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TECHNICAL MANUSCRIPT 39

RESPIRATORY EXPOSURE OF ANIMALS TO MICROORGANISMS WITH THE HENDERSON APPARATUS

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Orrin T. Miller

Gardner G. Gremillion

Safety Division
OFFICE OF THE SAFETY DIRECTOR

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FOREWORD

A study of the behavior of infectious disease agents as aerosols is basic to a comprehensive understanding of infectious disease processes. However, the equipment necessary for such studies is often beyond the resources of the small research laboratory. The Henderson Apparatus, as described here, provides a safe, simple means of conducting significant aerobiological research at a cost within the reach of many small laboratories.

ABSTRACT

The Henderson Apparatus has been in use for many years for the study of infectious disease transmission by the respiratory route. It is a simple, inexpensive apparatus for exposure of small animals to dynamic aerosols. This report presents detailed information concerning necessary equipment and techniques for conducting this type of experiment in a safe manner. References are made to commercial sources of equipment where these are pertinent.

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I. INTRODUCTION

The Henderson apparatus[1,2,3] is a device for studying the infectivity and virulence of microorganisms suspended in air as particulates. The apparatus has been named for Dr. David W. Henderson, MRE, Porton, England, who helped develop the original apparatus and introduced it to Fort Detrick workers during World War II.

The transmission of infectious diseases by air-borne microorganisms is well recognized, and consequently, animal aerosol exposure systems are coming into widespread use. Most equipment for studying air-borne microorganisms is large, expensive, and complex in operation. In contrast, the Henderson Apparatus is relatively small, simple, inexpensive, and easy to operate. The use of the Henderson Apparatus is limited to the study of dynamic aerosols. That is, the aerosol is generated in an air stream at one end of the apparatus and is then carried past the experimental animals and through the sampling station. The stability of microorganisms suspended in air, their decay rates,* settling rates, and other conditions requiring time cannot be studied in the apparatus.

The first model (Model 1) of the Henderson Apparatus was equipped with one animal exposure port and operations were carried out with the apparatus mounted on a bench or portable table. Hemostats were used to open and close each of the four hose connections at the sampling point in the circuit. Safety for the operators was obtained by the use of respirators, protective clothing, rubber gloves, etc.

Since the first models were introduced, a number of minor changes have been made by various workers. During the past few years, Fort Detrick engineers have studied the Henderson Apparatus carefully and as a result a number of major modifications have been made, resulting in Models 2 and 3. The four-port exposure tube and supporting equipment have been placed in ventilated cabinets for the protection of operating personnel. The hemostats have been replaced with valves and the manometers have been replaced with gauges. All other equipment, such as the animal holding devices, has been redesigned to improve operating techniques and to promote safety. In its final form, the animal exposure apparatus is a versatile, efficient mechanism for the study of infectious air-borne microorganisms with an acceptable degree of safety.

Auxiliary equipment and techniques that can be used with the Henderson Apparatus, such as ventilated animal cages and ultraviolet cage racks, air filters, and methods of decontamination with steam-formaldehyde, have also been developed during this time.

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* Decay Rate - The absolute reduction in numbers of microorganisms suspended in the air. A combination of settling and death of the microorganisms.

Model 2 (Figure 1) is a recirculating unit with filters, vacuum pumps, and an air drier mounted beneath the cabinet. The Henderson Apparatus is permanently fixed within the cabinet. Outside services required for operation are electrical power and compressed air.

Model 3 (Figure 2) was developed to reduce cost, to simplify construction and operation, and to give flexibility so that the apparatus can be readily mounted and used in any ventilated cabinet. Model 3 was designed as a nonrecirculating system with standard laboratory air pressure and vacuum being supplied to the system. The spray head, exposure tube, animal holders, and air samplers are identical in the two units. Although the Model 3 Henderson can be used in any ventilated cabinet, the most suitable cabinet for use with either model is the ventilated cabinet designed specifically for housing the Henderson Apparatus.

II. THE CABINET

The ventilated cabinet for housing the Henderson Apparatus (Models 2 and 3) is constructed of stainless steel and is 68 inches long by 40 inches high by 26 inches deep with a three-piece sloped front and a straight back (Figures 1 and 2). There are glass[a] or plastic[b] viewing panels in the upper slope and two sets of glove ports in the middle slope; the lower portion is a solid panel. All piping enters the cabinet through gasketed connections or through lines welded into the cabinet walls. The cabinet has four removable panels, two on the back and one on each end. These panels permit the attachment of autoclaves, air locks, pass-through tunnels, or other cabinets (Section VII).

Exhaust air from the cabinet is usually filtered through a standard 300-cfm bacteriological cabinet filter[c] before the air is discharged into the building exhaust system.[4] A vacuum gauge[d] is mounted on the cabinet to show that one half to one inch of water negative air pressure is being maintained within the closed cabinet. Germicidal ultraviolet radiation and spot or fluorescent lights are provided. A fixed steam-formaldehyde injector system may be installed in the cabinet for sterilizing the interior of the cabinet on completion of operations.

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a. Heat-tempered glass is used. One type is Herculite glass, made by Pittsburgh Plate Glass Company, Pittsburgh, Pennsylvania.
b. HT-CR-39, Cast Optics Corp., Hackensack, N.J.
c. Two layers of 50 FG (PF 105) filter medium are placed on each side of the filter frame. Filter medium in the cabinet air exhaust filter is sterilized and replaced yearly.
d. Magnehelic, F.W. Dwyer Mfg. Co., Chicago, Illinois.

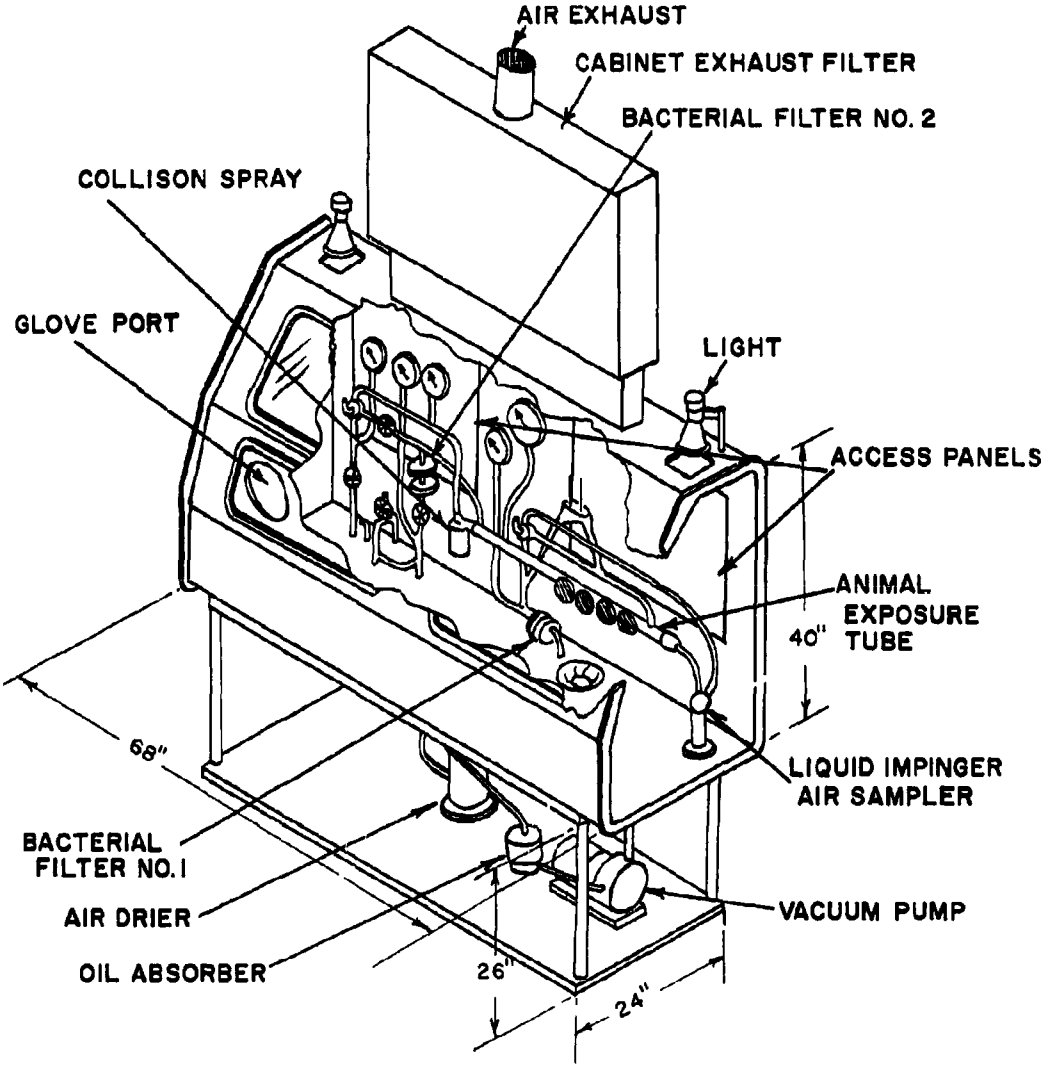

Figure 1. Henderson Apparatus, Model 2 Installed in Special Ventilated Cabinet.

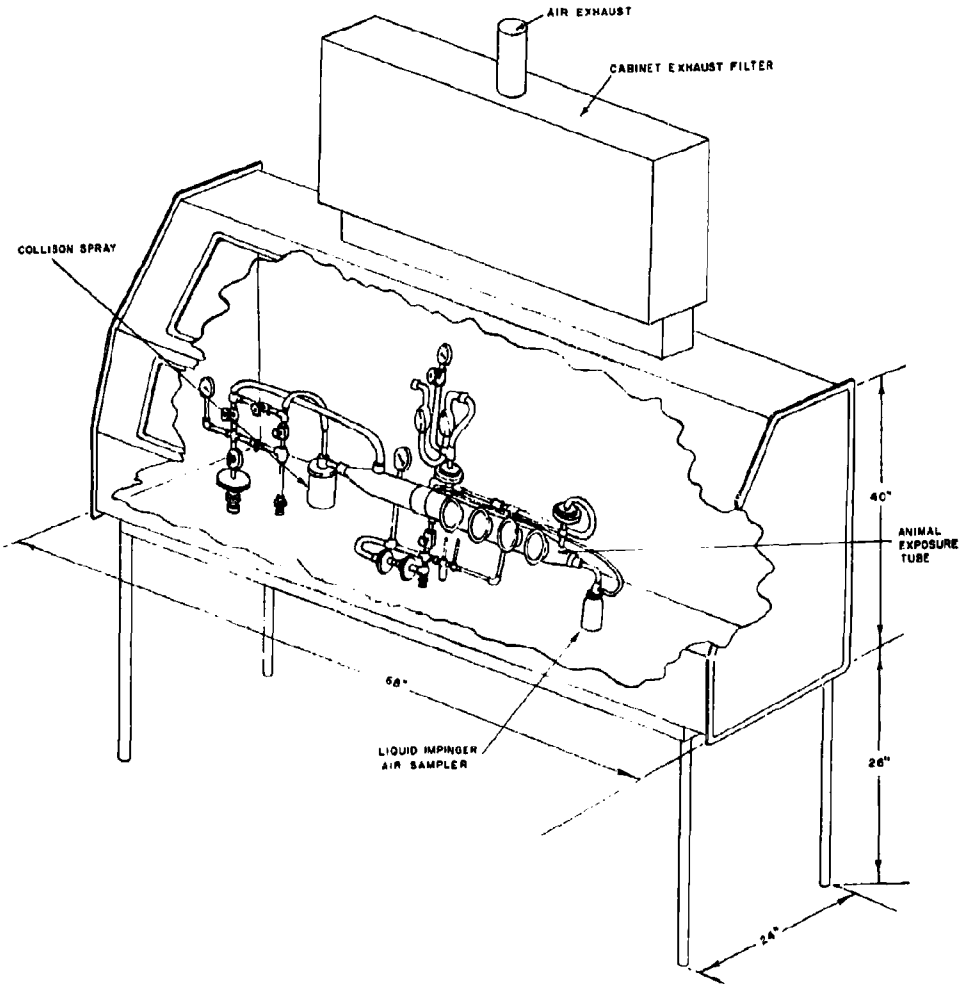

Figure 2. Henderson Apparatus, Model 3 Installed in Special Ventilated Cabinet.

III. THE HENDERSON APPARATUS

A. THE AEROSOL GENERATOR

A modified Collison spray apparatus is used to generate the aerosol. This consists of a glass jar containing a spray head and approximately 150 milliliters of liquid. The air ejection holes of the spray head nozzle must be above the liquid level in the jar. Compressed air flowing through the nozzles siphons the liquid up through the capillary-sized holes in the base of the spray nozzle. The larger particles impinge on the sides of the glass jar and fall back into the liquid. The fine particles (90 per cent, 1 to 10 microns in diameter) are carried with the air stream into the exposure tube. An Aerotec tube*, which is a small cyclone-type separator, can be placed in the line between the spray generator and the exposure tube to remove most particles above seven microns in diameter, or aerosol generators other than the Collison spray apparatus can be used to generate the aerosol. Equipment that will generate much more exact and controlled particle sizes, such as the British spinning disc,[5] is expensive and difficult to standardize and operate.

B. AIR SAMPLER, LIQUID IMPINGER TYPE

The standard capillary-orifice liquid impinger[6,7] (long or short stem), which is often called the all-glass impinger (AGI)** or Porton impinger, is usually used. This sampler, described by Rosebury,[8] may have limiting orifices that permit sampling at several rates between 0.1 and 12.5 liters of air per minute.

C. CALIBRATED CAPILLARY TUBES

The volume of air exhausted from the exposure tube is controlled by two calibrated capillary tubes. One capillary is constructed to give an airflow of 12 standard liters per minute and the other to give 16 standard liters per minute at a pressure differential of 14 ± 1 inches of mercury. Both are fabricated from capillary tubing with approximately one-millimeter-diameter bore. The capillary tubes are placed in line with an airflow meter and then trimmed in length to deliver the specified volume (one to two inches long, approximately). These calibrated capillary tubes are often referred to as critical orifices, although they are not critical orifices in the true sense of the word.

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* Aerotec Company, Greenwich, Conn.

** Ace Glass Company, Vineland, New Jersey. Molded samplers of the same type are available from Danielson Manufacturing Company, Danielson, Conn.

D. BYPASS SELECTORS

1. Three-Way Selector

The three-way selector used to direct airflow is a double-palm, button-operated valve.*

2. Valves

All needle valves, pressure reduction valves, and solenoid valves are commercially available.

E. ANIMAL EXPOSURE TUBE

The animal exposure tube is made of aluminum and is two inches in outside diameter and 35 inches long with four animal exposure cups along its length (Figure 3). Metal animal-holding tubes are equipped with a snap-type retaining ring for holding animals in place during exposure. The mouth and nose of the animal are exposed to the passing aerosol by inserting the head of the animal through a rubber diaphragm and locking the animal-holding tube to the exposure cup of the aerosol tube (Figure 4). Air samples are usually taken immediately before and after exposure of the animals. Air pressure within the exposure tube is maintained slightly less than air pressure within the cabinet. The cup covers are kept in place except when animals are being exposed. Special holders have been developed for holding mice or rats (Figures 4 and 5). For larger animals, such as monkeys or rabbits, a special exposure box replaces the standard exposure tube (Figure 6).

F. FILTERS

The low-pressure filters (1.7 cubic feet per minute) have an outside diameter of four inches and utilize four layers of 50 FG spun-glass filter medium.[9] These filters must be sealed in place by pouring N-700 Neoprene sealing compound** around the filter opening, or by bolting them together with flanges. The filter media should be cut so that no edges protrude before sealing. Filter media are changed when excessive resistance to airflow interferes with operation.

G. GAUGES

Gauges required are for vacuum and pressure. These are standard commercially available items.

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* Model BV308, Modernair Corporation, 400 Preda St., San Leandro, Calif.
** Protective Coating Inc., Tampa, Florida.

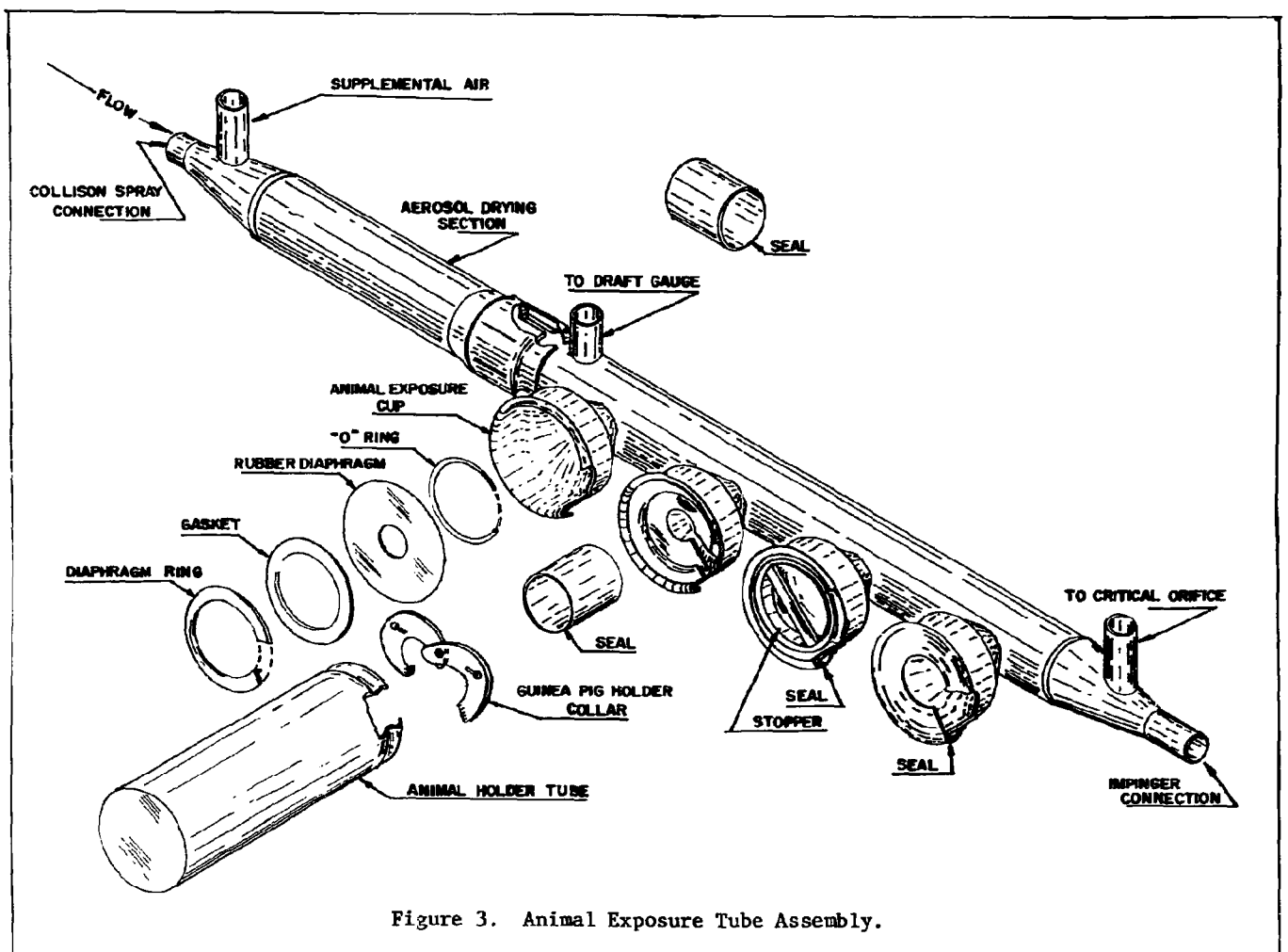

Figure 3. Animal Exposure Tube Assembly.

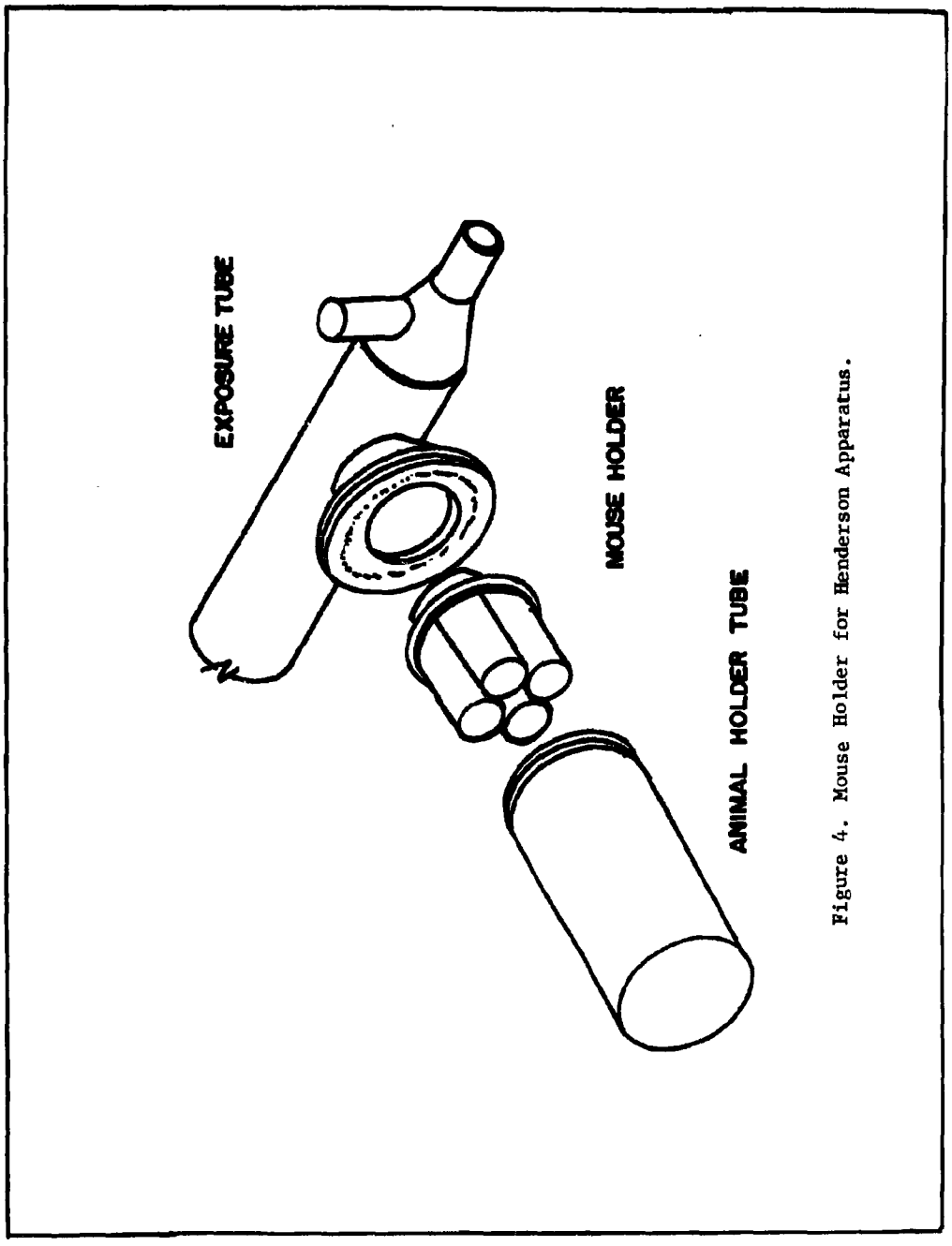

Figure 4. Mouse Holder for Henderson Apparatus.

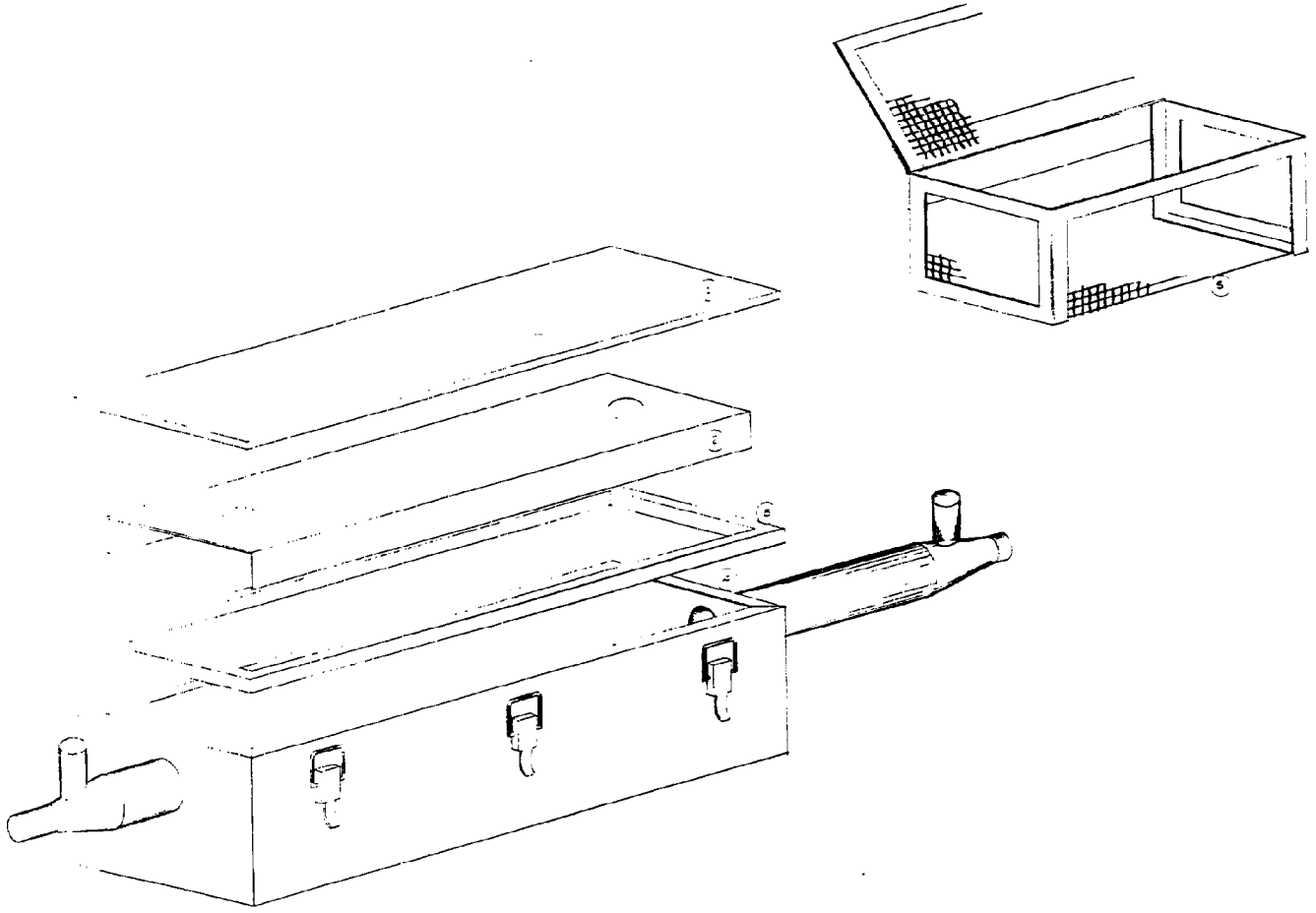

Figure 5. Animal Exposure Box Assembly for Henderson Apparatus.

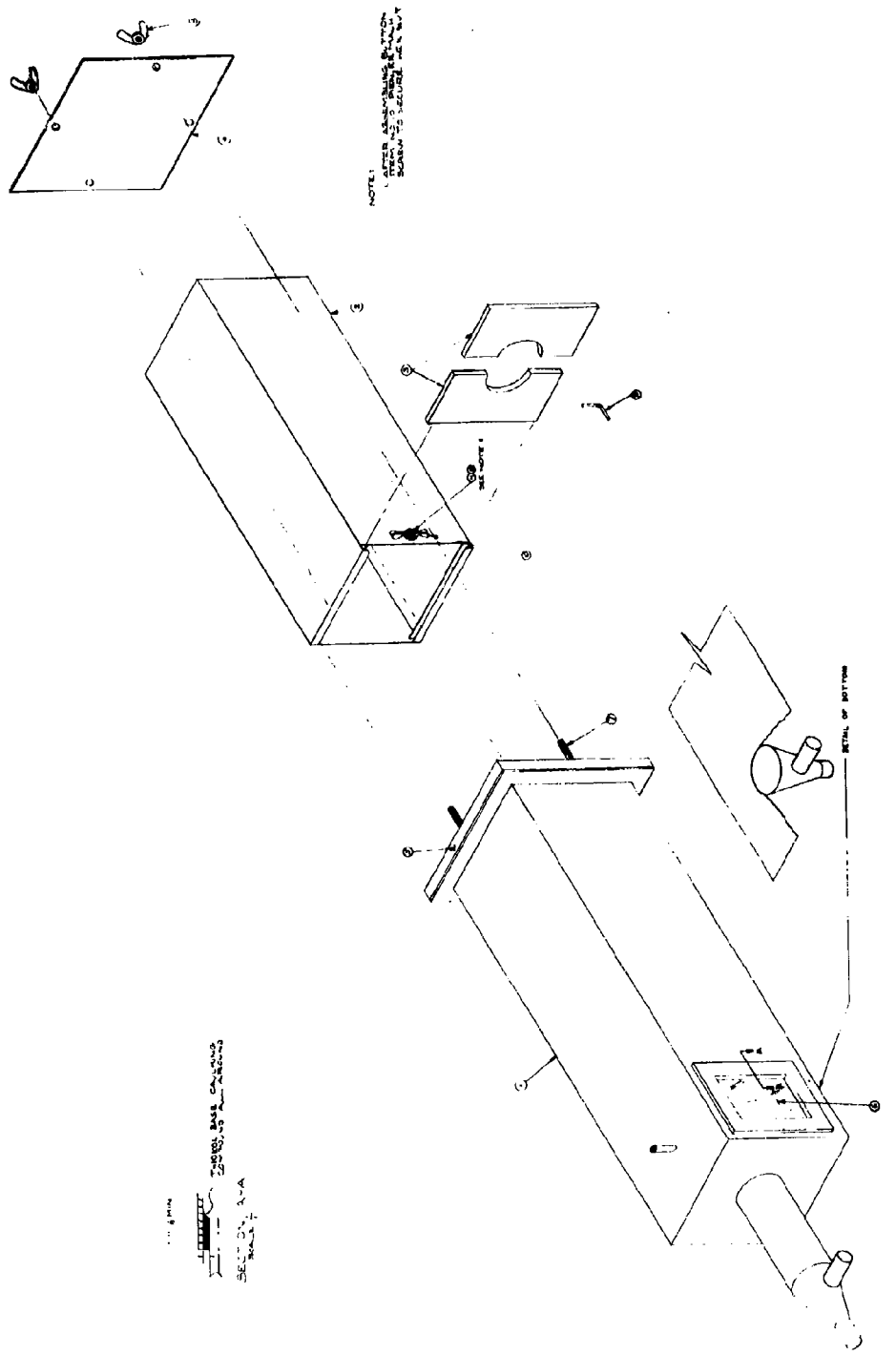

Figure 6. Large Animal Exposure Box Assembly.

H. HUMIDITY CONTROL

There is insufficient experimental data available pertaining to the evaporation rates of the various suspending media that are used with the apparatus to make definite statements pertaining to the complete drying of an aerosol. However, by utilizing the humidity chart below, one can calculate whether evaporation is possible.

TABLE I. MASS OF WATER VAPOR IN SATURATED AIR[a/]

Temperature, °F	Grams Water Per Cu. Meter Air	Temperature, °F	Grams Water Per Cu. Meter Air
60	13.27	72	19.69
62	14.19	74	20.98
64	15.17	76	22.34
66	16.20	78	23.78
68	17.30	80	25.31
70	18.45	82	26.91

a. Lange's Handbook of Chemistry, 9th edition, 1956, Handbook Publishers Inc., Sandusky, Ohio, pp. 1412.

To convert these values to more familiar units, one must divide the mass of water vapor in the saturated air at the respective temperatures by 1000. The value is then expressed as grams per liter. By spraying for a predetermined time and recording the weight change of the Collison jar, the weight of suspending medium aerosolized per unit of time can be determined.

The amount of aerosolized medium must not be more than the difference between the mass of water vapor in the saturated air and the amount actually present. This amount actually can be determined by multiplying the respective saturation values from Table I by the present relative humidity.

Suppose we have air entering at 70°F and 60 per cent RH. We also have been aerosolizing the suspending medium for one hour and by the change in weight of the Collison jar we find that in the hour we sprayed 10 grams of material. Therefore, approximately 0.167 gram per minute have been aerosolized

$$\left(\frac{10 \text{ grams}}{1 \text{ hour}} \times \frac{1 \text{ hour}}{60 \text{ minutes}} \right).$$

By referring to Table I, we find that at 70°F the mass of water in saturated air is 18.45 grams per cubic meter. Dividing by 1000 yields 0.0185 gram per liter. The apparatus uses 28 liters per minute, so the maximum amount of water vapor would be 0.518 gram. However, with a RH of 60 per cent there is 0.311 gram present in the entering air. Then the maximum amount that could be aerosolized would be 0.518 minus 0.311, or 0.207 gram per minute. By comparing the actual mass aerosolized with 0.207 gram per minute, one can feel confident that the particles can evaporate if given the proper time.

I. SEQUENCE OF EVENTS

The organisms to be aerosolized are suspended in a suitable liquid diluent. The aerosol is created from the suspended microorganisms with compressed air. The air passes to the spray head at a constant pressure of 26 pounds per square inch gauge.

Mixing and drying of the aerosol takes place as it passes through the first portion of the exposure tube. Animal exposure cups located toward the end of the animal exposure tube, allow animals to be exposed to the dry aerosol. Samples of the aerosol are collected at the end of the animal exposure tube by liquid impinger air samplers. Animal exposure and sampling are not concurrent.

The aerosol that has passed through the exposure tube is then filtered at a rate of 28 liters per minute through the low-pressure filters.

The relative humidity of the air in the circuit at the exhaust from the animal exposure tube is checked by a wet and dry bulb thermometer before and during each experiment. This reading and other data are recorded on the data sheet for each exposure.

J. CALIBRATION OF THE HENDERSON APPARATUS

In actual operation, the total flow of air passing through the Henderson apparatus is controlled by passage through two critical orifices. These critical orifices are short lengths of capillary glass tubing that limit the volume of air passing through them and thereby maintain a constant flow in the manifold even though there may be slight variations in the vacuum.

From a study of the flow diagram of the Henderson Apparatus (Figures 7 and 8), it is apparent that the flow of air divides after passing through the exposure tube. A portion of the air always moves past the wet and dry bulb thermometers and down to the exhaust filters. This portion of air (a) always passes through limiting orifice 1 (16 liters per minute), which is located immediately after the wet and dry bulb thermometers. The

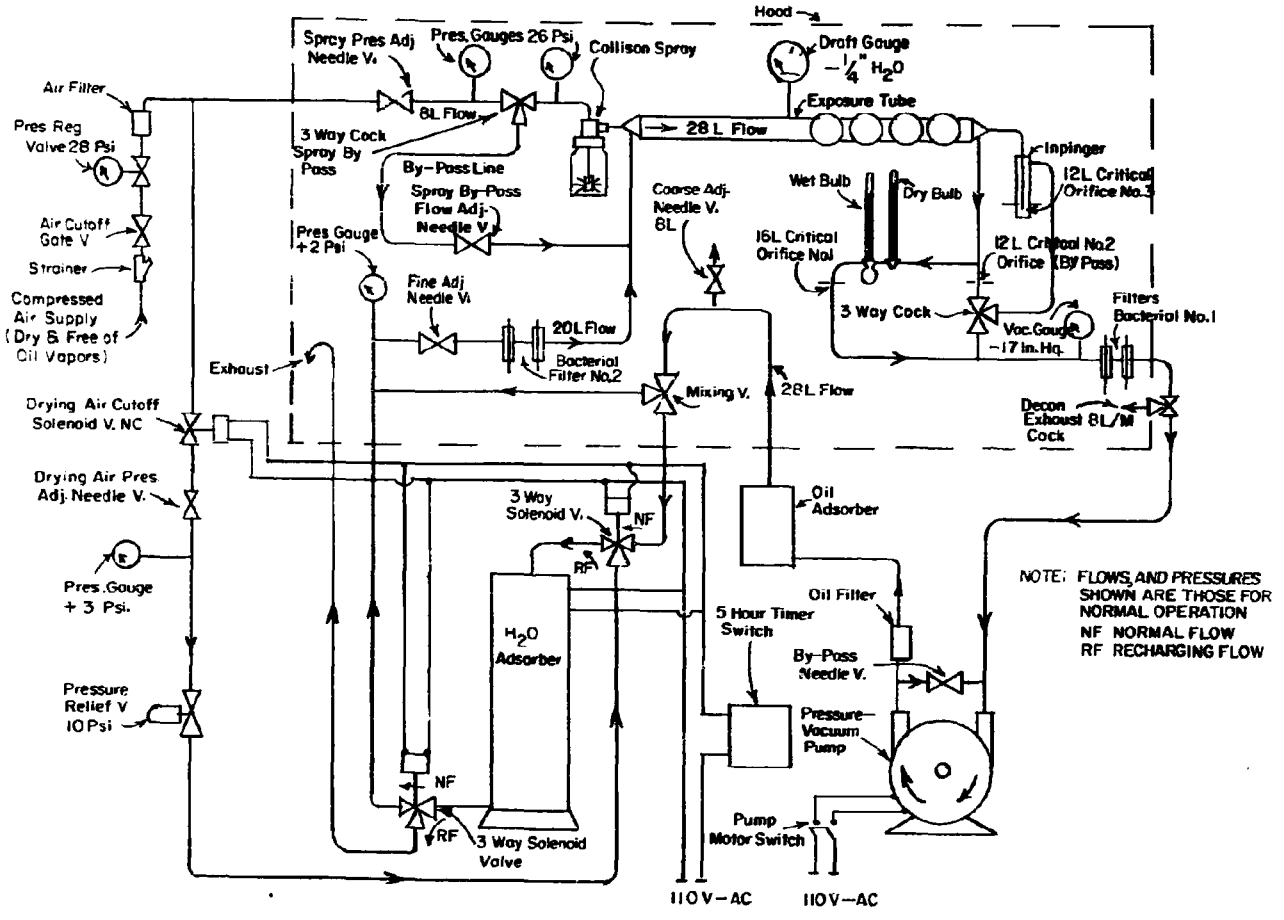

Figure 7. Flow Diagram of Henderson Apparatus, Model 2.

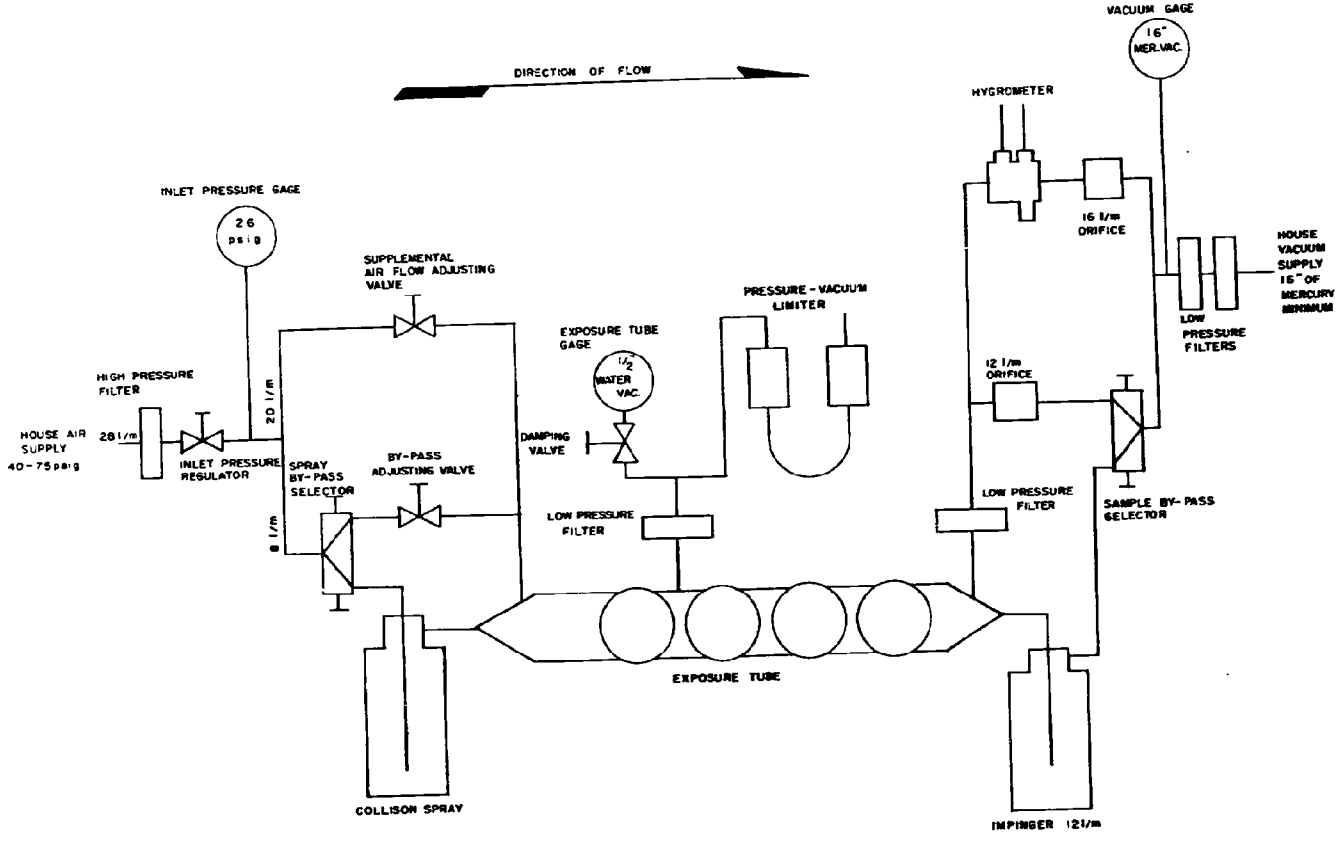

Figure 8. Flow Diagram of Henderson Apparatus, Model 3.

rest of the air (b) either passes through the impinger (which acts as a limiting orifice), or else through limiting orifice 2 (12 liters per minute) if the sample bypass selector is set for bypass. The total flow of 28 liters per minute is the sum of these two portions (a plus b) of air reaching the exhaust filters.

It is important to determine exactly how much air passes through the critical orifices in a given time. Critical orifice 1, positioned immediately after the wet and dry bulb thermometers, should be selected to allow 16 liters per minute of air to pass. The rate of airflow through critical orifice 2 should be approximately equal to the rate of air flow through the impinger. This is necessary because the flow of air normally passing through the impinger will be shunted through critical orifice 2, when the sample bypass selector is set up on bypass. If the rates of air flow through critical orifice 2 and through the impinger differ greatly, the dynamic aspects of the aerosol will change as the sample bypass selector is switched. The flow rate of air passing through the critical orifices can be controlled by selecting the proper length of glass capillary tubing.

IV. OPERATING PROCEDURES

A. MODEL 2

1. General Operating Procedures

Model 2 is a recirculating unit that operates independently of outside services except for electric power and compressed air. Air at the rate of 28 liters per minute is recirculated through the system by a heavy-duty rotary pump* located under the ventilated cabinet. The capacity of the pump should be such as to meet a continuous operation requirement of 28 standard liters per minute at minus 17 inches of mercury (Figures 7 and 9).

A slight amount of oil vapor is created by the operation of the vacuum pump and must be removed before the air is returned to the system. Oil vapor is removed with an oil filter**. The air then passes through an air drier*** where water vapor is removed and the relative humidity is brought to the desired percentage. The air drier is regenerated by a two-hour backwash of the unit at the end of the day's operations. The dried air, before entering the exposure tube, passes through the low-pressure No. 2 filter to assure complete removal of microorganisms.

Compressed air at the rate of eight liters per minute passes through two pressure regulating valves prior to entering the Collison spray head. The droplets from the Collison spray head pass into the exposure tube and mix with the dried filtered air from the vacuum pump. An aerosol of microorganism-bearing particles is formed, the particle size of which is partly dependent upon and can be controlled by the relative humidity of the air entering the exposure tube.

2. Specific Operating Procedures

The Model 2 is operated according to the following protocol:

(a) Under usual working conditions, the spray head assembly and liquid impinger air samplers are the only parts of the apparatus that require sterilization in the autoclave. The exposure tube and sampling system can be sterilized by formaldehyde, alcohol, beta-propiolactone, or quaternary ammonium compounds, as outlined in Section VII. If formaldehyde is used, it must be washed out of the exposure tube before proceeding with subsequent experiments.

(b) The flow of approximately eight liters per minute of air through the Collison spray head should be checked as described in Section V.

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* Gast Manufacturing Co., Benton Harbor, Michigan
** Type 360, Fisher Governor Co., Marshalltown, Iowa
*** Lectrodryer, Pittsburgh Lectrodryer Co., 500 32nd St., Pittsburgh, Penna.

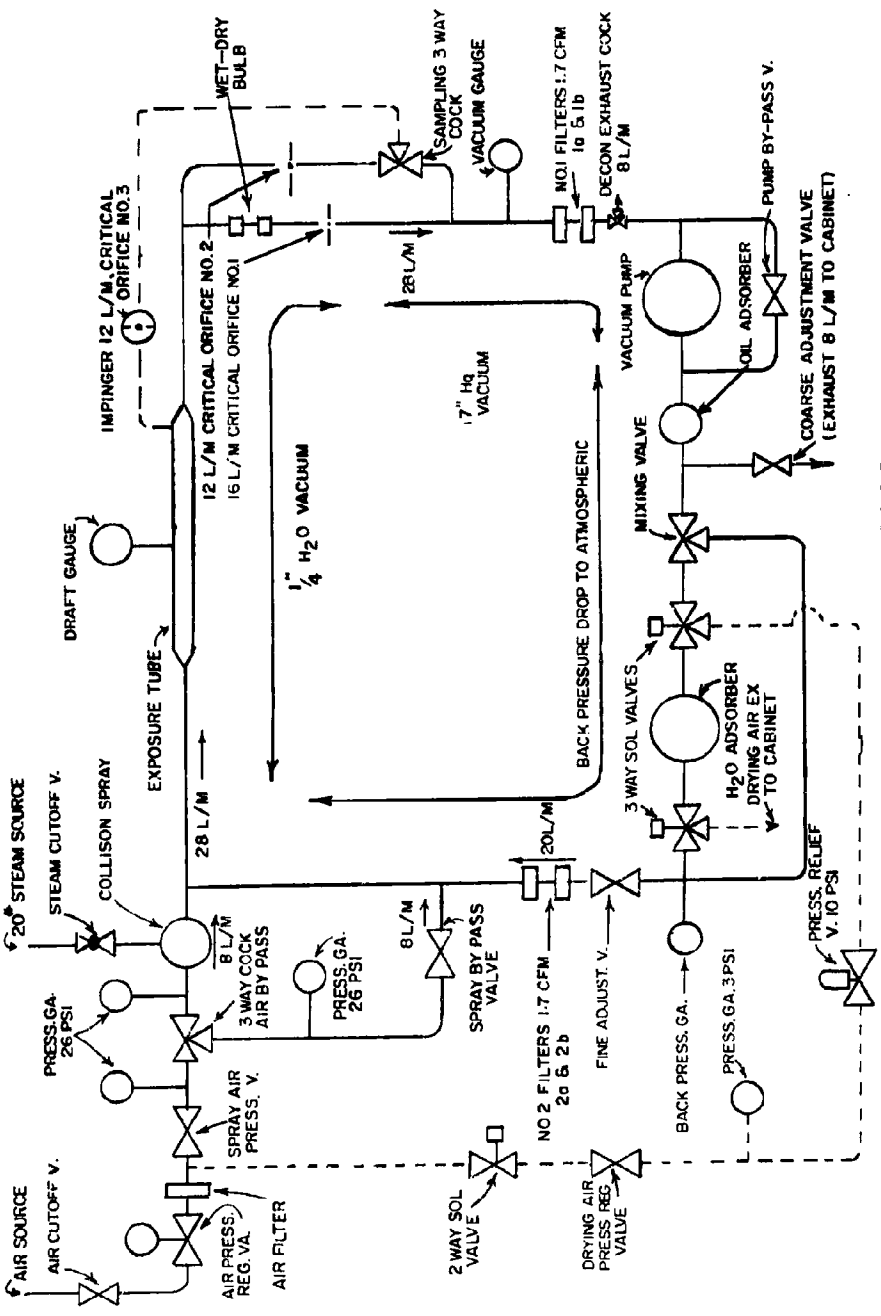


Figure 9. Circuit Diagram of Henderson Apparatus, Model 2.

(c) Check critical orifices for 12 liters per minute and 16 liters per minute air flow as described in Section V.

(d) Check impinger for 12 liters per minute flow as described in Section V.

(e) Test cabinet gloves by filling gloves with water and examining for leaks.

(f) Replace gloves on cabinet and turn on the cabinet exhaust blower. Check the vacuum gauge on the cabinet to see that the cabinet is operating at one inch reduced pressure (water).

(g) Open coarse and fine adjustment valves.

(h) Switch on vacuum pump.

(i) Turn on compressed air to spray head; adjust pressure to 26 psi.

(j) Adjust vacuum from vacuum pump to 17 inches of mercury.

(k) Close coarse adjustment valve until needle of draft gauge on the exposure tube floats at zero, then open valve for slight reduced pressure.

(l) Close fine adjustment valve until draft gauge on the exposure tube reads minus one fourth to minus one half inch of water.

(m) Turn spraying air cock to bypass and adjust needle valve in bypass until draft gauge on the exposure tube again reads minus one fourth to minus one half inch of water.

(n) Check sampling procedure by turning spraying air to spray head. Then turn sampling cock to the impinger. Draft gauge reading should remain steady at minus one fourth to minus one half inch of water.

(o) Turn spraying air to bypass and sampling cock to bypass.

(p) If conditions are stabilized the apparatus is ready for use.

(q) After use, reactivate the air-drying unit by setting the timer for two hours of operation. The timer automatically energizes the solenoid valves controlling the airflow through the drier, the heating element in the drier, and the cabinet exhaust blower. In this drying operation, compressed airflow through the heating element and the warm air removes the water from the desiccant and vents into the cabinet. The air is then exhausted from the cabinet through the exhaust filters. At the end of the drying cycle the timer automatically returns the system to normal operating conditions.

B. MODEL 3

1. General Operating Procedures

Model 3, has a nonrecirculating air system and was designed for use in a laboratory where air pressure and vacuum are available. This model can be used in the standard cabinet or it can be mounted in any ventilated cabinet that has air pressure and vacuum available. Air pressure required is from a minimum of 40 psig to a maximum of 75 psig, at a flow of one cubic foot per minute. Minimum vacuum required is 16 inches of mercury at a flow of 1 cubic foot per minute (Figures 9 and 10).

Pre-operation check list:

(a) High-pressure filter bolts tight.
(b) Supplemental airflow adjusting valve closed.
(c) Bypass adjusting valve closed.
(d) Spray bypass selector set for bypass.
(e) Collison spray bottle filled to correct level.
(f) Damping valve fully open.
(g) Pressure-vacuum limiter filled with water to slightly above the one sixteenth-inch holes in bottom tubes.
(h) Center joint of exposure tube is air-tight.
(i) Hygrometer has sufficient water in wet bulb.
(j) Orifices are clean.
(k) Impinger is attached and filled to correct level.
(l) All four low pressure filters are sealed.
(m) The four animal ports are closed with the port covers or with empty animal holders.
(n) All connections are tight and the rubber tubing is not pinched or rotted.

2. Specific Operating Procedures

The Model 3 is operated according to the following protocol:

(a) Turn on air pressure and adjust the inlet pressure regulator so that the gauge reads 26 psig.

(b) Turn on the vacuum supply and quickly switch the spray bypass selector from "bypass" to "spray" and adjust the supplemental airflow adjusting valve so that the exposure tube gauge reads one half inch of water vacuum. (Check the inlet pressure regulator; readjust, if necessary, to maintain 26 psig.)

(c) Switch the spray bypass selector from "spray" to "bypass" and quickly adjust the bypass adjusting valve to maintain one half inch of water vacuum on the exposure tube gauge. The inlet pressure gauge should remain at 26 psig. The vacuum gauge should read at least 16 inches of mercury negative pressure.

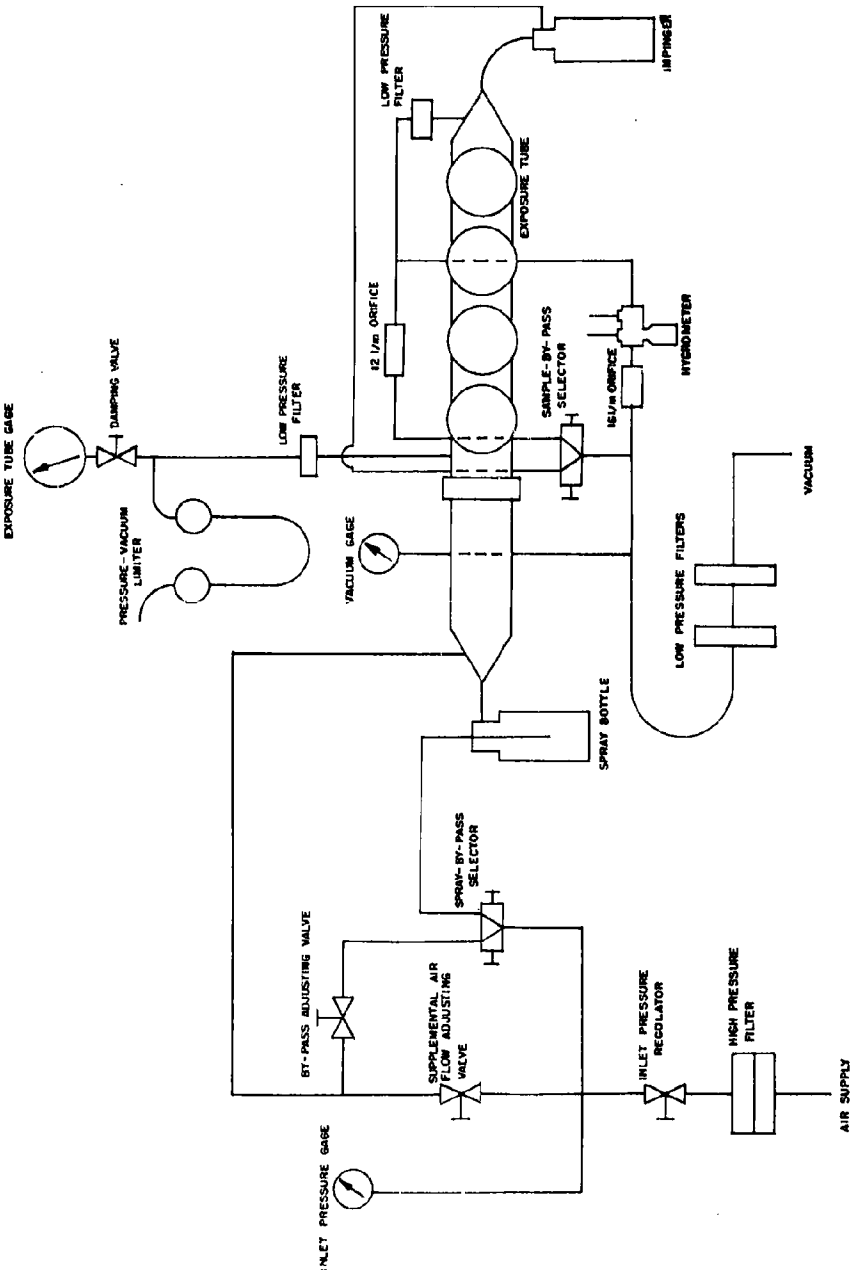


Figure 10. Circuit Diagram of Henderson Apparatus, Model 3.

(d) Switching the sampling bypass selector in the vacuum line between "sample" and "bypass" should not change any of the gauge readings after allowing a few moments for the system to balance. However, due to variations in impingers, the exposure tube gauge reading may vary slightly. This should be compensated for by adjusting the supplemental airflow adjusting valve.

(e) The damping valve should not be used unless the exposure tube gauge fluctuates violently because of the breathing of the animals. Care should be taken that this valve is never completely closed.

The system maintains equilibrium once the gauges have been set at the operating conditions of 26 to 27 psig inlet air pressure, 16 inches of mercury vacuum minimum, and approximately one half inch of vacuum (water) within the exposure tube. However, when animals are used, occasional adjustments of one valve may be necessary.

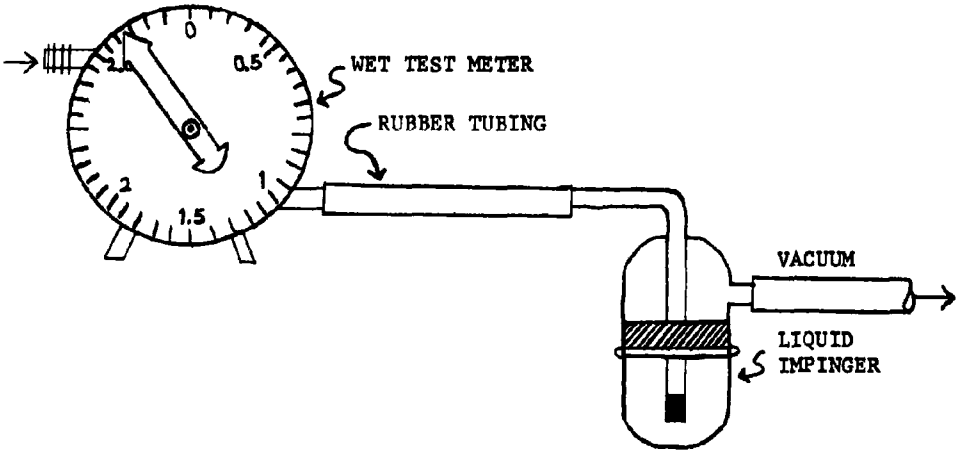
Model 3 may be removed from the cabinet to allow use of the cabinet for other purposes. Model 2 does not provide this flexibility.

V. CALIBRATION OF EQUIPMENT

A. CALIBRATION OF LIQUID IMPINGER AIR SAMPLERS

1. Procedure

The standard liquid impinger requires calibration only once. Each time it is used it should be thoroughly cleaned and the top matched to the bottom. For checking flow, attach a rubber vacuum hose to the outlet of a wet test meter and connect to the top inlet of a liquid impinger. Attach a second length of rubber vacuum hose to the impinger side arm and to a source of vacuum in excess of 17 inches of mercury. The building vacuum (20 to 26 inches negative mercury) is turned on, and the length of time required for 12 liters of air to flow through the impinger is recorded. Four such intervals are observed. The readings are averaged and the rate of flow per minute is calculated.



2. Calculations

Example: 12 liters flow

	56.0 seconds
	56.2 seconds
	56.1 seconds
	56.1 seconds
Average	56.1 seconds

If it takes 56.1 seconds for 12 liters of air to pass through the impinger, then in one minute the volume of air passing through the impingers can be calculated as follows:

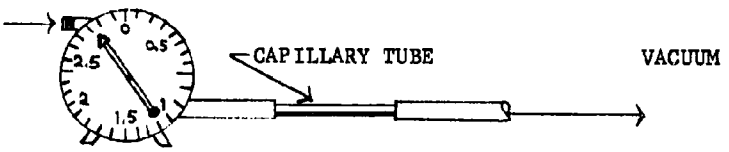
Example

$$\frac{12 \text{ liters}}{56.1 \text{ seconds}} \times \frac{60 \text{ seconds}}{\text{minute}} = 12.8 \text{ liters per minute}$$

The calculated volume of air and the number assigned to the impinger are recorded.

B. CALIBRATION OF CAPILLARY LIMITING ORIFICES

It is necessary to know the rates of flow of air through the capillary orifices. These can be calibrated in the same manner as the impingers. It is recommended that a number of each size be calibrated at one time because these small glass capillary tubes are easily broken. All orifices should be acid-cleaned at regular intervals to remove deposited particles that may restrict the airflow.



C. CALIBRATION OF THE AIRFLOW THROUGH THE SPRAY HEAD

The flow rate of air through the spray head is important for maintaining a satisfactory aerosol concentration.

1. Procedures

Place 50 to 120 milliliters of sterile suspension in the atomizer jar and attach the spray head that is to be calibrated. One jet of the spray head is opened when aerosolizing vegetative cells, but all three jets are used when aerosolizing bacterial spores. The spray head is placed in the Henderson cabinet and the primary air source of 26 psi is attached to the spray head. A rubber tube is brought from the spray head bottle outlet to the pressure side of the wet test meter. With standard operating air pressures, the rate of flow through the spray head is calculated in the same manner as

for the impingers. The rate of flow through each spray head will differ somewhat, but eight liters per minute is found to be the optimum, and the flow should not vary considerably from this value.

D. CALCULATION OF SPRAY FACTOR

The spray factor (SF) is the ratio of the number of organisms in the aerosol to the number of organisms in the suspension from which the aerosol was produced. This factor is determined by the following formula:

$$SF = \frac{\text{Microorganisms per liter of aerosol (a)}}{\text{Microorganisms per liter of liquid suspension (b)}}$$

when

$$(a) = \frac{\text{Average plate count x dilution x vol. imp. fluid}}{\text{Total volume of aerosol sampled}}$$

(b) = Average count of organisms per milliliter before and after spraying, multiplied by 1000 (10^3).

A spray factor must be determined for each type of liquid suspension sprayed since the viscosity of the fluid affects the rate of spray. It must also be determined if the spray head or air flow are changed.

If successive exposures are made with the same suspension and spray head, calculation of the spray factor is not essential for each exposure. However, a calculation of spray factor from the data provided by each exposure does provide a good check on the condition of the spray head. A spray factor between 1×10^{-6} and 2×10^{-6} is considered good.

E. CALCULATION OF SUSPENSION CONCENTRATION

Calculation of the concentration of the microorganisms required in liquid suspension in order to control the respiratory dose that the animal will receive is determined by the following formula:

$$\text{Suspension Concentration} = \frac{\text{Infective dose (c)}}{\text{Spray Factor (d)}}$$

When

(c) = The number of microorganisms per liter of air required to bring about infection of the animals. This number is determined experimentally from ID_{50} or LD_{50} test data.

(d) = Spray factor (as calculated above).

For example, the spray factor, as determined from cloud sampling, is assumed to be 1.4×10^{-6}. This is determined by spraying a liquid suspension of known bacterial concentration, determining the number of microorganisms in the aerosol produced, and calculating as in Section V.

Assume further that the infective or lethal dose for a guinea pig is 10 inhaled organisms. For guinea pigs weighing 350 to 450 grams assume an approximate respiratory volume per minute to be 0.15 liter (Table II). Therefore, the number of microorganisms per liter of aerosol required for an infective or lethal dose will be 10/0.15 or 66 organisms. Substituting in the formula to determine the required concentration of the suspension to be sprayed, we have:

$$1.4 \times 10^{-6} = \frac{66}{x}$$

$$x = \frac{66}{1.4 \times 10^{-6}}$$

$x = 4.7 \times 10^{7}$ organisms per liter of suspension
or 4.7×10^{4} organisms per milliliter of suspension.

If we expose a guinea pig to an aerosol produced from the above suspension, it should receive a dose of 10 organisms in one minute.

Table II gives the approximate respiratory volume per minute in cubic centimeters for various animals, based on weight.

TABLE II. MEASUREMENT OF THE RESPIRATORY VOLUMES OF LABORATORY ANIMALS[10/]

Animal	Method[a/]	Wt, grams	Resp/Min	Resp Vol/Min, cubic centimeter
Mice	1	Min. 12	84	11.1
		Max. 26	230	35.8
		Avg. 19.8	163.4	24.54
Mice	2	Min. 12		20.5
		Max. 25		35.5
		Avg. 20.7		28.1
Mice	3	Min. 16		11.4
		Max. 21		24.0
		Avg. 18.6		17.7
Cotton Rats	1	Min. 49	75	22.8
		Max. 130	115	71.4
		Avg. 76.8	94.5	39.6
Hamsters	1	Min. 65	33	33.3
		Max. 134	127	82.8
		Avg. 91.6	73.6	60.9
Hamsters	3	Min. 74		25
		Max. 121		65
		Avg. 95.7		47.8
White Rats	1	Min. 63	66	49.8
		Max. 152	114	101.2
		Avg. 112.8	85.5	72.9
Guinea Pigs	1	Min. 274	69	100
		Max. 941	104	382.2
		Avg. 266	90.3	155.6
Guinea Pigs	3	Min. 274		87
		Max. 941		329
		Avg. 477		154.1
Rabbits	4	Min. 792		270
		Max. 3090		1208
		Avg. 2069		800

Animal	Method [a/]	Wt, grams	Resp/Min	Resp Vol/Min, cubic centimeter
Monkeys	5	Min. 2050	31	311
		Max. 3080	52	1410
		Avg. 2682	40	863
Man	5	Min. 55,700	10.5	4900
		Max. 82,100	18.3	12200
		Avg. 68,500	14.2	8732

a. Method 1. Using oscilloscopic respirograph.
Method 2. Using head valve method and collecting expired air over mercury column.
Method 3. Using tracheal valve method and collecting expired air over mercury column.
Method 4. Using tracheal valve method and collecting expired air in automatic water trap.
Method 5. Using oscilloscopic respirograph and recording with a camera on a constantly moving film.

Methods 1 and 5 seem to be most accurate.

The following formula may be used to determine an approximate respiratory volume of a small laboratory animal:

Resp. Vol. per Min In cubic centimeters = $2.10 \times (\text{wt. in grams})^{3/4}$

VI. AEROSOL SAMPLING

The steps in the procedure for taking samples of the bacterial aerosol to determine the spray factor are as follows:

(a) Four or more liquid impinger air samplers containing 20 milliliters of collecting medium each and two drops of antifoam, if required, are placed in the cabinet.

(b) The dilution of the microorganisms to be sprayed is made in the suspending medium and placed in the glass jar. The total volume should not exceed 150 milliliters. The level of liquid in the spray jar should be about one half inch below the side orifices of the spray head.

(c) The spray jar is connected to the exposure tube.

(d) The three-way sampling cock is adjusted to allow 12 liters per minute of aerosol to flow through the sampling impinger. The total circuit flow remains 28 liters per minute, which includes a flow of 16 liters per minute through limiting orifice 1 (Figures 7 and 8).

(e) The sampling time is usually one minute. At the end of this time, the three-way cock is adjusted to divert the 12 liters per minute flow through critical orifice 2.

(f) The spray bypass selector is switched to bypass the Collison spray head.

(g) The airflow regulating valves in the circuit are opened for air wash.

(h) The impinger samplers are wiped with a suitable disinfectant and passed through the air lock and taken to a bacteriological cabinet for assay.

VII. ANIMAL EXPOSURE PROCEDURE

Animals are exposed according to the following protocol:

(a) The apparatus is assembled and tested as described in Section IV.

(b) The spray head glass jar with the sterilized suspension is replaced by one containing the proper concentration of the microorganisms.

(c) Liquid impinger samples as described in Section VI are usually taken before and after animal exposure. The liquid impinger samples serve as a check on the aerosol concentration.

(d) Animals are taken from their cages, secured in the animal holding tubes, and placed in the air lock of the cabinet.

(e) The operator of the exposure tube places the animals to be exposed in position so that the nose of the animal is inserted into the exposure tube through the rubber diaphragm of the exposure cup opening.

(f) The draft gauge is checked for steady pulsation, which shows regular breathing. Regulate fine adjustment valve, if necessary, to maintain minus one fourth to minus one half inch (water) in the exposure tube.

(g) Position the spray bypass selector to spray and start timing the exposure. Adjust pressure regulating valve on spray head to maintain 26 psig.

(h) The end of the exposure time is taken from the first action that is taken to switch off the spray.

(i) After the exposure time has elapsed, the spray bypass selector is set on the bypass position and clean air is allowed to sweep through the exposure tube for 30 seconds.

(j) The nose of the animal is withdrawn and a piece of sponge or cloth saturated in an appropriate disinfectant solution is swept up and over the nose of the animal.

(k) Replace port cover in the exposure cup.

(l) The apparatus is ready for the next exposure test or for aerosol sampling.

(m) Aerosol sampling: Follow procedure outlined in Section VI.

(n) The animals are moved into the transfer cabinet, removed from their holding tubes, and placed in ventilated cages (Figure 11).

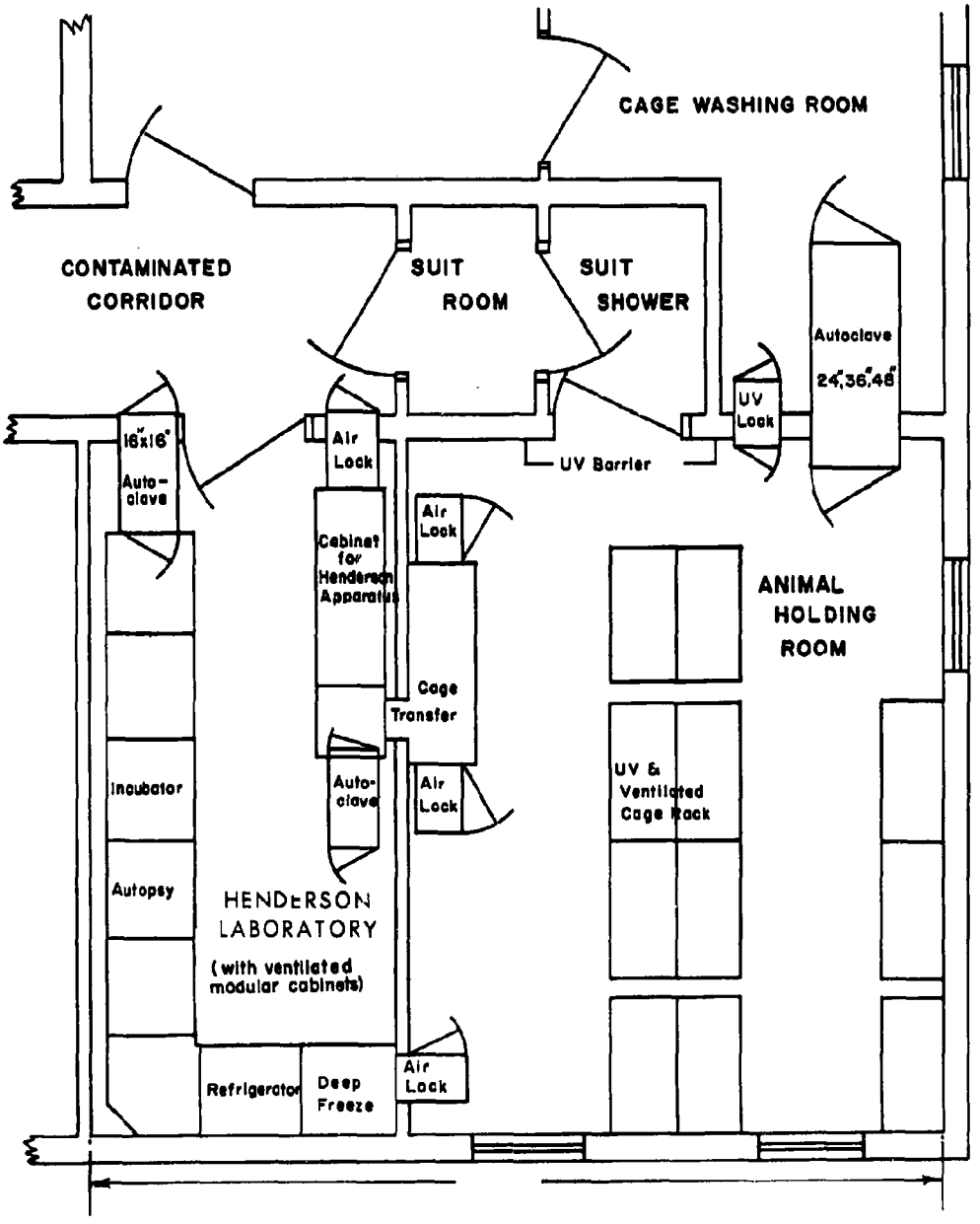


Figure 11. Suggested Room Layout for Respiratory Challenge of Animals.

(o) After completion of the experiment, the atomizer jar with connections are removed from the apparatus, placed in the attached autoclave, and treated for 20 minutes at 250°F (121°C).

(p) It is now necessary to decontaminate the cabinet and exposure apparatus. The extent of the decontamination will depend upon the future use of the apparatus. Between tests of the same microorganism, the apparatus can be cleaned sufficiently to reduce viable contamination so that interference will not be encountered during succeeding tests. Air-washing of the exposure tube will generally be sufficient; however, if steam is available in the cabinet it should be passed through the exposure tube. The steam, after passing through the system, is exhausted into the cabinet. The filters are dried by air-washing. Between tests with different microorganisms or at such times as there will be a delay of 24 hours or more between tests, the cabinet and apparatus should be more thoroughly sterilized. The Collison spray jar is removed and replaced with a container of formaldehyde-methanol solution* or other disinfectant treatment as outlined in Section IV. The formaldehyde-menthanol solution is sprayed through the system and valves are opened and closed to insure that the disinfectant enters all lines. After 10 to 30 minutes (depending upon the organism), the Collison spray jar containing formaldehyde solution is removed and the system is flushed with air. Steam, if available, may be passed through the system to remove the formaldehyde vapors.

Permanent cabinets should have a standard steam-formaldehyde system installed. This system can be used to sterilize the interior of the cabinet, or the cabinet may be sterilized by a formaldehyde vaporizer** if the cabinet is not equipped with a permanent steam-formaldehyde injector system. One milliliter of formaldehyde-methanol solution per cubic foot of cabinet space, including one milliliter for each cubic foot per minute of air being exhausted from the cabinet, should be vaporized into the cabinet each minute for a 30-minute period. After sterilization, remove all gloves from the cabinet and air-wash until the formaldehyde vapors have been removed. The exhaust blower should be kept in operation until the cabinet has been sterilized and air-washed. Equipment such as the animal holding tube will be autoclaved before removal from the cabinet system.

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* Formaldehyde-methanol solution: 37 per cent formaldehyde - 5 parts; methanol - 3 parts; adjust to pH 5.0 by the addition of sodium hydroxide.

** Hydromist Vaporizer, Model H, Arnold Laboratories, 7103 Laurel Canyon Blvd., North Hollywood, California.

VIII. LABORATORY DESIGN FOR USE WITH THE HENDERSON AEROSOL APPARATUS

In general, it is much more hazardous to operate the aerosol apparatus and to expose animals to infectious aerosols than to conduct standard bacteriological and virological procedures. If aerosol studies are planned that involve testing microorganisms highly infectious for man, it is desirable to have laboratories designed and equipped to provide adequate protection for the laboratory technicians, animal caretakers, and other building personnel.

Figure 11 shows a laboratory designed for aerosol studies and the respiratory challenge of animals.[11] This layout allows the introduction of animals and equipment into the cabinet through an ultraviolet air lock and the transfer of the animals to the animal room cage transfer cabinet by means of a pass-through box. The attached autoclave allows equipment to be autoclaved or treated with ethylene oxide gas[12,13] before it is removed from the cabinet. All standard laboratory operations such as diluting and plating, and the autopsy of animals, are carried out in the ventilated modular cabinet system shown in the laboratory.

The frequent association of laboratory infections with animal handling,[14] warrants special attention to the procedures and equipment used for holding aerosol-exposed animals. Cross-infection of aerosol-exposed animals frequently occurs with certain infectious microorganisms, thereby imperiling the validity of the experiment as well as providing evidence of potential hazard to the animal handler.[15-18] The need for special cages and cage racks will depend upon the organism under study. In some cases, exposed animals may be placed in solid-sided metal cages with mesh tops (Figure 12) and the cages kept on cage racks equipped with ultraviolet (Figure 13) lamps. These lamps, mounted in reflectors, provide a radiation barrier across the top of each cage, thereby preventing the outward escape of most air-borne organisms.[19] When still greater safety is necessary, the animals may be held in ventilated cages (Figure 12) until they no longer shed significant numbers of organisms from their fur or in their excretions.

Respiratory protection is the most important type of protection for the animal caretakers. Therefore, it may be desirable to provide a secondary change room leading to the animal quarters. This change room may be supplied with various types of respiratory protection depending upon the microorganism being studied. Several types of dust respirators are satisfactory, but if UV cage racks are used, safety goggles or shields must be worn to prevent UV eye burns. Standard military gas masks are effective, but give limited vision. A ventilated personnel hood* is very satisfactory because it provides good respiratory protection and skin and eye protection from UV (2537Å) radiation.

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* Snyder Manufacturing Company, New Philadelphia, Ohio.

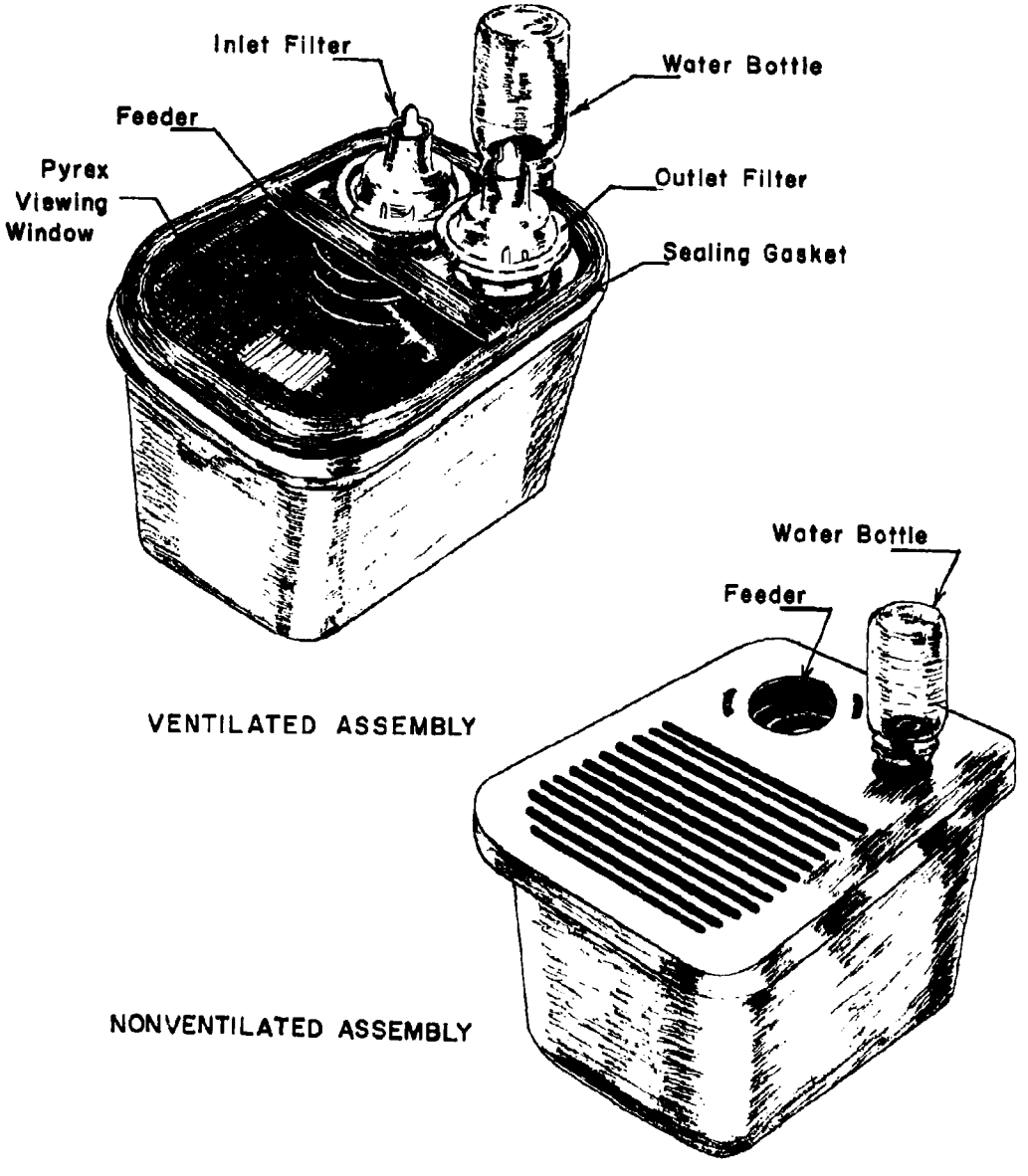

Figure 12. Ventilated and Nonventilated Animal Cage.

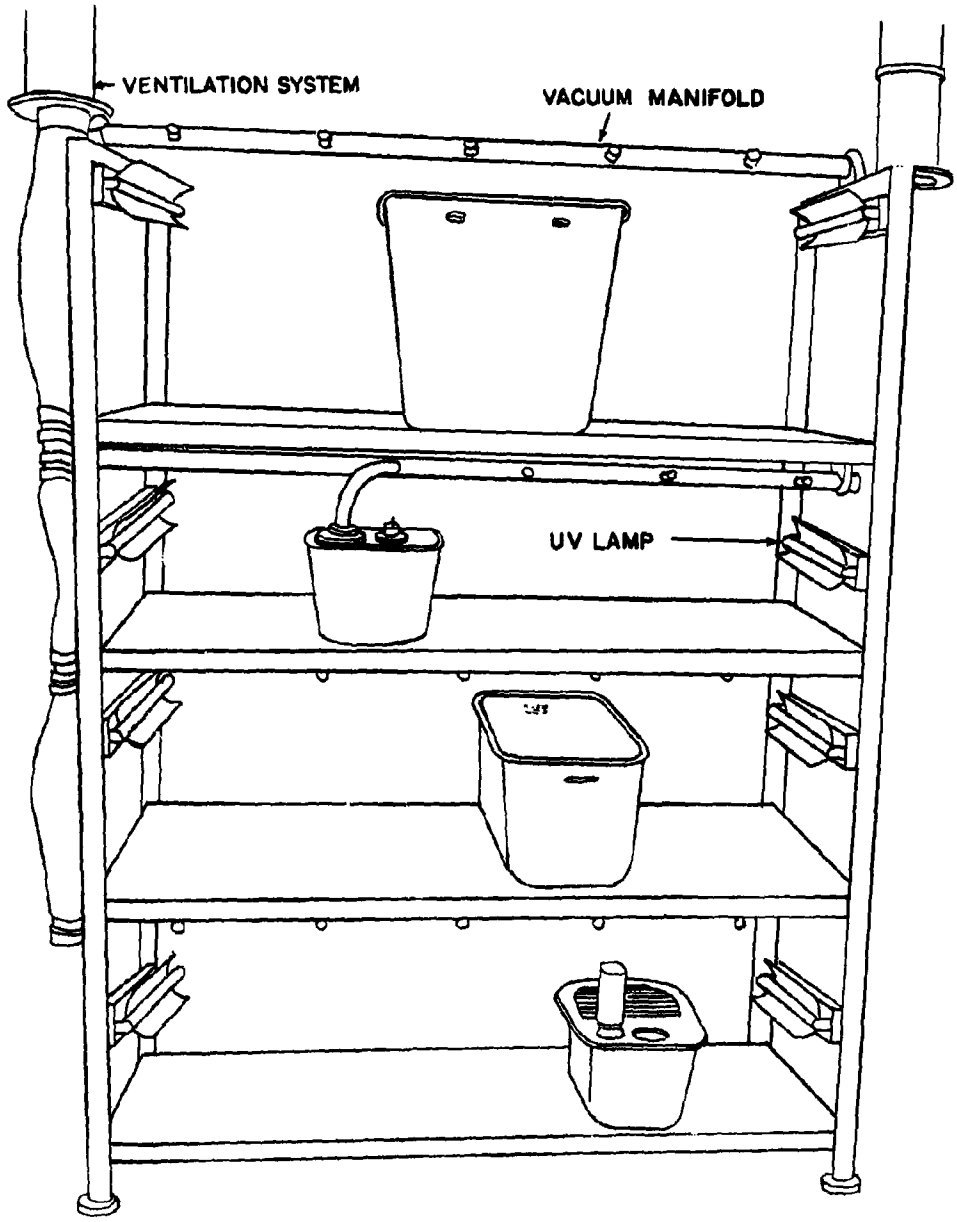

Figure 13. Cage Rack with UV and Ventilation Manifold.

When extremely infectious microorganisms are being studied and when ventilated cages are not available, and sometimes even when ventilated cages are available, it may be desirable to have the animal caretakers wear ventilated suits.* These suits are made of lightweight plastic and are easily donned and removed. Conditioned air supplied to the suits make them comfortable to wear and no more confining than the ventilated head hood. Figure 14 shows a design for a change room equipped with a disinfectant shower. The disinfectant used to sterilize the ventilated suit will vary with the microorganism being studied, but for the most resistant agents (Bacterial spores), two per cent peracetic acid is recommended.

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* Snyder Manufacturing Company, New Philadelphia, Ohio.

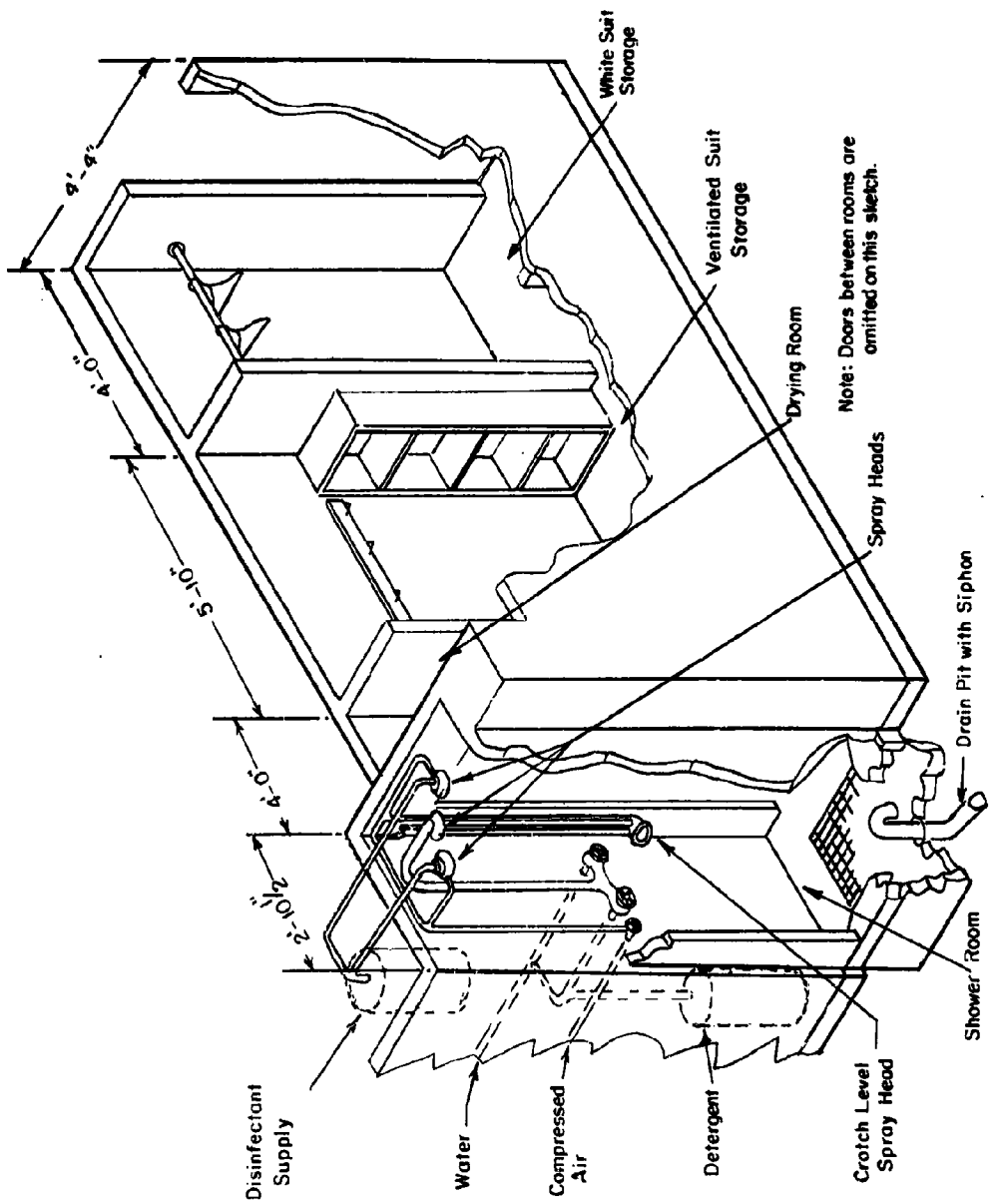

Figure 14. Change Room Equipped with Disinfectant Shower.

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APPENDIX

AUXILIARY EQUIPMENT LIST

A. HENDERSON APPARATUS

Commercial sources of the Henderson Apparatus: S. Blickman and Co., Weehawken, New Jersey, and Prime Eng. Inc., Buckeystown, Maryland.

B. CABINET FILTER

The cabinet exhaust filter, as shown in Figure 1, filters organisms from the air that is pulled from the cabinet by the exhaust blower. The filter housing is available from S. Blickman and Co., Weehawken, N.J. The fiberglass filter medium is marketed as PF-105 Filterdown by the Corning Glass Co., Corning, N.Y. or as FG-50 by the American Air Filter Co., Louisville, Kentucky. It is also available from Pittsburgh Plate Glass Co., Pittsburgh, Pa.

C. ONE-CFM AIR STERILIZER*

An electric air incinerator (one cubic foot per minute) has been developed at Fort Detrick. Essentially, it consists of a 750-watt transformer and an insulated coil of three fourths inch stainless steel tubing, which acts as a resistance heater and a channel for passage of the air.

D. WASTE COLLECTION TANK

It is sometimes desirable to have a waste collection and decontamination tank installed in the liquid waste removal system of the safety cabinets where infectious work is done. One such apparatus, designed at Fort Detrick, has a 20-gallon capacity. It is steam-heated, and utilizes a water jacket for rapid cooling of the effluent before discharge to other waste lines.

E. HENDERSON CABINET

Commercial source for cabinets is S. Blickman and Co., Weehawken, N.J.

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* Cremmillion, G.C., Miller, L.F., and Bodnar, C.A. "An electrical incinerator for sterilization of small volumes of air," Appl. Microbiol 6:274-276, July 1958.

F. AIR LOCKS FOR CABINETS

Air locks should be used with safety cabinets. With these air locks fitted on the ends of the cabinets, material can be inserted into or removed from the cabinet without contaminating the room. They are so constructed to provide UV irradiation of objects placed in the air locks.

G. VENTILATED ANIMAL CAGES

These cages, designed to be used with UV cage racks, reduce the possibility of cross-infection among infected laboratory animals and protect animal handlers. The cages vary in size depending on the type of animal being used. Each cage is equipped with a tight-fitting cover with provisions for supplying feed, water, and ventilation for the animals. Ventilated and nonventilated animal cages are shown in figure 12 of the text.

H. UV CAGE RACKS

These cage racks are four shelves high, mounted on casters, and can be equipped with air-exhaust connections and UV fixtures. Ventilated animal cages are placed on the shelves of the UV cage racks with connection of each cage to the air-exhaust system. The ends of each shelf contain UV fixtures capable of irradiating the ventilated animal cages. A typical installation is shown in Figure 13 of the text.