STUDIES ON HOST-VIRUS INTERACTIONS IN THE CHICK EMBRYO–INFLUENZA VIRUS SYSTEM*

I. Adsorption and Recovery of Seed Virus

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Most studies on the interrelationships between animal viruses and their respective hosts have been restricted to an analysis of the response to infection of the host animal as a whole, or of the affected organs. If one could study host-virus interactions in individual host cells, or groups of identical cells, it is obvious that much could be learned regarding the mechanism of infection. There are not many animal host-virus systems available at present which lend themselves as readily to such analyses as do bacterial host–virus systems, but the entodermal layer of the allantoic sac of the developing chick embryo infected with influenza virus provides an approach to such a system, although it is far from ideal.

In the analysis of the mechanism of infection of the cells lining the allantoic cavity of the chick embryo by influenza virus, one may reason, *a priori*, that the infectious process is divided into at least four major steps: (*a*) adsorption of the virus onto the host cells; (*b*) entrance of the virus into the cells;[1] (*c*) propagation of the virus within the host cells;[1] and (*d*) liberation of the newly formed virus from the cells. This division is supported, in part, by a number of previous studies which have been concerned predominantly with one or the other of the phases of the infectious cycle (1–3).

It is the aim of this series of studies to analyze further the individual steps

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[1] Although it has not been definitely proven that the influenza viruses propagate within the host cells, the following points may be cited in favor of such an assumption: intracellular multiplication has been demonstrated for other agents of this class, and it is unlikely that the influenza viruses are exceptional in this respect. The presence of antibody in the allantoic fluid does not prevent propagation of virus in the allantoic membrane (5). Irradiated, interfering influenza virus, and possibly also fully active virus, exerts a profound effect on the functions of the host cells in that it prevents further growth of the allantoic sac (3), conceivably by interaction with an enzyme system within the cell necessary for both virus and host cell growth.

of the infectious process. The first paper is concerned particularly with the adsorption of the seed virus onto the cells of the allantoic sac. The data to be presented here have in part been extracted from experiments conducted for various other purposes, which will be discussed in subsequent papers of this series (4, 5). They show that on the average, about 70 per cent of the injected virus is adsorbed onto the host cells, yet only a small percentage of the adsorbed virus can be demonstrated as active virus in suspensions of the allantoic membranes in spite of attempts to improve the yield by various means, or to find the missing virus in other structures of the egg. Thus the suggestion appears justified that the major portion of the infecting agent, upon intimate contact with the host cells, is so bound or becomes so altered as to be undetectable by infectivity titrations. The small fraction of active virus demonstrated in the tissue suspensions may represent virus which remains on the surface of the membrane and which possibly does not increase in quantity.

Materials and Methods

Preparations of Virus.—The PR8 strain of influenza A was mainly employed in these experiments, and only a few tests with the Lee strain of influenza B are included. The seed virus was prepared by inoculation of 0.2 ml. of infected allantoic fluid in dilution 10^{-7} into 10-day-old chick embryos by the allantoic route. After incubation of the eggs at 36–37°C. for 48 hours, they were chilled for 1 to 2 hours at 4°C., and the allantoic fluids were collected. They were distributed in 1 ml. amounts into ampules, which were sealed in an oxygen flame. After quick freezing, the ampules were stored in a dry ice chest. If 10^8 or 10^9 ID_{50} was required for injection, freshly prepared seed was used (5).

For experimental purposes, the seed culture was diluted in broth so as to contain the desired number of ID_{50} per 0.2 ml. inoculum, and injections were made by the allantoic route into 12-day-old chick embryos. Following incubation of the eggs, aliquots of the allantoic fluids were collected during the constant periods[2] (2), after removal of the shell over the air sac, by needle and syringe without previous chilling of the eggs. When quantitative data were needed, the remainder of the allantoic fluid was poured off and measured in a graduate cylinder. The allantoic membranes were harvested during the first 3 hours after infection in the case of the PR8 strain, and during the first 5 hours in the case of the Lee strain, corresponding to the constant periods as obtained in membrane suspensions[2] (4). After removal of the allantoic fluid as described, a cross-cut was made with scissors into the shell membrane and the underlying chorioallantoic tissue, the embryo, yolk sac, and albumen were discarded with precautions to sever the connections of the allantoic sac close to the unwanted structures. The allantoic sac at this stage of the embryonic development adheres to the shell membrane, and thus offers the opportunity to wash it thoroughly *in situ*. The eggs were each filled with sterile buffered saline solution (40 to 50 ml.), agitated, and the wash fluid poured off. After

[2] Following infection, a certain amount of seed virus is found in the allantoic fluid and in suspensions of allantoic membrane. This quantity remains constant for various periods of time until a sudden rise in infectivity is noted. The period of constancy varies, depending on the type of virus used and the material tested. In the allantoic fluid, the constant periods extend over 5 to 6 hours in the case of influenza A, and over 8 to 10 hours in the case of influenza B virus. The corresponding figures for suspensions of allantoic membranes are 4 and 6 hours, respectively.

repetition of this procedure, the membranes were removed from the shells, pooled, and washed twice more in Petri dishes containing approximately 50 ml. of buffered saline solution each. The tissue was then emulsified in chilled semimicro Waring blendors either immediately or after various periods of storage in the cold, as will be described. For each membrane, 5 ml. of buffered saline containing 100 units of penicillin and 100 μg. of streptomycin per ml. was used. Each suspension was prepared from at least four and usually six membranes. Blending was carried out as a rule for 3 minutes, but was varied in a few instances, as will be described. Some of the suspensions were titrated in chick embryos without centrifugation; the majority, however, were clarified by spinning at 2000 R.P.M. for 20 minutes.

Titrations of Virus.—The virus preparations were diluted in tenfold steps in broth containing 100 units of penicillin and 100 μg. of streptomycin per ml. Ten- or 11-day-old chick embryos were injected with 0.5 ml. of the various dilutions. The embryos were further incubated at 36–37°C. for 72 hours when 0.4 ml. of allantoic fluid was removed from each egg, transferred to individual test tubes, and tested for hemagglutinating activity as an indication of the presence of virus by addition of 0.2 ml. of a suspension of 1 per cent chicken red cells. The 50 per cent infectivity end-points were determined according to Reed and Muench. For titrations of the seed used in the various experiments, ten embryos were employed per dilution, for the experimental preparations, five each. However, since most of the data recorded in this paper are part of experiments to be discussed subsequently, usually two to five titrations during the constant periods were available which were combined for this analysis. Thus, the figures given for the experimental groups are based on titrations with at least ten and sometimes with up to twenty-five eggs per dilution.

Calculations.—In order to determine the degree of adsorption of the injected virus onto the host cells, the following data became available in the tests: (*a*) the amount of seed virus injected; (*b*) the quantity of free virus in the allantoic fluid during the constant period; *i.e.*, the ID_{50}/ml. found multiplied by the number of milliliters collected from these eggs. Subtraction of (*b*) from (*a*) corresponds to the amount of virus presumably adsorbed. The total amount of virus in the allantoic membrane was calculated by multiplying the ID_{50}/ml. by the volume of suspension (5 ml. per egg membrane).

EXPERIMENTAL

A few data on adsorption of influenza virus onto the cells lining the allantoic sac have been included in previous publications employing either the hemagglutination test (1, 3) or infectivity titration for assay (2). Additional information concerning this phase has been accumulating in subsequent experiments devised for other purposes (4, 5). As can be seen in Table I, in twenty-seven experiments employing the PR8 strain and eleven using the Lee strain, adsorption varied within certain limits as determined by the difference between the number of ID_{50} injected and the number of ID_{50} recovered free in the allantoic fluid of the injected eggs during the constant period. Average adsorption amounted to about 70 per cent of the injected seed virus, with a maximum at 96 and a minimum at 42 per cent.

It was expected that the degree of adsorption would vary to some extent with the quantity of virus injected. However, within the range of ID_{50} employed as inoculum, the average percentage of adsorption did not differ significantly, as seen in Table II, and the same extent of variation was noted regardless of whether 10^9 or 10^2 ID_{50} was injected.

The difference between the amount of seed virus injected and the quantity of virus recovered in the allantoic fluids of the injected eggs during the constant periods was relatively small in terms of logarithmic units, as seen in Table III. The average difference amounted to log 0.40, minimally 0.24 and maximally 0.63. The accuracy of such infectivity titrations has been studied extensively by Knight (6), who found on statistical analysis of replicate titrations of preparations of influenza virus that the chances are 19 out of 20 that differences in end-points of 0.37 and 0.62 logarithmic unit are significant in titrations employing ten embryos and five embryos per dilution, respectively. In the

TABLE I

Adsorption of Influenza Virus by the Allantoic Sac

Strain	No. of tests	Adsorption of injected virus		
		Average	Maximal	Minimal
		per cent	*per cent*	*per cent*
PR8	27	71	95	44
Lee	11	73	96	42

TABLE II

Relation of Concentration of Inoculum to Degree of Adsorption

Range of inocula (ID_{50})	Strain of virus			
	PR8		Lee	
	No. of tests	Adsorbed	No. of tests	Adsorbed
		per cent		*per cent*
$10^{8.0}$–$10^{9.9}$	7	65	1	81
$10^{6.0}$–$10^{7.9}$	7	71	1	72
$10^{4.0}$–$10^{5.9}$	8	80	4	87
$10^{2.0}$–$10^{3.9}$	5	64	5	62

experiments presented, the results of the seed titrations, using ten embryos per dilution, were compared with the combined results of two to five titrations of various allantoic fluids collected from the injected eggs during the constant periods. These employed five embryos each per dilution, and were corrected for the volume of fluid found in order to obtain the total amount of virus recovered. Although the recorded differences may seem to fall into the border region of statistical significance, the following points should be taken into account in judging the validity of the conclusions tendered: (*a*) All experiments showed differences in the same direction. These included the sixteen tests shown in Table III, which involved titrations of both the allantoic fluids and of suspensions of allantoic membranes, and an additional twenty-two

experiments in which only the allantoic fluids were studied. Thus, in thirty-eight experiments, the same trend was noted. (*b*) The results of the several five embryo titrations of the allantoic fluids harvested during the constant periods of the individual experiments, which were corrected for the total volume collected and which were combined for this analysis, agreed within log 0.25 on the average, with a maximal spread of log 0.50. On occasion, if only

TABLE III

Comparison between Virus Calculated to Be Adsorbed onto and Virus Actually Found in the Allantoic Membrane

Strain of virus	ID_{50} injected	ID_{50}* found in allantoic fluid	Difference log	ID_{50} adsorbed‡	ID_{50} found in allantoic membrane	Adsorbed virus	Injected virus
						per cent	*per cent*
PR8	$10^{9.60}$	$10^{8.97}$	0.63	$10^{9.48}$	$10^{7.60}$	1.3	1.0
	$10^{9.27}$	$10^{8.85}$	0.42	$10^{9.06}$	$10^{7.78}$	5.2	3.2
	$10^{9.23}$	$10^{8.89}$	0.34	$10^{8.97}$	$10^{7.16}$	1.6	0.9
	$10^{9.15}$	$10^{8.52}$	0.63	$10^{9.08}$	$10^{7.70}$	4.7	3.5
	$10^{9.09}$	$10^{8.72}$	0.37	$10^{8.82}$	$10^{7.53}$	5.1	2.8
	$10^{8.15}$	$10^{7.82}$	0.33	$10^{7.87}$	$10^{6.09}$	1.7	0.9
	$10^{8.09}$	$10^{7.83}$	0.26	$10^{7.72}$	$10^{6.30}$	3.8	1.7
	$10^{7.15}$	$10^{6.90}$	0.25	$10^{6.79}$	$10^{5.35}$	3.7	1.6
	$10^{7.09}$	$10^{6.70}$	0.39	$10^{6.55}$	$10^{5.32}$	3.0	1.8
	$10^{6.36}$	$10^{5.80}$	0.56	$10^{6.22}$	$10^{4.40}$	1.5	1.1
	$10^{6.10}$	$10^{5.77}$	0.33	$10^{5.83}$	$10^{4.50}$	4.8	2.5
	$10^{6.09}$	$10^{5.72}$	0.37	$10^{5.82}$	$10^{4.11}$	2.0	1.1
	$10^{5.93}$	$10^{5.69}$	0.24	$10^{5.56}$	$10^{3.90}$	2.6	0.9
	$10^{4.43}$	$10^{3.98}$	0.45	$10^{4.27}$	$10^{2.70}$	2.7	1.8
Lee	$10^{4.36}$	$10^{4.03}$	0.33	$10^{4.13}$	$10^{2.29}$	1.6	0.9
	$10^{4.51}$	$10^{3.91}$	0.60	$10^{4.38}$	$10^{2.40}$	1.1	0.8
Average.....			0.40			2.8	1.6

* ID_{50} per ml. multiplied by the volume of allantoic fluid collected.

‡ Determined by the difference between the values shown in columns 2 and 3.

two titrations were performed, identical results were obtained. Thus, these results fell well within the range of reproducibility given by Knight for employing this number of embryos per dilution, in spite of the additional variable in measuring the total volume of allantoic fluid present in the injected eggs. The amounts of fluid found in individual eggs as well as the averages of pooled fluids from different groups of eggs in an individual experiment showed variations within a range of from 8 to 10 ml. Small quantities of allantoic fluid were retained by the eggs, but these appeared negligible considering the large volumes of fluid present in 12-day-old embryos. This error possibly was

counteracted by the addition to the allantoic fluids of small amounts of blood, or the occasional inadvertent admixture of some amniotic fluid. (*c*) The same order of difference between the amount of seed virus injected and that recovered was noted in experiments in which the quantity of seed varied over a wide range; *i.e.*, the difference was independent of the number of tenfold dilutions required to reach the end-point of infectivity.

These various considerations of the limitations of the technics employed fail to discredit the conclusion that the average value of adsorption of 70 per cent is probably nearly correct. In order to establish this value more firmly, it was thought advisable to determine the amount of virus that could be demonstrated in suspensions of allantoic membranes following adsorption. However, when such tissue preparations were titrated in chick embryos simultaneously with the allantoic fluids collected from the same eggs, it was found that only a small percentage of the amount of virus calculated to be adsorbed could be demonstrated. As seen in Table III, only between 1 and 5 per cent of the adsorbed, or 0.8 to 3.5 per cent of the injected virus could thus be found. The limitations of experimental procedure and accuracy discussed above hold, of course, also for these tests. Additional sources of variation are to be found in the fact that the allantoic sacs may, to some extent, vary in size at this stage of development. Furthermore, this tissue may not be separable in its entirety, and small fragments of it may escape with the other structures of the egg when they are discarded. However, these additional sources of error could hardly account for the extent of the discrepancies in the virus assay.

Attempts to clarify this problem may be divided into several steps: efforts (*a*) to demonstrate an antiviral effect in allantoic fluid; (*b*) to improve the handling of the tissue and with it, the yield of virus; (*c*) to search for an inhibitor of virus activity in the tissues; (*d*) to demonstrate a loss of virus in the process of preparing membrane suspensions; (*e*) to find virus in other structures of the egg as a result of possible rapid resorption of the agent; and (*f*) to determine whether the non-adsorbed seed virus differed from the adsorbed agent in properties leading to the combination with the host tissue.

It was considered unlikely that allantoic fluids of 12-day-old embryos possess antiviral activities in view of the stability of infectivity in this medium (7), and the constancy of the virus titer in the allantoic fluid during the first few hours after infection (2). In agreement with this past experience, addition of known amounts of virus to normal allantoic fluid of 12-day-old chick embryos *in vitro* and incubation at 37°C. for 1 to 6 hours, did not reduce the infectivity as compared with that of control preparations of virus in broth. This result is in agreement with recent observations by Svedmyr (8) and by Hardy and Horsfall (9) who demonstrated the presence in normal allantoic fluids of a component which may prevent hemagglutination but apparently does not affect infectivity.

All attempts to increase the yield of virus from allantoic sacs have failed. It did not matter whether the suspensions of the tissues were prepared immediately following harvest or after the membranes had been stored in the cold at 4°C. for 24 hours, or at −15°C. for several days; the results of the titrations in chick embryos gave figures well within the accuracy of the technic. Variation in the method of emulsification of the tissue, likewise, failed to increase the virus titer. Grinding of the tissue with mortar and pestle, using alundum as abrasive, was inferior to the use of the Waring blendor. Varying the time of blending, it could be seen (Table IV) that after 1 minute, and regularly after 3, maximal titers were obtained, and further treatment in the blendor did not release significant quantities of additional virus. Microscopic examination of smears prepared from such suspensions revealed only occasional individual or small clusters of intact cells. Blending for 3 minutes was adopted

TABLE IV

Effect of Variation in the Time of Grinding in the Waring Blendor on the Yield of Virus (PR8) from Allantoic Membranes

Preparation No.	ID_{50} per ml.			
	Run in Waring blendor for			
	1 min.	3 min.	6 min.	10 min.
1	—	$10^{0.97}$	$10^{0.93}$	—
2	—	$10^{0.97}$	$10^{0.80}$	—
3	$10^{2.70}$	$10^{2.68}$	—	$10^{2.96}$
4	$10^{1.93}$	—	—	$10^{1.80}$
5	$10^{4.80}$	$10^{5.47}$	—	$10^{5.82}$

as the routine procedure. Repeated freezing at −70°C. and thawing at 37°C. prior to emulsification did not alter the results. Some of the crude tissue suspensions were exposed to intense sonic vibration[3] for periods up to 60 minutes. This procedure, which does not affect the titers of infectivity and hemagglutination of virus contained in allantoic fluid, did not improve the yield in the tissue emulsions (Table V), although the debris sedimentable by low speed centrifugation at 2000 R.P.M. for 20 minutes was minimal as compared to the sediment obtained from non-treated suspensions, indicating the more complete emulsification of the tissues. Crude suspensions were kept at 37°C. for 1 to 2 hours to effect any possible elution of virus from tissue fragments or dissociation of virus aggregates, again to no avail. Suspensions of tissue were incubated at 37°C. for 4 hours with 0.01 per cent trypsin.[4] The virus titers of

[3] The sonic oscillator used was manufactured by the Raytheon Manufacturing Company, Boston.

[4] The amount of trypsin used was sufficient to digest in 1 hour at 37°C. a 2 per cent solution of gelatin to such an extent that it no longer solidified at 4°C.

the digested preparations were similar to those of the controls not treated with the enzyme. When centrifuged and non-centrifuged suspensions of allantoic membrane were compared in their infectivity, no significant differences in titer were noted (Table VI), and the sediments contained less activity than the supernate. Centrifugation at 2000 R.P.M. for 20 minutes was, therefore, adopted as routine procedure. The virus titer in suspensions of membranes was quite stable at 4°C., and it did not matter whether titrations were done

TABLE V

Effect of Sonic Vibration on Yield of Virus (PR8) in Suspensions of Allantoic Membranes

Preparation No.	ID_{50} per ml.				
	Sonic vibration for				
	0 min.	3 min.	10 min.	30 min.	60 min.
1	$10^{1.80}$	$10^{1.80}$	$10^{1.47}$	—	—
2	$10^{3.80}$	$10^{3.93}$	$10^{3.80}$	—	—
3	$10^{3.47}$	—	$10^{3.47}$	—	—
4	$10^{2.68}$	—	—	$10^{2.13}$	$10^{2.80}$
5	$10^{2.80}$	—	$10^{3.13}$	$10^{3.42}$	$10^{2.80}$
6	$10^{2.93}$	—	$10^{2.97}$	$10^{2.80}$	$10^{2.78}$
7	$10^{5.28}$	—	$10^{5.47}$	$10^{5.80}$	$10^{5.20}$

TABLE VI

Effect of Centrifugation on Titer of Virus (PR8) in Suspensions of Allantoic Membranes

Preparation No.	ID_{50} per ml.	
	Not centrifuged	Centrifuged 20 min. at 2000 R.P.M.
1	$10^{3.47}$	$10^{3.13}$
2	$10^{3.47}$	$10^{3.80}$
3	$10^{2.80}$	$10^{2.68}$
4	$10^{2.93}$	$10^{2.96}$
5	$10^{5.13}$	$10^{5.47}$

immediately following preparation or after storage for 1 to 2 weeks at 4°C. (Table VII). This stability permitted repetition of titrations in case unexpected or questionable results were obtained in the first assay.

Another set of experiments was directed toward the demonstration of inhibitors of virus activity in the tissues. To avoid admixture of red blood cells or fragments thereof, as well as of serum which frequently contains a non-specific inhibitor of the hemagglutinating activity (10–12), membranes were perfused with buffered saline solution before harvest. Comparison of suspensions of perfused and non-perfused membranes did not reveal a significant difference in

virus titer. Addition of known amounts of virus to normal membranes immediately before blending or after their emulsification and subsequent titration of the preparations gave titers closely similar to those obtained with the same amount of virus added to broth in the same proportion.

As a further possibility, it was considered that virus may be eluted from the tissue in the process of preparation. Although elution has not been demonstrated as yet from the allantoic sac of the living chick embryo, it does occur when the tissue is killed by formalin (13). It was possible, therefore, that during the washing of the membrane, conditions became favorable for elution. However, titration of the first and second wash waters yielded quantities of virus corresponding to about 1 and 0.1 per cent, respectively, of the total amount in the allantoic fluids, indicating that the amount of virus found in the wash water most likely corresponded to the amount of allantoic fluid left in the eggs when washing commenced.

TABLE VII

Stability of Suspensions of Allantoic Membranes Infected with Influenza Virus (PR8)

No. of test	Time after preparation	ID_{50} per ml.		
		Suspension No.		
		1	2	3
	days			
1	1	$10^{2.68}$	$10^{2.96}$	$10^{4.96}$
2	7	$10^{2.68}$	$10^{2.93}$	$10^{4.82}$
3	14	$10^{1.93}$	$10^{2.80}$	$10^{4.80}$
4	18	$>10^{2.50}$	—	—

Finally, attempts were made to demonstrate virus in structures other than the allantoic membrane, in view of the possibility that some of the virus might become rapidly resorbed. However, no evidence for such an occurrence has been obtained in the past (3), and the present experiments have confirmed these data. No virus was found in the blood withdrawn from the embryos in the first few hours after infection. Suspensions of embryo and yolk sac contained less than 1 per cent of the activity of the allantoic fluids of the corresponding eggs, and it is likely that this amount reflected the degree of contamination of the test tissues with residual allantoic fluid during harvest.

The uniformly unrevealing tests presented above suggested that the seed virus left free in the allantoic fluid during the constant period might differ from that adsorbed onto the tissues by its combining properties. However, when allantoic fluids, collected 2 hours after injection of high titered seed, were employed for subculture, it was found that again about 70 per cent of the virus was adsorbed within 1 hour after injection. The constant period, likewise,

conformed to the usual experience in that 6 hours elapsed until new generations of virus were released into the allantoic fluid.

DISCUSSION

The data presented have shown that regardless of the quantity of virus used, about 30 per cent of the influenza virus injected into chick embryos by the allantoic route remains free in the allantoic fluid of the infected eggs. Since all attempts to demonstrate in allantoic fluid an inhibitor for the infectivity of virus particles have failed, it must be concluded that the remaining 70 per cent of the seed virus has been adsorbed onto the tissues of the allantoic sac. Adsorption of influenza virus onto red cells usually is far more complete (14) probably as a result of the fact that the virus and adsorbing cell are both suspended and intermixed in the same medium, offering a greater chance for the agents to collide. In the allantoic sac system, contact between virus and host cells can be made only at the fluid-tissue interface. The equilibrium between adsorbed and free virus is established usually within one-half to 1 hour after infection (2).

Attempts to demonstrate the adsorbed virus in suspensions of allantoic membrane met with only partial success. It has been impossible to detect more than 1 to 5 per cent of the amount of virus, and all efforts to improve the yield have failed. These included: emulsification of the tissues in the Waring blendor for extended periods of time; exposure of the crude suspensions to intense sonic vibration which resulted in more complete emulsification; digestion of the crude suspensions with trypsin; incubation of the emulsions at 37°C. for 1 to 2 hours to effect elution of virus from tissue debris; perfusion of the tissue prior to harvest in order to avoid admixture of inhibitors possibly present in the blood stream; search for inhibitors of viral activities in suspensions of normal allantoic sacs; and, finally, exclusion of possible resorption of virus and spread to other structures of the chick embryo. None of these tests has furnished a clue to the missing virus.

There remain three possibilities to be considered: (*a*) The methods of emulsification are not effective enough as yet to disrupt all the infected cells or to free all the virus from fragments of the tissue, so that it escapes detection. (*b*) Although emulsions of the tissue do not reveal an inhibitor for virus activity, the intact cells may be able to cause inactivation of some of the seed virus. In that case, the missing virus would have to be considered waste, and the active virus found in the tissue would correspond to the "infecting" agent. Finally, (*c*), the seed virus, upon the intimate contact with the host cells which leads to propagation, becomes, as a result, altered in such a way that it can no longer be demonstrated. The active virus, in this case, would represent "superficially adsorbed" virus which does not increase in quantity. This last point gains in importance, in view of the fact that a major portion

of the active virus in the membrane is accessible to the action of neutralizing antibody, as will be demonstrated in a subsequent paper of this series (5). The last point appears to be borne out, too, by results obtained in bacterial host–virus systems in which it has been found impossible, thus far, to recover the infecting agent from the host cell in the early stages of the infectious cycle (15).

SUMMARY

Upon injection of active influenza A or B virus into the allantoic cavity of the developing chick embryo, an average of only 70 per cent of the agent was adsorbed onto the tissue, as measured by the difference between the quantity of virus injected and that found free in the allantoic fluid of the injected eggs during the constant period. The degree of adsorption was similar, regardless of whether 10^9 or 10^2 ID_{50} of active virus was injected.

Attempts to demonstrate the adsorbed virus in suspensions of the infected tissue met with partial success only in that not more than 1 to 5 per cent of the amount calculated to be adsorbed was actually found. All efforts to increase the yield of virus have failed. These results led to the suggestion that the seed virus, which participates in the propagation, becomes altered in such a way that it no longer may be demonstrated by infectivity titrations, whereas the active virus found represents superficially adsorbed virus, which does not multiply.

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