THE DEMONSTRATION OF ONE-STEP GROWTH CURVES OF
INFLUENZA VIRUSES THROUGH THE BLOCKING EFFECT
OF IRRADIATED VIRUS ON FURTHER INFECTION*

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Previous studies (1, 2) have shown that the mechanism of infection of susceptible host cells by influenza virus can be divided into at least four separate phases: (I) the adsorption of the virus onto the host cells (2-4); (II) the development of changes in the host cells which alter their function (decrease or discontinuation of host cell multiplication) (2) and exclude certain other viruses from the cells (interference phenomenon) (5-9)—changes which are possibly concomitant with entrance of the virus into the host cells; (III) increase of the virus in association with the cell; and (IV) release of the newly formed virus from the infected tissue to spread the infection to other susceptible cells.

More is known about the first and second phases than about the third and fourth, especially if one includes the available information on the hemagglutination phenomenon in phase I. The present study has therefore been directed chiefly toward an analysis of the later steps in the infectious process, the intracellular propagation of the virus and its release from the infected cells. Attempts were made to obtain "one-step growth curves"¹ similar to those reported for the propagation of bacterial viruses by Ellis and Delbrück (10) and Delbrück and Luria (11). The technic used by these authors had to be modified for application to the chick embryo-influenza virus system. With these modifications the experiments have revealed that multiplication of the influenza viruses is based apparently on general principles similar to those affecting the propagation of the bacterial viruses.

Methods and Materials

Seed Culture of Virus.—The PR8 strain of influenza A and the Lee strain of influenza B were employed in these experiments. The agents were passed by the allantoic route in high

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¹ A one-step growth curve represents a single cycle in the increase in virus after infection of a susceptible host. By means of it one can estimate the yield of virus from a given number of host cells, all of them infected within a period of time purposely limited to allow for the association of the infectious agent with the cells.

dilution (10^{-7}) to 10 day old chick embryos by a technic previously described (6). After 48 hours of incubation at 36 to 37°C. the allantoic fluids were collected and stored in small volumes in glass-sealed ampules in a dry-ice cabinet. The seed culture was rapidly thawed immediately before use.

General Growth Curve Technic.—Adequate numbers of 11 or 12 day old chick embryos (the older embryos were found to be the more satisfactory) were inoculated with 0.2 ml. of suitably diluted seed culture by the allantoic route (usually $10^{-5.5}$ for PR8 and $10^{-6.0}$ for Lee) and returned to the incubator. Further injections, which will be described in the text, were given in 0.5 ml. amounts. Groups of five to six of the inoculated embryos for each experimental series were removed from the incubator at given time intervals and 3 to 4 ml. aliquots of allantoic fluid were withdrawn by needle and syringe from each of the eggs without previous chilling. The blood-free samples were pooled according to groups. The remainder of the allantoic fluids was poured off into a graduated cylinder with precautions to avoid rupture of the amnion or yolk sac. The average volume of allantoic fluid per egg was thus determined. The fluids were kept at 4°C. until the last groups were harvested. The preparations were then assayed for their content of active virus by titration in 10 day old chick embryos. Repetition of titrations of some of the fluids which had been stored at 4°C. for 1 week agreed well with the titers originally obtained.

Titration of Virus.—The allantoic fluids were diluted in multiples of ten with brain-heart infusion broth to which 100 units of penicillin and 50 gamma of streptomycin had been added per ml. This precaution was taken since the nature of the experiments did not permit adequate tests for bacteriological sterility prior to the titration. The diluted materials were injected in 0.5 ml. amounts into the allantoic cavity of groups of five normal 10 day old chick embryos. After incubation for 72 hours at 36–37°C. the individual allantoic fluids from these embryos were harvested and tested for their capacity to agglutinate chicken erythrocytes. To 0.4 ml. of fluid, 0.2 ml. of a 1 per cent suspension of thrice washed chicken red cells was added. The test mixture was incubated at 4°C. and read in terms of the pattern formed by the cells at the bottom of the tubes (12). The 50 per cent infectivity end point was determined according to the results of the hemagglutination test by the method of Reed and Muench.

Irradiation of Virus.—The infected allantoic fluids were dialyzed for 24 hours in cellophane bags against buffered saline solution of pH 7.0 in the approximate ratio of one part of fluid to twenty parts of saline solution, with repeated stirring of the bath, in order to remove the greater part of the urates (6). The dialyzed fluids were next exposed to ultraviolet light according to a method previously described (6). They were assayed for their interfering and hemagglutinating capacities as also outlined previously (1).

EXPERIMENTAL

Growth Curves of Influenza Viruses in Untreated Chick Embryos

Injection of 1000 to 10,000 ID_{50} of influenza virus into the allantoic sac of 11 to 12 day old chick embryos did not lead to complete adsorption of the virus onto the cells lining the allantoic sac, a certain amount of the virus, usually between 10 and 30 per cent, remaining free in the allantoic fluid. With the establishment of equilibrium in $\frac{1}{2}$ to 1 hour, the residual free amount of virus remained constant for a variable number of hours, depending on the strain of virus used. In the case of the PR8 strain of influenza A, 6 hours elapsed after injection, and with the Lee strain of influenza B, 9 to 10 hours before the concentration of virus in the allantoic fluid commenced to rise.

In some experiments the increase in titer during the next few hours was quite rapid, then lessened and rose again steeply later, suggesting a certain periodicity in virus production. In other instances the quantities of active virus increased only very slowly. Figs. 1 and 2 summarize the results of several experiments each with the PR8 and Lee strains. The mean values found in the allantoic fluids at various hours after infection have been linked by solid lines.

FIG. 1. Increase in the PR8 strain of influenza A virus in chick embryos (five experiments). The mean titers are indicated by the open circles.

These data suggested that the virus increased in association with the cells for about 6 hours in the case of the PR8 strain, and for about 9 hours in the case of the Lee strain before the newly formed agents were released from the injected cells. Thereafter the findings became quite irregular. This variability was presumed to be due to a varying degree of additional adsorption of freshly released virus onto susceptible cells, and hence the apparent titer was not considered an indication of the actual amount of virus produced. This conception was borne out by the experiments described in the next section.

The Effect of Heterologous Irradiated Virus on the Growth Curve of Influenza Virus

The variations just mentioned have been largely excluded by an adaptation of the "one-step growth technic" already mentioned, which had been de-

veloped for the study of bacterial viruses (10, 11). By this technic bacteria are exposed for a brief period to sufficient bacteriophage to infect most of them with a single particle. Further infection is then checked by dilution. In the supernate of centrifuged aliquots of the infected bacterial suspensions the amount of free virus is determined by the plaque method. The suspension is then incubated and aliquots are tested at intervals for the "infectious centers" present. For a certain fixed period, depending on the bacteriophage used, the number of plaques remains constant. During this constant period the virus is

FIG. 2. Increase in the Lee strain of influenza B virus in chick embryos (four experiments). The mean titers are indicated by the open circles.

multiplying within the host. Then there is a sudden rapid increase in the amount of detectable virus, followed by a new plateau. From the data obtained, it is possible to estimate the yield of virus per bacterium.

This technic obviously could not be applied without modifications to the chick embryo-influenza virus system. The following procedure was adopted:—

To obtain "single infection" of cells small doses of active virus were injected at the onset of the experiments; *i.e.*, usually 1000 to 10,000 ID₅₀. Then, to interrupt the process of adsorption and to prevent further infection of susceptible cells a second injection was given consisting of 0.5 ml. of allantoic fluid containing irradiated *heterologous* virus. This was sufficient to produce the interference phenomenon in all remaining susceptible cells (6) and to reduce, if not prevent, further adsorption of active virus from the seed culture as well as from the yield. The allantoic fluids of

groups of five to six eggs thus treated were harvested at various time intervals and the content of active virus was assayed by titration in 10 day old chick embryos.

The assumption that the relatively large amounts of irradiated heterologous virus would decrease or prevent the adsorption of the relatively low concentrations of newly formed virus upon release from the infected cells was based on experiments reported in previous publications (1, 2, 6). These earlier data were obtained mainly by using the hemagglutination reaction, which, however, permits detecting the adsorption of only fairly large amounts of virus. Some direct titrations of infectivity for chick embryos were required, therefore, to determine the adsorption of relatively small quantities of virus. Accordingly, groups of 11 day old chick embryos were injected with either 0.5 ml. of irradiated preparations of virus, or with normal allantoic fluid. Three hours later, a second injection of about 10^5 to 10^6 ID₅₀ of active homologous or heterologous virus was given. The allantoic fluids of these eggs were collected after further incubation of 2 to 3 hours and pooled according to groups. The infective titers for chick embryos of these fluids multiplied by the volume of allantoic fluid collected represented the total recovery. A comparison of this quantity with the total amount of virus injected revealed no significant decrease of virus if the eggs had been injected previously with irradiated virus, in contrast to the markedly lower recovery of virus in those groups which had received normal allantoic fluid prior to injection.

Examples of the results of the procedure are shown in Table I. They confirm the previous finding that there is little, if any, adsorption of active virus in the allantoic sac of eggs previously injected with large amounts of the irradiated agents.

Using the technic as described, "one-step growth curves" were obtained which resembled closely those of bacterial viruses. As can be seen in Figs. 3 and 4, the content of active virus in the allantoic fluids remained constant for 6 to 9 hours, respectively, depending on whether the PR8 or Lee strains were studied. This amount represented non-adsorbed virus from the inoculum. Thereafter, the concentration of active virus rose steeply for 2 hours in the case of the PR8, and for 2 to 3 hours in the case of the Lee strain. From then on the active virus titer remained on this higher plateau up to the 24th hour after infection, when the experiments were terminated. The figures show the data of five separate experiments each, with closely similar concentrations of active seed virus. The means for the various determinations are connected by the solid lines. A comparison of Figs. 1 and 3, and Figs. 2 and 4, gives emphasis to the supposition that in the absence of blocking by heterologous irradiated virus, some of the newly formed active virus is adsorbed onto the allantoic sac upon its release from the cells.

Varying the concentration of active virus in the inoculum over a wide range did not result in significant alterations in the "one-step growth curves." The constant period for the PR8 strain remained 6 hours, as seen in Fig. 5. Similar results were obtained with the Lee strain except that in this case the

TABLE I
*Prevention of Adsorption of Active Virus by the Injection of Irradiated Virus
 Prior to Infection*

1st Injection	2nd Injection		Result of harvest		
Irradiated allantoic fluid	Active virus	ID ₅₀	Allantoic fluid ml.	ID ₅₀ /ml.	Total ID ₅₀ recovered
PR8.....	PR8	10 ^{6.44}	7.6	10 ^{4.63}	10 ^{6.51}
Lee.....	PR8	10 ^{6.64}	9.2	10 ^{4.47}	10 ^{6.43}
Normal.....	PR8	10 ^{6.64}	7.7	10 ^{3.84}	10 ^{4.73}
PR8.....	Lee	10 ^{6.46}	7.0	10 ^{4.47}	10 ^{6.33}
Lee.....	Lee	10 ^{6.40}	6.8	10 ^{4.53}	10 ^{6.36}
Normal.....	Lee	10 ^{6.40}	7.3	10 ^{3.30}	10 ^{4.17}

FIG. 3. One-step growth curve of the PR8 strain of influenza A (five experiments). The mean titers are indicated by the open circles.

FIG. 4. One-step growth curve of the Lee strain of influenza B (five experiments). The mean titers are indicated by the open circles.

constant period was regularly 9 hours. When $10^{1.9}$ ID₅₀ of active PR8 virus were injected the titrations of the harvests had to be begun with undiluted allantoic fluids. These, however, contained sufficient concentrations of non-adsorbed irradiated heterologous virus (Lee) to cause interference in the low dilutions of the titrations. It was necessary, therefore, to add to the dilutions of the harvested allantoic fluids rabbit anti-Lee serum to prevent the interfering effect. The serum did not apparently influence the increase in quantity of the active PR8 virus.

FIG. 5. One-step growth curves following injection of various concentrations of active virus.

The "one-step growth curves" were practically alike when either the usual amounts of heterologous virus (undiluted allantoic fluid) or five times that amount of virus (concentrated by high-speed centrifugation) were injected one-half hour after infection. The individual end points of the various titrations did not differ significantly except for the slight variations inherent in the reaction. Such experiments furnished additional evidence that the amount of heterologous irradiated virus injected in the various tests described in this paper was sufficient to prevent extensive adsorption of the freshly released virus. Upon five-fold dilution, on the other hand, the amount of heterologous irradiated virus was insufficient to prevent additional adsorption.

In one experiment, the interval between infection and administration of the irradiated virus was varied and its effect on the "one-step growth curve"

studied. The embryos were infected with active PR8 virus and received irradiated Lee virus, 1, 3, and 6 hours after infection. The "one-step growth curves" for the 1 and 3 hour series were practically alike. However, after 6

FIG. 6. Growth curves following injection of irradiated heterologous virus at various hours after infection.

hours, the heterologous virus was given at the beginning of the first step and consequently some further cells became infected during the period before the irradiated virus was injected. Thus, the plateau finally reached was approximately 100-fold higher than that obtained in the 1 and 3 hour groups. This experiment is summarized in Fig. 6.

From the data presented in Figs. 3 and 4, attempts were made to calculate the quantities of virus produced.

The calculations were necessarily hampered by the inaccuracies inherent in the methods of virus assay and, to a lesser extent, by the technical difficulties in the quantitative collection of allantoic fluid. However, when the various experiments were combined which involved comparable amounts of virus as inoculum, although from different seed cultures (PR8) or similar dilutions of the same seed cultures (Lee), the calculations gave some information at least as to the order of magnitude of the production of virus in the cells of the allantoic sac. The calculations shown in Table II summarize five experiments each with the two respective strains. With each experiment the seed was titrated by the injection of tenfold dilutions into eight to ten eggs each. The amount injected in the experiments was calculated from the results of these titrations. The free virus in the allantoic fluids during the constant period represented virus from the inoculum which had not been adsorbed. This amount has been calculated by multiplying the mean ID₅₀ per ml. during the first 6 hours in the case of the PR8 virus, and during the first 9 hours in the case of the Lee strain, with the average volume of allantoic fluid harvested. This value sub-

tracted from the inoculum constituted the amount of virus adsorbed. The average total amount of virus after the step (10 to 24 hours after infection with PR8, and 12 to 24 hours after administration of active Lee virus) minus the active virus found in the allantoic fluid during the constant period represented the amount of virus produced. This value divided by the ID_{50} adsorbed corresponds to the number of ID_{50} produced per one ID_{50} adsorbed. As can be seen in Table II, these values fell between 45 and 79, with an average of 63 in the case of PR8 virus, and between 25 and 55, with an average of 36, for the Lee virus.

In view of the variations already described, the apparent difference in yield of PR8 and Lee viruses cannot be taken as absolute and it is possible that a

TABLE II
Calculation of Yield of Virus (Five Experiments Each)

	PR8 (ID_{50})		Lee (ID_{50})	
	Average	Range	Average	Range
(1) Inoculum.....	1540	1200- 2000	5250	4000- 7900
(2) Recovered during constant period.....	600	300- 1000	1100	1000- 1250
(3) Adsorbed; (1)-(2).....	940	590- 1500	4150	3000- 6800
(4) Recovered after the step..	59,500	32,500-96,000	149,000	106,000-230,000
(5) Total produced; (4)-(2)...	58,900	32,010-95,500	147,900	105,000-228,900
(6) $\frac{ID_{50} \text{ produced (5)}}{ID_{50} \text{ adsorbed (3)}}$	63	45- 79	36	25- 55

larger series of tests would have lessened these differences. As pointed out, the calculations should be taken only as an indication of the order of magnitude of virus production.

Depression of Yield of Virus by the Homologous Irradiated Agent

The earlier experiments were conducted with heterologous irradiated virus because it was anticipated that under certain conditions the interfering effect of the non-adsorbed irradiated virus would have to be neutralized by immune serum, as discussed above. This turned out to be a more fortunate choice than anticipated because it was found, subsequently, that homologous irradiated virus exerted a marked depressing effect on the step size in the growth curve experiments. As can be seen in Figs. 7 and 8, the step in the PR8 tests (two experiments) was strikingly delayed and decreased in size. In the four Lee experiments no step occurred in two, and only a slight rise in virus titer in the others. These experiments were performed simultaneously with tests in which heterologous irradiated virus was utilized, and the fluids giving the depressor effect were tested against heterologous active virus. Both tests gave the usual one-step curves. Thus it is apparent that homologous irradiated

virus may have a profound effect on the production of active virus in cells which had been infected prior to the administration of the irradiated agent, or

FIG. 7. Depression of the yield of virus by homologous irradiated virus (two PR8 experiments).

FIG. 8. Depression of the yield of virus by homologous irradiated virus (four Lee experiments). The results of tests utilizing the homologous irradiated virus are indicated by the various triangles; of those utilizing the heterologous irradiated virus by the various circles.

its release from such elements. This effect is not shared by heterologous irradiated virus.

The degree of depression in the yield of virus by homologous irradiated virus decreased gradually with the increase in the interval between infection and administration of the inactivated agent. Fig. 9 shows the results of an experiment with the Lee strain. It is to be noted that the size of the step increased gradually, but even when injected 4 hours after infection homologous irradiated

virus still depressed the yield to some extent. When given after 6 hours a one-step growth curve was obtained similar to that noted following injection of heterologous irradiated virus 1 hour after infection.

FIG. 9. Depression of the yield of active virus by homologous irradiated virus injected at various hours after infection.

FIG. 10. Depression of the yield of active virus by decreasing amounts of homologous irradiated virus.

The inhibitory effect of the homologous inactivated agent still was very marked when the irradiated allantoic fluid was diluted fivefold before injection. Upon further dilution in fivefold steps the effect decreased, as shown in Fig. 10 and the growth curve finally became comparable to those obtained in untreated chick embryos (Fig. 2.).

The agent responsible for the decrease in yield of virus was sedimentable, together with that responsible for the hemagglutinating property, by centrifugation of the preparation at 20,000 R.P.M. for 20 minutes. The supernatant fluid was ineffective in that it failed to depress the yield of active virus and the

growth curves resembled those shown in Figs. 1 and 2, obtained with infected eggs which received no further treatment. Irradiation of the homologous virus for prolonged periods of time likewise eliminated the inhibitory effect. In this case the interfering capacity had been destroyed by the irradiation but the hemagglutinating capacity was left partially intact (1).

It has not been possible as yet to determine whether the inhibitory effect is consequent on reduction of the amount of increase of the virus, associated with cells, or whether it is due to prevention or delay in its liberation. However, an experiment with the Lee strain carried over 48 hours did not disclose a delayed rise in virus titer. Longer intervals have not been tested. Attempts to determine the amounts of active virus in suspensions of carefully washed allantoic sacs harvested at various intervals after infection did not meet with success: only irregular results were obtained.

DISCUSSION

In a previous publication (2) it has been pointed out that there exist striking similarities between certain bacterial viruses and the agents of epidemic influenza in their host-virus interrelationships. These similarities were apparent in the conditions of adsorption of the viruses onto the host cells, the various aspects of the interference phenomenon between the inactivated agents and the active viruses, and the effects of the agents on the functions involving the propagation and metabolism of the host cells. The present study extends this agreement to the general appearance of the growth curves given by these agents. After entrance of the virus into the host bacterium, it multiplied within for a definite period of time which was found to be characteristic for each strain of bacterial virus studied (11). This period of propagation in association with the cell seems, likewise, characteristically different for the influenza viruses. In the case of the PR8 strain, it lasts about 6 hours, and in that of the Lee virus, about 9 hours. A few preliminary tests with other strains of influenza virus indicated that they may require shorter, or intermediate, periods of time for intracellular multiplication.

No information is available as to what happens within the cells during this latent period. Attempts to demonstrate a rise in the content of virus in suspensions of emulsified allantoic sacs harvested during the latent period have failed to produce reliable results, partly on account of technical difficulties such as incomplete liberation of the virus from the tissue and adsorption of the virus onto tissue debris, red cells, etc., partly, perhaps, for biological reasons; *i.e.*, the virus may pass through developmental stages in which it lacks infectivity as yet, and therefore cannot be demonstrated by the available technics.

At the end of the period of propagation the newly developed virus is released from the infected cells. This period is relatively short in the case of the bacterial viruses (11), which is in keeping with their faster growth cycle. In the

influenza tests, this period lasts for 2 to 3 hours until all the virus to be released from the infected cells has come away into the allantoic fluid. The amount of bacterial virus produced under standard conditions of culture of the host cells is again characteristic of the individual strain of virus (11). It is not possible, as yet, to make a similar statement in regard to the influenza viruses, since the technics of virus assay are not sensitive enough. The use of five chick embryos per dilution in infectivity titrations leads to results which may be accurate within $\log 0.6$ (13), and the accuracy of such titrations can be increased by the use of greater numbers of eggs per dilution or by performing a greater number of parallel titrations and determinations of the mean. The present experiments gave practically the same results as this latter method since all titrations of the allantoic fluids obtained during the constant period, and again of those collected after the plateau was reached, could be combined for the final evaluation. In addition the Lee experiments were all conducted with the same seed culture using the same dilution, so that the results of the five tests could be combined. In consequence the calculations gain somewhat in accuracy. However, all that can be said at present is that the number of ID_{50} of influenza virus produced per one ID_{50} adsorbed is of the order of 50. It is possible that the yield of PR8 virus exceeds somewhat that of the Lee strain. The number of virus particles constituting one ID_{50} has not been definitely determined. According to Friedewald and Pickels (14), who used centrifugally purified preparations, it amounts to about ten virus particles. In view of the fact that only part of these will be adsorbed, that some of them may have been inactivated by the methods of concentration, that possibly not all sedimentable material was virus, and that the experimenter has to do with the amount of virus infecting only 50 per cent of the eggs, the suggestion seems not amiss that one particle of influenza virus may cause infection of the chick embryo.

It appears most likely that the influenza virus freshly yielded into the allantoic fluid is released by the destruction of the entodermal cells of the allantoic sac, just as happens when bacteria are lysed by bacteriophage. It has been observed in infections with influenza virus that the epithelial lining of certain sections of the respiratory tract of ferrets and mice is largely destroyed (15-17). No studies of the lesions encountered in the allantoic sac of the chick embryo have been published except for a brief account by Burnet (18) who found only minor changes in the entodermal layer of the allantoic sac, save for damage of occasional individual cells or groups of cells. A few attempts to study the histological appearance of some of the allantoic sacs from the growth curve experiments have not been revealing. The number of infected cells was presumably very small since the infecting virus dose was limited to 1000 to 10,000 ID_{50} in most of the experiments, and further infection was prevented by the irradiated virus. However, when the allantoic sacs of chick embryos were examined 48 or 72 hours after injection of active virus only, that is to say at a

time when the titer of virus in the allantoic fluid had reached its peak, the entodermal epithelium appeared largely destroyed or severely damaged. It remains to be seen whether this destruction of the entodermal cells occurs concurrently with the appearance of fresh virus in the allantoic fluid, or whether the virus comes away as a result of some other process and the host cells are destroyed later. This question gains in importance in the light of the observations made on the depression of yield of active virus by the homologous irradiated agent, to be discussed further on.

An inhibiting effect has been demonstrated by Delbrück (19) in certain interference experiments with two distinct, active, bacterial viruses. The result of exclusion of the heterologous agent was a decrease in the yield of virus multiplying within the host cells, whereas exclusion of homologous virus was apparently without inhibitory effect. Addition of antiserum against the heterologous agent largely prevented the inhibition. The amount of suppression decreased also with the increase in the interval between infection of the bacteria and addition of the heterologous virus. This inhibitory effect in bacterial virus infections has many similarities with that now observed in the influenza system except that in the latter case the homologous virus produced the inhibition, not the heterologous, and furthermore the preparations had been inactivated by irradiation with ultraviolet light. Because homologous virus was the effective agent, it was impossible to test for neutralization of the inhibitory influence with specific immune serum, since residual free antibodies would have affected freshly produced active virus upon its release from the entoderm into the allantoic fluid. In the one-step growth experiments based upon the use of heterologous irradiated virus, injection of the corresponding antiserum did not increase the size of the step, indicating that the heterologous agent had no inhibitory effect.

The inhibition is presumably mediated by the virus particle but its mechanism is unknown. It may hinder the multiplication of the virus or prevent its release from the cells. It should be recalled in this relation that the preliminary injection of irradiated virus prevents infection by homologous and heterologous virus equally well (6). Conceivably, when introduced after infection, the homologous irradiated virus may still enter the cell and interfere with some stage of the development of the active virus, whereas the heterologous agent is unable to follow this course because a stage in the infectious cycle has been reached in which the heterologous agent cannot participate.

SUMMARY

After allantoic injection of chick embryos with a known amount of influenza virus, the process of adsorption of the agent onto host cells and infection of them can be interrupted at a given time by the administration of large quantities of heterologous virus inactivated by irradiation. A sudden great increase

in the amount of free virus in the allantoic fluid occurring after 6 hours in the case of the PR8 strain, and 9 hours in that of the Lee strain, indicates that the untreated virus associated with the host cells has multiplied. The length of the period preliminary to this increase remains the same even though the concentration of the original inoculum is varied over a wide range. Since administration of the irradiated virus leaves no susceptible host cells, because of the interference phenomenon, and further adsorption of active virus is minimized or entirely prevented, practically the entire new increment of virus can be found in the allantoic fluid and assayed; for every ID_{50} adsorbed about 50 ID_{50} are released. Homologous irradiated virus, on the other hand, when injected after infection of the allantoic sac, reduces the yield of virus to a more or less considerable extent. Some inhibitory effect can still be observed when the homologous irradiated virus is given several hours after infection. This effect is linked to the virus particle and destroyed by prolonged irradiation.

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