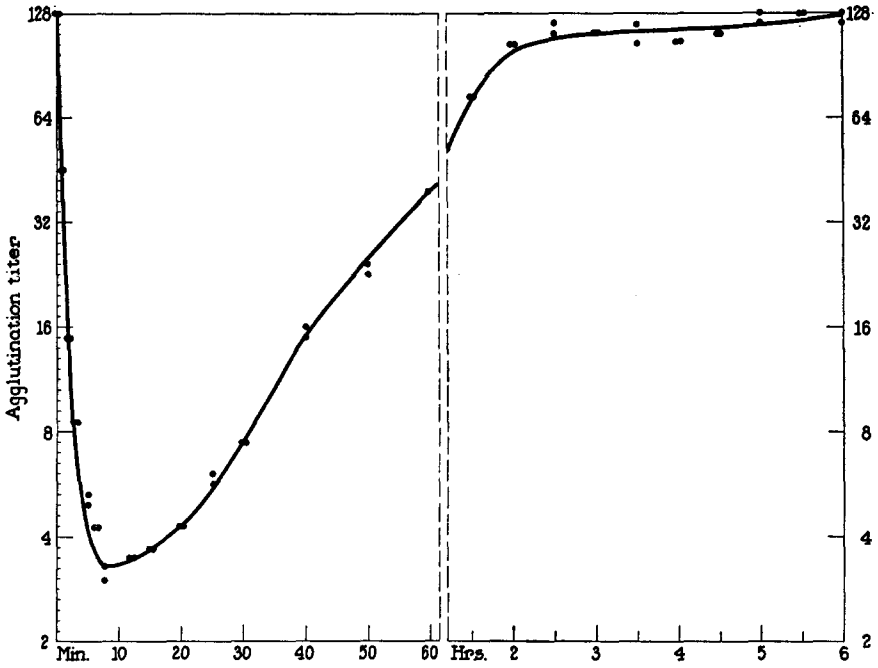

FIG. 1. Supernatant agglutination titers on a suspension of Lee allantoic fluid after the addition of chicken red blood cells. For this experiment the final dilution of virus preparation was 1:2, the final concentration of red cells was 1.5 per cent, and the adsorption was carried out at 23°C.

of chicken red cells were brought to room temperature and were then thoroughly mixed. 5 cc. samples were removed at intervals, the suspension being well mixed each time. The red cells in the samples were quickly sedimented at low centrifugal speed, and the supernatant fluids were pipetted off the cells. After the collection of all the samples the supernatant fluids were tested in duplicate for agglutination titer.

The results of these titrations are plotted in Fig. 1 and show that the influenza hemagglutinin was rapidly adsorbed from suspension. 9 minutes after the addition of red cells 97 per cent of the agglutinin present had been adsorbed

on the cells, and this was the point of maximum adsorption. From this time on the hemagglutinin slowly reappeared in suspension until at 6 hours' time the agglutinin titer of the supernatant fluid was back to its original value.

The same type of adsorption experiment, using the same concentration of red cells, was tried with a preparation of the PR8 strain of influenza virus. The graphic representation of this test is shown in the lowest curve of Fig. 2. With

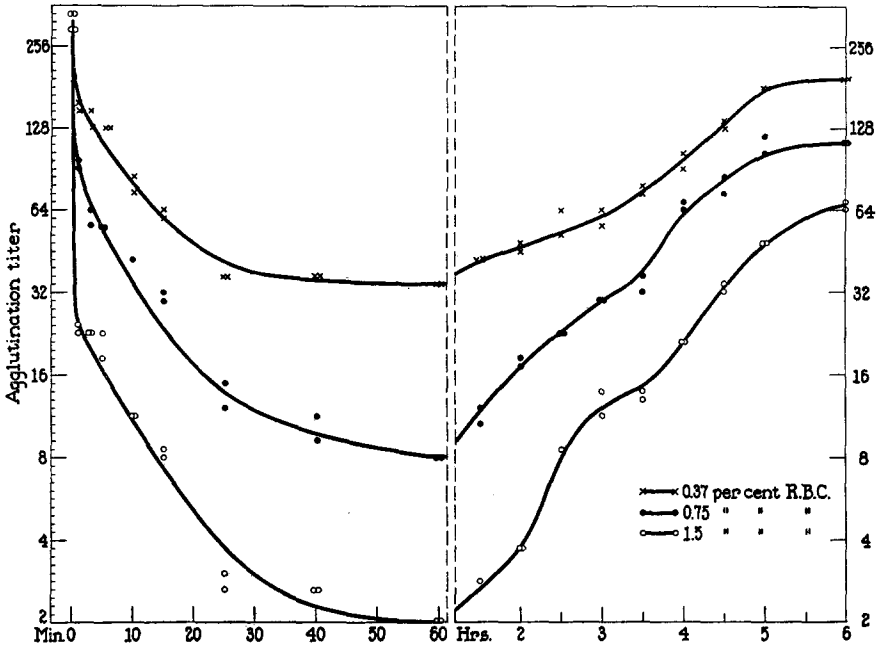

FIG. 2. Supernatant agglutination titers on suspensions of the PR8 virus strain after the addition of different concentrations of red cells. The final dilution of virus in each case was 1:2, while the concentration of red cells was 1.5 per cent, 0.75 per cent, and 0.37 per cent. The reactions were carried out at 23°C.

the PR8 strain maximum adsorption of the agglutinin did not take place for 1 hour, and following this the elution of the active agent was not so rapid, nor so complete, as occurred with the Lee strain at this temperature. At the end of 6 hours only 25 per cent of the hemagglutinin had reappeared in suspension.

These two types of curves, with adsorption maxima at different times, and with different speeds of elution, were characteristic of the respective virus strains, and repeated experiments of this type always gave similar results.

Adsorption and Elution of Influenza Hemagglutinins with Various Concentrations of Red Cells.—In Fig. 2 are shown the adsorption curves obtained when different concentrations of red cells were added to the same preparation of PR8

virus. Equal volumes of 0.75, 1.5, and 3.0 per cent red cell suspensions were added to PR8 allantoic fluid, and the supernatant titers were determined as before. Regardless of the concentration of cells used the time of maximum adsorption was the same, about 1 hour. With large concentrations of cells the maximum adsorption was more complete than with small concentrations, but correspondingly the subsequent elution of agglutinin was less complete in 6 hours when more cells were used. This experiment was carried out at room temperature (23°C.).

Adsorption of the PR8 Virus Hemagglutinin at Various Temperatures.—In order to test the effect of temperature on the adsorption-elution phenomenon a preparation of PR8 virus was used, and the reaction was carried out at 4°, 27°, and 37°C. Equal volumes of virus preparation and a 3 per cent suspension of red cells were both brought to corresponding temperatures before mixing together. Samples were taken as before, and the supernatants were all tested in duplicate for agglutination titer at room temperature. The results are shown graphically in Fig. 3.

To evaluate the results of this experiment it is easiest to consider the different phases of the adsorption and elution separately. The speed of the initial adsorption in the first minute was practically the same at the different temperatures. The difference in speed of adsorption at 4°C. and at 27°C. was very small for as long as 30 minutes. The most striking variation occurred in the time of maximum adsorption of the agglutinin. At 4°C. this was approximately 5 hours, at 27°C. the maximum occurred at 25 minutes, and at 37°C. this point fell between 3 and 5 minutes. Likewise when the degree of adsorption which occurred at the maximal points is considered, the percentage of total agglutinin adsorbed at 4°C. was found to be 99.5 per cent, at 27°C. it was 98.8 per cent, while at 37°C. only 87 per cent was adsorbed. In the elution phase of the experiment the differences found are also marked. At 4°C. less than 1 per cent of the agglutinin adsorbed had eluted in 18 hours. At 27°C. the elution was much more rapid but still very incomplete at 6 hours' time, only 25 per cent of the agglutinin having returned to the supernatant. The elution at 37°C. was much more rapid and was very nearly complete at 6 hours' time. A change in temperature from 4°C. to 37°C. greatly speeds up the elution phase of the reaction, and the curve obtained with PR8 virus at 37°C. resembles that found with Lee virus at 23°C. (Fig. 1).

Effect of Adsorption and Elution of Hemagglutinins on the Red Cells.—When chicken red cells are agglutinated by preparations of influenza virus, the clumps of red cells formed are very fragile and are easily broken up by gently shaking the tube which contains them. When the clumps are broken up so that the cells form a well dispersed suspension again, frequently the cells will agglutinate just as they did before and under suitable conditions such reagglutination may be demonstrated many times.

In the previous experiments on the adsorption of PR8 virus hemagglutinins at different temperatures (Fig. 3) some of the adsorbing cells were removed at various times for the testing of their agglutinability at different stages in the adsorption. For this purpose 2 cc. of the mixed suspension were used for each test. The cells and supernatant were thoroughly mixed with a pipette until

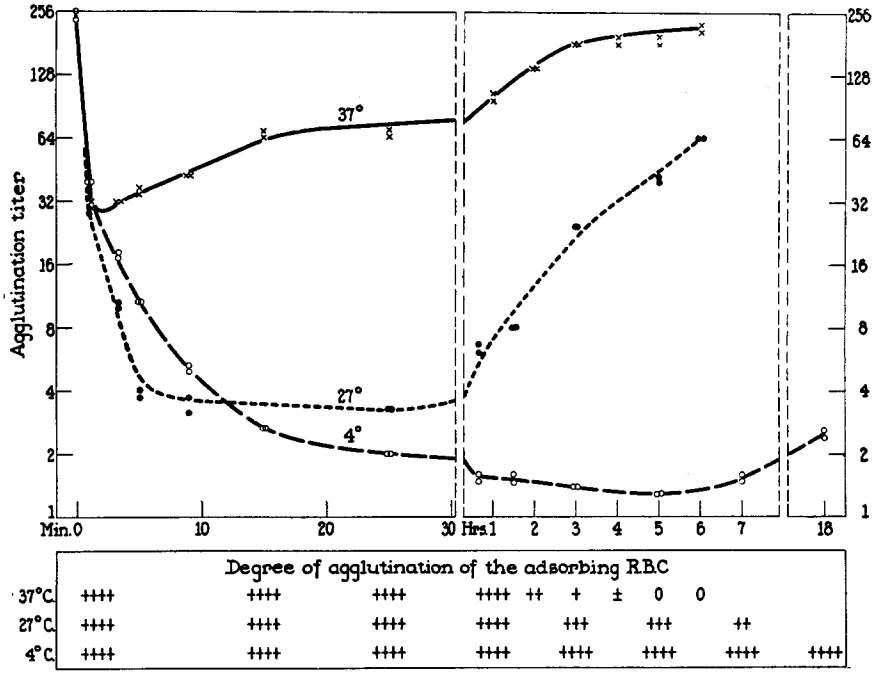

FIG. 3. Supernatant agglutination titers on suspensions of the PR8 virus strain. The adsorptions were carried out at 4°, 27°, and 37°C. The final dilution of virus suspensions was 1:2, and the concentration of cells was 1.5 per cent. At the bottom is indicated the degree of agglutinability of the adsorbing cells when removed from the suspension at various times. ++++ indicates the maximum degree of agglutination usually seen, and zero indicates no agglutination.

the suspension was even and showed no clumps, and then was put into a regular agglutination test tube and the degree of agglutination (or reagglutination) was read in 75 minutes. The results of these tests are recorded on the bottom of Fig. 3. Four plus indicates the maximum degree of agglutination usually seen under any conditions, and lesser degrees of aggregation are recorded as three plus to plus-minus. Zero indicates that the cells did not agglutinate at all. When the adsorption was carried out at 4°C., the cells retained their full capacity to agglutinate for at least 18 hours, and, as has been pointed out previously,

the cells retained almost all of their adsorbed agglutinin for this time. When the adsorption was carried out at 27°C. the agglutinability of the cells decreased as a significant amount of agglutinin was eluted, but aggregation still occurred after 7 hours' exposure to the virus. At 37°C. the ability of the cells to agglutinate disappeared at 5 hours. Cells removed from the 37°C. preparation also failed to agglutinate when suspended in fresh virus preparations of either the PR8 or the Lee strain.

Red cells which were mixed with Lee allantoic fluid at room temperature also showed with time a similar decrease in capacity to agglutinate. After 4 to 5 hours' exposure to the virus suspension, the cells failed to reagglutinate at all, either in saline or in fresh virus suspensions of the same or heterologous strains.

While the foregoing tests showed that cells which were exposed for long periods to virus suspensions lost their agglutinibility, it was decided to see if such inagglutinable cells were capable of adsorbing freshly added virus agglutinin. A 1.5 per cent suspension of red cells was added to a preparation of the Lee strain of influenza virus, and after 4 hours the cells were removed and added to a fresh suspension of virus prepared from the same strain, and the supernatant agglutinin titers were measured as before. Fresh red cells were added to more of the same virus suspension as a control on the adsorption. The results of these two adsorption tests are shown in Fig. 4. The control adsorption with fresh red cells gave the usual result, while the previously treated cells failed to adsorb a significant amount of agglutinin from the new virus suspension. The failure of these treated cells to adsorb any agglutinins on second exposure was not due to the fact that the cells were "saturated" with agglutinin, because in the first exposure the agglutinating agent had been almost completely eluted from the cells.

In a similar manner it has been shown that cells exposed to PR8 virus for 4 to 6 hours also fail to adsorb agglutinins from fresh suspensions of the same strain. In addition, it was found that red cells exposed to the PR8 strain failed to adsorb agglutinins from suspensions of Lee virus and vice versa.

These experiments show that the exposure of chicken red cells to either strain of virus alters the cell in such a way that it is no longer agglutinable and no longer has the power to adsorb influenza hemagglutinins. It also seems likely that a certain amount of agglutinin must be fixed to the cell in order for agglutination to occur. The loss of agglutinability and the elution of the agglutinin are parallel phenomena.

Activity of the Hemagglutinin Following Its Adsorption and Elution from Chicken Red Cells.—The following experiment was performed to determine whether the agglutinin which has once been adsorbed on red cells and subsequently eluted is still capable of further similar activity. 1.5 cc. of packed red cells was added to 100 cc. of Lee allantoic fluid. Samples were removed periodically, the cells sedimented, and the supernatant fluids removed. After 3 hours

all of the cells were removed from the remaining virus suspension, and fresh, packed cells were added in sufficient quantity so that the final concentration was 1.5 per cent. Samples were removed for another 3 hours, the second lot of cells was removed, and a third time fresh red cells were added. This suspension was sampled for a final 3 hours. All of the supernatant fluids were tested in duplicate for agglutination titer at one time. The results of this experiment

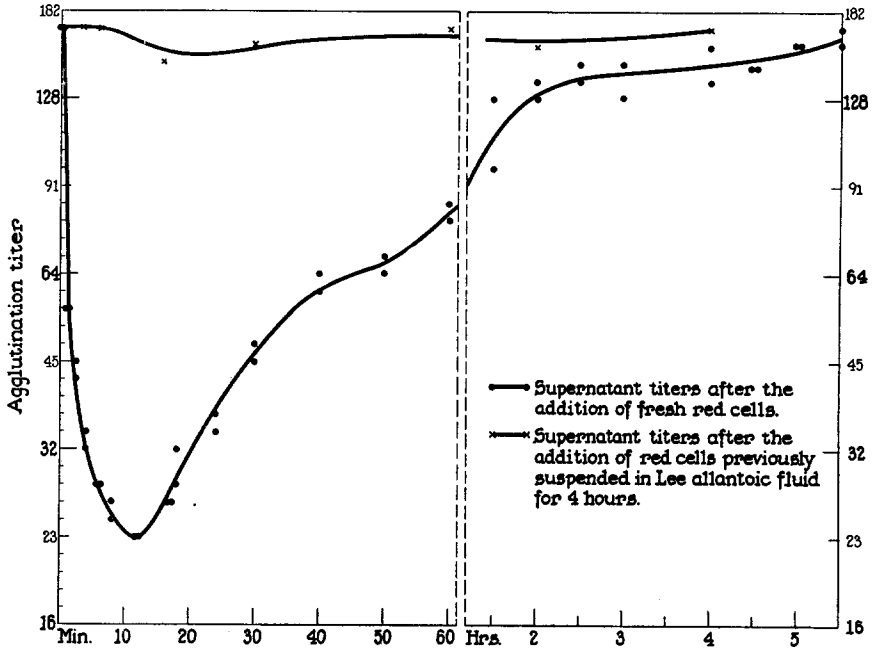

FIG. 4. Supernatant agglutination titers on suspensions of the Lee strain of influenza virus after the addition of fresh red cells and cells which had previously been exposed for 4 hours to Lee virus. The final concentration of virus was 1:2 in each case, while that of the cells was 0.75 per cent. The tests were carried out at 24°C.

are shown in Fig. 5, and they clearly indicate that the activity of the agglutinin is not exhausted by the process of adsorption and elution, and except in a minor degree no activity is lost in the process.

Stability of the Red Cell Factor.—Stroma was prepared from chicken red cells by homogenizing a concentrated suspension in a Waring mixer. The sediment from the fragmented cells was washed repeatedly in distilled water and in saline until no red color remained. When this stroma was added to virus suspension the hemagglutinin was rapidly adsorbed and elution began to occur after 10 to 15 minutes when the Lee strain of virus was used. The adsorption curves were quite similar to those obtained with the use of intact cells.

Chicken red blood cells were heated to 65°C. for ½ hour. At this temperature the cells were completely lysed, and the stroma was used for adsorption tests. The adsorption curves were similar to those obtained with intact cells. When the cells were heated at 100°C. for 5 minutes, adsorption and elution still occurred with the stroma, but the elution of the agent was much less marked than with the use of intact cells. In all of the experiments with stroma, heated

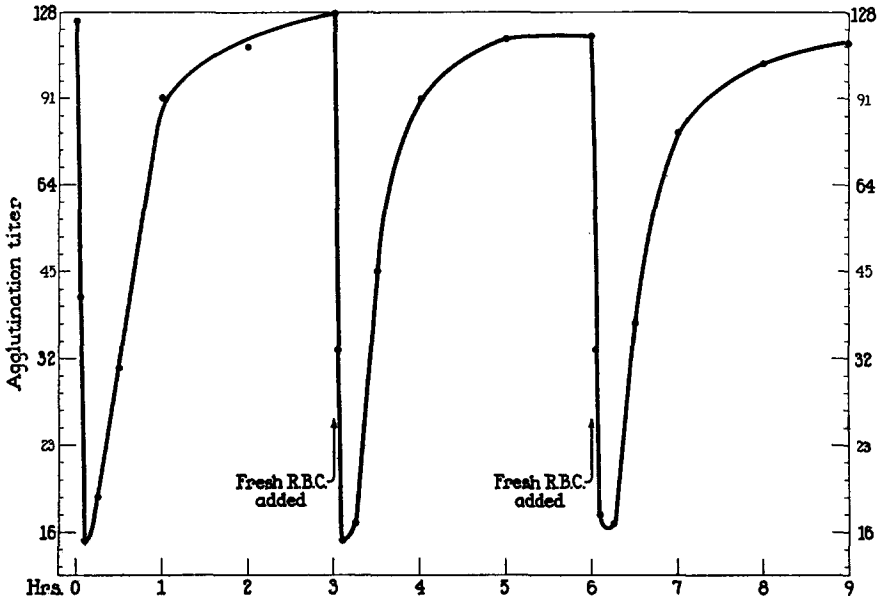

FIG. 5. Supernatant agglutination titers on a suspension of the Lee strain of influenza virus, to which fresh red blood cells were added three times at three hourly intervals. The final concentration of the virus suspension was approximately 1:1 throughout, while the concentration of cells was maintained at 1.5 per cent. The test was carried out at 24°C.

or unheated, a much larger amount of substance was necessary to produce a good adsorptive effect than if intact cells were used.

Stability of the Virus Hemagglutinins.—In order to determine the degree of heat stability of the influenza hemagglutinins, suspensions of PR8 and Lee virus were heated at 50°C., 55°C., and 60°C. for 1 hour. Samples were removed periodically and tested for agglutination titer. Each sample was also given intranasally in full concentration to six mice. The effect of heating on the parallel *in vitro* and *in vivo* tests is given in Table I. A temperature of 50°C. for 1 hour does not detectably affect the agglutination titer. At 55°C. about half the agglutinating activity is lost from each suspension in 1 hour. At 60°C. the inactivation is greatly enhanced. This is an average result, but the exact

degree of inactivation at a given temperature varies with different lots of virus. The loss of infectivity does not parallel the drop in agglutination titer.

TABLE I

Inactivation of Influenza Virus Hemagglutinins and Infectivity by Heat

Strain	Temperature	Agglutination titer						
		Length of time at given temperature (min.)						
		0	1	5	15	30	45	60
	°C.							
PR8 (A)	50	447*	479*	447*	479*	447*	479*	479*
	55	479*	338*	362*	315	223	194	223
	60	447*	169	9	6	5	3	<2
Lee (B)	50	194*	169*	194*	194*	182*	208*	208*
	55	208*	147*	128	128	104	97	91
	60	194*	56	<2	<2	<2	<2	<2

All samples were inoculated intranasally in mice, 0.05 cc. of undiluted material.

* Indicates those groups of mice in which death or lung lesions occurred as a result of the inoculation.

TABLE II

Inactivation of Influenza Virus Hemagglutinins and Infectivity by Formaldehyde

Strain	Concentration of formalin	Agglutination titer			
		Length of time of exposure to formalin			
		30 min.	1 day	3 days	8 days
	per cent				
PR8 (A)	None	256*	239*	256*	208*
	0.1	294*	138	128	91
	0.5	223	147	52	<2
	1.0	194	97	<2	<2
Lee (B)	None	147*	128*	128*	128*
	0.1	128*	138	128	128
	0.5	138	138	104	52
	1.0	120	104	34	<2

All samples were inoculated intranasally in mice, 0.05 cc. of undiluted material.

* Indicates those groups of mice in which death or lung lesions occurred as a result of the inoculation.

A preparation of PR8 virus was heated at 55°C. for 30 minutes. No infectivity was then detectable, and about half of the agglutinating power was lost. Red cells were added to this suspension, and the adsorption of the remaining agglutinin was measured. Adsorption and elution occurred in a manner similar to that obtained with untreated virus suspensions.

To find out the effect of formaldehyde on the hemagglutinins, again suspensions of the PR8 and Lee strains were used. Formalin was added to aliquots of each virus to a final concentration of 0.1, 0.5, and 1.0 per cent. After ½ hour the various suspensions were tested for agglutination titer. They were retested at 1, 3, and 8 days, the suspensions being stored at 4°C. Parallel tests for in-

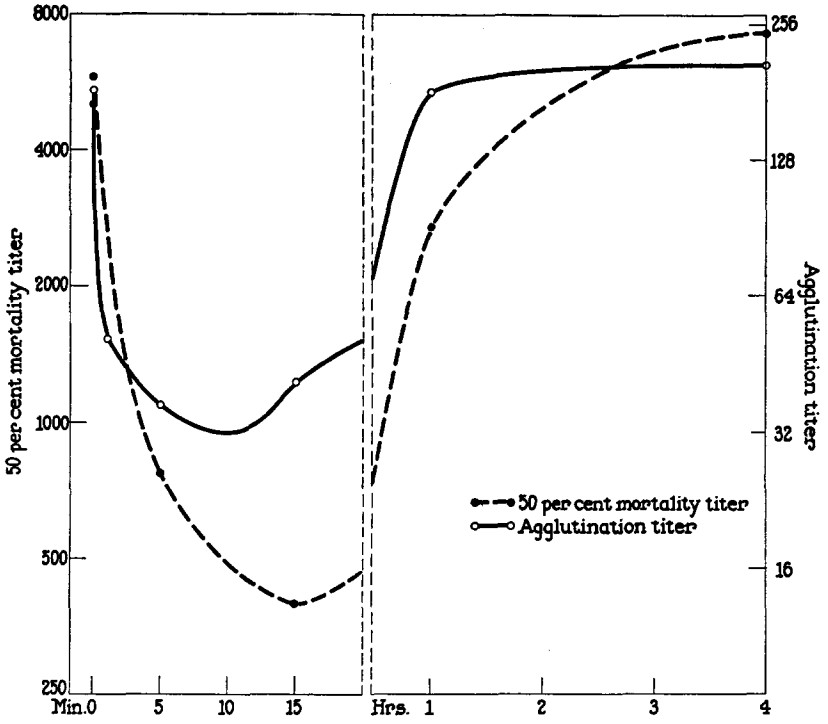

FIG. 6. Supernatant agglutination titers and 50 per cent mouse mortality titers on a suspension of the Lee strain of influenza virus, to which chicken red cells were added. The final concentration of the virus suspension for the test was 1:2 and of the cells 0.75 per cent.

fectivity were run by inoculating mice with 0.05 cc. of the undiluted material. The results are recorded in Table II from which it can be seen that formaldehyde has a slow inactivating effect on the agglutinin and a rapid and marked effect on the infectivity.

Formalin was added to suspensions of PR8 virus in such amount that after the addition of an equal volume of 1.5 per cent red cells the concentration was 0.1, 0.5, 1.0, and 2.0 per cent. Titrations were run on the supernatants up to 4 hours. Formalin concentrations of 0.1 and 0.5 per cent appreciably retarded the adsorption and elution of agglutinin. In the presence of 1.0 and 2.0 per

cent formalin, however, the agglutinin was rapidly adsorbed and there was no evidence of subsequent elution.

Correlation between the Adsorption and Elution of Virus Hemagglutinins and Infectious Activity.—While it has already been shown that chicken red cells adsorb virus infective activity from a suspension (2, 3), it has not been shown whether or not the infective particle is subsequently eluted from the cells. For this purpose a preparation of Lee virus and an equal volume of 1.5 per cent red cells were mixed. Samples were withdrawn at various intervals, from which the red cells were removed and the supernatant fluids were tested for agglutination titer *in vitro* and for mortality titer in mice. The two series of titers obtained are shown in Fig. 6. The agglutination titrations gave the usual type of curve with maximum adsorption at about 10 minutes, when approximately 80 per cent of the agglutinin had been removed. The 50 per cent mortality titers showed a maximum adsorption at 15 minutes when 95 per cent of the original infectivity was removed. In 4 hours' time both the agglutination titer and the mouse mortality titer of the supernatant fluid returned to their original values. Both forms of activity began to elute at about the same time. This result adds one further bit of evidence in favor of the view that the infective activity and the agglutinating activity in a virus suspension are very closely associated and may constitute parts of the same particle.

DISCUSSION

The foregoing data are much too meager to permit any definite conclusions to be drawn regarding the mechanism of agglutination of chicken red cells by suspensions of influenza virus. The facts do, however, suggest two analogies which may be of some assistance in planning a further attack on the problem.

The adsorption of bacteriophage onto the susceptible bacterial cell is very similar to the reaction we have described. In this case the bacteriophage particle is believed to attach to specific receptor points on the bacterial surface, and in instances in which the receptors have been isolated they have proved to be polysaccharides (8). Cells which do not possess the receptor points do not adsorb the phage. With bacterial viruses, however, the adsorption of the phage particle is followed by multiplication and then release of new particles. Here the analogy ends since there is no evidence so far of the ability of influenza virus to multiply in the presence of avian red cells.

The curves of adsorption and elution of influenza hemagglutinins suggest that the interaction of hemagglutinin and virus occurs in two phases: first, a combination (rapid even at low temperatures) and second, some alteration of the cells accompanied by a separation of the modified cells and the agglutinin. The latter phase appears to be considerably retarded at low temperatures. The modification of the cells in the second phase renders them incapable of combining with more agglutinin, while the released agglutinin is apparently unchanged.

The analogy of this reaction with the interaction of enzymes and substrates seems worthy of note. It is the generally accepted view that the first stage in enzyme action, at least in most cases, is a combination of enzyme and substrate. Then the substrate is chemically changed, whereupon the enzyme and the altered substrate dissociate, and the enzyme is free to adsorb and alter more substrate. It is clear that if one were able to make a measurement of the amount of free enzyme present during this process, the amount of free enzyme should be low in the initial stages after combination had occurred in the presence of an excess of substrate. In the later stages of enzyme action when the substrate has been largely used up, free enzyme should appear again in quantity approaching the initial concentration, since there is insufficient substrate to combine with all the enzyme present.

In this analogy the agglutinin corresponds to the enzyme, which is not used up, while the substrate corresponds to the substance at the receptor point on the red cell, which is destroyed during the process of agglutination, rendering the cell incapable of further adsorption. The sensitivity of the hemagglutinin to heat and to formalin is consistent with a substance of protein nature. The cellular substrate, on the other hand, is very resistant to heat, and this stability suggests that it may be a non-protein substance.

The obvious approach to the problem of the nature of this agglutination reaction is to try to isolate from the red cell the receptor substance and then to see what effect the virus suspensions have on it.

The simplest explanation of the actual aggregation of cells in the presence of the agglutinin is to view the latter as forming a bond between the cells. If the agglutinating particles were bi- or trivalent and there were multiple receptor points on the red cells, one could visualize how a network or clump of cells could be built up. The main evidence for this view is the fact that the agglutinability of the cells and the amount of virus hemagglutinin adsorbed are parallel phenomena.

Since the infective agent in a virus suspension was adsorbed and eluted in a manner similar to that of the hemagglutinin, it is clear that there is a close association between these two types of activity. Whether or not these two agents occur on the same particle or on different particles, and whether or not the two forms of activity are completely separable is not yet known. So far the agglutinin may be obtained without virus activity, but we have not yet been able to inactivate the agglutinin without also inactivating the virus.

It seems likely that the agglutination of red cells by influenza hemagglutinins may have some counterpart in natural infection of susceptible cells with the virus. While there is no evidence at all concerning this problem at present, it may be that susceptible cells possess a surface receptor similar to that of the red cell and that in infection this substance is altered by the hemagglutinin, thus injuring the cell membrane and providing a point of attack for the infective particle.

SUMMARY

A number of experiments were performed on the adsorption of influenza hemagglutinins on chicken red blood cells, from which the following conclusions were drawn:—

1. When chicken red blood cells and preparations of influenza viruses were mixed together, the influenza hemagglutinins present were rapidly adsorbed onto the cells. After varying lengths of time, dependent on the conditions of the experiment, the adsorbed hemagglutinins began to elute from the cells. With the Lee strain at 23°C. and the PR8 strain at 37°C. almost all of the adsorbed agglutinin was released in 4 to 6 hours.

2. When the number of red cells used for adsorption was increased, the speed and degree of adsorption of the hemagglutinins increased. The time of maximum adsorption of hemagglutinins was the same, regardless of red cell concentration, and with the larger amounts of red cells the speed and degree of elution was decreased.

3. When adsorption of PR8 virus agglutinins was carried out at 4°C. the adsorption was rapid and nearly complete. When the reaction was carried out at higher temperatures (27° and 37°C.), the adsorption was equally rapid but was progressively less complete with rise in temperature. At 4°C. the maximum adsorption was not reached for 5 hours; at 27°C. it was reached in 25 minutes; and at 37°C. the greatest degree of adsorption was attained between 3 and 5 minutes. The amount of elution observed at 4°C. at 18 hours was negligible, but the degree of elution increased with temperature so that at 37°C. almost all of the adsorbed agglutinin was released in 6 hours' time.

4. Red cells which had adsorbed and then fully eluted the agglutinin were not capable of adsorbing a detectable amount of fresh agglutinin. In addition, such cells would no longer agglutinate even though exposed to fresh virus suspensions.

5. The hemagglutinin of influenza B virus was capable of being adsorbed on and eluted from several successive lots of chicken red cells without appreciable loss of agglutinating activity.

6. The hemagglutinins of the PR8 and Lee strains were rapidly inactivated at 60°C. The presence of active virus was not necessary for the occurrence of the adsorption-elution reaction on chicken red cells.

7. The activity of the portion of the red cells responsible for the adsorption of the hemagglutinins persisted, though in reduced amount, even after heating for 5 minutes at 100°C. Hemagglutinins were adsorbed and eluted from red cell stroma.

8. The infective agent in influenza virus suspensions was adsorbed by chicken red cells simultaneously with the adsorption of hemagglutinins. 95 per cent of the infective agent was removed from suspension by the red cells after contact for 15 minutes. From then on the infective agent was gradually released from

the red cells. After 4 hours the 50 per cent mortality titer of the supernatant fluid was as high as at the beginning of the experiment.

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