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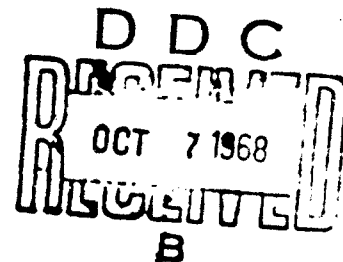
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VIABILITY OF RESPIRATORY VIRUSES IN THE AIR

[Following is the translation of an article by V.V. Vlodavets and R.A. Dmitriyeva, Institute of General and Communal Hygiene imeni Sysina, AMN USSR, Moscow, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No. 9, 1966, pages 30-34. It was submitted on 8 July 1965. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

For establishing the mechanism for the spreading of droplet-aerosol viral infections in collectives, it is important to clarify the kinetics of inactivation of respiratory viruses in the air of closed premises and the influence of various factors of the external medium on the inactivation rate. It is known from the literature that during the process of death of microorganisms in the air of closed premises at a stable room temperature, an important influence is exerted by the relative humidity and the chemical components, contained in the disperse phase of the aerosol (Webb, 1959, 1963; Hemmes, 1959; Vlodavets, 1962; Harper, 1963, etc.).

The present work is devoted to a study of the survival of the influenza virus and the adenovirus in the droplet phase of an aerosol at a various relative air humidity.

For creation of the aerosol we used an allantoic culture of the A1 influenza virus (Pan strain) with an infectious titer of $10^{-6.5} - 10^{-8}$ ID₅₀ for chick embryos (EID₅₀). The adenovirus aerosol was formed by spraying a culture fluid containing adenovirus type 5 (infectious titer $10^{-4.5} - 10^{-5.5}$ CPD₅₀).

Dispersion of the virus-containing fluids was performed with the help of a Barkovskiy glass sprayer for 2 minutes (output of the sprayer 0.16 ml/min) in a type E hermetic chamber, volume 500 liters. Five minutes after the spraying a kinetically stable polydisperse aerosol was created in the chamber. The size of the majority of the droplets in the aerosol, determined by the method of Fuks and Petryanov (1933), fluctuated between 0.8-1.2 microns.

Recovery of air samples was done with the help of a Rechmenskiy bacteria trapping device (1952) after 5 and 30 minutes and 1, 2, 3, 4, 5, and 6 hours after creation of the viral aerosol. Up to 10 liters of air was investigated in each sample. For trapping the influenza virus we used 2.5 ml of sugar broth as the adsorbing liquid in the Rechmenskiy device, and for trapping the adenovirus - medium No. 199. The presence of the influenza virus in the air samples was determined after 2 days on

10-11 day old chick embryos, and the adenovirus - based on the cytopathic effect on a transplanted line of HeLa cells.

A low relative humidity was created by means of forcing air into the chamber through an absorption column containing calcium chloride. An increase of relative humidity was achieved by means of spraying 2-15 ml of sterile tap water into the air of the chamber.

The data obtained testified that when the stated liquid was dispersed in the air of the chamber, an aerosol was created which contained biologically active influenza virus or adenovirus. In separate experiments active influenza virus was determined in the air over a period of 4-6 hours after creation of the aerosol, and adenovirus - over a period of 2-4 hours.

In the experiments with both influenza virus and with adenovirus, a regular lowering was established in the concentration of active virus during the period of observation, as well as a specific, quite clearly expressed, influence of the relative air humidity on the degree of inactivation of viruses in the droplet phase of an aerosol.

With the A1 influenza virus 39 six-hour tests were performed at various relative humidities (Table 1). It was established that at a low air humidity (18-20%) the influenza virus was detected in the air of the chamber over a period of 6 hours after dispersion, while in separate tests after 6 hours the titer of the trapped virus in 10 liters of air was $10^{-1.45}$ - $10^{-1.5}$ EID₅₀. Upon increasing the relative humidity up to 20-40% the influenza virus was constantly determined in the air over a period of 4 hours, while in separate tests small concentration of it could be detected after 6 hours following the spraying of the viral suspension. A considerable lowering in the survival of the influenza virus in the aerosol was observed when the relative humidity was increased from 40 up to 60%, and especially at a humidity of 60-70%. In the majority of the tests conducted under these conditions active virus was determined in the air over a period of 1-2 hours, and in 2 tests - only for 5 and 30 minutes after creation of the aerosol. With a further increase of the relative air humidity from 70 to 90% the time of stay for the influenza virus in the aerosol increased up to 2 hours, and in individual tests small concentrations of the virus were detected 4 hours after spraying. With a relative humidity above 90% the influenza virus was trapped in the air over a period of 1-2 hours.

Thus, the activity of the influenza virus in an aerosol was preserved longest at low indices of relative humidity; the most rapid inactivation of the virus took place at a relative humidity of 60-70%.

With the type 5 adenovirus we performed 19 five-hour tests (Table 2). It was noted that in contrast to the influenza virus, the adenovirus aerosol was detected longer (quite regularly for a period of 3 hours, and in

one test - for 4 hours) at high indices of humidity (62-84%). A lowering of the relative humidity led to the decrease in the survival of the adenovirus in the air. With a relative air humidity of 37-56% the adenovirus was constantly determined in the air of the chamber over a period of 2 hours, and in 2 tests at an air humidity of 37 and 46% the virus was detected for 3 hours. At a humidity of 22-33% a still more rapid inactivation of the adenovirus took place: Active virus was trapped in the air only for an hour.

The data obtained testify that low indices of relative humidity promote the rapid inactivation of adenovirus in the droplet phase of an aerosol.

We did not study the influence of the spraying process on the survival of the influenza virus and the adenovirus, nor the influence of various relative humidities in the first seconds and minutes of existence of the aerosol. Several ideas concerning these processes may be obtained on the basis of indirect indices - differences in the initial titer of the influenza virus and the adenovirus in the virus-containing fluid and the titer of the virus in the first air sample, which was taken 5 minutes after creation of the aerosol. The difference noted between the initial titer of the influenza virus and the adenovirus and the titer in the first air sample may be explained by the dilution of the virus in the large volume of air in the chamber, the partial settling of the large droplets from the aerosol and the inactivation of viruses under the conditions of an aerial medium (see Tables 1 and 2).

The differences between the initial titer of the influenza virus and the titer in the first air sample were significantly greater than the corresponding differences with the adenovirus. Even under conditions of low air humidity, which are most favorable for the influenza virus, it comprised 3.9 lg, and for the adenovirus at 50-70% humidity, that is under optimum conditions for it, only 1.6 lg. Under unfavorable conditions these differences are expressed still more sharply, comprising 5.7 lg for the influenza virus at a relative humidity of 50-60%, and only 2.2 lg for the adenovirus at low indices of inactivation humidity. In spite of the considerable differences in the percentages in the first minutes of the aerosol's creation, the curves of inactivation for the influenza virus and the adenoviruses turned out to be quite close. But in connection with the considerably higher content of influenza virus in the allantoic culture ($10^{-6.5}$ - 10^{-8} EID₅₀), active influenza virus was detected in the aerosol for a longer period of time in comparison with the concentration of adenovirus in the virus-containing fluid ($10^{-4.5}$ - $10^{-5.5}$ CPD₅₀). Without a doubt these differences would have been more distinct had there not been such a significant inactivation of the influenza virus at the moment of dispersion and during the first minutes after the formation of the aerosol.

Already in the 40's study began on the influence of relative air humidity on the survival of the influenza virus, however, the results

obtained by different authors bore a contradictory nature. Thus, according to the data of Borecky (1955) a high relative humidity is most favorable for preserving the activity of the influenza virus. Opposite opinions are maintained by Loosli et al (1943), Hemmes et al (1960), Yakovleva and Shandurin (1962), Harper (1963). They consider that the infectiousness of the influenza virus in an aerosol is preserved longest at a low relative air humidity. Finally, based on the data of Lester (1948) and Schechmeister (1950) the most rapid inactivation of the virus in the air takes place at a relative humidity of 50-60%, while at lower and higher indices of humidity more favorable conditions for the survival of the virus are created.

Our results support the opinion of those authors who consider a low relative air humidity favorable for the survival of the influenza virus. At the same time they come close to the data of Lester (1948) and Schechmeister (1950). According to our experiments, a 50-70% relative humidity was most unfavorable for the influenza virus. With a further increase of humidity the survival rate of the influenza virus increases only somewhat, but in the droplet phase of an aerosol at a relative humidity lower than 30% the influenza virus preserved its activity for many hours.

In recent years the influence of meteorological factors on influenza incidence has been studied quite intensively. Without denying the importance of meteorological factors, which doubtlessly exert a certain influence on the susceptibility of the host to an infectious agent, it should be kept in mind that infection with influenza takes place exclusively in closed premises. Therefore, it can be proposed that the conditions of the aerial medium in these premises (microclimate) exert a significant influence on the interrelationship between the micro- and the macroorganism, and correspondingly on influenza incidence in susceptible collectives. As is known, outside of large epidemics caused by new variants of the virus, influenza incidence is highest in the fall-winter season. During this period in connection with the switching on of heating in homes and communal premises there is a lowering of relative air humidity, that is, conditions are created which promote the prolonged survival of the influenza virus in an aerosol, and correspondingly there is an increase in the probability of infection of susceptible personnel. In this manner it can be considered that the relative air humidity of the premises is an important factor of the external environment, exerting a significant influence on the droplet-aerosol mechanism of transmission of the causative agent of the infection.

On the basis of our investigations, and also the data from a number of authors, it can be recommended that during epidemic outbreaks of influenza, in premises where the air can be controlled the relative air humidity be maintained within the limits of 50-70%. This measure, in our opinion, should lead to the rapid inactivation of the influenza virus in the air of premises and a decrease in the danger of the aerosol infection of susceptible contingents.

While the survival rate of the influenza virus in an aerosol has been subjected to quite an intensive study, the preservation of the adenovirus in the droplet phase of an aerosol to all intents and purposes has not been studied. There is only information concerning the stability of the adenovirus in the droplet phase of an aerosol (Vlodavets and Dmitriyeva, 1964) and its inactivation in this medium under the influence of short-wave ultraviolet radiation (Dmitriyeva, 1964; Jensen, 1964).

The nature of the influence of relative humidity on the survival of the influenza virus and the adenovirus in the droplet phase of an aerosol is different. The influenza virus preserves its activity best of all at a low relative humidity, and the adenovirus - at a high. Its most rapid inactivation sets in at 22-33% relative air humidity. The inactivating effect of various relative air humidities on different viruses was also shown in the investigations by Buckland and Tyrrell (1962). They studied the influence of humidity on viruses, applied in the form of small droplets on the surface of glass.

Conclusions

1. These investigations demonstrated the possibility of creating an aerosol of influenza virus and adenovirus by means of dispersing a virus-containing suspension in the air of an aerosol chamber. The stability of the aerosol established determined to a significant degree the length of preservation of the activity of viruses under the conditions of an aerial medium. ~~In its turn~~ the relative air humidity at a constant room temperature of 18-22°C exerts a significant influence on the rate of inactivation of viruses in the air.

2. The nature of the influence of relative air humidity on the survival of the influenza virus and the adenovirus in the droplet phase of an aerosol is different. The influenza virus preserves its activity longest at low indices of humidity (below 30%), while the adenovirus survives best of all at a high humidity (70-80%). The most rapid inactivation of adenovirus takes place at a humidity of 22-33%, and influenza virus - at a relative humidity of 50-70%.

3. During periods of influenza outbreaks it can be recommended that in premises with controlled air, in addition to the generally accepted hygienic measures, the relative humidity should be maintained within the limits of 50-70%. This will contribute to the inactivation of the infectious agent in the air and decrease the possibility of susceptible persons becoming infected.

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Table 1

Influence of relative humidity on the survival of influenza virus in the air

No. of tests	Relative humidity (in %)	Initial titer of virus (in EID ₅₀)	Titer of influenza virus of 10 liters of air after various periods of time					
			5 min	30 min	1 hr	2 hrs	4 hrs	6 hrs
4	18--20	8.2	4.3	3.3	2.5	1.8	0.9	0.8
3	20--30	7.6	3.0	2.5	2.3	0.9	0.6	-
4	30--40	7.5	2.8	2.4	1.6	1.6	0.6	-
5	40--50	7.6	2.6	1.3	1.0	0.6	-	-
7	50--60	7.9	2.2	1.7	0.9	0.7	-	-
4	60--70	7.1	3.0	2.6	1.0	-	-	-
4	70--80	7.0	3.1	2.6	1.3	0.8	-	-
8	80 and higher	7.5	3.4	2.3	1.5	0.7	-	-

Note: Virus titers, expressed in negative logarithms of EID₅₀, represent the average geometrical values.

Table 2

Influence of relative humidity on the survival of adenovirus in the air

No. of tests	Relative air humidity (in %)	Initial titer of virus (in CPD_{50})	Titer of virus in aerosol	Titer of virus in 10 liters of air after various periods of time					
				30 min	1 hr	2 hrs	3 hrs	4 hrs	5 hrs
5	22-33	4.3	1.9	1.2	0.55	In 1 test in titer of 0.5	-	-	-
8	37-56	4.6	2.4	1.6	1.66	0.43	In 2 tests in titer of 0.5	-	-
5	62-84	4.5	2.7	1.9	1.4	0.6	0.35	-	-

Note: Virus titers, expressed in negative logarithms of CPD_{50} , represent the average geometrical values.