THE PATHOLOGY OF CHRONIC RESPIRATORY DISEASE IN THE RAT

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ABSTRACT

THE PATHOLOGY OF CHRONIC RESPIRATORY

DISEASE IN THE RAT

by William Ellis Giddens, Jr.

Research was conducted to study the anatomy, microbiology, and pathology of the nasal cavity, middle ear, trachea and lungs of laboratory rats. The study included 149 weanling (3-4 weeks old), adult (8-9 weeks old) and aged (7-16 months old) rats randomly selected from germfree, defined flora, conventional and 3 chronic murine pneumonia-affected colonies (CMP-1, CMP-2, CMP-3). Gross and microscopic observations were conducted on all rats and viral and bacteriologic examinations were conducted on selected rats from each colony.

The normal structure of the respiratory tract of germfree, defined flora and conventional rats was characterized. Subepithelial lymphocytic tissue was scant in the respiratory tract of germfree rats, but it increased progressively in defined flora, conventional and chronic murine pneumonia-affected colonies.

In the CMP-1 group there was severe purulent rhinitis and otitis media in rats of all ages. Slight tracheitis and peribronchial lymphocytic infiltration were observed in adult and aged rats. The most commonly isolated organism was Mycoplasma sp. Inoculation by aerosol of CMP-1 tracheal and lung suspensions into 2 series of weanling rats and mice resulted in severe rhinitis and otitis media and moderate patchy pneumonia only in the second series of rats.

In the CMP-2 group, which consisted only of aged rats, there was severe chronic purulent rhinitis and pneumonia with extensive peribronchial lymphocytic cuffing and bronchiectasis. Bronchopneumonia and lobar pneumonia were occasionally observed. Diplococcus pneumoniae was the most commonly isolated organism. Experimental inoculation was unsuccessful in producing respiratory disease.

In the CMP-3 group, rhinitis, otitis media, and pneumonia were observed in both weanling and aged rats. Bronchiectasis was commonly observed in aged rats. Two organisms frequently isolated were Mycoplasma sp. and Pasteurella pneumotropica. Exposure of rats and mice to aerosols of tracheal and lung suspensions was unsuccessful in producing respiratory disease.

It is concluded that the essential etiologic factor in CMP is probably prolonged and continuous irritation and/or antigenic stimulation of the bronchial wall. Factors which are believed to affect this are: (1) the species and strains of microorganisms present, (2) environmental factors, such as ammonia fumes and dust, (3) the delicate construction of the rat bronchus, and (4) genetic susceptibility.

THE PATHOLOGY OF CHRONIC RESPIRATORY DISEASE IN THE RAT

By

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Dedicated to my wife

Huda

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INTRODUCTION

> "When people respire, they raise their chest because the motive principle of the organ described resident within the chest causes an identical expansion of this organ. When it dilates the outer air must rush in as into a bellows...But, as the increase of bulk causes the organ to dilate, so diminution causes contraction, and when it collapses the air which entered must pass out again...The numerous canal-like ducts in the lung, into which it passes, have each a blood vessel lying alongside, so that the whole lung is thought to be full of blood. The inward passage of air is called respiration, the outward expiration, and this double movement goes on continuously just so long as the animal lives and keeps this organ in continuous motion; it is for this reason that life is bound up with the passage of the breath outwards and inwards."
>
> Aristotle, 384-322 B.C.

For centuries after this early and remarkably perceptive observation of pulmonary structure and function, men have sought to unravel the mysteries of the lung. Much knowledge has been gained by studying laboratory animals, notably the rat.

The laboratory rat is plagued, unfortunately, by a rather high incidence of insidious respiratory diseases. The purpose of this work was to establish some morphologic and microbiologic parameters of the normal and the diseased respiratory tract in the rat.

The specific objectives were to study the morphologic and microbiologic aspects of the respiratory tract of germfree, defined flora, conventional, and chronic murine pneumonia-affected rats in order to:

1. Define the variation in morphology of the nasal cavity, middle ear, trachea and lungs of rats raised under the widely differing environments of these colonies, and to describe the pathogenesis of the lesions.

2. Isolate and identify bacteria and viruses and to relate these to the lesions present.

3. Experimentally reproduce and describe chronic murine pneumonia in rats and mice by aerosol inoculation of tracheal and lung suspensions of each of the chronic murine pneumonia-affected groups.

LITERATURE REVIEW

Previous reviews of chronic respiratory disease in rats have emphasized either the bacteriology or the pathology, or have considered the lungs and neglected the upper respiratory tract. For these reasons this review attempts to cover in detail the anatomy, microbiology, and pathology of the nasal cavity, middle ear, trachea, and lungs.

The Nasal Cavity and Middle Ear of the Rat

Anatomy. Kelemen and Sargent (1946) reported that the nasal cavity of the rat was divided by the nasal septum into 2 choanae, which were connected by an opening in the ventral aspect of the septum. Two systems of turbinates were present: (1) the nasal turbinate, arising from the nasal bone, and the maxillary turbinate, from the lateral wall of the nasal cavity, were both located in the rostral part of the cavity. They were simple coils covered with columnar epithelium. Opposite the rostral end of the maxillary turbinate was the vomeronasal organ, which achieves its maximum size in the rat; (2) the ethmoid turbinates consisted of 4 endoturbinates and 2 ectoturbinates and were located in the posterior nasal cavity. They originated from the ethmoid bone and were covered by olfactory epithelium. The 4 endoturbinates were grossly visible after the nasal septum had been removed. The 2 ectoturbinates could only be seen grossly after the endoturbinates had been removed. The frontal sinus was absent. The maxillary sinus was present from the

posterior level of the nasal and maxillary turbinates to the rostral level of the ethmoid turbinates.

Detailed studies of the normal middle ear of the rat could not be found. Rüdinger (1870) described the eustachian tube of the rat as containing numerous acinar glands. Ladman and Mitchell (1955) described these structures in the mouse as tubuloacinar glands. The terminal portions were composed of sero-acinar cells, the apices of which contained periodic acid-Schiff (PAS)-positive granules. The ducts were lined with columnar epithelial cells, some producing mucin and others containing cilia. They thought the glands served to lubricate the eustachian tube and to act as accessory salivary glands.

Kobayashi (1955) described the articulations of the auditory ossicles of the rat and other animals. In the rat the incudomalleolar and incudostapedial joints are true articulations.

Microbiology. Limited studies of the normal microbial flora of the nasal cavity and middle ear have been made. Nelson (1930b) was unable to isolate any organisms from the middle ear of 22 normal rats. Strangeways (1933) isolated Streptobacillus moniliformis from septicemic and arthritic mice which had been inoculated with blood from rats. The blood was obtained by killing the rats "with a sharp blow on the head of sufficient violence to cause blood to flow freely from the nose and mouth". Strangeways soon identified the organism as a resident of the rat nasopharynx and isolated it from 7 of 14 "normal" rats.

Williams et al. (1967) reported that from the nasal cavities of 29 conventional rats, Bordetella bronchiseptica, Pasteurella pneumotropica, Mycoplasma spp., Streptobacillus moniliformis, and Staphylococcus albus

were each isolated at least 10 times. In the middle ear, S. moniliformis, Mycoplasma spp., Alcaligenes faecalis, and hemolytic Streptococcus spp. were isolated from at least 5 of the 29 rats. Unfortunately, the morphologic changes in the upper respiratory tract were not described. In 30 rats from a barrier-sustained colony, no organisms were isolated from the middle ear, while Neisseria spp., Staphylococcus aureus, and Micrococcus spp. were isolated at least 7 times from the nasal cavity.

Flynn (1967), in a survey of the microbial flora of retired breeders from 10 colonies (both specific-pathogen-free and conventional), isolated Pasteurella pneumotropica from 90%, and Pseudomonas aeruginosa from 70%, of the nasopharynges examined. He noted that there was also a "high incidence" of these organisms in the lungs. Mycoplasmas were isolated from rats of only 1 colony, and S. moniliformis was not mentioned.

The rat and the mouse are very susceptible to nasal infections and to chronic otitis media, also known as suppurative otitis (McCordock and Congdon, 1924) or middle ear disease (Freudenberger, 1932). Nelson (1937a,b,c; 1940a,b) called the upper respiratory complex "infectious catarrh". The otitis media occasionally involves the inner ear, producing a peculiar tilting of the head, lack of coordination, and a bizarre rotation of the body when the rat is picked up by the tail. The condition is often called labyrinthitis (King, 1939), and such animals are referred to as "twisters" (Nelson and Gowen, 1930). Mice are also susceptible to labyrinthitis and the twisting syndrome. This condition is a separate entity from rolling or spinning disease (Gorril, 1956), a cerebral inflammation, or the mutants pirouette (Deol, 1956), mutants varitint-waddler (Deol, 1954), shaker-1 (Deol, 1956; Grüneberger et al., 1940), shaker-2 (Deol, 1954), waltzer (Deol, 1956), and jerker (Deol, 1954), which are hereditary diseases.

McCordock and Congdon (1924) observed that 23 of 2700 rats in their colony had signs of "twisting". Of 152 discarded rats and retired breeders, 79 had otitis media, while in younger rats killed "in their prime", only 9 of 42 (22%) were affected. Nelson and Gowen (1930) reported that 69% of laboratory rats had suppurative otitis media, of which 50% also had nasopharyngitis. In rats 3 to 4 months old, 32% had suppurative otitis media, of which only 1% had nasopharyngitis. They believed that otitis media occurred prior to nasopharyngitis and was probably due to a plugging of the eustachian tube or to the propulsion of bacteria into the tympanic cavity when the rat sneezed or coughed.

Freudenberger (1932) observed that otitis media was about twice as common in rats of the Wistar strain (36%) as in those of the Long-Evans strain (17%). The incidence in 3-week-, 12-week-, and 1-year-old rats was 12%, 15%, and 43%, respectively. Slightly more males (28%) than females (24%) were involved.

It has long been noticed that rats fed diets deficient in vitamin A had a higher than normal incidence of upper respiratory and pulmonary infections (Daniels et al., 1923; Bradford, 1928; Turner, 1928, 1929). Daniels et al. (1923) proposed that this was due to a lowering of the natural resistance so that rats became increasingly susceptible. Bradford (1928) observed that all of 31 rats fed diets deficient in vitamin A had otitis media, while only 3 of 13 rats fed a normal diet were similarly affected.

Reports prior to 1930 indicated that a number of organisms were present in the middle ear of rats with otitis media. They were: a mucoid bacillus (Bradford, 1928), gram-negative cocci (Turner, 1928)

and bacilli (McCordock and Congdon, 1924; Turner, 1928; Nelson, 1930a), Staphylococcus aureus (Turner, 1929; Nelson, 1930a), diphtheroids (Nelson, 1930a), streptococci (Nelson, 1930a), and Bacillus actinoides (Nelson, 1930a). Nelson (1957) later reported that Bacillus actinoides was, in fact, Streptobacillus moniliformis. Haberman et al. (1954) isolated S. moniliformis from 33 of 160 wild Norway rats kept under simulated natural conditions. They reported that lesions of otitis media were common.

Matheson et al. (1955) isolated no organisms from the middle ear of rats less than 14 days old, but 1 or more organisms were isolated from 93.3% of rats over 30 days old. The 3 organisms most commonly isolated were Pasteurella multocida, Diplococcus pneumoniae and Streptobacillus moniliformis. Of these, the only organism with a seasonal fluctuation in incidence was D. pneumoniae, which was more common in the winter.

Nelson (1937a) described an outbreak of infectious catarrh in mice and reported (1937b) the presence of "coccobacilliform bodies", 0.3 to 0.4 in diameter, in nasal and middle ear exudates and in inoculated chick embryo tissue cultures. He reproduced the disease (1937c) by inoculating the tissue cultures into normal mice. These bodies proved similar to the "pleuropneumonia-like organisms" of Klieneberger (1935). Edward (1947) described and reproduced a similar condition in mice.

Nelson (1940a,b) transmitted infectious catarrh to normal rats by instilling 10 to 12 drops of exudate from the middle ear of diseased rats. Bordetella bronchiseptica and Actinobacillus muris (S. moniliformis) were the most frequently isolated organisms. After 12 passages, these organisms could no longer be isolated, even though the disease could still be transmitted. "Coccobacilliform bodies" could be seen in Gram-stained

exudates from 91% of the nasal and 97% of the middle ear cavities examined. These organisms, which Nelson later (1948) identified as mycoplasmas, could be grown in chick embryo tissue cultures. Suspensions of infected tissue culture produced infectious catarrh on nasal inoculation into normal rats. Nelson (1955) defined infectious catarrh as a disease of rats and mice caused by mycoplasmas affecting primarily the upper respiratory tract and middle ear and, occasionally, the lungs. Vasenius and Tiainen (1966) recovered mycoplasmas from the middle ear of 40 of 42 rats with otitis media. The same organisms were often present in the nasal cavity.

Clinical Signs and Pathology. Pronounced clinical signs of infectious catarrh were often absent in rats (Nelson, 1955). Sniffling, torpor, pilo-erection and reddish-brown to yellow exudate around the eyes and nares were sometimes present (Dolowy et al., 1960). "Twisting", as previously described, was usually present if there was labyrinthitis (Nelson and Gowen, 1930).

Gross lesions are difficult to observe in the nasal cavity due to its inaccessibility. Kelemen and Sargent (1946) and Kelemen (1948) described the histologic changes of the nasal cavity in 40 apparently healthy rats. Only 16 were histologically normal; the remaining 24 had (in decreasing order of frequency) suppuration, fibrin formation, excess mucus, and hemorrhage. Similar changes were observed in the maxillary sinus. In 4 rats the choanae were completely blocked by exudates, which frequently contained foreign bodies, probably of food origin. Massive lymphocytic tissue and follicles were present in the submucosa of the lateral nasal wall. Kelemen (1948) observed that the nasal cavity of

the rat was predisposed to the development of inflammation because an antechamber was formed by the ethmoid turbinates. The pharyngeal duct emptied into this chamber. At this point there were connections between the anterior and posterior parts of the nasal cavity, the right and left choanae, and the nasal and pharyngeal cavities. The inadequacy of clearance accounted for the accumulation of foreign bodies and exudates. No such inflammatory changes were present in the nasal cavities of germfree rats (Kelemen, 1959).

In a later study of the nasal and paranasal cavities of 25 germfree and "ex-germfree" rats, Kelemen (1962) noted that all were free of lesions except 2: 1 had lymphocytic infiltrations of the nasal submucosa and another had excess production of mucus.

On examination of the middle ear, Nelson and Gowen (1930) reported that otitis media was diagnosed by the presence of an exudate in the middle ear cavity. The nature of the exudate varied markedly both as to consistency and amount, not only from rat to rat but from ear to ear in cases of bilateral infections. More commonly, either a thin, distinctly mucoid material or a thick, creamy, purulent exudate was present. The cells in the exudate were chiefly neutrophils with smaller numbers of various mononuclear cells. In some instances there was a distinct outer bulge in the tympanic membrane. At times the tympanic mucosa was thickened. It was possible to diagnose correctly the presence of exudate by the degree of resistance given by the tympanic membrane to the scissors point upon puncture. The normal membrane gave a slight "click".

In "twisters", Nelson and Gowen (1930) reported that the tympanic and petrous bones were always involved on the side to which the rats

turned. The bony framework of the tympanic bulla was soft, filled with pus, and enlarged.

In histologic examination of rats with otitis media, McCordock and Congdon (1924) observed destruction of the mucosa of the middle ear and its replacement by granulation tissue. The tympanic cavity filled with pus and resulted in osteitis of the bony framework with liquefaction and absorption of mature bone and formation of new bone. There was often an osteomyelitis of the auditory bullae (mastoiditis). An occasional complication was extension of the inflammation into the inner ear, with destruction of the semicircular canals, vestibular structures, and cochlea. They observed exudate in the eustachian tube and nasopharynx and postulated that the infection progressed from the nasal cavity through the eustachian tube to the middle ear and then to the inner ear.

Disease Control. Treatment of rats with infectious catarrh has been attempted by Dolowy et al. (1960) and Vasenius and Tiainen (1966). Dolowy et al. used 10 mg. of oxytetracycline hydrochloride per ounce of drinking water for 5 days and reported a decrease in the incidence of clinical signs from 75.4% to 30%. The signs of severe labyrinthitis disappeared in 8 of 19 rats. Vasenius and Tiainen (1966) also used oxytetracycline and reported that 7 of 20 rats and 3 of 4 mice recovered from the disease.

Nelson and Gowen (1931) developed a colony of rats free of otitis media by selecting for breeding stock only those offspring from parents who had no lesions. They were not able to eliminate chronic murine pneumonia by using these methods. King (1939) outlined a method for elimination of labyrinthitis based on the natural resistance of captive gray

Norway rats. Of 3000 such rats, only 6 had clinical signs of labyrinthitis. The offspring from albino rats were removed immediately after birth and foster-suckled on gray Norway mothers. These rats were then used as breeding stock to establish a new colony. After 100 generations signs of labyrinthitis had disappeared, even though otitis media and pneumonia were occasionally present. Significantly, 2 gray rats who foster-suckled albino offspring which had remained with their own mothers for several hours after birth developed characteristic signs and lesions of labyrinthitis.

Other methods of eliminating enzootic upper respiratory tract infections will be discussed in the section on pneumonia.

Methods of detecting rats with otitis media other than by necropsy have been explored by Grice et al. (1955) and Greselin (1961). Grice et al. (1961) used radiographs of the head to screen affected rats. In dorsoventral radiographs normal rats have clear spaces signifying the tympanic cavity. Rats with otitis media have diffuse opacities. Grice et al. reported a good correlation with the result of postmortem examination, but they made no mention of histologic examination.

Greselin (1961) used a modified otoscope to examine the tympanic membrane. He noted in infected rats that the tympanic membrane was distended and darker, and the vessels near the hammer were congested.

The Trachea and Lung of the Rat

Anatomy. The rat has 3 lobes in the right lung, 1 in the left, and 1 intermediate lobe which lies approximately in the midline. Green (1935) referred to the lobes as the right superior, middle, inferior lateral and inferior medial lobes and the left lobe. Innes et al. (1967) called

them the right apical, cardiac, diaphragmatic and azygos lobes, and the left lobe. Hillerbrand (1965) proposed the following Latin names: the lobus apicalis dexter, lobus cardialis dexter, lobus diaphramaticus dexter, lobus accessorius, and the pulmo sinister. In this thesis, the English equivalent of these names will be used.

The ventral aspect of the right diaphragmatic lobe was interrupted by the presence of a fissure which ran deeply into the lobe and often gave the impression of 2 lobes instead of 1 (Lauche, 1958). Donaldson (1923) reported that there was a decrease in the lung weight as a percentage of body weight from 1.0% at 5 Gm. of body weight to 0.57 to 0.62% at 25 Gm., and 0.32 to 0.34% at 400 Gm.

Innes et al. (1967) reported that the right main bronchus was short and abutted from the trachea at a near right angle, while the left bronchus descended obliquely into the hilus of the left lobe. The right bronchus immediately gave off a short trunk, the epiarterial branch, which went to the right apical lobe. The trachea and bronchi contained cartilagenous plates in their walls but the bronchi lost these when they entered the lungs (Miller, 1911). Lauche (1958) observed that the tracheal cartilages often were calcified in adult rats and mice.

Robinson (1889) described the embryonic development of rat and mouse lungs. In the 6- to 8-day-old rat embryo, the lungs began their development as a furrow in the ventral portion of the anterior gut. A bud was formed which grew into the mesenchyme and ramified by dichotomous budding. Unlike the human embryonic trachea, that of the rat gave off 3 branches, the third going to the right apical lobe. According to Stewart (1923) the bronchial tubes were lined by columnar epithelium which slowly became cuboidal as alveolar buds branched off the bronchi. He believed that

they were flattened by the gasping movements of the fetus or neonate. The cytoplasm of the lining epithelial cells was very plastic and flattened to yield what he believed were non-nucleated plates lining the alveoli. Some cells were thought to desquamate, leaving the capillary endothelium exposed to the alveolar space.

Bensley and Groff (1935-1936) reported that the cuboidal epithelium lining the alveoli persisted up to the 20-day fetus and was flattened by respiratory movements just before, during, or after birth. Sorokin (1961) obtained a different conclusion by studying organ cultures of lung tissue from 13- to 19-day-old fetuses. The columnar epithelium lining the bronchi became more cuboidal as branching proceeded. It became attenuated as the alveoli formed. Intracytoplasmic glycogen concentrated in the budding terminal epithelium but disappeared as the epithelial cells became attenuated. He concluded that the epithelial flattening occurred spontaneously and was not caused by respiratory movements.

Sorokin et al. (1959) compared the in vitro development of fetal rat and guinea pig lung cultures. Much of the differentiation that occurred prenatally in the guinea pig lung cultures (the gestation period is 68 days) occurred postnatally in rat lung cultures (the gestation period is 21 days). The "critical period" in the rat lung (when the glycogen disappeared and the alveolar epithelium became flattened) was 26 days after birth, or 113% of the gestation period. In contrast, the "critical period" for the guinea pig was 47 days after birth, or 67% of the gestation period.

Lauche (1958) reported that the large bronchi of rats as well as mice and guinea pigs contained relatively few mucous glands. In the

smaller bronchi there were none. The larger bronchi were lined by ciliated pseudostratified columnar epithelium. The mucosa of the smaller bronchi was composed of villous folds lined by nonciliated epithelium. The bronchi usually divided into 2, or rarely 3, terminal bronchioles which proceeded into the respiratory bronchioles. The ramification of respiratory bronchioles into alveolar ducts, alveolar sacs, and alveoli was similar to that seen in the human lung. Yevich (1965) reported that there are usually 1 or 2 orders of respiratory bronchiole. They were very short and opened directly into alveolar ducts. This caused inhaled particles to be deposited in the alveoli, instead of the bronchioles, as in most other mammals.

Guieysse-Pellisier (1937) described sac-like dilations in the bronchi with enormus hypertrophy of the cilia, so that they were taller than the cells from which they originated. The ciliated cells were interspersed among nonciliated cells, so the ciliary movement was ineffective in transporting particles and mucus.

The presence of lymphocytic tissue and even of lymph nodes in the lung was recognized by early investigators but was generally regarded as pathological. The investigations of Arnold (1880), however, established it as a constituent of the normal lung. Studying tissue from man, dog, cat, rabbit, and guinea pig, he observed the lymphocytic tissue to be located principally in subpleural, perivascular, and peribronchial locations.

Miller (1911) found that the rat, unlike most other animals, had no cartilage in the bronchial walls but had abundant peribronchial lymphocytic tissue. It often surrounded the bronchus and penetrated the tunica muscularis to lie just beneath the bronchial epithelium. The lymphocytic

tissue was peribronchial, periarterial, perivenous, and subpleural in location. It occurred as nodules, follicles, or smaller masses of lymphocytes.

The lymphocytic tissue was often found in association with the larger divisions of the bronchi. When located along a straight bronchus it was usually situated between the bronchus and its accompanying bronchial artery. If along the pulmonary artery, it was situated between that vessel and the adjoining alveoli. Isolated aggregations of lymphocytic tissue were found, on serial sectioning, to be located near vessels or bronchi not present in the plane of the first section. The pleura had numerous lymph vessels and occasional aggregations of lymphocytes but no nodules or follicles. The amount of pulmonary lymphocytic tissue increased with age. Miller ascribed this to the constant inhalation of irritating particles. Miller believed that pulmonary lymphocytic tissue served as a filter of lymph circulating through the lungs, and also served as a center to which macrophages carried their collected materials.

Warren and Drinker (1942) reported that lymph flow from the lung was reduced when the lungs were quiescent or if there be intense artificial respiration. Lymph flow was greatly increased if the oxygen level of the air decreased or if there be pulmonary venous hypertension. If the latter be severe enough, the lymph soon resembled blood in color. Miller (1947) stated that the lymph vessels did not extend into the alveolar walls but ended as blind sacs near the alveolar ducts. Policard (1960) expressed his belief that the lymphatic vessels in the rat did not end as blind sacs in the terminal portions of the bronchi but continued into the alveolar wall, though in a much attenuated form.

Guieysse-Pellisier (1927) studied the pulmonary lymphocytic tissue of wild and domestic rats, as well as other species. He divided pulmonary lymphocytic tissue into 2 types: the diffuse, located chiefly in the alveolar walls, and the compact, in the bronchial and vascular adventitia. The diffuse lymphocytic tissue was able to proliferate more rapidly than the compact when irritated. The amount of compact lymphocytic tissue in the bronchi and vessels varied greatly, from being absent to entirely encasing the structure around which it was located. Eosinophils were commonly present in the pulmonary lymphocytic tissue.

Andreasen (1943), in a study of the thymus, spleen, and lymph nodes of growing rats, observed that the weight of these organs increased at the same rate as the body weight up to the age of 3 to 4 months. Then there was a marked involution of the thymus. The lymph nodes remained constant in weight, while the spleen increased as the rat grew older.

Han (1961) described the ultrastructural characteristics of the lymph node of the rat. Reticular cells were of 3 types: nondifferentiated, those associated with collagen fibers, and those with phagocytic vacuoles. The nondifferentiated cells appeared to differentiate into fiber-associated reticular cells, phagocytic reticular cells, plasma cells, or into lymphoblasts. "Collagen fibers" (the argyrophilic fibers of light microscopy) were invariably surrounded by the cytoplasm of reticular cells.

Katzberg (1954), in a study of the mast cells in rat lymphoreticular tissues, noted that mast cells were common in the cortex and medulla, but not the germinal centers, of most lymph nodes. They were reported in the alveolar wall of the normal rat lung by Kay et al. (1967).

Takino (1932) was able to easily distinguish bronchial arteries in the rat from pulmonary arteries because the former were located in close proximity to the bronchi. Verloop (1949), in a detailed study of the circulation of the rat lung, observed that the right bronchial artery came from the right internal mammary artery in half of the rats studied and from the right costo-cervical trunk or the right superior intercostal artery in the other half. The left bronchial artery always originated from the left internal mammary artery. The bronchial arteries contained very few elastic fibers after entering the lung parenchyma and always were located in close proximity to their respective bronchi. The bronchi were surrounded by a layer of connective tissue containing abundant lymphocytic tissue. In this wall the bronchial arteries wound themselves in long spirals around the bronchi, often accompanied by thick nerve bundles. Only capillary anastomoses between the pulmonary and bronchial arteries were present. The pulmonary arteries replaced the bronchial arteries in supplying the bronchi when the bronchi became very small. Within the lung parenchyma, bronchial arteries could be differentiated from pulmonary arteries because they were muscular and lacked elastic tissue, ramified in the bronchial wall, and were usually accompanied by nerve bundles.

Guieysse-Pellisier (1937) and Innes et al. (1956) described the presence of myocardial fibers in the adventitia of the pulmonary veins in the lungs of rats. These striated fibers were present in both large and small veins. Berkeley (1893), in a study of the innervation of blood vessels in the rat, reported that the supply of nerves to the bronchial vessels was more abundant than that to the vessels of any other organ except perhaps the ovary.

The scientific literature concerning the nature of the alveolar wall is both voluminous and fascinating. Some who contended that the alveolar wall was not lined by nucleated epithelial cells (such as Stewart, 1923, and Geever et al., 1943) were opposed by those who declared that a continuous epithelial lining was present (such as Shea, 1936, and El Gazayerli, 1936). Part of the controversy was due to the limitation of light microscopy. This controversy was dramatically resolved by Low (1952), who published the first electronmicroscopic study of the alveolar wall of the rat and demonstrated that extremely flattened epithelial cells lined the alveoli.

Bertalanffy and LeBlond (1955) described 4 types of cells in the alveolar wall of the rat: the vacuolated alveolar lining cells, which had a mean life of 29.4 days per cell, the nonvacuolated alveolar lining cells, with a mean life of 8.1 days per cell, the endothelial cells and the circulating blood cells. The alveolar lining cells were described ultrastructurally in the rat by Watrach and Vatter (1959). The vacuolatd cells contained numerous osmiophilic laminated inclusions.

The arithmetic mean thickness of the blood-air barrier of the rat alveolar wall was reported by Wiebel and Knight (1954) to be 1.25 μ. A geometric model of the barrier, in the form of a corrugated membrane, was derived by them, using morphometric techniques.

Gross (1961) described the reticulin of the rat alveolar wall as moderately delicate, smoothly contoured fibers, uniform in thickness with short sidebranches or loops extending 2 to 4 μ from the axial strands.

The origin and purpose of the alveolar macrophage have received considerable attention by investigators. Carleton (1927) stated, on

the basis of tissue culture and histopathologic studies, that the alveolar macrophage was derived from the lining epithelium. Bensley and Groff (1935-1936) and Shea (1936) agreed with this theory, but Lang (1925), El Gazeryerli (1936) and Geever et al. (1943) maintained that the alveolar macrophage must be a mesenchymal component of the reticuloendothelial system since it was phagocytic. Lang (1925) introduced the term "septal cell" to designate the nucleated cell on the wall of the alveolus which gave rise to the alveolar macrophage. He insisted that, although it sometimes appeared to line the alveolar wall much as epithelium did, it was a connective tissue cell and a component of the reticuloendothelial system.

Bell (1943) described the proliferation of the alveolar lining cells under a wide variety of conditions, such as jaagziekte in sheep, chronic passive congestion, and lipid pneumonia. Their hypertrophy and hyperplasia was believed to be caused by acute inflammations, circulatory disturbances, and the presence of exudate in the alveoli (Geever et al., 1943).

Brundelet (1965) studied the dust-clearance mechanism in rats by exposing them to trypan blue and carmine red dyes. The alveolar macrophages phagocytosed the particles, passed into the connective tissue septa of the alveolar walls, and circulated between connective tissue fibers until they reached the peribronchial lymphocytic tissue. From here they seemed to migrate into the bronchial lumina to be passed out by the mucociliary mechanism.

Beaver et al. (1963) have described the frequent appearance, even in germfree rats, of focal collections of "foamy" alveolar macrophages. The cytoplasm of these cells contained lipid vacuoles and occasionally

even some granular pigment. Yang et al. (1966) called the condition "multifocal histiocytosis" and noted that grossly the foci appeared as brownish-white nodules 1 to 3 mm. in diameter. The cells contained lipid in their cytoplasm, and some cells took stains specific for phospholipids and cholesterol. About 15% of the cells contained iron-positive granules. Cholesterol clefts were occasionally seen in the cellular aggregations. Multifocal histiocytosis was observed in 16 of 191 males and 29 of 224 females. It was not observed in rats less than 15 months old. The cause of this condition was unknown.

Limited histochemical studies of the rat lung have been made. Fredricsson (1956) found that non-ciliated bronchiolar epithelial cells (the so-called Clara cells) contained very high alkaline phosphatase activity. This was believed to be related to their possible role in surfactant production. Similar alkaline phosphatase activity, observed in the septal cells, was attributed to their phagocytic properties. Tyler and Pearse (1965) observed the same intense enzymatic activity in these cells. They employed a variety of oxidative enzymes to study the alveolar wall and concluded that the glycolytic scheme and the pentose pathway were more active pathways of carbohydrate metabolism than the tricarboxylic acid cycle.

Microbiological Flora. Only limited microbiological studies of normal rat lungs have been made, and these have usually been in connection with the investigation of spontaneous respiratory diseases. In a study of the pulmonary flora of calves, rabbits, guinea pigs, mice and rats, Jones (1922) found in 36 samples from 6 rats that a streptothrix was isolated 1 time, B. subtilis and "cocci" 2 times, and "molds" 8 times.

He could decrease the number of organisms recovered by moistening the bedding and the feed. He recovered streptothrix from the straw and hay and concluded that dusty feed and bedding were the sources of most organisms in the lungs. He recovered streptothrix organisms from the bronchial lymph nodes of 2 of 3 guinea pigs examined.

Bordetella bronchiseptica has been isolated from both normal (Griffin, 1955) and pneumonic (Hoskins and Stout, 1919; Pankevicus et al., 1957; Winsser, 1960) rats.

Pasteurella pneumotropica was first isolated by Jawetz (1950) from the pneumonic lungs of mice. Flynn (1967) reported that Pasteurella pneumotropica and Pseudomonas aeruginosa were common isolates from the lungs of retired breeders from conventional and specific-pathogen-free colonies.

The upper and lower respiratory tracts of rats and mice have been reported to often harbor Mycoplasma spp. (Edward, 1940; Nelson, 1948) and latent infections could be activated by injecting intranasally a suspension of normal lung tissue (Sullivan and Dienes, 1940; Nelson, 1948), sterile broth (Lutsky and Organick, 1966), or by ligating the bronchus (Klieneberger-Nobel and Cheng, 1955).

In a study of 30 conventional and 29 barrier-sustained rats, Williams et al. (1967) isolated at least 18 different species of bacteria from the lungs, trachea, nasal cavity, and middle ear. Isolated from conventional but not barrier-sustained rats were Bordetella bronchiseptica, Pasteurella pneumotropica, Streptobacillus moniliformis, Pseudomonas aeruginosa, and Alcaligenes fecalis. The frequency of isolation and distribution of these organisms in the 29 conventional rats was as follows:

	Lung	Trachea	Nasal Passages	Middle Ear
Bordetella bronchiseptica	14	10	11	0
Pasteurella pneumotropica	10	0	12	0
Streptobacillus moniliformis	7	0	10	8
Mycoplasma spp.	11	0	13	5
Pseudomonas aeruginosa	7	0	9	0
Alcaligenes fecalis	0	3	0	7

Pathology and Microbiology

Chronic Murine Pneumonia. Chronic murine pneumonia (CMP) is probably the most serious problem in maintaining healthy rat colonies. It not only complicates experimental work on lung diseases (Cruickshank, 1948; Innes et al., 1956) but also seriously handicaps chronic toxicity studies (Gray, 1963).

Known also as bronchiectasis (Passey, 1936; Klieneberger and Steabben, 1940; Cruickshank, 1948), bronchopneumonia (Tunnicliff, 1916), pulmonary suppuration (Moise and Smith, 1928), chronic rat pneumonia (Gray, 1963), endemic pneumonia (Nelson, 1955), and enzootic bronchiectasis (Nelson, 1963), it is a disease affecting rats and mice. It differs from other roden pneumonias in that it is a

> "chronic pulmonary disease of rats that is characterized by aggregates of lymphoid tissue around bronchi and bronchioles, bronchitis, and bronchiectasis" (Newberne et al., 1961).

It is also frequently associated with upper respiratory tract and middle ear infections.

Microbiology

The earliest report of CMP is said to be that of Klein (1903), who isolated a diphtheroid from the consolidated lungs of white rats. In the hepatized portions,

> "the alveoli and infundibula, and bronchi were filled with, and distended by, fibrinous exudation, red and white blood corpuscles, and continuous masses of the above microbe."

Subcutaneous injection into rats and guinea pigs resulted in localized abscesses. The morphologic and cultural characteristics of the organism appeared identical with those of the diphtheria bacillus, but diphtheria antitoxin failed to neutralize the pathogenic action of the organism. Klein named the organism Bacillus muris.

Mitchell (1912) isolated B. muris from laboratory rats with "pneumonitis, consolidation and abscess formation". The abscesses varied in diameter up to 2 cm. On microscopic examination a bronchopneumonia was found. The bronchi and alveoli were filled with neutrophils, fibrin and desquamated epithelium. The larger caseous portions of the lung consisted of degenerated material with caseous detritus scattered throughout. Large numbers of Gram-positive diphtheroids were present in the lesions. Some abscesses were present in the kidneys, spleen and liver. Intrapleural and subcutaneous injection produced abscess formation or severe inflammation at the site of inoculation. Intranasal injection produced typical lung lesions 20 to 30 days postinoculation.

No other known reports of B. muris infection exist, and the organism is not listed in standard bacteriology reference books (Breed et al., 1957; Topley and Wilson, 1964).

Hoskins and Stout (1919) isolated Bordetella bronchiseptica from the nasal cavity, trachea, lungs and blood of rats with a "distemper-like disease" that was probably CMP. Smith et al. (1930) isolated from rats with "lung abscesses" a number of organisms: an unidentified coccus, a Gram-negative bacillus, Bordetella bronchiseptica, and a fusiform

bacillus. Pankevicius et al. (1957) reported the isolation of B. bronchiseptica from both normal and CMP-affected rats.

Streptobacillus moniliformis was isolated from 20 of 60 white rats with acute or chronic bronchopneumonia by Tunnicliff (1916), who called the organism a streptothrix. In none of 24 normal rats could the organism be isolated from the lungs. Intraperitoneal injection produced severe pneumonia in 4 of 5 rats with death resulting in 2.

On the other hand, Strangeways (1933) recovered the organism from the nasopharynx of 7 of 14 normal rats. Tunnicliff's failure to isolate S. moniliformis from normal but not pneumonic rats was probably due to the fact that she attempted isolations from the lung only.

A Gram-negative filamentous organism was isolated by Jones (1922) from rats with chronic pneumonia. Jones noted in these rats that involved lobes were shrunken. Practically all the mature rats that he examined had lesions. Jones noted the close resemblance of his isolate to Bacillus actinoides. Jones also isolated Bordetella bronchiseptica, cocci, and streptothrices. In a few instances nothing was isolated. As has already been mentioned, the organism called B. actinoides was later identified as S. moniliformis (Nelson, 1957).

Klieneberger (1935) found that all 7 investigated strains of Streptobacillus moniliformis contained what she considered to be symbiotic organisms resembling those causing bovine pleuropneumonia. She described these as "pleuropneumonia-like organisms" (Mycoplasmas) and called them L_1 and L2, after the Lister Institute where she worked. A "pleuropneumonia-like organism resembling L_1" was later found (Klieneberger and Steabben, 1937) in the lungs of 17 of 19 rats with bronchiectasis. In only 2 of these 17 was S. moniliformis isolated.

Concurrently, Nelson (1937a,b,c) was conducting his investigations on infectious catarrh of mice caused by "coccobacilliform bodies", and Sullivan and Dienes (1939) described a pneumonia in mice caused by mycoplasmas. In the latter the infection appeared to be latent in mice and could be activated by intranasal inoculation of suspensions of human pathologic tissues or of mouse lung, causing death in as little as 3 or 4 days. Edward (1940) encountered a similar situation in inoculating suspensions of human tissue into mice and was subsequently able to isolate mycoplasmas from normal, uninoculated mice. The lungs of pneumonic mice had focal, multifocal or lobar areas of gray-red consolidation. Histologically, there was peribronchial and perivascular proliferation of lymphoreticular cells.

In 1938 Dienes described his experiments with S. moniliformis and concluded that Klieneberger's symbiote was actually a variant strain of S. moniliformis and that the variation between the 2 in the morphology of the colonies and the individual organisms was largely due to the conditions of isolation and growth. Klieneberger (1940) repudiated Dienes' theory and maintained that the L_1 organism was a true symbiont of S. moniliformis.

Klieneberger and Steabben (1940), in a study of the incidence of mycoplasmas in rats, isolated the organisms from 138 of 251 laboratory rats and 1 of 17 wild rats. Of the 251 laboratory rats, 108 had bronchiectasis. Only 1 of the 17 wild rats had lung lesions, and this was the one from which mycoplasmas were isolated. Mycoplasmas were rarely isolated from rats less than 2 months old but were isolated from 100% of rats over 12 months old. Attempts to reproduce the disease by inoculating cultures of mycoplasmas into rats were not successful.

Nelson described infectious catarrh in rats (1940a), reproduced it by nasal inoculation of tissue culture fluids (1940b), as previously mentioned, and identified the causative agent as a mycoplasma (1948). Although he noted that infectious catarrh involved principally the upper respiratory tract and middle ear (the incidence of otitis media and rhinitis being 87% and 91%, respectively, of inoculated rats), pneumonia was not uncommon (20%). Nelson suspected, however, that the etiologic agent of infectious catarrh was different from that of CMP, because it was possible (Nelson and Gowen, 1931) to eliminate infectious catarrh in a colony of rats by selective breeding without affecting the incidence of CMP.

In an attempt to isolate the etiologic agent from rats, Nelson (1946a) induced pneumonia in mice by intranasal inoculations of lung suspensions from rats with CMP but which were free of mycoplasmas and infectious catarrh. Nelson inoculated mice because he was unable to find rats free of CMP. The disease in mice progressed slowly after an incubation period 7 to 14 days and was attended by a variable mortality rate which reached 5% in 5 weeks and 33% in 22 weeks. In diseased mice the incidence of pneumonia was 96%, otitis media 94%, and rhinitis 30%.

The pneumonia-producing agent passed through Berkefeld V filters but not N filters, suggesting that its size was comparable to that of the pox viruses. Its concentration in the lung was such that 10% lung suspensions could be diluted 10^7 times and still transmit the disease. Smears of the sediments of centrifuged lung suspensions had argyrophilic spherical particles smaller than the elementary bodies of vaccinia. The agent did not grow in embryonated eggs. It was inactivated after 1 week of storage at 40 C. in buffered saline but was able to survive at least

13 weeks in dry ice. It was largely removed from suspension on centrifugation at 9000 rpm for 30 minutes.

Nelson (1948) inoculated apparently normal young rats with the presumed pneumonia-producing agent but the incidence of pneumonia in inoculated rats was not significantly greater than in uninoculated rats. Mouse inoculation tests of the rats used indicated that the pneumonia-producing agent was widely dispersed throughout the colony from which his experimental rats came and that it was acquired by young rats within 7 to 14 days after birth by way of the upper air passages as the result of maternal contact. In 1951 Nelson established a colony of rats from germfree stock and was able to reproduce the disease in them, but he stated that further collaborative studies should be made.

Nelson called the disease in rats endemic pneumonia (Nelson, 1948) but later changed it to enzootic bronchiectasis (Nelson, 1957). He considered the etiologic agent to be a virus (Nelson, 1955). On the basis of his many years of work with chronic respiratory diseases in rats, Nelson considered chronic respiratory disease in rats to consist of 2 diseases: (1) infectious catarrh, caused by Mycoplasma pulmonis and producing rhinitis, otitis media and, less often, pneumonia in rats and mice, and (2) enzootic bronehiectasis, caused by a virus, causing rhinitis and CMP in rats and mice and otitis media in mice. This view was presented by Nelson on numerous occasions (Nelson, 1955, 1957, 1958, 1962, 1963, 1967) and has been generally accepted by other workers (Innes et al., 1956; Innes et al., 1957; Newberne et al., 1961; Joshi et al., 1961; Foster, 1962; Innes et al., 1967).

A different view was held by Klieneberger-Nobel (1962). Although she could not reproduce CMP in rats by inoculating mycoplasma

intranasally, the close connection between the presence of the organisms and the bronchietatic lesions led her to suspect a cause-effect relationship (Klieneberger and Steabben, 1937, 1940). Some additional factor was thought to be necessary to render the mycoplasma pathogenic. In 1955 Klieneberger-Nobel and Cheng, in a study of experimental ligation of the bronchus, found that, although mycoplasmas could not be isolated from normal rat lungs, when the bronchus was ligated and bronchiectasis developed, mycoplasmas could invariably be isolated from the mucus that collected in the dilated bronchi. Similar results were obtained by Ventura and Domardzki (1967).

Pankevicus et al. (1957) found that 14 of 25 rats from their CMP-affected colony harbored mycoplasmas in the lungs. Rats free of CMP were obtained and were inoculated intrabronchially with these mycoplasmas. Pulmonary infections were established and the organisms were passed 3 times in this manner. Although Pankevicius et al. had no difficulty in isolating mycoplasmas from the lungs of inoculated rats, they never observed any lesions suggestive of CMP. They concluded that the role of the mycoplasma in the etiology of CMP was highly questionable.

Joshi et al. reviewed (1961) the literature of CMP and reported (1965) the isolation of a virus from the lungs of 80% of rats examined. The agent, on electronmicroscopic examination, appeared to be round and about 110 Å in diameter, although no photographs of it were shown. It could only be isolated in rat embryonic skin cells, and a cytopathogenic effect was evident only if the cell cultures were incubated first at 37 C. for 4 to 6 days and then at room temperature for 2 to 5 days (Joshi et al., 1964). Inoculation into axenic and cesarean obtained, barrier-sustained (COBS) rats produced focal peribronchial collections of

lymphocytes. They reported that no peribronchial lymphocytic tissue was present in the lungs of uninoculated COBS or axenic rats. The agent survived for 48 hours at 25 C., for 2 to 3 weeks at 7 C., and for at least 6 weeks at -20 C.

Joshi et al. (1965) isolated a mycoplasma from 36% of normal rat lungs and 30% of pneumonic rat lungs. Intranasal inoculation of rats and mice with these organisms resulted in nasal discharge in the mice but no peribronchial lymphocytic infiltration in either.

Clinical Signs and Pathology

The incidence of CMP in rat colonies varies greatly. The data from reported surveys are summarized in Table 1.

Nelson (1955) and Innes et al. (1956) observed that most diseased rats exhibited few if any clinical signs, although in advanced stages roughened hair coat and loss of weight were obvious. Labored and audible breathing did not usually appear until the disease was quite advanced. Newberne (1961) noted that the disease was progressive to death, although Nelson (1955) reported that death rarely occurred before rats were 10 or 12 months old.

Morphologic descriptions of the lesions of CMP have been made by Hektoen (1915-1916), Moise and Smith (1928-1929), Passey et al. (1936), Saxton and Kimball (1941), Cruickshank (1948), Innes et al. (1956), Newberne et al. (1961), Gray (1963) and Innes et al. (1967). The many descriptions vary only in minor details or use of terminology, so a composite account of the lesions of CMP will be presented.

Innes et al. (1956) reported that the gross lesions of CMP were variable and may even be absent. When present the lesions may be discrete

Table 1. Incidence of chronic murine pneumonia in rat colonies

No. of Rats Examined	Ages	Percentage Affected	Reference
251	Not given	51	Passey et al., 1936
487	700 days	75	Wilens and Sproul, 1938
		(36.9)	
228	1 month (14)	0	Klieneberger and Steabben,
	1-4 months (84)	10	1940
	4-8 months (71)	31	
	8-12 months (33)	85	
	12-24 months (26)	100	
200	Not given	43.5	Cruickshank, 1948
433	All ages	91.4	Innes et al., 1956
500	1 year	27	Newberne et al., 1961
360	1 year	14	Newberne et al., 1961

and affect only a part or all of the lobe of a lung, or they may be disseminated. The involved areas were gray to red, indurated, and somewhat shrunken. The lobe often had an irregular appearance and was rubbery in consistency. The consolidated tissue cut easily and the exposed surface was flat, dry and homogeneous. As animals became older, the bronchi became dilated with inspissated exudate and had peribronchial lymphocytic proliferation. These appeared on the pleural surface as nodular pink to gray protuberances. Gray (1963) reported that the right apical and cardiac lobes and the accessory lobe were the most severely involved. Nelson (1963) reported that in experimentally induced cases of CMP (enzootic bronchiectasis) the 3 right lobes and the accessory lobe were more commonly involved, while in experimentally induced infectious catarrh, the left pulmonary lobe became involved most. He thought this variation in response to be of diagnostic value. Newberne et al. (1961) found that the right cardiac lobe was not often involved. Innes et al. (1956), in a study of naturally occurring cases, found no significant difference in the tendency of different lobes to be involved.

The earliest change to be observed histologically in the lungs was lymphocytic infiltration around bronchi and blood vessels (Innes et al., 1956). The lymphocytic tissue encroached upon the bronchial epithelium causing it to be attenuated in size (Newberne et al., 1961). There were patchy areas of interstitial pneumonitis with increased numbers of eosinophils and alveolar macrophages (Innes et al., 1956; Newberne et al., 1961). There was slight atelectasis around the bronchi. Innes et al. (1956) stated that bronchopneumonia was rare, but occasionally a picture simulating bronchopneumonia was produced by the combination of atelectasis and interstitial pneumonia.

A mucopurulent exudate appeared in the bronchi. The inflammation often produced a squamous metaplasia of the bronchial epithelium (Newberne et al., 1961). Passey et al. (1936) reported that in some cases this had been mistaken for neoplastic transformation. The exudate became caseous in appearance, as it became inspissated. The lymphocytic tissue around the bronchi extended peripherally to eventually surround even the bronchioles. This peribronchial and peribronchiolar zone of inflammation involved the smooth muscle, and it became somewhat attenuated, being replaced by fibrous tissue. There was atelectasis of the alveoli around the bronchi (Gray, 1963).

In the late stages of the disease, there was marked bronchiectasis with peribronchial atelectasis. In some instances the atelectatic alveoli had the appearance of fibrous tissue encapsulating the caseopurulent debris in the bronchial lumen. Ventura and Domardzki (1967), in their study of bronchiectasis due to experimental bronchial ligation, reported that the peribronchial elastic fibers became irregular, distorted and even absent after bronchial ligation. Atrophy and fibrosis of the peribronchial smooth muscle also occurred. The bronchial epithelium became desquamated, attenuated, underwent squamous metaplastic changes, or developed a hyperplastic papillary appearance (Newberne, 1961). Several theories have been proposed to explain the development of bronchiectasis:

1. Moise and Smith (1928) proposed that bronchiectasis was due to the plugging of the bronchi by mucus followed by the growth of microorganisms. Klieneberger and Cheng (1955) and Ventura and Domardzki (1967) found that when the bronchi were ligated, there was rapid invasion of the obstructed lobes by mycoplasmas.

2. Passey et al. (1936) suggested that lack of exercise might act as a predisposing factor because the animal was deprived of the opportunity for the full use and expansion of its lungs. This might result in the stagnation of normal mucous secretion which would eventually become infected.

3. Cruickshank (1948) suggested that the bronchial obstruction was due to large polypoid lymphocytic infiltrations circumscribing and constricting the bronchial lumen. He believed that mucous secretion was the result, not the cause, of this obstruction.

4. Gray (1963) stressed the loss of the musculoelastic membrane by inflammation as well as deciliation of the bronchial epithelium and hyperplasia of goblet cells. This allowed mucus to accumulate in the bronchi. Gray, in contrast to most workers, could find no correlation between the degree of peribronchial lymphocytic infiltration and the development of bronchiectasis. He noted that in some rats, extensive peribronchial lymphocytic cuffing was the only lesion, while in others there was too little infiltration to account for the extensive pneumonic involvement present. Innes et al. (1956) believed that germfree rats were free of pulmonary lymphoid tissue and that lymphocytic hyperplasia was the initial response of the lung to the virus described by Nelson (1946).

Innes et al. (1956) noted that there was lymphocytic infiltration of the alveolar walls. Foamy alveolar macrophages were in abundance, often clustered together and filling alveolar spaces. Gray (1963), in contrast, found that the major change in the alveoli was the mucopurulent filling of the air spaces.

Lemon (1967) observed, in a longevity study of CMP-affected and normal rats, that occasionally the large peribronchial lymphocytic aggregates appeared to show a lymphosarcomatous transformation that was often multifocal. In rats free of CMP, there were very few such lesions.

In 1967 Tucker and Wyatt reported the frequent presence of birefringent particles and fibers in the alveolar macrophages and bronchial and alveolar spaces of CMP-affected rats. They were able to produce these same substances by scratching a cube of rat feed with a pointed instrument. They demonstrated by Ouchterlony gel diffusion plates that serum from CMP-affected rats formed a precipitation band against coconut meal present in the cubed rat feed. They concluded that the rats had inhaled particles from the rat pellets and were forming antibodies against the proteins in the particles. They postulated that there may be an immunologic basis to CMP similar to bagassosis and other pneumoconioses.

Disease Control

Haberman et al. (1963) reported that sulfamerazine given daily in the feed at the rate of 5.0 mg. per 20 Gm. of feed eliminated rhinitis, otitis media, and bronchiectasis in the third through the tenth generation of rats, although almost all of the control rats had lesions. Chlortetracycline offered at the same concentration as sulfamerazine had no effect on the incidence of lesions. Haberman et al. (1963) were unable to transmit CMP to mice by intranasal inoculation of lung suspensions from treated rats.

Gray (1963) fed a newly developed and unidentified sulfonamide to rats at varying concentrations for periods up to 1 year. No significant difference was observed in the incidence of CMP in control and in treated groups.

Nelson (1951) obtained axenic rats from the Lobund Institute of the University of Notre Dame and used them as breeding stock to start a colony which was maintained in strict isolation. He was able to produce rats free of CMP in this colony. Innes et al. (1957) reported that rats from this colony were used to start other colonies, also free of CMP. Foster (1958) had identical results in starting a colony with cesarean-derived offspring foster-suckled by axenic mothers. A careful system of barriers against reinfection by airborne or other routes was described.

Other Viral Pneumonias and Similar Conditions

In 1945 Andrewes and Glover reported the isolation of a pneumotropic virus pathogenic for mice. The virus persisted for long periods in the lungs of infected mice. It was infectious in high dilutions, was relatively stable, and would pass a membrane with an average pore diameter of 450 mμ. When inoculated into mice the predominant reaction in the first 10 days was an exudation of neutrophils and proteinaceous fluid. The lungs had a gray color, and they called the disease "grey lung disease". The neutrophils were soon replaced by alveolar macrophages and perivascular and peribronchial cuffs of lymphocytes. These features persisted, although not necessarily unchanged, throughout the life of the mouse (Niven, 1950).

Laboratory rats, when inoculated with mouse lung suspension, had pulmonary lesions only rarely, although the virus could be passed in them up to 20 times (Andrewes and Glover, 1945). In cotton rats, however, inoculation produced a similar response to that seen in mice (Niven, 1950). Andrewes and Niven (1950) observed that chlortetracycline injections completely suppressed the development of pneumonia in inoculated mice.

In 1957 Vrolijk et al. described an agent which they isolated from wild and laboratory rats with lung lesions resembling those of grey lung disease. They were able to detect the agent by mouse inoculation. Electronmicrographs of partially purified suspensions showed virus-like particles with a size range of 200 to 250 mμ.

A pathogenic agent was recovered from the lungs of several wild rats by Nelson (1949a), who called the disease wild rat pneumonia. It was later demonstrated in a few naturally infected albino rats (Nelson, 1967). It was transmitted to mice by nasal instillation and regularly produced pneumonia. Deaths occurred 7 to 14 days after inoculation with mortality rates up to 58%. The lungs of infected mice had pink to red, patchy, smooth areas of consolidation with marked edema. The histologic reaction was largely limited to the alveoli and their walls with little or no bronchial involvement. Lymphocytes and large mononuclear cells predominated, but neutrophils were usually inconspicuous. Nasal injections in albino rats resulted in a similar but less acute pneumonia.

The causal agent was not cultivable in vitro or demonstrable by light microscopy. It was resistant to penicillin and streptomycin and could pass through a Berkefeld V filter. The agent was regarded as a pneumotropic virus probably somewhat below the size range of elementary bodies (Nelson, 1949b).

Nelson (1967) stated that the agents of grey lung disease and wild rat pneumonia were so similar that they should probably be regarded as strains of a single virus.

Marmion and Hers (1963) noted certain similarities between the grey lung virus (wild rat pneumonia virus) and Mycoplasma pneumoniae (the Eaton agent) and suggested that consideration of a mycoplasmal rather than a viral cause of grey lung disease might be rewarding. Nelson (1967) continued, however, to view the causal agent as a virus.

Gay and Attridge (1967) studied the fine structure of an agent which resembled the grey lung virus in its sensitivity to tetracyclines. The agent was observed in the alveolar macrophages of mice which had been inoculated with lung suspensions from rats with CMP. The organisms bore strong resemblance to those in pneumonia of gnotobiotic mice caused by infection with Mycoplasma pulmonis (Lutsky and Organick, 1966; Organick et al., 1966). Gay (1967) compared grey lung disease in intranasally inoculated mice with pneumonia caused by the "rat pneumonia agent". The 2 were identical in all respects. There was peribronchial lymphocytic cuffing, and large numbers of alveolar macrophages were present in the alveoli and bronchioles. Both agents were susceptible to tetracyclines but not to penicillin, streptomycin, chloramphenicols, or sulfonamides. Both agents were identical morphologically. They consisted of coccobacilli 0.3 to 0.6 μ in diameter lying free in the alveolar spaces or attached to the surface of alveolar macrophages. On electron-microscopic examination the organisms were oval-shaped structures. The mean dimensions for the rat pneumonia agent were 533 x 160 mμ and for the grey lung virus, 520 x 143 mμ. They were bounded by a 110 Å unit membrane which was separated from the internal material by a clear space

80 to 90 Å wide. This clear space, and the failure of the agents to grow on mycoplasma media, were the main factors which prevented them from being identified as mycoplasmas. Gay considered the 2 agents to be identical and referred to them as "mycoplasma-like agents".

In 1962 Wenzel reported the occurrence of a spontaneous bronchial and interstitial pneumonia in laboratory rats. Particles measuring 0.3 to 0.4 mμ in diameter were present in the cytoplasm of tracheal and bronchial epithelium. He regarded them as elementary bodies probably of the psittacosis-lymphogranuloma type. Nelson (1967) implied that Wenzel did not satisfactorily rule out the possible association of mycoplasmas.

Pneumonia Due to Infection With Corynebacterium kutscheri

A disease caused by infection with Corynebacterium kutscheri, known also as pseudotuberculosis (Kutscher, 1894; Bongert, 1901; LeMaistre and Thompsett, 1952) and Corynebacterium pseudotuberculosis (Fauve et al., 1964; Pierce-Chase et al., 1964) was first described in 1894 by Kutscher. He isolated the etiologic agent C. kutscheri, also known as Bacillus pseudotuberculosis murium (Kutscher, 1894), Corynethrix pseudotuberculosis murium (Bongert, 1901) and Corynebacterium murium (Topley and Wilson, 1964) from caseous tissue in the lung of a mouse. The pathogenicity for the mouse was described by Kutscher (1897), Bongert (1901), Lawrence (1957), Bicks (1957), and Fauve et al. (1966). The disease was usually inapparent (Tuffery and Innes, 1963). Existence of the infection was often discovered by finding a few caseopurulent foci in the lungs or lymph nodes of an otherwise normal mouse (Dingle, 1956). Fauve (1964) and Pierce-Chase et al. (1964) described a considerable variation in the susceptibility of different strains of mice to C.

kutscheri infection. Mild epizootics may take place in laboratory stocks of mice, especially if there be other resistance-lowering conditions such as cortisone injection (LeMaistre and Thompsett, 1952), irradiation (Schechmeister and Adler, 1953), or other infections (Wolff, 1950; Lawrence, 1957).

Outbreaks of the disease in colonies of rats have usually been reported only when there were other resistance-lowering factors such as cortisone injections (LeMaistre and Thompsett, 1952) or vitamin deficiencies (Gundel et al., 1932; Seronde, 1954, 1956; Zucker and Zucker, 1954; Zucker et al., 1956). Attempts to reproduce the disease experimentally in normal rats have been successful (LeMaistre and Thompsett, 1952; Vallee and Levaditi, 1957), unsuccessful (Kutscher, 1897; Bongert, 1901), or partially successful in that focal lesions developed but tended to heal (Seronde, 1954). Only when rats were injected with cortisone (LeMaistre and Thompsett, 1952) or fed vitamin-deficient diets (Seronde, 1954) could progressive and rapidly fatal infections be induced. Althought there are reports in which spontaneous lesions have been reported in rats (Vallee and Levaditi, 1957; Tuffery and Innes, 1963; Pestana de Castro et al., 1965), it has been stated that the rat is not susceptible to infection with C. kutscheri (Kutscher, 1897; Bongert, 1901; Dingle, 1956), especially if fed a complete diet (Seronde, 1954).

In 1968, however, Giddens et al. described a spontaneous outbreak of pneumonia due to infection with C. kutscheri in a commercial rat colony. Clinical signs were emaciation, hyperpnea, nasal and ocular encrustations, humping of the back, sluggishness, and roughness of the hair coat. Caseopurulent foci, 1 to 15 mm. in diameter, were observed primarily in the lungs and occasionally in the liver, kidneys and

subcutis. Corynebacterium kutscheri was consistently isolated from organs with lesions. Typical lesions were experimentally reproduced in some, but not all, rats inoculated with cultures of C. kutscheri by the intranasal, intraperitoneal, and oral routes.

Inapparent infections are a serious problem, for they can manifest themselves when animals are experimentally stressed. Tuffery and Innes (1963) have suggested radiation or cortisone injections as ways to unmask these inapparent infections in establishing a new colony. Weisbroth and Scher (1967) have described a close correlation between serologic antibody titers against C. kutscheri and the presence of lesions at necropsy in a colony of mice with C. kutscheri infection.

Pneumonias of Miscellaneous Etiology

Innes et al. (1956) and Nelson (cited by Innes et al., 1967) have emphasized the rarity of acute bacterial pneumonias in rats. Innes et al. (1956) described an acute epizootic characterized by fatal bronchopneumonia, myocarditis, and hepatic necrosis. Pure cultures of Pasteurella multocida were isolated from lesions, but attempts to reproduce the disease experimentally in young rats were not successful.

Wheater (1962) described the occurrence, in an SPF rat colony, of widespread infection of the trachea, and occasionally the lungs, with P. pneumotropica. Microscopic lesions in the trachea associated with the organism were slight and consisted of subepithelial infiltration with neutrophils, lymphocytes and plasma cells.

Glaser and Wood (1951) described the experimental reproduction of streptococcal pneumonia. Group A-hemolytic streptococci were inoculated intrabronchially into rats, and an acute pneumonia of lobar distribution

developed. The mortality rate was over 50%, and survivors had a high incidence of pulmonary abscesses.

Gunn and Nungester (1936) described the experimental reproduction of diplococcal pneumonia in rats. Some of their rats had CMP, and they noted that these were refractory to experimental infections. Nungester et al. (1955), in a study of the response of guinea pigs and rats to infection with D. pneumoniae or B. anthracis, observed that in guinea pigs there was neutrophilic infiltration without liquefaction, while in rats small abscesses formed very readily.

Ford (1965) described 2 natural outbreaks of pneumonia in Long-Evans rats caused by infection with Diplococcus pneumoniae type 8. Histologically there was an acute to subacute purulent bronchopneumonia. Ford emphasized the importance of proper diagnosis in differentiating this condition from CMP.

Hoskins and Stout (1919) and Smith et al. (1930) reported the isolation of Bordetella bronchiseptica from rats with CMP. Pankevicus et al. (1957) isolated the same organism from both CMP-affected and normal rats. Winsser (1960) experimentally inoculated rats and mice with cultures of B. bronchiseptica and produced acute to subacute bronchopneumonia, resulting in the death of some mice.

Linhartuva (1956) reported that of 20 rats treated with cortisone, 13 developed a pneumonia caused by Pneumocystis carinii. He was unable to transmit it to untreated rats and concluded that there was a latent infection which was unmasked by injections of cortisone.

Innes et al. (1967) described the frequent occurrence of granulomas in the lungs of otherwise normal older rats. Identifiable organisms or matter could not usually be found. They suggested, as did Fricsay

(1956), that these might be due to migrating larvae of Trichosomoides crassicauda, which inhabits the urinary bladder.

Innes et al. (1956, 1958) noted the frequent occurrence of spicules of bone in the alveoli of rats. The bone contained no vessels, Haversian systems, or periosteum, and it was concluded that they represented a fortuitous aspiration of the powdered dust of the food pellets and were particles from the fish-bone meal used in making the pelleted diet. There was no inflammatory reaction around the particles.

Engel and Grüneberg (1960) described a hereditary congenital disease in rats related to a recessive lethal gene. The condition was characterized by hypertrophy of the thoracic cartilages and rigidity of the thorax. Bronchi did not lengthen, and alveoli did not form. Lung differentiation was severely impaired.

Summary

It is evident that in spite of the excellent and persistent work of Nelson at the Rockefeller Institute, Klieneberger-Nobel at the Lister Institute, and the contributions of dozens of other workers around the world, chronic respiratory disease in rats is incompletely understood. It is still a serious problem and often complicates routine experimental work. It seriously limits the value of the rat for long-term studies. Much research is still needed to define the nature and interrelations of the various factors that cause respiratory diseases in the rat.

MATERIALS AND METHODS

Objectives. The specific objectives of this research were to study the morphologic and microbiologic aspects of the respiratory tract of germfree, defined flora, conventional, and CMP-affected rats in order to:

1. Define the variation in morphology of the nasal cavity, middle ear, trachea and lungs of rats raised under the widely differing environments of these colonies, and to describe the pathogenesis of the lesions.

2. Isolate and identify bacteria and viruses and to relate these to the lesions present.

3. To experimentally reproduce and describe CMP in rats and mice by aerosol inoculation of tracheal and lung suspensions of each of the CMP-affected groups.

Source of Animals. Rats were obtained according to the schedule in Table 2. The germfree rats were of the Sprague-Dawley strain* and were from cesarean-derived, isolator-maintained stock. They were germfree on the basis of the standards established by Wagner (1956). The defined flora rats were from the same colony and stock as the axenic rats but were maintained in isolators to which organisms described in 1965 by Schaedler et al. for mice (2 strains of lactobacilli, 1 strain of

* The A. R. Schmidt Co., Inc., 2826 Latham Drive, Madison, Wisc.

Table 2. Origin of rats used for morphologic and microbiologic studies

	Morphologic Study						Microbiologic Study
	Weanling Rats		Adult Rats		Aged Rats		
	Age	No. & Sex	Age	No. & Sex	Age	No. & Sex	No. & Age
Germfree	28 days	5M,5F	8 weeks	5M,5F	12. mo.	4M,3F	2 aged
Defined flora	21-28 days	10M	8 weeks	4M,5F	12 mo.	5M,5F	10 aged
Conventional	21 days	8M,2F	8 weeks	8M,2F	7 mo.	1M,9F	5 adults 5 aged
CMP-affected (CMP-1)	21 days	8M,2F	8-9 weeks	5M,5F	16 mo.	5M,5F	10 adults
CMP-affected (CMP-2)					11 mo.	4M,6F	9 aged
CMP-affected (CMP-3)	21 days	7M,3F			12 mo.	10F	10 aged
TOTAL	21-28 days	50	8-9 weeks	39	7-16 mo.	57	51 (2-16 mo.)

M = male
F = female

anaerobic streptococcus Group N, 2 strains of bacterioides, and 1 coliform (SLF) strain) had been added. In addition, a micrococcus had contaminated the isolators. Except for the addition of these organisms, the rats were raised in isolators under the same conditions as the germfree rats.

The conventional rats originated from the same colony and stock. They were started by transferring rats from the defined flora isolators to rooms which had been cleaned and sterilized with peracetic acid spray. These "subcolonies" were usually maintained for about 1 year, then destroyed, the rooms disinfected, and new stock added. Each room was supplied with filtered air under positive pressure, and the rats in it were cared for by an attendant who had contact with those rats only. All feed was autoclaved before being used.

One of the CMP-affected colonies (CMP-1)* was established in 1932 from rats of the Wistar strain. It had been continuously maintained as a closed colony. Rats were used for testing the toxicity of chemicals. The colony had a history of widespread CMP, and previous histologic examination of rats in our laboratory about 6 months prior to this work had confirmed the presence of typical lesions.

The second CMP-affected colony (CMP-2)** was of the Sprague-Dawley strain. It was a commercial colony which sold rats to academic and industrial laboratories. It had been believed affected with CMP for many years.

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* Biochemical Research Div., The Dow Chemical Co., Midland, Mich.

** Rawley Farms, 45805 West Ann Arbor Road, Plymouth, Mich.

The third CMP-affected group (CMP-3)* was from 2 rooms each containing approximately 500 breeding rats. The rooms were production rooms in the same commercial colony from which the axenic, defined flora, and conventional rats were obtained. The rooms had been disinfected and repopulated with defined flora rats approximately 1 year prior to the time this study was made. About 4 to 5 months later, widespread CMP was noticed in the older rats.

Microbiologic Methods. From 2 to 10 rats were selected from each colony and euthanatized with ether. The skin was reflected from a single mid-ventral incision and the exposed muscle tissue was flamed with a gas burner. The abdominal and ventral thoracic walls were removed, and 1 lung and approximately 1 cm. of the trachea were removed, using separate heat-sterilized scissors and forceps for each procedure. The lung and tracheal tissues were placed in sterile jars. The skin and lower jaw were removed from the decapitated head, which was then flamed with a bunsen burner. Separate sterile capillary pipettes were used to infuse approximately 5 ml. of brain-heart infusion broth into the tympanic cavity on both sides of the head and into the nasal cavity. These were then aspirated and infused into sterile tubes containing cotton swabs. The samples were frozen at -20 C. until ready for culturing. Some samples were split and cultured before and after freezing to determine the effects of freezing on the viability of the organisms.

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* The A. R. Schmidt Co., Inc., 2826 Latham Drive, Madison, Wisc.

The lungs and trachea were separately ground in Ten Broeck grinders using Hank's Balanced Salt Solution (BSS) at a dilution of approximately 1:5. The samples were inoculated according to the schedule in Table 3.

The blood agar consisted of 7% bovine red blood cells in tryptose agar.* Brain-heart infusion (BHI) semisolid was prepared from standard dehydrated media* to which was added 0.15% agar. The streptobacillus medium was either 20% horse serum in BHI agar (as recommended by Williams et al., 1967) or 10% bovine red blood cells in tryptose agar.* The latter was prepared and after inoculation was incubated in 5% CO_2 at 37 C. according to Robinson (1963).

Rat cell lines were started with tissues from 15- to 20-day-old fetuses from Sprague-Dawley females. These females came from a colony started in 1962 with cesarean-derived offspring foster-suckled on germ-free mothers. Periodic histologic examination of aged rats from this colony indicated an absence of peribronchial lymphocytic cuffing or bronchiectasis.

The female rats were anesthetized with ether, and the fetuses were aseptically removed and placed in petri dishes. Skin, kidneys, and lungs were removed and placed in separate beakers containing balanced salt solution (BSS) with 200 units of penicillin, 200 mcg. of streptomycin, and 50 units of polymyxin B per ml. The tissues were minced and washed twice in the above solution. They were then incubated in 0.25% trypsin in BSS at 20 C. for 30 minutes. The supernatant was discarded, fresh trypsin added, and the suspension incubated at 5 C. for 15 hours. Next the cells were washed twice in BSS. In the final dilution, 1 ml. of

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* Difco Laboratories, Detroit, Mich.

Table 3. Inoculation procedure for microbiologic samples

System Inoculated / Source of Sample	Bacterial Media				Cell Cultures					Animal Inoculations	
	Blood Agar	PPLO Agar	Strepto-bacillus Medium	BHI Semi-solid	Rat Embryonic Skin Cells	Rat Embryonic Kidney Cells	Rat Embryonic Lung Cells	HeLa Cells	Human Epithelial Carcinoma (HEP-2) Cells	Mouse (two passages)	Rat (two passages)
Nasal washings	+	+	+	+							
Tympanic cavity washings	+	+	+	+							
Tracheal suspension	+	+	+	+	+	+	+	+	+		
Lung suspension	+	+	+	+	+	+	+	+	+		
Pooled tracheal and lung suspensions	+	+	+	+	+	+	+	+	+	+	+

packed cells was suspended in 200 ml. of the growth medium, which was Medium 199* with 10% lamb serum and the antibiotics previously described. This suspension was distributed into glass screw-cap tubes (1.5 ml. each) and milk dilution bottles** (12 ml. each). Tubes were incubated in slant racks at 37 C. Monolayers were produced in 3 days with lung and skin cells and in 5 days with kidney cells. Lung and skin cell cultures could usually be subcultured every 7 to 10 days. Kidney cells did not grow well after the first or second passage, so primary cells were usually used.

HeLa cells and HEP-2 cells (Human Epithelial Carcinoma Type Two) were obtained from the Michigan Department of Public Health. Hank's BSS with 5% lamb serum, 0.5% lactalbumin hydrolysate,*** and 0.05% yeast extract*** (with the antibiotic concentrations described above) was the growth medium. Monolayers were produced in 3 days and subcultured every 7 to 10 days.

The maintenance medium for all cell cultures was EBME* with 2% lamb serum and the antibiotics previously described.

For subculturing, a mixture of 0.25% trypsin and 0.06% ethylene-diamine-tetra-acetic acid (EDTA) in BSS (Madin and Darby, 1958) was used. The monolayers were rinsed once and then incubated at 37 C. for 5 to 10 minutes with the trypsin-EDTA solution. The suspension was centrifuged for 10 minutes at 600 rpm. The supernatant was poured off and the packed cells were reseeded into previously described growth media at a ratio of

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* Grand Island Biological Co., Grand Island, N.Y.

** No. 2-943, Fisher Scientific Co., Detroit, Mich.

*** Difco Laboratories, Detroit, Mich.

1:3 and then distributed into tubes and bottles as previously described.

Tracheal and lung suspensions were pooled from each rat to be sampled, and an antibiotic concentration 5 times that previously described was added. Then, 0.2 ml. of each suspension was inoculated, according to the schedule outlined in Table 3, into 2 cell culture tubes after monolayers were established. Occasionally Kanamycin sulfate* at 200 mcg./ml. was added when the presence of mycoplasmas was suspected in the tissue. The cultures were examined at 2-day intervals after inoculation. Two series of rat embryonic skin cells were inoculated and 1 series was removed from the incubator at 4 to 5 days after inoculation and incubated for 4 to 5 days at room temperature, after the method described by Joshi et al. (1964). Cell cultures were incubated for 7 to 10 days after inoculation. One serial passage was made before samples were considered negative for cytopathic changes. Occasionally, cell cultures were grown on coverslips, which were then removed at selected intervals for histologic examination. They were fixed in Bouin's fixative and stained with the May-Grunwald-Giemsa stain using procedures described in the Manual of Histologic and Special Staining Technics of the Armed Forces Institute of Pathology, Washington, D.C. (1957).

The tracheal and lung suspensions from all rats of a single source were pooled. Samples of these pooled suspensions were inoculated into media according to the schedule in Table 3. The remaining suspension was used to inoculate male rats and mice, 21 to 28 days old, which were obtained from stocks free of the lesions of CMP. Approximately 5 of each were placed in chemically sterilized, transparent plastic cages covered

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* Kantrex, Bristol Laboratories, Syracuse, N.Y.

with filter caps of compressed fiberglass.* The volume within the covered cage was approximately 0.97 cubic feet. From 2 to 4 ml. of the trachea-lung suspension were extended to 10 ml. with distilled water. This extended suspension was sprayed into the covered cage with a nebulizer,** using compressed nitrogen gas at 15 pounds per square inch. This nebulizer produces infectious aerosols in which the majority of the particles are 1 to 5μ in diameter (Kundsin, 1968). It took 3 to 4 minutes to nebulize the extended trachea-lung suspension. The animals were left in the chamber a total of 10 minutes. The rats and mice were then separated and housed in cages covered with compressed fiberglass caps. Approximately 5 rats and 5 mice were housed in a separate room under identical conditions as controls.

At 4 to 5 weeks after inoculation, all rats and mice were euthanatized with ether, and gross and microscopic observations of the nasal cavity, middle ear, trachea and lungs were conducted. Pooled samples of lung tissue from the rats and mice were inoculated into bacterial and tissue culture media according to the schedule in Table 3. A second series of rats and mice was inoculated with the pooled suspension of lungs from the inoculated animals from the first series. This second series was maintained 4 to 5 weeks, then euthanatized, and examined in the manner described above.

Gross and Microscopic Examination of Tissues. Rats were euthanatized with ether. The trachea and lungs were exposed, and the trachea was infused with fixative until the lungs filled approximately 3/4 of the

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* Lab Cages, Inc., Hackensac, N.J.

** Model 40 Nebulizer, The DeVilbiss Co., Toledo, Ohio

thoracic cavity. Tissues were fixed in 10% buffered formalin, glutaraldehyde, or Bouin's fixative. Two transverse nasal sections were made, at 1/2 and at 3/4 of the distance from the external nares to the medial canthi. Two transverse sections of the middle ear were made, directly through the external ears and tympanic membranes, and at a point approximately 5 mm. rostral to this site. Two transverse sections of the trachea were made. Three transverse sections of the lungs were made: the first included the right apical and right cardiac lobes and the left lung at a plane just caudal to the heart; the second and third sections were approximately 5 and 10 mm., respectively, caudal to the first. In some rats parts of the lungs were removed for microbiologic examination.

The nasal and middle ear sections were cut after the fixed head, with the lower jaw and skin removed, had been soaked in a decalcifying solution* for 24 to 36 hours. All tissues were washed in tap water, dehydrated in ethyl alcohol, cleared in xylene, and embedded in paraffin. Sections were generally cut at 7μ (some nasal and middle ear sections were cut at 10μ) and stained with hematoxylin and eosin for general observation. Gomori's trichrome stain for collagen and smooth muscle, Wilder's reticulum stain for reticular fibers, Verhoeff's elastic stain for elastin, the Giemsa stain for mycoplasma and elementary bodies, and the Gram stain for bacteria were occasionally employed, using procedures described in the Manual of Histologic and Special Staining Technics of the Armed Forces Institute of Pathology, Washington, D.C. (1957).

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* Cal-Ex, Fisher Scientific Co., Fair Lawn, N.J.

Quantitative Histologic Techniques. Three transverse sections of lung from each rat were examined to determine the amount and distribution of pulmonary lymphocytic tissue. Each bronchus and bronchiole was classified into groups according to the number of lymphoreticular cells (primarily lymphocytes with occasional plasma cells and reticular cells) in peribronchial and peribronchiolar locations, using the scheme in Table 4. The classifications were:

slight (0-5 lymphocytes)
mild (6-15)
moderate (16-50)
severe ($>$ 50)

The total number of bronchi and bronchioles in each group was multiplied by the natural logarithm of the approximate median number of that group. Adding these figures and dividing by the total number of classified bronchi and bronchioles yielded the natural logarithm of the average number of lymphocytes per bronchus or bronchiole, hereafter referred to as the peribronchial lymphocytic index (PLI).

The PLI was computed for each rat and each age group from all the colonies sampled. Using the analysis of variance with least significant difference, comparisons were made between rats of similar ages from the different colonies.

Table 4. Computation of the peribronchial lymphocytic index (PLI)

Classification	Number of Peribronchial and Peribronchiolar Lymphocytes	Approximate Median	Natural Logarithm of Median	Number of Bronchi and Bronchioles in Each Classification
Slight	0-5	3	1.10 (M1)	n_1
Mild	6-15	10	2.30 (M2)	n_2
Moderate	16-50	33	3.50 (M3)	n_3
Severe	> 50	100	4.60 (M4)	n_4

Peribronchial lymphocytic index (PLI) = $\frac{\sum (mn)}{\sum n}$

n = number of bronchi and bronchioles in each group

m = natural logarithm of median in each group

RESULTS

A table of the experimental groups and their corresponding accession numbers in the Michigan State University Department of Pathology is presented in Appendix 1. A list correlating the morphologic and microbiologic observations of the nasal cavity and middle ear is presented in Appendix 2, and of the trachea and lungs in Appendix 3. A summary of the more commonly isolated organisms is given in Table 5.

A. The Nasal Cavity and Middle Ear

Germfree and Defined Flora Rats. The more rostral section of the nasal cavity (taken halfway between the medial canthi of the eyes and the end of the nose) included the nasal and maxillary turbinates, the maxillary sinus, and the vomeronasal organ (Figure 1). The ventromedial floor of the cavity is covered by stratified squamous epithelium, which changes at the level of the vomeronasal organ to simple and/or pseudostratified columnar ciliated epithelium (the respiratory epithelium) on the turbinates and lateral walls (Figure 2). On the nasal septum the epithelium is all pseudostratified, columnar and ciliated. This ciliated layer persists on the walls and septum of the nasal cavity except in the most dorsal part, between the septum and the nasal turbinate, where it changes to the olfactory epithelium. The septal epithelium contains more goblet cells and cilia than that of the turbinates or lateral walls. The lamina propria contains numerous large sinuses filled with blood or lymph and lined by endothelium. These lie close to the epithelial basement membrane

Table 5. Microorganisms most commonly isolated from the respiratory tract of defined flora, conventional (Conv.), and 3 chronic murine pneumonia-affected colonies (CMP-1, CMP-2, CMP-3)

	Defined Flora (5)*	Conv. (10)				CMP-1 (10)				CMP-2 (9)				CMP-3 (10)			
	N**	N	E	T	L	N	E	T	L	N	E	T	L	N	E	T	L
Diplococcus pneumoniae						1	3	1	1	3	5	3	3				
Pasteurella pneumotropica						1									2	1	1
Mycoplasma sp.						6	6	4	3					1	1	2	3
Staphylococcus aureus		2								1					1	1	
Micrococcus sp.	3	6	2	3	1	2	2	1		2	2	1		6		1	1
Corynebacterium kutscheri						3					1						

* The number of rats examined is enclosed in parentheses.

** N = nasal cavity; E = middle ear; T = trachea; L = lung.

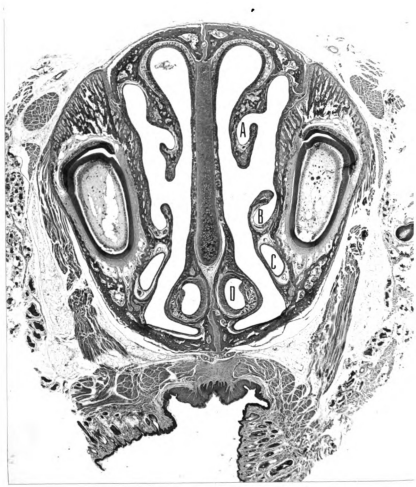

Figure 1. Transverse section of the nasal cavity taken halfway between the medial canthi of the eyes and the tip of the nose. Four-week-old defined flora rat. Note the nasal (A) and maxillary (B) turbinates, the maxillary sinus (C) and the vomeronasal organ (D). H & E stain. x 15.

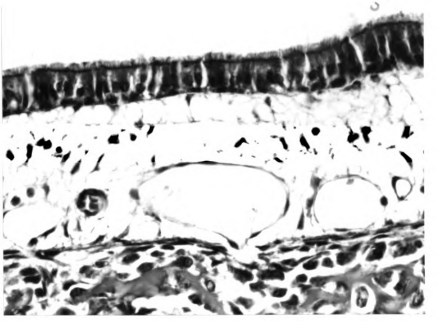

Figure 2. Respiratory epithelium in nasal cavity of 4-week-old germfree rat. Notice subepithelial lymph and blood vessels. H & E stain. x 560.

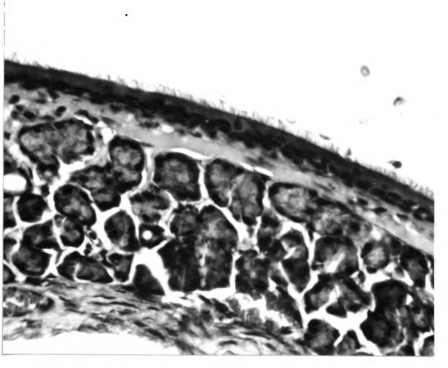

Figure 3. Tubuloalveolar glands of the respiratory submucosa. Nasal cavity of 4-week-old defined flora rat. H & E stain. x 560.

throughout the nasal cavity, being larger and more numerous on the turbinates. Beneath the respiratory epithelium there are numerous tubuloalveolar glands with a homogeneous eosinophilic or amphophilic cytoplasm (Figure 3). Glands are scarce in the olfactory lamina propria at this leve, but numerous nerve fibers are evident. No pigment granules were observed in the olfactory epithelium.

At the level of the second transverse section (3/4 of the distance from tip of nose to the medial canthi), some or all of the following ethmoid turbinates are visible (from dorsal to ventral): endoturbinate 1, ectoturbinate 1, endoturbinate2, ectoturbinate 2, and endoturbinates 3 and 4 (Figure 4). Olfactory epithelium covers all ethmoid turbinates and the wall of the nasal cavity down to the level of endoturbinates 3 and 4. Ventral to this level the medial portions of the endoturbinates 3 and 4 are covered with olfactory epithelium, and the lateral portions and the wall of the nasal cavity are covered with respiratory epithelium. The nasal septum is covered with olfactory epithelium down to its most ventral part where the septum ends abruptly, allowing the 2 choanae to connect. At this tip the septum is lined by stratified squamous epithelium. In the lamina propria of the olfactory epithelium throughout the nasal cavity at this level there are numerous nerve fibers and some glands (Figure 5). The glands are mucoserous and tubular or tubuloalveolar in type. They resemble those found beneath the respiratory epithelium except that the proportion of mucin-producing cells is greater. The maxillary sinus is lined by ciliated columnar epithelium, beneath which are extensive serous tubuloalveolar glands.

The olfactory epithelium is not ciliated but does contain a layer of olfactory hairs 2 to 3μ long which project into the nasal cavity

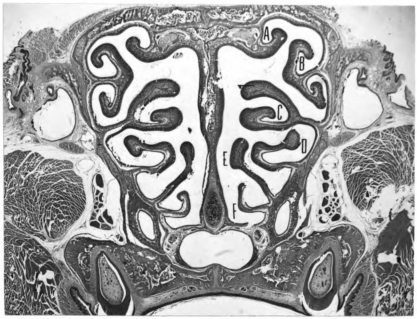

Figure 4. Transverse section of the nasal cavity taken 3/4 of the distance from the tip of the nose to the medial canthi of the eyes. Four-week-old germfree rat. Note the endoturbinate 1 (A), ectoturbinate 1 (B), endoturbinate 2 (C), ectoturbinate 2 (D), endoturbinate 3 (E) and endoturbinate 4 (F). H & E stain. x 7.

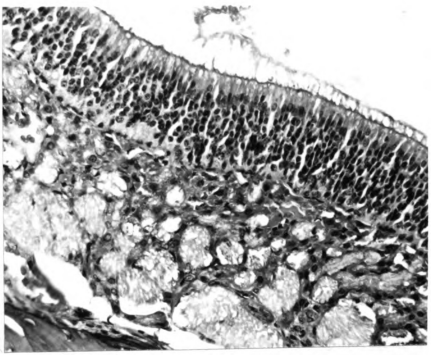

Figure 5. Olfactory epithelium and subepithelial nerves of the nasal cavity of 8-week-old germfree rat. H & E stain. x 350.

out of the ends of the olfactory cells. These olfactory hairs are immersed in a thin film of fluid from 2 to 6 microns in thickness which is probably produced by the olfactory glands. This thin film often detaches from the epithelium in tissue sections (Figure 6).

Subepithelial lymphocytic tissue is scarce in the nasal cavity of germfree and defined flora rats. It is usually present bilaterally beneath the respiratory epithelium of the wall of the posterior nasal cavity. Shortly after the nasal septum opens to connect the choanae, 2 shelflike prolongations of the tip connect with the lateral nasal walls on either side to form the beginning of the nasopharynx. Subepithelial lymphocytic tissue is usually present in the wall of the nasopharynx (Figure 7).

There was approximately twice as much subepithelial lymphocytic tissue in aged rats as in weaned rats. There was no difference in the amount of lymphocytic tissue in germfree and defined flora rats.

In both germfree and defined flora rats there was no evidence of inflammation in the nasal cavity. No difference was observed between the 3 rats from which Micrococcus sp. had been isolated (Appendix 2a), and any others in the germfree or defined flora groups. Occasionally small quantitites of mucoserous fluid were observed around the turbinates.

The 2 transverse sections through the external auditory meatus and a plane 5 mm. rostral to that meatus include the oropharynx, nasopharynx, eustachian tube, tympanic cavity, auditory ossicles, tympanic membrane, and external auditory meatus (Figures 8 and 9). As the nasopharynx proceeds caudally from the nasal cavity it is lined by columnar and pseudostratified columnar ciliated epithelium that contains numerous goblet cells. Large numbers of mucous glands lie ventral to the nasopharynx.

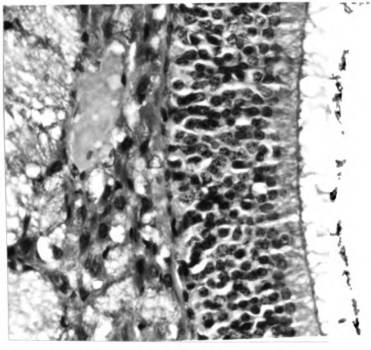

Figure 6. Olfactory mucosa and thin film of fluid which bathes olfactory hairs. Nasal cavity of 8-week-old germfree rat. H & E stain. x 560.

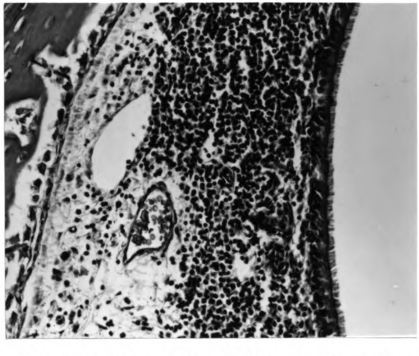

Figure 7. Subepithelial lymphocytic tissue of the nasopharynx. Four-week-old germfree rat. H & E stain. x 350.

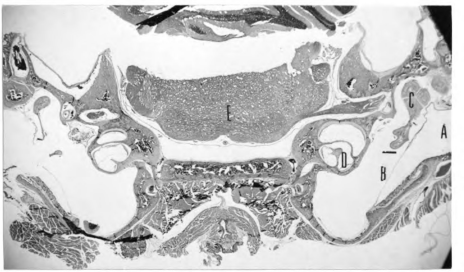

Figure 8. Transverse section through the external auditory meatus (A) and tympanic membrane (B) of 4-week-old defined flora rat. Note auditory ossicles (C), cochlea (D) and pons (E). H & E stain. x 7.

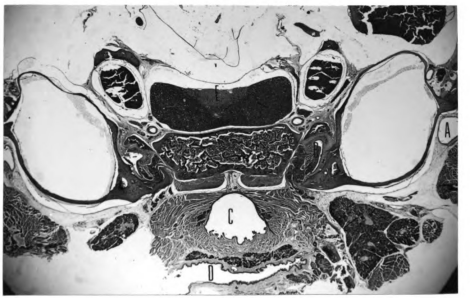

Figure 9. Transverse section approximately 5 mm. rostral to tympanic membrane. Four-week-old defined flora rat. Note tympanic cavity (A), eustachian tube (B), nasopharynx (C), oropharynx (D), and pituitary gland (E). H & E stain. x 7.

They empty into the oropharynx below them (Figure 10). Dorsal and lateral to the nasopharynx are mucoserous glands that empty into it. These glands are clustered around, but mainly dorsal to, the eustachian tubes as they proceed from the walls of the nasopharynx. Caudal to this point, the epithelium lining the ventral part of the nasopharynx changes to stratified squamous type, in preparation for the union with the oropharynx.

The eustachian tube proceeds dorsolaterally from the nasopharynx to the tympanic cavity. Its shape resembles that of an "S" as it curves laterally upon leaving the nasopharynx, then dorsally and caudally around the lateral edge of the occipital bone and then lateroventrally as it enters the tympanic cavity. It is lined throughout by ciliated columnar epithelium which contains numerous goblet cells. As the eustachian tube proceeds dorsally about midway between the nasopharynx and the tympanic cavity, the mucoserous glands, which surround and empty into it and the nasopharynx, are replaced by a collar of hyaline cartilage. This collar accompanies the eustachian tube to where it enters the medial side of the tympanic cavity (Figure 11). The epithelium lining the eustachian tube becomes cuboidal as it nears the tympanic cavity and usually becomes low cuboidal to simple squamous as it enters and lines the cavity. Both goblet cells and cilia are lost as the epithelial cells decrease in height. In some rats ciliated cuboidal epithelium with goblet cells was present along the medial wall of the tympanic cavity around the entrance of the eustachian tube.

The tympanic cavity is lined by low cuboidal to simple squamous epitheoium and beneath it is loose connective tissue with large numbers of thin-walled blood vessels. Beneath this connective tissue is the osseous framework which circumscribes and protects the cavity. The tympanic

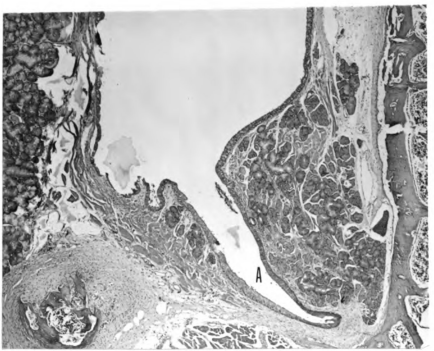

Figure 10. Nasopharynx of 4-week-old defined flora rat. Note eustachian tube (A) and its mucoserous glands. H & E stain. x 56.

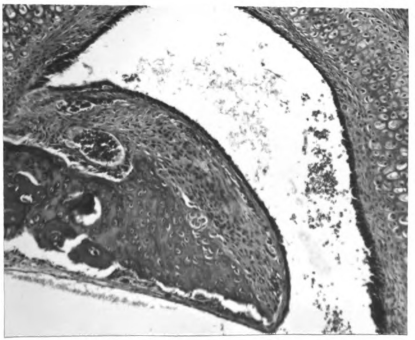

Figure 11. Entrance of eustachian tube into tympanic cavity of 4-week-old germfree rat. Note hyaline cartilage (the blood is a postmortem artefact). H & E stain. x 140.

membrane is on the lateral side. In the caudal part of the cavity, the bones of the middle ear, the malleus, incus and stapes, connect the tympanic cavity to the vestibule of the inner ear (Figure 12). These bones are extremely dense, having little myeloid tissue. The tympanic membrane consists of cutaneous, connective tissue and mucous layers (Figure 13). The mucous layer is a continuation of the epithelial lining of the tympanic cavity and is of simple squamous or low cuboidal type. The middle connective tissue layer appears to be a thin band of collagen. The cutaneous layer is continuous with the stratified squamous epithelium lining the external auditory meatus. It contains no adnexa and is only 1 to 2 cells thick. The entire membrane is 10 to 25μ thick.

Occasionally, a thin mucoserous fluid was observed in the ventral parts of the tympanic cavity and in the eustachian tube. No inflammatory or other cells were present in germfree or defined flora rats. No differences were observed between germfree and defined flora rats.

Conventional Rats. No lesions were observed in the nasal cavity or middle ear of weanling conventional rats. In adult and aged conventional rats an increased amount of mucoserous fluid was occasionally observed in the tympanic cavity and around the nasal turbinates (Figure 14). Increased subepithelial lymphocytic tissue was observed in the nasal cavity (Figure 15) and nasopharynx. In some rats this lymphocytic tissue had infiltrated the respiratory epithelium, and there was a reduction in the height of cells with a corresponding loss of cilia. Goblet cells were increased in number. Mucin had collected in the nasopharynx of 1 aged rat, and scattered neutrophils were present in it. In several aged rats the eustachian tube contained small quantities of mucoserous fluid.

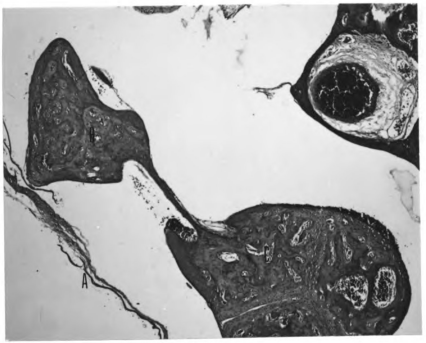

Figure 12. Middle ear of 4-week-old germfree rat. Note tympanic membrane (A), malleus (B) and incus (C). H & E stain. x 140.

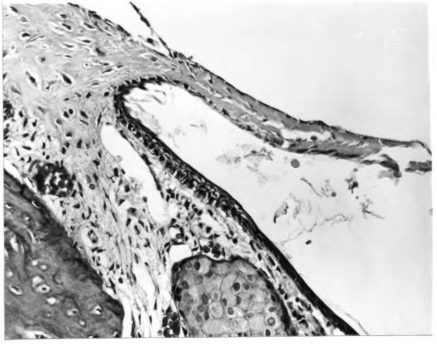

Figure 13. Tympanic membrane of 4-week-old germfree rat. Note stratified squamous layer, connective tissue layer, and tympanic mucosal layer. H & E stain. x 350.

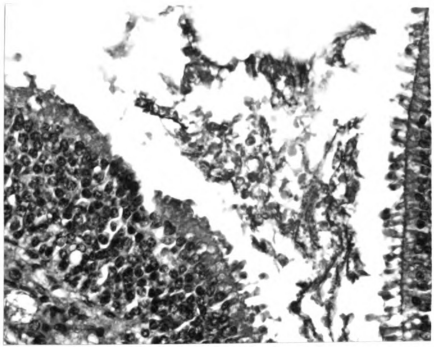

Figure 14. Mucoserous fluid and cellular debris between nasal septum and turbinate of 8-week-old conventional rat. H & E stain. x 560.

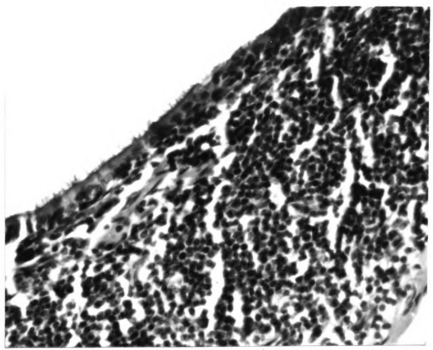

Figure 15. Subepithelial lymphocytic infiltration in the nasal cavity of 1-year-old conventional rat. H & E stain. x 560.

Other than these observations, there was no evidence of rhinitis or otitis media.

CMP-1 Rats. All of the weanling rats from CMP-1 were affected with severe purulent rhinitis and otitis media. At the level of the nasomaxillary turbinates a purulent or mucopurulent exudate collected, particularly in the crevice dorsolateral to the maxillary turbinate and both lateral and medial to the nasal turbinate (Figure 16). Subepithelial lymphocytes were increased in numbers, although they were not usually in definite aggregations. Both lymphocytes and neutrophils had infiltrated the epithelial layers. In many areas cilia were absent, or decreased in numbers, but goblet cells were generally increased in numbers (Figure 17). The ethmoid turbinates were also the site of severe inflammation (Figure 18). Masses of neutrophils were often seen in a serofibrinous or mucous exudate. Destruction of cilia and increased numbers of goblet cells were common. Neutrophilic and lymphocytic infiltration of the turbinate mucosa and submucosa was widespread (Figure 19). Often the epithelial layer seemed to disappear in areas of severe neutrophilic and lymphocytic infiltration. Edema and, occasionally, hemorrhage were present in the mucosa of both nasomaxillary and ethmoid turbinates.

The maxillary sinus often contained neutrophilic exudate in its lumen, with edema and neutrophilic and lymphocytic infiltration in its mucosa and submucosa.

All weanling rats from CMP-1 were affected with bilateral otitis media (Figure 20). The inflammation with neutrophilic accumulation was most severe in the tympanic cavity but often was observed in the eustachian tube and nasopharynx (Figure 21). The mildest lesions consisted

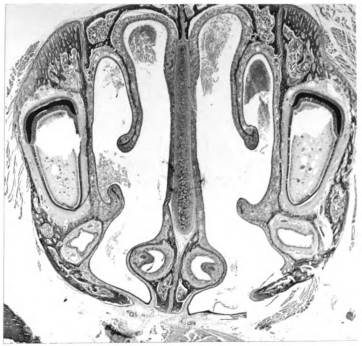

Figure 16. Mucopurulent nasomaxillary rhinitis in 3-week-old CMP-1 rat. H & E stain. x 15.

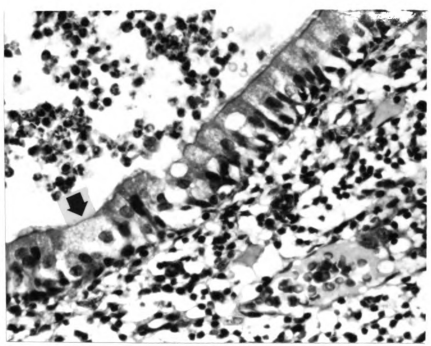

Figure 17. Subepithelial lymphocytic infiltration and neutrophilic exudate in nasal cavity of 3-week-old CMP-1 rat. Note increased number of goblet cells (arrow). H & E stain. x 560.

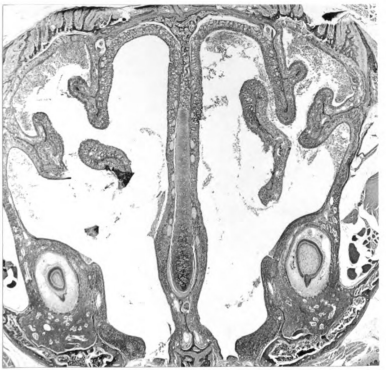

Figure 18. Mucopurulent ethmoid rhinitis in 3-week-old CMP-1 rat. H & E stain. x 15.

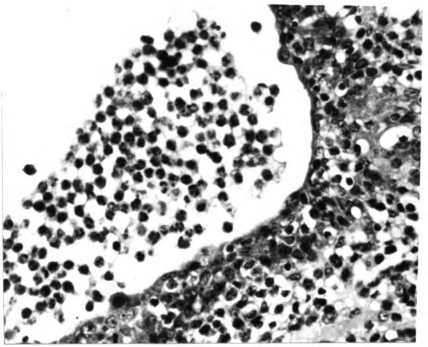

Figure 19. Neutrophilic and lymphocytic infiltration of nasal mucosa in 3-week-old CMP-1 rat. H & E stain. x 350.

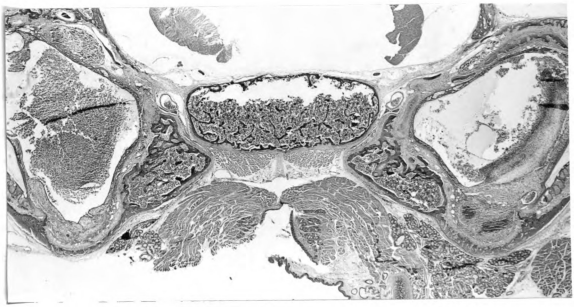

Figure 20. Bilateral purulent otitis media in 3-week-old CMP-1 rat. H & E stain. x 15.

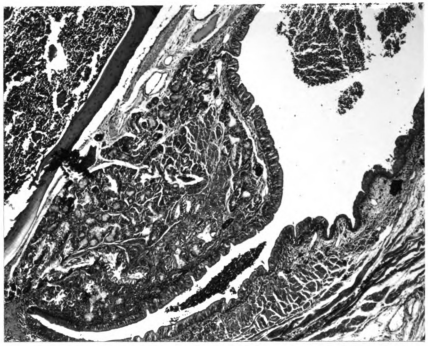

Figure 21. Purulent exudate in eustachian tube and nasopharynx of 3-week-old CMP-1 rat. H & E stain. x 56.

collections of small numbers of neutrophils in the cavity and slight infiltration of the tympanic mucosa. Edema and thickening of the mucosa and metaplasia of the mucosa to a columnar ciliated form with numerous goblet cells were also observed (Figure 22).

More severe involvement was characterized by necrosis of the lining epithelium (Figure 23) and filling of the tympanic cavity with neutrophils. Granulation tissue (Figure 24), arising from the tympanic submucosa, often had proliferated and filled the tympanic cavity. Small cystic glands, lined by cuboidal epithelium and containing mucous or serous fluid, were often seen in the submucosa or surrounded by granulation tissue. They were occasionally connected to the tympanic mucosa, as though they had begun infolding of the latter. The tympanic membrane was greatly thickened on the mucosal side, but no changes occurred on the cutaneous side (Figure 25). The malleus and incus were often surrounded by granulation tissue (Figure 26) or a pool of neutrophils (Figure 27).

In adult and aged rats the lesions were similar but more chronic. The nasal submucosa often contained massive lymphocytic aggregations (Figure 28) that encroached upon the epithelium, which was reduced in size and devoid of cilia. In some places neutrophils had infiltrated into olfactory and other submucosal glands, which were greatly dilated (Figure 29).

In the nasopharynx and eustachian tube the mucosa often appeared stratified, although intercellular bridges and keratin were not seen. Lymphocytic infiltration of the submucosa of the pharynx and eustachian tube was extensive. Purulent exudate was often seen in the eustachian tube and nasopharynx, probably arising from the tympanic cavity.

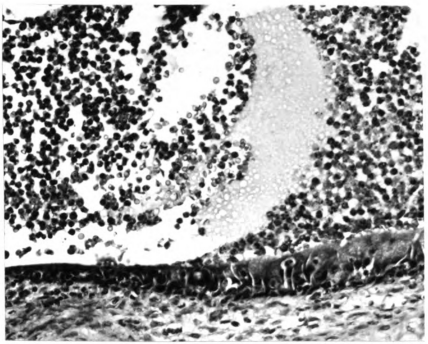

Figure 22. Transformation of epithelium of tympanic mucosa to columnar ciliated form. Purulent otitis media in 3-week-old CMP-1 rat. H & E stain. x 350.

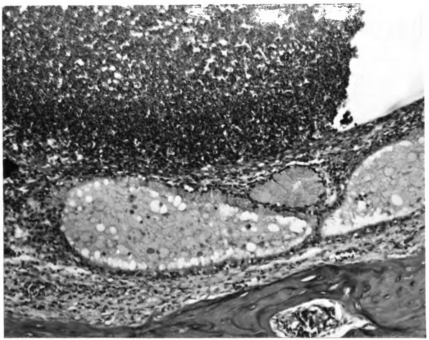

Figure 23. Neutrophilic infiltration and necrosis of tympanic mucosa in 3-week-old CMP-1 rat. H & E stain. x 140.

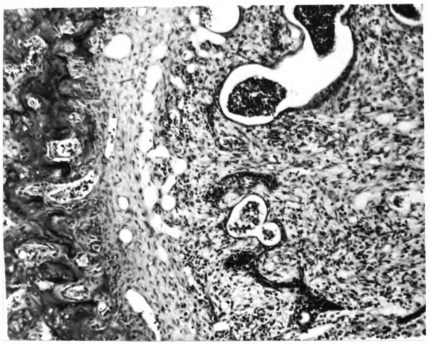

Figure 24. Neutrophilic aggregation in gland-like cysts and proliferation of granulation tissue in otitis media of 3-week-old CMP-1 rat. H & E stain. x 140.

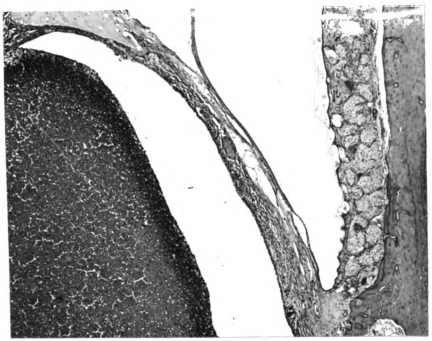

Figure 25. Purulent exudate and inflammatory thickening of tympanic membrane in otitis media of 3-week-old CMP-1 rat. H & E stain. x 56.

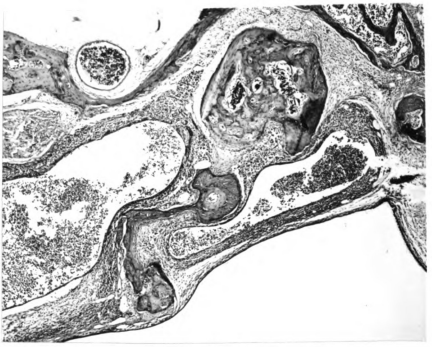

Figure 26. Beginning proliferation of granulation tissue around auditory ossicles in otitis media of 3-week-old CMP-1 rat. H & E stain. x 56.

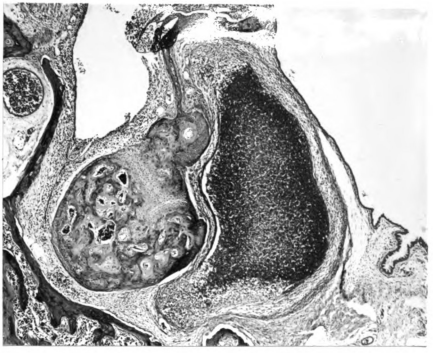

Figure 27. Beginning liquefaction necrosis of auditory ossicles in otitis media of 3-week-old CMP-1 rat. H & E stain. x 56.

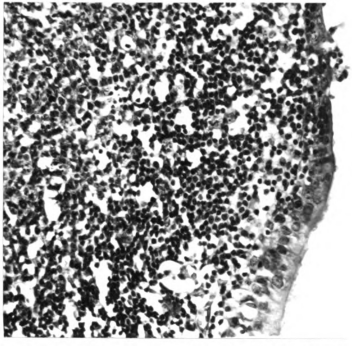

Figure 28. Massive subepithelial lymphocytic tissue in 8-week-old CMP-1 rat. Note encroachment of epithelium by infiltrating lymphocytes. H & E stain. x 350.

Figure 29. Neutrophilic infiltration of nasal submucosal glands of 8-week-old CMP-1 rat. H & E stain. x 560.

The tympanic mucosa was occasionally stratified and squamous, with sheets of keratin being formed (Figure 30). Rarely, squamous metaplasia of the submucosal glands was seen. The continuity of the mucosa was often interrupted by liquefaction necrosis or by proliferation of granulation tissue from the submucosa. The auditory ossicles were frequently surrounded by a wide zone of inflammatory tissue that contained cholesterol clefts (Figure 31).

The submucosal glands formed by infolding of the tympanic mucosa were more prominent than in the weanling rats. The acini were usually mucous in type but were occasionally mucoserous or serous. The cells lining the acini occasionally retained their cilia. Large mononuclear cells with vacuolar cytoplasm were often present in the acini or in the surrounding inflammatory tissue. In chronic cases the inflammation extended dorsally and laterally into the loose adipose connective tissue around the bony framework of the inner ear. Cholesterol clefts were often seen in this inflamed loose connective tissue (Figure 32). They were often surrounded by mucoserous exudate and large vacuolar mononuclear cells (Figure 33).

Occasionally the inflammatory tissue appeared to press upon the bone and cartilage causing a slight bowing in toward the labyrinth (Figure 32). Direct extension of the inflammation into the labyrinth was not observed, however. In only 1 instance, that of an aged CMP-1 rat, did the otitis extend through the tympanic membrane and involve the external ear (Figure 34).

CMP-2 Rats. In colony CMP-2, only aged rats were available for study. In all of these there was chronic purulent rhinitis (Figure 35) and

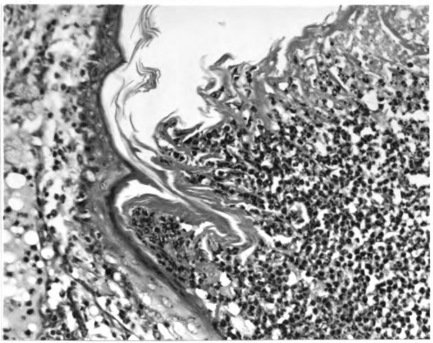

Figure 30. Stratified squamous metaplasia and keratin formation in wall of tympanic cavity in otitis media of 8-week-old CMP-1 rat. H & E stain. x 350.

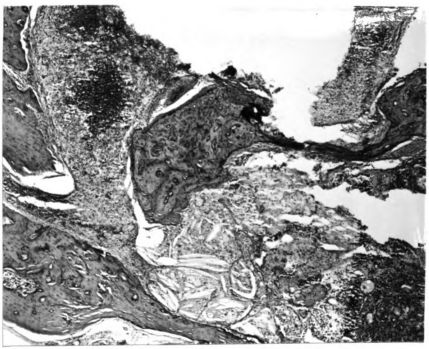

Figure 31. Inflammatory tissue and cholesterol clefts around auditory ossicles in otitis media of 8-week-old CMP-1 rat. H & E stain. x 140.

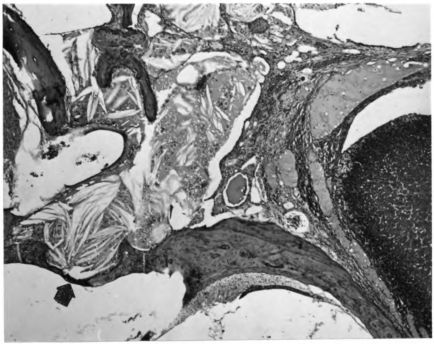

Figure 32. Cholesterol clefts in otitis media of 16-month-old CMP-1 rat. Notice bowing of bone toward inner ear (arrow). H & E stain. x 56.

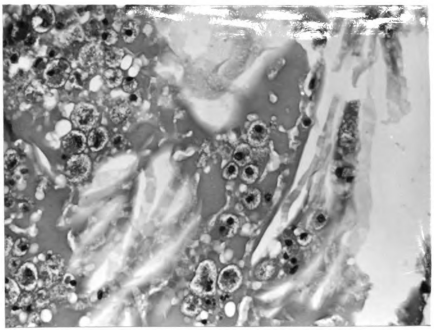

Figure 33. Magnification from Figure 32. Vacuolar mononuclear cells and eosinophilic exudate surrounding cholesterol clefts. H & E stain. x 140.

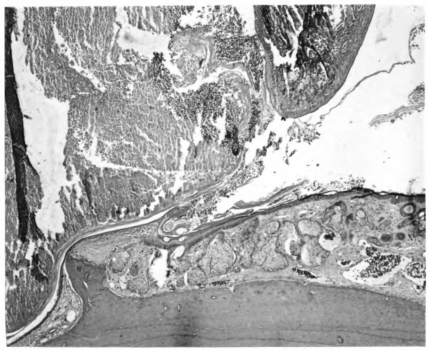

Figure 34. Extension of otitis media through tympanic membrane to external auditory meatus. Sixteen-month-old CMP-1 rat. H & E stain. x 56.

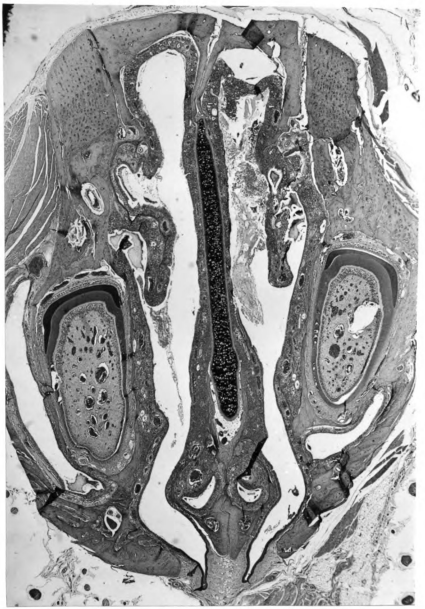

Figure 35. Chronic purulent rhinitis in 1-year-old CMP-2 rat. Note accumulation of exudate in nasal cavity. H & E stain. x 15.

chronic otitis media (Figure 36). The histologic changes were similar to those seen in adult and aged CMP-1 rats, except that the rhinitis was more severe in CMP-2 rats. Larger quantities of purulent exudate were present in the nasal cavity, and more subepithelial lymphocytic infiltration was present.

CMP-3 Rats. In colony CMP-3, 5 of 10 weanling rats had purulent rhinitis and 3 of these had bilateral otitis media. The nature and severity of these inflammations were similar to that of weanling CMP-1 rats. Of 10 aged rats in colony CMP-3, 8 had purulent rhinitis. Of these, 4 had otitis media. Both the rhinitis and otitis were less severe than in CMP-1 or CMP-2 rats. The otitis was characterized by moderate neutrophilic infiltration and mild connective tissue proliferation. One rat, however, had extensive fibroplasia and both tympanic cavities were filled with connective tissue and occasional remnants of submucosal glands containing mucus or inflammatory cells (Figure 37).

B. The Trachea and Lung

Germfree and Defined Flora Rats. The trachea is lined by ciliated pseudostratified cuboidal or columnar epithelium. In the very cranial part, at the level of the thyroid gland, there are scattered mucous and serous glands in the submucosal tissue, between the cartilaginous rings and the epithelium (Figure 38). Individual mast cells and lymphocytes are occasionally seen. Both cilia and goblet cells increase as the trachea proceeds caudally. The submucosal glands are absent where the trachea bifurcates.

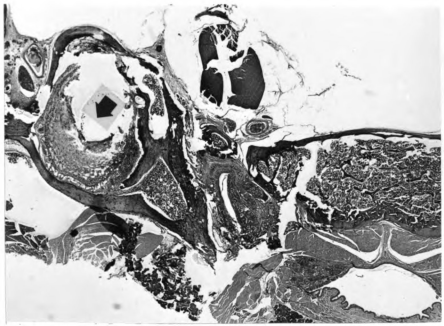

Figure 36. Chronic otitis media in 1-year-old CMP-2 rat. Note exudate and granulation tissue in tympanic cavity (arrow). H & E stain. x 15.

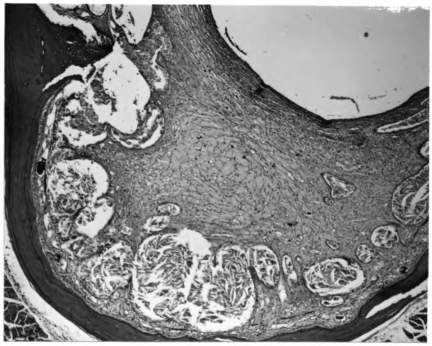

Figure 37. Chronic otitis media in 1-year-old CMP-3 rat. Note filling of tympanic cavity with inflammatory tissue and cystic gland-like structures. H & E stain. x 56.

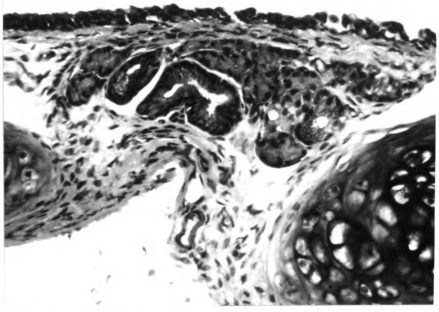

Figure 38. Tracheal mucosa and submucosa of 4-week-old germfree rat. Note serous and mucous glands. H & E stain. x 560.

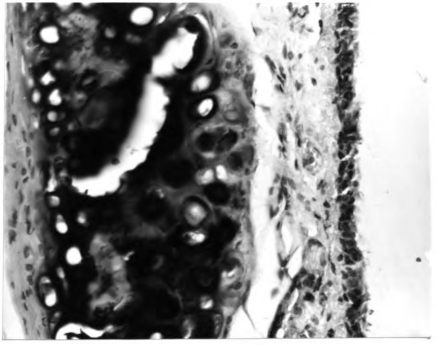

Figure 39. Mineralized tracheal cartilage of 13-month-old germfree rat. H & E stain. x 350.

The tracheal cartilage is hyaline in type. In weanling rats there is no mineralization, but in adult and aged rats, most or all of the cartilaginous rings are mineralized (Figure 39).

After the trachea bifurcates the bronchi quickly lose their cartilaginous supports. As they enter the lung parenchyma they are devoid of glands and cartilage, but do contain occasional aggregations of lymphocytic tissue (Figure 40.). These bronchi are accompanied by bronchial arteries and their related nerves, pulmonary arteries and pulmonary veins, the walls of the latter containing striated cardiac muscle fibers.

Peribronchial lymphocytic tissue was observed in all rats regardless of age or colony, the differences being more quantitative than qualitative. These aggregations of lymphocytes were surprisingly large in germfree and defined flora rats, even in weanlings, and were almost always located at the bifurcation of the bronchi or between a bronchus and an adjacent blood vessel. The cells were almost entirely small and medium lymphocytes (Figure 41). The lymphocytes appeared to collect first outside of the peribronchial smooth muscle layer and, as their number increased, to infiltrate between the smooth muscle and the bronchial epithelium. This subepithelial infiltration was relatively slight in germfree and defined flora rats.

The bronchi are lined by columnar or cuboidal ciliated epithelium. There are no peribronchial glands or cartilage. Beneath the basement membrane of the epithelium, there is a thin layer of collagenous connective tissue which joins the epithelium to the peribronchial smooth muscle.

Goblet cells and lymphocytic tissue are very scarce in medium and small bronchi. There is no obvious difference except diameter between

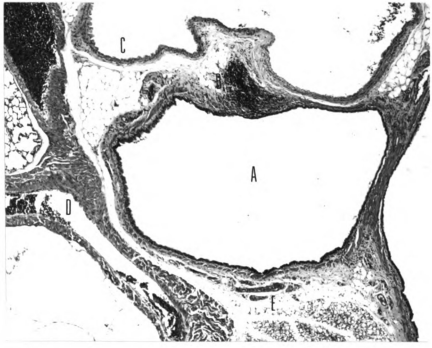

Figure 40. Bronchus (A) of a 4-week-old germfree rat with peribronchial lymphocytic aggregation (B). Note pulmonary artery (C), pulmonary vein with wall of cardiac fibers (D), and bronchial artery with adjacent nerve (E). H & E stain. x 56.

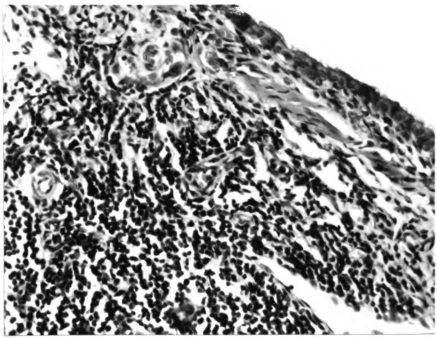

Figure 41. Small and medium lymphocytes in peribronchial lymphocytic tissue of 4-week-old germfree rat. H & E stain. x 560.

large, medium and small bronchi and bronchioles. They are lined by ciliated cuboidal to columnar epithelium, although bronchioles often have nonciliated epithelium.

Occasionally clusters of alveolar macrophages characterized by a vacuolar cytoplasm were observed in the alveolar spaces of older rats (Figure 42). They represent the "multifocal histiocytosis" of Yang et al. (1966). Both vacuolar and nonvacuolar macrophages and some mucus were occasionally observed in bronchial lumens (Figure 43), probably being transported up the tracheobronchial tree by ciliary movement.

Conventional Rats. The trachea of conventional rats was very similar to that of germfree and defined flora rats. There were slightly more subepithelial lymphocytes in adult and aged rats, but these were diffuse and were never seen in aggregations or encroaching upon the epithelium. More goblet cells were seen in the tracheal epithelium, especially in aged rats.

The lungs of conventional rats were more varied in appearance than those of germfree or defined flora rats. Most conventional rats resembled the latter, but 1 weanling rat, 4 adult rats, and 4 aged rats had varying degrees of mild pneumonia.

In the weanling rat the entire accessory lobe was consolidated and gray-red. Microscopically, in the affected part there was peribronchial lymphocytic infiltration, atelectasis, filling of the bronchial lumens and alveoli with mucus and moderate infiltration of this mucus with neutrophils (Figure 44).

In adult and aged conventional groups, only 1, an aged rat, had gross lesions of "multifocal histiocytosis". These consisted of several

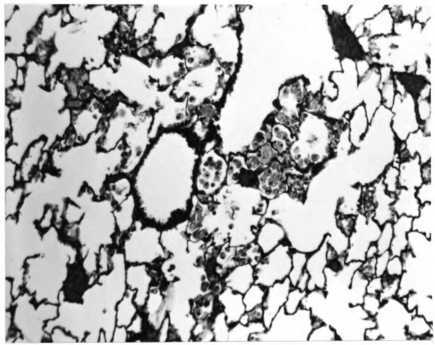

Figure 42. Vacuolar macrophages in alveoli of 1-year-old germfree rat. H & E stain. x 140.

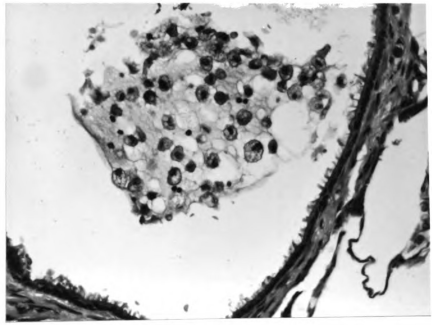

Figure 43. Vacuolar and nonvacuolar macrophages in bronchial lumen of 1-year-old germfree rat. H & E stain. x 350.

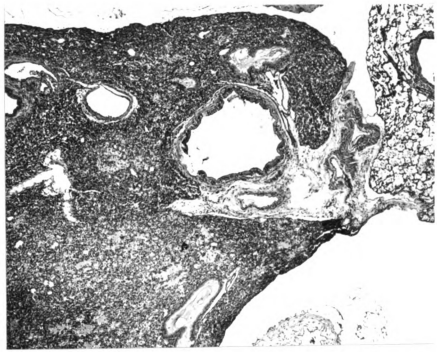

Figure 44. Peribronchial lymphocytic infiltration and mucopurulent consolidation in accessory lobe of 3-week-old conventional rat. H & E stain. x 56.

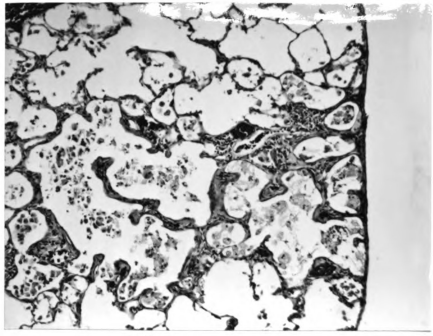

Figure 45. Subpleural aggregation of alveolar macrophages in 1-year-old conventional rat. Note thickened alveolar walls. H & E stain. x 140.

gray-white spots 0.5 to 1.0 mm. in diameter scattered over the dorsal and ventral surfaces of both lungs. Microscopically the aggregations of vacuolar macrophages were clustered in alveoli which often had thickened walls (Figure 45). These aggregations were more subpleural than hilar in location. They often included macrophages with a brownish iron-containing pigment.

In 4 adult rats there were occasional focal areas of pneumonia characterized by interstitial lymphoreticular infiltration and filling of the alveoli with alveolar macrophages and occasional neutrophils. Some of these foci contained an angular birefringent crystalline material that had a light green color (Figure 46).

Occasional bronchi and blood vessels were surrounded by lymphocytes in some adult and aged conventional rats (Figure 47). Most rats, however, had little peribronchial lymphocytic tissue and resembled germfree or defined flora rats.

CMP-1 Rats. The trachea in weanling CMP-1 rats was normal, but in adult and aged rats there occasionally was severe lymphocytic infiltration of the submucosa with encroachment of the epithelial layer, reduction in height of epithelium, and loss of cilia (Figure 48). Slight mucopurulent exudate was often present in the tracheal lumen. Most of the rats in group CMP-1 had no gross lesions (Figure 49). On the pleural surfaces of some adult and aged rats there were gray-white foci 0.5 to 1.0 mm. in diameter. The principal microscopic lesion observed in the lungs of all rats in CMP-1 was increased peribronchial lymphocytic infiltration. This was often the only lesion observed, no corresponding change being present in the alveoli. The lymphocytic infiltration occasionally

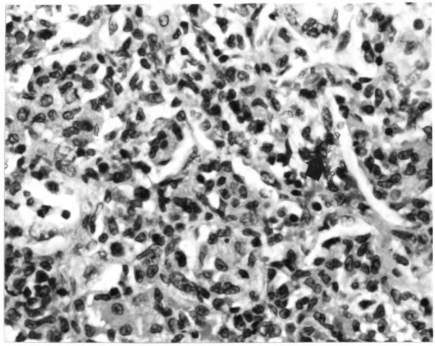

Figure 46. Mild focal pneumonitis in 8-week-old conventional rat. Note angular crystalline material (arrow) in inflammatory tissue. H & E stain. x 560.

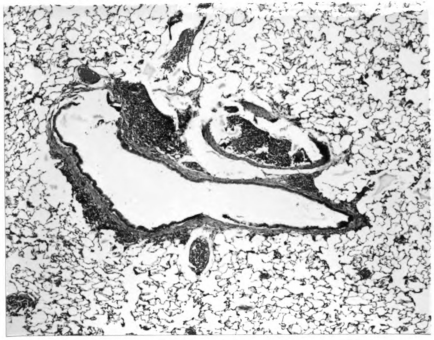

Figure 47. Peribronchial lymphocytic infiltration in 8-week-old conventional rat. H & E stain. x 56.

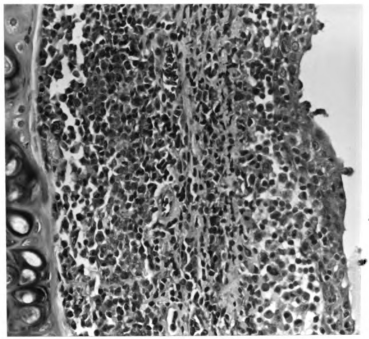

Figure 48. Subepithelial lymphocytic infiltration in trachea of 16-month-old CMP-1 rat. Note reduction in height of tracheal epithelium and loss of cilia. H & E stain. x 350.

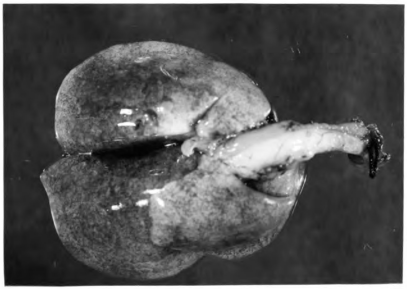

Figure 49. Absence of gross lesions in dorsal view of lungs of 16-month-old CMP-1 rat. Lungs were distended by intratracheal infusion of formalin.

protruded into the bronchial lumen causing a partial stenosis (Figure 50). The cells in these infiltrations were mostly lymphocytes, but frequently there were pigment-laden macrophages, neutrophils, plasma cells or reticulum cells.

Frequently, focal aggregations of vacuolar macrophages were observed in alveoli. The alveoli were, however, generally free of lesions.

One aged rat had several abscesses 4 to 11 mm. in diameter in the cranial part of the left lung. These consisted of a central core of caseopurulent debris, surrounded by a zone of neutrophils, then a zone of degenerating alveolar macrophages and compressed alveoli, and finally a connective tissue capsule (Figure 51).

CMP-2 Rats. Of the 10 aged rats in this group, only 1 had pronounced gross lesions. In this rat there were occasional dark gray spots of "multifocal histiocytosis" on the dorsal surface and some light gray central areas 1 to 2 mm. in diameter surrounded by reddish-gray peripheral zones (Figure 52). On the ventral surface the entire accessory lobe and the right cardiac lobe were occupied with numerous bronchiectatic cavities (Figure 53). The ventral portion of the right diaphragmatic lobe was reddish-gray and hepatized. There were adhesions of both right and left lungs to the diaphragm. The bronchial lymph nodes were enlarged.

Some rats in CMP-2 had the dark gray spots previously described and associated with "multifocal histiocytosis". A few had irregular gray areas of consolidation.

In the trachea, microscopically there was extensive subepithelial lymphocytic infiltration. Masses of lymphocytic tissue often protruded out into the tracheal lumen, causing a severe reduction in the height of the epithelium. In these areas there was disappearance of the cilia.

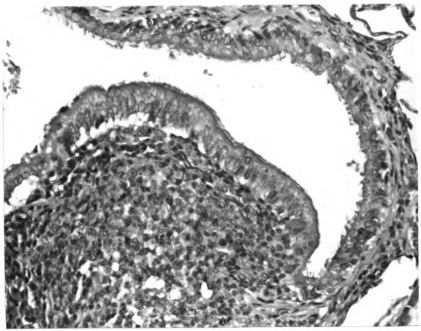

Figure 50. Peribronchial lymphocytic aggregation causing partial obstruction of lumen in 16-month-old CMP-1 rat. H & E stain. x 350.

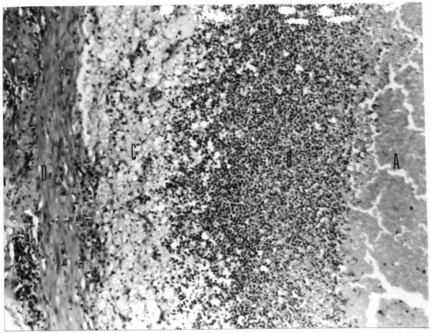

Figure 51. Wall of abscess in 16-month-old CMP-1 rat. Note caseous (A), neutrophilic (B), vacuolar macrophage (C) and connective tissue (D) zones. H & E stain. x 140.

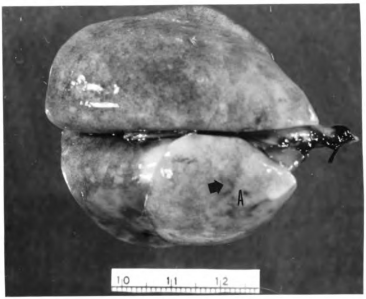

Figure 52. Dorsal view of lungs of 11-month-old CMP-2 rat. Note dark gray foci of "multifocal histiocytosis" (A) and red rings surrounding light central area (arrow). Lungs were distended by intratracheal infusion of fixative.

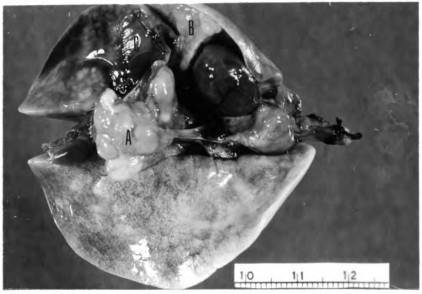

Figure 53. Ventral view of above lungs. Note bronchiectasis in accessory lobe (A) and right cardiac lobe (B) and red-gray hepatization of right diaphragmatic lobe (C).

In the lungs there was generally marked peribronchial lymphocytic infiltration (Figure 54). This was often accompanied by a filling of the adjacent alveoli with mucus and the subsequent infiltration of this mucus with alveolar macrophages and neutrophils (Figure 55). This process appeared to continue and eventually result in consolidation of the alveolar tissue. In many areas there was a bronchopneumonia, with purulent exudate filling the alveoli and the bronchial lumens, often unaccompanied by mucus or peribronchial lymphocytic infiltration. These bronchopneumonic areas were at first discrete but occasionally coalesced to involve an entire lobe, producing the red to gray hepatization seen in the ventral right diaphragmatic lobe of Figure 53. In the periphery of this lobe the exudate was more serofibrinous and less neutrophilic (Figure 56), resulting in flooded alveoli with intact alveolar walls.

Bronchiectasis was occasionally observed in CMP-2 rats (Figure 57). The dilated bronchi usually contained a mucopurulent exudate. The bronchial epithelium was often hypertrophic with extensive goblet cell production and very long cilia (Figure 58). Occasionally papillary hyperplasia of the bronchial mucosa was observed (Figure 59). Squamous metaplasia with keratin formation, as reported in the literature, was not observed.

CMP-3 Rats. Except for slight lymphocytic infiltration of the trachea and occasional clusters of vacuolar macrophages in some rats, no gross or microscopic lesions were observed in the trachea or lungs of weanlings from this group.

Gross lesions were observed in the lungs of 8 of the 10 aged rats. These were usually gray areas of consolidation (Figure 60), often

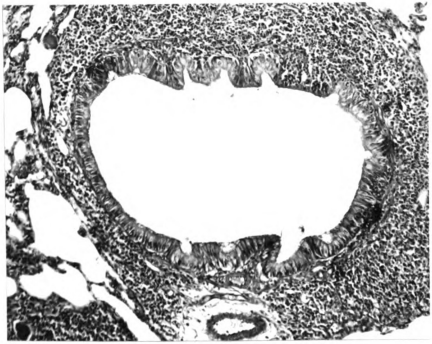

Figure 54. Peribronchial lymphocytic infiltration in 11-month-old CMP-2 rat. H & E stain. x 140.

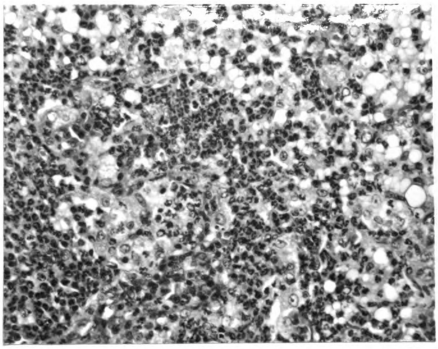

Figure 55. Alveoli filled with mucus, alveolar macrophages and neutrophils in 11-month-old CMP-2 rat. H & E stain. x 350.

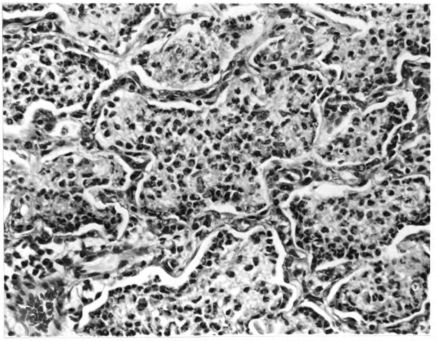

Figure 56. Alveoli filled with fibrinopurulent exudate in 11-month-old CMP-2 rat. Note preservation of alveolar architecture. H & E stain. x 350.

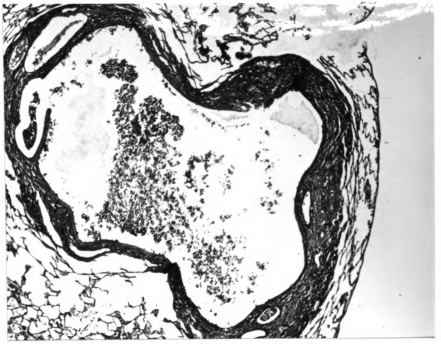

Figure 57. Bronchiectasis in 11-month-old CMP-2 rat. H & E stain. x 56.

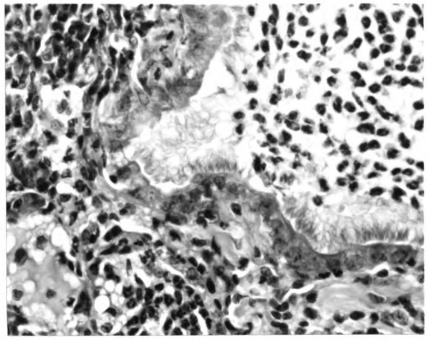

Figure 58. Hypertrophy of cilia in mucosa of bronchiectatic lesion of 11-month-old CMP-2 rat. H & E stain. x 560.

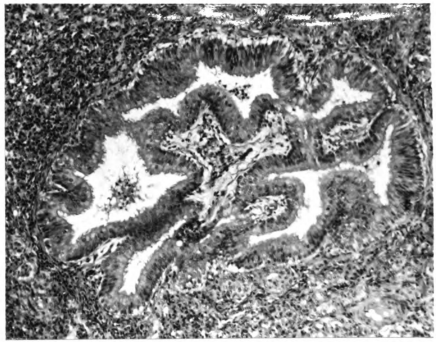

Figure 59. Papillary hyperplasia of bronchial mucosa in 11-month-old CMP-2 rat. H & E stain. x 140.

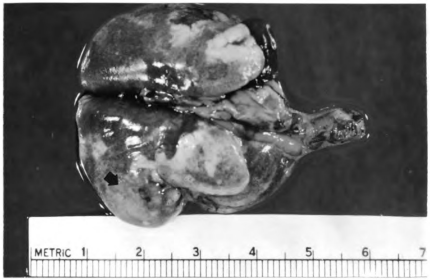

Figure 60. Dorsal aspect of the lungs of 1-year-old CMP-3 rat. Note gray areas of consolidation (arrow).

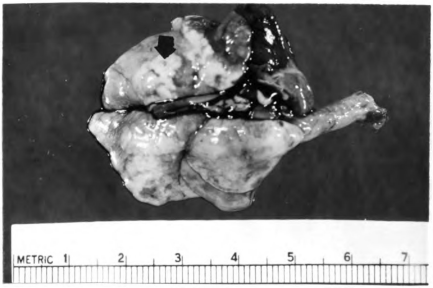

Figure 61. Dorsal aspect of the lungs of 1-year-old CMP-3 rat. Note gray consolidated areas surrounding dark centers (arrow).

containing dark centers, representing bronchi, surrounded by thick cuffs of lymphocytic tissue (Figure 61). Bronchiectasis was usually characterized by the presence of yellow foci varying in diameter from 1 to 5 mm. These were occasionally extensive and involved entire lobes (Figure 62).

In the trachea, microscopically, there was mild to severe tracheitis characterized by subepithelial lymphocytic infiltration and epithelial encroachment. The lungs were characterized by widespread peribronchial lymphocytic infiltration. Usually the bronchial lumen contained no exudate, but occasionally mucopurulent exudate filled the lumen. This was particularly true of, and seemed a prelude to, the greatly dilated bronchi (Figure 63).

Although the bronchial wall was occasionally destroyed in the bronchiectatic process, it was usually intact and was lined by columnar ciliated epithelium with numerous goblet cells. Occasionally stratified epithelium was observed (Figure 64), but squamous metaplasia and keratin formation did not occur. Papillary hyperplasia of the bronchial mucosa was often present. There was occasionally an infolding of the mucosa to form gland-like structures.

The alveolar tissue was often filled with mucus, alveolar macrophages, and neutrophils. There was frequently epithelialization of the lining cells so that they resembled acinar glands. These gland-like structures often contained goblet cells and ciliated epithelium similar to the bronchus.

Alveoli near bronchiectatic lesions and not filled with exudate were often atelectatic (Figure 65).

Figure 62. Note extensive bronchiectasis in dorsal region of left lung of 1-year-old CMP-3 rat.

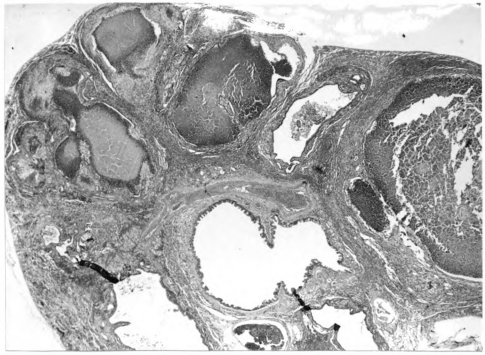

Figure 63. Tissues from rat in Figure 62. Note the bronchiectatic cavities filled with mucopus. x 15.

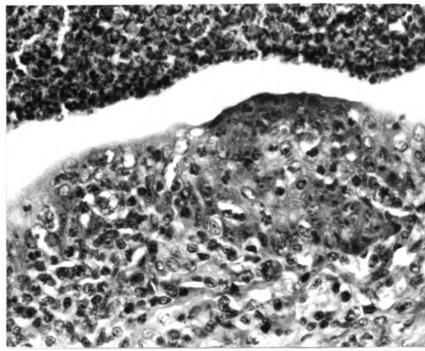

Figure 64. Stratified epithelium in mucosa of bronchiectatic lesion of 1-year-old CMP-3 rat. H & E stain. x 560.

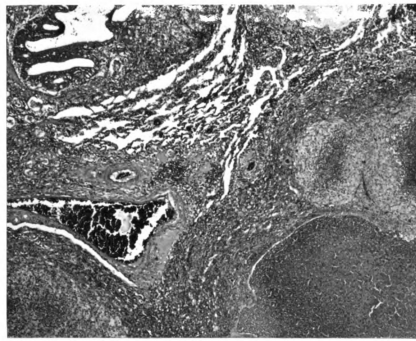

Figure 65. Atelectasis of alveoli around bronchiectatic lesions of 1-year-old CMP-3 rat. H & E stain. x 140.

In 2 aged rats there were numerous scattered granulomas in the alveolar tissue ranging in size from 25 to 300μ (Figure 66). These consisted of macrophages, lymphocytes, giant cells and occasional neutrophils. Most contained no extrinsic material, but in some, foreign bodies were evident. These consisted of fragments of bone (Figure 67), light green curved sheets or fibers approximately 7 to 8μ in diameter and 1200μ long (Figure 68), brown sheets or fibers approximately 3 to 4 in diameter and 1000 to 1100μ long, and large plant fibers composed of several compartments or cells, each about 25 to 30 long and 20μ wide (Figure 69). Several granulomas contained what appeared to be partially digested forms of these foreign particles.

The Peribronchial Lymphocytic Index. The PLI for each rat is presented in Appendix 3. The means of the PLI are shown in Figure 70. There was a significant difference between germfree or defined flora rats and all CMP-affected rats in all 3 age groups, the difference tending to increase markedly in older rats. The values for conventional rats at all ages were between gremfree and defined flora rats on the one hand and CMP-affected rats on the other. Only in the adult group did conventional rats have a significantly different score from germfree or defined flora rats. They were also significantly different from adult CMP-1 rats and from aged CMP-2 and CMP-3 rats.

The means and standard deviations of the PLI are shown in Figure 71. One can readily see the difference between the within-group variation in conventional and CMP-affected rats as opposed to germfree or defined flora rats. The 2 latter groups were far more uniform.

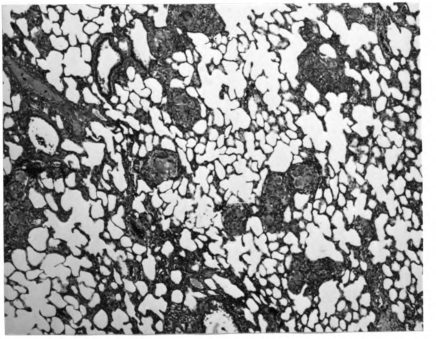

Figure 66. Scattered granulomas in lung of 1-year-old CMP-3 rat. H & E stain. x 56.

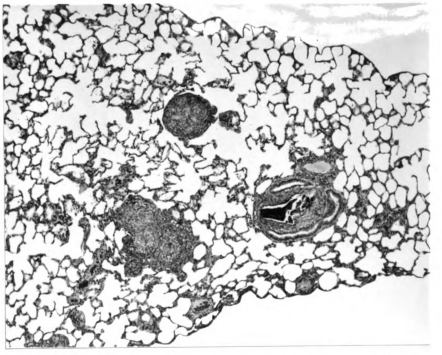

Figure 67. Fragment of bone in granuloma of lung of 1-year-old CMP-3 rat. H & E stain. x 56.

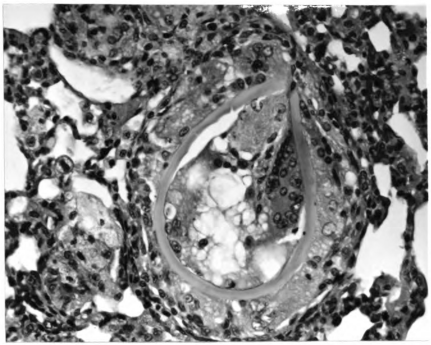

Figure 68. Curved fiber or sheet of foreign material in pulmonary granuloma of 1-year-old CMP-3 rat. H & E stain. x 560.

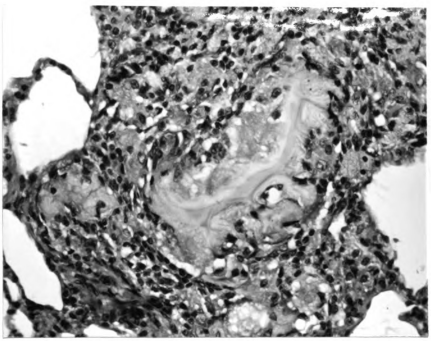

Figure 69. Compartmented foreign material in pulmonary granuloma of 1-year-old CMP-3 rat. H & E stain. x 560.

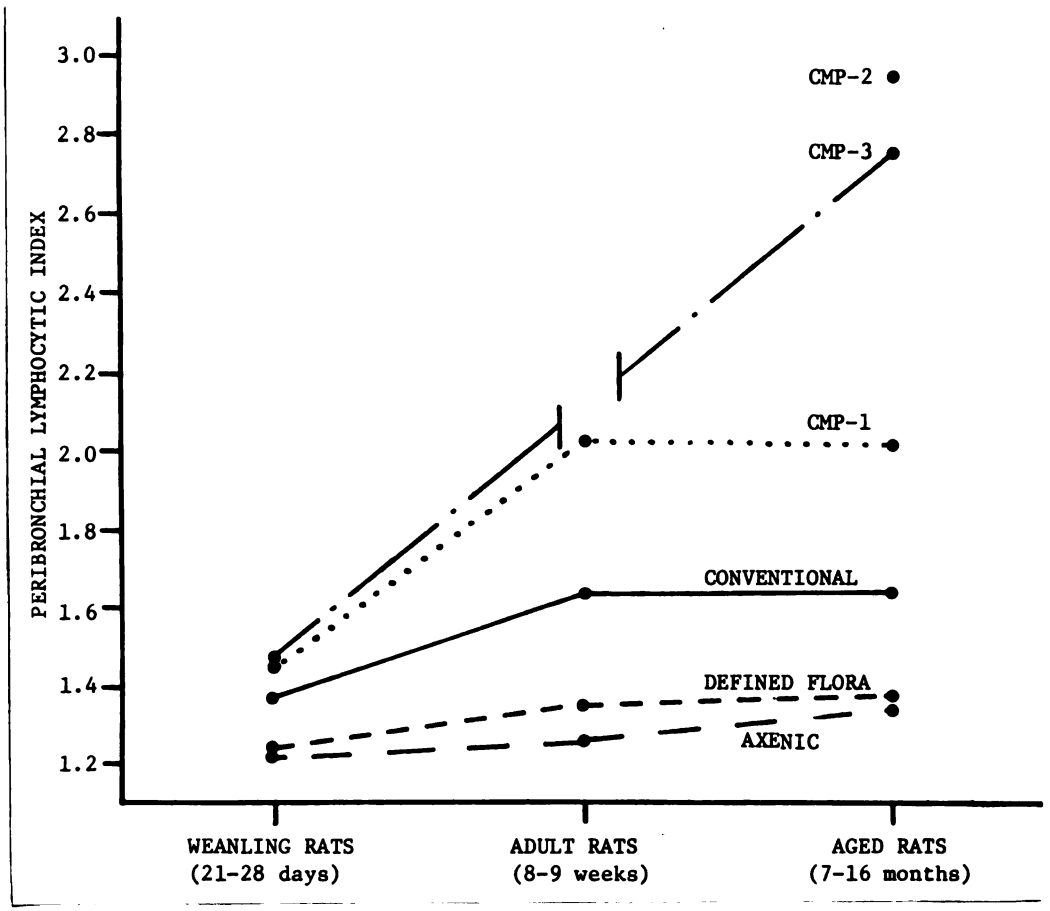

Figure 70. Means of the peribronchial lymphocytic index in germfree (axenic), defined flora, conventional, CMP-1, CMP-2, and CMP-3 colonies.

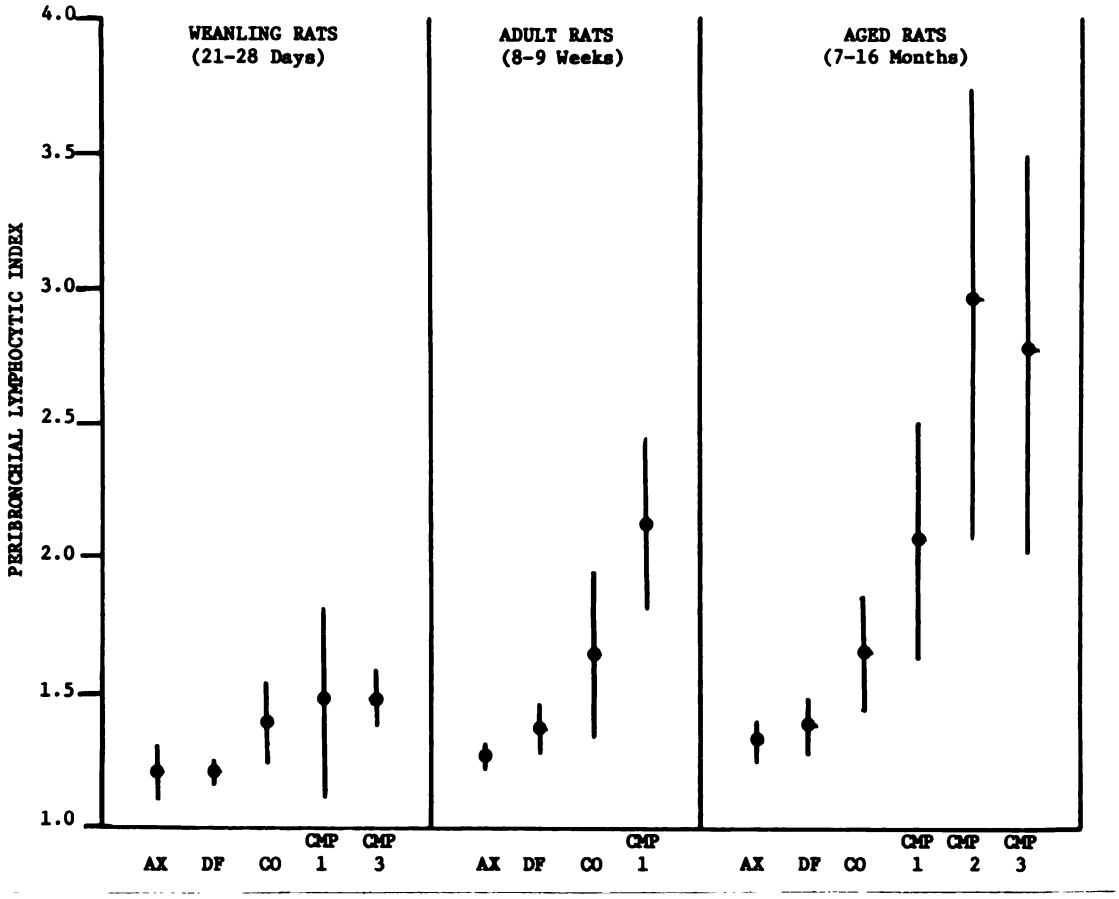

Figure 71. Means and standard deviations of the peribronchial lymphocytic index in germfree (AX), defined flora (DF), conventional (CO), and CMP-1, CMP-2, and CMP-3 colonies.

Results of Aerosol Inoculation. Table 6 summarizes the results of aerosol inoculation of pooled tracheal and lung suspensions. No gross lesions were observed in any of the inoculated animals. Four of 5 rats, in the second series inoculated with CMP-1 suspensions, had purulent rhinitis (Figure 72), and all 5 had otitis media (Figure 73). Neutrophilic exudation was characteristic of both inflammatory processes. They resembled the lesions seen in weanling CMP-1 rats.

Pulmonary lesions in these same rats were less advanced. Peribronchial lymphocytic infiltration (recorded only if lymphocytic tissue circumscribed more than half of a bronchus) was extensive in some rats (Figure 74), but it was patchy in distribution, as were most other lesions.

Inoculation of suspensions from CMP-2 rats was unsuccessful in producing respiratory disease. In rats inoculated with CMP-3 suspensions there was some peribronchial lymphocytic infiltration of 4 of 5 rats. Purulent rhinitis and otitis media developed in 1 rat, which also had mild focal bronchopneumonia.

Although mycoplasmas were originally present in CMP-1 and CMP-3 suspensions, none was recorded from inoculated animals of either the first or second series.

Table 6. Summary of observations of aerosol inoculation of weanling rats and mice with tracheal and lung suspensions of CMP-1, CMP-2, and CMP-3 rats (number under each lesion is the number of animals with that lesion)

						Trachea	Lungs					
			No. in Group	Rhinitis	Otitis media	Lymph. infil.	Epi. necrosis	Peribron. lymph. infil.	Bron. exudate	Bronchiectasis	Alv. Macros.	Alv. Neutrophils	Bacterial isolations
		C*	4	0	0	0	0	0	0	0	1	0	Streptomyces sp.
CMP-1	Mice	E**	4	0	0	0	0	0	0	0	1	0	No growth
1st		C	4	0	0	0	0	0	0	0	0	0	Micrococcus sp.
series	Rats	E	5	0	0	0	0	1	1	0	4	2	Micrococcus sp.
		C	6	0	0	0	0	0	0	0	0	0	S. aureus
CMP-1	Mice	E	5	0	0	0	0	0	0	0	0	0	No growth
2nd		C	5	0	0	0	0	0	0	0	1	0	No growth
series	Rats	E	5	4	5	4	2	5	2	0	4	2	No growth
		C	4	0	0	0	0	1	0	0	1	0	Bacillus sp.
CMP-2	Mice	E	5	0	0	0	0	0	0	0	0	0	No growth
1st		C	2	0	0	1	0	0	1	0	1	1	Micrococcus sp.
series	Rats	E	3	0	0	0	0	1	0	0	2	1	B. bronchiseptica, Micrococcus sp.
		C	3	0	0	0	0	0	0	0	0	0	No growth
CMP-2	Mice	E	6	0	0	0	0	0	0	0	0	0	P. pneumotropica
2nd		C	5	0	0	0	0	0	0	0	0	0	Micrococcus sp.
series	Rats	E	5	0	0	0	0	1	0	0	0	0	No growth
		C	4	0	0	0	0	0	0	0	1	0	Micrococcus sp.
CMP-3	Mice	E	5	0	0	0	0	0	0	0	0	0	No growth
1st		C	5	0	0	0	0	0	0	0	1	0	E. coli
series	Rats	E	5	1	1	1	0	4	1	0	5	2	No growth
		C	3	0	0	0	0	0	0	0	0	0	No growth
CMP-3	Mice	E	5	0	0	0	0	0	0	0	1	0	Micrococcus sp.
2nd		C	5	0	0	0	0	0	0	0	0	0	No growth
series	Rats	E	5	0	0	0	0	0	0	0	1	0	No growth

* C = control ** E = exposed

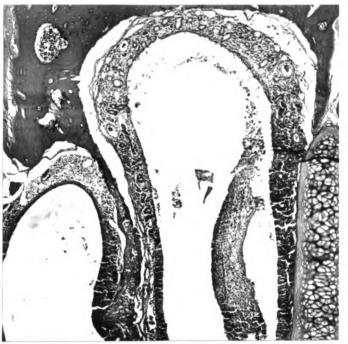

Figure 72. Purulent rhinitis in 7-week-old rat from second series inoculated with tracheal and lung suspensions of CMP-1 rats. H & E stain. x 56.

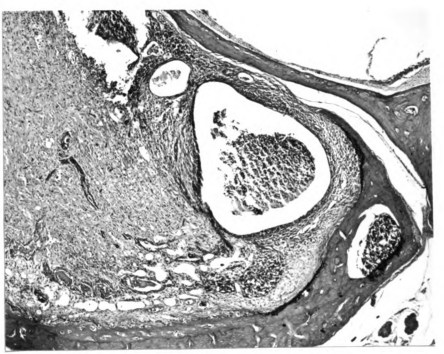

Figure 73. Same rat as above. Purulent otitis media. H & E stain. x 56.

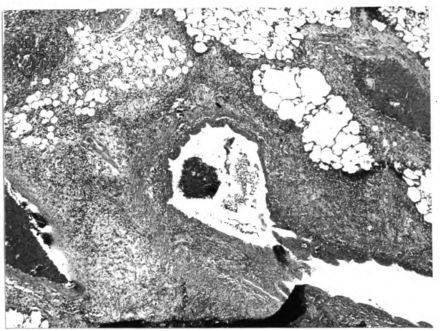

Figure 74. Peribronchial lymphocytic infiltration and bronchitis in rat from second series inoculated with CMP-2 suspensions. H & E stain. x 56.

DISCUSSION

The Nasal Cavity and Middle Ear. Of the various bacteria isolated from the nasal cavity and middle ear (Appendix 2), Micrococcus sp., Streptococcus viridans, Streptococcus sp., Escherichia coli, and Staphylococcus aureus were isolated only from defined flora and conventional rats or from these and the CMP-affected rats. Proteus sp. and Pseudomonas sp. were isolated 3 times and Herellea sp. twice in the 58 samples from the nasal cavity and middle ear of CMP-affected rats.

Diplococcus pneumoniae was isolated 12 times, Pasteurella pneumotropica 3 times, Corynebacterium kutscheri 3 times, and Mycoplasma sp. 14 times from the CMP-affected rats. Each of these 4 organisms is known to inhabit the rat respiratory tract, and each may have contributed to the development of the lesions present. Diplococcus pneumoniae and Mycoplasma sp. were the 2 most commonly isolated from each of the 3 CMP-affected groups. These 2 organisms are probably primary in initiating upper respiratory inflammation in the colonies examined.

In individual rats within these 3 groups there was no clear correlation between the presence of organisms and lesions. Often no organisms were isolated from rats with extensive lesions.

The lesions were similar in each of the 3 CMP-affected groups. A common pathogenesis, dictated more by architecture than causative agent, seemed to prevail. In both the nasal cavity and middle ear the earliest lesions were increased numbers of goblet cells, mucoserous exudate accumulating in lumens, and neutrophilic infiltration. As the neutrophils

increased in numbers they infiltrated the mucosa and submucosa. Increased mucoserous exudate was often accompanied by ciliary destruction, causing exudate to accumulate.

At this point the pathogenesis differed in the nasal cavity and the middle ear, the former having both oral and pharyngeal outlets for its exudates, and the latter having none, save the eustachian tube which is small and poorly placed. Although exudate accumulates around the turbinates and in the maxillary sinus, there are ventral outlets which allow an excess eventually to escape. Adult and aged CMP-1 rats had less exudate in the nasal cavity than weanling rats. In some rats with severe otitis media, the only evidence of rhinitis was subepithelial lymphocytic infiltration. A resistance probably developed gradually in chronic infections and caused a decrease in the severity of lesions in the nasal cavity. Judging by the extensive lymphocytic infiltration, the resistance is probably humoral.

In the middle ear the eustachian tube enters the tympanic cavity at a point midway between its dorsal and ventral limits, and the exudate which collects in the ventral parts cannot escape. Once the bacterial irritation is initiated, it starts a chain of events which can probably proceed after the infection is no longer present. Mucoserous exudate, produced by both goblet cells and the infolding of the epithelium to form glands, offers some protection to the mucosa but cannot escape and probably becomes a source of nutrients for further bacterial growth or, infiltrated with neutrophils, a source of irritation itself. It is also not surprising that frequently no organisms could be isolated from rats with otitis media. The irritation extends into the loose connective tissue with its lipocytes, causing cholesterol crystals to form, and provokes

exuberant granulation tissue, often filling the tympanic cavity and isolating the neutrophilic exudate in the gland-like remnants of the tympanic mucosa. In consideration of this view, data comparing the incidence of otitis media with rhinitis (Nelson and Gowen, 1930) are not to be taken too seriously, unless histologic criteria are used.

No extension of the inflammation to the inner ear was observed, even though several CMP-1 rats had signs of "twisting". The dysequilibrium is probably due to the inflammatory swelling of the tympanic cavity with subsequent pressure on the labyrinth.

The Trachea and Lungs. The prevailing concept of the etiologic agent of CMP is that it is a virus (Innes et al., 1967; Nelson, 1967), despite the fact that this virus has never been isolated. Its presence has been ascertained by inoculation of suspensions of pneumonic lung tissue into mice. The disease has never been convincingly reproduced in rats. Nelson (1951) made a passing reference to having reproduced it in rats, but indicated that additional work was needed. The photomicrographs published by Joshi et al. (1965) of CMP induced by inoculating germfree rats with their "virus" are unconvincing. They show as evidence of CMP the same amount of peribronchial lymphocytic tissue as was seen in some germfree rats in the present study. Their Figure 6, supposedly of an inoculated animal with "marked peribronchial lymphoid infiltration", actually shows the trachea or major bronchus with a tracheobronchial lymph node surrounded by adipose tissue. Their agent, by electronmicroscopic examination, was said to be 110 Å in diameter, approximately half the size of the smallest known animal virus.

Nevertheless, this paper was taken seriously, and every effort was made to isolate an agent in rat embryonic skin cells, following the procedures recommended. No cytopathogenic effect (CPE) was ever observed. All other cell culture lines also failed to develop CPE on inoculation.

The accepted pathogenetic concept of CMP is peribronchial lymphocytic infiltration leading eventually to bronchiectasis. As can be seen in the PLI graphs (Figure 71 and 72), there is a quantitative increase in the PLI as rats are exposed to more organisms in their environment. There is a small amount of peribronchial lymphocytic tissue in germfree rats, even when fed on water-soluble chemically-defined diets (Giddens et al., 1966). From this baseline amount, increased lymphocytic tissue is a response to irritation and, probably chiefly, to the antigenic nature of the irritant.

On the basis of an extensive literature review and more than 1 year of work, it is my conclusion that CMP is a complex disease, initiated by a variety of interrelated factors. The essential factor is prolonged and continuous irritation and/or antigenic stimulation of the bronchial mucosa. Several factors are believed to affect this:

A. Of first importance is the microorganisms which colonize the upper respiratory or tracheobronchial structures. Those most commonly isolated solely from CMP-affected rats in this study were Mycoplasma sp., Diplococcus pneumoniae, and Pasteurella pneumotropica. Other organisms not isolated in this research but probably also significant are Streptobacillus moniliformis, Bordetella bronchiseptica, and the "virus" of Nelson (1948) and the grey lung virus of Andrewes and Glover (1945), both of which are believed by some (Gay, 1967) to be mycoplasmas. It

is unlikely that one and only one particular organism is responsible for all cases of CMP. Certain of these may be active in some colonies while entirely different ones are present in others. A synergistic action of 2 or more species is likely. In both CMP-1 and CMP-3 rats at least 2 of the aforementioned organisms were present.

Some organisms, such as the mycoplasma, probably grow more readily in the nasal cavity and middle ear and spread slowly to the tracheobronchial tree (Nelson, 1955). This seems particularly to be the case in the CMP-1 group, where severe rhinitis and otitis media were accompanied by mild or moderate peribronchial lymphocytic infiltration. The same pattern was seen in the second series of rats inoculated with aerosols of CMP-1 rats. This selected pattern of growth probably explains why mycoplasma were not isolated from the inoculated animals since only the pooled lungs were examined microbiologically.

The presence of peribronchial lymphocytic infiltration accompanied by relatively few alveolar lesions as seen in the CMP-1 rats contrasts strongly with the extensive alveolar and bronchial lesions seen in CMP-2 rats. In these D. pneumoniae was the dominant organism. The lesions were often typical of purulent bronchopneumonia, or of lobar pneumonia.

Whichever organism becomes established in the respiratory tract probably has its own characteristic pattern of lesions, although peribronchial lymphocytic infiltration is probably an inherent part of all. Much overlapping undoubtedly occurs.

B. Equally important are those factors which aid in the establishment of tracheobronchial infections:

1. The degree of crowding, the sanitary standards and level of ammonia fumes, the temperature and humidity of the animal rooms, and

the air circulation are surely contributory to the establishment of respiratory infections in rats.

2. The rapid respiratory rate of the rat, coupled with his proximity to the bedding, water and feed, often cause particles to be inhaled, as is demonstrated in Figures 66, 67, 68, and 69. The larger particles are probably expectorated in normal rats, but in those with severe CMP, as in Figure 66 through 70, the ciliary movement and ability to cough may be impaired and particles cannot be removed so easily. This would explain why foreign body granulomas developed only in severely affected CMP-3 rats, even though germfree, defined flora and conventional rats were maintained on the same kind of food and bedding. It is probably the explanation for the presence of such particles in the CMP-affected rats described by Tucker and Wyatt (1967), being thus the result and not the cause of CMP.

The alveolar aggregations of vacuolar macrophages have been named "multifocal histiocytosis" by Yang et al. (1967), an unfortunate designation becuase the cells do not resemble normal histiocytes. They are distended alveolar macrophages, and if one accepts the theory of Schultz (1959), they are derived from the granular pneumocytes. Since these cells are believed to secrete surfactant, it is tempting to speculate that the vacuoles are filled with surfactant, excess production of which is stimulated by focal irritation. The presence of occasional iron-containing pigment, probably hemosiderin, in some cells is further support for the focal irritation theory, most likely caused by small inhaled particles, possibly bacteria and/or dust from the litter or food or droplets of water. This would explain why the condition was more common in conventional and CMP-affected rats than in germfree or defined flora

rats. The only rat lung in the germfree or defined flora group from which an organism was isolated (Streptococcus sp. from #076623) was also the only one with gross and microscopic lesions of the condition.

C. A third and major factor is probably the structure of the very thin-walled rat bronchus, which is devoid of cartilage and glands. It contains relatively little collagen and smooth muscle in relation to its rather large diameter. Once exudate begins to accumulate, its removal becomes difficult, especially when peribronchial lymphocytic infiltration encroaches upon the mucosa, and there is reduction in the size of the epithelium and a loss of cilia. Other parts of the bronchus produce increased amounts of mucus in response to the irritation, and this mucus accumulates in the bronchial lumen, eventually filling even its related alveoli. This mucostatic process predisposes to even further bacterial growth. Air cannot pass into such bronchi and alveoli and, as the thorax expands on inspiration, these bronchi are probably dilated by the negative pressure in the surrounding alveoli. The extra space in the dilated bronchus is probably filled by mucus from higher up in the tracheobronchial tree, due to both gravity and the inrushing air. Eventually the bronchus is permanently dilated by the constant presence of this exudate, coupled with the inflammatory weakening of the bronchial wall.

This theory of the pathogenesis of the bronchiectasis explains why the most ventral lobes are predisposed to such a lesion.

Once an infection becomes established, the rat in question probably acts as a miniature aerosol generator, producing a constant but low concentration of organisms in the air it exhales. If newborn rats are placed in contact with such a rat, they probably develop a similar but

low grade infection. The development from this inauspicious and inapparent beginning to grossly evident bronchiectasis probably takes 3 to 12 months. If this theory be true, research in which weanling rats were inoculated intranasally with 1 large exposure to organisms and then held for 3 to 4 weeks should not be taken too seriously in evaluating the pathogenesis of a particular organism if the results are negative.

D. A fourth factor is probably genetic susceptibility. Certainly there is a difference in the incidence of CMP in different colonies. The rats used for inoculation of suspensions from CMP-1 rats came from the same stock as those of the germfree, defined flora, conventional and CMP-3 rats. Perhaps the reason why CMP developed in the CMP-1 inoculated rats is due more to the increased susceptibility of the inoculated strain than to the presence of particular organisms in the suspensions.

SUMMARY AND CONCLUSIONS

Research was conducted to study the anatomy, microbiology, and pathology of the nasal cavity, middle ear, trachea and lungs of laboratory rats. The study included 149 weanling (3-4 weeks old), adult (8-9 weeks old) and aged (7-16 months old) rats randomly selected from germfree, defined flora, conventional and 3 chronic murine pneumonia-affected colonies (CMP-1, CMP-2, CMP-3). Gross and microscopic observations were conducted on all rats and viral and bacteriologic examinations were conducted on selected rats from each colony.

The normal structure of the respiratory tract of germfree, defined flora and conventional rats was characterized. Subepithelial lymphocytic tissue was scant in the respiratory tract of germfree rats, but it increased progressively in defined flora, conventional and chronic murine pneumonia-affected colonies.

In the CMP-1 group there was severe purulent rhinitis and otitis media in rats of all ages. Slight tracheitis and peribronchial lymphocytic infiltration were observed in adult and aged rats. The most commonly isolated organism was Mycoplasma sp. Inoculation by aerosol of CMP-1 tracheal and lung suspensions into 2 series of weanling rats and mice resulted in severe rhinitis and otitis media and moderate patchy pneumonia only in the second series of rats.

In the CMP-2 group, which consisted only of aged rats, there were severe chronic purulent rhinitis and pneumonia with extensive peribronchial lymphocytic cuffing and bronchiectasis. Bronchopneumonia and

lobar pneumonia were occasionally observed. Diplococcus pneumoniae was the most commonly isolated organism. Experimental inoculation was unsuccessful in producing respiratory disease.

In the CMP-3 group, rhinitis, otitis media, and pneumonia were observed in both weanling and aged rats. Bronchiectasis was commonly observed in aged rats. Two organisms frequently isolated were Mycoplasma sp. and Pasteurella pneumotropica. Exposure of rats and mice to aerosols of tracheal and lung suspensions was unsuccessful in producing respiratory disease.

It is concluded that the essential etiologic factor in CMP is probably prolonged and continuous irritation and/or antigenic stimulation of the bronchial wall. Factors which are believed to affect this are: (1) the species and strains of microorganisms present, (2) environmental factors, such as ammonia fumes and dust, (3) the delicate construction of the rat bronchus, and (4) genetic susceptibility.

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APPENDICES

Appendix 1. Identification of experimental animals with accession numbers of the Department of Pathology at Michigan State University

	Weanling Path. No.	Adult Path. No.	Aged Path. No.
Germfree	076681-076690	076691-076700	076701-076707
Defined Flora	076601-076610	076611-076619	076621-076630
Conventional	076641-076650	076651-076660	076661-076670
CMP-1	075445-075454	077510-077519	075465-075474
CMP-2	---	---	076078-076087
CMP-3	076631-076640	---	076710-076719

Appendix 2a. Morphologic and bacteriologic observations of the nasal cavity and middle ear of aged germ-free (076706-707) and defined flora (076621-625) rats

	Rat Numbers						
	076706	076707	076621	076622	076623	076624	076625
Nasal Cavity:							
Purulent exudate	0*	0	0	0	0	0	0
Subepithelial lymphocytic infiltration	0	0	0	+***	+	+	0
Epithelial necrosis	0	0	0	0	0	0	0
Bacterial isolations	N**	N	Micro-coccus sp.	N	Micro-coccus sp.	N	Micro-coccus sp.
Middle Ear:							
Purulent exudate	0	0	0	0	0	0	0
Epithelial necrosis	0	0	0	0	0	0	0
Bacterial isolations	N	N	N	N	N	N	N

* 0 = absent ** N = no microorganisms isolated *** + = slight

Appendix 2b. Morphologic and bacteriologic observations of the nasal cavity and middle ear of adult (076656-660) and aged (076661-665) conventional rats

	Rat Numbers									
	076656	076657	076658	076659	076660	076661	076662	076663	076664	076665
Nasal Cavity:										
Purulent exudate	0*	+	+	0	0	0	0	0	0	0
Subepithelial lymphocytic infiltration	+**	+	+	0	+	+	+	+	+	+
Epithelial necrosis	0	0	0	0	0	0	0	0	0	0
Bacterial isolations	N***	Micrococcus sp.	Micrococcus sp.	Escherichia coli, Streptococcus viridans	Streptococcus viridans	Micrococcus sp.	Staphylococcus aureus, Micrococcus sp.	Micrococcus sp.	Staphylococcus aureus	Micrococcus sp.
Middle Ear:										
Purulent exudate	0	0	0	0	0	0	0	0	0	0
Epithelial necrosis	0	0	0	0	0	0	0	0	0	0
Bacterial isolations	N	N	N	Escherichia coli	Micrococcus sp.	N	Micrococcus sp.	N	N	N

* 0 = absent ** + = slight *** N = no microorganisms isolated

Appendix 2c. Morphologic and bacteriologic observations of the nasal cavity and middle ear of adult CMP-1 (077510-519) rats

	Rat Numbers									
	077510	077511	077512	077513	077514	077515	077516	077517	077518	077519
Nasal Cavity:										
Purulent exudate	0*	++***	+++	+	++	+	+	+	+++	+++
Subepithelial lymphocytic infiltration	+++**	+++	+++	+++	+++	+++	++	++	+++	+++
Epithelial necrosis	0	0	+	0	0	0	0	0	++	+
Bacterial isolations	Pasteurella pneumotropica, Mycoplasma sp.	Mycoplasma sp.	Micrococcus sp., Escherichia coli, Streptococcus sp.	Micrococcus sp.	Escherichia coli	Mycoplasma sp., Corynebacterium kutscheri	Diplococcus pneumoniae, Mycoplasma sp.	Proteus sp.	Mycoplasma sp., Corynebacterium kutscheri	Mycoplasma sp., Escherichia coli
Middle Ear:										
Purulent exudate	0	++	+++	0	0	+	+++	+++	+++	+++
Epithelial necrosis	0	+#	+++	0	0	++	+++	+++	+++	+++
Bacterial isolations	Mycoplasma sp.	Mycoplasma sp., Diplococcus pneumoniae	Mycoplasma sp., Micrococcus sp., Proteus sp.	Micrococcus sp.	N##	Mycoplasma sp.	Diplococcus pneumoniae, Mycoplasma sp.	Diplococcus pneumoniae	Mycoplasma sp.	N

* 0 = absent ** +++ = severe *** ++ = moderate # + = slight ## N = no microorganisms isolated

Appendix 2d. Morphologic and bacteriologic observations of the nasal cavity and middle ear of aged CMP-2 (076079-087) rats

	Rat Numbers								
	076079	076080	076081	076082	076083	076084	076085	076086	076087
Nasal Cavity:									
Purulent exudate	++*	++	+++	++	+++	+++	++	+++	+++
Subepithelial lymphocytic infiltration	+++**	+++	+++	+++	+++	+++	+++	+++	+++
Epithelial necrosis	0***	0	0	0	0	++	0	0	0
Bacterial isolations	Diplococcus pneumoniae, Staphylococcus aureus	Diplococcus pneumoniae	Diplococcus pneumoniae	Micrococcus sp.	Micrococcus sp., Pseudomonas sp.	Pseudomonas sp.	N	N	N
Middle Ear:									
Purulent exudate	+++	+++	+++	+++	0	+++	+++	+++	+++
Epithelial necrosis	+++	+++	+++	+++	0	+++	+++	+++	+++
Bacterial isolations	Diplococcus pneumoniae	Diplococcus pneumoniae, Micrococcus sp.	Diplococcus pneumoniae	Micrococcus sp.	N#	Corynebacterium kutscheri	Diplococcus pneumoniae	Diplococcus pneumoniae	N

* ++ = moderate ** +++ = severe *** 0 = absent # N = no microorganisms isolated

Appendix 2e. Morphologic and bacteriologic observations of the nasal cavity and middle ear of aged CMP-3 (076710-076719) rats

	Rat Numbers									
	076710	076711	076712	076713	076714	076715	076716	076717	076718	076719
Nasal Cavity:										
Purulent exudate	++*	0	+#	+++##	+	+++	0	+++	+	+
Subepithelial lymphocytic infiltration	++	++	++	+++	+++	+++	0	+++	++	+
Epithelial necrosis	0**	0	0	+	0	+	0	+	0	0
Bacterial isolations	Micrococcus sp., Pseudomonas spp.	Micrococcus sp., Pseudomonas sp., Proteus sp.	Pseudomonas sp., Proteus sp.	N	Mycoplasma sp.	N	Micrococcus sp., Herellea sp.	Streptococcus viridans, Herellea sp.	Micrococcus sp.	Streptococcus viridans, Micrococcus sp.
Middle Ear:										
Purulent exudate	++	0	0	0	0	++	0	++	+	0
Epithelial necrosis	0	0	0	0	0	+	0	++	+	0
Bacterial isolations	N***	Pasteurella pneumotropica	N	N	N	N	N	N	Pasteurella pneumotropica	Mycoplasma sp., Staphylococcus aureus

* ++ = moderate ** 0 = absent *** N = no microorganisms isolated # + = slight ## +++ = severe

Appendix 3a. Morphologic and bacteriologic observations of the trachea and lungs of aged germfree (076706-707) and defined flora (076621-625) rats

	Rat Numbers						
	076706	076707	076621	076622	076623	076624	076625
Trachea:							
Lymphocytic infiltration	0*	0	0	0	0	0	0
Epithelial necrosis	0	0	0	0	0	0	0
Bacterial isolations	N**	N	N	N	N	N	N
Lungs:							
Peribronchial lymphocytic index	1.30	1.24	1.37	1.41	1.45	1.26	1.53
Bronchial exudate	0	0	0	0	0	0	0
Bronchiectasis	0	0	0	0	0	0	0
Vacuolar macrophages	0	0	0	0	++***	0	0
Perivascular cuffing	0	0					
Bacterial isolations	N	N	N	N	Strepto-coccus sp.	N	N

* 0 = absent ** N = no microorganisms isolated *** +++ = severe

Appendix 3b. Morphologic and bacteriologic observations of the trachea and lungs of adult (076656-660) and aged (076661-665) conventional rats

	Rat Numbers									
	076656	076657	076658	076659	076660	076661	076662	076663	076664	076665
Trachea:										
Lymphocytic infiltration	0*	0	0	0	0	0	0	0	+	0
Epithelial necrosis	0	0	0	0	0	0	0	0	0	0
Bacterial isolations	Micrococcus sp.	Streptococcus viridans, Micrococcus sp.	N	N	Streptococcus viridans	Corynebacterium sp.	N	N	Micrococcus sp.	N
Lungs:										
PLI	2.13	1.69	1.40	1.66	2.10	1.61	1.55	1.70	1.66	2.12
Bronchial exudate	0	0	0	+	0	0	0	0	0	0
Bronchiectasis	0	0	0	0	0	0	0	0	0	0
Vacuolar macrophages	+**	++#	0	+++##	+	0	0	+++	0	0
Perivascular cuffing	+	+	0	+	+	0	0	+	0	0
Bacterial isolations	N***	Micrococcus sp.	N	N	N	N	N	N	Streptococcus viridans	N

* 0 = absent ** + = slight *** N = no microorganisms isolated # ++ = moderate ## +++ = severe

Appendix 3c. Morphologic and bacteriologic observations of the trachea and lungs of adult CMP-1 (077510-519) rats

	Rat Numbers									
	077510	077511	077512	077513	077514	077515	077516	077517	077518	077519
Trachea:										
Lymphatic infiltration	+++*	++#	+++	++	++	++	++	++	++	++
Epithelial necrosis	0**	0	0	0	0	0	0	0	0	0
Bacterial isolations	Pseudomonas sp.	Mycoplasma sp.	Proteus sp.	N	N	Mycoplasma sp.	Diplococcus sp., Micrococcus sp.	N	Mycoplasma sp.	Mycoplasma sp.
Lungs:										
PLI	2.06	1.82	1.82	1.82	2.70	2.00	2.28	2.08	2.38	2.49
Bronchial exudate	0	0	0	0	++	+##	0	0	0	+
Bronchectasis	0	0	0	0	++	+	0	0	0	+
Vacuolar macrophages	0	++	0	0	0	++	++	++	++	++
Perivascular cuffing	0	++	++	+	++	+	+	++	++	++
Bacterial isolations	N***	Mycoplasma sp.	Mycoplasma sp.	N	N	N	Mycoplasma sp.	Diplococcus pneumoniae	N	N

* +++ = severe ** 0 = absent *** N = no microorganisms isolated # ++ = moderate ## + = slight

Appendix 3d. Morphologic and bacteriologic observations of the trachea and lungs of aged CMP-2 (076079-087) rats

	Rat Numbers								
	076079	076080	076081	076082	076083	076084	076085	076086	076087
Trachea:									
Lymphocytic infiltration	+*	+++	+++	+++	+	+	+	+++	+++
Epithelial necrosis	0**	0	0	+	0	0	0	++	0
Bacterial isolations	N***	N	Diplococcus pneumoniae	Diplococcus pneumoniae	Diplococcus pneumoniae	N	N	Micrococcus sp.	N
Lungs:									
PLI	2.66	2.42	2.34	3.68	1.84	4.00	3.70	3.72	2.16
Bronchial exudate	+	+	+	+++	+	++	++	++	+
Bronchiectasis	+	0	+	+++	0	+++	+++	+++	+
Vacuolar macrophages	++#	+	0	++	0	+++	+++	+++	+
Perivascular cuffing	+++##	+++	+++	+++	0	+++	+++	+++	+
Bacterial isolations	N	N	Diplococcus pneumoniae	N	Diplococcus pneumoniae	Bacillus sp.	N	Diplococcus pneumoniae	N

* + = slight ** 0 = absent *** N = no microorganisms isolated # ++ = moderate ## +++ = severe

Appendix 3e. Morphologic and bacteriologic observations of the trachea and lungs of aged CMP-3 (076710-719) rats

	Rat Numbers									
	076710	076711	076712	076713	076714	076715	076716	076717	076718	076719
Trachea:										
Lymphocytic infiltration	+*	+	+	+	+	+++	+	+++	+	+
Epithelial necrosis	0**	0	0	0	0	+	0	+	0	0
Bacterial isolations	N***	N	Pasteurella pneumotropica	Micrococcus sp.	N	Mycoplasma sp.	N	N	Mycoplasma sp.	Staphylococcus aureus
Lungs:										
PLI	2.06	2.34	3.90	2.21	2.43	3.56	2.78	3.62	3.24	1.97
Bronchial exudate	0	++##	+++	0	0	+++	0	+++	0	0
Vacuolar macrophages	+++#	+++	+++	++	++	+++	0	+++	+	0
Perivascular cuffing	+++	+++	+++	+	+	+++	+	+++	+	+
Bacterial isolations	N	N	Pasteurella pneumotropica	N	Micrococcus sp.	Mycoplasma sp.	N	Mycoplasma sp.	Mycoplasma sp.	N

* + = slight ** 0 = absent *** N = no microorganisms isolated # +++ = severe ## ++ = moderate

VITA

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1961: Doctor of Veterinary Medicine
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Iowa State University
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1966: Master of Science
Department of Pathology
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1968: Doctor of Philosophy
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Board Certification:

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Professional Posts:

1961: 1st Lieutenant (VC) U. S. Army
Commanding Officer
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Professional Posts, cont.

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1963: Captain (VC) U. S. Army
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Professional Societies:

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Research:

Giddens, W. E., Jr.: The Pathology of Silo Gas Toxicosis in Pigs. M. S. Thesis, Michigan State University, East Lansing, Mich., 1966.

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