The Pulmonary Clearance of Bacteria by Calves and Mice

L. E. Lillie and R. G. Thomson*

SUMMARY

Using a modified aerosol generator, white mice and calves were exposed to aerosols of viable Staphylococcus aureus and Pasteurella haemolytica and the clearance of the inhaled organisms by the lungs of the experimental animals was measured. Fifty-seven percent of inhaled S. aureus were cleared in two hours by the mouse lungs, 79% were cleared in four hours and 93% were cleared in eight hours. Fifty-six percent of inhaled P. haemolytica were cleared in two hours by the mouse lungs, 76% were cleared in four hours and 93% were cleared in eight hours. Seventy percent of inhaled S. aureus were cleared in two hours by the calf lungs, 90% were cleared in four hours and 95% were cleared in eight hours. Seventy-five percent of inhaled P. haemolytica were cleared in two hours by the calf lungs, 90% were cleared in four hours and 92% were cleared in eight hours.

RÉSUMÉ

A l'aide d'un générateur d'aérosols modifié, les auteurs exposèrent des souris blanches et des veaux à des aérosols de Staphylococcus aureus et de Pasteurella haemolytica viables; ils mesurèrent ensuite le taux d'élimination des microbes inhalés par les poumons de ces animaux d'expérience. Les souris éliminèrent 57% des S. aureus en deux heures, 79% en quatre heures et 93% en huit heures. Elles éliminèrent 56% des P. haemolytica en deux heures, 76% en quatre heures et 93% en huit heures. Les veaux éliminèrent 70% des S. aureus en deux heures, 90% en quatre heures et 95% en huit heures. Ils éliminèrent 75% des P. haemolytica en deux heures, 90% en quatre heures et 92% en huit heures.

INTRODUCTION

The lung clearance technique has been used by several workers to measure the reaction of the respiratory defense mechanisms, especially alveolar macrophage and mucociliary clearance of infectious and noninfectious particles under various debilitating conditions (7, 8, 9, 11, 17, 18, 19, 20, 21, 22, 23, 26). Measurement of lung clearance requires that living animals be exposed to aerosols of a size and character such that the particles are able to penetrate into and deposit on the surfaces of the alveoli and the nonciliated bronchioles. The number and activity of particles detectable at various post exposure times are then measured and the percentage clearance is calculated.

Laurenzi and co-workers found that 45% of inhaled *Staphylococcus aureus* were cleared by mouse lungs in one hour; 70% were cleared in two hours; 88% were cleared in four hours; and 97% were cleared in eight hours (19, 22). Other workers used this clearance pattern as a standard to compare the effects of various debilitating influences (19, 20). Cortisone, hypoxia, alcohol, cigarette smoke, barbiturates, carbon black, and *Escherichia coli* endotoxin have been shown to reduce the clearance

*Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Present address of the senior author is Department of Microbiology, Pathology, and Public Health, School of Veterinary Science and Medicine, Purdue University, West Lafayette, Indiana, U.S.A. 47907.

This work was supported by the Canada Department of Agriculture and the Ontario Department of Agriculture and Food, and was carried out in partial fulfillment of requirements for the degree of Master of Science.

Submitted July 16, 1971.

of inhaled *S. aureus* by mouse lungs (19, 20, 21).

Green and Kass found that *Staphylococcus albus* was cleared more rapidly by mouse lungs (94% in four hours) than was *S. aureus* (88% in four hours) or *Proteus mirabilis* (70% in four hours) (4). These authors also determined that cold, as an environmental stress, did not affect clearance rates, but that wetting and exposure to cold significantly reduced clearance.

Sellers *et al* (27) and Goldstein *et al* (9) have shown that exposure to virus several days previously will severely retard lung clearance of inhaled bacteria. Influenza virus reduced the clearance of inhaled *S. aureus* in mice from 80% cleared in four hours to —20% cleared (i.e. 120% retained or an increase of 20% in the number of bacteria originally inhaled) (18). Reovirus also reduced the clearance rates of inhaled *S. aureus* from 15% retained in four hours to 52% retained (9).

The clearance of gram-negative bacteria has been studied by several workers. *Pasteurella pneumotropica* was cleared more slowly than *S. aureus* by mouse lungs and with greater variation in the clearance pattern (11). *Pseudomonas aeruginosa* multiplied rapidly for the first hour after inhalation and only then did clearance begin (17). Rylander found that guinea pig lungs reduced the viability of inhaled *E. coli* rapidly but that actual physical removal of the bacteria from the lungs occurred much more slowly (26). Mycobacteria, in contrast to most other species, are cleared very slowly by the lung (23). This experiment is an attempt to measure the lung clearance of *Pasteurella haemolytica* and the first attempt to measure bacterial clearance in the bovine lung.

MATERIALS AND METHODS

The Aerosol Exposure Apparatus

The aerosol exposure apparatus used in this experiment is a half scale model of the machine designed by Laurenzi and Guarneri (13) (Figs. 1, 2). It consists of a rectangular plexiglass and sheet metal chamber 36 inches long, 18 inches wide, and 16 inches deep. An eight inch exposure port is situated in the centre of one long side and a plexiglass glovebox is situated on the opposite side. Two plexiglass doors connect the interior of the chamber with the interior of the glovebox and the interior of the glovebox with the outside. The edges of the doors are lined by rubber gaskets. The exposure port is sealed with heavy polyethylene film during mouse exposures.

A primary air flow is produced by an electric air compressor operated at ten pounds per square inch. Air is driven (sequentially) through a four-ply manifold of copper tubing, through rubber tubing to four air flow meters and thence to each of four DeVilbiss number 40 glass nebulizers. Each glass nebulizer is attached by clamps to one of four cylindrical plexiglass intake tubes 12 inches in length and three inches in inside diameter. A circular plexiglass baffle approximately 2.5 inches in diameter is placed in the lumen of the plexiglass cylinders six inches from the

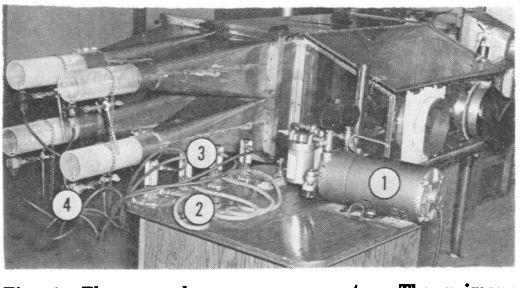

Fig. 1. The aerosol exposure apparatus. The primary air flow is generated by the air compressor (1), and moves through the four ply manifold (2), the air flow meters (3) and aerosolizes the bacterial suspension in the wells of the nebulizers (4).

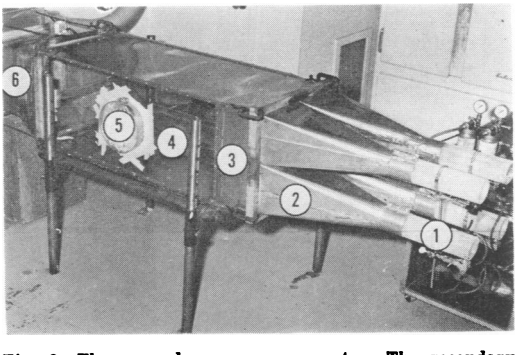

Fig. 2. The aerosol exposure apparatus. The secondary air flow is generated by the fan and the consequent negative internal pressure. The dilute aerosol moves through the air intake tubes (1), the individual mixing chambers (2), the common mixing chamber (3), past the experimental animals in the exposure chamber (4) (exposure port (5)), through the filter (6), and exits via the fan housing and the exhaust duct.

upstream end. The upper half of the nebulizer is placed into the lumen of the tube through a one inch hole in the bottom of the tube 1.5 inches from the upstream end and secured by metal clamps. The upstream openings of the cylinders are covered by several layers of cotton gauze held by elastic bands.

The plexiglass tubes fit snugly into metal sleeves which open into conical individual mixing chambers which are 18 inches in length, three inches in diameter at the upstream end and rectangular 7.5 by nine inches at the downstream end. These converge into a common mixing chamber 12 inches long, 18 inches wide, and 16 inches high. The common mixing chamber is fastened to the upstream end of the exposure chamber by four metal C clamps and a rubber gasket.

The downstream end of the exposure chamber fits into a rectangular sheet metal cone 14 inches long and narrowing to a 10.75 inch square. The downstream end of the cone fits into an absolute filter[1]. Downstream of the filter is a 15 inch exhaust fan driven by a 0.5 horsepower electric motor and a flexible metal tube four inches in diameter and eight feet long which ducts the exhaust air to the exterior of the building. A solenoid pressure manometer is inserted in the upstream end of the exhaust duct and connected to the air compressor at the upstream end of the system so that if the fan ceased functioning the decrease in air pressure would turn off the compressor and prevent the escape of aerosol into the room.

When operating, the machine consists of a primary and a secondary air flow (Fig. 3). Eight ml of the bacterial suspension are placed in the well of each of the nebulizers and are aerosolized by the primary air flow. The nebulizers are calibrated to deliver a particle size distribution of (19, 22):

3.3 — 5.5 μ	14%
2.0 — 3.3 μ	26%
1.0 — 2.0 μ	53%
1.0 μ or less	7%

The aerosol then enters the plexiglass intake tubes and is mixed with, dried by and becomes part of the secondary air flow. The secondary air flow is produced by the fan at the downstream end of the system

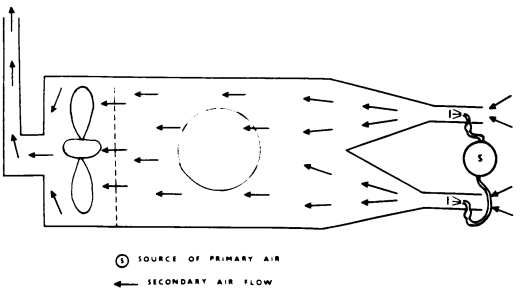

Fig. 3. Air flow diagram (of the aerosol exposure apparatus).

and the consequent negative pressure created inside the exposure chamber which sucks room air into the intake tubes at the rate of approximately 100 linear feet per minute. Particles of larger size and mass will have sufficient inertia that they will not bend with the airstream and will thus be impinged on the baffles in the intake tubes and removed from the aerosol.

The aerosol then moves through the mixing chambers, past the experimental animals in the exposure chamber, and out through the absolute filter. Relative humidity within the exposure chamber is monitored by a wet and dry bulb hygrometer.

Preparation of the Bacterial Suspensions

A coagulase positive strain of *S. aureus* (FDA 209P) was obtained from G. A. Laurenzi and J. J. Guarneri[2]. The organism was stored on 5% beef blood agar slopes at —70°C. until used. Single colony inocula were placed into tubes containing 5 ml of trypticase soy broth (TSB Difco[3]) and incubated overnight at 37°C. One ml quantities of the resulting broth culture were used to inoculate 100 ml quantities of TSB in 250 ml Erlenmeyer flasks. These were incubated for 16 hours at 37°C in a shaker water bath. After incubation, 45 ml quantities of the 16 hour broth cultures were placed in 50 ml centrifuge tubes and centrifuged at 6000 RPM for 25 minutes in a Sorval Superspeed Centrifuge[4] (Relative

[1]Flanders Filters Inc., Riverhead, New York.

[2]Then of the New Jersey College of Medicine and Dentistry, Jersey City, New Jersey.

[3]Difco Laboratories, Detroit, Michigan.

[4]Ivan Sorval Incorporated, Norwalk, Connecticut.

TABLE I: Number of Mice Exposed Per Trial

Trial	1	2	3	4	5	6	7
S. *aureus*	50	57	89	97	97	98	—
P. *haemolytica*	52	90	79	70	70	70	74

Centrifugal Force = 4340 G's), washed in 0.1 molar potassium phosphate buffer (pH 7.3-7.5), centrifuged again and then resuspended in 12 ml of diluent (either phosphate buffer or distilled water). The resulting bacterial suspension had a mean density of 7.7 x 10^9 (± 2.87 x 10^9) organisms per ml.

A laboratory strain of *P. haemolytica* (biochemical type A, serotype 1) was obtained from E. L. Biberstein[5]. The organism was grown on 5% beef blood agar plates and stored in 5% dextrose in hermetically sealed vials at —70°C until use. Several smooth colonies (as determined by the crystal violet technique (31) which demonstrated pronounced haemolysis were inoculated directly into 200 ml quantities of brain heart infusion broth (BHI Difco[3]) in one litre Erlenmeyer flasks. *P. haemolytica* has a high oxygen requirement and the larger flasks provided greater surface area and therefore increased aeration (30). These were incubated at 37°C in a shaker water bath for eight to nine hours. After incubation, 45 ml quantities of the BHI broth cultures were placed in 50 ml centrifuge tubes and centrifuged at 6000 RPM for 25 minutes. The pellet was resuspended in 5 ml of 0.1 molar potassium phosphate buffer (pH 7.3-7.5) to give a mean suspension density of 9.22 x 10^9 (± 9.23 x 10^9) organisms per ml.

All bacterial suspensions were prepared immediately prior to use.

Exposure and Sampling of Mice

Adult white mice weighing 25-30 grams were obtained from the Ontario Veterinary College Research Farm. During the exposure the mice were placed in individual half inch wire mesh cages, 2.5 x 2.5 x 4 inches in size. These cages were designed to prevent crowding, to allow complete freedom of movement and to cause as little air turbulence as possible. From 50 to 100 mice per trial were placed in the exposure chamber of the aerosol apparatus (Fig. 4, Table I).

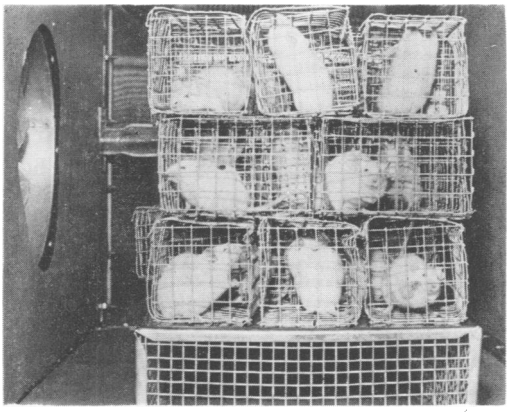

Fig. 4. A group of caged mice in the exposure chamber (viewed from the upstream end).

After each exposure, the mice were arbitrarily divided into approximately equal groups. One group was killed immediately after the exposure and the other groups were killed at one, two, three, four, six, eight, 12, 24 and 48 hours post-exposure.

A total of 488 mice divided into six separate trials were exposed to aerosols of *S. aureus*. A total of 505 mice divided into seven separate trials were exposed to aerosols of *P. haemolytica*.

The mice were killed by clamping the neck with a curved haemostat and were placed in a disinfectant bath (Dettol[6] or Kem-25[7]) for several minutes. The thorax was then opened and the lungs removed by transection of the main stem bronchi. The lungs were placed in individual glass grinders and homogenized with 5 ml of sterile phosphate buffer. Tenfold dilutions to 10^{-3} in buffer tubes were prepared from the homogenate. The serial dilutions and the original homogenate (for convenience referred to as 10^{-0}) were then plated onto 5% beef blood agar using the drop technique (24) adapted so that each drop consisted of 0.05 ml instead of 0.02 ml. Following overnight incubation at 37°C, individual colonies could be distinguished and contaminant colonies, when present, were easily identified. Colonies were counted from those dilutions yielding a count of

[5]College of Veterinary Medicine, University of California, Davis, California.

[6]Reckitt & Colman (Canada) Ltd., Lachine, Quebec.
[7]Kem-San Limited, Oakville, Ontario.

from 30 to 300 colonies. In cases where the lowest dilution (i.e. the homogenate) yielded fewer than 30 colonies, this number was recorded. An American Optical electronic touch counter and a Quebec Counter were used to count the bacterial colonies.

The most probable number of colony forming units (C.F.U.) in each lung was calculated by using the mean count of the four drops multiplied by the dilution factor. The mean number of C.F.U. per lung was then calculated for the group of mice killed at each post-exposure time. The group mean number of C.F.U. was expressed as a percentage of the number of C.F.U. present immediately after the exposure (0 hour), and referred to as the group mean percentage retention. Percent clearance then equaled 100% minus the percent retention.

Exposure and Sampling of Calves

Predominantly male calves (two animals were female) ranging in age from two to four months and varying in weight from 125 to 200 pounds were used for the calf studies. These calves were obtained either shortly after birth from the Ontario Agricultural College dairy herd or from local cattle markets. The calves were raised in pens holding two calves per pen until shortly before use. They were then assembled in a barn adjacent to the aerosol exposure facility. Prior to exposure the calves were weighed and temperatures, cardiac rates and respiratory rates taken. Insofar as possible, calves of similar weights were used in the same trial. No attempt was made to assess the immunological status of these calves.

The calves were exposed by placing their heads inside the large exposure port and closing a polyethylene hood around the neck by means of a drawstring (Fig. 5). During the exposure the calves were restrained in a portable wooden chute placed adjacent and perpendicular to the long axis of the exposure chamber. Three or four calves were exposed sequentially (in as short a time as possible) in each trial to the same bacterial suspension under the same operating conditions. All exposures were of 30 minutes duration. The reference calf, i.e. the 0 hour calf, was usually exposed last. Thus if any deterioration occurred in the bacterial suspension during the trial, it would be reflected in a low

Fig. 5. A calf with its head in the exposure chamber (viewed from the upstream end).

deposition values rather than false low clearance values. The possibility of an increase in the density of the suspension during the 1.5 to two hours of each trial was considered to be low because the bacterial pellet was washed and then suspended in a non-nutrient buffered medium. Following exposure, the calves were returned to the adjacent barn. Either two or three calves were used to determine each post-exposure clearance value.

Calves were killed by high voltage electrocution either immediately following exposure or at two, four, six and eight hours post-exposure. The head was maintained in an elevated position until a tracheal plug could be inserted thus preventing contamination by retrograde flow of rumen contents. As soon as the current was disconnected the neck was flooded with Dettol or Kem-25 disinfectant solution and the trachea and larynx exposed and isolated. A sterile wooden tracheal plug was inserted and secured by sterile string. The skin of the animal was flooded with disinfectant solution and the right lateral thoracic wall removed. Care was taken not to incise the abdominal organs. The heart, lungs, mediastinum, esophagus and trachea were removed, placed in a sterile tray and taken to the processing laboratory. Samples of liver, kidney, spleen, bronchial lymph node and heart's blood were taken for bacteriological culture. The lungs were separated by transection of the main bronchi and weighed.

Eleven 10 gram samples of lung were excised as shown in Fig. 6 and placed in sterile plastic cups. Occasionally plum colored patches of atelectasis or consolidation were present in some sampling areas. In this case, alternate areas were sampled.

Each lung sample was individually homogenized with 100 ml of sterile phosphate buffer (pH 7.3-7.5) in a sterile Waring blender. The homogenates were diluted, plated and counted as described above. The most probable number of colony forming units (C.F.U.) in each 10 gram sample of lung was then calculated by using the mean of the four drops multiplied by the dilution factor. The mean number of C.F.U. per gram of lung for each region sampled and for the lung as a whole was then calculated. The kidney, spleen, liver and heart's blood were plated onto blood agar to determine if the inhaled microorganisms were moving beyond the lung.

RESULTS

The deposition of *S. aureus* in the lungs of the experimental mice averaged 95.90 x 10^3 ± 29.60 x 10^3 organisms per lung (Range 66.55 x 10^3 — 146.20 x 10^3 organisms per lung). The deposition of *P. haemolytica* in the experimental mice averaged 38.56 x 10^3 ± 19.56 x 10^3 organisms per lung (Range 3.77 x 10^3 — 60.40 x 10^3 organisms per lung).

The deposition of *S. aureus* in the lungs of the experimental calves averaged 178.87 x 10^3 ± 138.37 x 10^3 organisms per gram of lung (Range 5.79 x 10^3 — 455.64 x 10^3 organisms per gram of lung). No difference was observed in the deposition of *S. aureus* in the different regions of the calf lung except for the posterior tip of the diaphragmatic lobes which were consistently lower than the mean for the whole lung. *S. aureus* were not found in the sampled organs other than the lungs.

The deposition of *P. haemolytica* in the lungs of the experimental calves averaged 44.42 x 10^3 ± 72.63 x 10^3 organisms per gram of lung (Range 9.95 x 10^3 — 192.00 x 10^3 organisms per gram of lung). No difference was observed in the deposition of *P. haemolytica* in the different regions of the calf lung except for the posterior tip of the diaphragmatic lobes which were consistently lower than the mean for the whole lung. With one possible exception (thought to be due to technical error) *P. haemolytica* were not found in the sampled organs other than the lungs.

The clearance of the inhaled bacteria is shown in Table II and Figs. 7 to 10.

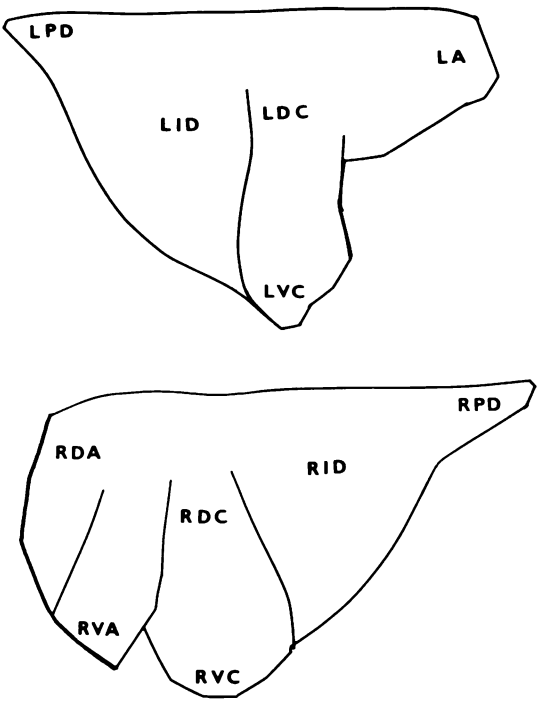

Fig. 6. The sampling sites of calf lungs. LA — left apical, LVC — left ventral cardiac, LDC — left dorsal cardiac, LID — left intermediate diaphragmatic, LPD — left posterior diaphragmatic, RVA — right ventral apical, RDA — right dorsal apical, RVC — right ventral cardiac, RDC — right dorsal cardiac, RID — right intermediate diaphragmatic, RPD — right posterior diaphragmatic.

DISCUSSION

The preparation of a bacterial suspension for aerosolization and the aerosolization of the suspension subjects the microorganisms to severe environmental stresses. These stresses consist mainly of fluctuations in relative humidity and temperature (15), exposure to oxygen (1, 6, 33), and to changes in molecular bound water due to dehydration and rehydration (33). The resistance of a bacterium to the stresses of aerosolization is related to its resistance to other forms of stress (10).

The results of this experiment suggest that *S. aureus* withstood these stresses much better than did *P. haemolytica*. This impression is in agreement with those of Hatch (14) and Zentner (32) that gram-negative bacteria are generally more susceptible to the stresses of aerosolization than are gram-positive bacteria. In this investigation the density of the *S. aureus* suspensions was usually lower than the

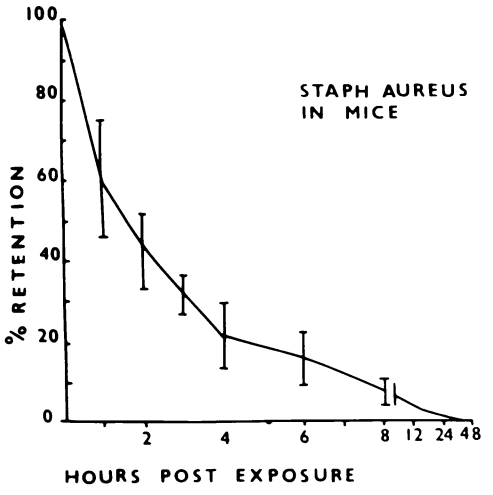

Fig. 7. The clearance of Staphylococcus aureus by mouse lungs from 0-48 hours post exposure.

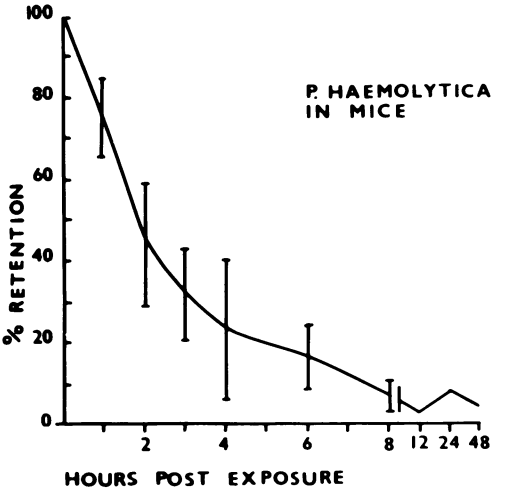

Fig. 8. The clearance of Pasteurella haemolytica by mouse lungs from 0-48 hours post exposure.

density of the *P. haemolytica* suspensions while the deposition of *P. haemolytica* was usually lower than the deposition of *S. aureus*.

The reason for the lower deposition of *P. haemolytica* in the lungs of mice was not determined but was surmised to be due to a greater death loss during aerosolization and also to a possible tendency towards clumping because of the mucoid capsule.

P. haemolytica has more exacting cultural requirements than most other bacteria (30) and therefore more care was required to produce a uniform suspension than with *S. aureus*. The broth cultures of *P. haemolytica* in this experiment were much more varied in terms of numbers of organims than were the cultures of *S. aureus* thus accounting for the high standard deviation ($9.22 \times 10^9 \pm 9.23 \times 10^9$ organisms per ml) which resulted because of one exceptionally heavy culture.

To our knowledge this experiment represents the first measurement of the clearance of *P. haemolytica* by the lung. The only previous report of clearance studies with a Pasteurella species is that of Goldstein and Green (8) who studied the clearance of *Pasteurella pneumotropica* in mice. Other workers (2, 3, 4, 16, 27, 29) have reported on the infectivity of aerosols of *Pasteurella pestis, Pasteurella tularensis, Pasteurella multocida,* and *P. haemolytica,* but have not measured the rate of lung clearance. The clearance of *P. haemolytica* in mouse lungs is similar in pattern to but slightly slower in rate during the

TABLE II: Mean Percent Retention[a] of Bacteria in Lungs of Mice and Calves

Hours Post Exposure	Mouse Lungs				Calf Lungs			
	S. aureus		P. haemolytica		S. aureus		P. haemolytica	
0	100%	S.D.[b]	100%	S.D.	100%	S.D.	100%	S.D.
1	60.5%	13.77	76.0%	9.54	—		—	
2	42.7%	9.0	44.4%	15.42	29.8%	7.54	25.0%	6.94
3	31.8%	4.35	32.0%	11.83	—		—	
4	21.1%	8.12	23.9%	17.16	9.9%	3.14	10.5%	3.65
6	16.0%	6.33	18.0%	8.04	16.0%	3.02	10.9%	5.06
8	7.5%	3.52	7.5%	4.13	5.0%	.95	8.2%	2.39
12	3.6%	1.26	3.8%	.09	—		—	
24	0.7%	.41	8.3%	7.48	—		—	
48	0.2%	.13	4.8%	2.67	—		—	

[a] Clearance equals 100 % minus percent retention
[b] Standard deviation

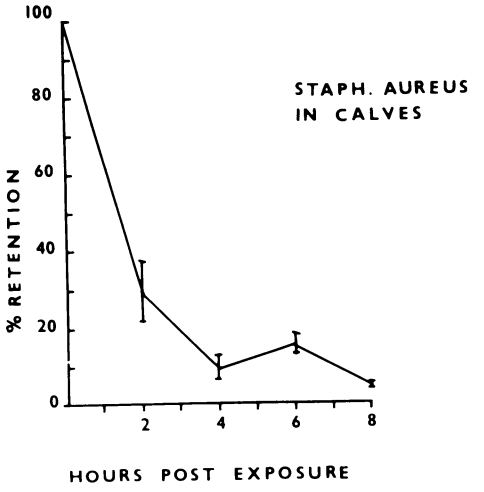

Fig. 9. The clearance of Staphylococcus aureus by calf lungs from 0-8 hours post exposure.

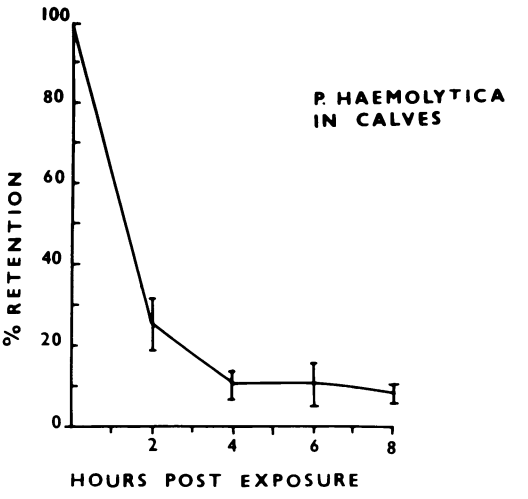

Fig. 10. The clearance of Pasteurella haemolytica by calf lungs from 0-8 hours post exposure.

first six hours than the clearance of *S. aureus*. The *P. haemolytica* clearance rates are similar in pattern to but are more rapid (by 2 to 10% per hour) than the rates reported by Goldstein and Green (8) for *P. pneumotropica* in mice. The standard deviations were also greater for *P. haemolytica* indicating a greater individual variation in the response of the mouse lungs to *P. haemolytica* than in the response to *S. aureus*.

The clearance of *S. aureus* by mouse lungs in this experiment was similar in pattern to but slightly slower in rate (by 5 to 10% per hour) than the clearance observed by Laurenzi and Guarneri (22). The present results probably represent small technical differences and essentially agree with and confirm the results of these earlier workers.

Both *S. aureus* and *P. haemolytica* were cleared at slightly more rapid rates in the calf than in the mouse lungs. The reason for this may be that the bovine lung is continually exposed to inhaled ruminal microorganisms (5, 25) which may produce a state of continual alveolar macrophage activation (7), or that the bovine alveolar macrophages have greater metabolic and enzymic activity. At two hours post-exposure 12.86% fewer *S. aureus* could be recovered from the calf lungs than from the mouse lungs. At four hours this difference was 13.38%. At later post-exposure times these differences become less marked. This was felt to be due to the limitations of the experimental technique in that a much greater number of calves would be required to measure accurately the more subtle clearance rates which occur at longer post-exposure times.

P. haemolytica frequently increases in numbers and dominates the nasal flora in cattle stressed by transit (28). By means not completely understood, these organisms enter the lung, multiply and induce a pneumonic response. Several possible methods exist by means of which nasal flora may reach the lung, one of which is in the form of droplet nuclei either aspirated from the nasal flora or inspired in air exhaled from surrounding animals also experiencing a rise in nasal *P. haemolytica*. Droplet nuclei are recognized as important sources of both viral and bacterial respiratory diseases of man. The observations of Grey and Thomson (12) suggest that *P. haemolytica* moves from the nose to the lung in the tracheal air in the form of droplet nuclei. Aerosol exposure — lung clearance studies therefore are a method of studying one of the probable links between a proliferation of the nasal flora and pulmonary invasion of pasteurella.

The present experiment demonstrates that *S. aureus* and *P. haemolytica* are inactivated by the normal bovine lung. Many workers have demonstrated that stress factors similar to those connected with "Shipping Fever" do allow inhaled bacteria to colonize and multiply in the lung. Goldstein and Green (8) demonstrated this

fact using *P. pneumotropica* in mice. The aerosol exposure-lung clearance model, using *P. haemolytica* and calves or more mature cattle offers a useful experimental system for the study of bovine pneumonia. By altering the debilitating conditions imposed on the experimental animals, either singly or in combination, the environmental conditions seen in the natural disease may be reproduced and their effects on the defense mechanisms of the lung measured.

Use of the lung clearance technique in large animals requires several adaptations. In previous experiments large numbers of small animals were used to compute each point on the clearance curve. In this experiment only a few animals were used to compute each point. However, many samples were taken from each animal. In this experiment each animal was exposed consecutively instead of simultaneously as with the laboratory species. Modifications to the body of the aerosol exposure apparatus will permit future exposures to be carried out simultaneously.

ACKNOWLEDGMENTS

The aerosol exposure apparatus was constructed and calibrated by the School of Engineering of the University of Guelph, Guelph, Ontario under the direction of Professors J. Scott and D. Pattie, and Mr. C. Hutchins. The excellent technical assistance of Mr. Brendon McCann and Miss Mary Grosso, and Doctors F. Harris and J. Richardson (then undergraduate veterinary students) is gratefully acknowledged.

REFERENCES

1. **BENBOUGH, J. E.** Death mechanisms in airborne **E. coli**. J. gen. Microbiol. **47**: 325-333. 1967.
2. **BERENDT, R. F.** Some physiological and immunological properties of **P. pestis** recovered from aerosols. J. Bact. **75**: 217-223. 1958.
3. **CARTER, G. R.** Some remarks on shipping fever in Canada. Can. J. comp. Med. **20**: 289-293. 1956.
4. **COLLIER, J. R., T. L. CHOW, MAXINE M. BENJAMIN and A. W. DEEM.** The combined effect of infectious bovine rhinotracheitis virus and **Pasteurella haemolytica** on cattle. Am. J. vet. Res. **21**: 195-198. 1960.
5. **COLLIER, J. R. and C. F. ROSSOW.** Microflora of apparently healthy lung tissue of cattle. Am. J. vet. Res. **25**: 391-393. 1964.
6. **COX, C. S. and F. BALDWIN.** The toxic effect of oxygen upon the aerosol survival of **Escherichia coli B.** J. gen. Microbiol. **49**: 115-117. 1967.
7. **FERIN, J., G. URBANKOVA and A. VICKOVA.** Pulmonary clearance and the function of macrophages. Archs envir. Hlth **10**: 790-795. 1965.
8. **GOLDSTEIN, E. and G. M. GREEN.** Alteration of the pathogenicity of **Pasteurella pneumotropica** for the murine lung caused by changes in pulmonary antibacterial activity. J. Bact. **93**: 1651-1656. 1967.
9. **GOLDSTEIN, E., G. M. GREEN and C. SEAMANS.** The effect of silicosis on the antibacterial defense mechanisms of the murine lung. J. infect. Dis. **120**: 210-216. 1969.
10. **GOODLOW, R. J. and F. A. LEONARD.** Viability and infectivity of microorganisms on experimental airborne infection. Bact. Rev. **25**: 182-187. 1961.
11. **GREEN, G. M. and E. H. KASS.** The influence of bacterial species on pulmonary resistance to infection in mice subjected to hypoxia, cold stress and ethanolic intoxication. Br. J. exp. Path. **46**: 360-366. 1965.
12. **GREY, C. L. and R. G. THOMSON. Pasteurella haemolytica** in the tracheal air of calves. Can. J. comp. Med. **35**: 121-128. 1971.
13. **GUARNERI, J. J.** Personal communication to R. G. Thomson. 1966.
14. **HATCH, M. T. and R. L. DIMMICK.** A study of dynamic aerosols of bacteria subjected to rapid changes in relative humidity. Proc. First Intern. Symp. Aerobiol. Berkely, Calif. 1963.
15. **HATCH, M. T. and R. L. DIMMICK.** Physiological responses of airborne bacteria to shifts in relative humidity. Bact. Rev. **30**: 597-602. 1966.
16. **HETRICK, F. M., S. C. CHANG, R. J. BYRNE and P. A. HANSEN.** The combined effect of **Pasteurella multocida** and Myxovirus Parainfluenza 3 upon calves. Am. J. vet. Res. **24**: 939-947. 1963.
17. **JACKSON, A. E., P. M. SOUTHERN, A. K. PIERCE, B. D. FALLIS and J. P. SANFORD.** Pulmonary clearance of gram-negative bacilli. J. Lab. clin. Med. **69**: 833-841. 1967.
18. **KASS, E. H., G. M. GREEN and E. GOLDSTEIN.** Mechanisms of antibacterial action in the respiratory system. Bact. Rev. **30**: 488-496. 1966.
19. **LAURENZI, G. A., L. BERMAN, M. FIRST and E. H. KASS.** A quantitative study of the deposition and clearance of bacteria in the murine lung. J. clin. Invest. **43**: 759-768. 1964.
20. **LAURENZI, G. A. and J. J. GUARNERI.** A study of the mechanisms of pulmonary resistance to infection: the relationship of bacterial clearance to ciliary and alveolar macrophage function. Am. Rev. resp. Dis. **93**: 134-141. 1966.
21. **LAURENZI, G. A., J. J. GUARNERI and R. B. ENDRIGA.** Important determinants in pulmonary resistance to bacterial infection. 7th Conf. Resp. Dis. Aspen 1964 Med. Thorac **22**: 48-59. 1965.
22. **LAURENZI, G. A., J. J. GUARNERI, R. B. ENDRIGA, and J. P. CAREY.** Clearance of bacteria by the lower respiratory tract. Science **142**: 1572-1573. 1963.
23. **LOUDEN, R. G., LINDA R. BUMGARNER, JACQUELINE LACEY and G. K. COFFMAN.** Aerial transmission of mycobacteria. Am. Rev. resp. Dis. **100**: 165-171. 1969.
24. **MILES, A. A. and S. S. MISRA.** The estimation of the bactericidal power of the blood. J. Hyg., Camb. **38**: 732-748. 1938.
25. **MULLENOX, C. H., M. J. ALLISON and J. R. SONGER.** Transport of aerosolized microorganisms from the rumen to the respiratory system during eructation. Am. J. vet. Res. **25**: 1583-1594. 1964.
26. **RYLANDER, R.** Pulmonary defense mechanisms to airborne bacteria. Acta physiol, scand. supp. **306**: 1-89. 1968.
27. **SELLERS, T. F., J. SCHULMAN, C. BOUBIER, R. MCCUNE and E. D. KILBOURNE.** The influence of influenza virus infection on exogenous staphylococcal and endogenous murine bacterial infection of the bronchopulmonary tissues of mice. J. exp. Med. **114**: 237-256. 1961.
28. **SAUNDERS, J. R. and D. T. BERMAN,** Epizootiological studies of shipping fever II. Exposure of calves to **Pasteurella** and para-influenza 3 virus. Can. J. comp. Med. **28**: 57-62. 1964.
29. **THOMSON, R. G., M. L. BENSON and M. SAVAN.** Pneumonic pasteurellosis of cattle: microbiology and immunology. Can. J. comp. Med. **33**: 194-206. 1969.
30. **THORPE, B. D. and S. MARCUS.** Phagocytosis and intracellular fate of **Pasteurella tularensis** II in vitro studies with rabbit alveolar and guinea pig alveolar and peritoneal mononuclear phagocytes. J. Immun. **93**: 558-565. 1964.
31. **WESSMAN, G. E.** Cultivation of **Pasteurella haemolytica** in a chemically defined medium. Appl. Microbiol. **14**: 597-602. 1966.
32. **WHITE, P. C. and J. B. WILSON.** Differentiation of smooth and nonsmooth colonies of **Brucellae**. J. Bact. **61**: 239-242. 1951.
33. **ZENTNER, R. J.** Techniques of aerosol formation. Bact. Rev. **25**: 188-193. 1961.
34. **ZENTNER, R. J.** Physical and chemical stresses of aerosolization. Bact. Rev. **30**: 551-557. 1966.