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Exposure Chamber for 66 Mice Suitable for Use with the Henderson Aerosol Apparatus

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The Henderson apparatus (D. W. Henderson, J. Hyg. **50**:53, 1952; O. T. Miller and G. G. Gremillion, *In* Technical Manuscript 39, U.S. Army Biological Laboratories, Fort Detrick, Md., 1963) has become standard equipment for exposing a variety of animals to microbiological aerosols. When small animals such as mice are used, it is advantageous to aerosolize many animals in a single study. The standard small-animal exposure chamber supplied with the Henderson apparatus accommodates only 16 mice, necessitating repeated periods of nebulization. This produces an unavoidable variation in the character of the aerosol delivered to the different groups of mice. To circumvent this problem, we have devised a small-animal exposure chamber which holds 66 mice. It is designed for use with

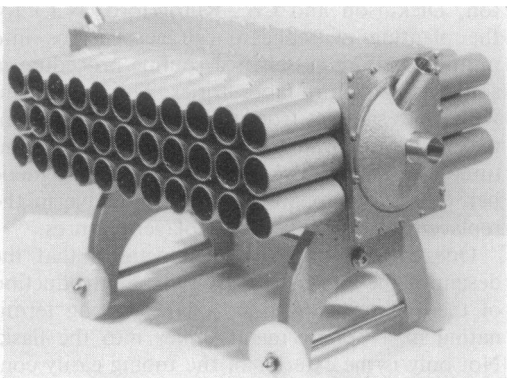

FIG. 1. *Photograph of the mouse exposure chamber.*

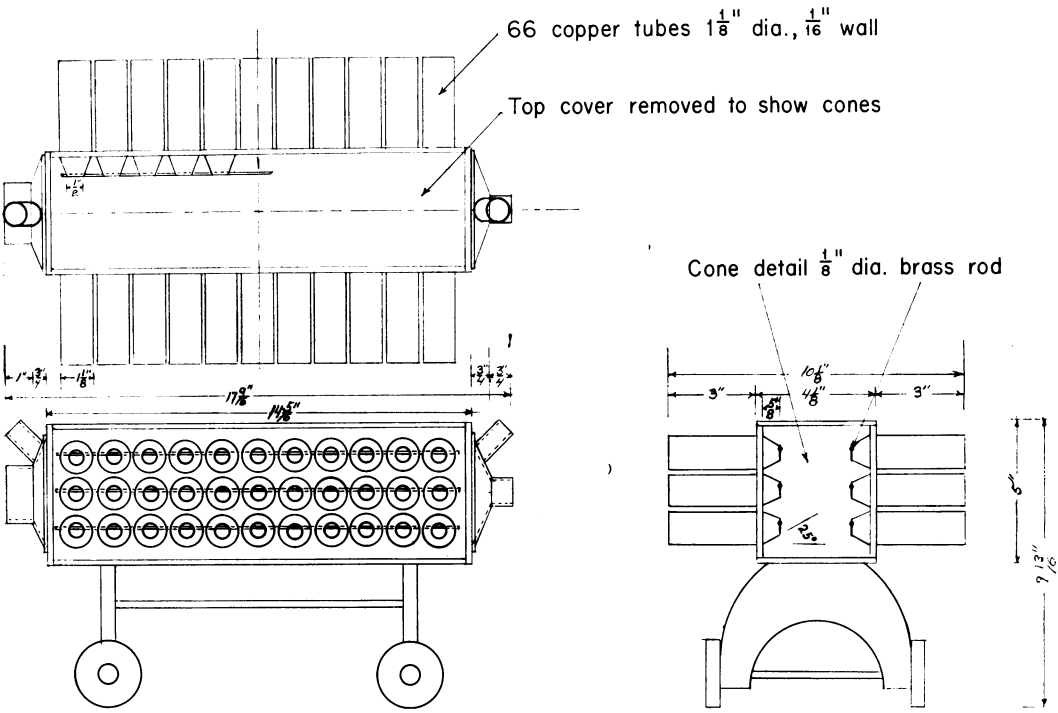

FIG. 2. *Scaled diagram of the mouse exposure chamber.*

TABLE 1. *Evaluation of 66-port mouse exposure chamber adapted for Henderson aerosol apparatus*[a]

Animal port no.	Expt no. 1	Expt no. 2	Expt no. 3	Animal port no.	Expt no. 1	Expt no. 2	Expt no. 3
1	2.03	2.24	1.02	34	2.01	3.10	1.16
2	1.53	0.81	1.12	35	1.78	<0.01	0.40
3	—[b]	1.75	1.10	36	1.61	1.48	0.83
4	1.50	4.50	5.00	37	3.35	1.80	0.67
5	3.31	0.77	3.10	38	3.27	1.85	0.84
6	2.14	2.40	1.38	39	3.00	3.50	0.93
7	1.71	3.75	2.00	40	2.60	1.20	—
8	1.52	1.00	1.40	41	4.50	—	1.89
9	3.50	4.50	0.75	42	1.50	1.57	2.60
10	3.27	3.05	3.11	43	1.91	3.10	4.60
11	2.02	2.18	1.22	44	4.20	3.30	1.58
12	3.30	—	1.01	45	2.40	2.09	0.85
13	3.51	—	2.40	46	0.93	4.60	2.16
14	2.00	3.50	—	47	3.50	4.80	0.90
15	2.17	3.60	—	48	1.40	5.40	2.11
16	1.49	<0.01	2.08	49	0.80	7.40	1.30
17	2.66	—	3.80	50	4.28	3.60	1.74
18	2.98	—	1.10	51	2.40	2.32	1.00
19	1.82	3.41	4.60	52	1.03	2.18	4.70
20	2.82	0.96	2.85	53	2.86	4.20	5.60
21	2.71	2.52	2.10	54	3.00	2.93	2.95
22	1.61	3.60	1.14	55	2.63	3.60	2.80
23	1.30	2.90	1.00	56	—	0.61	2.40
24	2.00	3.20	1.88	57	2.29	5.90	1.07
25	2.22	1.13	1.80	58	1.35	4.20	5.70
26	1.56	2.64	1.76	59	2.76	3.65	3.80
27	3.00	1.44	0.80	60	2.05	3.90	1.11
28	—	0.91	2.05	61	2.40	3.60	2.40
29	2.28	3.25	0.97	62	4.00	3.00	2.43
30	2.40	3.03	0.06	63	1.71	4.20	7.10
31	1.55	2.99	1.40	64	1.42	3.66	4.80
32	2.54	3.24	2.11	65	3.80	3.80	1.23
33	1.68	3.30	0.64	66	1.15	4.90	2.05

[a] *P. aeruginosa* "310" was nebulized for 30 min. The bacterial counts per whole mouse lung ($\times 10^5$) were taken at "time-zero." For experiments 1, 2, and 3, respectively, the means ± SD ± SE were 2.333 ± 0.915 ± 0.115; 4.557 ± 3.195 ± 0.409; and 2.102 ± 1.460 ± 0.180. The spray factors were 5.15×10^{-6}, 2.42×10^{-6}, and 2.05×10^{-6} for experiments 1, 2, and 3, respectively. The average impinger values were 7.6×10^5/ml, 1.35×10^6/ml, and 8.8×10^5/ml for experiments 1, 2, and 3, respectively.

[b] Blank spaces represent samples technically unsatisfactory or dead animals.

the Henderson apparatus and enables the simultaneous exposure of a large number of animals to the same aerosol.

Description of the exposure chamber. The chamber is constructed of brass plate [3/16 inch (0.47 cm) thick], copper tubing [1 1/16 inch (2.96 cm) inside diameter] with aluminum outlets on either end, nylon wheels, and stainless-steel axles (Fig. 1 and 2). The larger end connects with the aerosol drying section of the exposure tube assembly of the Henderson apparatus, whereas the smaller end is for the impinger connection. The openings directed diagonally upward on either end of the chamber connect to the draft gauge (larger end) and to one of the critical orifices (smaller end). The inner extremity of each animal exposure tube tapers to a 1/2 × 3/8 inch (1.27 × 0.95 cm) elliptical opening which projects 3/4 inch (1.90 cm) into the aerosol chamber. After the mice are placed in the tubes, styrofoam plugs (1 inch long) are inserted behind them to maintain their noses forward into the inner opening, and rubber stoppers are placed over the outer openings to make an air-tight closure. The aerosol exposure is then carried out as with the standard Henderson exposure chamber.

Description of the experiments. Female Swiss-Webster strain mice (20 to 22 g) were exposed for 30 min to aerosols of *Pseudomonas aeruginosa* (strains "DB" and "310") in a manner similar to that previously described (A. E. Jackson, P. M. Southern, A. K. Pierce, B. D. Fallis, and J. P.

TABLE 2. *Evaluation of standard 16-port mouse exposure chamber of the Henderson aerosol apparatus*[a]

Animal port no.	Expt no. 1	Expt no. 2	Expt no. 3
1	5.00	1.80	1.55
2	3.00	1.02	2.88
3	1.72	0.74	2.94
4	1.80	1.05	2.45
5	0.42	0.76	1.47
6	0.60	0.74	2.50
7	1.06	0.26	2.15
8	1.34	1.63	1.98
9	0.67	0.70	1.85
10	0.68	1.29	2.30
11	0.52	1.99	4.30
12	1.76	2.04	4.90
13	0.43	1.14	2.51
14	0.73	2.00	2.90
15	0.79	1.64	3.50
16	0.85	3.00	1.30

[a] *P. aeruginosa* "DB" was nebulized for 30 min. The bacterial counts per whole mouse lung ($\times 10^5$) were taken at "time-zero." For experiments 1, 2, and 3, respectively, the means $\pm$ SD $\pm$ SE were $1.336 \pm 1.143 \pm 0.285$; $1.363 \pm 0.673 \pm 0.168$; and $2.593 \pm 1.112 \pm 0.238$. The spray factors were 1.78×10^{-6}, 3.23×10^{-6}, and 2.95×10^{-6} for experiments 1, 2, and 3, respectively. The average impinger values were 1.7×10^6/ml, 2.3×10^6/ml, and 4.55×10^6/ml for experiments 1, 2, and 3, respectively.

TABLE 3. *Evaluation of particle size distribution with the Andersen aerosol sampler*[a]

Andersen plate no.	16-Port chamber	66-Port chamber
1	6	6
2	5	9
3	5	10
4	12	18
5	942	>2,427
6	786	840

[a] Colonies per plate per 30-sec sample of *P. aeruginosa*.

Sanford, J. Lab. Clin. Med. **69**:833, 1967). Immediately after nebulization (time-zero), the mice were removed from the chamber and were killed by luxation of the neck. The lungs were removed aseptically, weighed, ground with sterile mortars and pestles, and 10-fold serial dilutions were made in sterile saline. Samples were quantitatively cultured on eosin-methylene blue agar plates. Results obtained with the 66-port chamber were compared with similar experiments using the standard 16-port chamber. Elapsed time from the end of nebulization until all mouse lungs were removed never exceeded 20 min.

The results are shown in Tables 1 and 2. Although there is a variation in bacterial counts of approximately $1.5 \times \log_{10}$, the vast majority of counts are within one $\log_{10}$ deviation, and the results obtained by use of the standard 16-port chamber are essentially similar. Table 3 contains data comparing the relative density of various particle sizes obtained by sampling the impinger outlet of both exposure chambers with an Andersen cascaded sieve-type aerosol sampler (A. A. Andersen, J. Bacteriol. **76**:471, 1958). With this sampler, plates 5 and 6 collected particles, at least 95% of which were 2 μ or less in diameter. Both aerosol chambers contained almost all particles in this size range.

Conclusion. The foregoing data demonstrate that this 66-port aerosol exposure chamber is suitable for use with the Henderson apparatus and enables the simultaneous exposure of a large number of animals.

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