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THE PROBLEM OF DETERMINING THE TOTAL MICROBIAL CONTENT OF AIR

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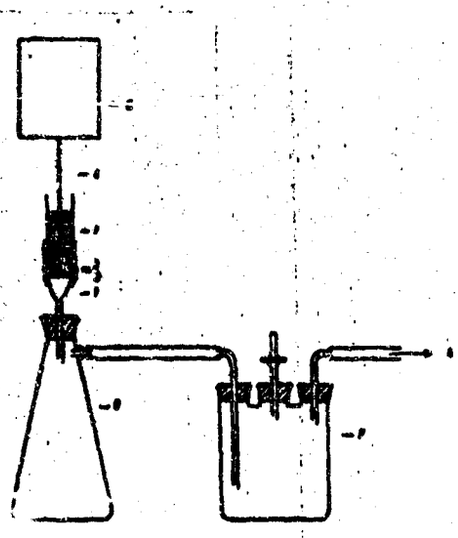
The concentration of suspended solid and liquid particles in the atmosphere can be quite accurately determined with present-day methods. On the contrary, determinations of the microbial content of air produced values that were much too low. This was caused, partly, by the low efficiency of certain sampling devices for aerial microbes, but mostly because these microorganisms were cultured on media.

Cultural methods are selective methods. Yet, the world of microorganisms contains an immense variety of physiological forms. Although a very large number of species is present, only very few are able to grow under standardized conditions. It is known that ultraviolet irradiation inactivates microorganisms, but that visible light "reactivates" them. Many microorganisms, widely distributed in nature, are strictly anaerobic and can only be cultivated under specialized, anaerobic techniques. Other microorganisms, e.g. many parasitic forms, multiply only on spezialized media which are highly selective and which inhibit the growth of other forms, be they aerobic or anaerobic. Consequently, when aerial microbes do not form colonies on media prepared in the laboratory, it does not mean they are dead.

Microorganisms frequently adhere to each other or to dust or aerosol particles. Such an aggregation is difficult to disperse. There may be 20-50 cells present, but they form only a single colony on the medium.

In addition to conventional media, these considerations led to the use of methods which allow the direct enumeration of microorganisms isolated from air. The only problem is the presence of solid, non-biological particles. When aerosol particles are collected on glass slides, the "dirt effect" is so large that only some of the larger mold spores and pollen grains can be counted with accuracy, but none of the numerous bacteria which are also present.

In order to achieve "clean" air samples, a modified Cholodny method (Cholodny 1928), developed in the Microbiological Laboratory of the Institute of Aerobiology, was tested. The method consists of concentrating the microorganisms, suspended in a liquid, by filtration and washing them. The apparatus used is shown in Fig. 1.



1. STEFI 3 (upper and lower part)
2. MF 30
3. MF 15
4. stirrer
5. motor for stirrer
6. suction flask
7. Woulff flask
8. vacuum pump

Fig.1. Apparatus used for concentrating and washing suspensions of Microorganisms

A suspension of aerial microorganisms is prepared by impinging and fixing in formol. It is then added to the upper part of a STEFI 3 apparatus (Sartorius Membranefilter Co.), provided with a MF 30 grid membrane filter and a MF 15 membrane filter beneath. The combination of two membrane filters reduced greatly the rate of flow. In this manner and by stirring constantly only a relatively small portion of suspended cells came in contact

with the surface of the filter and adhered to it. The suspension was then reduced in volume to 2-3 ml. Soluble dirt particles were removed by adding several portions of sterile distilled water. The volume was again reduced. Finally, the suspension of aerial microbes, reduced in volume and washed, is made up to exactly 3 ml. Direct counts are made by covering a glass slide with an adhesive tape in which round holes of 9.6 mm diameter were cut with a cork borer. When the tape is attached to the glass slides the holes must be well centered. With a bloodsugar pipet, 0.01 ml of the microbial suspension is placed into the hole of the tape and evenly distributed. After drying, the tape was removed, the preparation was stained with 0.001% acridine orange solution and examined by fluorescence microscopy. "Dirt particles" appear orange-red when stained with acridine orange and viewed by fluorescence microscopy. If the preparation is heavily contaminated by dirt, the microorganisms appear as green particles. If the preparations are more or less "clean", many cells stain red, but this is a concentration-effect which can be eliminated by washing the stained and dried preparations with distilled water by letting it run under the coverslip. Quantitative counts are made by counting 10 fields in two directions, moving the center of the field by 1 mm each time. We used a Zeiss Standard Universal Microscope with a 40X objective and a 12.5X ocular in our investigations. In order to obtain accurate results it is necessary to use sterilized liquids and carefully cleaned glassware.

In all experiments, numbers of cells observed by this particular method were 10^3 - 10^4 times greater than numbers of colonies obtained by the method of impinging and cultivating suspensions, described by Petras (1966). For example, on one day, six air samples were collected by impinging in a rabbit stall of the Institute of Aerobiology. Cultivation on a medium yielded 29.7 - 42.1 colonies per liter of air. The Chclodny method yielded 38,200 - 52,600 cells per liter of air. In January of 1967, on a certain day, air was sampled outside the institute, despite the unfavorable season of the year. There were present several thousand cells, in one case more than 10,000 cells, per liter of air. Media, invariably, yielded considerably fewer than one colony per liter of air. To exclude the possibility that these amazingly large numbers of microorganisms were produced by contaminated impingers, experiments were performed in which cell-free air, prefiltered through a MF 30 membrane filter, was passed through the impinger solution. In these experiments, not more than 2-3,000 cells per liter of air were obtained and used as a "base line figure".

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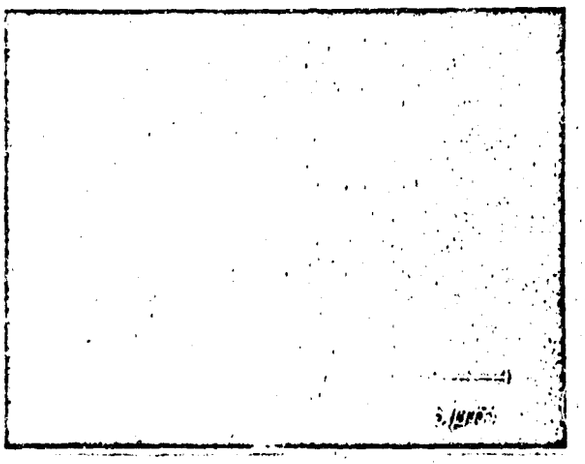

Fig.2. Typical Cholodny preparation of aerial microbes.

Fig.3. Cholodny preparation of spore-forming micro-organisms.

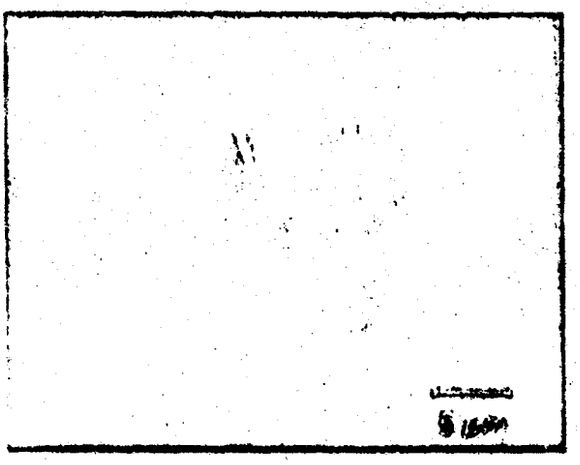

Fig.4. Microorganisms adhering to a dirt particle. Upon staining, bacteria are mainly green, dirt is mainly red and clearly distinguishable from the background.

Jannasch and Jones(1959) arrived at similar conclusions when they tested seawater samples. They counted 9,000 times more cells than colonies per given volume of sample. In the Microbiological Laboratory of the Institute of Aerobiology, experiments were performed in which only clearly recognizable microorganisms were counted. Despite poor color contrast, the picture seen resembled Figures 2 and 3. Starving cells tend to become invisible, cannot be seen in a light microscope and cannot be properly recognized. Also, cells adhering to dirt particles, despite efforts to clean up the preparation, cannot be exactly identified(Fig.4). This means, the true number of microorganisms present is probably somewhat larger than the number counted. The number of truly "dead" cells will have to be determined by other methods.

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