Device for Inhalation Exposure of Animals to Spores. (25677)

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Several systemic mycoses begin as upper respiratory or pulmonary infections following inhalation of fungi. The fungi, presumably, are inhaled as spores in air-borne dust from colonies of pathogenic fungi growing in the patient's environment. In experimental studies it sometimes is desirable to simulate these conditions of natural exposure to fungi. Elaborate and expensive instruments have been designed to disperse estimated numbers of spores or particles in air to which animals are experimentally exposed. Recognized disadvantages of these include cost, large size, inconvenience of operation and contamination of the laboratory. The quantitative aspects of exposure are unpredictable because counts of the spores per unit volume of air in such an instrument do not measure numbers which actually enter the lungs.

Methods. The device described herewith is at first a culture flask and subsequently an exposure chamber. It overcomes the disadvantages pointed out above, and simultaneous exposure of 10 mice permits an adequate sampling of animals for accurate estimation of number of viable spores which actually enter the lungs. Its design can be modified to accommodate larger animals and for use with atomized suspensions of microorganisms or for dusts of selected particle size.

Results. Fig. 1 is a photograph of the device in use as a culture flask. It is a 1 liter heavy wall pyrex flask with 10 tubular side arms fused (ring-sealed) at a 30° angle above the horizontal into ports spaced equidistantly around the flask at a level of 4 cm above the base. Each side arm is a pyrex tube of 2 mm wall thickness, 21 mm inside diameter and 14 cm length, constricted to a 1 cm opening at one end. This end extends about 2 cm into the flask beyond the seal. The mouth of the flask and of each side arm is closed by a cotton stopper. The lower end of each side arm also may be closed by a cotton stopper on an applicator stick which extends beyond the distal end of side arm. The flask is filled to a depth of 2-3 cm with an agar medium, it is sterilized in the autoclave, the agar surface is seeded with a suspension of spores, the flask is incubated until mature spores are abundant and the device is then ready for its role as an exposure chamber.

Fig. 2 is a semidiagrammatic drawing showing (for clarity) only 2 of the 10 side arms. It illustrates use of the device as an exposure chamber by replacement of the cotton stopper in the mouth of the flask by a unit for creating air currents over the surface of the culture. This is a #10 stopper through which pass 2 glass tubes. The upper end of the shorter tube is bent at a right angle and attached to a rubber balloon capable of expansion to 100 ml capacity before elastic changes. The longer tube is bent to a right angle but at a point above the first so that it can be rotated. It is attached by rubber tubing to a 100 ml syringe. The tube is held in position by 2 rubber collars (not shown in the drawing) and the portion which passes through the stopper is lubricated with glycerine. The lower end of this tube is curved to direct a jet of air across the surface of the fungus culture. This unit is wrapped in paper and sterilized in the autoclave.

In preparation for use of the device as an exposure chamber, and before insertion of the air-circulating unit, a mouse is placed in each of the side arms. It is pushed to the bottom so that its nose fills and extends beyond the open end of the tube and it is held in place by a cotton plug. The upper end of the side arm is then closed by a rubber stopper. The air circulating unit is now put in place.

When the apparatus is fully assembled with mice in place an attendant produces air turbulence over the culture by pumping 50 ml of air into and out of the chamber by regular strokes of the syringe plunger. A second attendant, by rotation of the tube through which air is pumped, directs an air jet uniformly over all parts of the culture to disperse spores throughout the chamber. Changes in

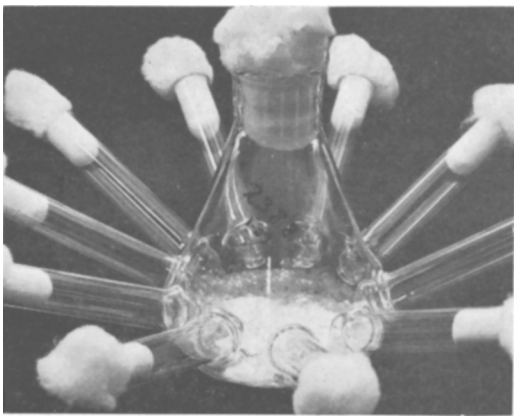

FIG. 1. Device in use as a culture flask.

air pressure are minimized by the ballast action of the balloon which must be nearly empty when the syringe plunger is at the 50 ml mark and must be attached in such a way that it never acts as a flap valve to close the tube. It must expand freely so that it will hold 75-100 ml of air without stretching and will thus prevent changes of air pressure within the flask.

Pumping of air should be continued for a period which will vary with species and strain of fungus, number of spores disseminated and severity of exposure desired. The mice should be left in place for 2-3 minutes after air turbulence has subsided to allow partial settling of air-borne spores. The device may be placed in a safety hood during mouse exposure but in use we have not found the experimental

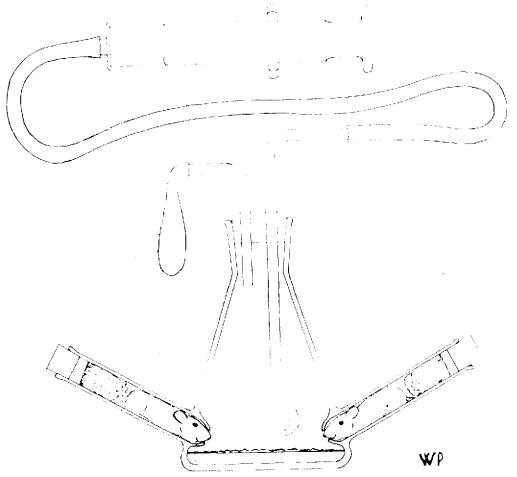

FIG. 2. Use as an exposure chamber.

fungus in Petri dishes exposed during the entire procedure. The closed system is an essential safety feature of this device but it is obvious that there must be some contamination of at least the anterior portion of the animal's head. Flow of contaminated air over the rest of the body is reduced by the stopper back of the animal and by equalization of pressure achieved by expansion of the balloon. Contamination can be reduced further by swabbing mice with a disinfectant. At completion of exposure mice are removed, cotton stoppers are replaced in side arms and the apparatus is sterilized in the autoclave. If mice must remain within this closed system longer than 10 minutes, a larger flask should be used in the design in order to prevent depletion of O_2 and subsequent changes in the animals' respiration rates and depth of inhalation.

Our device permits simultaneous exposure of 10 mice to an atmosphere containing dry spores of a fungus. The numbers of viable spores which actually enter the lung can be estimated retrospectively by killing 5 mice taken from alternate side arms, grinding the lungs, plating this tissue and counting fungus colonies after incubation. The instrument can be adapted by changes in design to produce an "atomized" mist of microorganisms in droplets or a dust of selected particle size. Its design can be changed to expose larger animals without loss of its simple, self-contained and safety features.

The device was made to specifications supplied by the authors in the Glass Shop of the Nat. Inst. of Health.

Summary. A culture flask with 10 side arms is converted by insertion of a unit for blowing air across a fungus culture into an exposure chamber. Ten mice or other animals can be exposed simultaneously with minimal contamination to an atmosphere containing dry spores. The design can be adapted to exposure of larger animals and to use with dusts, mists and gases. Superior features of the device are: it is small and portable, it is simple in construction and operation, it is a closed system, and contamination of fur of animals is minimal.

Received February 15, 1960. P.S.E.B.M., 1960, v103.