ONE-STEP GROWTH CURVES OF VARIOUS STRAINS OF INFLUENZA A AND B VIRUSES AND THEIR INHIBITION BY INACTIVATED VIRUS OF THE HOMOLOGOUS TYPE*

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Studies on the propagation of influenza viruses in the chick embryo (1) have shown that under certain conditions one-step growth curves may be obtained which are similar in principle to those observed in bacterial host-virus systems (2, 3). Upon allantoic injection of either the PR8 strain of influenza A or the Lee strain of influenza B virus, only part of the active virus is adsorbed by the cells lining the allantoic cavity. The amount of residual free virus of the seed, which can be assayed in the allantoic fluid of the injected eggs by infectivity titrations, remains constant for a period of 5 to 6 hours in the case of the PR8 strain, and for 8 to 9 hours in that of the Lee strain. After this constant period, the virus titer increases as a result of liberation of newly formed virus from the infected tissue. Part of the released virus is adsorbed immediately upon some of the remaining uninfected host cells, and the rise in titer in the allantoic fluid is consequently relatively slow. However, if one induces the interference phenomenon in the remaining susceptible cells by injection of large amounts of heterologous virus inactivated by irradiation with ultraviolet light (4, 5), most of the liberated virus remains free in the allantoic fluid and a rather sharp increase in infectivity is noted at the end of the constant period. The release of virus extends over 2 to 4 hours. Thereafter, the infectivity titer of the allantoic fluids remains stationary at the new plateau, since no additional host cells have become infected and consequently no further virus has been propagated.

The use of irradiated heterologous virus for the blockade of the remaining susceptible host cells, as indicated above, permits the study of a single infectious cycle from adsorption of the seed to liberation of the new generations of virus. However, with injection, following infection, of homologous, rather than heterologous, irradiated virus, a marked reduction in the yield of virus was noted. This effect might be due to inhibition of virus production in, or its release from, the host cells. Evidence for the former alternative will be presented elsewhere (6).

In the experiments to be reported, growth curves of several strains each of

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influenza A virus, including one strain of swine influenza virus, were compared with each other and with those of three strains of influenza B virus. It will be shown that all strains of influenza A virus exhibited constant periods of 5 to 6 hours, whereas 8 to 10 hours elapsed before influenza B virus was released from the infected host cells. It also will be demonstrated that the inhibitory effect of the homologous irradiated virus, when injected after infection with active virus, is a type-specific and not strain-specific property. There exist, however, reciprocal quantitative differences in cross-inhibition by irradiated influenza A and swine influenza viruses.

Materials and Methods

Viruses.—The following strains of influenza virus were used: PR8 (7), WS (8), Melbourne (9), F99 (10), and L_7 47 (11) of Type A; S15 of swine influenza (12); and Lee (13), ES (14), and Saha (15) of Type B. The methods used for the preparation of seed, the irradiation of virus with ultraviolet light, and the assay of infectivity have been fully described (1). No changes in technic have been introduced except for the use of concentrated irradiated virus preparations for some of the experiments. This concentration was achieved by high speed centrifugation of the infected allantoic fluids at 14,000 R.P.M. for 1 hour, and resuspension of the sedimented virus in a fraction of the supernatant fluid to effect a four- or eightfold concentration. Dialysis of the concentrate and irradiation were carried out in the manner previously described.

Growth Curve Technic.—Adequate numbers of 12-day-old chick embryos were infected by the allantoic route with 1,000 to 10,000 ID_{50} of virus (0.2 ml. inoculum), and returned to the incubator. One hour later a second injection was given, by the same route, of 0.5 ml. of preparations of homologous or heterologous irradiated virus. Following further incubation at 36–37°C., 5 to 6 eggs of each series were removed at 1 to 2 hourly intervals for harvest of the allantoic fluids. These were collected by means of needle and syringe, without previous chilling of the eggs. The fluids of corresponding eggs were pooled and stored at 4°C. until titrations for infectivity could be made in 10-day-old chick embryos. The results of the titrations are expressed as the number of ID_{50} per ml. of allantoic fluid.

EXPERIMENTAL

Comparison of Various Strains of Influenza A and B Viruses.—One-step growth curves were obtained with five strains of influenza A and one strain of swine influenza virus. In these experiments, irradiated Lee virus was employed for the blockade of the remaining uninfected cells of the host tissue. For obtaining the growth curves of three strains of influenza B virus, preparations of irradiated influenza A virus (PR8) were injected as the blocking agent 1 hour after infection. The growth curves obtained with the various strains of influenza A and swine influenza virus, on the one hand, and of the three strains of influenza B virus, on the other, resembled each other closely. Since such curves for the PR8 and Lee strains have been published in detail, it suffices here to tabulate the essential findings. As can be seen in Table I, the extent of adsorption of seed virus varied from 63 to 90 per cent. These figures were obtained by determining the amount of virus injected (titration of the seed), and subtracting from these values the quantities of virus found free in the

allantoic fluids of the injected eggs during the constant periods. The difference was considered as the amount of virus adsorbed. The percentage of seed virus thus calculated to be adsorbed varied with individual strains (PR8 or Lee) from test to test over a fairly wide range (42 to 96 per cent), as will be shown elsewhere (16). This variability in all likelihood is caused by the inaccuracies inherent in the methods of assay employed. The variations in the percentage of adsorption observed in the experiments recorded in Table I cannot be interpreted, therefore, as an indication of differences in the various strains used.

The constant periods extended over 5 to 6 hours in the case of all the influenza A and swine influenza strains, and over 8 to 10 hours in the case of the influenza B viruses. It appears from this observation that the constant period is

TABLE I

One-Step Growth Curves with Various Strains of Influenza A and B Virus

Strain	Type	No. of experiments	Seed virus adsorbed	Constant period	Release period	ID_{50} virus released per ID_{50} adsorbed
			per cent	*hrs.*	*hrs.*	
PR8	A	8	71	5–6	2–3	63
WS	A	1	90	6	4	104
Melbourne	A	2	68	5–6	2–3	60
F99	A	2	74	5–6	3–4	77
$L_7$47	A	1	72	5	3	48
S15	Swine	3	83	6	3–4	81
Lee	B	7	73	8–9	3–4	36
ES	B	2	63	8–9	3–4	40
Saha	B	1	89	10	3	31

characteristic for each of the two types, but that there exist no marked differences in this respect among the various strains of one type.

The time required for the release of virus from the infected host cells is again somewhat variable from experiment to experiment. Thus, no consistent differences have been discernible between influenza A and B strains, nor within each type.

Finally, the yield of virus, *i.e.* the number of ID_{50} liberated per ID_{50} of seed adsorbed (1), also varies over a fairly wide range, probably again because of the technical difficulties inherent in the methods of assay of influenza virus. However, it seems quite definite that there exists a difference between the influenza A and B strains, in that the latter always show a distinctly lower yield of virus than the former. It should be pointed out that the amount of virus liberated into the allantoic fluid does not reflect the total virus production (6).

The Inhibitory Effect of Homologous Irradiated Virus.—In another set of

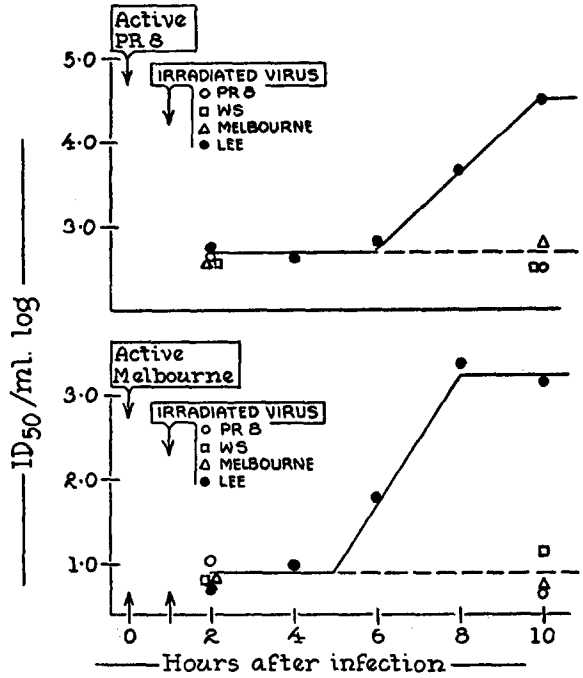

FIG. 1. The inhibitory effect of irradiated virus of various strains of the homologous type on one-step growth curves.

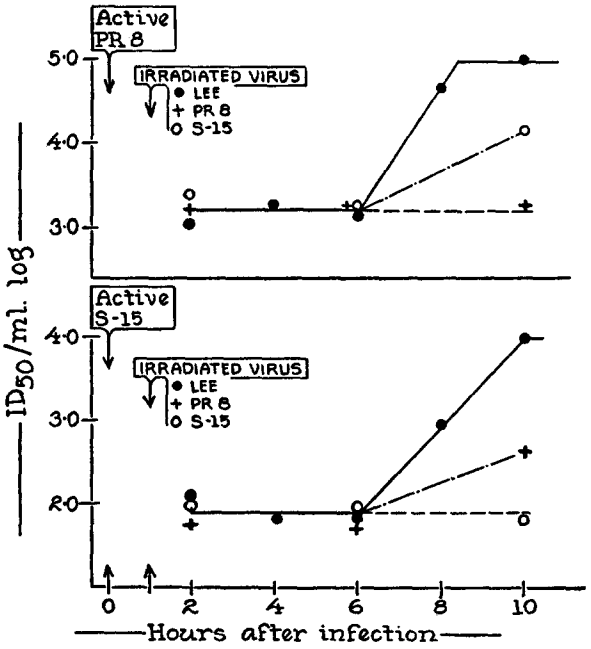

FIG. 2. Partial cross-inhibition of one-step growth curves between irradiated PR8 and S15 strains of influenza virus.

experiments, the inhibitory effect of irradiated virus of the homologous type was studied. In these tests, the blocking agents were prepared from centrifugally concentrated suspensions of virus, since undiluted, inactivated allantoic fluids did not produce complete inhibition of the step with regularity. As can be seen in Fig. 1, the irradiated preparations of the PR8, WS, and Melbourne strains inhibited equally well the appearance of newly formed active virus in the allantoic fluids of the eggs infected with active PR8, Melbourne, or, not shown in the figure, with WS virus. On the other hand, when using irradiated concentrated Lee virus for the blocking, the usual sharp steps in the virus concentration were obtained after constant periods of 5 to 6 hours.

These experiments indicated that the inhibitory effect was type-specific but not strain-specific. In this connection, it was of interest to study whether the behavior of swine influenza virus was like that of human influenza A strains. As shown in Fig. 2, irradiated concentrated S15 virus produced an inhibitory effect in growth curve experiments with active PR8 virus, but to a lesser extent than the irradiated homologous PR8 strain. Conversely, the step in S15 growth curves was completely inhibited by irradiated S15 virus, but only partially by inactivated PR8 virus. Repetition of the experiment again showed only partial cross-inhibition.

DISCUSSION

The data presented reveal that as far as comparison between strains of types A and B is concerned, there exist marked differences in the constant periods; *i.e.*, in the periods in which the virus presumably multiplies in conjunction with the host cells. All influenza A strains studied showed constant periods of 5 to 6 hours, the B strains of 8 to 10 hours. Furthermore, the quantity of virus released into the allantoic fluid for every ID_{50} of seed virus adsorbed was found distinctly larger in the case of influenza A than in that of influenza B virus. No consistent differences are apparent in comparing the constant periods of various strains of one type. It must be emphasized, however, that the methods employed for the quantitative harvest of the allantoic fluids and the assay of virus activity are relatively inaccurate, and that the time intervals chosen for testing are rather widely spaced. It is possible, therefore, that smaller differences in the constant periods among various strains of one type may well have escaped detection.

The fact that influenza virus of type A has a shorter constant period appeared to furnish an explanation of earlier reports that, upon simultaneous injection of approximately equal concentrations of both influenza A and B viruses, the former outgrew the latter and, consequently, the influenza B strains became undetectable by the methods employed (17). However, recent observations indicate (18) that, on occasion, a mixture of the PR8 and Lee strains may be carried through nine allantoic passages apparently, without the loss of the influenza B virus. On the other hand, unpublished experiments conducted in this labora-

tory showed that passage through eggs of a mixture of four strains of influenza A virus (PR8, WS, Melbourne, and F99) resulted after three transfers in a culture which contained, as far as demonstrable, only the Melbourne strain, in spite of the fact that the constant periods of these strains extend over similar periods of time. It was felt that a slight numerical advantage of one strain in the mixed seed used for the first passage might have led to increasingly greater advantages in the subsequent passages, so that the other strains were gradually prevented from producing sufficient concentrations of virus to become demonstrable by the hemagglutination test. It is possible, particularly in the light of the apparent simultaneous passage of influenza A and B strains (18), that factors other than those discussed may determine the survival or loss of strains in mixed infections.

It has long been apparent that there exist certain cross-relationships between the human influenza A and swine influenza viruses. Common antigenic components among these agents have been demonstrated in neutralization and immunization tests (19–21), by inhibition of hemagglutination (22, 23), and the soluble complement-fixation antigens of the two groups of viruses appear indistinguishable by the methods employed (24, 25). It was not surprising, therefore, that the growth curve experiments failed to reveal marked differences between influenza A and swine influenza viruses. Cross-inhibition of virus propagation by injection, following infection, of irradiated virus likewise indicated a close relationship. However, in this case, the inhibitory effect upon propagation of the PR8 strain was more pronounced with the homologous irradiated PR8 virus than with the irradiated S15, and conversely. The strains of influenza A virus, on the other hand, appeared to be identical in this respect.

SUMMARY

One-step growth curves of five strains of influenza A, one strain of swine influenza, and three strains of influenza B virus have been analyzed.

The influenza A and swine influenza strains showed constant periods of 5 to 6 hours before newly formed virus was liberated from the infected cells, whereas 8 to 10 hours elapsed in the case of the influenza B strains.

The yield of virus in the allantoic fluids, *i.e.* the number of ID_{50} released for every ID_{50} of seed virus adsorbed, was consistently higher in the case of the influenza A and swine influenza strains than in that of the influenza B viruses.

Interruption of the cycle by injection of inactivated virus subsequent to infection can be achieved by any of the strains of the homologous type. However, cross-tests between influenza A and swine influenza virus led only to partial inhibition of virus growth.

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