AD-783 573

CONCERNING INACTIVATION OF VIRUSES IN AIR

R. A. Dmitriev

Foreign Technology Division
Wright-Patterson Air Force Base, Ohio

14 June 1974

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UNCLASSIFIED

Security Classification

AD-783573

DOCUMENT CONTROL DATA - R & D

Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

ORIGINATING ACTIVITY (Corporate author) Foreign Technology Division Air Force Systems Command U. S. Air Force	2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED 2b. GROUP

3 REPORT TITLE

CONCERNING INACTIVATION OF VIRUSES IN AIR

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

Translation

5 AUTHOR(S) (First name, middle initial, last name)

R. A. Dmitriev

6 REPORT DATE 1973	7a. TOTAL NO. OF PAGES 14	7b. NO. OF REFS 14
8a CONTRACT OR GRANT NO b. PROJECT NO c d T74-03-07	9a. ORIGINATOR'S REPORT NUMBER(S) FTD-MT-24-727-74 9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	

10 DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited.

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY Foreign Technology Division Wright-Patterson AFB, Ohio

13 ABSTRACT

06

DD FORM 1 NOV 65 1473

UNCLASSIFIED

Security Classification

EDITED MACHINE TRANSLATION

FTD-MT-24-727-74 14 June 1974

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By: R. A. Dmitriev

English pages: 9

Source: Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii, Nr. 4, 1973, pp. 21-25

Country of Origin: USSR
Requester: FTD/PDTR
This document is a SYSTRAN machine aided translation, post-edited for technical accuracy by: SSgt Michael L. Seidel
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В в	*В в*	V, v	Т т	*Т т*	T, t
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Д д	*Д д*	D, d	Ф ф	*Ф ф*	F, f
Е е	*Е е*	Ye, ye; E, e*	Х х	*Х х*	Kh, kh
Ж ж	*Ж ж*	Zh, zh	Ц ц	*Ц ц*	Ts, ts
З з	*З з*	Z, z	Ч ч	*Ч ч*	Ch, ch
И и	*И и*	I, i	Ш ш	*Ш ш*	Sh, sh
Й й	*Й й*	Y, y	Щ щ	*Щ щ*	Shch, shch
К к	*К к*	K, k	Ъ ъ	*Ъ ъ*	"
Л л	*Л л*	L, l	Ы ы	*Ы ы*	Y, y
М м	*М м*	M, m	Ь ь	*Ь ь*	'
Н н	*Н н*	N, n	Э э	*Э э*	E, e
О о	*О о*	O, o	Ю ю	*Ю ю*	Yu, yu
П п	*П п*	P, p	Я я	*Я я*	Ya, ya

* ye initially, after vowels, and after ъ, ь; e elsewhere. When written as ё in Russian, transliterate as yё or ё. The use of diacritical marks is preferred, but such marks may be omitted when expediency dictates.

FOLLOWING ARE THE CORRESPONDING RUSSIAN AND ENGLISH

DESIGNATIONS OF THE TRIGONOMETRIC FUNCTIONS

Russian	English
sin	sin
cos	cos
tg	tan
ctg	cot
sec	sec
cosec	csc
sh	sinh
ch	cosh
th	tanh
cth	coth
sch	sech
csch	csch
arc sin	$\sin^{-1}$
arc cos	$\cos^{-1}$
arc tg	$\tan^{-1}$
arc ctg	$\cot^{-1}$
arc sec	$\sec^{-1}$
arc cosec	$\csc^{-1}$
arc sh	$\sinh^{-1}$
arc ch	$\cosh^{-1}$
arc th	$\tanh^{-1}$
arc cth	$\coth^{-1}$
arc sch	sech^{-1}
arc csch	csch^{-1}
rot	curl
lg	log

CONCERNING INACTIVATION OF VIRUSES IN AIR

R. A. Dmitriev

Institute of General and Municipal Hygiene mena. Sysina, AMN of Academy of Medical Sciences the USSR, Moscow (entered 15/11 1972).

Although the role of the air medium in the propagation of respiratory virus infections is commonly known, the mechanism of their transmission is still not completely studied. The respiratory viruses enter the air during a coughing and sneezing of sick people. In this case, the large drops of aerosol rapidly settle, while fine drops long remain suspended and are moved by draughts, causing illnesses in susceptible contingents. In connection with this, the question concerning the effect of the different factors of the air medium on the inactivation of viruses in an aerosol is of significant interest. According to literature, one of the factors which affects the inactivation of viruses in air, is the relative humidity. So, a series of researchers noted rapid inactivation of the influenza virus with average and high indices of relative humidity [1, 2, 5, 6, 10, 11, 13]. Furthermore, there are reports about the effect of relative humidity on parainfluenza virus [12], adenoviruses [2, 12], policmylitis viruses [5, 6], vesicular stomatitis, small-pox,

Venezuelan encephalomyelitis [5], the virus of measles [7, 8].

We studied the effect of different relative humidity indices on the processes of inactivation of parainfluenza viruses, respiratory-syncytial virus, adenoviruses, enteroviruses and the virus of Newcastles's disease in the aerosol drop phase.

Experimental studies were conducted on the following models of viruses, the infectious activity of which was expressed in negative logarithms: the parainfluenza virus (type 3) 6.5-7.0 log of $QPD_{50/0.2\ ml}$; respiratory-synctial (strain Randall) 3.0-3.8 log of $QPD_{50/0.2\ ml}$; virus of Newcastle's disease (strain V_1) 7.0-7.5 log of $ID_{50/0.2\ ml}$; an adenovirus of type 5 (standard 22f, isolated from the defecations of people, ill with infectious hepatitis) 6.5-7.0 log of $QPD_{50/0.2\ ml}$; ECHO 7.0 log of $QPD_{50/0.2\ ml}$.

The investigations were conducted in an experimental chamber 500 l in volume in which, with the aid of a Barkovsky system glass atomizer, for 2 min. a virus-containing liquid was dispersed (productivity of the atomizer, 0.37 ml/min). Samples of air in the amount of 20 l were taken with the aid of a Rechmenskij bacterial trap after 5 and 30 min., 1, 3, 5, 7, and 24 hours after the dispersion of the virus suspension. 3 ml of medium No. 199 with antibiotics (penicillin and streptomycin) was used as a catching liquid in bacterial trap.

Isolation and titration of the viruses in samples was conducted by conventional methods: the ECHO-7 virus - on the primarily triturated culture of the kidneys of monkeys; the viruses of paragrippa, respiratory-synctial virus and adenoviruses - on a rewoven line of cells of HeLa; the virus of Newcastles's disease - on 9-day chick embryos. On each breeding of sample they were used on 4 culture tubes of tissue or on 4 chick embryos. Final results were considered according to the cytopathic effect with adenovirusaes on the 21-28th day, with the ESNO-7 virus after 10 days, with respiratory-syncytial virus during

10-12 days, whereupon to accelerate the appearance of a cytopathic effect, a change of the medium in the infected test tubes was conducted after each 5 days [3]. For the development of the paragrippa virus on the 7th day after infection a hemadsorption reaction was established, for the development of the virus of Newcastle's disease an RGA [PГA - hemagglutination reaction] was established with the allantois liquid of the infected embryos after 2 days of cultivation. The inactivation of viruses in air was judged according to loss of infectious activity by them.

Dynamics of the inactivation of viruses in an aerosol with different atmospheric humidity.

Virus	Relative humidity (in %)	Titer of Virus								
		In initial suspension	In aerosol 5 min. after atomization	After different periods following dispersion						
				30 min	1 h	2 hours	3 hours	5 hours	7 hours	24 hours
ECHO-7	20–25	7.0	1.75	0.9	0.9	—	0	0	0	0
	50–55	7.0	2.85	2.05	1.35	—	0.35	0.3	0.15	0
	80–85	7.0	3.75	2.55	1.95	—	0.96	0.6	0.5	0.15
Type 5 Adeno-virus										
Standard Strain	20–25	7.0	2.16	1.65	0.66	—	0	0	0	0
	50–55	6.75	3.26	2.75	1.66	—	1.16	0.41	0.38	0.25
	80–85	6.9	3.6	3.0	2.4	—	1.3	1.0	0.35	0.33
Strain 22f	20–25	7.0	3.8	3.0	2.12	—	1.25	0.75	0.25	0
	80–85	7.0	3.75	3.25	2.8	—	1.75	1.0	0.5	0
Newcastles diseases	20–25	7.0	5.2	4.5	4.0	—	3.5	2.5	1.6	
	50–55	7.3	4.8	3.5	3.25	—	2.3	1.5	0.5	—
	80–85	7.25	5.0	3.25	3.2	—	2.45	0.96	0	—
Parainfluenzal	20–25	6.75	2.7	1.35	0.8	0.45	0.3	0	0	—
	50–55	6.9	1.8	0.45	0.33	0	0	0	0	—
	80–85	7.0	2.05	1.2	0.7	0.2	0	0	0	—
Respiratory-syncytial	20–25	3.5	1.3	Hp	Hp	0	0	0	0	—
	50–55	3.6	1.3	Hp	Hp	0	0	0	0	—
	80–85	3.35	0.8	Hp	—	0	0	0	0	—

Note. Titer of viruses is expressed in negative logarithms of the $ЛД_{50/0.2\ мл}$ and is the geometrical mean of 5-7 sets of experiments at each every index of relative humidity.
The designations: Nr - virus is revealed only in the undiluted samples; - the titer was not determined.

The investigations were conducted with 3 indices of relative humidity: 20-25, 50-55, and 80-85%. Air temperature varied from 19 to 22°.

As the results of the conducted investigations showed, there were varying degrees of inactivation of different viruses. The influenza viruses and respiratory-syncytial virus were inactivated most rapidly in the air of the chamber, more prolonged time managed to detect adenoviruses, the enteroviruses and the virus of Newcastle's disease. Relative humidity had on the degree of the inactivation of viruses in aerosol and essential effect. Thus, adenoviruses (both strains) and the ECHO-7 virus were most rapidly inactivated with low indices of relative humidity. With humidity within the limits of 20-25% these viruses could be determined in air of the chamber only for hour. On the contrary, average and high indices of relative humidity contributed to the most prolonged conservation of infectious activity of these viruses in air. Thus, with atmospheric humidity of 50-55% both viruses were detected in the chamber for 7 hours, and with humidity 80-85%, in certain cases even for 24 hours after the creation of the aerosol (see the table).

Different results were obtained during the study of dynamics of inactivation in air of the paragrippa and respiratory syncytial viruses. On one hand, they turned out to be slightly stable under conditions of the air medium, on the other they are less susceptible to the effect of different relative humidity. The parainfluenza virus was inactivated most rapidly with average and high relative air humidity (it was detected only for 1-1 1/2 hours), while with low relative humidity the degree of inactivation was considerably lower and the virus could be determined in the air of the chamber for 2-3 hours (see the table).

The respiratory-cyncytial virus possessed even less stability in an aerosol state. In air of its chamber it basically could be seen for 5-30 min. after the dispersion of virus suspension. With low relative humidity, the titers of virus were somewhat higher than with average and high humidity, which testified to the favorable effect of low humidity. With humidity within the limits 50-55 and 80-85%, respiratory-syncytial virus was determined only

in undiluted suspensions. The short period, during which respiratory-syncytial virus could be seen in the air of the chamber, connected, on one hand, with the high lability of this virus, on the other - with the low infectious titer of the initial virus-containing liquid utilized for creation of the aerosol (see the table).

The virus of Newcastles's disease unlike the parainfluenza and respiratory-syncytial viruses possessed high stability in the drop phase of an aerosol and like them, was less subject to the effect of different relative humidity. The differences in the inactivation of the virus of Newcastles's disease in air under the action of relative humidity were insignificant and varied, as a rule, within the limits of 1-2 hours. However, the data obtained testified to the clearly expressed tendency toward the greatest inactivation of the virus at high and average relative humidity and greater stability at low humidity (see the table). Thus, with humidity 50-55%, the virus of Newcastle's disease regularly could be detected in the air for 5-6 hours, and with humidity 20-25% for 7 hours. Moreover, with low humidity the titer of the virus on the average after 7 hours following dispersion of the suspension was 1.6 log ID_{50}/0.1 ml.

It should be noted that the greatest effect of relative humidity developed in the first seconds and minutes of the existence of the virus aerosol. The differences in the titers of the virus in the atomized virus-containing suspension and the sample of air obtained after 5 min. after dispersion testified to this (see the table). So, under the effect of low relative humidity the virus ECHO-7 was inactivated to the greatest degree - a difference in titers of more than 5 logarithms, for the adenoviruses this difference was 4-4.5 logarithms. Under the unfavorable conditions, i.e., with high and average relative humidity, the difference in titers for

the parainfluenza virus reached 5 logarithms, and for the virus of Newcastle's disease it reached 2-3 logarithms.

The obtained results testified, that many viruses can long retain infectious activity under conditions of the air medium at room temperature, therefore, during a determined time they can constitute a threat to susceptible contingents. It is necessary to note that the viruses which long retain infectious activity in air (adenoviruses, enteroviruses and the virus of Newcastle's disease) settle on the surface of surrounding subjects which, in turn, can be a secondary source of the infection of people. Thus, the dried drops of aerosol during dry retraction can be raised from surfaces (dust phase of aerosol) into the air and be moved under the effect of its currents, supporting the constant circulation of viruses in closed rooms. In the propagation of adenoviruses, enteroviruses and the virus of Newcastle's disease, both the drop and dust phase of aerosol, and infected surfaces can have significance. Parainfluenza and respiratory-syncytial viruses are unstable in the external medium, and therefore their propagation is connected basically with the air-drop and contact course of transmission.

The most important factor of the air medium which affects the process of virus inactivation in the aerosol state is the relative humidity, especially in the first minutes of aerosol formation. During this period, such factors as the inactivation and the process of sedimentation exert the greatest effect on lowering in the infectious activity of viruses. However, Miller and Artenstein [12] as showed, who marked atomized suspensions of the parainfluenza virus and adenoviruses by fluorescein, and then studied the changes in the content of color and infectious activity of viruses in air, lowering the quantity of viruses occurred due to inactivation, and, to a lesser degree, due to sedimentation.

The mechanism of the inactivation of viruses in air, dependent on the average and high relative humidity, is not clear. With low relative humidity rapid dehydration of nucleic acids occurs, which as a final result leads to the inactivation of viruses in an aerosol state [14]. This hypothesis was confirmed by the experiments of Jong and Winkler [9] on the polio virus model and its nucleic acid, in which it was shown that the inactivation of the virus in air was primarily connected with a change in the structure of nucleic acid. However on the basis of these investigations it is not possible to explain the more rapid inactivation of a number of viruses during high relative humidity, and significant stability with low. These literature make it possible to assume that the inactivation of viruses in air, dependent on its relative humidity, to a considerable extent is connected with the structure of virus particle. Thus, the nucleoproteins of myxo- and paramyxoviruses (influenza, parainfluenza, Newcastles's disease virus, respiratory-syncytial virus) are surrounded by a secondary shell which contains proteins, lipides, carbohydrates, and other cell components. Apparently, this shell safeguards viruses from the disastrous action of low relative humidity. On the contrary, adeno- and enteroviruses do not have a similar shell, as a consequence of which, with low humidity, they rapidly are inactivated. Certain confirmation of this hypothesis are the investigations of Benbough [4]: on the model of the purified Semliki virus the author has shown that the removal of protein and salts does not affect the stability of virus with low relative humidity, but accelerates its inactivation with high, while carbohydrates, especially inosite, raise the stability of virus with low humidity and do not affect infectious activity with high.

Thus, the results of the investigations conducted affirm, that the prolonged conservation infectious activity by the viruses of in air have a determined epidemiological significance and should be considered in conducting various hygienic and disinfecting measures.

Conclusions.

1. In closed living and community rooms, many viruses can long retain infectious activity in air. The more stable under these conditions are the adenoviruses, the enteroviruses and the Newcastle's disease virus, but the parainfluenza viruses and respiratory-syncytial virus are inactivated sufficiently rapidly. The relative humidity has a significant effect on the degree of virus inactivation in the air.

2. Adenoviruses and enteroviruses are inactivated in the course of 1 hour at low relative humidity and long (for 7-24 hours) retain infectious activity with high and average atmospheric humidity; the parainfluenza viruses, respiratory-syncytial virus and the virus of Newcastles's disease are inactivated most rapidly with high and average relative humidity and are more stable with low.

3. The degree of inactivation of different viruses in the aerosol drop phase under the effect of relative humidity is connected, apparently, with the structure of virus particles.

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Stability of a number of viruses was studied under experimental conditions. Adenoviruses, enteroviruses and virus of Newcastle disease proved to be the most stable under conditions of room temperature and relative humidity, under such conditions parainfluenza and respiratory-syncitial viruses were inactivated much more rapidly. A significant effect on the extent of viral inactivation in aerosol condition was produced by relative air humidity. At low relative air humidity adenoviruses and enteroviruses were inactivated in the course of one to two hours, whereas at high and moderate humidity it was possible to detect them in the chamber for 7 to 24 hours. Parainfluenza, respiratory-syncitial and Newcastle disease viruses were rapidly inactivated at high and medium relative air humidity, and—to a lesser extent--at low humidity.

The character of inactivation of various viruses in droplet aerosol phase under the effect of relative humidity was apparently associated with the structure of viral particles.