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Infectivity of influenza virus aerosols

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The PR8 strain of influenza virus can remain viable in ageing aerosols for considerable periods (Harper, 1961). However, viability of micro-organisms determined by their growth *in vitro* may not indicate their ability to infect animals via the respiratory route (Schlamm, 1960; Hood, 1961). If influenza is normally transmitted via this route it is epidemiologically important to establish whether any correlation exists between viability as measured by *in vitro* or *in ovo* methods and respiratory infectivity for a susceptible animal host. Previous reports on respiratory infectivity of aged influenza virus aerosols by Edward, Elford & Laidlaw (1943), Loosli, Lemon, Robertson & Appel (1943*a*) and Loosli, Robertson & Puck (1943*b*) lack such a comparison.

Recently developed *in vitro* techniques for influenza virus assay (Fazekas de St Groth & White, 1958*a*), for holding aerosols for long periods (Goldberg, Watkins, Boerke & Chatigny, 1958) and for assessing physical decay of aerosols (Harper, Hood & Morton, 1958) facilitate a study of this kind. This paper describes an attempt to determine whether two strains of influenza virus lose respiratory infectivity with ageing in aerosols.

MATERIALS AND METHODS

Two mouse-passaged strains of influenza virus, PR8 (type A) and Asian (Singapore, type A2), were used. Preparation of viral suspensions *in ovo*, production, holding, sampling and physical decay measurement of aerosols were similar to those described by Harper (1961) except that dialysed casein was replaced by 0·2% (w/v) gelatin in the suspending and collecting fluids and a stainless steel drum of 500 l. capacity was used for holding the aerosols. Several batches of suspension were used, storage time (in solid CO_2) before use varied from 9 to 62 days for the PR8 strain and 1 to 40 days for the Asian strain. Virus titres of suspensions used for generating aerosols and of aerosol samples were determined *in vitro* using the egg-membrane piece technique (Fazekas de St Groth & White, 1958*a*). Membrane pieces (MP) from 13-day-old eggs were used for the PR8 strain and from 12-day-old eggs for the Asian strain. Frequent parallel assays were made by allantoic injection *in ovo*. The titres of the virus suspensions used were from 100 to 400×10^6 EIU50/ml., 7 to 50×10^6 MP50/ml. for the PR8 strain and 40 to 200×10^6 EIU50/ml., 3 to 50×10^6 MP50/ml. for the Asian strain.

Aerosols were held at approximately 23° C. under dry (20% R.H.), medium (50% R.H.) and wet (80% R.H.) conditions. Respiratory infectivity (LD50 and ID50) of the aerosols was obtained by exposing 18–22 g. mice, in groups of 8–10 for

periods ranging between 10 sec. and 20 min., in a manner similar to that previously described (Hood, 1961) for exposing guinea-pigs. After exposure the mice were held for 3–4 weeks in individual isolation boxes and deaths recorded. The lung-retained minute volume for mice of this weight inhaling particles of the size generated in the apparatus was shown to be *ca.* 7 ml. (Harper & Hood, 1962). The dose of virus particles retained by the mouse was obtained by the product of: 7 × exposure time (min.) × viable virus/ml. of aerosol.

In some experiments mice surviving 3–4 weeks after exposure to small doses of virus were bled and sera examined for the presence of haemagglutination-inhibiting influenza virus antibodies against the homologous strain of virus. Since negative control sera were < 5 in titre, antibody titres of > 20 were accepted as evidence of infection.

RESULTS

PR8 *strain of influenza virus*

Determination of viable virus by the MP method resulted in aerosol viabilities similar to those reported by Harper (1961) (Table 1). Virus titre ratios—*in ovo*/MP—for suspensions and aerosol samples did not differ significantly and were

Table 1. *Mouse respiratory LD50 of PR8 influenza virus aerosols*

Suspension*	Aerosol conditions: Temp. (°C.)	R.H. (%)	Viability (%)	Age (hr.)	LD50 (in MP50 units)	Relative potency whole egg/egg membrane piece of aerosol sample
A	23·3–25	15–21	40	0	4 (0·7–10)	3 (1–8)
A	—	—	8	20	3·5 (1·1–12)	6 (3–12)
A	—	—	160	0	1·2 (0·5–3)	7 (3–15)
A	—	—	12	20	7·8 (3·3–18)	5 (2–10)
B	—	—	74	0	2·9 (1·4–10)	4 (1–9)
B	—	—	11	20	2·2 (0·9–4·4)	8 (3–22)
B	—	—	13	20	2·1 (0·3–6·7)	—
B	—	—	44	0	1·6	—
B	—	—	12	20	1·4 (0·5–3)	—
C	22–25	52–55	30	0	2·8 (1·3–6·5)	8 (4–15)
C	—	—	4	1	4·4 (1·8–15·6)	—
D	—	—	77	0	7·3 (2·3–59)	2 (1–4)
D	—	—	1	2	3·5 (1·1–4·8)	8 (4–17)
C	23·3–26·6	78–85	52	0	4·8 (1·9–38)	6
C	—	—	2	4	2·2 (0·8–14)	—
C	—	—	48	0	2·4 (1–6)	—
C	—	—	1	4	1·1 (0·5–3·5)	8 (4–17)
C	—	—	44	0	1·7 (0·4–∞)	8 (4–15)
C	—	—	0·1	4	2 (0·2–∞)	—
E	—	—	35	0	4·7 (1–3917)	20 (8–53)
E	—	—	4	2	2·8 (1–∞)	—

Weighted mean LD50 = 2·6 (2–3) MP 50 units.
0 hr. = *ca.* 3 sec.
Figures in parentheses are 95% confidence limits.
* Relative potency (whole egg/egg membrane piece) of 10 suspension samples = 11·8 (range 2·4–31).

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similar in range to those reported by Finter & Armitage (1957), and Fazekas de St Groth & White (1958*b*) for this virus. Thus, no change in the relative sensitivity of the ageing aerosol virus to the two methods of assay was found.

The mouse respiratory LD50 for aerosols aged 3 sec. was not significantly different from those at 2 hr. (50% R.H.), 4 hr. (80% R.H.) and 20 hr. (20% R.H.). All were within the range of 1–8 MP50 units. The weighted mean of the LD50's for all aerosol ages was 2·6 MP50's with 95% confidence limits of 2–3·4. Mouse deaths occurred between the sixth and fifteenth day after exposure.

The presence of haemagglutination-inhibiting influenza antibodies in blood sera of surviving mice indicated that the ID50 was about 0·03–0·04 MP50 for all aerosol test conditions, i.e. about 1–2% of the LD50 (Table 2).

Table 2. *Respiratory infectivity (mouse) of PR8 influenza virus*

Aerosol conditions			
R.H. (%)	Age (hr.)	Mouse dose (MP50)	Mice infected (%)
18	20	0·08	90
49	0	0·02	100
50	0	0·04	40
55	1	0·08	90
85	0	0·04	90
85	0	0·05	90
85	2	0·04	60
85	4	0·1	60
85	4	0·01	0

0 hr. = *ca.* 3 sec.

The Asian strain of influenza

The virulence (lethality) of the Asian strain was less than that of the PR8 strain and suspension titres several-fold lower. To obtain aged aerosols containing sufficient viable virus to enable mouse respiratory LD50's to be determined with reasonable exposure times more concentrated (25 and 50% allantoic fluid) suspensions had therefore to be used. These suspensions were unstable and had relatively short storage lives. It was thus rarely possible to repeat tests with any one batch. The LD50's obtained ranged from 3 to 75 MP50's but this variation was not associated with age or R.H. of the aerosols (Table 3). Statistical assessment showed the data to be heterogeneous (unlike those obtained with PR8) and the relevant heterogeneity factor had to be used when calculating the 95% confidence limits of the weighted mean LD50. These limits were 6·4–17 and the weighted mean was 10 MP50's. A few tests of surviving mice for blood sera antibody titre indicated ID50's of *ca.* 0·5 MP50, i.e. about 5% of the LD50 for all types of aerosol (Table 4). The *in ovo*/MP titre ratios confirmed that previously found with the PR8 strain, i.e. no change in the relative sensitivity of ageing virus from aerosols to the two assay methods. Aerosol viability of the Asian strain was similar to the PR8 strain under similar test conditions.

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Table 3. *Mouse respiratory LD50 of Asian influenza virus aerosols*

Suspension*	Aerosol conditions: Temp. (°C.)	R.H. (%)	Viability (%)	Age (hr.)	LD50 (in MP50 units)	Relative potency whole egg/egg membrane piece, of aerosol sample
1	21–23	13–26	45	0	8 (4–20)	13 (6–32)
2	—	—	20	20	41 (21–493)	9 (4–22)
3	—	—	71	0	41 (14–169)	8 (5–12)
3	—	—	12	20	7 (2–82)	24 (10–72)
4	—	—	100	0	26 (9–143)	—
5	—	—	61	0	75 (32–358)	—
5	—	—	21	20	6 (2–16)	13 (8–21)
4	21·7–24	49–51	27	0	9 (0·4–1807)	15 (8–31)
4	—	—	8	1	6 (2–67)	9 (5–18)
5	—	—	37	0	45 (22–409)	—
5	—	—	3	1¼	6 (4–12)	—
6	—	—	39	0	3 (1–16)	—
6	—	—	13	1	11 (3–347)	—
3	20·6–25	82–88	29	0	17 (5–326)	22 (12–43)
3	—	—	7	4	8 (2–99)	2 (0·3–7·8)
3	—	—	3	4	3 (1–18)	8 (3–25)
4	—	—	73	0	21 (11–55)	—
5	—	—	103	0	33 (9–156)	—
5	—	—	13	¾	15 (5–162)	17 (10–29)

Weighted mean LD50 = 10 (6–17, with the relevant heterogeneity factor), MP 50 units.
0 hr. = *ca.* 3 sec.
Figures in parentheses are 95 % confidence limits.
* Relative potency (whole egg/egg membrane piece) of 10 suspension samples = 14·8 (range 4·3–30).

Table 4. *Respiratory infectivity (mouse) of the Asian strain of influenza*

Aerosol conditions: R.H. (%)	Age (hr.)	Dose MP50's	% mice infected	ID50
21	0	1·9	80	—
21	20	0·6	44	—
26	0	—	—	1·6 (0·6–4·7)
26	20	—	—	0·3 (0·2–0·5)
57	0	0·5	60	—
50	2	0·8	90	—
80	0	0·4	55	—
80	4	0·2	80	—
85	2	—	—	0·2

0 hr. = *ca.* 3 sec.

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CONCLUSIONS

The ability of ageing aerosols of two strains (PR 8 and Asian) of influenza virus to infect mice via the respiratory route is paralleled by their ability to grow *in vitro* (MP) and *in ovo*. The mouse respiratory LD 50 of the viruses does not change significantly during ageing of aerosols up to 20 hr. under the conditions tested. There is also no apparent change in the ID 50. Thus, the changes in viability of the viral aerosols (determined by MP and *in ovo* methods) were a direct indication of the respiratory infectivity and virulence of the aerosols for mice.

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