PULMONARY DISEASE IN RATS

A Survey with Comments on "Chronic Murine Pneumonia" *

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The albino rat is less susceptible to acute pulmonary infections, both natural and induced, than other rodents used for laboratory work, and therefore is not commonly used in experimental bacteriologic and virus studies. If ideal conditions of breeding are established, including strict control over general hygiene, nutrition, and disease, rats can be bred which show remarkable freedom from epizootic infections, and in which acute pneumonia does not play a prominent rôle. Acute pulmonary infections in rats, caused by Pasteurella, *Brucella bronchiseptica,* and pneumococci, have occurred with epizootic spread and high mortality, but such outbreaks are uncommon. Nelson (who has conducted the most extensive investigations on pulmonary disease in rats in the United States) mentioned,[1] in 1953, that in 20 years he has observed only one acute epizootic outbreak. One of us (J.R.M.I.) had a similar experience while in charge of an "Animal Breeding Farm" in England (1940–46), in which a self-contained colony of rats was established, productive of more than 20,000 rats a year. However, in our own laboratories in recent years, two acute epizootics of pneumonia have occurred, one caused by type II pneumococci, and the other, mainly by Pasteurella.

In all laboratories in which large numbers of rats are used experimentally, morbidity (less importantly, mortality) due to chronic pulmonary disease is a perennial problem. It may be more striking in commercially obtained rats, for the animals are then of unknown clinical history, and reared under conditions not defined by experimental users. Although the nature of the disease has long been known, until recently it was a confused complex. Pathologists who examine many rats are familiar with the common macroscopic finding of involvement of a part or whole of a lobe, or of several lobes of the lungs by areas of induration and bronchiectasis. Most diseased rats exhibit few, or no, clinical signs of respiratory disease, although in advanced cases there may be snuffles, torpor, rough hair, and dyspnea. These signs are recognized as being unreliable by those dealing with rats, and the lesions are thus usually dismissed at necropsy as incidental findings or cursorily noted as of little moment. This is particularly

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true in experiments not specifically concerned with the lungs, or on occasions when necropsies are done by those with limited experience. In view of the incidence of pulmonary disease in rats, it is remarkable that it is so rarely mentioned as a complicating factor considering the many experiments in which rats are used. We give our own data later, but a few published figures on the incidence can be quoted[1-5] (Table I), and these must be given serious consideration.

TABLE I
Incidence of Chronic Murine Pneumonia

Source	No. of rats examined	Percentage affected
Passey *et al.* (U.K.)[2]	251	51
Cruickshank (U.K.)[3]	200	43·5
Klieneberger and Steabben (U.K.)[21]	268	40
Wilens and Sproul (U.S.A.)[5]	487 (all over 700 days of age)	75
Ratcliffe (U.S.A.)[4]	Not given	75

Whenever the bulk of work with rats concerns chronic toxicity, or toxicopathologic, studies with particular reference to pulmonary damage caused by chemical compounds, the incidence and severity of natural disease become of conspicuous significance. From this standpoint, thousands of rats have been used by our laboratories in a multitude of experiments, and it is apparent to us that the problem of chronic pulmonary disease has been mostly ignored. The opening sentence of a paper by Cruickshank[3] (1948) stated: "In the course of some tentative experimental work on lung diseases it was found that the stock laboratory rats employed suffered from a spontaneous disease of the lungs which made the experiments *useless*." His experience in England and his opinion are not unique. In work of our own type, the difficulty of determining changes caused by a toxic inhalant material from those resulting from, or complicated by, natural disease sometimes has been insuperable. We have thus raised the issue that rats might advisedly be discarded for work involving toxic inhalants, unless the animals are derived from a colony in which chronic pulmonary disease does not exist; and Nelson[6] has shown that it can be eradicated. Unless this is achieved, a more suitable, small, laboratory rodent might be the hamster, which is as fecund as the rat, and seems to be relatively free from chronic pulmonary disease, although susceptible to acute infections.

Survey of Chronic Pulmonary Disease in Rats at Chemical Corps Medical Laboratories

METHODS

Many observations have been accumulated from the large numbers of rats (a total of 3,646 in the years 1953 and 1954) that are necropsied in connection with toxicopathologic experiments. For comparative purposes, however, selected groups of rats (totaling 433) were killed for special examination of the lungs. These were rats which to all appearances were clinically healthy, the younger of which would normally have been issued to investigators. Apart from one group of aged rats, all animals were bred in the Medical Laboratories colony. After sacrifice (usually with ether) the lungs were examined in a collapsed state, or after ligating the trachea before the chest was opened. Sections were made of the lungs of all animals and, along with the hundreds of others from past years, these sections form the basis for our later definitions of the lesions of this disease. All sections were stained with hematoxylin and eosin, and in selected instances with Wilder's reticulum stain, Weigert's elastica, and phosphotungstic acid-hematoxylin. Bacteriologic cultures were made at various times, but not as a routine procedure.

FINDINGS *

Based on gross observations and histologic examination, a general assessment was made of lesions in the lungs related to different age groups, and the results are given in Table II.

Because of the variation in the numbers of animals in the different groups, the different times of the year when the rats were killed, differences in husbandry, and other variables, exact comparisons are difficult.

* *Remarks on Some Normal Anatomical Features of the Rat Lung.* In the rat (also the hamster and mouse) the right lung has three main lobes (apical, cardiac, azygos, and a small intermediate caudal one) exceeding in anatomical mass the left lung, which is a single lobe. There are no fundamental differences between rodents in the descending arborization of the respiratory tree. The right main bronchus is short and abuts from the trachea at a near right angle, while the left main bronchus descends obliquely into the hilus of the left lung; the right main bronchus immediately gives off a shorter trunk for the most cranial (apical) lobe of the lung—the so-called epi-arterial branch. Cartilaginous plates are found in the walls of secondary bronchi of rabbits and guinea-pigs, but, in the rat, mouse, and hamster, cartilage fades from the walls of the bronchi as soon as they enter the lungs. The adventitia of the pulmonary veins in the lungs of the rat and mouse shows a peculiar normal feature of striated muscle fibers contiguous with those of the heart.[7-9] These cardiac muscle fibers are present in the larger intrapulmonary branches, but may be seen in small veins (Fig. 1). In rabbits and guinea-pigs such fibers are reputed to surround only the short extrapulmonary veins. This might have some physiologic significance, but has been given little attention, other than in the German literature on histology. It might, however, be of some significance pathologically, in the sense that infections with a predilection for heart muscle could spread by contiguity to the lungs. (See work by Pappenheimer and Daniels[10] on a transmissible "rickettsial" disease of mice causing myocarditis.)

Some general conclusions can be drawn. The figures are in agreement with the experience of other workers[1-5] on the high incidence of a disease which may not kill, or even cause any apparent disturbance in health, and which becomes more frequent with advancing age. It is apparent that the macroscopic appearances alone may not lead to the detection of this disease. Of 433 rats of all ages, 216 showed normally pink crepitant lungs (69 of these were 3 weeks or less old).

Likewise, the number considered normal histologically would be reduced if a more critical examination were adopted. For example, if microscopic collections of lymphoid tissue were considered pathologic,

TABLE II

Range of Normality in Lungs of Rats

Group	Age	Numbers	Gross		Microscopic
			Normal	Pneumonia	"Excess" lymphoid tissue*
A	Under 3 weeks	19	19	0	2
B	3 weeks	50	50	0	50
C	2 months	39	18	21	18
D	3–4 months	7	4	3	4
E	4 months (diet exp.)	95	11	84	11
F	5–6 months	22	14	8	10
G	7–12 months	25	21	4	20
H	12–15 months	103	35	68	28
I	15–17 months	23	12	11	12
J	Over 12 months (commercial)	50	32	18	24
	Totals	433	216	217	179

* In lungs which were grossly normal. Grossly normal lungs which were devoid of "excess" lymphoid tissue were found in only 37 rats.

the number of so-called normal lungs would be smaller. Lymphoid tissue in any amount is unusual (yet occasionally seen) in suckling rats, but microscopic aggregations were observed in all of the 50 newly weaned (21 days) rats. In rats with chronic disease there is always marked lymphoid participation. This lymphoid tissue involves the walls of the bronchi, and is found in perivascular locations, particularly about arterioles and venules. There is proportionately less involvement of the bronchioles. Involvement of the bronchial tree varies in degree from small to massive aggregations of lymphocytes, infiltrating the deeper parts of the bronchial mucosa and pushing aside and obliterating all structures, with cleavage and disappearance of the normal elastic and muscular fibers (Fig. 2). Examination of the stained sections,

simply by the naked eye, shows the bronchial arborizations outlined by broad, blue-stained, interrupted sleeves (Fig. 3). This lymphoid nodular lesion, which might well be referred to as a follicular bronchitis, was present in very many rats without an associated bronchopneumonia. Hence, we can understand the views previously expressed about its rôle in the production of bronchiectasis (e.g., Passey *et al.*[2] and Cruickshank[3]). These data indicate the extremely high incidence of all stages of a chronic disease complex, which may be found in almost any group of our laboratory rats and in other colonies as well.*

However, the criteria which were used to compose Table II serve only as a rough separation of normality from abnormality. If a more strict yardstick is utilized, we find that the lungs of most adult rats are significantly abnormal. (There was no difference found by us between our own rats and those obtained commercially.) If comparisons are to be made by others between our figures and their own, they will be largely invalid unless comparable detailed histologic examinations are made.

An illustration of the importance of this conclusion can be found in some toxicity experiments made in our own laboratories. Previously, there had been preliminary reports that chronic bronchitis ensued after protracted inhalation exposure to diborane (B_2H_6) in low concentrations. It was manifest that such a conclusion would be difficult to substantiate, inasmuch as the "normal rat" has in most instances some degree of chronic bronchitis, and studies were made to clarify this. Four groups of 20 rats were selected according to age (5 weeks, 9 weeks, 6 months, and 1 year old). These groups were divided equally so that 10 animals served as untreated controls and 10 were exposed to diborane (6 ppm.), 4 hours per day, 5 days per week, for 6 weeks. At the end of this period all animals were sacrificed and the lungs subjected to detailed histologic examination. A sample of the protocol used in the critical histologic evaluation of the lungs is given in Table III, and needs little comment. Each feature was noted as present or absent, and, when applicable, graded from 1 to 4. We were unable to confirm the conclusion that chronic bronchitis was a toxic effect, and in only one respect was a predictable difference noted between treated and untreated rats. Foamy macrophages were found more frequently, and in greater numbers, in the alveolar spaces of animals exposed to diborane, a finding for which we have no explanation. It should be

* An examination, for instance, of the lungs of 50 male and female rats, reputed to be breeders over 15 months old, received from a well known rat colony in England, revealed an incidence of 11 with marked macroscopic lesions, and 28 with histologic lesions, including excess lymphoid tissue.

TABLE III
Schema for Evaluation of Histologic Changes in the Lungs

	1	2
Lymphoid infiltration, bronchial tree		
Mucosal		
Disruption of basement membrane		
Submucosal		
Bronchial musculature		
Follicle formation		
Limited to large bronchi		
Limited to small and medium bronchi		
Limited to bronchioles		
Extra-cartilaginous		
Perivascular lymphoid		
Large vessels		
Small vessels		
Thickening of alveolar walls		
Inflammatory		
Hyaline		
Patchy		
Diffuse		

	1	2
Alveolar spaces		
Acute exudate		
Chronic exudate		
Macrophages		
Patchy		
Diffuse		
Pleural thickening		
Fibrosis		
Focal		
Diffuse local		
Diffuse general		
Bronchial dilatation		
Bronchiolar dilatation		
Exudate in bronchi		
Destruction of bronchial epithelium		
Epithelial regeneration		
Epithelial hypertrophy		

noted, however, that such foamy macrophages often are found in the alveolar spaces (Fig. 4) either singly or in solid masses, infrequently containing a brownish pigment, in all age groups from suckling rats on, and in otherwise healthy animals.

Comments on "Chronic Murine Pneumonia"

Macroscopic Appearances

The lesions may be discrete and affect a part or all of a lobe, or they may be disseminated. If discrete, the involved area is gray to red, indurated, and somewhat depressed. When a whole lobe is involved, it is shrunken, has a cobbled surface, and is rubbery. Disseminated foci generally are small, sharply circumscribed, reddish brown, millet-like masses (Fig. 5). These areas of induration cut easily and the exposed surfaces appear flat, dry, and homogeneous. There may be cyst-like spaces, which are actually dilated bronchi filled with mucoid or mucopurulent material. The lesions progress slowly, and may be of long duration before the lung is affected to the extent of causing respiratory incapacity, which in rats is still difficult to measure in a clinical sense. In late lesions, the affected lobe, or lobes, are markedly distorted by nodular masses which often appear as pinkish or pearl-gray protuberances. These marked bronchiectatic areas are filled with caseous débris (inspissated exudate), and superficially may resemble abscesses (Fig. 6). These latter lesions have not been particularly frequent in our own experience, but in our laboratories, rats used for experiment and necropsied are seldom old animals. It is noteworthy that pleural adhesions or empyema are relatively rare findings, an observation which will be commented upon later. It is rare, indeed, to find a chest cavity in the rat in which the entire thoracic contents are matted together. The severity of the morbid process is not necessarily reflected by severe clinical signs, and many observers have expressed astonishment at the extent of pulmonary disease in rats which appeared in good condition when alive. As the disease advances, however, unthriftiness may develop, with roughened hair, loss of weight, snuffles, and wheezing. There is no tendency for any particular lobe to be affected more frequently, as shown by the lobe involvement in 100 diseased rats (Table IV).

Microscopic Appearances

From the foregoing, it can be seen that much of the histologic picture can be accounted for by the variation in the type and degree of lymphoid infiltration. That this lesion begins very early in life is apparent from finding microscopic lymphoid collections in some suckling rats. This is almost a universal effect by the time of weaning. Involve-

ment of the bronchial tree is first noted in its proximal parts, but as the rat ages, the process extends to embrace the peripheral components. Bronchiolar disease at this stage can be said to be relatively mild. The lymphoid tissue implicates all layers of the bronchus and at its height is of a massive kind, in which formation of primary follicles always is found.

Perivascular collections of lymphocytes occur as early as peribronchial involvement, and there need be no quantitative correlation between the two. Perivascular infiltration is most conspicuous about small vessels—arterioles and venules — and frequently large cuffs and sleeves are found (Fig. 7). In younger rats, eosinophils are fairly numerous and pigment-laden phagocytes sometimes may be present.

TABLE IV
Involvement by Lobes in 100 Rats with Gross Lesions of Chronic Murine Pneumonia

Left lobe	25
Right cranial lobe	29
Right middle lobe	30
Right caudal lobe	31
Right intermediate lobe	26
Total number of lobes involved	141

Parenchymal involvement by lymphocytes tends to be focal at first, in the form of a patchy chronic interstitial pneumonitis (Fig. 8). Locally diffuse lesions soon make their appearance, sometimes a whole lobe showing inflammatory thickening of the alveolar septa. Inflammatory cells within the alveolar spaces usually are less conspicuous. The presence of foamy macrophages (sometimes with pigment) has been mentioned. The changes of bronchopneumonia seem to be relatively *infrequent*. Far more often a picture simulating bronchopneumonia is produced by a combination of atelectasis and interstitial pneumonia. It is true that focal abscesses are observed occasionally, but the correctness of the conclusion stated is suggested by the unique rarity of adhesions or empyema.

As the process advances with age, the picture becomes more complex. Dilatation of the bronchial tree in a segmental fashion and accumulation of secretions increase, and there is more and more atelectasis. Peribronchial fibrosis can be discerned readily, but what, at first glance, may appear to be extensive pulmonary fibrosis, may turn out to be marked atelectasis (Fig. 9). The bronchiectatic process may continue to a stage in which a lobe, or a whole lung, consists of multilocular spaces surrounded by collapsed and compacted parenchyma (Fig. 10). It is in the more advanced stages that bronchiolectasis may appear.

Many authors have commented upon the occurrence of squamous metaplasia of the bronchial epithelium, although it has not been common in our experience. It may be limited to a segment, or be widespread, even extending into alveolar ducts and alveoli, and its true nature should be recognized. Passey *et al.*[2] (and others) have pointed out that such metaplasia was mistaken for metastases by Fibiger[11] in his pioneer work on the relationship of a supposed gastric carcinoma to the parasite *Gongylonema neoplasticum*. This change, with keratinization, may be very prominent and has some superficial resemblance to epidermoid carcinoma.

Etiology and Pathogenesis of Chronic Murine Pneumonia

The history of work on the etiology of chronic murine pneumonia is reminiscent of that on distemper in dogs. For years, the murine disease was commonly contended (or assumed) to be of bacterial origin, although the exact etiology was uncertain, and its pathogenesis was not clear. The most extensive investigations have been those of Nelson[1,12-17,19-20] at the Rockefeller Institute, New York, who demonstrated that the disease was not primarily bacterial in origin, and has shown recently that the disorder is initiated in the young rat by a virus. Saxton and Kimball[18] suggested that the cause is not directly or indirectly related to dietary deficiency factors. It seemed, however, that a comparison of rats on a standard cube diet should be made with animals raised on a relatively simple but adequate diet. To this end, 100 rats were weaned at 21 days and divided into two groups. One group received our standard commercial cube diet and the other received bread and milk with weekly supplements of cod-liver oil. All animals were sacrificed at the age of 4 months and examined. Our findings indicated no significant differences as far as the lungs are concerned. However, it is worth mentioning that the rats fed bread and milk were beautiful smooth-coated animals; they were larger on the average but not obese, cleaner, and more active than the animals fed the standard diet.

Many attempts, over the past 40 years, have been made to prove a specific bacterial origin and a variety of microorganisms have been isolated, a few of which might be listed: a diphtheroid (*Bacillus muris*), a Gram-negative streptothrix (which was probably *Streptobacillus moniliformis*), *Br. bronchiseptica* (also once thought to be the cause of canine distemper), *Pasteurella muricida,* and *Streptobacillus moniliformis* (*Actinobacillus actinoides*).

Much work also has been carried out along the same lines by Miss

K. Wilson in the Bacteriology Section of our own laboratories, and the following is a brief summary of her findings. Reference has been made that in one epizootic outbreak, in which a large number of deaths occurred, type II pneumococci were isolated from the heart's blood and pulmonary lesions. After the epizootic subsided, 70 per cent of the survivors were found to be carrying the same organism in their throats. Six months later, in another outbreak, the predominating organism recovered was Pasteurella, although from some rats type II pneumococci also were isolated. The rats at that time were housed in the same room as rabbits, which are notoriously susceptible to pasteurellosis, and may have been the origin of the murine infection. Cultures were made also from 43 rats of varying ages from 5 weeks to over 1 year and a variety of organisms recovered, among which were *Br. bronchiseptica* (12 times), *Pasteurella multocida* (4 times), a diptheroid, staphylococci, and a hemolytic streptococcus, but no pleuropneumonia-like organisms at any time. The lungs of the very young rats were relatively free from pathogenic organisms.

Because of the high carrier rate and lack of more positive evidence, an etiologic relationship remained unsubstantiated. Without exception, these organisms (in the experience of other workers) were not capable of invoking the disease experimentally in its entire chronicity. It became apparent that the lesion was initiated by some other factor and then was followed by secondary bacterial invaders, for none of the bacteria isolated fulfilled the requirements of the original postulates of Koch. One great handicap in critical experimental study patently stemmed from the lack of rat colonies free from the natural disease.

The confusion of the earlier work on etiology has been clarified by Nelson.[16,19,20] He demonstrated that there are two independent diseases involved in the chronic respiratory complex of rats by eradicating one—the infectious catarrh—without an effect on the incidence of the chronic pulmonary disease.[17] The infectious catarrh, also a common disorder of the mouse, has been shown by Klieneberger and Steabben[21]* to be caused by pleuropneumonia-like organisms. This disease, occurring in young and adult rats, is of slow onset, long duration, and may involve the middle ear, occasionally with contiguous infection of the inner ear (labyrinthitis), which is manifested by circling and twisting in the living rat. In 1946, Nelson[14] presented experimental proof that the primary factor in chronic pulmonary disease of rats was a virus. He showed that the disease was transmissible

* Reference 1 also may be consulted.

to mice by a filterable suspension of lungs from affected rats, that the agent was effective at dilutions of 10^7, that it was removed by centrifugation at 9,000 r.p.m. for 30 minutes, and that, although it could not be cultivated in chick embryos, it remained active for 3 months in the frozen state (i.e., as long as it was kept). The infection was established regularly in mice by nasal instillation, and by direct contact, but bronchiectasis was not produced in mice kept as long as 32 weeks. The virus was demonstrated in practically all breeders, and was transmitted by the female to her young soon after their birth, infectivity being possible throughout the life span of the rat. There is no doubt that these two infections have coexisted in most breeding colonies (England and the United States), so that there can be infectious catarrh and chronic pneumonia or a combination in most colonies. More recently, Nelson has found that the mouse carries a similar virus naturally.[22]

The pathogenesis still is not clear, particularly in regard to bronchiectasis. It has been postulated by some that the bronchiectasis is due to plugging of the bronchi by mucus followed by the growth of microorganisms.[23] Others have felt that mucus secretion is a result, not the cause, of bronchial obstruction, the latter being initiated by massive lymphoid aggregates to the extent of forming polypoid masses circumscribing and constricting the bronchial lumen.[3] Thus it is that a combined viewpoint of obstruction and bacterial infection has become accepted as an explanation of bronchiectasis. Our contention, however, is that this concept does not explain adequately the production of bronchiectasis, because we have not been impressed that lymphoid proliferation, even in its most massive proportions, significantly constricts the bronchial lumen. This process may promote stasis by increasing the rigidity of the bronchial tree, but such stasis, uncomplicated by any other factors, would not be expected to produce more than very moderate dilatation of bronchi.

Patchy interstitial pneumonitis, occurring early in the course of chronic pulmonary disease, was mentioned earlier, and this is often a salient change in alveoli contiguous to the bronchial tree, accompanied by varying degrees of atelectasis. What one frequently observes then, particularly in the arborizations of the bronchial tree beyond the primary bronchus, is a process of lymphoid infiltration of the bronchial wall accompanied by peribronchial pneumonitis and atelectasis. The sequence of this is peribronchial fibrosis, and the picture produced is that of early bronchiectasis. The factor of stasis is now exemplified by a somewhat dilated structure with a swollen (hypertrophied) epithe-

lium, in which ectasia continues with concomitant increasing atelectasis. The chronic disease, as we know it, is thus a slowly progressive process over many months. This progression of tissue changes is more in keeping with those leading to bronchiectasis in man.*

Establishment of Rat Colonies Free from Chronic Murine Pneumonia

All workers who have been in charge of rat breeding colonies recognize the inherent difficulties in controlling chronic pulmonary disease. The work of Nelson[1,6,12-17,19,20,22] showed why some of those difficulties existed, in spite of possibly excellent conditions as to housing, cage design, diet and watering systems, animal husbandry, and the personnel handling the animals. None of these factors can have any direct significant influence on the presence of viral or other infections of such low virulence and protracted chronicity. Nevertheless, they are fundamental for the maintenance of an otherwise healthy colony. From personal experience (J.R.M.I.), none of the modern chemotherapeutic agents has a demonstrable prophylactic or therapeutic value. While prophylactic therapeutic measures by drugs might be possible in small colonies, they would be impractical for large ones with a production goal of more than 50,000 rats a year, unless medication were possible in drinking water or pelleted diet. In rats to be used for the study of experimental infection, such measures would be unwarranted and undesirable. Total eradication must be achieved by other measures, such as those proposed by Nelson.

Nelson[6] (1951) reported that, starting with young breeders, obtained by caesarian section and then hand-fed, a breeding colony of rats (not a germ-free one) was established for 2 years with absence of virus infection, pleuropneumonia-like organisms, and *Streptobacillus moniliformis*. Examination of adult year-old rats from this colony showed no evidence of disease in the lungs, middle ears, or nasal passages. Young rats from this colony were shown to be susceptible to the murine pneumotropic virus, with lesions induced identical to those of naturally occurring chronic pneumonitis. We suggest, if the breeding system of Nelson is tried, that a number of small autonomous units might be better than a single large one, because if disease prevention breaks down in one unit, then all is not lost. Finally, in such an operation, all principles, well known and proved by experience to be necessary for the successful large-scale breeding of any laboratory species, should be rigidly adhered to, viz., those concerned with nutrition, cage

* We realize that our descriptions almost certainly cover the chronic effects of both infectious catarrh and murine pneumonia.

sterilization, disease prevention, adequate ventilation, and record-keeping; all animal colonies should be quarantined to avoid possible contact with infection from wild vermin, and should have no physical proximity to experimental animals.

Summary

Acute epizootic infections in laboratory rats are relatively rare. Chronic murine pneumonia, on the other hand, is a perennial problem. With a high morbidity but low mortality, it is of considerable significance in experimental studies on the lungs, particularly in assessing the pathologic effects of toxic materials. The complex of chronic lesions may render the task of differentiating naturally from experimentally acquired lesions extremely difficult. The incidence of the clinically silent lesions reported in the literature ranges from 50 to 75 per cent of rats which appeared in normal health. Our figures are higher due to a more critical pathologic examination. The macroscopic and histologic appearances of the chronic pneumonia complex are described. Exception is taken to previous views on the pathogenesis of the lesion and consideration is given to recent studies by others on the etiology of the disease, particularly to its probable viral origin.

Addendum by the Senior Author

Establishment of Pulmonary Disease-Free Colony of Rats

After this paper was completed, an attempt was made to produce a colony of rats free from chronic murine pneumonia. Through the kindness of Dr. John B. Nelson (Rockefeller Institute, New York), 56 rats, 1 to 3 months old, were obtained on April 4, 1955. Eleven paired matings were set up. No attempt was made at establishing fertility records. By October 1 nearly 100 rats from the first, second, or third generation had been supplied the Armed Forces Institute of Pathology, Washington, D.C., where a separate colony of brother-sister matings has been started under Lt. Col. T. C. Jones, V.C.

The animals were not kept under strict isolation. All rats were kept on wire without bedding; paper was provided to pregnant animals for nests; the usual principles of hygiene and sterilization of cages were adopted; standard pelleted diets were given with a supplement of fresh tinned dog food to pregnant "pairs." Routine mortality of the progeny, which were weaned until October and which were over 1 day old, was almost nil. All original parents (the 11 pairs) were killed when about 9 months old, and when at least three or four litters had been born from each female. Many of the progeny now have been examined. Of the remaining rats from Dr. Nelson, 8 were killed at 3

months, and 26 at 6 and 7 months of age. Thorough routine necropsies and other examinations were made of all rats. The results were as follows:

The rats were free from Salmonella as determined by bacteriologic examination of feces, and were practically free from gastro-intestinal helminthic infestation. No middle ear infection occurred. Splenectomy of some rats revealed an absence of Bartonella infection. Examination of pieces of skeletal muscle, esophagus, and heart showed an absence of sarcosporidia and no myositis or myocarditis of any kind. In not a single rat were lesions found, macroscopically or microscopically, which bore resemblance to any feature of the complex of chronic pneumonia. The absence of peribronchial lymphoid tissue, except microscopic collections, supported our belief that such tissue is not a "normal species histologic difference for rats." (We think lymphoid proliferation in the rat lung may be the initial response to the Nelson rat pneumonia virus.) Bacteriologic examinations were made of the left pulmonary lobe of all rats (the four right lobes being reserved for histologic study); no organisms were recovered which were of undisputed pathogenic importance, and neither pleuropneumonia-like organisms nor *Br. bronchiseptica* were isolated (both species have been recovered many times within recent months from our "normal" stock colony).

We conclude that it is not a difficult matter to raise *"normal rats"* in a normal environment free from infections, particularly those which affect the lungs and are therefore of importance to workers studying toxic inhalants. There are no intrinsic difficulties in raising a disease-free rat colony. It costs no more to do so in terms of time, labor, and food. Further, compared to the mouse, the rat is an amazingly resistant animal.

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[*Illustrations follow*]

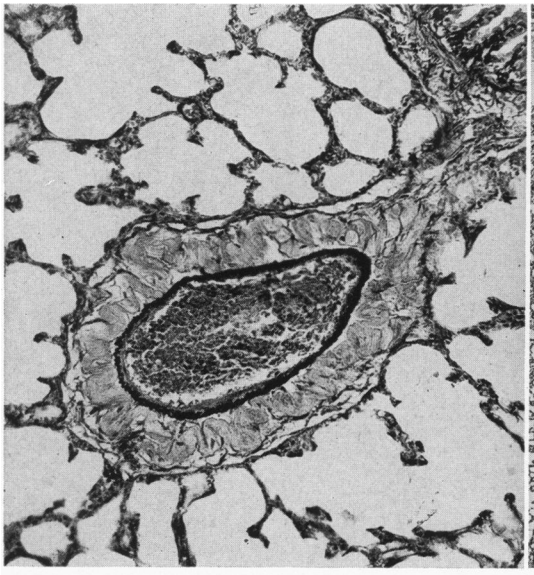
LEGENDS FOR FIGURES

FIG. 1. Lung, rat. A vein within the parenchyma shows striated cardiac muscle fibers in the adventitia. Weigert's elastic method. × 85.

FIG. 2. Lung, rat, 6 months old. Massive lymphoid aggregates around bronchi with disruption of the walls. Hematoxylin and eosin stain. × 35.

FIG. 3. Lung, rat, 2 months old. Low-power view of broad, irregular sleeves of lymphoid tissue outlining the bronchial tree. Hematoxylin and eosin stain. × 4.

FIG. 4. Lung, rat. Focal collections of foamy macrophages in the alveolar spaces of an otherwise normal structure. Hematoxylin and eosin stain. × 435.



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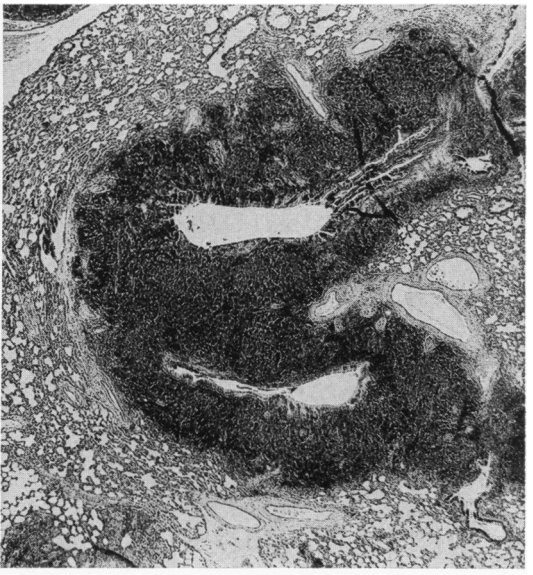

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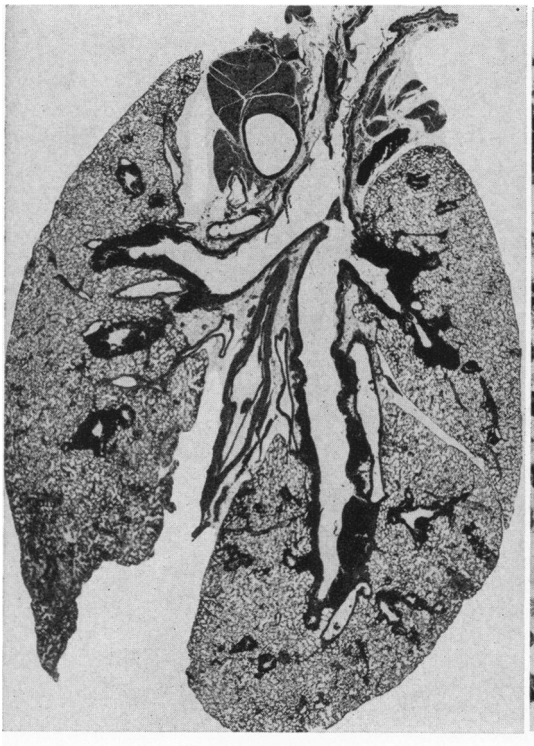

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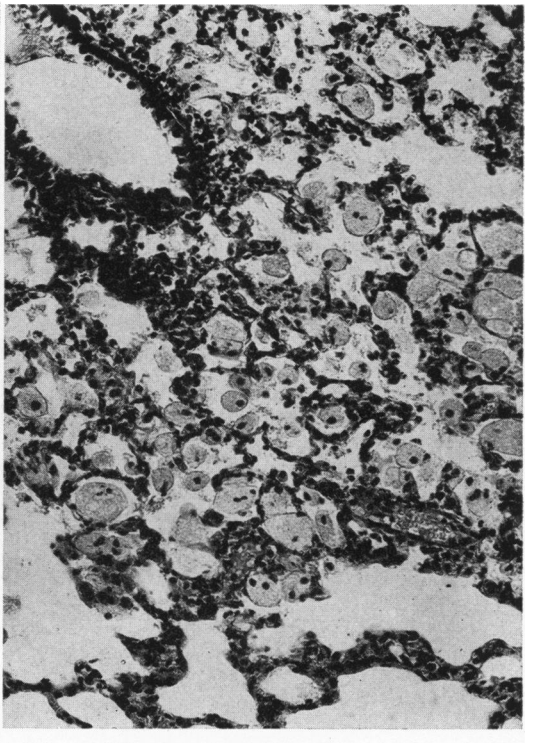

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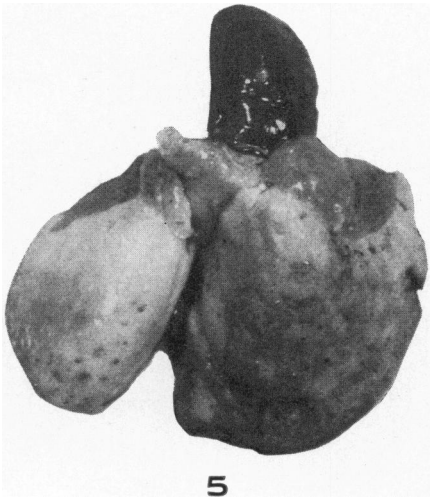

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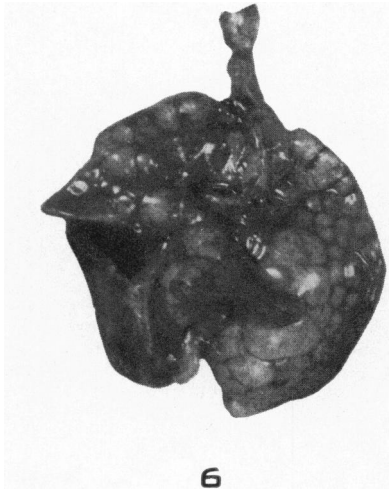

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FIG. 5. Lung, rat. Complete induration of right apical and middle lobes and apex of left lung; scattered miliary lesions in other lobes.

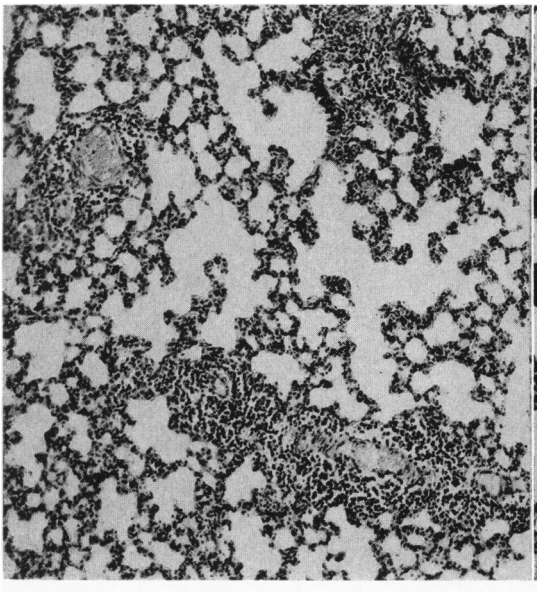
FIG. 6. Lungs, rat, 3 months old. Multiple lesions representing what appears to be a fulminating bronchiectasis; infrequent, but occurs in young animals.

FIG. 7. Lung, rat, 3 months old. Perivascular cuffing. Hematoxylin and eosin stain. × 85.

FIG. 8. Lung, rat, 3 months old. An area of interstitial pneumonitis. Hematoxylin and eosin stain. × 345.

FIG. 9. Lung, rat. Typical lesion of chronic pneumonia showing complete atelectasis of a lobe. Hematoxylin and eosin stain. × 35.

FIG. 10. Lung, rat. Advanced lesion of chronic pneumonia; all right lobes are involved in bronchiectasis. Hematoxylin and eosin stain. × 4.



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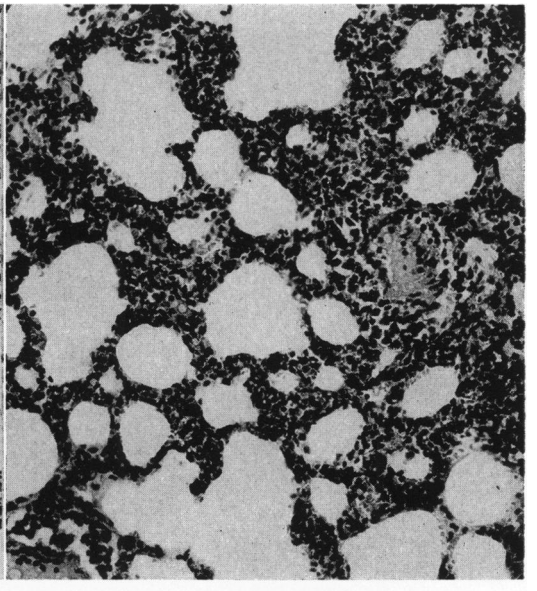

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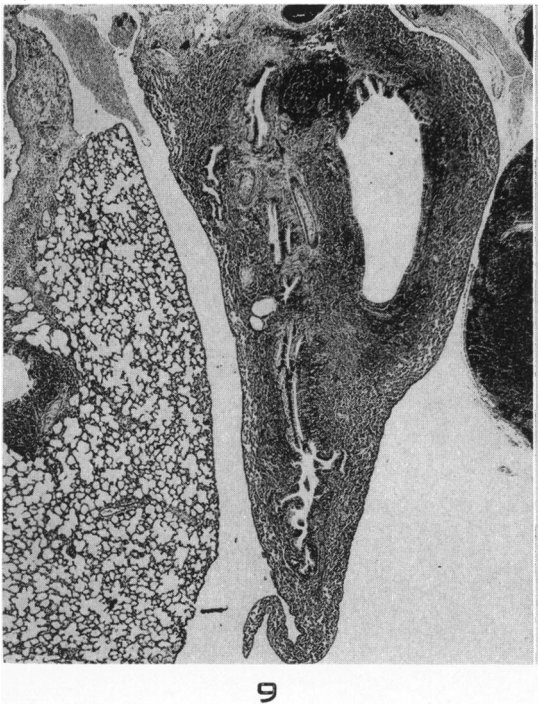

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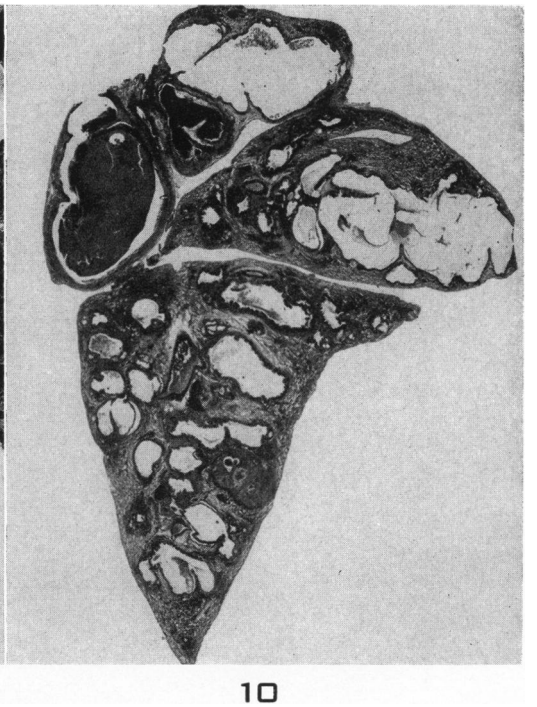

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