DETECTION OF THE MINIMUM CONCENTRATIONS OF INFLUENZA VIRUS IN THE DROPLET PHASE OF AN AEROSOL

AD648109
TT67-61205

Translation No. 1694

May 1966

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MAR 14 1967

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DETECTION OF THE MINIMUM CONCENTRATIONS OF INFLUENZA VIRUS IN THE DROPLET PHASE OF AN AEROSOL

[Following is the translation of an article by S. Ya. Gaydamovich and V. V. Vlodavets, Institute of Virology imeni D. I. Ivanovskogo, AMN USSR, and the Institute for General and Communal Hygiene imeni A. N. Sysina, AMN USSR, Moscow, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology), 1963, No 8, pages 349--353. It was submitted on 25 Jun 1962. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

Though there are several accounts on the stabilization of the influenza virus in protein-containing liquids and buffer solutions and the unfavorable influence of saline solutions and distilled water [6, 7, 9], the problem concerning the influence of the spray fluid on the stability of a viral aerosol has been studied very little. According to the data of I. B. Blok /1/, the influenza virus which is dispersed in a physiological saline solution injures the pulmonary tissue of mice to a lesser degree and has a lower titer of hemagglutinins than the same virus atomized (sprayed) in saliva. G. S. Yakovleva and S. V. Shandurin /5/ established that the influenza virus atomized in broth is considerably more persistent in an aerosol than when it is dispersed in a physiological solution.

In the present work we studied the feasibility of detecting the minimum concentrations of influenza virus in an aerosol, depending on the liquid in which an allantoic culture of the virus was cultivated and dispersed, and also the influence of the spray fluid on the amount of virus which is trapped.

Materials and Methods

Various dilutions of the Pan strain of A-prime influenza virus were dispersed in a 250 liter experimental chamber. This was done with the help of a modified Larkovskiy atomizer. Over a period of 2 minutes 0.3 ml of a virus suspension was dispersed in the air of the chamber. Various liquids were used for diluting the virus suspension (sugar broth, allantoic fluid of chick embryos, physiological saline solution, milk, native saliva, distilled water, phosphate buffer mixture). Immediately before each series of tests the allantoic culture of the influenza virus was diluted in 10-fold dilutions from 10^{-1} to 10^{-5}--10^{-6} depending on the liquid used. The tests were carried out at a temperature of 18--22.5° and a relative air humidity of 47--65%.

In the first test we selected the most diluted suspension of the virus for atomizing. After 5 minutes following the conclusion of the dispersion of the virus we took a 50 liter sample of air with the subsequent titration of the trapped virus on chick embryos /2/. Then we carried out a series of tests with lesser dilutions of the virus. The last experiment was with a dilution of 10^{-1}, and when using allantoic fluid for dilution the undiluted virus was dispersed last. Following the conclusion of a series of tests

with a particular liquid and the disinfection of the air in the chamber with short-wave ultraviolet radiation, we carried out the experiments with another liquid.

In all the tests the trapping of the viral aerosol was accomplished with the help of a Rechmenskiy bacteria trap and sugar broth was used as the adsorbent. According to our data /3/ this is the most effective method of trapping an aerosol of the influenza virus.

Results

As is seen from table 1, sugar broth is the liquid which is most favorable for the stabilizing of the influenza virus in an aerosol during the first period following the dispersion of the viral suspension. Somewhat worse results were obtained with the dilution and dispersion of the influenza virus in milk and the allantoic fluid of chick embryos. It should be noted however that the differences between these liquids are not great, and in general the results obtained are quite close to each other. Thus, following the dilution of a virus suspension in these liquids, the virus is detected in all the air samples when sprayed in a dilution of 10^{-3}, and when dilutions of 10^{-3}--10^{-4} are used it is trapped not only in a nondiluted substrate, but also in dilutions of it up to 10^{-2}--10^{-3}. In individual tests the influenza virus is detected in the air following dispersion of dilutions of 10^{-5}--10^{-6}, which testifies to the high sensitivity of the Rechmenskiy device for the indication of a virus aerosol.

The native saliva of man turned out to be less favorable for the atomization of the influenza virus. In all probability this is connected with the presence of inhibitor substances /4/ in it. Thus, when a virus suspension is atomized (sprayed) in saliva in a dilution of 10^{-2} the influenza virus is detected in the air of the chamber in all the samples, and with a dilution of 10^{-3}--10^{-4} in 5 tests out of 6. Worse results were obtained when distilled water was used; the influenza virus is trapped in all the tests when dispersed in a dilution of 10^{-1}, and in 5 tests out of 6 when atomized (sprayed) in dilutions of 10^{-2}--10^{-3}. The most toxic medium is the physiological saline solution. Following the dispersion of the virus in a 0.85% solution of NaCl the influenza virus is determined in the air in all the tests only when the very lowest dilution of 10^{-1} is used. In 3 tests with a phosphate buffer mixture we did not obtain clear results which would make it possible to characterize the influence of this liquid on the influenza virus in an aerosol. It can only be assumed that a phosphate buffer is mildly favorable for the influenza virus, and based on its effect is close to distilled water.

We made several theoretical calculations of the feasibility of detecting the influenza virus in an aerosol following its dispersion in various dilutions. In these calculations we proceeded from the assumption that the infection of a chick embryo is caused by introduction of one virus particle into the allantoic cavity. Here we did not take into consideration the physical loss of the aerosol due to the settling of the drops which contained the virus and the biological loss due to the inactivation of the virus under the conditions of the atmosphere; the stated processes go on particularly intensively during the first minutes of the aerosol's existence. The calculations of the minimum concentrations of an aerosol of influenza virus which may be detected in the air of an experimental chamber, without

considering the loss of the virus due to inactivation and settling, are presented in table 2.

They testify to the fact that when liquids are used for dilution which are favorable for the influenza virus (sugar broth, milk, allantoic fluid), the doses of virus which are recovered are close to the calculations, and in individual cases even exceed them somewhat. The latter may be explained by the considerable mechanical influence during the dispersion of the virus suspension. This is accompanied by the crushing of the drops when they strike the glass surface and as a result of this there is a disruption of the virus aggregates with the appropriate increase in the number of viral particles. This assumption is found in agreement with the experimental data of Zimmermann /11/, who considers that the change in the titer of the virus in a suspension is dependent on a change in the ratio of free viral particles and their aggregates.

The calculations cited show that in all cases in the presence of favorable conditions the influenza virus is determined with the help of a Rechmenskiy bacteria trap when a virus suspension in dilutions of 10^{-4}--10^{-5} is dispersed in the chamber. If all the liquids studied exerted a similarly favorable effect on the influenza virus in an aerosol, then it would be trapped to a similar degree without any dependency on the spray fluid. Only protein-less liquids would have a certain advantage, since in the absence of protein the evaporation of water is speeded up and the volume of the drop is reduced more rapidly. In other words, in a protein-less liquid and especially with the atomization of the virus suspension in distilled water, conditions would be created at which the virus particles would be found longer and in a greater concentration in a suspended state in the air of the chamber. Consequently, the influenza virus, diluted and dispersed in distilled water or a physiological solution could be determined in larger concentrations than the virus which is atomized in sugar broth or milk.

The tests carried out, however, clearly show that when protein-containing liquids (sugar broth, milk, allantoic fluid) are used as the spray fluid the influenza virus is determined following the atomization of large dilutions and in greater concentrations than following pulverization in solutions of inorganic salts (physiological saline solution, phosphate buffer mixture) and distilled water. These data testify to the favorable effect of protein-containing liquids on an aerosol of the influenza virus. The rapid lowering in the concentration of an aerosol in saline solutions takes place as a result of the inactivation of the influenza virus in the first minutes or even seconds following the creation of the aerosol. These data agree with the data of Ferry and Maple /8/ and Webb /10/, who showed on models of bacterial aerosols that the death of the microorganisms in the aerosol takes place in two phases: There is a significant dying off in the first minutes and even seconds following dispersion of the bacterial suspension and a slower dying off during the entire following period.

Thus, the results of our tests showed that sugar broth, milk and allantoic fluid used as the spray fluid, and to a lesser degree native saliva,

are a protective factor which promotes the preservation of the viability of the influenza virus in the first, most unfavorable, period of the transition from a liquid to an aerosol state.

Conclusions

1. Protein-containing liquids (sugar broth, milk, allantoic fluid) are considerably more favorable for preserving the viability of the influenza virus in an aerosol than distilled water and saline solutions (physiological saline solution, phosphate buffer mixture). Native saliva occupies an intermediate position.

2. The results of determining the influenza virus in an aerosol when liquids which are favorable for the preservation of the virus are used as the spray fluid are close to the theoretical calculations.

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Table 1

Trapping of an influenza aerosol depending on the nature of the dispersing liquid

Dispersion phase	Number of tests	Dilution of influenza virus	Trapping doses of virus (in dilutions of substrate)					
			undiluted	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
			Number of tests					
Sugar broth	6	10^{-6}	1	-	-	-	-	
	6	10^{-5}	2	1	-	-	-	
	6	10^{-4}	4	4	1	-	-	
	6	10^{-3}	6	4	3	2	-	
	6	10^{-2}	6	6	4	3	-	
	6	10^{-1}	6	6	6	6	3	
Milk	5	10^{-6}	1	-	-	-	-	
	5	10^{-5}	2	-	-	-	-	
	5	10^{-4}	4	2	-	-	-	
	5	10^{-3}	5	5	3	-	-	
	5	10^{-2}	5	4	4	4	1	
	5	10^{-1}	5	5	5	5	2	
Allantoic fluid	6	10^{-6}	1	-	-	-	-	-
	6	10^{-5}	-	-	-	-	-	-
	6	10^{-4}	2	1	-	-	-	-
	6	10^{-3}	6	5	1	-	-	-
	6	10^{-2}	6	4	4	2	-	-
	6	10^{-1}	6	6	5	4	1	-
	6	undiluted	6	6	6	6	3	3
Saliva	6	10^{-6}	-	-	-	-	-	
	6	10^{-5}	3	-	-	-	-	
	6	10^{-4}	5	1	-	-	-	
	6	10^{-3}	5	1	2	-	-	
	6	10^{-2}	6	3	3	2	-	
	6	10^{-1}	6	5	5	2	-	
Distilled water	6	10^{-6}	-	-	-	-	-	
	6	10^{-5}	1	-	-	-	-	
	6	10^{-4}	3	-	-	-	-	
	6	10^{-3}	5	3	-	-	-	
	6	10^{-2}	5	3	-	-	-	
	6	10^{-1}	6	6	6	3	-	
Physiological solution	6	10^{-5}	-	-	-	-	-	
	6	10^{-4}	1	-	-	-	-	
	6	10^{-3}	3	1	-	-	-	
	6	10^{-2}	5	4	1	-	-	
	6	10^{-1}	6	5	5	1	-	

Table 2

Approximate calculations of the feasibility of detecting an aerosol of influenza virus with the Rechmenskiy bacteria trap

Dilution of influenza virus	Theoretically possible maximum concentrations of trapped virus
Undiluted	10^{-4} -- 10^{-5}
10^{-1}	10^{-3} -- 10^{-4}
10^{-2}	10^{-2} -- 10^{-3}
10^{-3}	10^{-1} -- 10^{-2}
10^{-4}	Undiluted -- 10^{-1}
10^{-5}	" 0
10^{-6}	0