The in Ovo Production of Incomplete Virus by B/Lee and A/PR8 Influenza Viruses*

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With 6 Figures

The formation of non-infectious hemagglutinin or incomplete virus in chick embryos on serial passages of undiluted influenza virus infected allantoic fluids has been most extensively studied with the PR8 strain (2, 4, 9, 10, 11, 13, 15, 18, 21) but the phenomenon has been observed also with other myxoviruses although to a varying degree (1, 3, 5, 7, 19, 20).

It has been claimed, however, that the Lee strain of influenza B virus does not produce incomplete virus (6) or that it produces only very small quantities compared to other influenza viruses (7). This is in contrast to previous findings in this laboratory (11, 16, 17) and elsewhere (22) but a detailed investigation of the production of incomplete virus by the Lee strain has so far not been published.

The present paper records a growth curve study with the Lee strain showing the development of incomplete virus in the allantoic membranes and fluids on serial passage of undiluted virus infected fluids. A study comparing the behavior of the Lee and PR8 strains on undiluted passage in the allantois is also presented.

Materials and Methods

Virus preparations. The A/PR8 and the B/Lee strains were employed.

Standard Passages (St-P) were carried out as described earlier (15). Groups of five or more 11 day old embryos were inoculated allantoically with 0.2—0.5 ml. amounts of 10^{-5} or 10^{-6} diluted virus infected allantoic fluids. After incubation at 35°C (or 37°C) for 44 hours and subsequent chilling of the eggs the allantoic fluids were harvested and tested for hemagglutinating activity. The positive fluids were pooled and used as seed for continued transfer either immediately or after storage at +4°C for 1—5 days or at −60°C for longer periods of time. Such fluids which usually have maximal infectivity and hemagglutinating titers are referred to as standard virus (St).

* Dedicated to the Honor of the 60th birthday of Professor Sven Gard
Undiluted passages (UP) were initiated with freshly harvested Standard virus. Groups of five or more 11—12 day old chick embryos were inoculated allantoically with 0.5—1.0 ml. of undiluted standard fluids. After incubation at 35 °C (or 37 °C) for 16—21 hours, the eggs were chilled and the allantoic fluids were harvested and passed without dilution either on the day of harvest or after storage at +4 °C overnight. This procedure was repeated for the desired number of passages. The allantoic fluids from the successive passages of undiluted virus are referred to as 1st, 2nd, 3rd etc. undiluted passage (UP-1, UP-2, UP-3 etc.).

Infectivity titrations were carried out on the day of harvest or after storage at +4 °C overnight. Occasionally retitrations were made on samples which had been stored at −60 °C for several days. Serial tenfold dilutions of the virus suspensions were prepared in buffered saline and groups of five 11 day old chick embryos were inoculated allantoically with 0.2 ml. amounts of the various dilutions. After incubation for 72 hours at 35 °C the eggs were chilled and the allantoic fluids tested separately for hemagglutinin. The 50 per cent end-point was calculated according to Kärber. The titers are expressed as the number of 50 per cent infective doses (EID₅₀) per milliliter allantoic fluid.

Hemagglutinin titrations. The pattern method previously described (14) was employed. From each preparation of virus two different series of twofolds dilution were made in saline, each dilution in the one series being the geometrical mean of two adjacent dilutions in the other. To each tube was added equal amounts (0.5 ml.) of a 0.25 per cent suspension of chick red cells. The mixtures were kept at 20 °C for 1½ hours and at +4 °C overnight before reading. The number of agglutinating units (HA) per milliliter is expressed as the reciprocal of the final dilution of virus which agglutinated 50 per cent of the red cells added.

The infectivity/hemagglutinating ratio (I/A ratio) was calculated as the log difference between the infectivity and hemagglutinin titers.

Antibiotics. G-penicillin and streptomycin were added to allantoic fluids and to dilutions in sufficient amounts to give a final concentration of 200 units/ml. and 50 μg/ml., respectively.

Experimental

Growth curves — Lee strain of influenza B virus

The development of virus infectivity and hemagglutinins was studied in repeated harvests of a standard passage and the 11 first consecutive passages of undiluted allantoic fluid virus. The standard passage was initiated with a freshly harvested standard passage fluid which was diluted 10⁻⁶ and inoculated in 0.5 ml. amounts into the allantoic cavity of 120 10 to 13 day old embryos. After continued incubation at 35 °C groups of 5 eggs were removed from the incubator. The allantoic fluids were withdrawn and pooled groupwise. The membranes were harvested, washed in saline to remove the allantoic fluid, ground in a Waring blender and resuspended in saline to a 10 per cent suspension. The 44 hour harvest consisted of 20 eggs and the pooled fluids were used undiluted for the continued transfer.