THE CONSTRUCTION AND OPERATION OF EXPERIMENTAL ROOMS FOR THE STUDY OF AIR-BORNE INFECTION*

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In order to carry out adequately controlled studies on the effects of temperature and humidity on the behavior of bacteria and viruses suspended in the air under various experimental conditions, it became necessary to have available enclosed spaces in which any desired atmospheric state could be produced and maintained. The use of conventional methods of air conditioning is unsuitable for this purpose because all of these depend upon exchange of the air inside the experimental space with conditioned air. Hence, two identical air-tight, glass-walled rooms 8 feet × 10 feet and 8 feet high were built each within a separate air-conditioned shell which can be kept constantly at any temperature and relative humidity likely to be encountered in spaces occupied by human beings. Rapid circulation of the conditioned air over all surfaces of the inner room provides a sufficiently high rate of heat transfer to insure constant temperature conditions within that space. The relative humidity of the inner room can be maintained at the same level as the air of the outer shell or can be increased by the introduction of steam. Two rooms provide much greater flexibility in experimentation than would a single one since tests under completely different conditions can be conducted simultaneously and the use of one room for a long term experiment does not immobilize the entire equipment.

Construction

The floor plan of one of the rooms is shown in Fig. 1 and a side elevation in Fig. 2. The outer shell which is insulated with a 6 inch layer of rock wool contains three double glass windows and is entered by a heavy refrigerator type of door. Sufficient space is provided between the two chambers to allow passage around the inner room and the performance of experimental procedures. The walls and ceiling of the inner room are made of ⅛ inch window glass except for one ¼ inch plate glass section through which holes for air sampling and other purposes are cut. The glass plates are supported by and cemented to 2 × 4 wooden joists. Wood strips cover the junctions inside the room. The floor is made of cement and the door of sheet metal. All the inside surfaces except the glass are covered with several coats of vinylite paint which is relatively non-absorbent toward glycol and other vapors and which provides an effective seal of pores and cracks. A series of electrical outlets is installed in the inner room.

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Three wire mesh shelves on which settling plates or cages of mice may be exposed to the test atmospheres are placed along one wall at levels from the floor of 6 inches, 3 feet, and 6 feet 6 inches respectively. Small, tight fitting doors at each level provide access to the shelves (only one shelf is shown in the figures). Arm length gloves of rubber or canvas may be fitted to the inside of these doors when dangerous pathogens are dispersed into the experimental room. An air lock entrance is provided.

Scavenging equipment consists of a motor-driven blower which draws air at a rapid rate from the room and discharges it to the outside of the building, a complete change of air being accomplished in 2 to 3 minutes. Space is provided for the installation of disinfection apparatus at the point of exit of the air, for use in experiments involving highly infectious disease agents. Several devices for closing the scavenging ducts tightly were tested before a satisfactory one was found. This consists of a ¼ inch lucite disc 9 inches in diameter which closes against another plate of lucite surrounding the opening of the scavenging duct. A layer of sponge rubber is attached to one of the contacting surfaces and vaseline is applied to the other to insure a tight seal. The mechanism is operated by compressed air (see scavenging duct and scavenging vent in Fig. 1).

The conditioned air enters the outer room just above the ceiling of the inner chamber (Fig. 2), and is so directed by louvers in the grill that it flows over the ceiling and upper walls and returns around the lower walls and under the floor of the inner room to the exit grill placed near the floor of the outer conditioned space. The thermostat and humidistat are located just above this grill. The volume of air flow is 800 cubic feet per minute.

A schematic plan of the air conditioning machinery is shown in Fig. 3. Part of the air returning from both test rooms is dried by a single silica gel dehumidifier[1] which contains an automatic regenerating unit for the silica gel. The current of dried air is then divided between the conditioning chambers of the two test rooms. (Only one such chamber is shown in the drawing.) The thermostat and humidistat control valves determine whether the air entering each conditioning chamber shall be humidified or dehumidified, and heated or cooled. The return air is shunted by the proportioning valves directly back to the conditioning chamber unless further dehumidification is required in which case part or all of it is sent through the dehumidifier. Oiled fiber glass filters[2] help reduce the dust content of the air. A fresh-air intake duct is provided for the purpose of scavenging the air of the inner room or admitting fresh air to the system if so desired. A separate steam inlet controlled by its own humidistat is placed inside of the inner room so that its relative humidity can be raised above that of the conditioned air of the shell.[3] All adjustments are regulated by a pneumatic control system.[4]

Equipment for Purposes of Experimentation

Temperature and Humidity Recording.—A continuous record of the temperature and relative humidity prevailing in the outer chamber is made by a wet-dry bulb recording instrument[5] mounted on one wall of the shell. A psychrometric apparatus is also placed on a shelf inside the glass room which can be operated at will.

[1] Bryant Heater Company, Cleveland, Ohio.

[2] Owens-Corning Fiber Glass Corporation, Toledo, Ohio.

[3] No provision was made for reducing the relative humidity inside the inner room below that of the outer shell, because all the methods for removing water vapor also result in loss of bacteria and glycol vapor from the air.

[4] Johnson Service Company, Chicago, Illinois.

[5] Taylor Instrument Company, Rochester, New York.

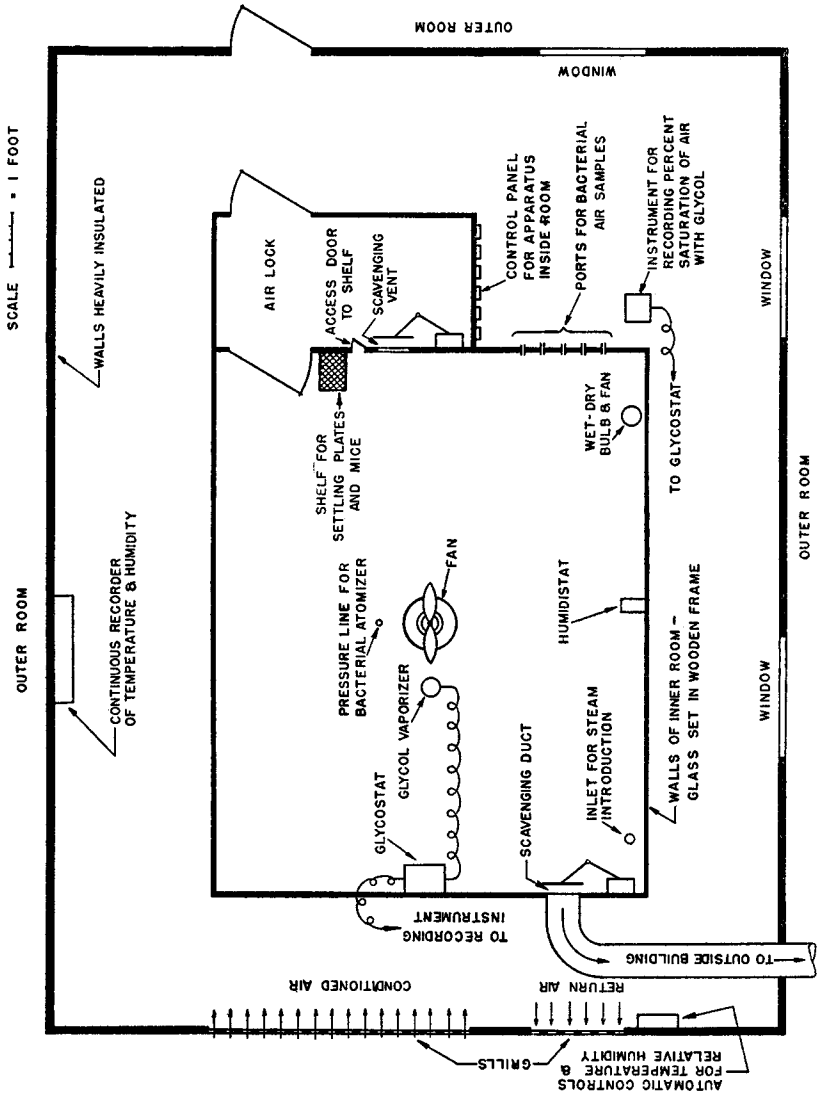

FIG. 1. Floor plan of experimental room.

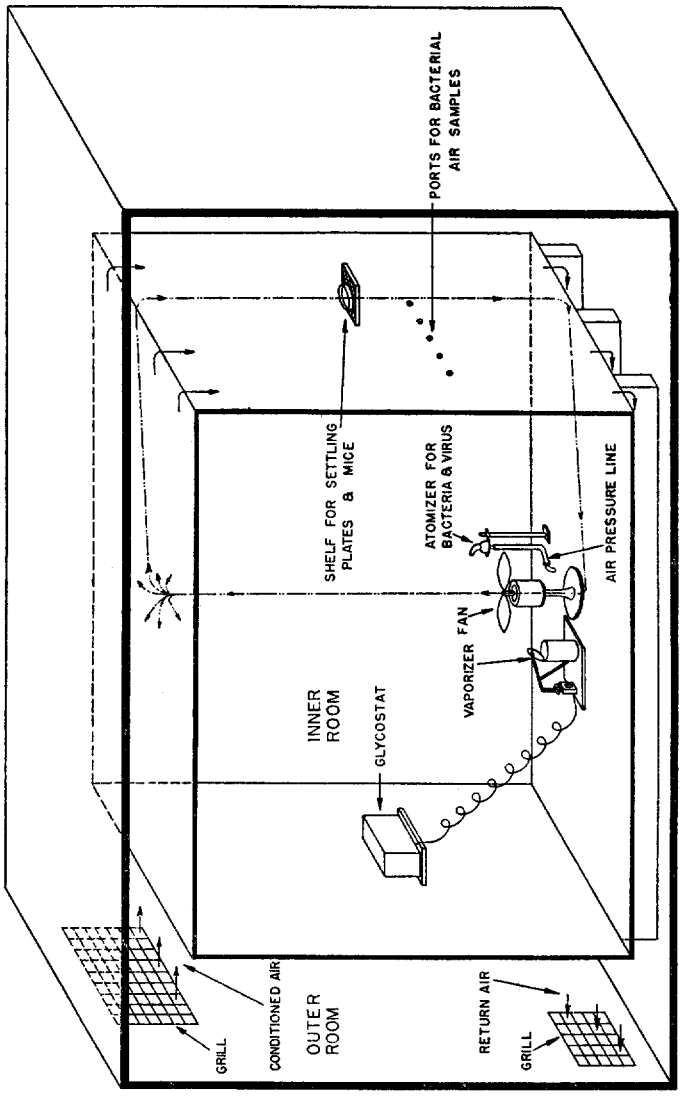

FIG. 2. Side elevation of experimental room. Only one shelf for settling plates is shown, and the doors and the airlock have been omitted for purposes of simplification.

Fan for Mixing Air of Inner Room.—A 20 inch, two-blade fan[6] placed in the center of the inner room produces mixing of the air by directing a current toward the ceiling which then disperses and returns along the walls and floor to the fan (Fig. 2).

Atomization of Pathogenic Agents.—A compressed air line extending under the floor to the center of the room provides a means of atomizing bacteria and viruses into the main ascending air current.

Air Sampling.—Provision is made for recovery of pathogenic agents from the air of the rooms by settling plates and bacterial air samplers (1–3). See Fig. 2. The latter are operated by a battery of suction pumps[7] located in the outer shell. In order to provide against possible aerial contamination resulting from

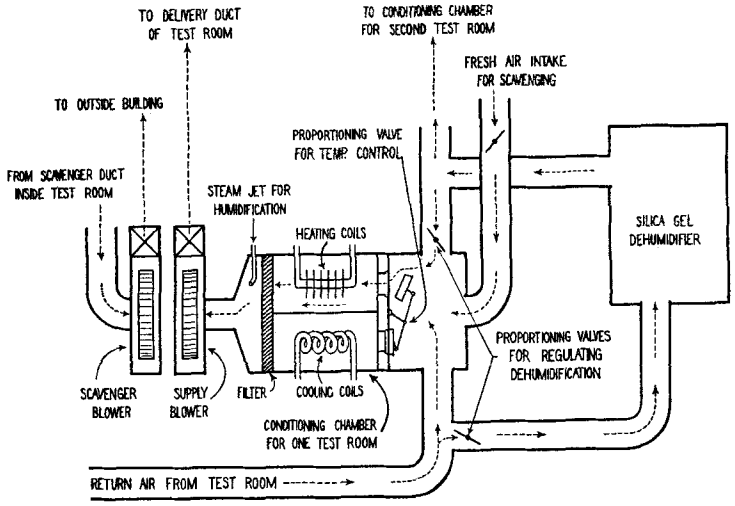

FIG. 3. Schematic plan of the temperature and humidity control system.

escape of pathogens through the bacterial air sampler, the air discharged by the pumps may be led to the outside of the building.

Glycol Vaporizer.—The introduction of glycol vapors into the atmosphere of the inner room is achieved by means of a simple vaporizer consisting of an electric light bulb immersed in a beaker of glycol placed in a cylindrical brass cup, the lid of which is opened and closed by a solenoid valve.

Glycostat.—Regulation of the concentration of glycol vapor is accomplished by means of a glycostat which controls the operation of the solenoid valve in the vaporizer (4).

Recording of Glycol Vapor Concentration.—A continuous record of the concentration of glycol in the air is made by means of a recording milliammeter[8] connected to the glycostat (5) (Fig. 1).

[6] Reynolds Electric Company, Chicago, Illinois.
[7] Welch Manufacturing Company, Chicago, Illinois.
[8] Esterline Angus Company, Indianapolis, Indiana.

Tyndall Beam for Detection of Glycol Mist.—An intense, collimated beam of light (not shown in the figures) directed across the inner room is employed to detect the presence of an aerosol in the room by means of the Tyndall effect.

All the apparatus is controlled from an instrument panel located outside the chamber.

Operation of the Chambers

When running continuously, temperature and relative humidity are maintained at remarkably constant levels with variations of not more than 1°F. and 2 per cent relative humidity, limits which are eminently satisfactory for the requirements of our experimental procedures. These conditions can be maintained for periods of a week or more. The relative humidity can be varied from 12 per cent to 95 per cent and the temperature from 50 to 100°F.

The range of temperature-humidity combinations attainable depends to a certain extent on the atmospheric conditions out-of-doors. With outdoor temperatures of 90 to 100°F., which are common during Chicago summers, the temperature within the chambers cannot be lowered to less than 55°F. When the outdoor dew-point is not higher than 65°F., the lowest dew-point which can be maintained inside the chambers is about 20°F., which corresponds to a relative humidity of about 12 per cent at 72°F., or 25 per cent at 55°F.[9]

Changes in atmospheric conditions can be produced quite rapidly. Relative humidity can be raised or lowered 20 to 30 per cent within almost as many minutes. Temperature equilibrium requires a longer period of time. If extreme conditions are required for an experiment the desired settings are made the previous night.

By leaving the door to the inner room open, equilibration of the relative humidity and temperature usually occurs within half an hour or less. This can be hastened by drawing air from the outer chamber through the inner one by means of the scavenging fans, with provision for a small access of fresh air to prevent formation of a negative pressure within the system. If, however, very low humidity is desired and the outside relative humidity is high, employing the scavenging system would upset the experimental conditions.

When the inner chamber is sealed, the rate of its air exchange with the outer shell becomes negligibly small. Thus relative humidities 20 to 30 per cent higher than those in the conditioned shell can be maintained in the closed inner room following a single introduction of sufficient steam to bring the humidity to the desired level. The temperature inside the sealed chamber follows that of the outer shell very closely. However, when operations such as glycol vaporization or steam introduction are carried on inside the inner room, its temperature may rise 1–3°F. above that of the outer space.

The fan for mixing the air of the inner room has been operated at various

[9] The dew-point is an index of the absolute amount of water vapor in the air and is independent of the temperature.

speeds from 100 R.P.M. to 500 R.P.M. by means of a variable transformer. These rates produce air currents immediately above the fan with velocities ranging from 50 ft./min. to 300 ft./min. The higher speeds produce more rapid mixing of the sprayed bacterial and virus suspensions, and have exhibited no deleterious effect on their viability for periods as long as 30 minutes.

Scavenging is accomplished in a manner designed to prevent the escape of bacteria from the inner chamber to the outer shell. First, the outer door to the air lock is opened. Then the following sequence of operations is carried out: (1) opening the fresh air intake; (2) turning on the scavenging fan; (3) opening the valve to the scavenging duct; (4) opening the scavenging vent connecting the inner room to the air lock. In this way a continuously decreasing pressure is maintained from the outer shell, through the inner chamber, to the scavenging duct. A series of four switches placed from left to right in the proper order, controls the mechanism. As an extra precaution for the protection of the operator these switches are placed outside the air-conditioned space. Repeated samplings of the air of the shell during and following experiments with hemolytic streptococci have failed to recover a single streptococcus. Furthermore, settling plates exposed and bubbler samples run in the closed air lock during an experiment in which the small doors, for the insertion of blood agar plates, were being constantly opened and closed, failed to recover more than a very rare bacterium of the kind present in the air of the inner room even though no sleeves over the doors were employed. During the 2 years in which these experimental rooms have been in operation no accidental infection with the pathogens employed (hemolytic streptococcus, pneumococcus, *Staphylococcus aureus*, influenza virus, etc.) has occurred.

Dispersion and Collection of Bacteria

With the atomizers employed for the dispersal of bacterial cultures into the air (6) spraying for 1 minute with air dried by passage through a calcium chloride tower, under a pressure of 100 mm. of mercury, results in delivery of approximately 0.3 cc. of a standardized bacterial broth suspension containing 400,000,000 bacteria per cc.[10] In the case of group C hemolytic streptococci, blood agar plates exposed for 5 minutes in such an atmosphere will yield 50 to 80 colonies. The bubbler samplers (2) collect on the average 600 to 1000 streptococci per cubic foot of air.

Consecutive 5 minute bubbler air samples started first at the beginning of the bacterial spray and repeated every minute thereafter tend to show a slight increase in number of bacteria per cubic foot for the first 2 minutes. During the next 10 minutes, the numbers recovered remain fairly constant after which there is a slow decrease in count which continues gradually over a period of

[10] The mass median diameter of the droplets delivered by these atomizers varies from 0.6 μ to 0.8 μ. We wish to thank Mr. Lawrence Sonkin of the Toxicity Laboratories, University of Chicago, for making these determinations for us.

many hours. On the other hand, the settling plates exposed for 5 minute periods at the same time intervals, collect a maximum quantity of bacteria in the first three plates and thereafter show a progressive diminution in numbers collected, which is more rapid that that exhibited by the bubblers. This difference in results between the two methods of air sampling is to be interpreted on the basis of the selective action of settling plates for the larger droplets and of the bubblers for the smaller ones (7).[11]

In order to determine whether the position chosen for the air-sampling ports (Fig. 1) provides accurate information as to the actual bacterial content of the air of the room, bubbler samplers were placed at various locations inside the room and operated at the same time as were those at the fixed ports. No significant difference in the samples taken at various stations was detected.

Vaporization and Control of Glycol Concentration

Vaporization of the glycol was produced, as described above, through heating the liquid by means of a light bulb controlled by a variable transformer. The voltage was adjusted before each experiment to produce approximately the desired rate of vaporization of glycol into the air (8). Thereafter, the glycostat, which was set so as to maintain a given percentage saturation regulated the amount of glycol dispersed into the space by means of a butterfly valve on the vaporizer outlet (not shown in Fig. 2). It was found that a period of an hour or more was required to establish a constant concentration of glycol in the air. This is due largely to the fact that a minute layer of glycol tends to adsorb on glass and other surfaces. After a certain amount of such condensation has taken place a steady state is reached in which a smaller output from the vaporizer is required to maintain a constant per cent saturation of the atmosphere. The amount of glycol deposited on these surfaces is too small to be visible or detected by touch. However, its presence can be demonstrated by means of the glycostat. If, at the end of each experiment, the chamber is completely swept out with clean air so that the glycostat no longer records any glycol in the atmosphere, re-sealing the room results in a slow release of sufficient vapor to produce a definite response by the glycostat. On account of this condensation of glycol on the walls of the experimental room and its subsequent re-evaporation it was found necessary to wash the entire surface of the room with water following each experiment.

Various Uses of the Experimental Rooms

These rooms have permitted a study of the effects of temperature and hu-

[11] It may be pointed out that under the conditions of these experiments where a constant and reproducible air current is maintained, the settling plate is a reliable air-sampling device. Plates exposed side by side for the same interval of time yield counts which agree with each other within ± 6 per cent. Furthermore, counts from consecutively exposed plates fall on a smooth curve, which can be duplicated very closely in successive experiments. Under field conditions, however, where air currents are highly random, the settling plate is a much less satisfactory index of the bacterial content of the atmosphere (7).

midity on air-borne bacteria and viruses in relation to their survival, infectiousness for animals, particle size, and susceptibility to the action of glycol and other lethal vapors. They have proved indispensable for the calibration of the glycostat and investigation of the behavior of glycol vapors. They have also been employed for studies on the dispersion into the air and distribution in the environment of respiratory pathogens by human subjects (8-12).

These chambers also lend themselves to the study of aerial infection in constantly flowing atmospheres. The scavenger fan can be operated during the course of an experiment so as to draw in a constant stream of fresh air, while a continuous introduction of either bacteria or glycol or both is maintained. Such a dynamic arrangement permits more accurate observation of transient phenomena which occur in the course of a few seconds.

Finally, these chambers make possible a study of the effects of environmental conditions on experimental animals with particular reference to their susceptibility to infection by various pathogens.

SUMMARY

This communication describes the construction and operation of two identical experimental rooms in which it is possible to produce and maintain a wide range of temperature and humidity with or without exchange of the room air. The ability to maintain a large air mass under constant conditions makes it possible to study the effects of different atmospheric states on air-suspended bacteria and viruses in relation to their survival, particle size, humidification, killing by lethal vapors, and host susceptibility. A brief description of the functioning of the rooms under experimental conditions is given.

We wish to acknowledge our indebtedness to Mr. Samuel R. Lewis and Mr. R. W. Shields of the engineering firm of Samuel R. Lewis and Associates, 100 West Monroe Street, Chicago, Illinois, who designed and installed the equipment. Dr. H. M. Lemon and Dr. C. G. Loosli also rendered valued assistance. For the engineering details we wish to refer the reader to Mr. Samuel R. Lewis.

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