THE SORPTION OF INFLUENZA VIRUS BY CHICKEN ERYTHROCYTES*

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The nature of the forces of attraction between cells and viruses is of obvious interest; and although erythrocytes probably are not the cells of primary importance in influenza virus infections, the attractive forces between them and influenza virus provide an experimental approach to the problem.

Previous reports presented data which indicate that the effect of influenza virus upon erythrocytes is influenced to a considerable extent by forces which appear to be of a physical-chemical nature. Failure of the WS strain of influenza virus to agglutinate sheep erythrocytes was shown to result from several sets of forces, some of which tended to agglutinate sheep erythrocytes and others of which tended to inhibit agglutination; over a narrow pH range the conditions could be controlled so that agglutination was sufficiently satisfactory to permit use of sheep erythrocytes for agglutination-inhibition tests (1). Furthermore, the WS strain of virus, which was in the phase of variation in which it agglutinated guinea pig but not chicken erythrocytes under usual test conditions, was shown to agglutinate erythrocytes of both species when the system was suitably buffered (2). Those data are of interest because in the former instance they showed that failure of the virus to agglutinate sheep erythrocytes was not due to lack of "receptors," as has been supposed (3), and in the latter instance they showed that the failure of the "O"-like phase of virus to agglutinate chicken erythrocytes was not due to the lack of affinity of the variant for that species of cells.

The present paper includes additional evidence of the influence of physical-chemical forces upon reactions between influenza virus and erythrocytes. The data indicate that the forces of attraction between virus and chicken erythrocytes are governed by the laws of mass action or, perhaps, by the laws of simple adsorption. Virus is sorbed[1] by erythrocytes in a manner which effects a pro-

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[1] The non-committal term *sorption* is employed throughout this paper in place of the more usual *adsorption* because experience in this laboratory indicates that absorption as well as

portional distribution of virus between erythrocytes and suspending fluid; the proportional distribution is maintained even when the quantity of erythrocytes employed is excessively large.

Materials and Methods

Virus.—Three egg-adapted strains of influenza A virus were employed,—the WS (4) and PR8 (5), and the recently isolated Smith (6). Virus was derived from pools of allantoic fluid obtained from eggs containing 12- to 14-day-old embryos, and which had been inoculated 2 days previously. All material used for egg inoculations contained approximately 250 units of penicillin per ¼ ml. inoculum.

Chicken Erythrocytes.—Cells used for sorption experiments or for hemagglutination tests were from 2 to 7 days old, and had been obtained aseptically by bleeding adult hens from the wing vein. No anticoagulant was used; immediately after withdrawal, the blood was diluted with approximately 40 volumes of saline, filtered aseptically through gauze, and then washed four times. Each of the first two washings was made with about 100 volumes of saline, the remaining washings with 30 to 40 volumes.

Sorption Experiments.—The mass of erythrocytes to be employed as sorbant was obtained by sedimenting the cells from the required volume of a ½ per cent to 2 per cent suspension of chicken erythrocytes; the packed cells (cooled to 0°C.) were suspended in the fluid to be sorbed, and the resultant suspension kept at 0°C. for 30 minutes. The suspensions then were centrifuged in an angle centrifuge for 5 minutes in the cold, and the sorbed supernatant fluids quickly removed. Throughout the experiments strict aseptic precautions were maintained and care was exercised to keep the temperature of the test mixtures between 0°C. and 4°C. until erythrocytes had been removed from the sorbed fluids.

Hemagglutination Tests.—Results were read on the basis of cell patterns after mixtures of 0.2 ml. volumes each of fluid, saline, and ½ per cent suspension of chicken erythrocytes had stood in Kahn tubes at room temperature for about 1 hour; tests with guinea pig cells were read after 1½ hours.

Tests for Egg Infectivity.—Tests were made by amniotic inoculation of hens' eggs containing 10- to 12-day-old embryos; the butt ends of the eggs had been removed and the chorio-allantoic membrane exposed 1 or 2 days previous to inoculation.

Dilutions of fluids to be tested were made in bacteriological culture broth; the serial dilutions were made with individual pipettes, in either threefold or tenfold steps. In most tests in which threefold dilutions were employed, three series were prepared, each from an aliquot of fluid to be tested, and duplicate eggs inoculated per dilution, per series (a total of 6 eggs for each dilution). When tenfold dilutions were employed, each was tested in a series of 10 eggs.

Two days after inoculation, the allantoic fluids were removed and tested in serial twofold dilutions for capacity to agglutinate chicken or guinea pig erythrocytes. Positive hemagglutination was assumed to indicate presence of virus; no agglutination, the absence of virus. Infectivity titers (ID_{50}) were determined by the cumulative 50 per cent end-point method of Reed and Muench (7). The test fluids all were cultured to determine the presence of bacterial contaminants.

EXPERIMENTAL

Sorption of Hemagglutinins from Influenza Virus Suspensions by Chicken Erythrocytes.—Tables I, II, and III include data from three experiments, each made with allantoic fluid infected with a different strain of influenza A virus;

adsorption may be involved in the reaction between influenza virus and erythrocytes. Perhaps neither term is correct.

TABLE I

Hemagglutinating Activity of WS-Infected Allantoic Fluid after Sorption with Varying Masses of Chicken Erythrocytes

Mass* of cells employed as sorbant	Initial twofold dilution of fluid									
	1	2	3	4	5	6	7	8	9	10
ml.										
0	++	++	++	++	++	++	++	++	+	0
0.1	++	++	++	++	++	++	++	+	0	0
0.2	++	++	++	++	++	++	++	+	0	0
0.3	++	++	++	++	++	+	0	0	0	0
0.4	++	++	++	++	++	+	0	0	0	0
0.5	++	++	++	++	++	+	0	0	0	0
0.6	++	++	++	++	++	+	0	0	0	0
0.7	++	++	++	++	+	0	0	0	0	0
0.8	++	++	++	++	+	0	0	0	0	0
0.9	++	++	++	++	+	0	0	0	0	0
1.0	++	++	++	++	0	0	0	0	0	0
2.0	++	++	++	+	0	0	0	0	0	0
3.0	++	++	+	0	0	0	0	0	0	0

* Expressed in terms of milliliters of a 1 per cent suspension of chicken erythrocytes from which the cells were sedimented.

Degree of agglutination: ++, complete; +, partial; 0, none.

TABLE II

Hemagglutinating Activity of Smith-Infected Allantoic Fluid after Sorption with Varying Masses of Chicken Erythrocytes

Mass* of cells employed as sorbant	Initial twofold dilution of fluid											
	1	2	3	4	5	6	7	8	9	10	11	12
ml.												
0	++	++	++	++	++	++	++	++	++	++	++	+
0.1	++	++	++	++	++	++	++	++	++	++	0	0
0.2	++	++	++	++	++	++	++	++	++	+		
0.4	++	++	++	++	++	++	++	+	0	0		
0.8	++	++	++	++	++	++	+	0	0	0		
1.6	++	++	++	++	+	0	0	0	0	0		
3.2	++	++	++	+	0	0	0	0	0	0		
6.4	++	++	++	+	0	0	0	0	0	0		

* Expressed in terms of milliliters of a ½ per cent suspension of chicken erythrocytes from which cells were sedimented.

Degree of agglutination: ++, complete; +, partial; 0, none.

in each instance constant volumes of allantoic fluid were sorbed with varying volumes of chicken erythrocytes.

It is clear from the data (Tables I, II, and III) that the capacity of chicken

erythrocytes to remove virus was somewhat dependent upon the strain of virus employed; the WS (Table I) and Smith (Table II) strains were rather similar in that relatively large masses of cells did not remove completely the hemagglutinating activity; in the case of the PR8-infected fluids (Table III), activity was removed completely.

In the presence of unsorbed hemagglutinins, the quantity of hemagglutinin removed per unit volume of erythrocytes did not increase in proportion to the increase in the mass of erythrocytes employed. For example, the cells from 0.1 ml. and 0.2 ml. (or an average of 0.15 ml.) of suspension (Table I) removed 50 per cent of available hemagglutinin from the WS-infected fluid (*i.e.*, re-

TABLE III

Hemagglutinating Activity of PR8-Infected Allantoic Fluid after Sorption with Varying Masses of Chicken Erythrocytes

Mass* of cells employed as sorbant	Initial twofold dilution of fluid										Log of egg ID_{50}
	0	1	2	3	4	5	6	7	8	9	
ml.											
0		++	++	++	++	++	++	++	++	0	5.57
0.25		++	++	++	+	0	0	0	0	0	
0.5		++	+	0	0	0	0	0	0	0	
1		+	0	0	0	0	0	0	0	0	
2		0	0	0	0	0	0	0	0	0	
4		0	0	0	0.	0	0	0	0	0	
8	0	0	0	0	0	0	0	0	0		4.0
16	0	0	0	0	0	0	0	0			2.7
32	0	0	0	0	0	0					2.61

* Expressed in terms of milliliters of a 1 per cent suspension of chicken erythrocytes from which the cells were sedimented.

Degree of agglutination: ++, complete; +, partial; 0, none.

duced the titer from 2^9 to 2^8). However, cells from volumes of 0.3 ml. to 0.6 ml., or an average volume of 0.45 ml., removed only about 37 per cent more activity than the 0.15 ml. average volume; increase in cell volume from the average 0.45 ml. to 0.8 ml. increased the quantity of virus removed only by 6 per cent; and increase in cell volume from 0.8 ml. to 2.0 ml. increased the quantity of virus sorbed by only 3 per cent. Thus, when the fluid was sorbed with the average volume of 0.15 ml. of cells, that 0.15 ml. unit volume removed 50 per cent of hemagglutinin, but when an aliquot of the fluid was sorbed with 3.0 ml. of the same lot of cells, each 0.15 ml. unit mass of that volume removed less than 5 per cent of hemagglutinin (*i.e.*, 3.0 ml., or twenty 0.15 ml. units, removed less than 99 per cent). Obviously, the sorbing mechanism of each 0.15 ml. unit volume of cells in the 3.0 ml. mass was only one-tenth as saturated

as the 0.15 ml. average volume which sorbed 50 per cent of the activity, yet in spite of that free sorbing mechanism, the hemagglutinin was not completely removed.

Similarly, it is clear from Table II that cells from 0.2 ml. of suspension removed 75 per cent of hemagglutinin from the Smith-infected fluid, but cells from twice the volume removed only 19 per cent additional activity; increasing the volume from 0.4 ml. to 0.8 ml. increased sorption by only 3 per cent. When one considers the quantity of virus removed per unit volume of erythrocytes, it is clear that the 0.2 ml. volume of cells removed 75 per cent of activity, but the 6.4 ml. volume of cells removed only about 99 per cent, or one–twenty-fifth the quantity of hemagglutinin per each 0.2 ml. volume; and in spite of that great excess of unsatisfied sorbing mechanism, the cells of the 6.4 ml. volume did not remove the hemagglutinin completely. It thus seems clear that forces exist in systems containing influenza virus suspensions and chicken erythrocytes which tend to keep virus in suspension even in the presence of an excess of erythrocytes.

The same phenomenon is evident in the case of the PR8-infected fluid (Table III), although all apparent hemagglutinating activity was removed from the fluids which were sorbed with masses of erythrocytes from volumes greater than 1 ml. However, removal of hemagglutinating activity was not an indication that virus had been removed completely; tests for egg infectivity of the fluids sorbed with the three largest volumes of cells showed that significant quantities of virus remained in suspension (Table III).

Failure to Remove Influenza Virus Completely from Suspension by Repeated Sorption with Chicken Erythrocytes.—The results of the previous experiment (Table III) showed that although the hemagglutinating activity of the PR8-infected fluid was completely removed by sorption with moderately large volumes of chicken erythrocytes, significant amounts of virus remained in suspension. It seemed desirable to determine whether or not repeated sorption would completely free allantoic fluid of virus as determined by infectivity for embryonated eggs.

Two PR8 allantoic fluids were employed; one differed from the other in that a fairly large precipitate had formed, the removal of which yielded a clear supernatant fluid with a significantly lower virus titer than the other. Both fluids were sorbed with sufficient erythrocytes to make 8 per cent suspensions; the high titer fluid was sorbed four times, and the low titer fluid, six times.

It is clear from the results included in Table IV that the first sorption of fluid "A" removed 97 per cent of the available virus (reduced the titer from approximately 10^9 to approximately $10^{7.6}$), but that three additional sorptions failed to remove the remaining 3 per cent of virus; the second sorption reduced the titer to only $10^{5.6}$, and two additional sorptions further reduced the titer

only to $10^{4.6}$. Similarly, the first sorption of fluid "B" (Table IV), reduced the titer from $10^{5.86}$ to $10^{3.67}$, but five additional sorptions effected a reduction in titer only to $10^{3.57}$. Thus, the data again indicate that a proportion of virus remained in suspension although repeatedly exposed to chicken erythrocytes, a large part of the sorbing mechanism of which was apparently free.

Repeated Sorption of Virus Which Previously Had Been Sorbed by Chicken Erythrocytes and Eluted in Saline.—The results of the foregoing experiments could be interpreted to mean either that two kinds of virus were present in the allantoic fluids employed, one kind of which had a greater affinity for eryth-

TABLE IV

Repeated Sorption with Chicken Erythrocytes of PR8-Infected Allantoic Fluids

Allantoic fluid	Log of egg infectivity titer (ID_{50}) of fluid before and after sorption						
	No. of sorptions						
	0	1	2	3	4	5	6
A	9.10	7.57	5.62	5.10	4.62		
B	5.86	3.67	—	—	—	—	3.57

TABLE V

Repeated Sorption with Chicken Erythrocytes of a Suspension of Virus Previously Sorbed on Chicken Erythrocytes and Eluted in Saline

Log of egg infectivity titer (ID_{50}) of the suspension before and after sorption			
No. of sorptions			
	1	2	3
8.50	6.32	5.75	4.50

rocytes than the other, or that the allantoic fluid contained inhibitory substances which were effective when the virus concentration was relatively low. The following experiment was made to test these points.

PR8-infected allantoic fluid was sorbed with chicken erythrocytes and the sorbed virus eluted in saline. The eluate (which contained only virus shown by previous test to possess an affinity for chicken erythrocytes) was then sorbed with chicken erythrocytes. The initial volume of eluate employed was 4 ml.; sorptions were made with cells from 2 ml. of a 2 per cent suspension.

The results (Table V) are quite similar to those obtained in the previous experiments made with whole allantoic fluid. The ID_{50} of the eluate before sorption was $10^{8.5}$ (Table V), and the first sorption reduced the titer to $10^{6.3}$. It is obvious that that reduction represents removal of more than 99 per cent of available ID_{50} units. However, the remaining 1 per cent of virus was not re-

moved by 2 subsequent sorptions. In other words, the sorbing mechanism of erythrocytes employed for the 2nd or 3rd sorption contained less than one hundredth as much virus as the erythrocytes used for the 1st sorption, but in spite of that large proportion of free sorbing mechanism, the virus was removed only partially.

It must be assumed that all of the virus in the eluate had had an affinity for chicken erythrocytes because it had been sorbed by chicken erythrocytes and eluted in saline shortly before the experiment was made. Moreover, that procedure should have eliminated inhibitory substances which might have been present in the allantoic fluid, unless the inhibitory substances had an affinity for erythrocytes, and like the virus, were sorbed and eluted.

Sorption of Allantoic Fluids, the Virus Content of Which Had Been Reduced by Dilution.—A possible objection to the presented data is that each new lot of erythrocytes used to sorb the same fluid added an additional amount of an inhibitory substance, the cumulative effect of which was sufficient to interfere with subsequent sorption of virus. In order to circumvent that objection, experiments were made in which the virus content of fluid was reduced by serial dilution, and each of the dilutions then sorbed with a unit volume of chicken erythrocytes. All such experiments, regardless of whether the fluids were diluted in normal allantoic fluid, saline, or bacteriological culture broth, showed the difficulties in removing virus completely from suspension by sorption with chicken erythrocytes. All tests seemed to show a relationship between quantity of virus sorbed to the quantity left in suspension. It was shown repeatedly that whereas 90 per cent or more of available virus could be removed from a concentrated virus suspension by a given volume of cells, that same volume of the same lot of cells was incapable of removing completely the virus from the same virus suspension which had been diluted to 10^{-6}. The following experiment is illustrative:

Six serial tenfold dilutions of PR8 allantoic fluid were prepared in culture broth. Equal volumes (3.6 ml.) of each of the dilutions and of the original fluid were sorbed with sufficient fresh chicken erythrocytes to yield 2 per cent suspensions; the sorbed fluids then were tested for egg infectivity. The results are summarized in Table VI.

It is clear from the data (Table VI) that regardless of the great differences in quantities of virus available, in no instance did the unit volume of chicken erythrocytes completely remove the virus. The virus appears not to have been sorbed in absolute amounts, but in amounts proportional to the quantity of virus available. For each tenfold increase in dilution of allantoic fluid there was an approximate tenfold decrease in quantity of virus sorbed; but, if it is borne in mind that the mass of cells employed was the same for each sorption, it is obvious that the cells employed to sorb the 10^{-1} dilution of fluid could have contained only one-tenth the quantity of virus as the cells used to sorb

the undiluted fluid, yet a significant amount of virus remained unsorbed; similarly, the cells used to sorb the 10^{-4} dilution could have contained only one ten-thousandth as much virus as the cells employed for sorption of the undiluted fluid, and again an appreciable quantity of virus remained in suspension.

The proportion of virus removed from suspension appears not to have been the same for all dilutions of allantoic fluid, but increased progressively as dilution increased from 10^{0} to 10^{-3}, and then decreased progressively as dilution increased from 10^{-3} to 10^{-6}; dilution of the allantoic fluid to 10^{-3} seems to

TABLE VI

Sorption of Serial Dilutions of PR8 Allantoic Fluid with a Unit Mass of Chicken Erythrocytes

Dilution of fluid	ID_{50} units of virus			Log $\frac{\text{Sorbed}}{\text{Not sorbed}}$
	Available*	Not sorbed	Sorbed‡	
10^{0}	10^{8}	3.65×10^{6}	9.64×10^{7}	1.422
10^{-1}	10^{7}	7.6×10^{4}	9.92×10^{6}	2.114
10^{-2}	10^{6}	4.6×10^{3}	9.95×10^{5}	2.335
10^{-3}	10^{5}	2.95×10^{2}	9.97×10^{4}	2.529
10^{-4}	10^{4}	6.3×10^{1}	9.94×10^{3}	2.199
10^{-5}	10^{3}	6.8×10^{0}	9.93×10^{2}	2.164
10^{-6}	10^{2}	2.0×10^{0}	9.8×10^{1}	1.690

* The ID_{50} of the undiluted fluid was determined and that value was used to calculate the ID_{50} of each dilution.

‡ Calculated as virus available less virus remaining in suspension after sorption.

have produced an optimal concentration of virus for the mass of erythrocytes employed. The variation in the proportion of virus sorbed is especially apparent when one considers the logarithm of the ratio of sorbed to unsorbed virus (Table VI).

The ratio of sorbed to unsorbed virus appears to have been related in a parabolic manner to the total quantity of virus available. The data (Table VI) are arranged in Graph 1 so that the logarithm of the total quantity (ID_{50}) of virus available is plotted against

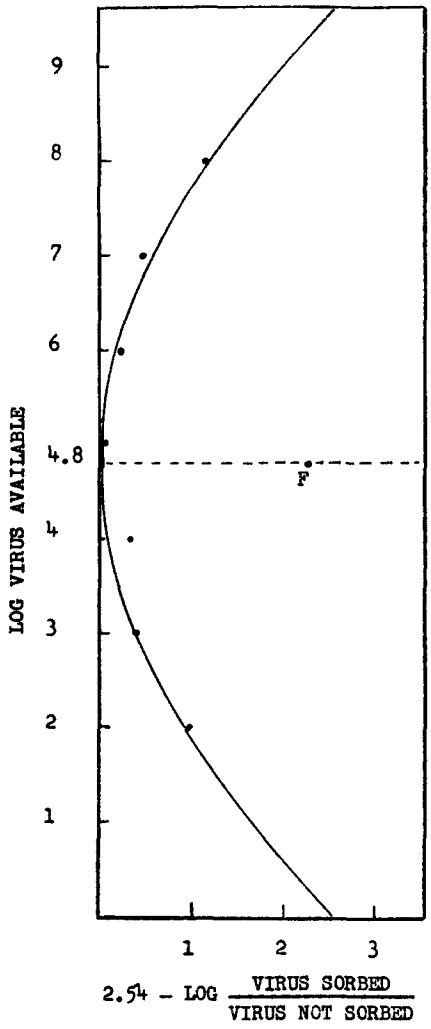
$$2.54 - \text{logarithm } \frac{\text{Virus sorbed}}{\text{Virus not sorbed}},$$

in which the factor 2.54 is the logarithm of the maximum ratio of sorbed to unsorbed virus, and which was estimated from a smoothed curve obtained when the logarithm of that ratio was plotted against the logarithm of the virus originally available.

The smooth curve in Graph 1 is the parabola

$$y^2 = 4ax$$

for which $a = 2.27$, and the axis of which is through the ordinate 4.8 (concentration of virus at which the ratio of sorbed to unsorbed virus was maximum).



GRAPH 1

The points, plotted from the experimental data, fall strikingly close to the parabola, and, accordingly, suggest that for a given mass of erythrocytes, the ratio of virus sorbed to virus remaining in suspension was a parabolic function of the total quantity of virus originally available in the suspension.

DISCUSSION

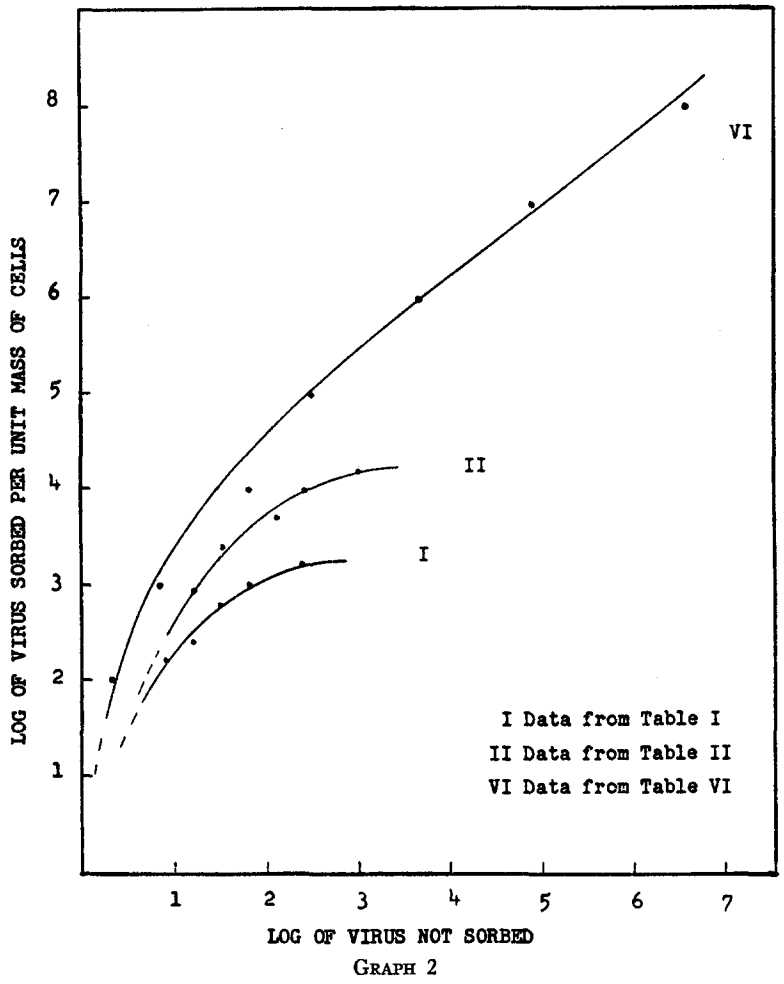
The data suggest that the sorption of influenza virus was governed by some orderly mechanism which effected a proportional relationship between quantities of virus which combined with erythrocytes and quantities which remained free in suspension. Failure of the excess erythrocytes to remove the virus more completely cannot be attributed to the presence of virus which had little or no affinity for chicken erythrocytes, to the accumulation of inhibitory substances derived from erythrocytes employed for previous sorption, or to inhibitory substances present in the allantoic fluids in which virus was suspended. It seems unlikely also that the results were due to decreased chance of contact between erythrocytes and virus particles incidental to decrease in virus concentration; erythrocytes are relatively large bodies, and in the numbers employed should have provided ample chance of contact; moreover, the same proportional distribution of virus between fluid and excess of erythrocytes occurred in the case of fluids of high or intermediate titers, as well as in the case of fluids of low titer.

The point cannot well be made that the virus which appeared to be unsorbed was actually virus which had been sorbed but subsequently eluted because of destruction of erythrocyte receptors. Care was taken to conduct the experiments at temperatures (0°C. to 4°C.) at which "destruction" of receptors occurs at a very slow rate. Furthermore, in those tests in which a great excess of erythrocytes was employed, any virus freed from the destroyed receptors should have had ready access to the numerous uncombined and undamaged receptors; however, the ratio of sorbed to unsorbed virus was the same in those instances as in the tests in which the receptor systems approached saturation. Moreover, tests were made to determine whether detectable virus was released during a 30 minute sorption period at 0°C. Aliquots were removed from the virus-erythrocyte suspension, and egg infectivity titers determined after 0, 10, 20, 30, and 40 minutes; the titer decreased rapidly during the first 10 minutes, then less rapidly until about 30 minutes, after which time it appeared to remain stationary during the remainder of the test period.

The orderly and proportional relationship between quantity of combined virus and quantity which remained free in suspension raises the question of whether or not the process was governed by the laws of simple adsorption, or perhaps by the laws of mass action.

In order to test the applicability of Freundlich's adsorption isotherm, the data presented in Tables I, II, and VI are arranged in Graph 2 so that the logarithm of the quantity of virus sorbed per unit mass of erythrocytes is plotted against the logarithm of the quantity of virus not sorbed; the data from Tables I and II are expressed in terms of hemagglutinating units, those from Table VI in terms of egg infectivity (ID_{50}) units. The fact that the plotted points (Graph 2) do not fall along straight lines but along fairly smooth curves

(the shapes of which suggest segments of parabolas) was to have been expected from Graph 1 in which the relationship of sorbed to unsorbed virus per unit mass of cells appears to have been a parabolic function of the total concentration of virus. If the adsorption isotherm applied in a strict sense, the graphs



GRAPH 2

should have been straight lines; but whether or not that fact eliminates the possibility of simple adsorption is problematic.

The data obviously do not lend themselves to an accurate quantitative analysis. Nevertheless, it is possible to obtain some idea of the relationships involved. The law of mass action is essentially an expression of relationships between reacting units, and although it is impossible to discuss the reaction

between influenza virus and chicken erythrocytes on the basis of molecular weights, it is possible to discuss it on the basis of reacting units and thus test the data in a qualitative manner. It must be assumed, however, that the units have a "valence" of one.

The total number of erythrocyte receptor units included in the mass of cells employed in the experiment summarized in Table VI may be estimated from the equation of the parabola shown in Graph 1, to have been approximately $10^{9.6}$,—if it is assumed that sorption of virus was limited by the total number of receptors present. On the same basis the total number of receptors not combined with virus (free receptors) may be estimated for any given instance to have been approximately $10^{9.6}$ less the number of virus units sorbed. Also, the number of combined virus-receptor units may be assumed to have been equivalent to the number of virus units sorbed. Then, if the law of mass action applies, the product of the number of units of free virus and the number of free receptor units, divided by the number of combined virus-receptor units should be a constant. The logarithms of the values obtained for the data from the experiment included in Table VI were 8.17, 7.49, 7.27, 7.07, 7.40, 7.44, and 7.91 for the concentrations of virus from 10^8 to 10^2 (Table VI), respectively. The suggestive parabolic arrangement is obvious, and when the value 7.07 (lowest value) is subtracted from each value, the resultant values are numerically the same as those obtained when the logarithm of the ratio of sorbed to unsorbed virus (Table VI) is subtracted from the value of the maximum ratio (Graph 1). Thus the "constants" would fall along the parabola shown in Graph 1.

The point could be made, therefore, that the reaction between influenza virus and chicken erythrocytes is a reversible[2] one governed by the laws of mass action, and in which the ratio of combined to free virus per unit mass of erythrocytes is a parabolic function of the total quantity of virus present.

SUMMARY AND CONCLUSIONS

Data are presented which indicate that the forces of attraction between influenza virus and chicken erythrocytes are governed by an orderly mechanism which effects a proportional distribution of virus between erythrocytes and suspending fluid. For a unit mass of erythrocytes, the ratio of combined (sorbed) virus to free (unsorbed) virus is sufficiently constant over a wide range of virus concentrations to indicate compliance with the laws of mass action, or, perhaps, with the laws of simple adsorption; however, the ratio of combined (sorbed) virus to free (unsorbed) virus appears to be a parabolic function of the total quantity of virus.

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[2] It is better to view the initial reversible reaction, which is the subject of the present paper, as being distinct from the reaction involved in the subsequent "elution" of virus.

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