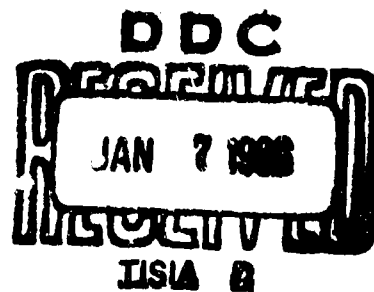
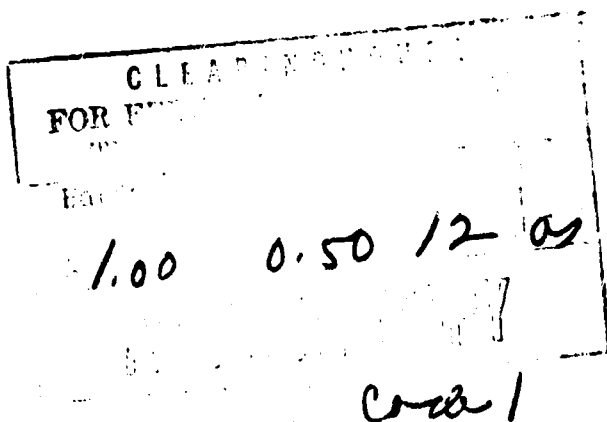


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DYNAMICS OF A BACTERIAL AEROSOL IN THE DUST AND DROP PHASE

[Following is the translation of an article by V. V. Vlodavets, Institute of General and Communal Hygiene imeni A. N. Sysina, USSR Academy of Medical Sciences, appearing in the Russian-language periodical Mikrobiologiya (Microbiology), No. 1, Vol XXXIII, 1964. Translation performed by Sp/7 Charles T. Ostertag Jr.]

The distribution of microorganisms under the conditions of an air medium is strongly tied in with the colloidal properties of the particles and the drops of the bacterial aerosol.

The length of occurrence of microorganisms in the air, diffusion, electrical charge, and their mixing by air currents are based completely on the laws of colloidal chemistry. Along with the physico-chemical processes peculiar to aerosols, in bacterial aerosols the death of the microorganisms is constantly taking place also. Therefore the concentration of viable bacteria in an aerosol changes both due to the settling of microorganisms (physical loss) and to their necrosis (biological loss).

The present article is a study of the dynamics of aerosols of Staphylococcus albus in the drop and dust phases which were artificially created in a hermetically sealed chamber.

Methods

Strain 284 of Staphylococcus albus, isolated from the air of inhabited premises, was used as the experimental model. The bacterial aerosol was created in a 250 liter experimental chamber of the static type. For obtaining the drop phase of the aerosol in the chamber, they dispersed a suspension, containing 200 million bacteria in 1 ml (according to the bacterial standard of turbidity), of staphylococci in a physiological solution. With the help of a Barkovskiy sprayer, a 0.12 ml bacterial suspension was dispersed. As a result of this a polydispersed aerosol was created and the main mass of the drops were within the limits of 2 to 10 microns.

For obtaining the dust phase of the aerosol, dust was removed from blankets by means of a vacuum cleaner. The dust obtained was screened twice through a No. 100 strainer and sterilized with dry heat at

150-160° for two hours. In a Petri dish, 0.8 - 1.0 g of dust was mixed with a dense suspension of staphylococci (40-50 billion bacterial cells in 1 ml) and dried in an incubator at 37° for 18-20 hours. The dried dust was removed from the surface of the dish and lightly pulverized in a mortar. For creating the aerosol a 20 or 30 mg suspension of dust was sprayed with the help of a powder dispenser.

The size of the dust particles fluctuated from 1 to 100 microns; the main mass was made up of particles from 3 to 40 microns. By burning in a muffle furnace it was established that the dust contained 46-47% inorganic and 52-53% organic substances. A more detailed method of obtaining the dust phase of a bacterial aerosol was described earlier (Vlodavets, 1960).

Changes in the concentration of the bacterial aerosol were determined by the method of bacterial settling on Petri dishes with MPA. It must be noted that even though the method of settling is less accurate than many aspiration methods, it has an advantage for static type aerosol chambers -- it does not disrupt the air regimen of the chamber. Sampling of air probes was performed in 10, 20 and 30 minutes, and 1, 2, 3, 4, 5, 6, 7, and 8 hours following the creation of the bacterial aerosol. Each dish was exposed for 10 minutes. Each test was repeated four times, after which the average indices were derived. The number of colonies of staphylococci growing in the first dish, which was exposed in 10 minutes following the spraying of the bacterial suspension, was conditionally taken as 100%.

In separate tests the initial and final concentrations of the bacterial aerosol were controlled with the help of aspiration devices: Rechmenskiy bacteria trap and the Krotov slit device.

Decreasing the air humidity in the chamber was achieved by introducing dry calcium chloride. For increasing humidity a crystallizing basin with warm water was placed in the chamber for 12-16 hours.

Results

Drop Phase of the Bacterial Aerosol

As a result of spraying a bacterial suspension in the chamber a bacterial aerosol with a quite high concentration was created. With the help of a Rechmenskiy bacteria trap, from 800 to 1,920 viable cells of staphylococci were determined in 1 liter of air. The maximum concentrations of bacteria were observed after creation of the aerosol, then the content of staphylococci in the air gradually decreased. In the opinion of a number of authors (Wells, Wells, 1936; Rechmenskiy, 1951, Klieve, Wasilewski, 1951; Vershigora, 1958, 1960; Yaroshenko, 1957; Webb, 1959, Sinelnikova, 1961) and according to our observations, staphylococci are

stable under conditions of an air medium, therefore a decrease in their concentration in the air is dependent mainly on the settling of bacterial drops. The process of cell necrosis has a relatively small significance.

With the dispersion of a suspension of staphylococci in various liquids (0.5 and 0.85% solutions of NaCl, distilled water, and an 0.85% solution of NaCl with the addition of horse serum) it was noted that the most favorable conditions for the prolonged maintenance of an aerosol were created by using distilled water. After eight hours in the air it was determined that 3.2% of viable bacteria remained from the initial concentration (table 1). Such a lengthy preservation of an aerosol is explained, in all probability, by the rapid evaporation of the external aqueous membrane of the bacterial drops which does not contain salts. As a result of this their size decreases rapidly and correspondingly the rate of settling. This is also due to the lack of a toxic effect of sodium chloride. However, the lowest concentrations of staphylococci in the beginning of the test also pointed to the possibility of the death of part of the bacteria as a result of a disruption of osmotic pressure. The most rapid decrease is in a concentration of an aerosol following dispersion of the suspension in a 0.85% solution of NaCl -- after eight hours only 0.17% of the bacteria from the initial concentration were detected. This is an indication of a certain toxic effect of NaCl on cells of staphylococci found in the condition of an aerosol, since as a result of the evaporation of water the concentration of salt in the bacterial drops increases sharply. An intermediate position in stability is occupied by aerosols of staphylococci obtained following dispersion of the bacterial suspension in an 0.85% solution of NaCl with the addition of serum or in an 0.5% solution of NaCl. These facts, obtained by the method of settling, were confirmed in a number of cases by aspiration methods: With the Rechmenskiy bacteria trap and the Krotov slit device.

When studying the behavior of the drop phase of a bacterial aerosol under various indices of humidity, a bacterial suspension was used which was prepared in an 0.85% solution of NaCl. In these tests, low humidity was understood as a relative humidity below 35%, medium humidity -- from 40 to 60%, and high humidity -- over 70%. The tests were conducted at room temperature which fluctuated from 18 to 22°.

As a result of the tests conducted it was established that a high concentration of Staphylococcus albus was preserved longest of all at a low air humidity (table 2). After eight hours 1.3% of the bacteria from the initial concentration were detected, while at medium and high indices of humidity 0.17 and 0.03% of Staphylococcus albus respectively were determined.

With a low air humidity the concentration of staphylococci was reduced more slowly, which can be explained by the speedier evaporation

of the external aqueous membrane of the bacterial drops, that is, by the rapid decreasing of their sizes and consequently, rate of settling. Thanks to the rapid evaporation of water the bacterial cells were damaged less and there was less of a manifestation of the toxic effect of sodium chloride on the cells of staphylococci. With an increased humidity the rate of evaporation of water was slowed down, thanks to which the aerosol of staphylococci was more rapidly exhausted, probably due to the settling of large and medium size drops.

For clarifying the effect of air humidity on the behavior of a bacterial aerosol, the first time following the spraying three series of tests were conducted at low, medium and high indices of humidity. Each series of tests was conducted in the course of one day when using one and the same bacterial suspension. Three successive samples were taken by the method of settling: Immediately after the conclusion of spraying the suspension of staphylococci, after 10 and after 20 minutes. The average results, obtained in one of the series of tests, are presented in figure 1.

With a high air humidity considerably more bacterial drops settled in the course of the first 10 minutes than with low and medium indices of humidity. In samples taken after 10 minutes, the results obtained were quite close with the various humidities. However, already in the next sample, taken after 20 minutes, the number of viable staphylococci determined at a high humidity was 39% lower than the data obtained at a low humidity, and 37% lower than at a medium humidity. These facts support the assumption that at a high air humidity, already in the first 10 minutes following the formation of the aerosol, a settling takes place of a considerable number of bacterial drops, as a result of which the concentration of staphylococci decreases in comparison with tests at medium and low indices of humidity.

Dust Phase of a Bacterial Aerosol

Following the dispersion of a bacterial dust of staphylococci there was noted a considerably more rapid settling of dust particles than when setting up tests with the drop phase of staphylococci (table 2). This was also confirmed with investigations of the air with the help of a Rechmenskiy bacteria trap. Moreover, individual staphylococci in the dust phase could be found in a suspended state for a more prolonged time interval and were detected after 6-8 hours from the moment of spraying.

At low and medium indices of humidity the rate of settling of particles of bacterial dust was very close. At high indices of humidity a more rapid reduction was observed in the concentration of staphylococci in the air of the chamber, apparently due to the speeding up of the settling of particles of bacterial dust. This is explained by the hygroscopicity of the dust particles which, by absorbing moisture, increased in size and weight, and also by the strengthening of the process of coagulation in the moist air which promotes the adhesion of dust

particles into larger aggregates. However, here the possibility cannot be excluded of a certain influence of humidity on the dying off of staphylococci following arrival in a humid atmosphere.

For studying the influence of various indices of humidity on the behavior of the dust phase of an aerosol, for the first time following spraying, tests were conducted with one and the same batch of bacterial dust with short exposure times (figure 2). The air samples were taken immediately after creation of the aerosol, and after 10 and 20 minutes. In the course of the first 10 minutes a considerable amount of particles of the bacterial dust settled down, mainly coarsely dispersed ones, as a result of which the concentration of staphylococci in the air was sharply reduced.

At a high air humidity, in the first 10 minutes somewhat more particles of the bacterial aerosol settled down than at low and medium humidity. But then in the following sample, taken 10 minutes following spraying, at a high air humidity the number of colonies of staphylococci was sharply lowered (correspondingly by 2.4 and 2.9 times) in comparison with indices from tests at medium and low humidity. In samples taken after 20 minutes, a further lowering was noted in the amount of staphylococci in comparison with the number of them determined at medium and low indices of humidity (correspondingly by 4.5 and 5.3 times). Thus, these tests supported the assumption that at a high air humidity the concentration of staphylococci in the dust phase of an aerosol was sharply decreased already in the first 30 minutes following creation of the aerosol, while the most considerable lowering of the concentration is observed after the first 10 minutes.

If it is assumed that bacteria in the dust phase are not bound with the dust particles but are freely suspended in the air, then the time of their stay in the aerosol state was equal to and maybe even exceeded the time of residence of bacterial drops in the air, since the latter, even after complete evaporation of the external aqueous film, preserved salt and other substances found in the drop on the surface of the bacterial cells. However, experimental data testifies to the fact that particles of bacterial dust settle down considerably more rapidly than bacterial drops. These observations serve as indirect proof of the bond of microorganisms in the dust phase of an aerosol with the dust particles. However, the nature of this bond still has not been thoroughly studied. It is suggested that in a dust phase of a bacterial aerosol the bacterial cells are bound primarily with coarsely dispersed particles of dust. This is testified to by the sharp lowering of the concentration of the aerosol, caused by the settling down, together with the large dust particles, of a considerable part of the bacterial dust in the course of the first 10 minutes after dispersion. However, the possibility

cannot be excluded here that a certain amount of bacteria in the dust phase are not bound with dust particles or lost this bond during dispersion. This can explain such a lengthy stay in the air of individual staphylococci.

Conclusions

1. In a 250 liter experimental chamber, the dynamics were studied of an aerosol of Staphylococcus albus in the drop and dust phase. After dispersion of the bacterial suspension or bacterial dust, the concentration of the aerosol gradually decreased. Viable cells of staphylococci were exposed in the air still after 6-8 hours.
2. Most suitable for the prolonged preservation of an aerosol of staphylococci in the air is dispersion of a bacterial suspension, prepared in distilled water, whereas an 0.85% solution of NaCl least of all promoted the lengthy preservation of staphylococci in the air. An intermediate position is occupied by an 0.5% solution of NaCl, and also an 0.85% solution of NaCl with the addition of horse serum.
3. The dust phase of an aerosol is kinetically less stable than the drop phase, which is apparently connected with the colloidal properties of particles of bacterial dust, their size and hygroscopicity. Therefore the liberation of air from particles of the dust phase proceeds much faster than from the drop phase of an aerosol.
4. A decrease in relative air humidity promotes an increase of the time of occurrence of staphylococci in the drop and dust phases of an aerosol. High humidity assists the settling down of bacterial drops as well as of particles of bacterial dust and the lowering of the concentration of the aerosol.

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Table 1

Influence of the dispersion phase on the duration of detection of staphylococci in an aerosol.

Time test was taken after spraying	0.85% solution NaCl		0.5% solution NaCl		0.85% solution NaCl + horse serum		Distilled water	
	Number of colonies	%	Number of colonies	%	Number of colonies	%	Number of colonies	%
10 min	2721	100	2901	100	2978	100	442	100
20 min	2065	76.3	2426	83.5	2219	74.5	368	87.2
30 min	1689	62.4	1939	66.9	1785	60.3	305	71.9
1 hr	1168	44.3	1364	47.1	1082	36.0	250	59.6
2 hr	536	19.9	715	24.7	492	16.3	188	44.7
3 hr	286	10.2	400	13.8	262	8.8	102	24.0
4 hr	161	5.5	173	6.9	144	4.9	64	14.8
5 hr	61	2.1	111	3.8	77	2.6	53	12.3
6 hr	25	0.91	54	1.8	52	1.7	30	6.9
7 hr	10	0.38	33	1.1	23	0.8	19	4.4
8 hr	5	0.17	21	0.74	17	0.6	13	3.2

Behavior of bacterial aerosols.

Time the test was taken after spraying	In the drop phase w/ air humidity				In the dust phase w/ air humidity							
	Low 23---32%		Medium 46---56%		High 79---91%		Low 23---31%		Medium 51---56%		High 82---93%	
	No	%	No	%	No	%	No	%	No	%	No	%
10 min	2336	100	2721	100	2607	100	2993	100	5180	100	1665	100
20 min	2032	88.9	2055	76.3	2092	80.0	1664	55.6	2957	57.1	609	37.1
30 min	1780	76.3	1689	62.4	1240	57.2	1116	37.5	2058	39.5	313	19.1
1 hr	1404	60.1	1168	44.3	953	36.0	445	14.5	711	13.5	71	4.3
2 hr	708	30.3	536	19.9	370	13.7	90	2.9	194	3.6	10	0.66
3 hr	548	23.4	286	10.2	55	3.0	36	1.2	75	1.4	4	0.31
4 hr	316	13.5	161	5.5	37	1.4	20	0.64	28	0.54	4	0.31
5 hr	176	7.5	61	2.1	14	0.54	14	0.46	14	0.26	2	0.14
6 hr	109	4.6	25	0.9	7	0.29	7	0.24	9	0.17	2	0.14
7 hr	39	1.6	10	0.36	4	0.17	4	0.13	6	0.11	1	0.08
8 hr	31	1.3	5	0.17	1	0.03	3	0.06	2	0.04	1	0.08

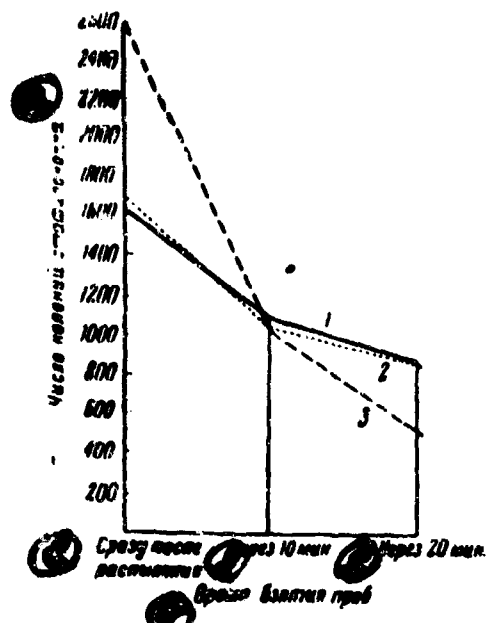


Figure 1. The influence of air humidity on the drop phase of a bacterial aerosol.

1 -- low humidity; 2 -- medium humidity; 3 -- high humidity.
a -- number of colonies of staphylococci; b -- time of taking sample; c -- immediately after spraying; d -- after 10 minutes; e -- after 20 minutes.
This legend applies to figure 2 also.

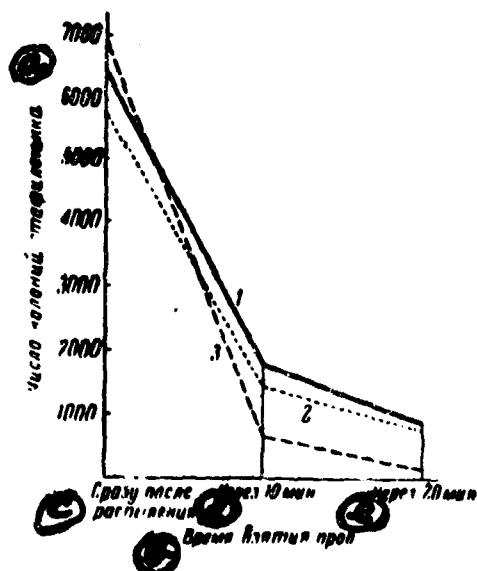


Figure 2. The influence of air humidity on the dust phase of a bacterial aerosol.

Legend is the same as figure 1.