



EXPERIMENTAL RESPIRATORY
INFECTIONS IN THE RAT

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ABSTRACT

EXPERIMENTAL RESPIRATORY INFECTIONS IN THE RAT

by Joe D. Burek

A total of 8 experiments were conducted exposing germfree and conventional rats to *Mycoplasma* sp., *Mycoplasma pulmonis*, *Bordetella bronchiseptica*, *Pasteurella pneumotropica*, and a combination of *Bordetella bronchiseptica* with *Pasteurella pneumotropica*. Approximately 200 rats were infected and a similar number of noninfected controls maintained. In each experiment, the acute and chronic aspects of exposure were studied. Infection was determined by isolation of the organism(s) and the presence of tissue lesions. Pneumonia was more severe in conventional than germfree rats after aerosol exposure to *B. bronchiseptica*. *Pasteurella pneumotropica* was never isolated from the lungs of conventional or germfree rats after aerosol exposure. A combined infection of *B. bronchiseptica* with *P. pneumotropica* produced lesions identical to *B. bronchiseptica* alone. No enhancement of *P. pneumotropica* occurred as a result of the combination. *Mycoplasma* sp. was not capable of producing respiratory infection by intranasal instillation or aerosol exposure of broth cultures or pig kidney cell cultures in conventional or germfree rats. Infection was not produced when the organism was placed directly into the trachea after surgical tracheotomy or when the organism was injected intravenously with or without agar. Current investigations

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demonstrated another mycoplasma species of rat lung origin that is capable of producing an acute to subacute suppurative bronchopneumonia in germfree rats. (Supported by NIH grant 5 R01 FR00383.)

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By

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Dedicated to

Janet

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INTRODUCTION

Respiratory diseases are a serious problem in the laboratory rat and have complicated experiments for many years. The most persistent problem is chronic pneumonia with its insidious onset, its prolonged course and eventually terminal outcome.

There has been an inability to definitively categorize, that is, to establish a definite etiology for all forms of the chronic respiratory disease complex in the rat. As a result, it is quite easy to lump all respiratory diseases into one broad classification such as "Chronic Respiratory Disease" or "Chronic Murine Pneumonia". However, to erradicate or control pneumonia it is essential to determine the primary agent, the source, and the mode of transmission of the pneumonia.

Objectives

The objectives of this research were to compare several types of infections in laboratory rats and determine microscopically how the rat responds to each. *Bordetella bronchiseptica* and *Pasteurella pneumotropica* were chosen to represent bacterial pathogens of the respiratory tract of rats. *Mycoplasma pulmonis* and *Mycoplasma* sp. were used to represent mycoplasma agents.

The specific aims of this research were:

1. To determine the pathogenicity and clinical signs of infections due to the above-mentioned organisms.

2. To determine and compare histopathology and microbiology during various stages of the diseases.

3. With the information obtained under objectives 1 and 2, to formulate methods of prevention and control.

LITERATURE REVIEW

A voluminous amount of literature is available on respiratory infection in the rat. Summaries and reviews have been presented by Nelson (1957), Nelson (1963), Innes *et al.* (1956), Joshi *et al.* (1967), Giddens (1968), Brennan *et al.* (1969), and Pankevicius *et al.* (1957). Much of the research that has been conducted has dealt with the mouse and, to a limited extent, the rat. This review is limited primarily to the major articles dealing with respiratory infections in the rat.

The current status of chronic respiratory disease in the rat consists of 2 major forms: (1) chronic murine pneumonia (CMP), which is a slow, progressive respiratory disease. It is characterized by few if any clinical signs and is believed to be of viral origin. Histologic examination of the lungs has demonstrated lymphocytic infiltration and hyperplasia. Only occasionally are other inflammatory cells noted. Bronchiectasis is not a consistent lesion but may be present; (2) infectious catarrh is a disease produced by *Mycoplasma pulmonis*. It is seen as a rhinitis, otitis media or labyrinthitis and occasional snuffling and sneezing. In advanced cases, exudates may be found around the eyes and nose. Histologically, there is inflammation of nasal passages and middle ear predominantly of lymphocytes and neutrophils. The lung lesions are often large areas of suppuration, lymphocytic and neutrophilic exudates in the bronchi and occasional bronchiectasis. The pneumonia is believed to be an extension of the upper respiratory disease.

The Mycoplasma Organisms

Mycoplasma is the only genus of organisms belonging to the Order Mycoplasmatales. They are highly pleomorphic, reproduce by breaking up of filaments into coccoid elementary bodies, lack a rigid cell wall and may be either parasitic or saprophytic.

The morphology of the mycoplasma organisms (pleuropneumonia-like organisms; PPLO) is rather difficult to describe because of the great amount of pleomorphism. The smallest reproductive units (minimal reproductive units) are the elementary bodies, granules or coccobacilliform bodies. They are spherical in shape and consist of a single cell that may range from 110 to 250 millimicrons, depending on the species. This is significant because these bodies will pass bacteria-retaining filters and may therefore be confused with virus particles or lead to some unjustified conclusion when tissue filtrates are used to infect experimental animals. The elementary bodies or "PPLO-cells" (Kleineberger, 1962), lack a rigid cell wall and are bounded by a triple-layered unit membrane as seen by electron microscopy and described by Tanaka *et al.* (1965). This lack of a rigid cell wall may explain the extreme pleomorphism but also accounts for the complete resistance of these organisms to penicillin and other cell wall inhibiting antibiotics. It may also aid one in understanding the reproduction of these organisms, which is accomplished by the formation of filaments which develop from the granules. The filaments break up into granules of various sizes which are in turn capable of filament formation. Each PPLO-cell may vary in size, but are identical insofar as cellular potential is concerned.

To isolate mycoplasma, an enriched media is absolutely essential. The culture media varies with the species to be isolated but, in general, all cell-free media contains similar constituents. The broth media is

prepared as a beef heart infusion peptone media that is enriched with 10 to 25% serum to provide the necessary proteins and fresh yeast extract to supply the needed sterols. It should be pointed out that one of the ways PPLO differ from bacteria is their requirement for cholesterol or a similar steroid. There are a few exceptions to the requirement of a high lipid media, but these are among the saprophytic species. The agar media is prepared in a similar fashion but agar is added to produce a semisolid to solid media.

Culturing is the only positive method for isolation, but several other techniques are available to aid in identification. Any direct examination for the organism is useless, but if the tissues or exudates are fixed in methyl alcohol and stained with Giemsa's stain, the elementary bodies may occasionally be seen (Kleineberger, 1962). Other useful aids include complement fixation, which is probably the best serologic method for identification, the use of fluorescent antibody techniques, darkfield microscopy, and electron microscopy.

A useful stain to use for colonies on artificial media is the Dienes stain* (Dienes, 1945) because it can be used on the colonies directly without previous fixation. A drop of the stain is spread over the surface of a coverslip and allowed to dry. The coverslip is then placed stain side down, onto the PPLO colonies and, after a 10- to 20-minute wait, to give the colonies time to take up the stain, the colonies are examined under the light microscope. They stain a blue to violet color, and the dark center of the colonies, along with the granules, are readily identified. Bacterial L forms also take up this stain but may lose the deep staining characteristics after a few minutes. This

*Methylene blue 0.5g.; Azure II 0.25g.; Maltose 2.0g.; Na_2CO_3 0.05g.; benzoic acid 0.04g.; distilled water 20.0ml.

fading does not always occur and, to positively differentiate PPLO from bacterial L forms, one must rely on cultural characteristics and serial passage in an antibiotic free media.

Mycoplasma pulmonis was first isolated by Klieneberger and Steabben (1937) from the lungs of rats with bronchiectatic lesions and from normal rats. This organism was defined at this time as L₃ (L for the Lister Institute where Klieneberger worked; has no relation to the bacterial L forms) and was isolated from the lungs of 17 of 19 pneumonic rats. In later research, the L₃ organism was isolated from the lungs of 138 rats from a group of 251 laboratory rats. Lung lesions were observed in 108 rats. Attempts to reproduce the disease failed. Their conclusion at this time was that there was no definite evidence for or against L₃ being the etiologic agent of bronchopneumonia in rats.

Nelson (1940a,b) reported otitis media and pneumonia in the albino rat and reported coccobacilliiform bodies in the cytoplasm of leukocytes. It was at this time that he coined the term "infectious catarrh" of the albino rat to refer to this particular infection. His attempts to reproduce the pneumonia with pure cultures of L₃ were fruitless, but he was able to reproduce the pneumonia with lung suspensions or with minced chick embryo tissue suspensions with the organism.

Further studies by Nelson (1946a,b) revealed "endemic pneumonia" to be present in the absence of PPLO. Clinically the signs were that of rhinitis with a mucopurulent exudate, and a low grade nasal irritation with little or no snuffling. Since PPLO were not isolated and this disease was in a rat colony free of infectious catarrh, a virus-like agent was thought to be the etiologic agent. Attempts to culture an organism in eggs or cell cultures were unsuccessful. This virus-like agent was transmissible by direct contact and was probably passed from the mother to the young.

In order to eliminate the disease, rats were derived by cesarean and artificially reared in barrier isolation. It was only by this method that Nelson (1951) was able to remove the virus-like agent and the PPLO from his colonies.

In a summary, Nelson (1958) concluded CRD in rats could be divided into 2 separable diseases. The first was infectious catarrh produced by PPLO. The lesions were a persistent exudative inflammatory response of the nasal cavity, middle ear, and lungs. The second disease was endemic pneumonia, or rodent bronchiectasis. The agent is probably a virus and produces a chronic bronchopneumonia with bronchiectasis. These 2 syndromes may exist independently or may be superimposed upon each other. Both are reproducible in SPF rats by lung suspensions, middle ear exudates and nasal washings (1963).

Klieneberger was not convinced by Nelson's conclusions. Klieneberger-Noble and Cheng (1955) found PPLO (L₃) to be associated with bronchiectasis in lobes of lungs that were ligated at the bronchi. Lungs were negative before surgery, but after ligation PPLO were found in high numbers in the stagnated exudate in the ligated, bronchiectatic lobes. This procedure was repeated by Ventura and Domardzki (1967) with similar results.

It was also noted by Klieneberger and Steabben (1940) that L₃ increased directly with lesion severity until the lungs became purulent. They were reduced in numbers or absent when the whole lobe became dry and caseous. Also observed at this time were rats between 1 to 3 months of age that had been subjected to the stress of castration or ovario-hysterectomy. The L₃ organism increased in frequency compared to stock rats of the same age. Eighteen of 23 developed lung lesions and 22 of 23 had the mycoplasma.

Although she was not completely swayed by Nelson, Klieneberger stated that , "All kinds of injections of L₃ cultures into rats, with or without agar, have so far not resulted in producing acute pulmonary disease." She does feel at this time that if the L₃ is the causative agent, an additional factor may be needed to render it pathogenic. However, the possibility of the L₃ agent being only a secondary invader is still plausible.

Other attempts to produce lesions with pure cultures of PPLO have also proven to be unsuccessful (Joshi *et al.*, 1965; Pankevicius *et al.*, 1957).

PPLO infections in rats and mice are apparently not passed from mother to fetus *in utero*. Research by Lemcke (1961) suggests that although the mothers had PPLO in the lung and nasopharynx, the young did not acquire the infection until later in life. As the age of the rat increased, the percentage of total rats that had a titer for PPLO increased. Within a few weeks of life, PPLO could occasionally be isolated from the nasopharynx, but not the lung.

In 1956, Innes *et al.* established the term chronic murine pneumonia (CMP) and suggested it as a better term than Nelson's enzootic bronchiectasis. The term CMP is now used to indicate the disease in rats described by Passey *et al.* (1936) and Innes *et al.* (1956), in which mycoplasma are not isolated and the lesions are characterized by a peribronchial lymphocytic infiltration with bronchiectasis occasionally noted in advanced cases.

The *Bordetella bronchiseptica* and *Pasteurella pneumotropica* Organisms

The etiology of CMP has long been a question that has remained unanswered. The primary role or even the secondary role of bacteria in

the etiology and pathogenesis of the classical chronic murine pneumonia is believed to be relatively insignificant (Giddens, 1968; Innes *et al.*, 1956; Joshi *et al.*, 1961; Nelson, 1957; Newberne *et al.*, 1961; Pankevicius *et al.*, 1957; Vrolijk, 1957). Even though the role of bacteria in CMP is dubious, mention should be made of bacteria that are found to be agents of distinct respiratory problems in the rat. Of these, *Bordetella bronchiseptica* and *Pasteurella pneumotropica* are frequently associated with the rat respiratory tract and will therefore be discussed and their pathological features alluded to.

Bordetella bronchiseptica is a motile, noncapsule producing Gram negative rod that is a common respiratory inhabitant of many animals. It may remain in the nasopharynx or more commonly in the trachea and be innocuous or it may progress to an acute infection and result in death within a few days.

In Bergey's Manual (1925 and 1957), Galli-Vallerio in 1896 is described as the first to isolate this organism from dogs with distemper and gave it the name of *Bacillus caniculae*. Lignieres (1901), in the same sources, described this organism as a *Pasteurella*, but Ferry (1911) again placed the organism in the genus *Bacillus*. The organism has been further classified as *Alcaligenes bronchisepticus* (Bergey's Manual, 1925), *Brucella bronchiseptica* and finally *Bordetella bronchiseptica* (Bergey's Manual, 1957).

Winsser (1960) has described *B. bronchiseptica* infections as being either latent, present as carriers, or in overt illness. He has described a catarrh in the nasal passage of laboratory animals related to this organism and has isolated it from nasal discharge and from the soft palate of animal caretakers. *Bordetella bronchiseptica* was isolated from a child that developed a mild whooping cough 12 days after receiving a

rabbit with snuffles. *Bordetella bronchiseptica* was also isolated from the rabbit after it accidentally died. He described the white rat as being quite resistant but that carrier states do occur since he was able to isolate the organism from the lungs of rats with no gross lesions.

Griffin (1955) found hemolytic *Streptococcus* and *B. bronchiseptica* (*Bacterium bronchisepticum*) to be the 2 most common isolates from guinea pigs with respiratory disease. The lesions produced were that of focal pneumonia with areas of congestion or consolidation. Ganaway *et al.* (1965) also described acute *B. bronchiseptica* pneumonia in a guinea pig colony where bronchopneumonia and death occurred overnight in several breeders.

Bordetella bronchiseptica, *Aerobacter cloacae* and *Staphylococcus albus* were isolated from pneumonic lungs of albino rats by Smith *et al.* (1930). The gross lesions consisted of abscess formation, single or multiple, and in one or several lobes. Bronchiectasis was occasionally noted along with adhesions. Nelson (1940a,b) isolated *B. bronchiseptica* along with *Actinobacillus muris* from rats. Pankevicius *et al.* (1957) found *Bordetella bronchiseptica* to be the most common bacteria isolated from pneumonic and grossly normal lungs in a stock colony of rats.

Six of 21 wild rats that were trapped at a small town dump contained *B. bronchiseptica* (Switzer *et al.*, 1966). Switzer also noted that skunks, foxes, raccoons, and cats were carriers of this organism.

Filion *et al.* (1967) reported a respiratory condition in young turkeys characterized by rhinitis, bronchitis and bronchopneumonia with *B. bronchiseptica* as the possible agent. Attempts to experimentally infect turkeys and chickens were successful, but mice were apparently resistant.

Bordetella has been noted as a pathogen of swine for several years (Phillips, 1943; Switzer, 1956; Duncan *et al.*, 1966; Ross *et al.*, 1967; Arskog, 1967). Switzer, in his attempts to reproduce atrophic rhinitis, has at times incriminated this organism as a possible agent (Harris and Switzer, 1968).

The trachea is the best location to attempt isolation and culture in most species (Ross *et al.*, 1967; Switzer *et al.*, 1966; Ganaway, 1965).

Pasteurella pneumotropica is a Gram negative, nonhemolytic rod that is differentiated from *Pasteurella multocida* in that it is both indol and urease positive and does not produce acid from mannitol or sorbitol (Wheater, 1967; Brennan *et al.*, 1965).

Pasteurella pneumotropica is a common isolate from the respiratory tract of rats and mice. Jawetz (1950) and Jawetz and Baker (1950) first isolated and characterized this organism and alluded to its pathogenicity. They described the infection as latent in the respiratory tract and felt that the organism generally would not produce clinical illness except under artificial conditions. In one study, Jawetz (1950) found the incidence in infected animals to be: white mice, 30 to 100%; white rat NIH, 87.5%; cotton rat, 25%. Generally, the natural spread of the organism from parent to offspring was not serious enough to result in spontaneous illness. However, his research indicated that latent *P. pneumotropica* infections increased susceptibility of experimental infection via intranasal instillation. *Pasteurella* free mice were less susceptible to intranasal infection.

The lesions in mice, described by Jawetz and Baker (1950), began as peribronchial alveolar pneumonitis with the alveoli filled with polymorphonuclear cells and some fibrin. There were areas of focal alveolar coalescence with some polymorphonuclear cells in the bronchi 48 hours

after experimental exposure. After 72 hours, gray hepatization began to occur with consolidation of upper lobes of lungs. Bronchi were filled with exudate. He described the lesion in Swiss mice as "...a picture of peribronchial consolidation with spreading and coalescence of involved areas." Their conclusion of resistance of young mice to infection was not related to subclinical infection but to "nonspecific hereditary factors."

Flynn *et al.* (1965) isolated *P. pneumotropica* from all but 1 of 11 colonies. The organism was isolated from nasal passages of 90% of the rats he examined and also isolated the organism from a "high incidence" of the lungs and from the uterus of several breeders.

Pasteurella pneumotropica was cultured on several occasions from mice by Hoag *et al.* (1962). Most isolations were from uteri and brain. The mouse colonies appeared normal.

Giddens (1968) isolated this organism from several rats with chronic murine pneumonia. Isolations from the nasal passage of one rat from one colony and from the middle ear of 2 rats, trachea of one and lung of one of another colony.

Spontaneous outbreaks of *P. pneumotropica* in rodents have been reported by Heyl (1963) and Wheeler (1967).

This organism is isolated almost entirely from rats and mice, but there is one report of the isolation of *P. pneumotropica* from a normal human respiratory tract (Henricksen and Jyssum, 1961; Henricksen, 1962).

Summary

While respiratory disease in the rat has received considerable investigative attention, still much needs to be examined regarding the disease. The pathogenesis of specific microorganisms in a defined animal

requires additional study. In addition, the interrelationship of different pathogens needs to be established. In this literature review, references to experimental work with germfree rats were not found.

MATERIALS AND METHODS

The germfree and other facilities at Barn 5, at the Veterinary Research Farm, were used to conduct these experiments. This research was part of a larger project on murine pneumonia and was carried out over a 2-year period.

Source of Animals

Two sources of experimental animals were used: (1) Germfree rats were procured from a Sprague-Dawley derived colony from Notre Dame University. The colony was originally cesarean-derived and maintained under germfree conditions for several generations. These rats were reared to maturity and bred to establish a germfree "subcolony" at Michigan State University. It is from this subcolony that rats were obtained for germfree studies; (2) Conventional rats used in the following experiments were obtained from a local breeder. They were originally cesarean-derived and were barrier-maintained.

Conventional rats were maintained while on experiment in clear plastic cages with autoclaved wood shavings. The plastic cages were disinfected by cleaning with a strong iodine preparation. Filter-tops made of compressed fiberglass were used to cover the cages and thereby reduce contamination via aerosol.

Source of Organisms

Both *Bordetella bronchiseptica* and *Pasteurella pneumotropica* were originally isolated from rat lung tissue. The source of these 2 organisms

was the Michigan State University microbiology collection.

Mycoplasma pulmonis, kon strain, was originally isolated from rat lungs by Klieneberger-Noble and maintained by the American Type Culture Collection. The A.T.C.C. #19612 was the source of this organism.

Mycoplasma sp., also of rat lung origin, was isolated from rats with chronic murine pneumonia. These rats were from the CMP-3 group used by Giddens (1968).

Exposure

Two methods of exposure were used to infect the rats with the different microorganisms.

1. Intranasal instillation. The rats were anesthetized with ether and a pipette was used to place 1 drop (0.05 ml.) of culture medium into each nostril. The rat was held in a vertical position to permit maximum use of the force of gravity to gravitate the broth medium down toward the lungs.

2. Aerosol exposure. All rats to be exposed were placed in a clear plastic cage that was covered with a fiberglass filter-top. A hole was cut in the filter-top to permit insertion of a nebulizer.* Five milliliters of broth were placed in the nebulizer and, with 15 pounds of pressure, an aerosol was created in the cage. Rats were left in the closed cage for 30 minutes to assure maximum exposure and then transferred to other cages.

Necropsy and Cultures

All animals were necropsied and cultured as follows: Rats were euthanatized with ether and placed in dorsal recumbency with all 4 legs

*DeVilbiss No. 640 nebulizer, The DeVilbiss Company, Somerset, Pa., U.S.A.

held out in extension. The skin was removed from the ventral aspect of the carcass as well as the entire cranium. The abdominal cavity was opened and the viscera examined for gross lesions. Next, the ventral rib cage was removed, exposing the thoracic viscera. A section of the left lobe was removed for culture. The trachea was then dissected from the surrounding cervical tissue and was transected at the pharynx. A sterile platinum loop was inserted into the trachea to obtain material for culture. This loop was then used to streak a blood plate and/or PPLO plate and to inoculate BHI broth. The trachea, lungs, and heart were then removed for fixation.

The tip of the nostrils were cut off with scissors. A platinum loop was inserted, the mucus collected, and the loop used to streak a blood plate and a PPLO plate and inoculate broth. The head was then placed in formalin.

Bacterial cultures from the lungs were obtained by taking a sterile piece of lung and streaking the cut surface over a blood agar plate. A portion of this lung was then macerated with the aid of scissors and placed in BHI (brain heart infusion) medium for 24 hours.

Mycoplasma cultures were obtained by first streaking a PPLO plate with the cut surface similar to the technique used for bacteria. This portion of lung was then ground in a tissue grinder and placed in PPLO broth medium and incubated for 3 to 4 days. At this time, 1 ml. of this inoculated broth was transferred to new PPLO media as well as streaking another PPLO plate. A total of 3 transfers in broth and PPLO agar plates were made on all PPLO samples. If no growth was recorded after the third transfer, the samples were considered negative for mycoplasma.

All tissues were fixed in 10% buffered formalin. The lungs were perfused with formalin by slowly inflating the lobes with a syringe

filled with formalin. The trachea was then tied off and the lungs placed in formalin.

Three sections were made through the lungs for histopathologic examination. The first section was taken through the apical and portions of the cardiac lobes as well as the apical portion of the left lobe. The second section included the remainder of the cardiac, a portion of the azygous lobe, and the midportion of the left lobe. The third section included the diaphragmatic lobe and the distal portion of the left lobe. Two cross sections were made of the trachea. The head was decalcified for 24 hours and 2 cross sections of the nostrils were made. The first section was 1/2 inch from the tip of the nose. The second section was 1/4 inch anterior to the medial canthus of the eyes.

The mycoplasma broth medium was prepared from the dehydrated Bacto PPLO Enrichment Broth from Difco Laboratories. It was enriched with 20% horse serum and 10% yeast extract. To this, thallium acetate and penicillin were added to retard bacterial growth.

The agar medium was prepared from the dehydrated Bacto PPLO Agar from Difco Laboratories. The constituents are identical to the broth medium plus the addition of 1.5% agar.

Experiments

The following experiments were conducted. In all the trials, control rats were given the sterile broth or tissue culture medium and a similar number of nontreated controls were maintained separate from the experimental animals, but under similar conditions. The experiments may be summarized as follows:

1. Sixteen conventional weanling rats were exposed to broth cultures of *Mycoplasma* sp. Eight were exposed by aerosol and 8 by intranasal instillation. The broth cultures were incubated for 3 days before exposing the rats. Rats were examined daily and necropsied after 14 days.

2. Eleven germfree 21-day-old rats were exposed to an intranasal instillation of *Mycoplasma* sp. growing for 5 days in pig kidney cell (PKC) cultures. Rats were necropsied at 2 weeks, 4 weeks, and 5 weeks.

3. Four conventional weanling rats were exposed to *Mycoplasma* sp. by surgically entering the trachea and placing 1 drop of broth culture directly into the trachea. Rats were euthanatized and necropsied at 4 weeks past infection.

4. Six conventional weanling rats were exposed to a broth culture of *Mycoplasma* sp. intravenously. The rats were observed for 4 weeks and then necropsied.

5. Twenty conventional rats were exposed to *M. pulmonis*, 10 by intranasal instillation of the organism growing 5 days in PKC cultures and 10 by aerosol exposure of the same culture. Rats were necropsied after 4 weeks.

6. Eleven conventional rats were exposed to a 24-hour broth culture of *B. bronchiseptica*. Necropsy was performed at selected intervals up to 2 months after exposure.

7. Same as experiment 6, using *P. pneumotropica*.

8. Eight conventional rats were exposed to a combination of *B. bronchiseptica* and *P. pneumotropica*. Necropsy was performed as in experiments 6 and 7.

RESULTS

Experiment 1

On daily examination clinical signs were not observed. Therefore, after 14 days, all rats were euthanatized. Necropsies were performed on each rat and cultures were taken from the rats as described previously.

Cultures from the nasal passage were negative for *Mycoplasma*. However, nasal contaminants were noted in nearly all rats, both experimental and controls. These contaminants were mainly a combination of *Micrococcus* and *Staphylococcus* sp.

Bacteria were not isolated from the trachea, but *Mycoplasma* sp. was isolated from one rat. All other rats were negative for *Mycoplasma*.

Cultures from the lungs of all rats were negative for bacteria and *Mycoplasma*.

On gross and microscopic examination, the nasal passage, trachea, and lungs were normal. There were no changes that were even suggestive of a response to the organism in any of the rats with one exception.

In one rat, from the group exposed by internasal instillation, *Mycoplasma* sp. was isolated from the trachea. The nasal passage and trachea were normal and histologic examination did not reveal microscopic changes. However, one portion of the lungs contained an area of bronchiectasis, lymphocytic proliferation and infiltration, peribronchial and perivascular edema, lymphangiectasis, lymphatics filled with lymphocytes, and hypertrophy of sections of bronchiolar epithelium. There were numerous alveolar macrophages within the lumen of the bronchi, with an

increased production of mucus. The alveolar walls were thickened in this area with edema and lymphocytes. Emphysema and atelectasis were also present in this localized area but did not involve much of the lung area. Because this reaction involved most of the left lobe of the lung and could be followed through several histologic sections at different levels of this lobe, this reaction is considered to be an early response of the lung to this particular microorganism (Figure 1).

Experiment 2

The 11 germfree rats that were exposed to intranasal instillation of *Mycoplasma sp.* growing for 5 days in pig kidney cell tissue cultures were euthanatized at 2 weeks, 4 weeks, and 5 weeks. No gross or microscopic lesions were observed in the nasal passage, trachea, or lung.

Experiment 3

Attempts to infect rats by surgical entrance into the trachea and thus admitting 1 drop of mycoplasma broth culture directly into the trachea were all negative both culturally and histopathologically.

Experiment 4

The results of intravenous inoculations with mycoplasma broth cultures, with and without agar, were also negative.

Experiment 5

The 10 conventional rats that were exposed to *M. pulmonis* by intranasal instillation of the organism growing for 5 days in PKC cultures were negative on culture and no tissue lesions were seen.

The 10 rats exposed by aerosol to the same culture were also negative.

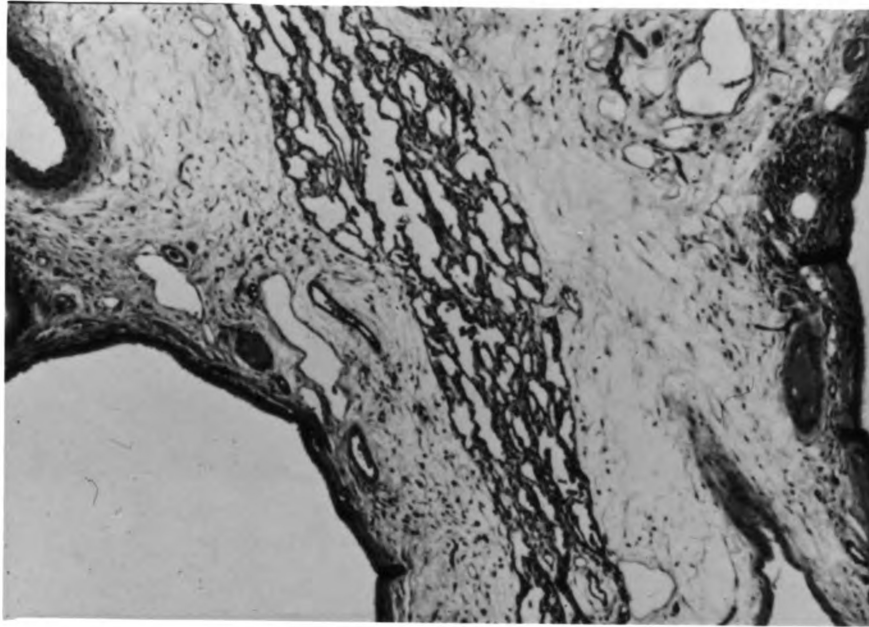


Figure 1. Distal portion of the left lobe of the lung from a 5-week-old rat, 14 days after intranasal exposure to *Mycoplasma sp.* Note the peribronchiolar edema, dilation of subepithelial lymph vessels, atelectasis and focal perivascular lymphocytic cuffing. H & E stain. x 40.

Clinical signs were not apparent in any of the rats. Gross and microscopic examination did not localize any abnormalities. Lymphocytic hyperplasia or infiltration around the bronchi was not marked.

Experiment 6

1. One week after aerosol exposure. All rats had rough hair coats, were anorectic and had respiratory distress. Food consumption was markedly reduced when compared to controls. Average weight was 75 grams, which compared to an average of 92 grams for control rats.

Two rats were killed at this time and were found to have *B. bronchi-septica* in their nasal passage, trachea and lungs (Table 1).

An increased amount of mucus or exudate was in the nasal passage and trachea upon gross examination. The lungs had numerous focal pale areas .1 to .3 mm. in diameter, suggestive of focal necrosis. Distribution of these areas was uniform throughout all lobes without predilection for any area of the lungs.

The nasal submucosa of both rats contained a few lymphocytes and an occasional neutrophil but did not appear significantly different from controls and was therefore considered normal. There was, however, an increased quantity of exudate in the nasal passage. This exudate consisted of lymphocytes, neutrophils and an occasional macrophage all in a mucous matrix.

The trachea of both rats did not contain microscopic lesions.

Microscopic examination of the lungs revealed numerous focal areas of bronchopneumonia. The pneumonic areas contained a core of lymphocytes and a few neutrophils. Many of these cells were necrotic. Around the core were numerous alveolar macrophages and lymphocytes and a few fibroblasts and neutrophils (Figure 2). In several areas the focal pneumonic lesions appeared to coalesce (Figure 3).

Table 1. Summary of the isolations of *Bordetella bronchiseptica* in conventional rats that were exposed to the organism by aerosol exposure

No. of animals in group	No. of weeks after exposure	Nasal Passage	Trachea	Lung
2	1	2	2	2
5	2	5	5	4
2	4	2	0	2
2	8	1	1	1

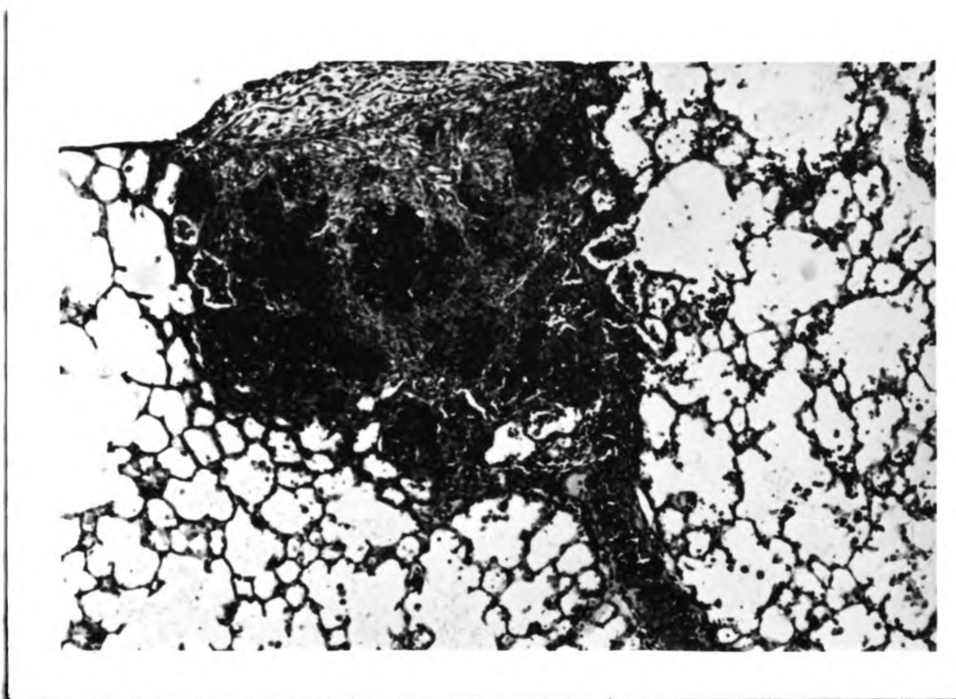


Figure 2. Focal area of bronchopneumonia with lymphocytes, neutrophils and macrophages in a 4-week-old rat, 7 days after aerosol infection with *B. bronchiseptica*. Note the areas of necrosis within the pneumonic lesion and the lack of cellular reaction next to the focal lesion. H & E stain. x 40.

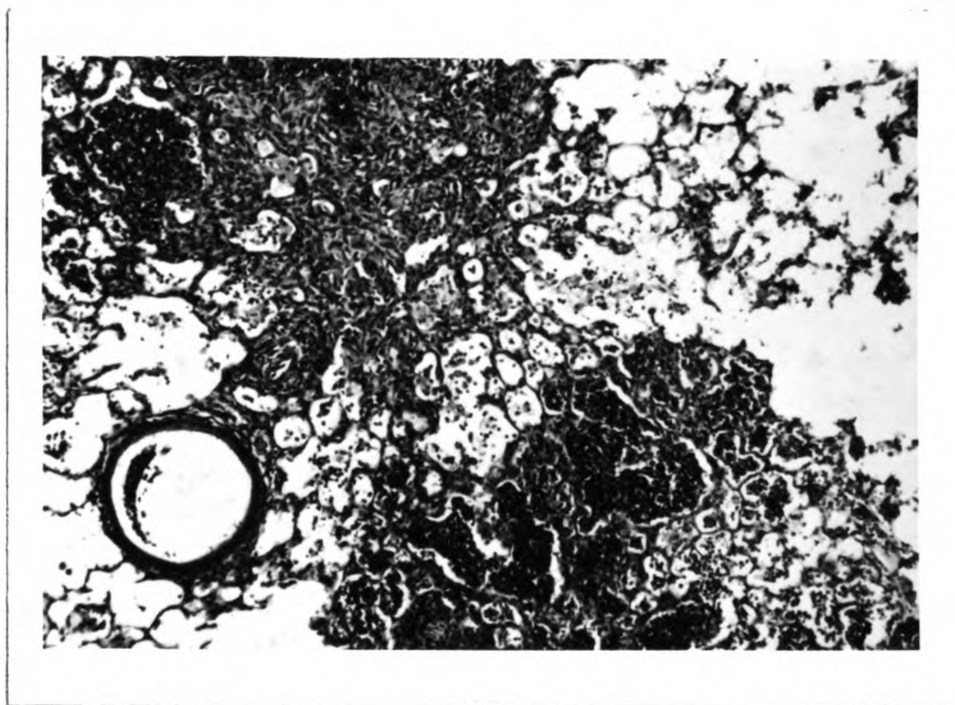


Figure 3. Lung from a 4-week-old rat, 7 days after aerosol exposure to *B. bronchiseptica*. Focal pneumonic lesion with coalescence and necrosis. H & E stain. x 40.

Bronchiolar epithelium in the region of the inflammatory reaction was hyperplastic. An exudate similar to that found in the nasal passage was in the lumen of several bronchioles.

2. Two weeks after aerosol exposure. Occasional sneezing was the only clinical sign observed. Food consumption was comparable to controls and average weights were lower than controls, but they were not significantly different.

Exudates were not observed around the nostrils, nor was there any sign of respiratory distress.

Five rats were euthanatized. All 5 contained *B. bronchiseptica* in the nasal passage and trachea. All but 1 had the organism in the lung (Table 1).

No gross lesions were observed in any of the 5 rats.

The nasal passage of 3 rats had a mild rhinitis characterized by lymphocytes and a few neutrophils in the submucosa and infiltrating between the nasal epithelial cells. An increase in nasal exudate was also in these rats, but not as extensive as was observed 1 week earlier.

Two rats had a mild lymphocytic infiltration along with a few neutrophils in the trachea. Generally, the trachea was considered normal.

All 5 rats had a moderate hyperplasia of lymphoid tissue along the bronchi and bronchioles. Three of the 5 had lymphoid infiltration along the bronchi and occasionally into the epithelial lining (Figure 4). The same 3 rats also had areas of bronchopneumonia with focal inflammatory infiltration and coalescence. There was a minimal amount of necrosis (Figure 5). Increased fibroblasts and evidence of collagen were noted by the use of trichrome stains. Bacterial colonies, some emphysema and extensive subepithelial lymphoid cuffing of bronchioles were noted in 2 of the pneumonic rats.

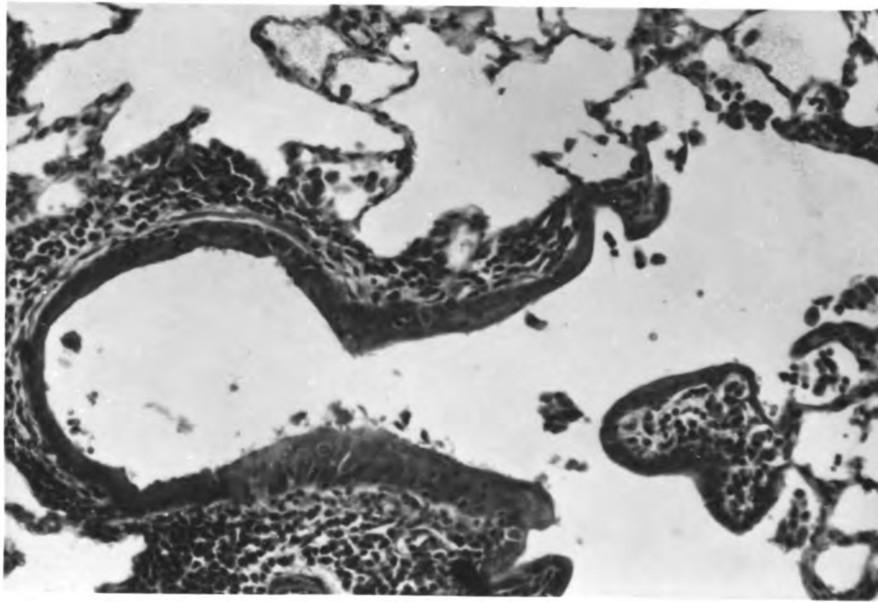


Figure 4. Minimal lymphocytic infiltration along bronchiole in a 5-week-old rat, 14 days after aerosol exposure to *B. bronchiseptica*. H & E stain. x 100.

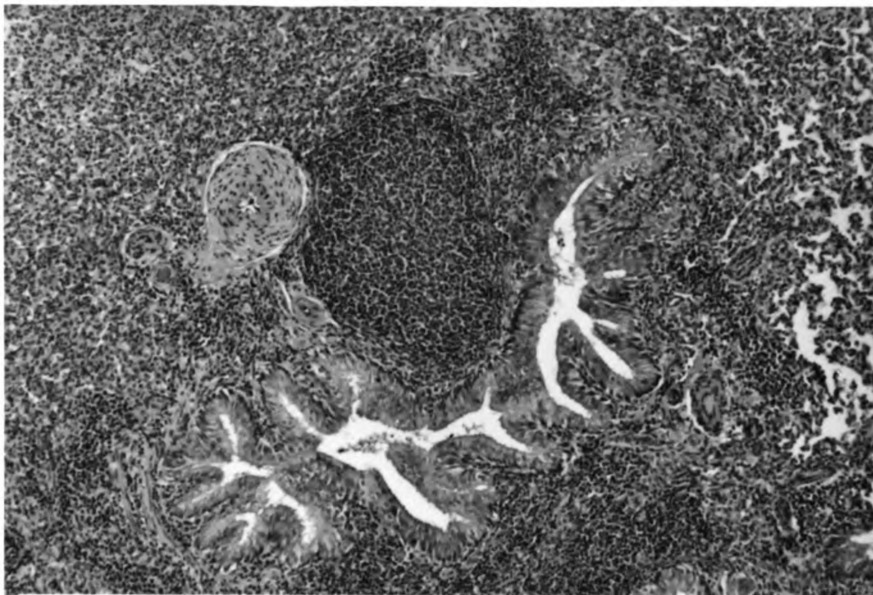


Figure 5. A large focal area of pneumonia with coalescence and hyperplasia of adjoining bronchiolar epithelium in a 5-week-old rat, 14 days after aerosol exposure to *B. bronchiseptica*. H & E stain. x 40.

3. Four weeks after aerosol exposure. By the end of 4 weeks, no clinical signs were observed and gross lesions were not noted after euthanasia of 2 rats.

Both animals contained *B. bronchiseptica* in their nasal passages and lungs. Both tracheas were negative upon cultural examinations (Table 1).

The nasal passage of 1 rat had a mild rhinitis with a moderate increase in lymphocytes diffusely below the epithelial surface. A few neutrophils were noted. The other rat was normal.

The trachea of both rats was histologically normal.

The lung of 1 rat was characterized by diffuse lymphoid infiltration along the major bronchi and arterioles. The lung parenchyma and alveoli were normal and did not differ from controls in their overall appearance.

The lung of the second rat contained numerous areas of regressing bronchopneumonic lesions. These areas were surrounded by alveolar macrophages and some connective tissue, fibroblasts and lymphocytes (Figure 6).

4. Eight weeks after aerosol exposure. Clinical signs had not been observed and the last 2 rats were examined. No gross lesions were noted.

One rat had *B. bronchiseptica* in the nasal passage, trachea and lung. The other rat was negative from all locations.

Both rats appeared to have a mild rhinitis characterized by infiltrations of lymphocytes and a few neutrophils. However, both controls also had a similar response in their nasal passage, leading one to the conclusion that this is a function of age rather than irritation from *Bordetella*.

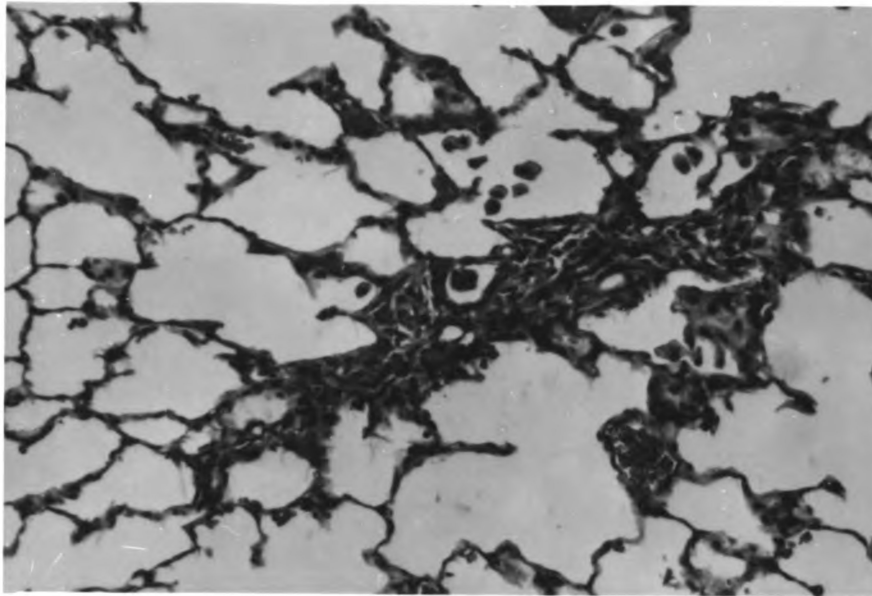


Figure 6. A 7-week-old rat lung, 4 weeks after aerosol exposure to *B. bronchiseptica*. Note the apparent regression of pneumonic lesions with lymphocytes and macrophages as the predominant cell types. H & E stain. x 100.

A few lymphocytes were noted in the trachea, but this was considered normal.

The lesions in the lungs of both rats were of focal areas of lymphoid hyperplasia or nodules with a reduction in the diffuse lymphocytic infiltration around most bronchi. A few small areas remained that appeared to be regressing pneumonic lesions. Multifocal histiocytosis were noted in these areas as well as in areas that appeared normal. The only diffuse lymphoid infiltration was around the large bronchioles and bronchi.

Experiment 7

1. One week after aerosol exposure. All rats were observed and no clinical signs were noted. Feed consumption was comparable to controls as were the average weights.

Two rats were euthanatized. There were no gross lesions. *Pasteurella pneumotropica* was isolated from the trachea of 1 rat (Table 2).

Nasal passage and trachea did not have microscopic lesions. The lung of 1 rat had focal lymphoid hyperplasia, but no evidence of pneumonia.

2. Two weeks after aerosol exposure. Five rats were observed and no clinical signs were noted. Feed consumption and average weights were comparable to controls. Of the 5 rats, only 1 was found to harbor *Pasteurella*. It was isolated from the nasal passage and trachea, but not the lung (Table 2).

Nasal passage and trachea of all 5 rats were normal.

On microscopic examination 2 rats had a distinct increase in lymphoid follicles as well as a mild increase in lymphoid elements between the basement membrane and epithelial cells. The other 3 rats were normal.

Table 2. Summary of *Pasteurella pneumotropica* in conventional rats that were exposed to the organism by aerosol exposure

No. of animals in group	No. of weeks after exposure	Nasal Passage	Trachea	Lung
2	1	0	1	0
5	2	1	1	0
2	4	1	1	0
2	8	0	1	0

3. Four weeks after aerosol exposure. No clinical signs were observed. Weights and feed consumption were similar to controls.

Of the 2 animals examined, 1 had the organism in the nasal passage and trachea (Table 2).

Microscopic examination of the upper respiratory tract did not reveal any significant changes. Both rats had an increased amount of lymphoid elements around the bronchi, but this did not differ from controls and was considered normal.

4. Eight weeks after aerosol exposure. The last 2 rats had no clinical signs after 8 weeks.

Both rats had areas of multifocal histiocytosis, that is, focal areas of alveolar macrophages in the alveoli but no evidence of pneumonia.

Nasal passage and trachea when compared to controls were normal.

Experiment 8

1. One week after aerosol exposure. Hair coats were rough, but none of the rats appeared anorectic, nor were any clinical signs apparent. Feed consumption was comparable to controls. Average weights of these rats were similar to controls.

Both rats were found to have *B. bronchiseptica* in the nasal passage, trachea and lungs (Table 3). However, *P. pneumotropica* was isolated from the trachea of both rats.

On gross examination, the nostrils had increased amounts of mucus. Microscopic examination demonstrated a mild rhinitis with the predominant cell the lymphocyte, with an occasional neutrophil and macrophage. There was evidence of inflammatory cells infiltrating into the nasal epithelium.

Table 3. Summary of the isolations of *Bordetella bronchiseptica* and *Pasteurella pneumotropica* in conventional rats that were exposed to the organisms by aerosol exposure

No. of animals in group	No. of weeks after exposure	Nasal Passage		Trachea		Lung	
		Borde- tella	Pasteur- ella	Borde- tella	Pasteur- ella	Borde- tella	Pasteur- ella
2	1	2	0	2	2	2	0
2	2	2	0	2	1	2	0
2	4	2	2	2	0	2	0
2	8	2	0	2	0	1	0

The trachea appeared normal on gross and microscopic examination.

Lungs were characterized by a diffuse lymphoid reaction around bronchi and a vasculitis. Focal pneumonitis was noted with lymphocytes and macrophages predominating and occasional neutrophils and fibroblasts observed. Coalescence was noted in several areas. This response was nearly identical to the lungs in the *Bordetella* group of rats.

2. Two weeks after aerosol exposure. Two rats were euthanatized. They did not differ from controls in feed consumption, average weights or clinical appearance.

Both rats harbored *B. bronchiseptica* in the nasal passage, trachea and lungs. Only 1 rat had *P. pneumotropica* present in the trachea (Table 3).

Some exudate was noted in the nostrils but a rhinitis was not present. The trachea had no gross lesions but a mild lymphoid infiltration was observed.

Lungs were characterized by focal lymphoid hyperplasia and infiltration around bronchi as well as a few focal lesions of lymphocytes and macrophages, and a few foci of coalescence (Figure 7).

3. Four weeks after aerosol exposure. Two rats were euthanatized. Both rats were clinically normal.

Cultures were positive for *B. bronchiseptica* in both rats from the nasal passage, trachea and lungs. *Pasteurella pneumotropica* was isolated from the nasal passage of both rats (Table 3).

The upper respiratory tract had no gross or microscopic lesions.

The lung of 1 rat had a diffuse as well as focal lymphocytic proliferation around bronchi (Figure 8). A few small foci of pneumonia were observed in both rats.

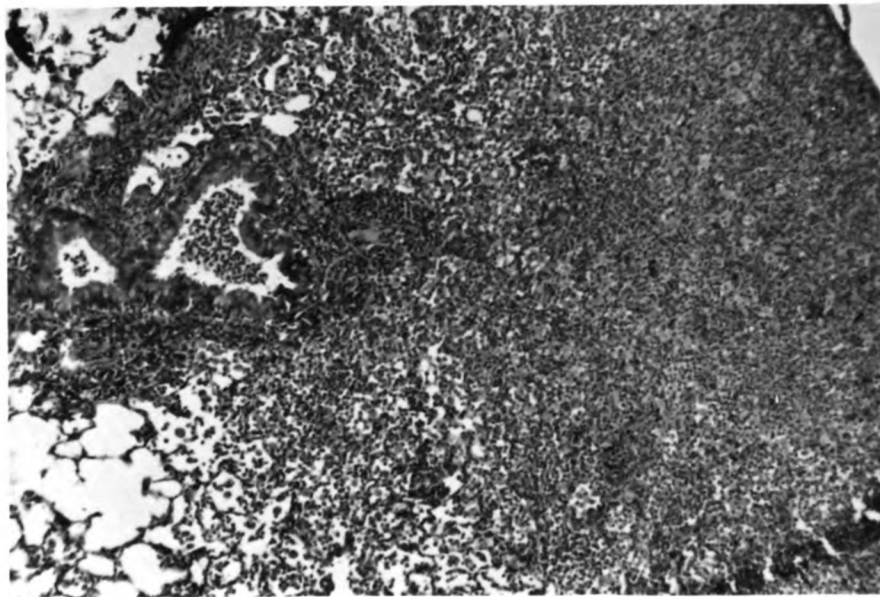


Figure 7. Extensive focal inflammatory exudate extending through alveoli with disruption of the normal alveolar pattern in a 5-week-old rat, 14 days after aerosol exposure to *B. bronchiseptica* and *P. pneumotropica*. H & E stain. x 40.

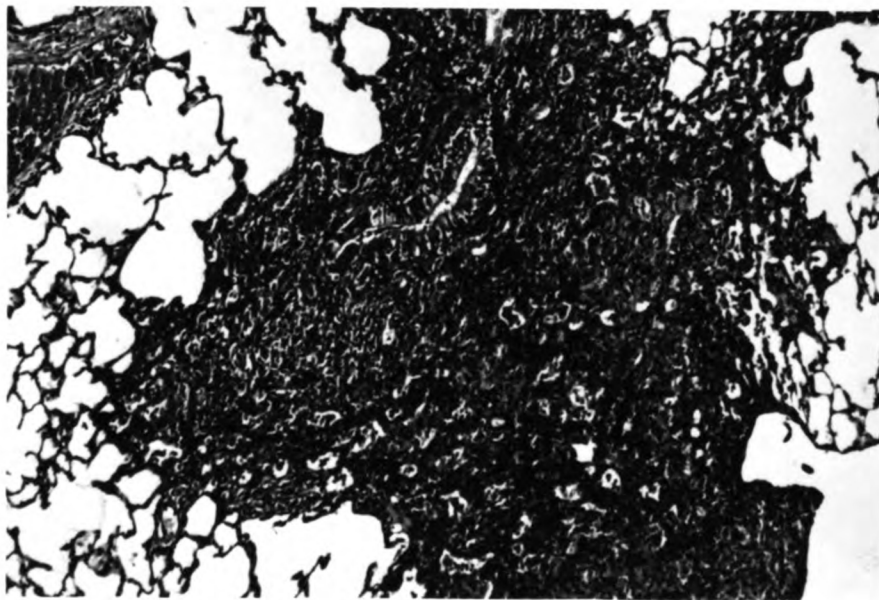


Figure 8. Lung from a 7-week-old rat, 4 weeks after aerosol exposure to *B. bronchiseptica* and *P. pneumotropica*. Note the extensive peribronchiolar lymphocytic infiltration and hyperplasia. H & E stain. x 40.

4. Eight weeks after aerosol exposure. The last 2 rats were clinically normal.

Bordetella was isolated from the nasal passage and trachea of both rats and the lung of one. *Pasteurella pneumotropica* was not isolated from either rat (Table 3).

Gross and microscopic lesions of the upper respiratory tract were not found.

Lung reaction was minimal. Lymphoid tissue was reduced around bronchi, and little evidence of pneumonia was present. Several areas of multifocal hyistiocytosis were observed.

DISCUSSION

Mycoplasma Infection in the Rat

Only 1 rat was found to contain Mycoplasma in its trachea. This rat was also the only animal to have distinct lesions in its lungs. However, since this is only one of many rats that were exposed to *Mycoplasma sp.* and *Mycoplasma pulmonis*, it is difficult to determine the significance of this finding.

There are several possible explanations for the failure of Mycoplasma to localize in the rat respiratory tract when pure cultures of the organisms are used: (1) the rats used may have had a resistance to infection by this organism; (2) the organism loses its virulence when passed in artificial media; (3) the Mycoplasma may be secondary invaders and are not capable of primary infections in the rat; (4) there are several strains of Mycoplasma that may be found in the rat respiratory tract and only certain strains of the same species are capable of producing respiratory infections; (5) certain age factors of the host may play a role; and (6) Mycoplasma may need a virus to become pathogenic. Any one or a combination of these may result in failure of infection to occur.

Bordetella bronchiseptica Infection in the Rat

Bordetella bronchiseptica was consistently isolated from the nasal passage, trachea and lungs after experimental exposure. The response was characterized by an acute pneumonic reaction which slowly regressed

over a period of 2 months. Although an increase in peribronchiolar lymphocytes was noted between 1 and 4 weeks after exposure, by 8 weeks the peribronchiolar tissue was similar to controls. Therefore, peribronchiolar lymphocytes can regress if the antigenic stimulus is removed. This would lead one to conclude that in CMP, where there is a chronic insidious increase in lymphoid tissue, there is apparently a constant antigenic stimulation preventing regression of the inflammatory cells.

Pasteurella pneumotropica Infection in the Rat

Pasteurella pneumotropica has been reported to produce experimental pneumonia. However, no isolations were made from the lungs of 11 rats exposed to *P. pneumotropica* by itself or from 8 rats exposed to both *B. bronchiseptica* and *P. pneumotropica* in combination. The organism could be isolated from the nasal passage and more commonly the trachea, thus indicating that the organism is capable of surviving in the rat respiratory tract. Even though the lung tissue was damaged by *Bordetella*, the *Pasteurella* was still not capable of lung invasion.

One might speculate that *P. pneumotropica* needs some added factor besides lung damage to become pathogenic. This factor could conceivably be a Mycoplasma, rather than a bacterium. It would be interesting to see if the 2 organisms together might be capable of lung invasion.

Another point to consider is the age at which the rats were exposed to *Pasteurella*. There may very well be an age predilection or a time when the rat is more sensitive to infection by these organisms. Data should be collected concerning uterine infections, early embryonal infections and susceptibility to repeated infections of the same organism.

Combined *Bordetella bronchiseptica* and *Pasteurella*
pneumotropica Infections in the Rat

The results from a combined infection of *Bordetella* and *Pasteurella* parallel the reaction of *Bordetella* alone. There was no increase in *pasteurella* isolations compared to infection with *Pasteurella* alone. There was no significant difference in lesions in the nasal passage, trachea or lung as compared to that with *Bordetella* alone.

SUMMARY AND CONCLUSIONS

A total of 8 experiments was conducted over a 2-year period, exposing germfree and conventional rats to *Mycoplasma sp.*, *Mycoplasma pulmonis*, *Bordetella bronchiseptica*, *Pasteurella pneumotropica*, and a combination of *Bordetella bronchiseptica* with *Pasteurella pneumotropica*. In each experiment, the acute and chronic aspects of the exposure were determined. Infection was determined by isolation of the organism(s) and the presence of tissue lesions. Where possible, the infections were compared to determine host response to each agent.

From these experiments, the following conclusions were made:

1. *Mycoplasma sp.* was not capable of producing respiratory infection in the rat with intranasal instillation or aerosol exposure of a broth culture. Even when the organism was placed directly into the trachea or when given intravenously, no lesions were produced.
2. *Mycoplasma pulmonis* did not produce respiratory infection when a 5-day growth of this organism in PKC cultures was inoculated intranasally.
3. After aerosol exposure to *Bordetella bronchiseptica* from a 24-hour growth in BHI, an acute to subacute multifocal bronchopneumonia was produced that regressed over a period of 2 months.
4. *Pasteurella pneumotropica* did not invade the lung after aerosol exposure to a 24-hour growth of the organism in BHI culture media.
5. A combination of *B. bronchiseptica* and *P. pneumotropica* infection produced lesions identical to *B. bronchiseptica* alone. No enhancement of *P. pneumotropica* occurred as a result of the combination.

REFERENCES

REFERENCES

- Arskog, R.: *Bordetella bronchiseptica* and piglet pneumonias. Nord. Vet. Md., 19, (1967): 58-61.
- Bergey's Manual of Determinative Bacteriology, 2nd ed. The Williams and Wilkins Company, Baltimore, 1925, p. 257.
- Bergey's Manual of Determinative Bacteriology, 7th ed. The Williams and Wilkins Company, Baltimore, 1957, p. 403.
- Brennan, P. C., Fritz, T. E., and Flynn, R. J.: Role of *Pasteurella pneumotropica* and *Mycoplasma pulmonis* in murine pneumonia. J. Bact., 97, (1969): 337-349.
- Dienes, L.: Morphology and nature of the pleuropneumonia group of organisms. J. Bact., 50, (1945): 441-458.
- Duncan, J. R., Ross, R. F., Switzer, W. P., and Ramsey, F. K.: Pathology of experimental *Bordetella bronchiseptica* in swine: atrophic rhinitis. Am. J. Vet. Res., 27, (1966): 457-466.
- Filion, R. par, Cloutier, S., Vrancken, E. R., and Bernier, G.: Infection Respiratoire du Dindonneau Causee par un Microbe Apparente au *Bordetella bronchiseptica*. Can. J. Comp. Med., 31, (1967): 129-134.
- Flynn, R. J., Brennan, P. C., and Fritz, T. E.: Pathogen status of commercially produced laboratory mice. Lab. An. Care, 15, (1965): 440-447.
- Ganaway, J. R., Allen, A. M., and McPherson, C. W.: Prevention of acute *Bordetella bronchiseptica* pneumonia in a guinea pig colony. Lab. An. Care, 15, (1965): 156-162.
- Giddens, W. E., Jr.: The pathology of chronic respiratory disease in the rat. Ph.D. Thesis (1968), Michigan State University.
- Griffin, C. A.: Bacterial diseases of some common laboratory animals. Proc. An. Care Panel, 6, (1955): 92-112.
- Harris, D. L., and Switzer, W. P.: Turbinate atrophy in young pigs exposed to *Bordetella bronchiseptica*, *Pasteurella multocida*, and combined inoculum. A.J.V.R., 29, #4, (April, 1968): 777-785.
- Heyl, J. G.: A study of *Pasteurella* strains from animal sources. Antonie van Leeuwenhoek, J. Micro., 29, (1963): 79-83.

- Henriksen, S. D., and Jyssum, K.: A study of some *Pasteurella* strains from the human respiratory tract. Acta. Path. Micro. Scand., 51, (1961): 354-368.
- Henriksen, S. D.: Some *Pasteurella* strains from the human respiratory tract. A correction and supplement. Acta. Path. Micro. Scand., 55, (1962): 355-356.
- Hoag, W. G., Wetmore, P. W., Rogers, J., and Meier, H.: A study of latent *Pasteurella* infection in a mouse colony. J. Inf. Dis., 111, (1962): 135-140.
- Innes, J. R. M., McAdams, A. J., and Yevich, P.: Pulmonary disease in rats. A survey with comments on "Chronic Murine Pneumonia". Am. J. Path., 32, (1956): 141-159.
- Jawetz, E.: A pneumotropic *Pasteurella* of laboratory animals. I. Bacteriological and serological characteristics of the organism. J. Inf. Dis., 86, (1950): 172-183.
- Jawetz, E., and Baker, W. H.: A pneumotropic *Pasteurella* of laboratory animals. II. Pathological and immunological studies with the organism. J. Inf. Dis., 86, (1950): 184-196.
- Joshi, N. N., Blackwood, A. C., and Dale, D. G.: Chronic murine pneumonia: A review. Can. J. Comp. Med., 25, (1961): 267-273.
- Joshi, N., Dale, D. G., and Blackwood, A. C.: Etiology of murine endemic pneumonia. Rev. Can. Biol., 24, (1965): 169-178.
- Klieneberger, E., and Steabben, D. B.: On a pleuropneumonia-like organism in lung lesions of rats, with notes on the clinical and pathological features of the underlying condition. J. Hyg., 37, (1937): 143-152.
- Klieneberger, E., and Steabben, D. B.: On the association of the pleuropneumonia-like organism L₃ with bronchiectatic lesions in rats. J. Hyg., 40, (1940): 223-227.
- Klieneberger-Nobel and Cheng, K. K.: On the association of the pleuropneumonia-like L₃ organism with experimentally produced bronchiectasis in rats. J. Path. Bact., 70, (1955): 245-246.
- Klieneberger-Nobel, E.: Pleuropneumonia-like Organisms (PPLO): Mycoplasmataceae. Academic Press, London/New York, 1962.
- Lemcke, R. M.: Association of PPLO infection and antibody response in rats and mice. J. Hyg., 59, (1961): 401-412.
- Nelson, J. B.: Infectious catarrh of the albino rat. I. Experimental transmission in relation to the role of *Actinobacillus muris*. J. Exp. Med., 72, (1940a): 645-654.
- Nelson, J. B.: Infectious catarrh of the albino rat. II. The causal relation of coccobacilliiform bodies. J. Exp. Med., 72, (1940): 655-662.

- Nelson, J. B.: Studies on endemic pneumonia of the albino rat. I. The transmission of a communicable disease to mice from naturally infected rats. *J. Exp. Med.*, 84, (1946a): 7-14.
- Nelson, J. B.: Studies on endemic pneumonia of the albino rat. II. The nature of the causal agent in experimentally infected mice. *J. Exp. Med.*, 84, (1946b): 15-23.
- Nelson, J. B.: Studies on endemic pneumonia of the albino rat. IV. Development of a rat colony free from respiratory infections. *J. Exp. Med.*, 94, (1951): 377-386.
- Nelson, J. B.: The etiology and control of chronic respiratory disease in the rat. *Proc. An. Care Panel*, 7, (1957): 30-40.
- Nelson, J. B.: Infection in laboratory animals with pleuropneumonia-like organisms. *J. Natl. Cancer Inst.*, 20, (1958): 911-919.
- Nelson, J. B.: Chronic respiratory disease in mice and rats. *Lab. An. Care*, 13, (1963): 137-143.
- Newberne, P. M., Salmon, W. D., and Hare, W. V.: Chronic murine pneumonia in an experimental laboratory. *Arch. Path.*, 72, (1961): 224-233.
- Pankevicius, J. A., Wilson, C. E., and Farber, J. F.: The debatable role of a pleuropneumonia-like organism in the etiology of chronic pneumonia in rats. *Cornell Vet.*, 47, (1957): 317-325.
- Passey, R. D., Leese, A., and Knox, J. C.: Bronchiectasis and metaplasia in the lung of the laboratory rat. *J. Path. Bact.*, 42, (1936): 425-434.
- Phillips, J.: *Alcaligenes (Brucella) bronchisepticus* as a factor in porcine pneumonia. *Can. J. Comp. Med. and Vet. Sci.*, 7, (1943): 58-59.
- Ross, F. R., Switzer, W. P., and Duncan, R. J.: Comparison of pathogenicity of various isolates of *Bordetella bronchiseptica* in young pigs. *Can. J. Comp. Med. and Vet. Sci.*, 31, (1967): 53-57.
- Smith, D. T., Bethune, N., and Wilson, J. L.: Etiology of spontaneous pulmonary disease in the albino rat. *J. Bact.*, 20, (1930): 361-370.
- Switzer, W. P.: Studies on infectious atrophic rhinitis. V. Concept that several agents may cause turbinate atrophy. *Am. J. Vet. Res.*, 17, (1956): 478-484.
- Switzer, W. P., Mare, C. J., and Hubbard, E. D.: Incidence of *Bordetella bronchiseptica* in wildlife and man in Iowa. *Am. J. Vet. Res.*, 27, (1966): 1134-1136.
- Tanaka, H., Hall, W. T., Sheffield, J. B., and Moore, D. H.: Fine structure of *Haemobartonella muris* as compared with *Eperythrozoon coccoides* and *Mycoplasma pulmonis*. *J. Bact.*, 90, (1965): 1735-1749.

- Ventura, J., and Domardzki, M.: Pathogenesis of experimental bronchiectasis in laboratory rats. Arch. Path., 83, (1967): 80-85.
- Vrolijk, H., Verlinde, J. D., and Braoams, W. G.: Virus pneumonia (snuffling disease) in laboratory rats and wild rats due to an agent probably related to grey lung virus of mice. Ant. V. Leeu., 23, (1957): 173-183.
- Wheater, D. W. F.: The bacterial flora of an SPF colony of mice, rats and guinea-pigs. Conalty, M. L.: Husbandry of Laboratory Animals (1967), Academic Press, pp. 343-360.
- Winsser, J.: A study of *Bordetella bronchiseptica*. Proc. An. Care Panel, 10, (1960): 87-104.

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