Laboratory Safety in Research With Infectious Aerosols

ARNOLD G. WEDUM, Ph.D., M.D.

THE MEANS by which man becomes infected in the laboratory depend to a considerable extent upon his experimental procedures. But in the absence of procedures that especially predispose to aspiration, penetrating self-inoculation, or dermal contamination, the most common method of infection is the inhalation of accidentally formed microbial aerosol.

How Accidental Infection Occurs

A 1950 survey of 1,342 laboratory-acquired infections in the United States revealed that recognizable accidents accounted for only 16 percent (*1*). The precipitating act, source, or means of infection was unknown in 80 to 84 percent of the cases. At Fort Detrick, exhaustive on-the-spot investigation of 90 laboratory illnesses occurring from 1953 to 1957 could reduce this unknown group to no less than 65 percent (*2*).

Common manipulations with the inoculating needle, pipette, syringe, centrifuge, lyophilizer, and blendor create bacteria-laden aerosolized particles suitable for inhalation. Table 1 provides illustrative data from a larger series of determinations concerning the number of such particles that may be recovered within 2 feet of the work area by air sampling (*3*, *4*). These numbers will be increased somewhat if arthrospores of *Coccidioides immitis* are used (*5*). Because most bacteria in the air occur in clumps (*6*), these numbers of particles can contain a human infectious dose, particularly if the operation is repetitious. Common laboratory accidents likewise liberate organisms into the air (table 2). Furthermore, accidents with petri plates, lyophilized ampoules, or a centrifuge may create microbial aerosols that can cause infection in persons stationed in other parts of the building, one or more floors away. A few selected episodes of this sort are included in table 3.

That only a small number of appropriate micro-organisms are required to induce illness in man is not always fully realized. Table 4 summarizes various published data. The indicated human infectious dose produced clinical disease in 50 percent or more of the volunteers. For such highly infectious

Table 1. Bacteria recovered by air sampling within 2 feet of the site of common bacteriological procedures

Procedure	Colonies obtained per operation
Removing tight cover of standard Waring blendor immediately after mixing culture	[1]
Opening lyophile culture tube	86
Decanting centrifuged fluid into flask	17
Inserting hot loop in culture flask	9
Removing dry cotton plug from shaken culture flask	5
Pipetting 1 ml. of inoculum to poured agar petri plate	3
Pipetting 1 ml. of culture into 50 ml. of broth	1

[1] Too numerous to count.

Dr. Wedum is director of Industrial Health and Safety, U.S. Army Biological Laboratories, Fort Detrick, Frederick, Md. *In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.*

micro-organisms as *Pasteurella tularensis* or *Coxiella burnetii*, 10 microbial units are enough to infect most unimmunized men.

In an effort to reveal how infection typically may be acquired during aerobiological research, a review has been made of 51 infections contracted during 1948 to 1959 at Fort Detrick among persons doing aerobiological studies with pathogenic micro-organisms. The causes and number of infections follow:

Cause of infection	*Number*
1. Unknown	28
2. Removed things from aerosol exposure chambers before exit door was tightly closed, without air washing or without adequate decontamination. (It is believed that several of the unknowns are referrable to this group.)	7
3. Self-inoculation with needle or glass	4
4. Accidental burst of external balloon used to equalize pressure in an aerosol chamber	2
5. Petri plates broken on floor when pushed through a double-ended autoclave	3
6. Forced ventilation of safety cabinet system turned off	2
7. Leak or break in arm-length glove attached to an aerosol chamber. (May be another explanation for unknown sources of infection.)	2
8. Leak in aerosol tank	1
9. Various techniques performed at an open laboratory bench	1
10. Without wearing a respirator, entered an animal room holding aerosol-exposed animals	1

NOTE: Actions 5, 6, 9, and 10 were contrary to laboratory regulations.

Table 2. Bacteria recovered by air sampling during common laboratory accidents

Accident	Colonies obtained per accident
One 50-ml. tube breaking in centrifuge and culture splashing side of centrifuge; air sampled 7 inches above centrifuge	1,183
One 50-ml. tube breaking in centrifuge but all 30 ml. of culture staying in trunnion cup	4
Accidentally breaking one ampoule of lyophilized nutrient broth culture on floor; air-sampled at nostril height, 18 inches each side of accident site, for 1 hour	491
Drop of culture falling 12 inches onto steel surface; air sampled within 2 feet of site	16
Petri plate cultures dropped on floor; air sampled 4 feet above floor, 70 feet from accident	9

Occupational exposure to infection. Variation in occupational frequency of infection seems to correspond to the degree of exposure to an aerosol produced by manipulation of pure culture. It is our impression that there are comparatively fewer infections among janitors, dishwashers, animal caretakers, and persons doing animal autopsy than among those persons directly handling cultures. The former inhale secondary microbial aerosols from sources such as floor dust, animal fur, and tissues. Surveys in our laboratories and elsewhere, as summarized in tables 5 and 6, seems to confirm this impression. The Fort Detrick figures include

Table 3. Episodes of single-source multiple laboratory infections

Disease	Probable source of infection	Persons infected	
		Maximum distance from source	Number infected
Brucellosis (*7*)	Centrifugation	Basement to 3d floor	94
Coccidioidomycosis (*8*)	Culture transfer, solid media	2 building floors	13
Coxsackie virus infection (*9*)	Spilled tube of infected mouse tissue on floor.	5 feet (estimated)	2
Louping ill virus infection (*10*)	Intranasal inoculation of mice	2 feet (estimated)	3
Murine typhus (*11*)	Intranasal inoculation of mice	6 feet (estimated)	6
Q fever (*12*)	Centrifugation	1st floor to 3d floor	47
Tularemia (*13*)	20 petri plates dropped	70 feet	5
Venezuelan encephalitis (*14*)	9 lyophilized ampoules dropped	4th floor stairs to 3d or 5th	24

NOTE: Numbers in parentheses are references.

Table 4. Human infectious dose

Micro-organism of—	Route of infection	Growth medium		Microbial units per human infectious dose
		Medium	Microbial units per ml.	
Malaria (*15*)	Intravenous	Blood	4×10^4	10
Q fever (*16*)	Inhalation	Egg yolk	[1] 1×10^{10}	[1] 10
Salmonellosis (*17*)	Ingestion	Beef broth	1×10^9	10^6
Scrub typhus (*18*)	Intradermal	Egg yolk	[1] 15×10^3	[1] 3
Syphilis (*19*)	Intradermal	Rabbit testis [2]	36×10^6	57
Tularemia (*20*)	Intradermal	Broth	1×10^{10}	10
Tularemia (*20*)	Inhalation	Broth	1×10^{10}	10
Venezuelan encephalitis (*21, 22*)	Subcutaneous	Egg	[1] 33×10^{10}	[1] 1
West Nile fever (*23*)	Intramuscular	Mouse brain	[1] 33×10^9	[1] 1

[1] In mouse or guinea pig infective units.
[2] Centrifuged resuspended preparation.

NOTE: Numbers in parentheses are references.

nonhospitalized and subclinical cases diagnosed immunologically. While many of the laboratory technical assistants at Fort Detrick are engaged in work of potentially great infectious hazard, part of their work is sufficiently repetitious so that standardized safety precautions can be instituted. Obviously, the procedures of the trained scientific personnel are less susceptible to standardization and the infectious risk consequently is not as easily controlled. Only personnel working in infectious disease laboratories were included in table 6. All had received nonviable specific vaccines whenever they were available.

Animal handling. The hazard to man of handling aerosol-exposed animals has not been accurately ascertained. However, the presumptive evidence that he could become infected is impressive. Monkeys and guinea pigs, whose whole bodies have been exposed to aerosolized particles 1 to 10 microns in diameter, will transmit infection to uninoculated cage-mate controls in the case of anthrax, brucellosis, plague, Q fever, tuberculosis, tularemia, and Venezuelan encephalitis. In many of these experiments the animals were held in artificially ventilated closed cages. Infection of cage-mate control animals often occurs after aerosol challenge. Table 7 summarizes information concerning this aspect of aerosol challenge.

Rosebury (*28*) concluded from his experiments, in which the aerosol-exposed test animals were air-washed for 10 minutes before placement with cage-mate controls, that "animal coat contamination under the experimental conditions of this work involves no serious hazard either to the operators or to the animals themselves." But later experiments have shown that guinea pigs bodily challenged with aerosol containing 490 *Brucella suis* per liter of air and then air-washed for 1 hour will transmit the infection to cage-mate controls during the first 24 hours of communal caging (*29*), presumably by bacterial shakeoff from hair or by exhaled bac-

Table 5. Percentage distribution of infection according to occupation

Occupation	Fort Detrick N=369	P & S survey [1] N= 1,286	Attack rate per year per 1,000 persons
Trained scientific personnel	58. 5	78. 1	1. 0
Laboratory technical assistants	21. 7	10. 3	. 4
Animal caretakers	2. 1		
Dishwashers	3. 8		
Janitors	0		
Administrative and clerical	3. 7	6. 7	1. 0
Maintenance employees [2]	7. 8		
Visitors, friends, etc	2. 4		
Students, not in research	0	4. 9	. 1
Total	100. 0	100. 0	. 5

[1] Pike and Sulkin survey, 1952 (ref. 24).
[2] Carpenter, electrician, engineer, garbage truck crewman, painter, plumber, sewage plant operator.

teria. Indicative of the potential of bacterial shakeoff from animal body hair are some experiments with spores of the nonpathogenic *B. subtilis* var. *niger*. This bacillus could be recovered daily for 9 days from the air of a ventilated cage containing one control monkey and one that had been bodily exposed in a separate aerosol chamber for 1 minute to 1.4×10^5 spores per liter of air (*26*). Guinea pigs similarly tested yielded the bacilli for 18 days (*29*). Hair of guinea pigs exposed to *Serratia indica* aerosol concentrations of 3×10^7 organisms per cubic foot released large numbers of organisms to the room air (*41*). Air washing after exposure to the aerosol is recommended before final caging of grouped animals. When only the head is exposed to the aerosol, it is our practice to wipe the head with a 2 to 3 percent solution of Lysol.

The importance of organisms excreted in the urine or feces must be considered in regard to infection of cage mates or the animal caretaker. An incomplete list of diseases in which the specific micro-organisms are excreted in the urine or feces of some laboratory animals includes anthrax, brucellosis, cholera, glanders, leptospirosis, lymphocytic choriomeningitis, melioidosis, plague, poliomyelitis, psittacosis, Q fever, salmonellosis, shigellosis, streptococcal infection, tetanus, tuberculosis, and tularemia. Many published experiments show that some of these organisms then become airborne.

Another bit of evidence that experimental animals are a potential source of infection for the experimenter may be found as the result of careful animal autopsy. This may reveal cross infection between two groups of animals, each inoculated with a different micro-organism and caged in the same room or air stream.

Table 6. History of occupational infection among 711 current laboratory personnel, Fort Detrick, Md.

Occupation	Percentage	
	Infected [1]	Not infected [1]
Trained scientific personnel	21	79
Laboratory technical assistants	18	82
Animal caretakers	12	88
Administrative and clerical	6	94
Maintenance employees [2]	6	94
Dishwashers	5	95
Janitors	0	100

[1] By the micro-organism under study in the laboratory.
[2] See table 5.

When the significance of the above experience is considered together with the effect of such variables as animal cage litter, humidity, number of organisms in the challenging aerosol, infectivity, virulence, and environmental stability of the micro-organism, augmentation of the test inoculum by inhalation or cannibalism, feeding, watering, cage-cleaning practices, and air movements, it is desirable to think critically about each step in the care of the test animals so that the methods may be suitable for the circumstances, not only for human safety but to safeguard experimental validity. Rather than test each total set of experimental conditions before proceeding with the research itself, it sometimes may be more safe and productive to use a system of individual caging, group caging for each challenging dose, ultraviolet irradiation barriers, or ventilated cages that will provide maximum security for the test and for the experimenter (*28, 41–47*).

Laboratory Rules and Procedures

The rules and techniques needed to handle pathogenic organisms safely are so varied, depending upon the agent, experiment, experimenter, and equipment, that it is not possible to do much more than refer to a few of the good reviews on the subject (*48–54*). Each laboratory contemplating formulation of a set of regulations would do well to examine these reviews and then adapt and adopt those that are suitable for the local situation. Some of the laboratory regulations most widely applicable to infectious agents are:

1. There will be no direct mouth pipetting of infectious or toxic fluids.
2. Pipettes will be plugged with cotton.
3. No infectious material will be blown out of pipettes.
4. No mixtures of infectious materials will be prepared by bubbling expiratory air through the liquid by a pipette.
5. Use an alcohol-soaked pledget around the

stopper and needle when removing a syringe and needle from a rubber-stoppered bottle.

6. Use only needle-locking hypodermic syringes.

7. Expel excess fluid and bubbles from a syringe vertically into a cotton pledget soaked with disinfectant or into a small bottle of cotton.

8. Before and after injection of an animal, swab the site of injection with a disinfectant.

9. Sterilize discarded pipettes and syringes in the pan into which they were first placed after use.

10. Before centrifuging, inspect tubes for cracks. Inspect the inside of the trunnion cup for rough walls caused by erosion or adhering matter. Carefully remove all bits of glass from the rubber cushion. A germicidal solution added between the tube and the trunnion cup not only disinfects the surfaces of both of these but also provides an excellent cushion against shocks that otherwise might break the tube.

11. Use centrifuge trunnion cups with screw caps or equivalent.

12. Avoid decanting centrifuge tubes. If you must decant, afterwards wipe off the outer rim with a disinfectant. Avoid filling the tube to the point that the rim becomes wet with culture.

13. Wrap a lyophilized culture vial with disinfectant-wetted cotton before breaking.

14. Never leave a discard tray of infected material unattended.

Table 7. Infection of cage-mate control animals

Etiologic agent of—	Animals	Aerosol challenge by—			Administration of challenge						
		Whole body	Head only	Nose, mouth	IP	SC	IV or IN	IM or IT	Oral	IC	ID or foot pad
Anthrax (*25, 26*)	Monkey	+	—	—	—	—	—	—	—	—	—
Anthrax (*25, 27*)	Guinea pig	+	—	+	0	—	—	—	—	—	—
Brucellosis (*28*)	Mice	0	—	—	—	—	—	—	—	—	—
Brucellosis (*29, 30*)	Guinea pig	+	0	—	—	—	—	—	—	—	—
Coccidioidomycosis (*31–34*) [1]	Monkey	—	0	—	—	+	—	—	—	—	—
Coccidioidomycosis (*31–34*) [1]	Guinea pig	—	[2] 0	[3] 0	0	—	—	[4] +	—	—	—
Coccidioidomycosis [1]	Dog	—	[2] +	—	—	—	—	—	—	—	—
Epidemic diarrhea (*25*)	Mice	—	—	—	—	—	—	—	[5] +	—	—
Infectious bronchitis (*25*)	Chicken	—	—	—	—	—	—	[4] +	—	—	—
Japanese B [1]	Mice	—	—	—	0	0	0	0	0	0	—
Meningopneumonitis (*28*)	Mice	+	—	—	—	—	—	—	—	—	—
Newcastle disease (*25*)	Chicken	+	—	—	—	—	—	—	—	—	—
Plague (*35*) [5]	Monkey	+	—	—	—	—	—	[4] +	—	—	—
Plague (*25, 36*)	Guinea pig	+	—	+	—	—	—	—	—	—	—
Poliomyelitis (*25*)	Monkey	—	—	—	—	—	[6] +	—	+	—	—
Psittacosis (*28*)	Mice	0	—	—	—	—	—	—	—	—	—
Q fever [1]	Guinea pig	+	—	—	—	—	—	—	—	—	—
Rift Valley fever [1]	Monkey	+	—	—	—	—	—	—	—	—	—
Rift Valley fever [1]	Mice	—	—	—	—	—	—	—	—	±	—
Rift Valley fever [1]	Lamb	—	—	—	0	—	—	—	—	—	—
Tuberculosis (*37*) [7]	Mice	—	—	—	—	—	+	—	—	—	—
Tuberculosis (*25*)	Guinea pig	+	—	—	+	+	—	—	—	—	—
Tularemia (*28*) [1]	Mice	0	—	—	0	0	—	—	—	—	—
Tularemia [1]	Guinea pig	+	0	—	0	0	—	—	—	—	—
Vaccinia (*38*)	Rabbit	—	—	—	—	—	—	—	—	—	[8] +
Vaccinia (*39*) [1]	Mice	—	—	—	0	0	0	—	0	0	0 [8] +
Variola (*40*)	Monkey	—	0	—	—	—	—	—	—	—	—
Vesicular stomatitis (*25*)	Mice	—	—	—	+	—	—	—	—	—	—
Venezuelan encephalitis (*25*)	Guinea pig	+	—	—	—	—	—	—	—	—	—
Venezuelan encepahlitis [1]	Monkey	—	[5] 0	—	—	—	—	—	—	—	—

[1] Experimental controls from various sources. [2] Challenge by dry arthrospores. [3] Challenge by wet and dry fragmented mycelia. [4] Intratracheal only. [5] Control animals in separate but adjacent cages. [6] IN only. [7] Wheater, D. W. F., and Russell, W.: Unpublished experiments. Imperial Chemical Industries, Ltd. Alderley Park, Macclesfield, Cheshire, England. [8] ID only.

NOTE: +=infection of cage-mate control, ±=available data inconclusive, 0=no infection of cage-mate control, —=no data. IP=intraperitoneal, SC=subcutaneous, IV=intravenous, IN=intranasal instillation, IM=intramuscular, IT=intratracheal, IC=intracerebral, ID=intradermal. Numbers in parentheses are references.

15. Sterilize all contaminated discard material.

16. Periodically clean deep freeze and dry ice chests in which cultures are stored to remove any broken ampoules or tubes. Use rubber gloves and respiratory protection during this cleaning.

17. Use rubber gloves when handling diagnostic serum specimens carrying a risk of infectious hepatitis.

18. Develop the habit of keeping your hands away from your mouth, nose, eyes, and face. This may prevent self-inoculation.

19. Avoid smoking, eating, and drinking in the laboratory.

20. Make special precautionary arrangements for oral, intranasal, and intratracheal inoculation of infectious material.

21. Give preference to use of operating-room gowns fastened at the back.

22. Evaluate the extent to which the hands may become contaminated. With some agents and operations, forceps or rubber gloves are advisable.

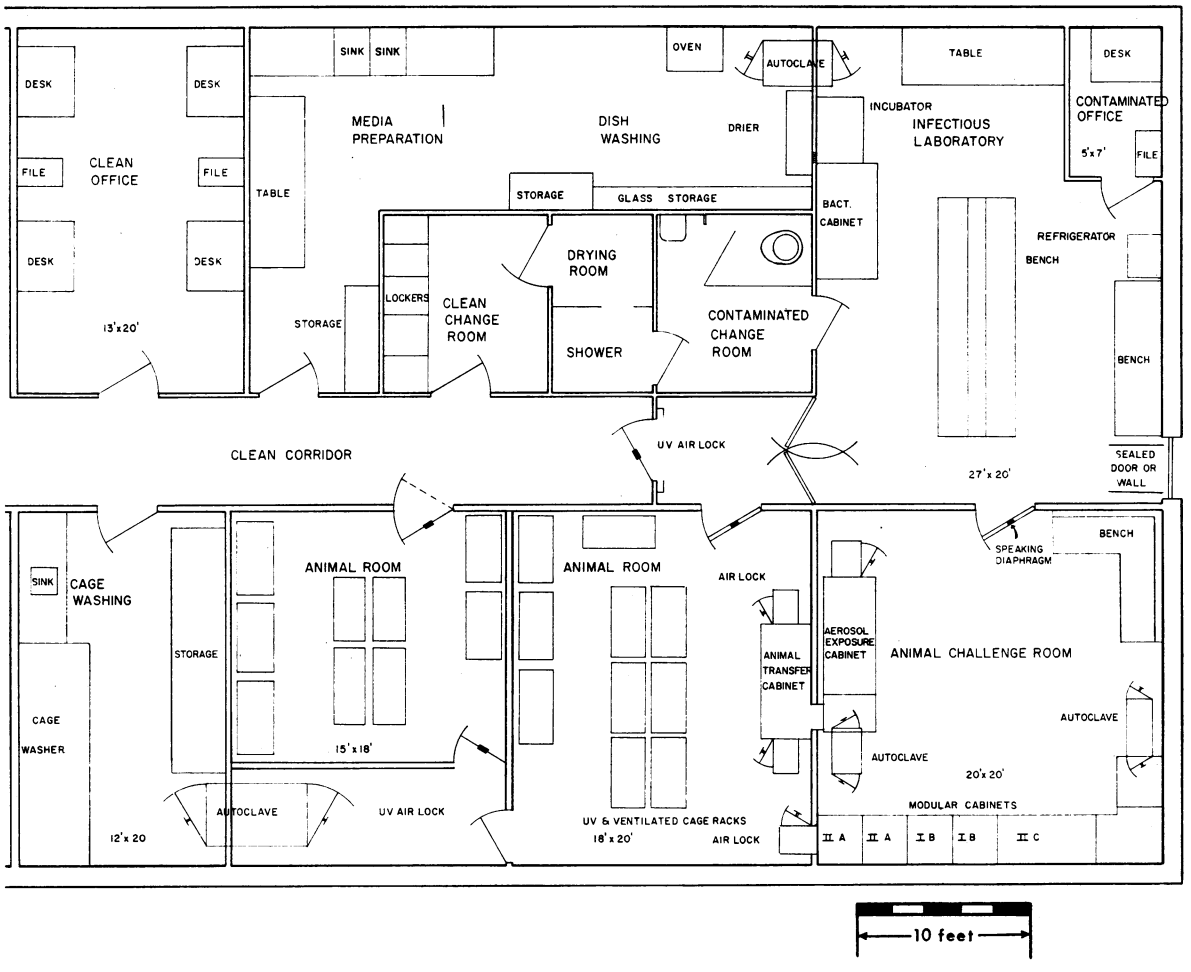
23. Wear only clean laboratory clothing in the dining room, library, and so forth.

24. Shake broth cultures in a manner that avoids wetting the plug or cap.

Building Design and Equipment

The design of the building and the equipment depend upon a preceding analysis and definition of the problem. Application to a specific laboratory will vary significantly with the microorganisms to be used, degree of protection by

Figure 1. Floor plan for laboratory experiments with aerosol-exposed animals



vaccination, type of experiments, experimental animals, volume of infectious material, experience and educational level of personnel, personalities, plans for the future, building structure, available or foreseeable equipment, finances, legal liability, and the extent to which political implications and public relations must be considered.

The details of floor plans and construction are so varied and voluminous as to prohibit their incorporation in this report. Figure 1 shows a small unit that will allow experiments with any organism using animals as large as monkeys. Other plans and suggestions have been reviewed and referenced elsewhere (*55*). For those concerned with the details of air filtration systems, as applied to microbiological laboratories, at least two excellent summaries are available (*56*, *57*). Their perusal is strongly recommended. Proposals and standards also are available for use of ultraviolet irradiation in the laboratory (*58–60*). A common deficiency in planning is failure to make advance policy decisions concerning the microorganisms, animals, and experiments that are to be permissible (*61*).

Table 8. Correlation of estimation of risk with recommendations for use of protective cabinets

Disease or agent	Cabinet system [1]	Single cabinets [2]	
	Aerosol studies	Aerosol studies	Other techniques
Brucellosis	+++		+++
Coccidioidomycosis	+++		+++
Russian spring-summer encephalitis	+++		+++
Tuberculosis	+++		+++
Monkey B virus	+++		++
Glanders	++	+++	+++
Melioidosis	++	+++	+++
Rift Valley fever	++	+++	+++
Arbo viruses, general		+++	++
Encephalitides, various		+++	++
Psittacosis	++	+++	++
Rocky Mountain spotted fever	++	+++	++
Q fever	++	+++	++
Typhus	++	+++	++
Tularemia	++	+++	++
Tularemia [3]		++	+
Venezuelan encephalitis [3]		+++	+
Anthrax	+++		+−
Botulism [3]	++	+++	+−
Histoplasmosis		+++	+−
Leptospirosis		+++	+−
Plague	+++		+−
Poliomyelitis	+++		+−
Rabies	+++		+−
Smallpox [3]	+++		+−
Typhoid		+++	0
Adeno and entero viruses		++	+−
Diphtheria [3]		++	0
Fungi, various		++	0
Influenza		+	+−
Meningococcus		++	0
Pneumococcus		++	0
Streptococcus		++	0
Tetanus [3]		++	0
Vaccinia [3]		++	0
Yellow fever [3]		++	0
Salmonellosis		+	+−
Shigellosis		+	+−
Infectious hepatitis			+−
Newcastle virus		+	0

[1] Fig. 2, 3, or equivalent. [2] Fig. 4. [3] For persons receiving live vaccine or toxoid.

NOTE: +++ mandatory; ++ strongly advised; + optional, but in absence of a cabinet a few infections will occur; +− depending upon technique and supervision; 0 not required.

Estimation of risk. To assist in answering some of the questions concerning policy (*61*) and to provide a basis for making other decisions and accepting or rejecting some of the suggestions referenced or outlined here and in table 8, an estimation of risk is offered for consideration. As a guideline, the following orders of decreasing magnitude of risk and decreasing complexity of precautionary measures are proposed for diseases of man and animals as studied in the laboratory.

1. Suitable for any type of experiment with any micro-organism and any animal up to the size of a chimpanzee.

2. Preparation of dry powders of infectious agents.

3. Dissemination of pathogenic microbial aerosols:

(*a*) Organisms highly infectious for man, producing a distressing disease for which there is an incompletely protective vaccine and only partially successful specific chemotherapy. The difficulty in treating such syndromes as pneumonic plague causes their aerosolized pathogenic agents to be included at this level of hazard, even though they are not as readily infective as the others.

(*b*) Organisms infectious for man, producing disease that is incapacitating but usually not serious when acquired in the laboratory, for which there is an incompletely protective vac-

Figure 2. Gastight cabinet system

cine and no specific chemotherapy. Although the organism of glanders is less infective and the disease may be treated with sulfadiazine, glanders should be included here because of the dangerous clinical syndrome produced.

(*c*) Toxins or organisms highly infectious for man, producing disease for which there is either effective vaccination or effective specific chemotherapy.

4. Laboratory studies not involving planned dissemination of aerosols. The subclassification would be the same as in 3 (*a*), (*b*), (*c*).

5. Dissemination of dry or fluid aerosols of organisms with comparatively low invasiveness, usually with no vaccine available, often subject to specific chemotherapeusis but sometimes capable of causing serious pneumonia. Staphylococcus, streptococcus, and pneumococcus are examples.

6. Laboratory studies not involving dry powders or planned dissemination of aerosols, with organisms of less serious risk because of various mitigating factors present to varying degrees, such as availability of vaccination, specific treatment, and low infectivity in the laboratory.

7. Minor infections:

(*a*) Nuisance diseases such as Newcastle virus conjunctivitis.

(*b*) Organisms seldom causing laboratory infection such as pneumococcus, streptococcus, staphylococcus, meningococcus, vaccinia virus, and diphtheria and tetanus bacilli.

8. Classroom demonstrations or student work with killed, stained preparations or with attenuated strains.

Complexity of equipment. The foregoing estimates of risk can be correlated with specific recommendations about procedures and equipment. Such correlations, in regard to use of protective cabinets, are shown in table 8.

Dry powders of infectious organisms are best handled in a continuous line of gastight cabinets, so constructed that materials or containers leaving the system are autoclaved or sterilized with gas (fig. 2). A germicidal trap or "dunk

bath" holding liquid disinfectant, through which an exiting container is passed under a baffle plate that prevents entry of air, cannot be depended upon to kill adherent dried microbial powder, although such tanks are reasonably effective against other contamination.

As a universal germicide for sterilizing the exterior surfaces of items as they are removed through an air lock, a 2 percent spray of peracetic acid is best, although its corrosiveness and toxicity require use of a subsequent thorough wash with water. If items are removed through a dunk bath, 0.5 percent sodium hypochlorite with 0.1 percent Nacconol (sodium alkyl aryl sulfonate) is a good universal disinfectant. We have used 5 percent phenol or 2 percent Roccal (alkyl dimethyl benzyl ammonium chloride) in this connection when items have been contaminated with liquid or dried vegetative-type pathogens. Scrubbing the items beforehand and as they pass through the dunk bath greatly improves the chances of achieving sterility.

Generation of infectious aerosols is safest in the gastight cabinet system, but variations are permissible, depending upon the organism. For instance, the dynamic aerosol system of Henderson (*62*) may be housed in a single ventilated cabinet with an ultraviolet-irradiated air lock for passage of animals and materials. Disinfectant may be applied in the air lock to exiting articles by an attached rubber glove. This arrangement is less suitable for anthrax and brucellosis organisms because their environmental resistance makes it hard to secure adequate decontamination of articles leaving the cabinet. The equipment is safer if an autoclave equipped for gas or steam sterilization is put at one end and an air lock at the other end. In the absence of the autoclave an unimmunized operator is likely to acquire tularemic infection if he does not wear an effective respirator or gas mask. It is at this point that diligent attention to technique becomes very important.

Animals challenged with infectious aerosols can be kept in the cabinet system or removed by means of a transfer box in which the attachment neck can be flushed out with germicide. Our tests show that retention of the germicide is necessary for 15 to 20 minutes under these operating conditions. After challenge, removal of the animals through an air lock is risky. After their noses or heads have been wiped with disinfectant and the animals air washed, they may exit in a tight cage or tight transfer box if they have to be carried any distance to the animal room.

A better method is to place the aerosol generator and cabinet so the animals may be passed directly through the cabinet and adjoining wall into the animal room, where they can be received by an animal attendant protected by a ventilated headpiece or gas mask (fig. 3). The animals can then be placed in ventilated cages or in open-top cages on ultraviolet-irradiated racks (*41*). The hazards associated with removing things from aerosol chambers were the most frequent known causes of infection during our aerobiological studies.

Our experience has led us to install an increasing number of gastight cabinets joined, to replace an equal or lesser amount of work space composed of separated individual cabinets.

Monkeys, especially chimpanzees, and larger animals difficult to handle in closed cabinet systems after aerosol challenge can safely be challenged, housed, and examined in an isolation

Figure 3. Caging system for aerosol-exposed animals

room if proper safeguards are available. These include a ventilated impermeable suit, ventilated head hood or gas mask for the workers, and isolation of the room by negative air pressure, air filters or air incinerator, ultraviolet irradiation of the room, and an ultraviolet air lock. A clothes change room may be needed or a germicidal shower for the ventilated suit. Peracetic acid (*63*) is recommended for such a shower. The use of isolation rooms is facilitated by the suitability of B-propiolactone (*64*, *65*) for disinfection of rooms and buildings and of ethylene oxide (*66*) for delicate instruments otherwise injured by autoclaving. Both chemicals are toxic and the recommended precautions (*65*) should be followed carefully. Steam formaldehyde is effective (*67*). Sedation of the monkey with Sernyl [piperidine, 1-(1-phenylcyclohexyl), Parke-Davis Co.] or a similar drug, 5 minutes before handling, by intramuscular injection of 1 mg. of drug per kg. of body weight, will reduce bites, scratches, and other accidents.

Before and after the actual experimental aerosolization of the culture, the usual laboratory manipulations of opening test tubes, flasks and bottles, pipetting, diluting and plating, inoculating and autopsying animals, grinding, operating a sonic vibrator, inoculating and harvesting eggs, and forced aerating of cultures all liberate enough organisms of some species to infect man.

To control these hazards and many others, the most useful, versatile, and effective piece

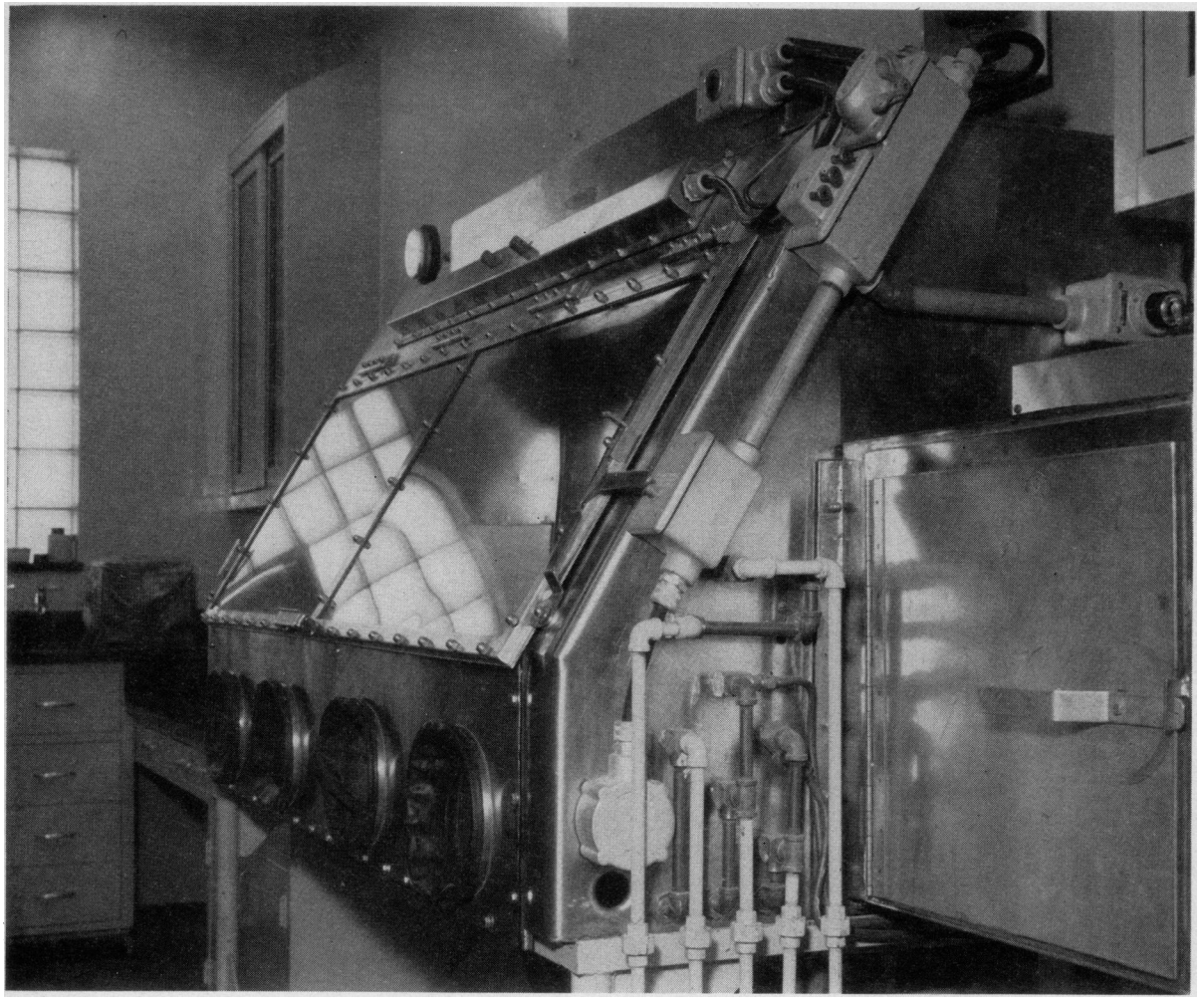


Figure 4. Microbiological safety cabinet

of equipment is the microbiological safety cabinet, also known as a safety hood, autopsy cabinet, ventilated work cabinet, glove box, or control cabinet (fig. 4). These have been made in a great variety of designs, but the more desirable features are:

1. Sufficient inward air flow at 50 to 100 linear feet per minute for an open-front cabinet, or approximately 1 inch of water negative pressure for a gastight cabinet, to prevent escape of airborne particulates created by procedures in the cabinets.

2. A glass or clear plastic viewing panel between the operator and the operation.

3. A filter, incinerator, or other device to remove or destroy micro-organisms in the exhaust air.

4. Internal cabinet surfaces resistant to chemical corrosion and heat, free of cracks or crevices that interfere with decontamination, constructed to withstand liquid or gaseous sterilization.

5. Ample working space that will minimize the need to remove contaminated material before completion of an operation.

6. A front panel, where the hands or attached gloves enter the cabinet, that can be closed during highly hazardous work or during decontamination.

7. Appropriate services such as electricity, gas, vacuum, air, light, ultraviolet irradiation, water, drain.

8. A ledge or lip at the front of the working surface to prevent spilled liquid or disinfectant from running out of the cabinet.

9. A pass-through box, air lock, dunk bath, gas chamber, or autoclave at one or both ends of the cabinet, or a blank plate that can be removed to permit such an attachment, for passage of materials in or out.

10. In a gastight cabinet, an air inlet filter with a valve or other airtight closure on the air inlet side of the filter housing is needed to maintain controlled air flow. An "absolute" filter with 99.99 percent bacterial filtering efficiency is recommended.

11. Cabinets with a removable glove port panel or equivalent opening for the hands, not gastight and operated without attached gloves, are best located at the back end of a room or in an L-shaped area to minimize the effect of extraneous air currents created by passers-by or by doors being opened, which may cause temporary back drafts of potentially infectious air to leave the protective cabinet.

The most comprehensive collection of photographs of these cabinets illustrating their many variations may be found in a recent extensive review of microbiological safety (*52*). An excellent summary on cabinets and miscellaneous other devices and procedures has been published (*48*).

The open bench top is suitable for aerosol studies of some micro-organisms if scrupulous attention is paid to safety details. The Henderson apparatus (*62*) or other aerosol generators of comparable size may be operated in the open if the operators use adequate respiratory protection and isolate the room. Such isolation should include negative air pressure in the room relative to adjoining spaces, provision for periodic decontamination of the entire room, removal of micro-organisms from exhaust air, and an air lock or barrier system of some sort at entrance to the room.

In many instances the open bench top likewise may serve during the pre- and post-aerosolization microbiological procedures (*50*, *53*). However, it is my opinion that there is greater risk than when a control cabinet is used.

Accessory Equipment

When preparing materials for aerosolization or during their examination afterwards, not always can all the equipment be conveniently operated in a control cabinet.

The centrifuge. Entirely aside from breakage of centrifuge tubes, normal operation of the centrifuge may produce microbial aerosols. As with so many other matters, attention to the fine points of technique makes the difference between hazard and safety. If the rim of the tube is wet with culture fluid, this culture is thrown out as an aerosol from either a smooth rim or from the liquid trapped between screw threads when a screw-capped tube is used (*68*). In a horizontal-type centrifuge, some of the liquid escaping from a tube or bottle cap runs down into the trunnion cup, but in an angle head more of it is flung into the air. For this reason, it has been suggested that it is undesirable to use an angle centrifuge for the routine

preparation of sputum concentrates (*49*). Table-model centrifuges enclosed in ventilated ultraviolet-irradiated boxes are used in some Swedish laboratories (*52*). Sometimes small centrifuges can be moved temporarily into a general purpose control cabinet. More elaborate housings are available for the refrigerated centrifuge (*69*). The Sharples super-centrifuge must be enclosed because of the aerosol generated.

Some interesting studies could be done to see how often trunnion cups and the centrifuge bowl should be sterilized. Much would depend on local circumstances.

The shaker. Use of a shaking machine for aerating flasks or tubes of culture by oscillation sooner or later results in breakage and aerosolization. Because the accident usually is not detected immediately, a considerable aerosol may be formed. This matter has not been studied as a simulated accident. Because of the obvious danger, various precautionary arrangements sometimes are used. Shakers may be enclosed in a ventilated cabinet, with or without ultraviolet irradiation (*48*, *69*). A simpler method, especially useful for small flasks, is to put the flasks, fixed in place by clips, springs, or rubber cut-out placements, into a leak-proof metal box covered with a fiberglass air filter and a viewing glass. Inspection before opening will determine whether there is breakage or a fallen plug or stopper that requires precautionary action. When shakers are in walk-in incubators the flasks can be placed in a leak-proof metal box on or surrounded by a disinfectant-soaked absorbent material.

The lyophilizer. The chief hazards associated with the lyophilizer (*70*) occur at the rubber sleeves and manifold outlets, at which points the hands or rubber gloves of the technician become grossly contaminated. Aerosols form when the dry product is handled or a tube of dried culture is opened or dropped. During the process of lyophilization the apparatus becomes contaminated internally and, in the absence of a filter in the air exhaust line to the vacuum pump, so presumably does the oil of the vacuum pump. The apparatus should be operated in a closed cabinet or designed so it can be sterilized without dismantling if it has been used with dangerous pathogens.

Medical Program

The number of infections known to occur during aerobiological research is the result of a combination of factors, such as infectiousness of the organism, number of persons potentially exposed, precautions taken, thoroughness of medical investigations of illness in laboratory personnel, and completeness of reporting.

The extent, quality, and easy availability of medical attention given without cost to personnel is an important element in recognition of laboratory-acquired illness. By law in many countries, occupational illness receives free medical care. The medical staff should provide vaccination, the necessary medical examinations, and precautionary hospitalization not only without charge to the employee but without loss of regular pay or of accrued vacation time. Otherwise, the employee is disinclined to report what in the early stages of disease appears to be a minor respiratory infection. A good policy is to regard every illness in an occupationally exposed person to be occupational until medically proved to be otherwise. This may not always be necessary or desirable, depending principally upon the organism under study. But it is mandatory, for instance, when conducting research on experimental pulmonary anthrax or experimental pneumonic plague. In the latter instance, antibiotic treatment delayed 24 hours after onset of the initial apparently trivial symptoms often results in death of the patient. Pulmonary anthrax likewise requires the earliest possible specific treatment.

Routine periodic serologic examination. When technically possible, serologic examination is recommended for all personnel as a means of revealing a more accurate infection rate and a correspondingly truer measure of the effectiveness of the control measures. Otherwise a subclinical infection may be unnoticed or passed off as a severe cold or mild attack of influenza. For those contemplating aerobiological research with an infectious agent producing a serious disease for which there is a poor vaccine or no vaccine and poor treatment, there is no better test than to go through the proposed procedures for a few weeks with virulent *Pasteurella tularensis* using unimmunized personnel. Hemagglutinin titers will detect the subclinical infec-

tions. Streptomycin therapy will control the clinical disease but will leave a wholesome respect for the potential hazards accompanying the research process.

REFERENCES

(*1*) Sulkin, S. E., and Pike, R. M.: Survey of laboratory-acquired infections. Amer J Public Health 41: 769–781 (1951).

(*2*) Wedum, A. G.: Policy, responsibility, and practice in laboratory safety. *In* Proceedings, Second Symposium on Gnotobiotic Technology. Notre Dame, Ind., University of Notre Dame Press, 1959, pp. 105–119.

(*3*) Wedum, A. G.: Bacteriological safety. Amer J Public Health 43: 1428–1437 (1953).

(*4*) Wolf, H. W., et al.: Sampling microbial aerosols. PHS Publication No. 686 (Public Health Monograph No. 60). U.S. Government Printing Office, Washington, D.C., 1959.

(*5*) Kruse, R. H.: Potential aerogenic laboratory hazards of *Coccidioides immitis*. Amer J Clin Pathol 37: 150–158 (1962).

(*6*) DuBuy, H. G., Hollaender, A., and Lackey, M. D.: A comparative study of sampling devices for air-borne microorganisms. Public Health Rep Supp. No. 184. U.S. Government Printing Office, Washington, D.C., 1945.

(*7*) Huddleson, I. F., and Munger, M.: A study of an epidemic of brucellosis due to *Brucella melitensis*. Amer J Public Health 30: 944–954 (1940).

(*8*) Smith, C. E.: The hazard of acquiring mycotic infections in the laboratory. Address delivered before the Epidemiology and Laboratory Sections, American Public Health Association annual meeting, November 2, 1950, St. Louis, Mo. Mimeographed.

(*9*) Shaw, E. W., Melnick, J. L., and Curnen, E. C.: Infection of laboratory workers with Coxsackie viruses. Ann Intern Med 33: 32–40 (1950).

(*10*) Rivers, T. M., and Schwentker, F. F.: Louping ill in man. J Exp Med 59: 669–685 (1934).

(*11*) Loffler, W., and Mooser, H.: Mode of transmission of typhus fever; study based on infection of group of laboratory workers. Schweiz Med Wschr 72: 755–761 (1942).

(*12*) Huebner, R. J.: Report of an outbreak of Q fever at the National Institutes of Health. Amer J Public Health 37: 431–440 (1947).

(*13*) Barbeito, M. S., Alg, R. L., and Wedum, A. G.: Infectious bacterial aerosol from dropped petri dish cultures. Amer J Med Technol 27: 318–322 (1961).

(*14*) Slepushkin, A. N.: Epidemiological study of a laboratory infection with the Venezuelan equine encephalomyelitis virus. Vop Virus (USSR) 4: 311–314 (1959).

(*15*) Boyd, M. F., and Kitchen, S. F.: On attempts to hyperimmunize convalescents from vivax malaria. Amer J Trop Med 23: 209–225 (1943).

(*16*) Tigertt, W. D., and Benenson, A. S.: Studies on Q fever in man. Trans Assoc Amer Physicians 69: 98–104 (1956).

(*17*) McCullough, N. B., and Eisele, C. W.: Experimental human salmonellosis. J Infect Dis 88: 278–289 (1951).

(*18*) Ley, H. L., Jr., Smadel, J. E., Diercks, F. H., and Paterson, P. Y.: Immunization against scrub typhus. V. The infective dose of *Rickettsia tsutsugamushi* for men and mice. Amer J Hyg 56: 313–319 (1952).

(*19*) Magnuson, H. J., et al.: Inoculation syphilis in volunteers. Medicine 35: 33–82 (1956).

(*20*) Saslaw, S., et al.: Tularemia vaccine study. I. Intracutaneous challenge. II. Respiratory challenge. Arch Intern Med 107: 689–714 (1961).

(*21*) Smith, D. G., Mamay, H. K., Marshall, R. G., and Wagner, J. C.: Venezuelan equine encephalomyelitis. Amer J Hyg 63: 150–164 (1956).

(*22*) Sutton, L. S., and Brooke, C. C.: Venezuelan equine encephalomyelitis due to vaccination in man. JAMA 155: 1473–1476 (1954).

(*23*) Southam, C. M., and Moore, A. E.: Induced virus infections in man by the Egypt isolates of West Nile virus. Amer J Trop Med 3: 19–50 (1954).

(*24*) Pike, R. M., and Sulkin, S. E.: Occupational hazards in microbiology. Sci Monthly 75: 222–227 (1952).

(*25*) Kirchheimer, W. F., Jemski, J. V., and Phillips, G. B.: Cross-infection among experimental animals by organisms infectious for man. Proc Animal Care Panel 11: 83–92 (1961).

(*26*) Phillips, G. B., Jemski, J. V., and Brant, H. G.: Cross infection among animals challenged with *Bacillus anthracis*. J Infect Dis 99: 222–226 (1956).

(*27*) Druett, H. A., Henderson, D. W., Packman, L., and Peacock, S.: Studies on respiratory infection. I. The influence of particle size on respiratory infection with anthrax spores. J Hyg 51: 359–371 (1953).

(*28*) Rosebury, T.: Experimental air-borne infection. Baltimore, Williams & Wilkins Co., 1947.

(*29*) Phillips, G. B., Broadwater, G. C., Reitman, M., and Alg, R. L.: Cross infections among brucella-infected guinea pigs. J Infect Dis 99: 56–59 (1956).

(*30*) Elberg, S. S., and Henderson, D. W.: Respiratory pathogenicity of brucella. J Infect Dis 82: 302–306 (1948).

(*31*) Blundell, G. P., Castleberry, M. W., Lowe, E. P., and Converse, J. L.: The pathology of *Coccidioides immitis* in the Macaca mulatta. Amer J Pathol 39: 613–630 (1961).

(*32*) Castleberry, M. W., Converse, J. L., and Del Favero, J. E.: Coccidioides transmission to in-

fant monkey from its mother. Arch Pathol 75: 459–461 (1963).

(*33*) Rosenthal, S. R., and Elmore, F. H.: Studies on the contagiousness of coccidioidomycosis. Amer Rev Tuberc 61: 106–115 (1950).

(*34*) Jacobson, H. P.: Coccidioidal granuloma. Calif. Western Med 29: 392–396 (1928).

(*35*) Meyer, K. F., and Larson, A.: The pathogenesis of cervical septicemic plague developing after exposure to pneumonic plague produced by intratracheal infection in primates. *In* Symposium proceedings, Haffkine Institute Diamond Jubilee, 1959. New Jack Printing Works Pvt. Ltd., Bombay, India, 1960, pp. 1–12.

(*36*) Druett, H. A., et al.: Studies on respiratory infection. II. Influence of aerosol particle size on infection of the guinea pig with *Pasteurella pestis*. J Hyg 54: 37–48 (1956).

(*37*) Gray, D. F., and Mattinson, M. W.: Detection of small numbers of tubercle bacilli from dispersed cultures, using mice, guinea pigs, and artificial media. Amer Rev Tuberc 65: 572–588 (1952).

(*38*) Duran-Reynals, F.: On the spontaneous immunization of rabbits to vaccine virus. J Immunol 20: 389–391 (1931).

(*39*) Briody, B. A.: Response of mice to ectromelia and vaccinia viruses. Bact Rev 23: 61–95 (1959).

(*40*) Hahon, N., and McGavran, M. H.: Air-borne infectivity of the variola-vaccinia group of poxviruses for the cynomolgus monkey, *Macaca irus*. J Infect Dis 109: 294–298 (1961).

(*41*) Phillips, G. B., Reitman, M., Mullican, C. L., and Gardner, G. D.: Applications of germicidal ultraviolet in infectious disease laboratories. III. The use of ultraviolet barriers on animal cage racks. Proc Animal Care Panel 7: 235–244 (1957).

(*42*) Gremillion, G. G.: The use of bacteria-tight cabinets in the infectious disease laboratory. *In* Proceedings Second Symposium on Gnotobiotic Technology. Notre Dame, Ind., Notre Dame Press, 1959, pp. 171–182.

(*43*) Horsfall, F. L., and Bauer, J. H.: Individual isolation of infected animals in a single room. J Bact 40: 569–580 (1940).

(*44*) Jemski, J. V.: Maintenance of monkeys experimentally infected with organisms pathogenic for man. Proc Animal Care Panel 12: 89–98 (1962).

(*45*) Lind, A.: Danger of TB infection to personnel in pathological laboratories. *In* Transactions NAPT Commonwealth Chest Conference, National Association Prevention of Tuberculosis, Session No. 7, 1958. Tavistock House North, Tavistock Square, London W.C.1, England, 1959.

(*46*) Wolochow, H., Chatigny, M., and Speck, R. S.: Studies on the experimental epidemiology of respiratory infections. VII. Apparatus for the exposure of monkeys to infectious aerosols. J Infect Dis 100: 48–57 (1957).

(*47*) Imperial Chemical Industries, Ltd.: Pharmaceutical research. Pharmaceuticals Division, Alderley Park, Alderley Edge, Macclesfield, Cheshire, England, 1957.

(*48*) Chatigny, M. A.: Protection against infection in the microbiological laboratory: Devices and procedures, advances in applied microbiology. Vol. 3. Academic Press, New York, 1961, pp. 131–192.

(*49*) Precautions against tuberculous infection in the diagnostic laboratory. Committee report. Monthly Bull Minist Health (London) 17: 10–18 (1958).

(*50*) Long, E. R.: The hazard of acquiring tuberculosis in the laboratory. Amer J Public Health 41: 782–787 (1951).

(*51*) Morris, E. J.: A survey of safety precautions in the microbiological laboratory. J Med Lab Techn 17: 70–82 (1960).

(*52*) Phillips, G. B.: Microbiological safety in U.S. and foreign laboratories. (Technical Study No. 35.) Industrial Health and Safety Division, U.S. Army Biological Laboratories, Frederick, Md., September 1961.

(*53*) Smadel, J. E.: The hazard of acquiring virus and rickettsial diseases in the laboratory. Amer J Public Health 41: 788–795 (1951).

(*54*) U.S. Navy Medical News Letter: Safety measures in the tuberculosis laboratory. AMA Arch Ind Health 13: 522–524 (1956).

(*55*) Wedum, A. G.: Control of airborne infection. Bact Rev 25: 210–216 (1961).

(*56*) Gilbert, H., and Palmer, J. H.: High efficiency particulate air filter units. U.S. Atomic Energy Commission, 1961.

(*57*) Becker, H. M., Buchanan, L. M., Hall, L. B., and Goddard, K. R.: Air filtration of microbial particles. PHS Publication No. 953. U.S. Government Printing Office, Washington, D.C., 1962.

(*58*) Hoffman, J. C., and Nagy, R.: Engineering fundamentals of air sanitation with ultraviolet light. Air Eng 2: 30–36, 43–45, 46–50 (1960).

(*59*) Wedum, A. G., Hanel, E., Jr., and Phillips, G. B.: Ultraviolet sterilization in microbiological laboratories. Public Health Rep 71: 331–336 (1956).

(*60*) Hart, D.: Bactericidal ultraviolet radiation in the operating room. JAMA 172: 1019–1028 (1960).

(*61*) Wedum, A. G., and Phillips, G. B.: Criteria for design of a microbiological research laboratory. J Amer Soc Heart Refrig Air Condit 6: 46–52, February 1964.

(*62*) Roessler, W. G., and Kautter, D. A.: Modifications to the Henderson apparatus for studying air-borne infections. J Infect Dis 110: 17–22 (1961).

(63) Trexler, P. C.: The sterilization of clean protective clothing with peracetic acid and beta-propiolactone. Bact 1958: 35 (1958).
(64) Allen, H. F., and Murphy, J. T.: Sterilization of instruments and materials with beta-propiolactone. JAMA 172: 1759–1763 (1960).
(65) Spiner, D. R., and Hoffman, R. K.: Method for disinfecting large enclosures with beta-propiolactone vapor. Appl Microbiol 8: 152–155 (1960).
(66) Schley, D. G., Hoffman, R. K., and Phillips, C. R.: Simple improvised chambers for gas sterilization with ethylene oxide. Appl Microbiol 8: 15–19 (1960).
(67) The practical aspects of formaldehyde fumigation. Committee report. Monthly Bull Minist Health (London) 17: 270–273 (1958).
(68) Whitwell, F., Taylor, P. J., and Oliver, A. J.: Hazards to laboratory staff in centrifuging screw-capped container. J Clin Pathol 10: 88–91 (1957).
(69) Reitman, M., and Wedum, A. G.: Microbiological safety. Public Health Rep 71: 659–665 (1956).
(70) Reitman, M., et al.: Potential infectious hazards of laboratory techniques. I. Lyophilization. II. The handling of lyophilized cultures. J Bact 68: 541–544, 545–548 (1954).

PUBLICATION ANNOUNCEMENTS

Address inquiries to publisher or sponsoring agency.

Young Adults and Their Parents. Public Affairs Pamphlet No. 355. By Harry Milt. January 1964; 28 pages; 25 cents. Public Affairs Pamphlets, 381 Park Ave. South, New York, N.Y., 10016.

Family Therapy—Help for Troubled Families. Public Affairs Pamphlet No. 356. By George Thorman. February 1964; 20 pages; 25 cents. Public Affairs Pamphlets, 381 Park Ave. South, New York, N.Y., 10016.

New Approaches to Mental Health Treatment for Labor and Low Income Groups. A survey. Report No. 2. By Frank Riessman. February 1964; 29 pages. National Institute of Labor Education Mental Health Program, 250 West 57th St., New York 19, N.Y.

The Rockefeller Foundation President's Review, 1963. 1964; 87 pages. The Rockefeller Foundation, 111 West 50th St., New York 20, N.Y.

Radiation Levels. Air, water and food. Symposium. By A. S. McLean, S. G. Burgess, E. Windle Taylor, R. Scott Russell, and E. R. Mercer. 1964; 57 pages; $2.25. The Royal Society of Health, 90 Buckingham Palace Road, London, S.W.1 England.

Maryland Department of Mental Hygiene. Annual statistical report for the fiscal year ending June 30, 1963. 1964. 51 pages. Maryland Department of Mental Hygiene, State Office Building, Baltimore 1, Md.

Standards and Recommendations for Hospital Care of Newborn Infants. 1964; 89 pages; $1.50. American Academy of Pediatrics, 1801 Hinman Ave., Evanston, Ill., 60204.

Dialogues. Behavioral science research approaches to selected mental health problems. Edited by Jerome Levy and Robert M. Hunter. November 1963; 79 pages. Western Interstate Commission for Higher Education. University East Campus, University of Colorado, Boulder, Colo.

A Clinical Approach to the Problems of Pastoral Care. Edited by Jerome Levy and Roma K. McNickle. January 1964; 108 pages. Western Interstate Commission for Higher Education, University East Campus, Boulder, Colo.

Mental Health Data Collection in the West. Problems of communication and professional development. Edited by Jerome Levy and Robert M. Hunter. December 1963; 78 pages. Western Interstate Commission for Higher Education. University East Campus, University of Colorado, Boulder, Colo.

Cancer Registration and Survival in California. 1963; 406 pages. California Tumor Registry, California Department of Public Health, 2151 Berkeley Way, Berkeley 4, Calif.

Essentials for Hospital Auxiliaries. 1963; $1.50. United Hospital Fund of New York, 3 East 54th Street, New York, N.Y.

World Health Organization

WHO publications may be obtained from the Columbia University Press, International Documents Service, 2960 Broadway, New York, N.Y.

Social Aspects in the Teaching of Obstetrics and Gynaecology. Fourth report of the WHO Expert Committee on Maternal and Child Health. WHO Technical Report Series No. 266. 1963; 22 pages; 30 cents; Geneva.

International Standards for Drinking-Water. Second edition. 1963; 206 pages; $5.25; Geneva.

Bibliography on Yaws, 1905–1962. 1963; 106 pages; $2.75; Geneva.

Bibliography on the Epidemiology of Cancer, 1946–1960. 1963; 168 pages; $3.25; Geneva.

Specifications for Reagents Mentioned in the International Pharmacopoeia. 1963; 267 pages; $6; Geneva.