AD 674493

TRANSLATION NO. 1092

DATE: July 68

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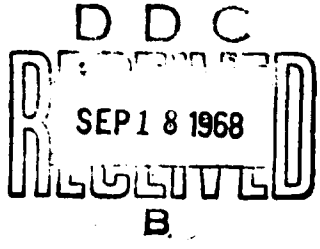

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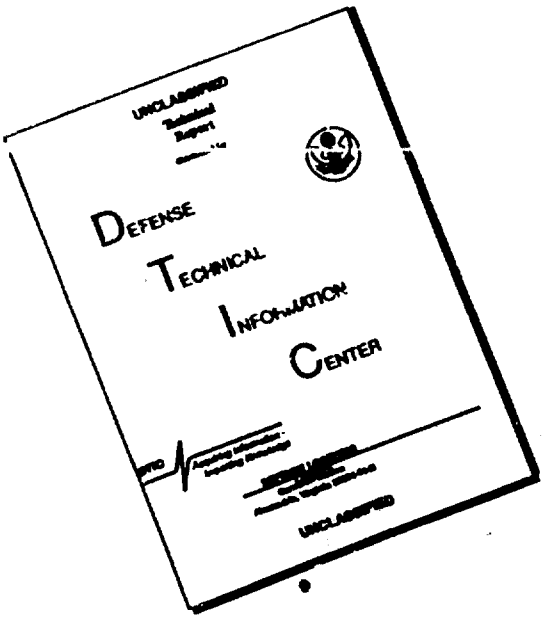

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Trans. V-1656
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Vlodavets, V. V.

Eksperimental'naya model' pylevoi fazy bakterial'nogo aerozolya

[Experimental model of a bacterial aerosol in the dust phase]

Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii 31(10):56-62. Oct. 1960.
448.3 Z4

(In Russian)

Since the close of the last century a considerable number of experimental works have been carried out in which artificial bacterial aerosols in the drop phase were studied, yet there are available only isolated reports on experiments conducted with the dust phase of bacterial aerosols. Moreover, methodology needed for setting up standard serial experiments with bacterial dust have not yet been developed at all.

Shtiben suggested inoculating ground chalk with the liquid culture of the tubercle bacillus. Absorbent cotton was saturated with this suspension, then dried, put in a bag and beaten out in a small chamber in which susceptible animals were present. For the purpose of obtaining artificially infected dust, Berov, Kouzov and Semich inoculated cement dust with a bacterial suspension in

Tsentral'nyi Institut Usovershenstvovaniya Vrachei [Central Institute for Advanced Training of Physicians]

Received at Editor's Office, Oct. 4, 1958.

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distilled water ([Bacillus subtilis [sennaya palochka - hay bacillus], pigmentaryy cocci) and with the spores of mold fungi, but Williams and Lidwell infected talcum with the spores of Bacillus subtilis. Experiments with bacterial dust infected by Staphylococcus were conducted by Spivak (citing Rechmenskii)], but in this work methodology applicable to the preparation of bacterial dust is not cited. Works of the last century include thee famous experiments conducted by [George] Cornet by means of beating rugs saturated with the sputum of tuberculosis patients in a room in which guinea pigs were kept.

In a recently published work by Vershigor it is pointed out that the dust phase of aerosol was prepared by means of infecting a lycopodium of talcum or dust of soil with a staphylococcal suspension. It seems to us that the use of a lycopodium the particles of which have similar dimensions is the more interesting [method].

The present work is devoted to the creation of a stable model of the dust phase of a bacterial aerosol in a static aerosol chamber for the purpose of conducting various investigations in the realm of air-borne infections.

Felted blankets from a boarding-house that had been in use for a long time served as a source of dust. Dust was extracted from the blankets with the aid of a vacuum cleaner. It contained a large quantity of lumps, large particles of hairs and fibers. Aiming to obtain finer and more uniform dust, it was sifted twice

through the standard sieve no. 100. [Begin p. 57] According to data obtained by burning the dust in a muffle furnace, 46.1 - 47.4% of the weight comprised inorganic and 52.6 - 53.9% organic substances. The dust obtained from the blankets contained also a considerable quantity of microorganisms - the white and citrus-yellow staphylococci [Staphylococcus albus, St. citreus, St. aureus], yellow sarchina [Sarcina lutea], gram-positive spore-forming bacilli, actinomycetes [and] mold fungi. In addition, there was a preponderance of coccal flora in all samples.

Moist sterilization of dust in an autoclave had to be rejected because it led to the formation of moist and solid lumps that were hard to pulverize. Apart from this, after a moist sterilization the dispersibility of the dust was considerably decreased. To effect sterilization the dust was spread in a thin layer on the bottom of a Petri dish and exposed to the action of a 150 - 160° [C] temperature for 2 hours. The dust acquired a brown shade that was darker as a result of the partial carbonization of the organic substances the quantity of which decreased in the different portions of dust by 0.76 - 1.82%. In sterilizing with dry heat there formed tiny, loose lumps of dust that scattered when ground lightly in a mortar. Sterilization at 160 - 170° [C] resulted in considerable carbonization and, therefore, it cannot be recommended.

We inoculated the dust with a culture of the white staphylococcus which was isolated from the air of a dwelling (strain

mm. 284). To infect 0.8-1 gm of dust we used the washings of a 24-hour staphylococcal culture [taken] from 5-6 test tubes of slanted agar, i.e. only 10-12 ml of the bacterial suspension. The density of the bacterial suspension that was determined according to bacterial standards equalled 40 to 50 billion bacterial cells per milliliter (the density of the bacterial suspension was determined by the dilution method).

The obtained staphylococcal suspension was poured in a Petri dish at the bottom of which was dispersed a thin layer of a batch [znaveska] of dust (0.8-1 gm) that had been sterilized and pulverized in a mortar. The dust and the bacterial suspension were mixed thoroughly with a glass rod, afterwhich the open [Petri] dish was placed in an incubator at 37° [C] for 18-20 hours. During this time-period the liquid evaporated, but the bacterial dust remained at the bottom of the dish in the form of a thick layer. The dry dust was removed from the surface of the dish with the aid of a scalpel. The dust obtained usually contained small and average-size lumps of a fairly loose structure. Hence the whole portion of the dust was subjected to slight crushing in a mortar.

It must be noted that in removing the dust from the Petri dish with a scalpel as well as in crushing it in a mortar, its particles entered the air in considerable quantities and, for this reason, these operations should be carried out in covered boxes on tables [or benches].

Since dust is very hygroscopic it was stored in an exsiccator on a layer of dried calcium chloride. In different experiments the moisture of dust that was dried in an incubator comprised 5.4 - 7.2%, yet after 4-5 days of storage in an exsiccator it was reduced to 2.3 - 2.7%.

For the purpose of creating a dust phase of bacterial aerosol, dust inoculated with staphylococcus was dispersed in a static aerosol chamber of a 250 liter capacity. A pulverflator the outlet tube of which was introduced into the chamber through an opening in a rubber plug was used for dust dispersion. The bacterial dust was weighed on analytical scales in the glass beaker of the pulverflator. Dispersion was carried out with the aid of an air current forced out by an air compressor, or with the aid of a rubber bulb. [rezinovaya grusha].

As a result of the dispersion of the batch of bacterial dust in the chamber, there was created a polydispersion bacterial aerosol in the dust phase; the size of the aerosol particles fluctuated within a wide range - from 1 up to 100 and even 250 mu, [Begin p. 56] with the fundamental mass being comprised of particles the size of 3 to 40 mu.

In conducting experiments with the dust phase of bacterial aerosol in an experimental chamber, two samples of air (taken by the sedimentation method) in dishes with meat-peptone agar were used in each experiment. Samples were extracted 10 and 20 minutes after the dispersion and each dish was kept [in the chamber] 10

minutes at a time. No air samples were taken during the first 10 minutes following the dispersion, because the largest fractions of bacterial dust settle during that time, and the intermixing of air in the chamber also takes place.

The sedimentation method used in aerosol chambers of the static type produces convincing comparative results, yet at the same time does not violate the dynamics of bacterial aerosol nor the aerial regime of the chamber, which does occur when aspiration methods are used to investigate bacterial aerosols.

Within the first 20-30 minutes after the dispersion there settled a considerable number of bacterial dust particles primarily on account of the macro-dispersion fraction of aerosol [grubodispersnoi fraktsii aerozolya]. After this the concentration of bacterial aerosol decreased considerably and an ever decreasing quantity of bacterial dust particles settled in the Petri dishes.

Every time an experiment conducted with bacterial dust was finished, the air in the chamber was disinfected with the aid of the bactericidal ultra-violet lamp BUV-15. In table 1 is shown the influence exerted by the duration of the action of ultra-violet rays upon the white staphylococcus during the dust phase of a bacterial aerosol. Although a staphylococcus in the dust phase is more resistant to the action of ultra-violet rays than in the drop phase of aerosol (Rechmenskii), a 3-4 minute exposure insured reliable disinfection of the air in the chamber.

During manipulations within the chamber (uncovering and

covering of the dishes), particles of settled bacterial dust rose from the bottom of the chamber leading to a second infiltration of the air. In order to prevent reaus;ension and to fix settled bacterial particles, a thin layer of vaseline or vaseline oil was applied to the bottom of the chamber. After conducting several experiments, the bottom of the chamber was contaminated by settled dust. The chamber was cleaned with the aid of a cotton tampon soaked in alcohol.

The dose of bacterial dust required for standard experiments was determined by weight. For this purpose several batches (of 5, 10, 20, 30 and 50 mg) were weighed out on analytic scales and then dispersed in the chamber. Subsequently only two batches of dust were taken - 20 and 30 mg [Begin p. 59] which were more suitable for experimental purposes. When 5 or 10 mg were used a considerable portion of the dust was retained in various parts of the pulverizator, and dispersion of 50 mg clouded up the experimental chamber too much.

Table 1

Influence of various exposures of the dust phase of bacterial aerosol to ultra-violet rays

Exposure to ultra-violet lamp BUV-15	10 minute exposure begun 10 minutes after dispersion		10 minute exposure begun 20 minutes after dispersion		Humidity (in %)	Temperature
	Number of colonies	% of survival	Number of colonies	% of survival		
Control dispersion....	1 520	100	912	100	64	20.5°
15 seconds.....	324	21.3	154	16,8	63	21°
30 "	126	8.2	114	12.5	63	21°
5 "	57	3.7	49	5.3	65	21°
1 minute.....	26	1.7	25	2.7	66	21.5°
4½ minutes.....	16	1	10	1	65	21.5°
1 [7] "	11	0.7	7	0.7	65	21.5°
[7] "	4	0.2	5	0.5	66	21.5°

In conducting serial experiments it is necessary to take a dose of bacterial dust that would insure the growth of 1500 to 3000 colonies of the white staphylococcus in a Petri dish containing meat-peptone agar. When the number of colonies was larger, calculation became more difficult, even though in air infection a more uniform distribution of bacteria has been noted on the surface of the nutrient medium. This permitted Shtern to recommend th: indicated method for the purpose of uniform inoculation of [Petri] dishes.

In order to establish the recurrence of the results, a series of experiments was conducted in which, after brief intervals of time, batches of the same portion of bacterial dust and of the same size were dispersed in the chamber within one working day.

Three series of experiments were conducted, with each series consisting of 5 individual experiments. Air samples were extracted by the method of sedimentation immediately after the dispersion, after 10 minutes and after 20 minutes. The dishes of each sample were exposed for 10 minutes. We used the average number of colonies as 100% and determined deviations from it. As a result of the experiments conducted, it was established that the greatest fluctuations in the number of colonies in one experimental series did not exceed 15-20% and the average deviation comprised 4.5%. Thus, fluctuations in the number of caught bacterial particles were somewhat higher than in the drop phase of bacterial aerosol in which they did not exceed 10-13%.

These fluctuations depended on the following factors:

1) the degree of staphylococcal inoculation of dust which in turn depended on the density of the bacterial suspension, the amount of dust and its mixing with the bacterial suspension;

2) on the homogeneity of the dust, on the presence of a large or small number of lumps and on the macro-dispersion particles in the batch;

3) on the similarity of dispersion conditions;

4) on strict observance of the time when Petri dishes are put in the chamber and when they are removed.

The above listed factors are of great importance in obtaining identical results when bacterial dust is dispersed from the same portion.

The bacterial dust prepared by the method described can be used in experiments only for a limited time, because of the rapid dying off of staphylococcus in artificially inoculated dust. In the first 2-3 days the process of dying off usually was insignificant, but in the days that followed, as can be seen in table 2, bacteria were dying off in considerable numbers.

In most cases bacterial dust was fit for experiments for 5-6 days and only rarely for 7-10 days.

Table 2

Influence of storage duration upon the survival of staphylococcus in bacterial dust

Duration of storage (in days)	10 minute exposure begun 20 minutes after dispersion	10 minute exposure begun 20 minutes after dispersion
1	2 048	1 138
3	1 868	1 012
4	1 526	940
5	1 200	796
6	964	508
8	620	368
9	552	300

For the purpose of obtaining convenient samples of bacterial dust that is suitable for serial experiments [and] could be used for an extended time, we performed lyophilization of it. In so doing, we proceeded from the position that drying of a bacterial culture [Begin p. 60] at low air pressure after it had been rapidly frozen causes practically no disorder in its biological properties and colloidal structure (Rechmenskii, Suvorova and

others).

After the bacterial dust that had been prepared by the method described above was dried in an incubator and ground in a mortar, it was poured in sterilized ampules in 30 mg quantities and exposed to lyophilic drying. Then a portion of the ampules with the bacterial dust was stored at room temperature (15-20° [C]), another portion was kept at 37° and a third one at 2-4°. Bacterial dust that was not subjected to lyophilization and was stored in an exsiccator at room temperature was used for the purpose of control. Observations were conducted for a period of 35 days from the day the lyophilic dust was prepared. In table 3 are cited the results of experiments on the preservation of the viability of the white staphylococcus in lyophilic dust under conditions of different storage temperatures. Every figure in table 3 is an average numerical indicator of the results of three individual experiments.

Table 3

Influence of lyophilic drying and of maintenance under different conditions upon the viability of staphylococcus in bacterial dust

Duration of storage (in days [24 hrs.])	Conditions of storage	10 minute exposure begun 10 minutes after dispersion	10 minute exposure begun 20 minutes after dispersion
1	In ampules.....	1 800	944
	Control.....	2 140	1 156
5	Room temperature	1 608	806
	36-37° [C].....	1 329	626
	2-4°.....	1 472	772
	Control.....	1 102	526
10	Room temperature	1 430	611
	36-37°.....	1 265	567
	2-4°.....	1 430	644
	Control.....	708	369
20	Room temperature	1 054	620
	36-37°.....	1 014	534
	2-4°.....	1 269	656
	Control.....	504	342
35	Room temperature	546	211
	36-37°.....	402	170
	2-4°.....	1 062	661
	Control.....	391	131

Before the experiment was set up the ampule was broken and its contents - bacterial dust - were poured into the beaker of a pulverizator. It must be taken into consideration that in breaking the ampule and in pouring out the dust invasion of the air by microorganisms is possible. The danger of an air invasion by pathogenic bacteria and viruses while work is conducted with lyophilic cultures has been pointed out also by Reitman and his fellow workers.

One day [24 hrs] after lyophilization the number of staphy-

lococcal colonies grown in dishes was smaller when lyophilic dust was dispersed than in the dispersion of dust serving as control. This, obviously, was due to retention of a portion of the dust in the beaker when the batch was taken and when it was poured into the ampules, as well as on the walls of the ampule itself.

After 5 days of storage a sharp decrease in the number of staphylococcal colonies was noted during the dispersion of the control portion of the dust and some decrease in the number of colonies when lyophilic dust was dispersed, besides, this process was more pronounced in the experiment conducted with the portion of dust stored at 37° [C]. The process of dying off of staphylococci increased slowly by the 10th day.

In the interval between the 10th and 20th days almost no dying off of staphylococci was observed in the portions of lyophilic dust. Only in the control portion stored in an exsiccator at room temperature was observed a considerable decrease in the number of colonies.

On the 20th day the process of staphylococcal dying was the least pronounced in the samples of lyophilic dust stored at 2-4° [C], mortality was somewhat higher in the portions of dust kept at room temperature, and a more drastic process of dying was observed at 37°. [Begin p. 61] A considerable decrease in the number of viable bacteria was noted also in control samples.

By the 35th day of storage further, considerable dying of staphylococci occurred in lyophilic dust stored at 37°, and, in

a lesser degree, this process manifested itself [also] at room temperature. Staphylococci were fairly well preserved at 2-4° [C].

In evaluating the results obtained, one can arrive at the conclusion that bacterial dust dried by the lyophilization method and stored at 2-4° can be utilized in setting up experiments for a period of 30-35 days. Lyophilic dust kept at 15-20° and at 37° can be used in experiments for over 20 days. Bacterial dust dried in an incubator and not subjected to lyophilic drying is suitable for experiments only 5-6 days and in rare cases 7-9 days.

The use of dust inoculated with staphylococcus and dried by the lyophilization method in setting up experiments with bacterial aerosol in the dust phase simplifies the work considerably and offers the possibility of conducting experiments with the same portion of bacterial dust for a long time.

Conclusions

1. Methodology for setting up experiments with bacterial aerosol in the dust phase has been developed. For this purpose sterilized dust was infected with a dense suspension of the white staphylococcus in a physiological solution and [then] dried. In dispersing 20 or 30 mg of bacterial dust in a chamber of a 250 liter capacity a fairly stable aerosol was obtained, its presence could still be detected 6-8 hours later.

2. A shortcoming of the method is the rapid dying off of the white staphylococcus in artificially infected bacterial dust,

as a result of which it is no longer fit for use in experiments at the expiration of 5-7 days or, more rarely, after 7-9 days.

3. When bacterial dust is dried by the lyophilization method, it can be used for experimental purposes for an extended time. In storing lyophilic dust at 2-4° ~~(c)~~ it can remain fit for serial experiments over 35 days, and when maintained at room temperature or at 37° - over 20 days.

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