QUANTITATIVE CHARACTERIZATION OF AEROSOLS

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Only a fraction of the extensive investigations of aerobiological techniques over the last decade has appeared in technical journals and adequate reviews of the literature are almost nonexistent. Consequently, an embarrassing array of possible subjects is available for discussion in a generalized paper relating to technique. The present paper will be limited to a brief discussion of the procedures which have become more or less standardized in several rather specialized laboratories for the estimation of the basic parameters of particulate biological aerosols.

Although field studies are essential for the evaluation of the significance of airborne infection as it relates to public health and as a problem in civil defense, research in aerobiology, medical or otherwise, depends primarily upon techniques for generating reproducible aerosols into controlled environments and for observing the subsequent behavior of these aerosols in time.

Cloud Chambers

For the study of static aerosols, i.e., aerosols which are not being continuously generated throughout the course of observation, cloud chambers of various geometries and for various purposes have been developed but the same basic principles of operation apply to all of them. At Fort Detrick we have made extensive use of cylindrical tanks with convex ends (Fig. 1).

The physical decay (or fallout) of a contained static aerosol depends to a large extent upon the area/volume ratio of the chamber, which should be as small as practical. The cylindrical shape provides nearly minimal decay. These tanks may be jacketed for the adjustment and control of temperature, and a drying tower and a source of steam permit similar control of relative humidity. The purpose of the fanning system is to produce a homogeneous distribution of the aerosol within the tank so that a minimal number of sampling points will yield a representative estimate of cloud concentration. The purging system permits rapid removal of the aerosol from the tank at the conclusion of a trial, thus increasing the number of trials which may be performed within a given period of time. Also, this system may be employed to reduce aerosol concentration rapidly to a point where animal exposure will not result in 100% infection. For investigations involving pathogenic organisms, the tanks must be air-tight. As an additional safety factor, they are operated under a pressure negative to that of the surrounding room. A simple dynamic system possessing certain limitations will be described later.

A fairly recent variation in the design of cloud chambers is the toroid or rotating drum described by Goldberg and associates (4). Figure 2 is a photograph of a 500-liter drum being installed within a housing cabinet. The tight cabinet not only provides safety for the operators but also permits the economic control of temperature. After the drum is filled with aerosol generated by an atomizer, it can be isolated from the rest of the system by valves. Rotation of the drum at a low angular velocity (½ to 5 rev/min) greatly reduces physical decay and changes which may occur in the biological properties of the aerosol can be studied over long periods of time. This applies only to small particle aerosols. Such a system, based on a 500-liter drum, has been adopted as a reference standard testing procedure by several laboratories in England, Canada, and the United States. Collaborative testing will be instituted in February of 1961 in six laboratories, each with an identical test system.

Samplers

General. The problem of obtaining a truly representative sample of the aerosol and one which is universally suitable for a variety of possible measurements has never been fully resolved. The number of types of samplers that has been developed is roughly equal to the number of investigators. A nearly ideal sampler for a biological aerosol would accept and retain, regardless of size, 100% of the particles within the air volume sampled, classify these particles into size groups at 1-μ intervals and count them while doing so, and maintain 100% viability so that subsamples of

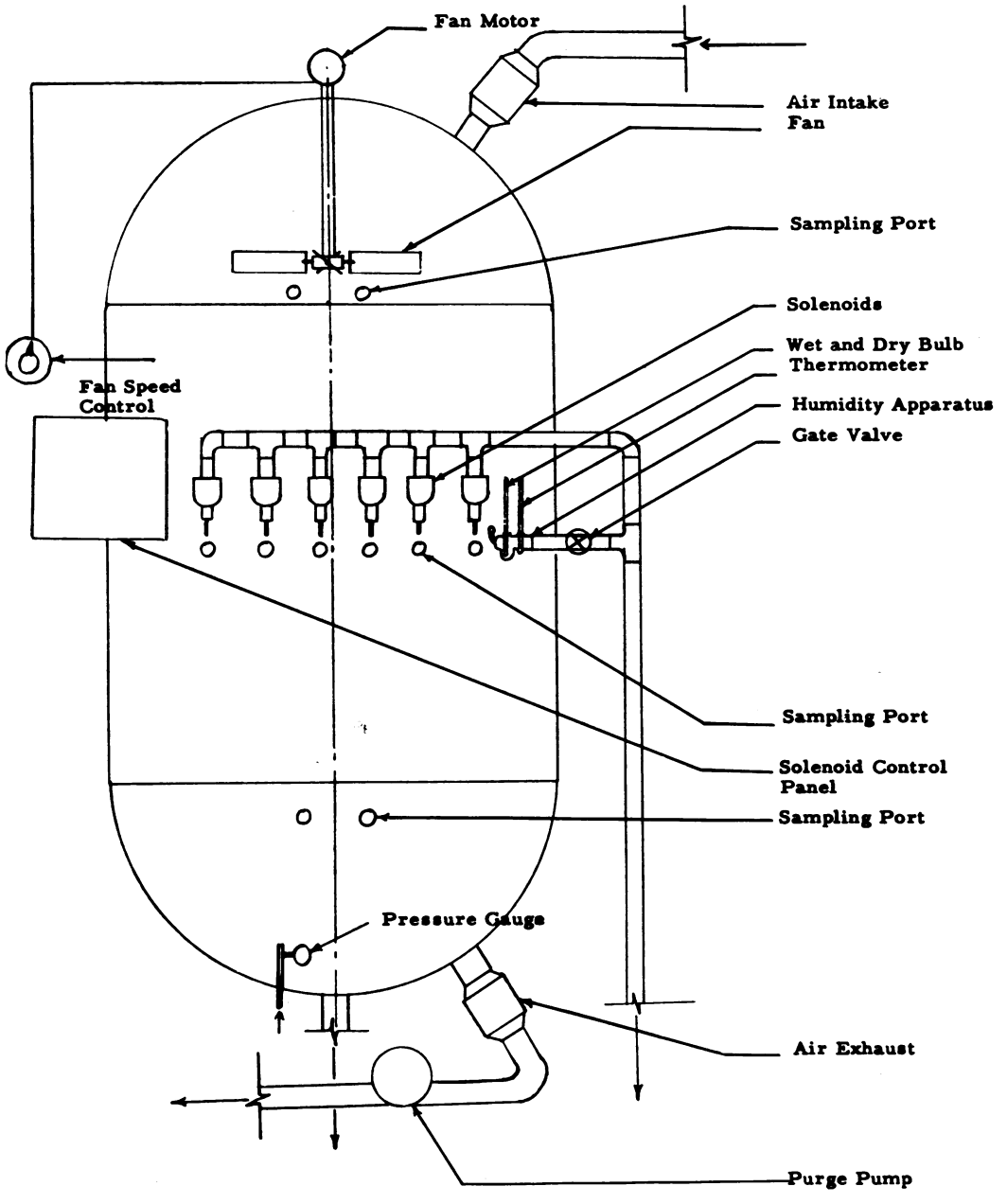

FIG. 1. *Cylindrical tank cloud chamber*

each particle size could be cultured and enumerated. Even with a sampler such as this, microscopy would still be required to determine the population of organisms within each particle for each size class and indirect techniques would be necessary for estimating the proportion of viable organisms within each size class. Such a sampler is not available, of course, but techniques and equipment have been developed which permit the obtaining of essentially all of the desired information, provided that the investigator is given sufficient time, funds, and hands.

Viable concentration. If there is such a thing as a standard sampler for estimating the viable con-

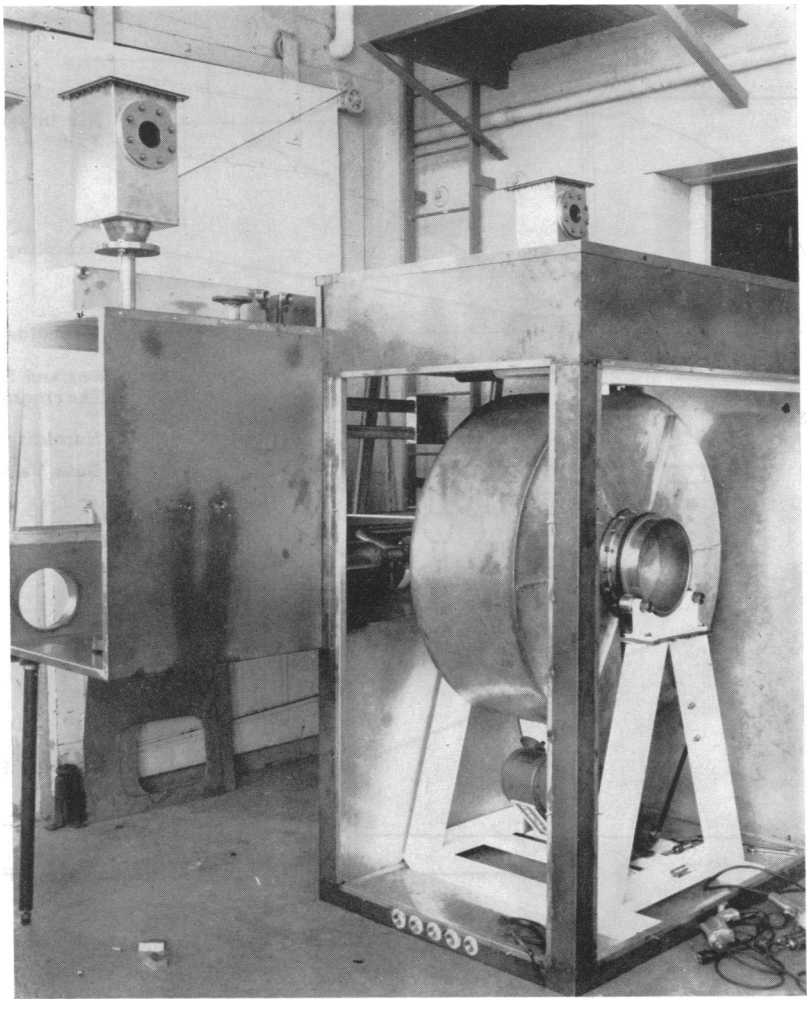

FIG. 2. *Toroid or rotating drum cloud chamber*

centration of organisms in aerosols, it is the so-called Porton impinger developed at the Microbiological Research Establishment at Porton, England, as a modification of the impinger sampler described by Greenburg and Smith (5). This and other impinger-type samplers have been variously modified and widely used (15). In all of the modifications, however, the incoming air leaves the tip of the intake tube at sonic velocity and particles are impinged on a collecting fluid. The sampler accepts particles up to about 20 μ in size and retains with high efficiency those down to less than $\frac{1}{2}$ μ in diameter. This high efficiency, however, applies only in the physical sense. Impingement at sonic velocity is rather drastic treatment and vegetative organisms suffer some mortality depending upon species, length of storage before use, age of aerosol, environmental conditions, and a number of other factors. In sampling systems generally, biological efficiency tends to vary inversely as physical efficiency and the choice of sampler depends upon the experimental objectives. Nevertheless, the Porton impinger, in its various modifications, is at present the most useful sampler we have for its particular purpose. Because of the conflicting characteristics of aerosol sampling systems and the wide variety of sampling objectives, many different types of samplers have been evolved for specialized purposes. Some of these are described below. One of the few adequate reviews of the literature in this field was published in 1959 by the U. S. Department of Health, Education, and Welfare (12) (*cf. also* 2).

Particle sizing. Many aerobiological phenomena

can be neither understood nor interpreted without a knowledge of the particle size distribution of the aerosol and the impinger-type sampler can, in itself, provide no information whatever concerning particle size distribution. The integrity of the particle is destroyed upon impingement and the resulting datum is simply "numbers of organisms per unit of sampler volume."

Many of the data in Dr. Goodlow's paper were obtained by collecting particles on a slide in a settling chamber, then staining, measuring, and counting microscopically. This method still provides the most accurate and complete information that can be obtained regarding the physical particle size spectrum of an aerosol but the tedium and the time required severely limit the number of trials or individual experiments which can be processed. Also, the data so obtained do not distinguish between living and dead organisms.

One of the earlier devices for obtaining particle size information fairly easily is the well-known Casella cascade impactor. This device separates an aerosol into four particle size fractions but the information obtained is limited to total particle mass per impactor stage and does not yield information concerning numbers of particles. The mass median diameter is of limited usefulness in biological investigations. A few years ago the Battelle Memorial Institute (10, 11) developed a six-stage straight-line impactor which not only provided two additional points for the estimate of mass distribution but was designed so that coated slides at the collecting stages could be easily removed and washed into growth medium for enumeration of the viable organisms. This procedure

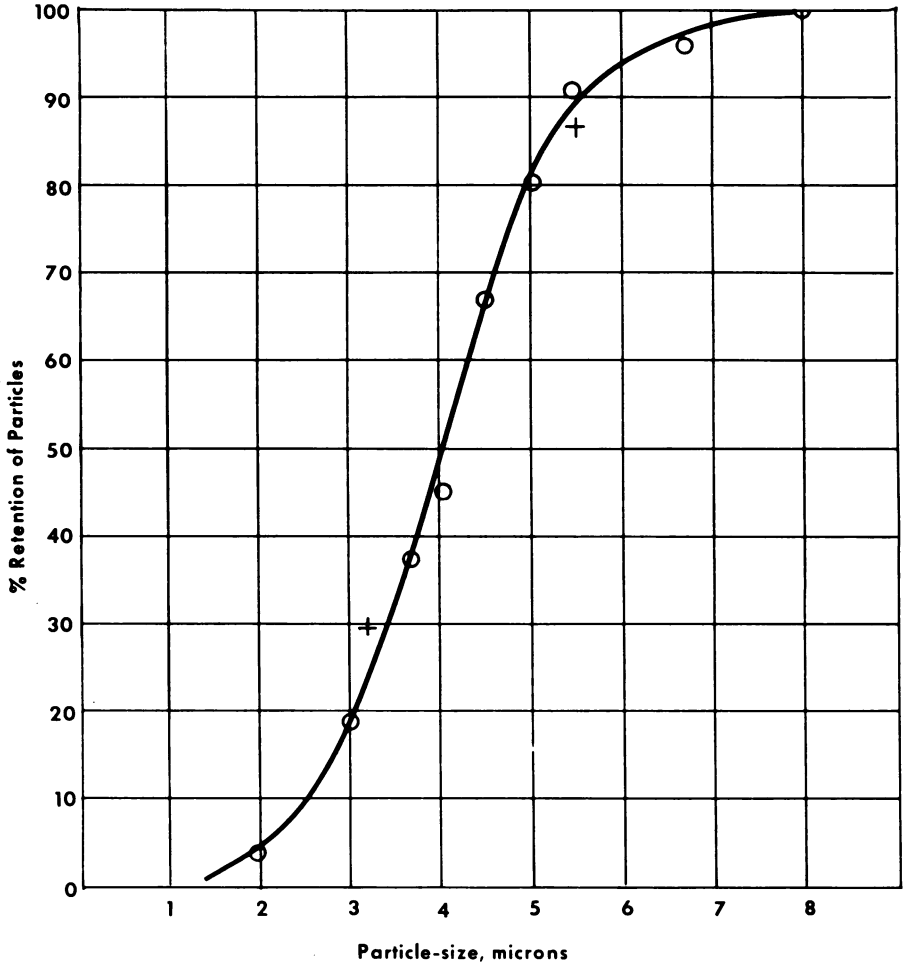

FIG 3. *Relation of particle size to particle retention by an impinger. (From May and Druett (9).)*

provides the median diameter of particles containing viable organisms (Viable Organism Median Diameter), which is critical in investigations of infectivity.

An additional labor-reducing refinement is the "single stage impactor" developed recently at Fort Detrick. These impactors can be designed to retain particles larger than any given diameter and permit smaller particles to pass on into a Porton impinger connected in series. Such samplers have been designed with cut-off points from 1 to 19 μ at 2-μ intervals. A series of such devices, requiring assay of only the Porton impingers, yields with minimal labor a reasonably precise estimate of the distribution of viable organisms throughout the particle size spectrum of the aerosol. Neither the Battelle impactor nor the single stage impactor provides information concerning numbers of particles.

One difficulty with all size classification techniques, which will probably never be resolved, is the tendency of investigators to forget that the separation of particle size fractions by physical means is invariably statistical, never absolute. This is illustrated in Fig. 3 by a graph (modified from May and Druett (9)) relating particle size to particle retention for specific conditions of impingement.

Particle counting. For the automatic counting of particles in an aerosol, the Armour Research Foundation (3) developed an electronic instrument which has had fairly wide publicity as the Aerosoloscope. This instrument, which measures light scattering, draws a cloud sample through a sensing chamber and not only counts the numbers of particles but classifies them into 12 size increments, and it will do this in a few seconds. In actual practice, however, the results fall considerably short of the ideal. The sensing chamber can handle reliably only aerosols of rather low concentration. Many samples must be diluted with clean air before reaching the sensing chamber and in our hands such results have not been quantitatively reproducible. Also the machine's response depends somewhat upon both the composition and the geometry of the aerosol particles. Consequently, a jagged irregular particle, for example, might be counted in one size classification, whereas a spherical particle of essentially the same diameter would record in a different size classification. This method therefore requires rather extensive calibration for each different type of aerosol and becomes as laborious as the routine use of microscopy. However, the instrument can indicate rapidly relative changes in cloud concentrations and can be very useful for such functions as monitoring physical decay. A number of other electronic instruments have been developed for similar purposes but the same general limitations apply. It can be concluded, at least for general purposes, that the automatic counting and size classification of particulate aerosols with quantitatively acceptable results is still in the future. Lacking true automation, we have an urgent need for a method of estimating the number of aerosol particles containing living organisms that is both technically simple and widely applicable. Here also we have not been notably successful. The Andersen sampler (1), which is now commercially available, is useful for this purpose but only with low concentration aerosols. Similar restrictions apply to techniques involving Millipore filters and sieve samplers.

Rate of Decay

In biological aerosol characterization the "rate of decay" is a highly critical parameter which is closely correlated with particle size but which fortunately is much more easily estimated. The decay parameter consists of two components: the rate of fallout, or physical decay, and the death rate of the organisms. The sum of the two, or total decay, is rather easily determined by estimating the viable concentration at various stages of cloud age. The determination of physical decay is a more difficult problem and has been most satisfactorily accomplished by the use of tracers. Historically, the first approach was by the addition of an inanimate material to the biological suspension prior to dissemination. Sodium fluorescein, for example, can be added to a bacterial slurry and subsequent aerosol samples assayed fluorophotometrically. This procedure has certain obvious disadvantages. Many organisms cannot tolerate the presence of a dye even in low concentrations. Also, the assumption is required that the dye and the organisms will be distributed identically or nearly so throughout the particle size spectrum of the aerosol. The array of available data, however, indicates this procedure to be, in general, quantitatively acceptable. A second approach is the employment of highly resistant organisms. Virtually all of the spores of *Bactillus subtilis* var. *niger* are known to remain viable as aerosols for long peri-

ods of time over a rather wide range of environmental conditions. The use of living organisms as tracers not only makes the assumption of identical distribution more reasonable, but generally permits quantitative enumeration at lower concentrations than is possible with inanimate substances. In recent years (6) cultures of test organisms have been grown in a radioactive medium, usually containing P^{32} (7). These tagged cells are then killed and added to live suspensions as tracers. They can be easily assayed for radioactivity in aerosol samples by well-known procedures. The use of an organism as its own tracer eliminates the usual objections to the tracer method. Biological decay is readily obtained as the difference between total decay and physical decay.

Although aerobiological investigations can serve many purposes, the intent of this Conference is such that the above procedures can be considered as leading ultimately to objectives of primarily medical interest. These might include the investigation of infection through a natural portal of entry, the pathogenesis of such infection, or the estimation of the potential of aerosols containing varying concentrations of pathogenic organisms to produce infection in animals or man. The latter datum cannot be quantitatively established without the application of at least some of the foregoing techniques whether the aerosol is generated experimentally or occurs naturally, for example, in a hospital ward.

Dose-Response Relationship

The final information needed to complete the study of an airborne infectious agent is the determination of the dose-response relationship in animals and, if possible, in man. Included also must be the effect upon this relationship of particle size, aging in aerosol, and diverse environmental conditions. Two principal methods have been generally employed for the exposure to aerosols of small experimental animals including monkeys. In one case the animals are held in restraining cages and then the entire unit is placed within the cloud chamber through a port designed for the purpose. After exposure the animals are air-washed to remove as much coat contamination as possible and

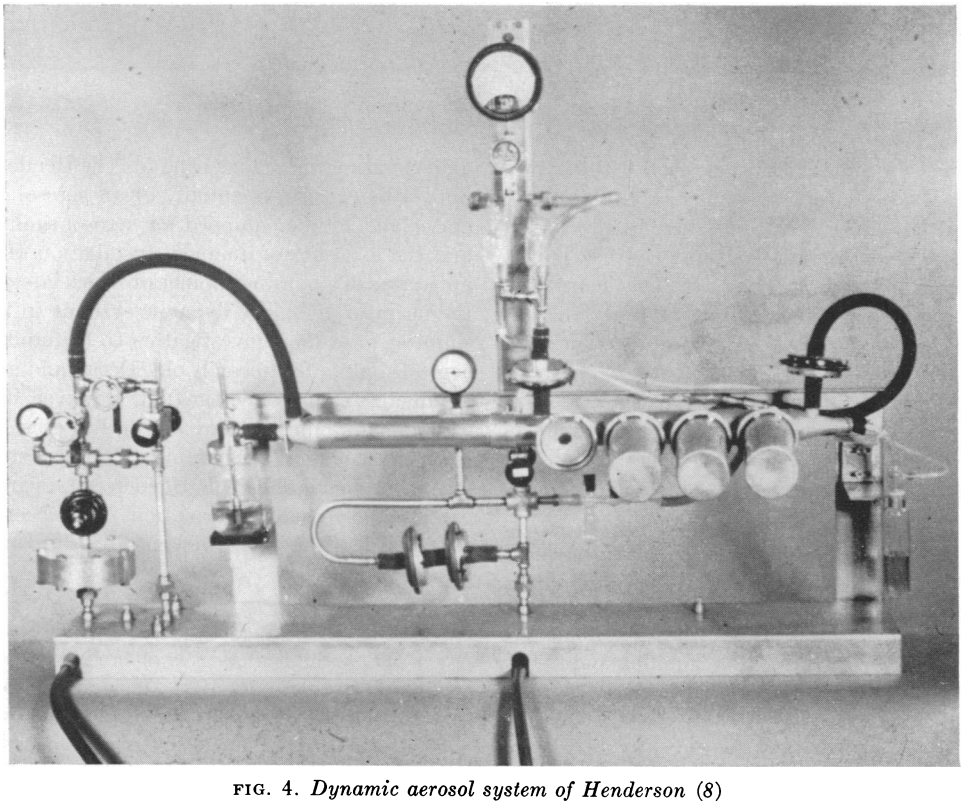

FIG. 4. *Dynamic aerosol system of Henderson (8)*

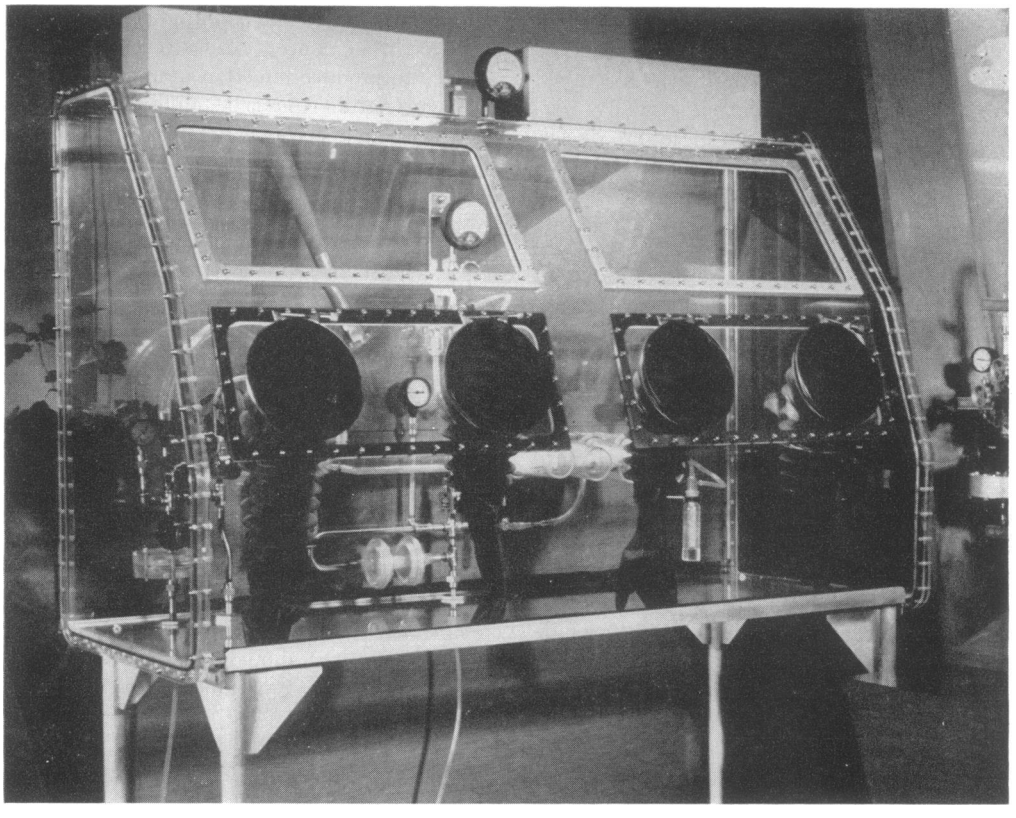

FIG. 5. *Another form of Henderson (8) dynamic aerosol system*

then held in individual cages, each with its own air supply, to prevent cross infection and biasing of results. This procedure is used routinely in the larger static aerosol units at Fort Detrick. Larger animals present individual problems depending upon the type of animal and the design of the test facility. In one of our static test units, which is a 40-ft steel sphere with a capacity of approximately one million liters, five cubicles have been constructed for the exposure of human volunteers through gas masks connected directly with the interior of the sphere. Quantitative medical studies (14) have been successfully conducted.

The second method of exposure is to thrust the animal's head through a rubber diaphragm into a tube through which the aerosol is flowing. This technique avoids generalized coat contamination and considerably reduces the handling and safety problems. It has been used with the smaller static aerosol units and also with dynamic systems such as those described by Henderson (8), which are simple and relatively inexpensive in construction and operating costs (Fig. 4 and 5). The Henderson apparatus consists essentially of an aerosol generator and a tube equipped for aerosol sampling and the exposure of animals. Installation of the apparatus in a housing cabinet provides safety for the operator (13). The dynamic systems in their simplest form limit investigators to the study of aerosols only a few seconds old. Decay and aging parameters cannot be estimated. However, if the experimental objectives are not in the aerosol per se but in the fate of the organism and the test animal after inhalation, the Henderson apparatus can provide valuable information. It has been extensively used in both England and the United States.

The prime problem in quantitating the dose-response relationship of an infective aerosol lies less in the mechanical aspects of exposure than in the interpretation of the data. The imprecision of quantal animal assay is well known even when working with toxic chemicals, for example, which can be dissolved and administered with a high de-

gree of precision. In the case of aerosol exposure, there is a considerable area of uncertainty even concerning the dose which is actually received by each animal. Both the volume of aerosol inhaled and the fraction of organisms retained may vary considerably from animal to animal, and this serves only to compound the difficulty. It is not the purpose of this paper to discuss the analysis of animal exposure data but consultation with a biometrician is recommended for both the design and analysis of quantitative animal experiments.

Summary of Critical Parameters

Summarized in Table 1 are the quantitative parameters which, at the present state of knowledge, are considered essential for an understanding of the "natural history" of airborne infectious organisms and for estimating their disease-producing potential.

There is no intent to imply that this entire list of parameters must be estimated for all aerosol investigations or that testing must always be conducted in large-scale, expensive aerosol systems. The pathogenesis, for example, of airborne infectious organisms can be effectively studied with a simple aerosol system and with minimal biological and physical measurements. Adequate aerosol testing facilities with low construction, installation, and operating costs are available but it should be recognized that such facilities can provide only limited or specialized information. For investigations of small particle aerosols requiring a versatile testing system suitable to a variety of experimental objectives, the standard testing system mentioned earlier, based on the 500-liter rotating drum, can be procured and installed at a cost of about 35 thousand dollars. This includes all of the control equipment and the housing cabinets. A dynamic system such as the Henderson apparatus, with its more limited capabilities, can be installed for approximately 5 to 6 thousand dollars. All of the indicated costs are based upon the assumption that a suitable building exists in which studies with highly infectious organisms can be conducted and that the usual facilities including steam and vacuum lines and an incinerator for sterilizing contaminated air are available.

TABLE 1. *Quantitative parameters affecting airborne infectious organisms*

1. Particle size distribution, as numbers of particles within each size class	NMD
2. Numbers of viable organisms within each size class	VNMD
3. Total decay rate	TDR
4. Physical decay rate	PDR
5. Biological decay rate	BDR = TDR − PDR
6. Dose-response curve for various particle sizes. Number of particles versus number of organisms.	
7. Effect of environmental factors.	
8. Prediction equations of cloud diffusion under various meteorological conditions.	

Conclusion

This discussion necessarily has been limited to a few of the highlights of the technology available for experimental aerobiology and even these have been treated only sketchily. Several of the references, however, contain extensive bibliographies.

In recent years investigations requiring the study of aerosols (chemical or biological) have undergone a marked expansion as evidenced by publications in the journals of appropriate disciplines and by symposia such as this Conference. Because of the intensive investigations of the problems arising from air pollution in and around the industrial complexes of this and other countries, procedures for the study of chemical aerosols have been widely published. This has been less true for biological aerosols and an urgent need exists for the publication of a monograph devoted to procedures for the study of airborne microorganisms.

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