AN APPARATUS FOR THE SAFE INOCULATION OF ANIMALS WITH DANGEROUS PATHOGENS

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(With Plate 3 and 3 Figures in the Text)

Introduction

During intranasal inoculation of virus and bacterial suspensions into anaesthetized mice there is a danger of dissemination of infective droplets into the air.

With dangerous pathogens such as the rickettsiae of typhus fever, or the viruses of lymphogranuloma venereum and psittacosis, great care must be taken by the operator against the dissemination and inhalation of infective droplets.

In most laboratories precautions against this air-borne spread of infection include the wearing of masks and the use of inoculation boxes of simple design. Most commonly the inoculation box is constructed of glass and metal, one side being left open or provided with holes through which the hands and arms can be introduced. Often no precautions are taken to sterilize the air in the box, and infective droplets can escape through the armholes and when the mice are transferred to their cages. A box of this kind was used in this laboratory for the intranasal inoculation of mice with the rickettsiae of murine typhus fever. That the risk of infection when this technique is employed is great, and that, moreover, the simple precautions taken were insufficient, was shown by the fact that within a month of starting the work, all four persons employed in the infection of mice by the pulmonary route became infected.

To minimize the risk to laboratory workers who employ the intranasal route of infection of mice with dangerous pathogens, an inoculation box was designed and constructed. This apparatus has proved very effective, and has, furthermore, given the opportunity of studying the extent of pollution of the air which accompanies intranasal inoculations.

Description of apparatus and method of use

The apparatus (Pl. 3, figs. 1, 2) consists of a steel and glass box 12–18 in. high, divided into two sections, each 20 × 20 in. Each section has a hinged glass lid sloping from behind forward. The total capacity is approximately 8·5 cu. ft. To prevent the escape of infective droplets from the box during the manipulation of the mice, powerful through-draught ventilation is provided by a gas burner and chimney. The burner is similar to an ordinary boiling ring and is placed in a chimney of stove-pipe tubing. Just above the burner is an inner steel tube closed at both ends. This deflects the hot gases into the narrow annular space between the inner and outer tubes. Both tubes get red hot near the burner and so ensure that all air drawn up the chimney becomes effectively heated. The burner should be lit 10–15 min. before starting inoculations, and the heat of the furnace checked by the red glow visible through the inspection window. As an additional safeguard against the spread of infective droplets, each compartment is provided with a quartz-jacketed Hanovia ultra-violet lamp which can be turned on after the mice are inoculated. The operator wears a surgical gown and rubber gloves. His hands are introduced through rubber sleeves into the left-hand compartment. The draught ventilation provided by the chimney is so strong that it is unnecessary to ensure an air-tight fit of the rubber sleeves round the elbows; they should fit well enough round the elbow to move in and out with the movements of the arms, but not tight enough to be uncomfortable. Anaesthetized mice are handed in through a door on the left. When inoculated, the mice are placed in glass jars which are then covered with perforated galvanized iron lids and pushed across into the right-hand compartment where they remain until they can safely be removed. The glass jars employed are 4½ in. deep and 5½ in. in diameter, and the right-hand compartment can accommodate 20–25 of them.

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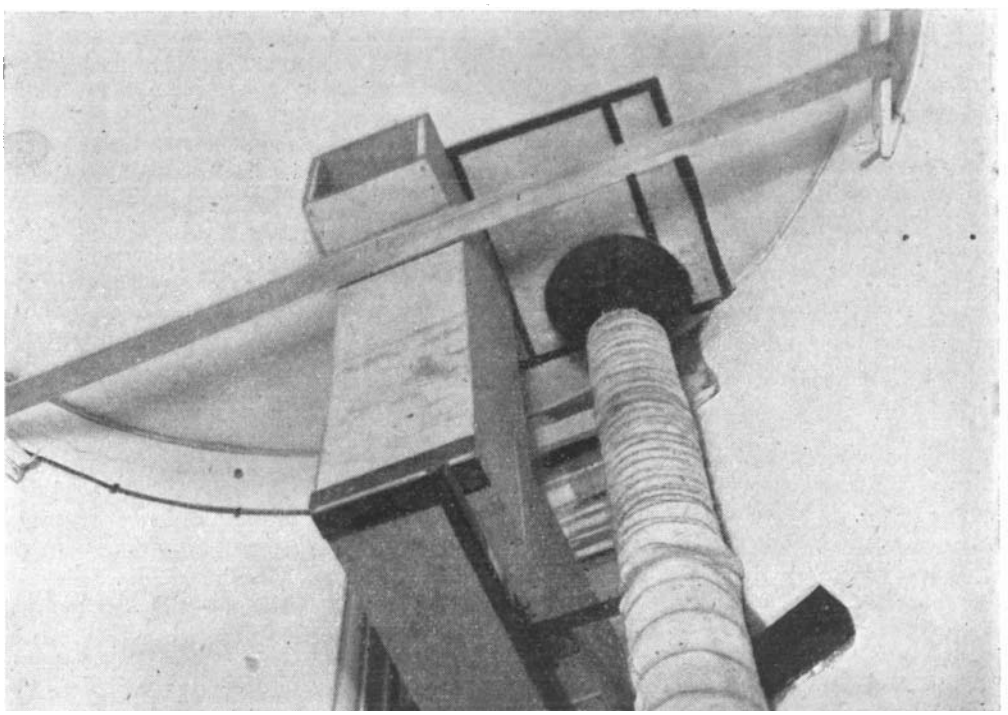
The glass jars should be marked with grease pencil according to the inoculum which the mice are to receive. They can be handed in through the sliding door in the left side of the box. Other apparatus such as syringes, pipettes, etc., is also handed in through this door. In the apparatus shown in Text-figs. 1 and 2 the sliding door moves backwards and forwards. Later it was found more convenient to have the sliding door move up and down, and to have an additional hinged tray for the introduction of anaesthetized mice. These improvements have been incorporated in a subsequent model, the construction of which is described in the appendix. When all the mice are inoculated, the operator's arms are withdrawn; and the gloves and surgical gown removed for sterilization. The ultra-violet lamps are turned on, and to ensure an adequate through draught when the doors into the left-hand compartment are closed, the diaphragm in the air inlet duct is opened. The mice are left in the box for 45 min. to 1½ hr., after which hinged doors in the front of the right-hand compartment can be opened and the mice removed. The mice are transferred to cages and the glass jars placed at once in a suitable receptacle which either contains an antiseptic solution or can be autoclaved immediately.



Text-fig. 1. Showing the top of the chimney and the duct containing the exhaust fan.

In the apparatus described in the appendix the lower horizontal part of the chimney passes through an outside wall. The remainder of the chimney including the furnace is outside the building. The whole apparatus can, however, be erected inside the laboratory. One of this kind has been in regular use for about four months with entirely satisfactory results. In this case the chimney carrying the gas burner terminates about 4 in. below the ceiling, which is protected by a chimney cowl and a sheet of asbestos (Text-fig. 1). An exhaust fan built in a duct which opens near ceiling level ensures the removal of fumes and hot air escaping from the chimney. In this type of apparatus a special ignition element is unnecessary. The furnace can conveniently be lighted with a taper introduced through a window in the chimney at a point immediately below the level of the gas burner. The experiments reported in this paper were performed in the apparatus with its furnace and chimney inside the laboratory.

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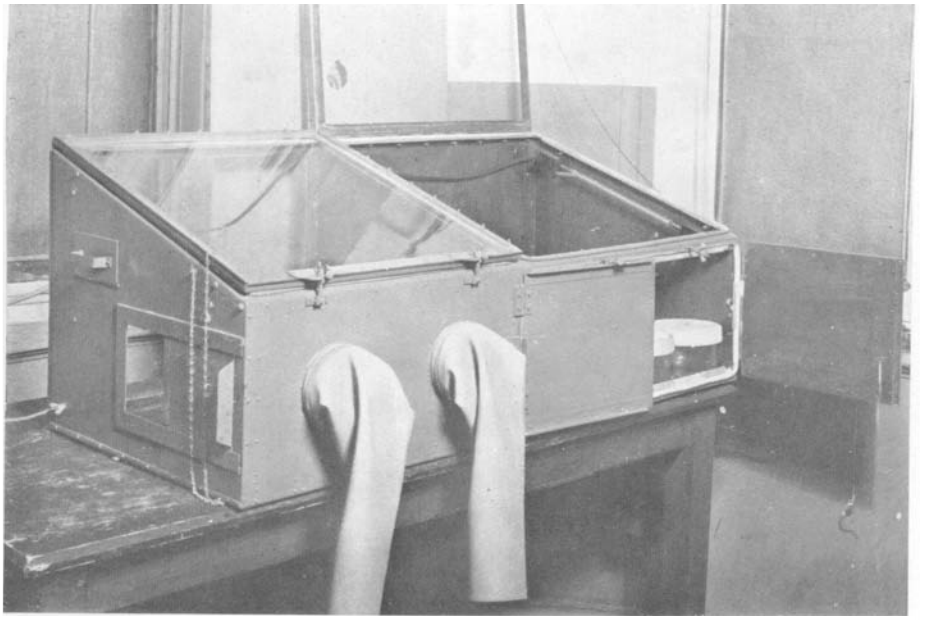

Fig. 1. The inoculation box with the sliding door on the left. The doors of the right-hand compartment are open.

Fig. 2. This figure shows the inoculation box in use.

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The degree of aerial infection during intranasal inoculation of mice and the effect of smooth anaesthesia

Groups of six mice were inoculated on the bench in a large laboratory, without any precautions against dissemination of droplets. A heavy suspension of *Chr. prodigiosum* (a 24 hr. slope culture emulsified in 5 c.c. nutrient broth) was used as inoculum. With one group great care was taken to ensure good anaesthesia, so that the inoculum (0·05 c.c.) was aspirated quickly and without bubbling. With a second group bad anaesthesia was induced so that bubbling occurred on the nose during the inoculation. Throughout the experiment 5 cu. ft. samples of air were taken at the rate of 1 cu. ft./mm. with a slit sampler (Bourdillon, Lidwell & Thomas, 1941), so placed that the air intake was on the same horizontal level as the operator's nose, and about 18 in. away from the mice being inoculated. The samples were taken on nutrient agar or Schlesinger agar plates, which were then incubated at 25° C. for 48 hr.

The results recorded in Table 1 show that in both cases recognizable infection of the air occurred, but that in the case of the badly anaesthetized mice the extent of pollution of the air was greater.

Table 1. *Droplet infection of laboratory air during intranasal inoculation of mice with* Chr. prodigiosum. *Numbers of infective droplets in 5 cu. ft. air samples*

	Good anaesthesia	Bad anaesthesia
Before inoculation	0	0
During inoculation (6 mice)	4	32
Next 5 min.	2	2

Table 2. *Dissemination of infective droplets by mice inoculated intranasally with* Chr. prodigiosum *in inoculation box. Mice inoculated during the first 5 min. period. Numbers of infective droplets in 5 cu. ft. air samples*

Time after commencement of inoculation min.	A. U.V.R. and ventilation during alternate 5 min. periods	B. No U.V.R. or ventilation
0– 5	496	864
10–15	28	1008
20–25	15	255
30–35	43	66
40–45	54	31

To determine the time after intranasal inoculation, during which mice can disseminate infective droplets, groups of ten mice were inoculated intranasally in the inoculation box with 0·05 c.c. doses of a heavy suspension of *Chr. prodigiosum*, and kept in the inoculation box in glass jars during the remainder of the experiment. In order not to interfere with the dissemination of droplets into the air of the box, the jars were left uncovered. The purpose of these experiments was also to determine the degree of atmospheric pollution which occurs. To prevent the escape of droplets through ventilation the gas burner in the chimney was therefore not ignited. In actual use with a dangerous pathogen such a procedure would, of course, not be adopted. Series of 5 cu. ft. samples of air from the box were taken with a slit sampler through a wide glass tube. In series B, 5 min. pauses between successive air samples were allowed. In series A, the ultraviolet lamps were switched on, the doors and lids of the box opened, and the windows of the laboratory opened during the 5 min. periods between samples. Previous experiments have shown that the opening of windows and doors, etc., will completely remove infective droplets present in the air at the time.

The results recorded in Table 2 show that *prodigiosum*-laden particles were recovered from the air for as long as 40–45 min. after inoculation of the mice. In the series A, where the air in the box was cleared of the *prodigiosum* between successive air samples, the counts per 5 cu. ft. fell rapidly, but their persistence in small numbers indicated that mice were actively disseminating infective particles throughout this period. The relatively high counts during the 30–35 and 40–45 min. samples in this series can be accounted for by the fact that during these times two mice which escaped from the jars, which for the sake of the experiment had been left uncovered, were very actively moving about in the vicinity of the intake tube

of the slit sampler. The large numbers of infective droplets present in the air during the first 25 min. after the inoculation is strikingly shown in these results.

It is apparent, therefore, that the greatest danger period is during the first 24 min. after inoculation. Infective droplets are present in the air for at least 45 min. after inoculation in many experiments, but the numbers, even when no precautions are taken to remove them, are very much smaller during the end of this period than earlier on. In experiments performed by C. H. Andrewes it has been found that 1½ hr. after the intranasal infection of mice, no more infective particles could be recovered from the air of the inoculation box. It can be concluded, therefore, that mice should be left in the box for at least 30 min. after inoculation and, preferably, 1½ hr.

The opportunity afforded by the inoculation box was also taken to determine whether significant contamination of the air occurs during pipetting of emulsions. In experiments performed by Andrewes, it was found that the manipulations such as are usually involved in making serial dilutions resulted in slight contamination of the air. The extent of this contamination is, however, negligible in comparison to that occurring during mouse inoculation.

The effect of ultra-violet rays and the furnace on the air contamination in the inoculation box

Experiments were performed in the inoculation box using mice infected intranasally with *Chr. prodigiosum* emulsions, or coarse mists of the same organism sprayed from a hand atomizer. About 0·5 c.c. of emulsion was sprayed on each occasion. Five cu. ft. samples of air from the box were taken with a slit sampler. In the control series of experiments the furnace and ultra-violet lamps were both off; in a second series the ultra-violet lamps were turned on for 2 min. periods between successive air samples while the furnace was off throughout, and in the third series the furnace was on. The results recorded in Table 3 were obtained in one experiment using the same emulsion for all three series, but fresh mice for each. They show that ultra-violet rays reduce the number of infective particles in the air significantly, but that the chimney is much more effective. The chimney alone is capable of clearing the air of the box almost completely within 7 min. after introduction of a heavy spray of bacteria-laden particles.

Table 3. *The effect of the furnace and ultra-violet rays on the numbers of infective droplets distributed into the air in the apparatus by inoculated mice or by a hand atomizer. Numbers of infective droplets in 5 cu. ft. air samples. Mice inoculated during the first 5 min.*

Time after commencement of inoculation or spraying min.	Control Furnace and U.V.R. off		Furnace on throughout		U.V.R. lamps on during 2 min. pause between samples	
	Inoc. mice	Spray	Inoc. mice	Spray	Inoc. mice	Spray
Before	0	0	0	0	0	0
0– 5	912	Conf.	73	Conf.	1200	Conf.
7–12	1392	Conf.	36	8	268	2096
14–19	912	Conf.	31	0	91	140

Conf. = confluent growth on plate.

Table 4. *The effect of the furnace on atomized* subtilis *spore emulsion. Numbers recovered from 5 cu. ft. air samples*

Time after commencement of spray min.	Sprayed into entrance of furnace	Sprayed 1/10th vol. outside box into top of room
Before	4	1
0– 5	1	292
5–10	?1	160

Tests of the sterilizing efficiency of the furnace

An emulsion of *B. subtilis* spores was sprayed into the mouth of the furnace, or, in control experiments, into the air of the laboratory near the top of the chimney. A total volume of 2·5 c.c. was atomized with a hand atomizer into the mouth of the furnace, but only one-tenth this volume was used in the control experiment. Samples of air were taken from the top of the chimney through wide glass tubing connected to a slit sampler. The counts obtained from 5 cu. ft. samples of air in a typical experiment are recorded in Table 4. It can reasonably be concluded that, as the furnace is so highly effective against heat-resistant

spores, non-sporing pathogens will be dealt with at least equally effectively. The experiment has been repeated taking air samples at the level of the operator's face, and using *Chr. prodigiosum* as well as *B. subtilis* as indicator organisms. The results have been similar.

The effect of the inoculation box in preventing dissemination of infective droplets into the air of the laboratory during intranasal inoculation of mice

Samples of air immediately outside the open slide door on the left and from inside the box were taken with a slit sampler. Five cubic ft. samples were taken during consecutive 5 min. periods and during the first 5 min. ten mice were inoculated intranasally, under ether anaesthesia, with a *Chr. prodigiosum* emulsion. In the control series the furnace and ultra-violet lamps were off. In the second series the furnace was on. The slide door was left open throughout the experiment. The results recorded in Table 5 show that, while the furnace is off, large numbers of infective droplets escape from the box. On the other hand when there is a through draught created by the furnace, no infective droplets were detected in the air of the laboratory, and, furthermore, the numbers in the box itself were rapidly reduced.

Table 5. *The efficiency of the apparatus in preventing the distribution of infective droplets into the air of the laboratory. Numbers of infective droplets in 5 cu. ft. air samples*

Time after commencement of inoculation min.	Control without furnace or U.V.R.		With furnace on	
	Outside box	Inside box	Outside box	Inside box
Before	0	—	0	—
0– 5	90	—	0	—
5–10	146	—	0	—
10–15	—	468	—	23
15–20	—	186	—	9

Discussion

The results described indicate clearly that during the intranasal inoculation of mice a significant dissemination of infective droplets into the surrounding air occurs. The number of infective droplets distributed into the air is greater if there is obvious bubbling during the inoculation, but even if the inoculum is aspirated quickly and smoothly, the numbers are large and they are being continuously disseminated for at least 45 min. The risk of infection to the operator when a dangerous pathogen is used must, therefore, be obvious. In the light of the results here recorded, the magnitude of this risk appears to be even greater than has generally been realized. The use of a specially constructed inoculation box of simple design has been found to overcome the danger of inhalation infection to a very large extent. An apparatus of this type has been in continuous use by four bacteriologists and four assistants over a period of 4 months. During this time a total of approximately 4000 mice have been inoculated intranasally with the rickettsiae of murine typhus. Four of the eight individuals have not contracted typhus. Of the other four, three were infected prior to the installation of the inoculation box, and within a month of commencing work involving the intranasal inoculation of rickettsiae into mice. None of those employed in typhus work during that first month escaped infection. During the first 4 months since the installation of the inoculation box only one case of typhus has occurred, although four of the individuals who had regularly used the apparatus during two of these months were non-immune. All those employed on typhus work have received several courses of vaccine, the relative ineffectiveness of which is shown by the laboratory infections which have occurred.

SUMMARY

An apparatus which prevents the distribution of infective droplets into the air of the laboratory during the intranasal inoculation of mice is described.

Experiments using *Chr. prodigiosum* as indicator organism are described to show the extent and duration of the droplet dissemination by inoculated mice, and the effect of the apparatus in reducing this dissemination.

We wish to express our thanks to Dr R. B. Bourdillon for many suggestions in the design of the apparatus, and to Mr C. D. Sutton for the photographs.

REFERENCE

BOURDILLON, R. B., LIDWELL, O. M. & THOMAS, J. C. (1941). *J. Hyg., Camb.*, **41**, 197.

APPENDIX

By A. J. G. HUBBARD

Construction of inoculation box

The main structure consists of 16 s.w.g. steel plating and $\frac{1}{4}$ in. glass panels. The details of construction are shown in Text-figs. 2 and 3.

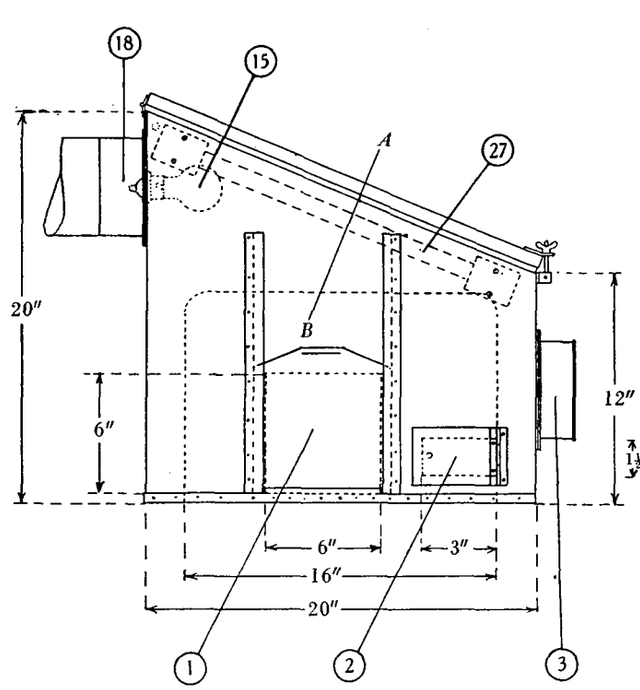
In the left-hand side of the box is an opening (1) 6×6 in. which can be closed by a sliding door. The door is most conveniently made to slide up and down in grooves on each side. A flat tray (2) hinged in front fits into a second opening 2×4 in. Armholes (3) are fitted with collars, over which fit loose rubber sleeves (4) made of $\frac{1}{32}$ in. sheet rubber. The sleeves taper slightly towards the free end, where the circumference is 14 in. The length of the sleeves is 18 in.

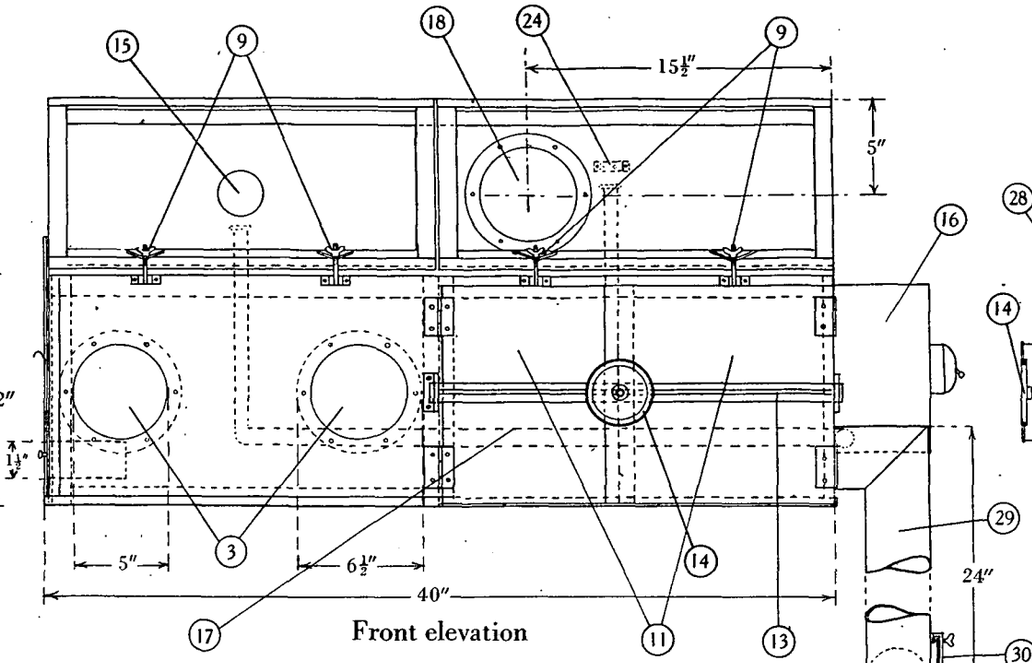
The lid (5) of each compartment consists of a $\frac{1}{4}$ in. glass panel (6 in section E–F) in a steel frame. To make the joint airtight the glass panels are clamped down in their frames on tubular rubber draught excluders (10) by steel clamping strips (7). The lids are hinged separately at the back with piano hinges (8) and clamped down in front with hinged wing nuts (9). The contacts between the lid and the frame of the box are made airtight by means of tubular rubber draught excluder (10).

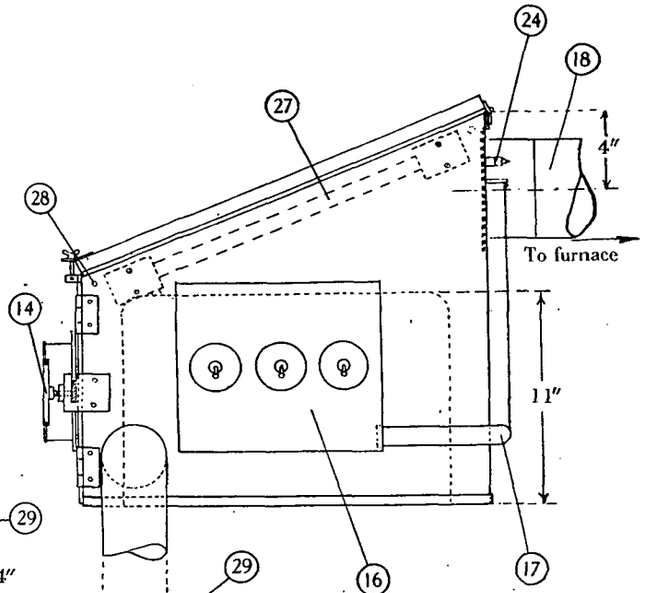
The front of the right-hand compartment consists of two steel doors (11) hinged at the side and overlapping about 1 in. in the mid-line. The details of construction of the joint between the glass panel (6) in its steel frame, the frame of the box (12) and the front doors (9) are shown in section E–F. A detachable steel bar (13) fits across the doors into slots at each side. A hand wheel (14) forces the doors against rubber seals (draught excluder) to ensure an airtight joint when the box is in use.

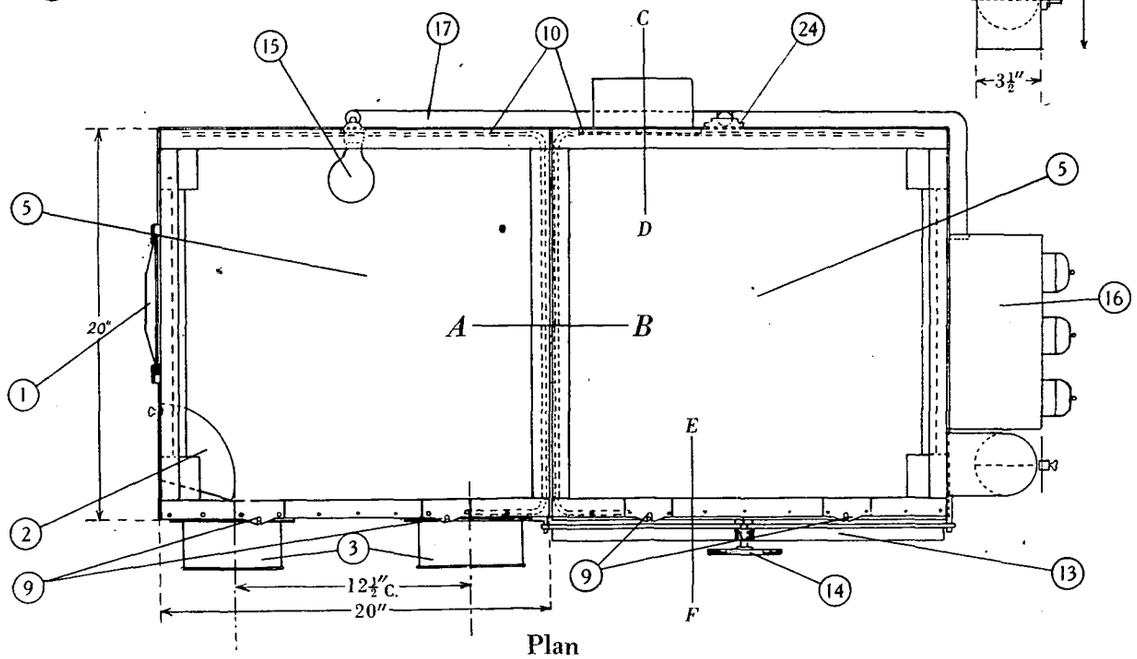
Light is provided by a 25 W. electric bulb (15) which is operated by one of the switches in the switch box (16), the wiring being conducted through the conduit (17).

An air outlet (18) 5 in. in diameter in the left upper quadrant of the back of the right-hand compartment communicates directly with the chimney furnace. The joint between the chimney and the box is made airtight by a rubber washer (19). The furnace consists of a gas ring (20) and two concentric steel pipes (21, 22) made of 16 s.w.g. steel plating. The inner pipe is sealed above and below except for a small aperture ($\frac{1}{4}$ in. diam.) to allow of air expansion in the inner pipe. When in operation the gas burner heats the lower parts of the concentric pipes to a red heat and creates a strong upward draught of air. The outer pipe of the furnace is continuous above and below with galvanized iron stove-piping. The whole chimney and furnace is covered with several layers of asbestos lagging. In the apparatus shown in the diagram, the lower horizontal part of the chimney passes through an outside wall, the remainder of the chimney including the furnace being outside the building. Special arrangements had, therefore, to be provided to









Text-fig. 2. Plan of inoculation box.

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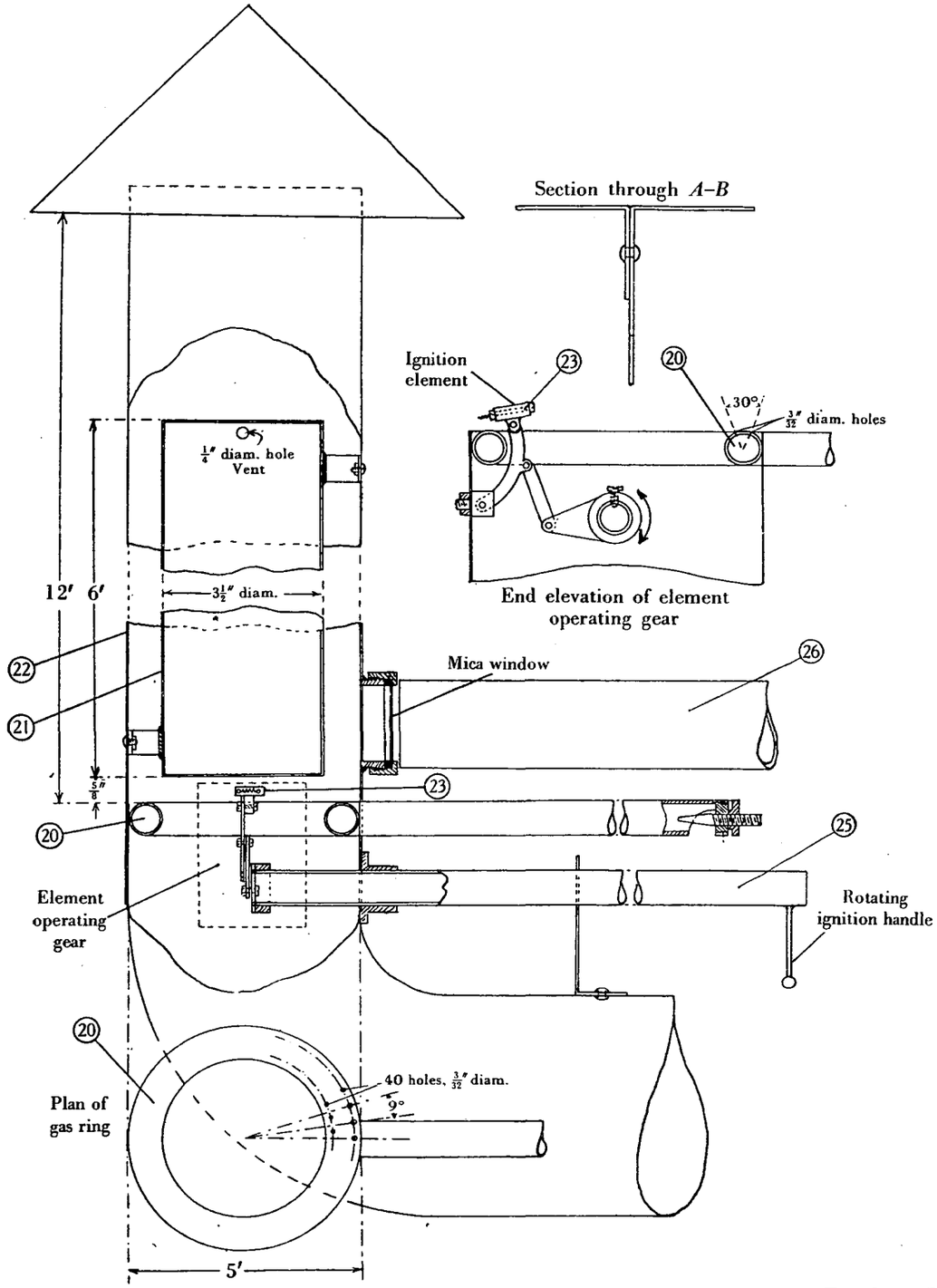
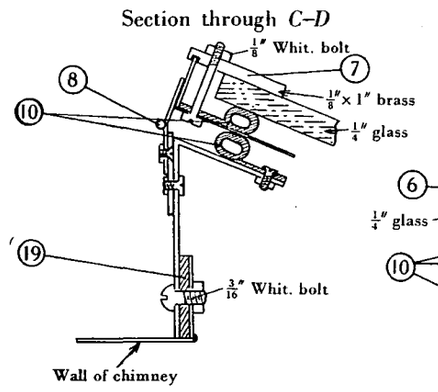
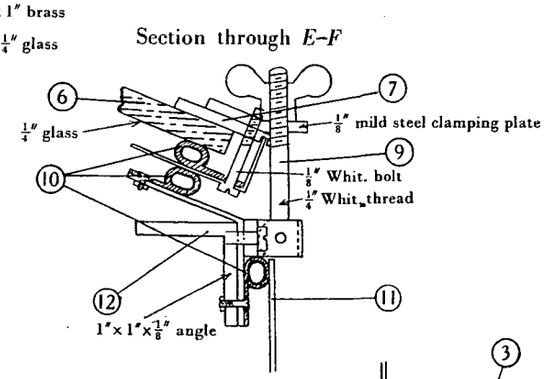
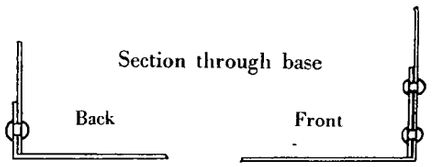
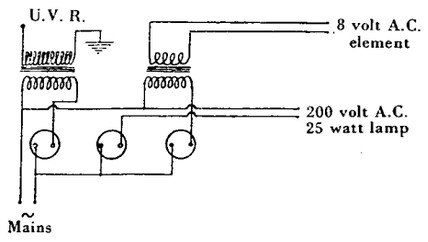


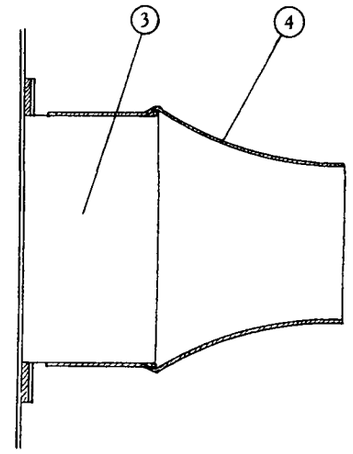








Details of wiring in Switchbox-16



Section through armhole showing rubber sleeve

Text-fig. 3. Plan of inoculation box.

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light the furnace. An 8 V. a.c. element (23) mounted on a hinged operating gear, such as shown in the figure, has proved effective. The switch for the element is fitted in the box (16). The wiring goes to a Steatite terminal block (24) and from here continues through the tubular ignition handle (25) to the element. The ignition handle is constructed of metal gas piping, and so constructed that, on rotation, the element is pushed over the holes of the gas burner. When the gas is ignited, the element is immediately withdrawn from the flame. A mica inspection window, visible through a tube (26) which passes through the wall, permits of the continuous observation of the furnace while the apparatus is in use.

Each compartment is fitted with a quartz jacketed Hanovia ultra-violet lamp (27). The transformer and switch for the ultra-violet lamps are fitted in the switch box (16). The wiring is conducted through the conduit (17) and enters the box at (28) (see right-side elevation).

An air inlet is provided by a duct (29). The aperture of the duct can be partially or completely occluded by a diaphragm operated by an outside handle (30).

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