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DETERMINATION OF THE PHYSICAL PARAMETERS OF VIRAL AEROSOLS

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DETERMINATION OF THE PHYSICAL PARAMETERS OF VIRAL AEROSOLS

Report I. Application of the Method of Continuous Ultramicroscopy to Elaborate Conditions for Using the Aerosol Chamber.

[Following is the translation of an article by A. I. Gromyko, I. Ya. Vlasenko and I. I. Terskikh, Institute of Virology imeni Ivanovskogo, AMN USSR and the Institute of Physical Chemistry, AN USSR, Moscow, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), No 6, 1966, pages 83-88. It was submitted on 21 May 1965. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

The dynamics and operational conditions of aerosol chambers, intended for the creation of infectious aerosols, have been studied by a number of investigators (Bolotovskiy, 1959; 1961; Gromyko, 1963; Danilov et al., 1964; Terskikh et al., 1958, 1961; Rosebury, 1947). Several premises have been developed for the reproduction of experimental aerosol infections. However, theoretical calculations, concerning the determination and dosimetry of virus-containing aerosols, have not been verified by visual measuring methods, therefore the problem dealing with the most precise and maximum controlled dosimetry of the various causative agents and vaccine antigens, introduced into the organism of an animal in the form of aerosols, remains open. In the present report we will present data, obtained with the help of a VDK continuous ultramicroscope, developed at the Institute of Physical Chemistry, AN USSR, and intended for the investigation of aerosol systems (Burshteyn, 1954; Deryagin and Vlasenko, 1948, 1949, 1951, 1953, 1955, 1959; Spurnyy et al., 1964).

Several types of ultramicroscopes exist for the determination of a countable concentration. One group is intended for determining the countable concentration in optically isolated volumes of an interrupted current of air. These are the ultramicron recorder of Vigdorchik (1939), the Belkin and Kosenko recorder (1951) and others. With the help of these devices it is possible to determine the number of aerosol particles in a unit of volume and the particle size of the aerosol based on the rate of particle settling in an isolated space.

The literature cites a number of essential advantages for the continuous ultramicroscope in comparison with the computation of particles in an isolated volume of air (Deryagin and Vlasenko, 1949). It also cites the advantages of the continuous ultramicroscope in comparison with other recording devices, created for the study of air contamination with solid and liquid aerosols (Deryagin and Vlasenko, 1951, 1953; Khukhrina and Vorontsova, 1955).

In the present report we will examine methodical problems, connected with the use of the continuous ultramicroscope, data on the time required to achieve the maximum equilibrium concentration of the virus-containing material in the chamber, and the time needed for the removal of the aerosol from the chamber after completion of the experiment. In report II we will present the results of studying the condition of the aerosol cloud and the importance of the shifts observed for determining the doses of infection with the aerosols.

The aerosols were created from 10-fold dilutions of a suspension of mouse lungs containing influenza virus (type A, Pr-8 strain) or ornithosis virus (Lori psittacosis strain). The suspensions of organs were prepared and cultivated on Martien broth and were inactivated by heating prior to investigation in the continuous ultramicroscope.

The virus-containing aerosol was created in the IVK-2 aerosol chamber (Terskikh et al., 1961) by the mechanical spraying of the stated materials. For spraying we used a metal sprayer (figure 1), prepared in the ornithosis laboratory, consisting of a metal jet installed in a glass globe. By means of changing the diameter of the discharge opening of the jet, through which the material being sprayed is fed, a change is made in the percentage composition of the polydisperse aerosol in such a manner that it primarily represents a finely dispersed aerosol, 80% of the particles of which have a diameter of 2--3 microns. It is known that it is mainly aerosol particles no greater than 3 microns in size which penetrate into the deep sections of the lungs (Burnshteyn, 1954; Shoshkes et al., 1950).

The degree of dispersion in the vent of the sprayer was determined microphotometrically, by applying the aerosol on a base with liquid petrolatum.

The VDK continuous ultramicroscope (figure 2) makes it possible to calculate the "outbursts", caused by particles, which in a specific period of time cross an illuminated zone in the direction of the path of vision of the microscope (Deryagin and Vlasenko, 1951 and 1953). The virus-containing aerosol is drawn from the air of the IVK-2 chamber into a cuvette in the devise at various times after completion of spraying. The rate of flow is regulated in such a way that in a minute no more than 50--100 "outbreaks" take place. After the recording of a specific number of particles (N = 50, 100) the calculation ceases and the volume of air (ω) passing during the time the particles were recorded is measured. The conimetric (calculated) concentration of substance (n) is calculated by the formula:

$$n = \frac{a \cdot N}{\omega},$$

where N - the number of calculated "outbreaks"; ω - the total volume of aerosol passing through the cuvette; a - the constant for the device at the given diaphragm opening.

The dispersed composition of the aerosol was determined by the sedimentation method (alternating with the conimetric calculation). For this a portion of the aerosol is drawn from the chamber into the cuvette. All the valves in the chamber are set in such a position that there is no channel in the cuvette and the time for the settling of the particles is measured. Knowing the path of settling H (equal to the diameter of the field of vision) and the time for the settling of particles t, the rate v was found. The radius of the particles was calculated by the Stokes --Cunningham formula:

$$r = \frac{-a\lambda + \sqrt{a^2 + \lambda^2 + 18\frac{\eta}{\gamma g}\cdot v}}{2}$$

where r - the radius of the particles; a - the constant; λ - length of the free range of gas molecules; γ - specific gravity of the substance of aerosol particles; g - speeding up of the force of gravity, equal to 980 cm/sec^2; η - viscosity of the medium (air $1.882 \cdot 10^{-4}$ g/cm · sec.).

Since the values a and λ were insignificant they were discarded, and the formula acquired the following form:

$$r = \sqrt{\frac{9}{2}\cdot\frac{\eta}{\gamma g}\cdot\frac{H}{t}}\ .$$

The specific gravity of the broth on which the virus suspension was prepared equalled 1 g/cm^3, and the diameter of the field of vision, corresponding to the diaphragm opening, was 1.3 mm. Placing these parameters into the formula we obtained:

$$r = \sqrt{\frac{9}{2}\cdot\frac{0.0001882\ g/cm\cdot sec\cdot 0.13\ cm}{980\ cm/sec^2\cdot 1\ g/cm^3\cdot t\ sec.}}\ .$$

In order to ensure the possibility of a rapid determination of particle dispersion under various conditions of the aerosol cloud, we reduce the last formula to the simplified expression:

$$r = 3.34\cdot 10^{-4}\,cm\sqrt{\frac{1}{t}}\ .$$

By substituting only the value of t, it is possible to compile a general table of values for the radius of particles, corresponding to the time for their settling (table 1).

By measuring only the time for the settling of the aerosol particles we obtained data on the dispersed composition of the aerosol in the chamber.

The design of the device made it possible to determine the dispersed composition of an aerosol by the most rapid method. With a lessening of illumination the eye is capable of registering only particles with a radius greater than the determined magnitude. By conducting the calculation of the particles at various positions of the photometric wedge, changing the intensity of the light pencil, it is possible to obtain data on the number of particles of different size. For this it is necessary that there be a preliminary graduation of the wedge according to the particles of the stated aerosol.

Data concerning the time for the achievement of the maximum equilibrium concentration in the IVK-2 aerosol chamber (Gromyko, 1963; Danilov et al., 1964; Terskikh, 1963; Terskikh and Gromyko, 1963) were obtained with the help of a continuous ultramicroscope (table 2). For a comparison we determined the time for the achievement of an equilibrium maximum concentration, stemming from the theoretical calculations proposed for operation in aerosol chambers (Bolotovskiy, 1961),

The volume (V) of the IVK-2 aerosol chamber was 220 liters. The virus-containing suspension was sprayed with air, entering the chamber at a rate of 38 liters a minute (L). We determined the time for the achievement of an equilibrium maximum concentration according to an equation which is relative to a case when the time of life of the aerosol considerably exceeds the time of "scavenging" of the chamber (by "scavenging" we mean a single exchange of air in the aerosol chamber). The main mass of particles coming out of the sprayer had a diameter of 2--3 microns, and the time of life of such particles considerably exceeded the time for the "scavenging" of the chamber. In connection with this we used the formula

$$t = \frac{2.3 \cdot V}{L} = 13.3 \text{ min.}$$

(Bolotovskiy, 1959, 1961), where t - time for the achievement of an equilibrium concentration of aerosol in the chamber.

Theoretically at this time the equilibrium concentration of the aerosol sets in. It makes up 90% of the maximum concentration; after 26.6 minutes the concentration will equal 99%, and after 39.9 minutes -- 99.9% of maximum.

By using the method of continuous ultramicroscopy for checking the time for the maximum saturation of the chamber with the aerosol it was established that an equilibrium concentration was created already in 10 minutes and it did not increase with further spraying. In practice the scattering over a period of 14 minutes matched up with the time for the achievement of the maximum equilibrium concentration. The spraying of a suspension of

ornithosis virus, adapted to the lungs of mice, over the period necessary to achieve the maximum equilibrium concentration made it possible to create an aerosol in which a 60 minute exposure led to the death of mice weighing 7--8 grams in 5--6 days.

With the help of the continuous ultramicroscope we visually determined the time for the removal of the virus containing aerosol from the chamber (table 3).

It was established that with a threefold renewing of the air in the chamber it was still possible to determine a quantity of aerosol particles which considerably exceeded the ordinary level of room air (background), while in previous works (Bolotovskiy, 1959, 1961) it was pointed out that a threefold exchange of air in the chamber was sufficient for the complete removal of the infectious aerosol from the air of the chamber. Only after a fivefold exchange were we not able to expose an increased number of particles. In order to be satisfied of the complete removal of the infectious aerosol, it was necessary to carry out the "scavenging" of the chamber 2--3 times longer than it was established experimentally. The probelms of disinfecting the withdrawn aerosol and the IVK-2 chamber were described earlier (Terskikh et al., 1961).

The experimental data presented demonstrate the expediency of using continuous ultramicroscopy for the purpose of studying biological aerosols. In particular they made it possible to develop a series of conditions for the use of the IVK-2 aerosol chamber.

Conclusions

1. For the study of the physical properties of infectious and vaccine aerosols it is possible to use the continuous ultramicroscope, based on the principle of calculating particles in an uninterrupted stream of air. It was demonstrated that it was feasible to use it for determining the concentration and particle size composition of a biological aerosol.

2. It was established that using the continuous ultramicroscope it is possible with sufficient accuracy to determine the time for the maximum saturation of the chamber with the aerosol. Corrections were introduced into the calculations made earlier.

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GRAPHIC NOT REPRODUCIBLE

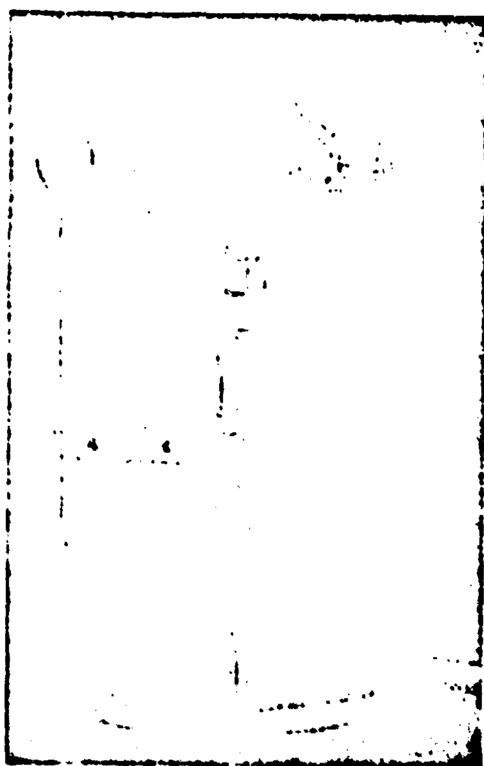

Figure 1. Metal sprayer.

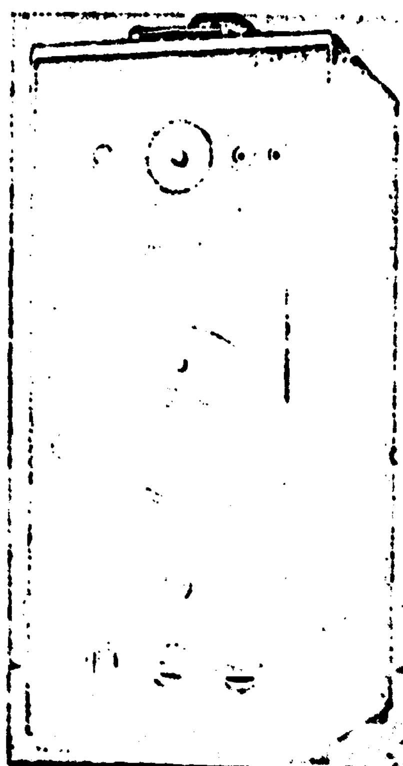

Figure 2. VDK continuous ultramicroscope.

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Table 1

Dependency between the rate of settling and the radius of the aerosol particles.

Time for settling (in seconds)	Radius of particles (in microns)	Time for settling (in seconds)	Radius of particles (in microns)
1	3.34	25	0.67
5	1.49	30	0.61
8	1.19	35	0.57
10	1.06	40	0.53
12	0.96	45	0.49
15	0.86	50	0.47
17	0.81	55	0.46
20	0.75	60	0.43
22	0.71		

Table 2

Dependency of the degree of chamber saturation with aerosol particles on the dispersion time.

Dispersion time (in minutes)	Number of aerosol particles (in 1 x 10* cm^3)				
	n_1	n_2	n_3	n_4	$n_{aver.}$
5	1.6	1.3	1.3	-	1.4
8	3.8	2.8	3.8	4.1	3.6
10	7.7	8.2	7.2	-	7.7
15	6.7	6.2	6.5	7.8	6.7
20	6.9	6.2	6.5	7	6.6
25	8.2	7.2	8.2	6.2	7.4

* Illegible (appears to be 5)

Table 3

Degree of removal of the aerosol from the chamber depending on the frequency of "scavenging".

Frequency of "scavenging"	Concentration of particles (1×10^5 cm^3)
Background (room air)	0.03
Number of particles before "scavenging"	7.75
Single scavenging	0.30
Triple "	0.15
Fivefold "	0.03
Tenfold "	0.03