STUDIES IN THE PATHOLOGY OF THE RESPIRATORY SYSTEM OF THE DOG IN VIRUS INFECTIONS

By

Adolf Michael Watrach, M.R.C.V.S.

Summary

The viral involvements of the respiratory system in animals and man form a morphologically distinct group of pathological conditions. The changes produced in the lung tissue by the action of viruses present a remarkable picture and have attracted much attention in the last three decades.

Among the canine viral infections, distemper appears to be the only disease entity which consistently shows its effects on the respiratory tract and displays pulmonary lesions characteristic of all viral pneumonias.

The present study was undertaken with the intent of supplementing and enlarging upon the available but scanty information on the pathology of the respiratory system in distemper. It embodies the results of observations on the morbid anatomy and histopathology of the entire tract and bacteriology of the lung. The material on which the findings are based comprises thirty naturally occurring and twenty experimental cases of the disease. In the course of the investigations, special attention has been paid to the histopathological aspect of pneumonia. Some of the changes observed involve the long debated issue of the ultimate nature of the alveolar lining and required for their interpretation additional, electron microscopic studies on the normal structure of the pulmonary alveolar wall.

Clinical signs of the disease and lesions in the respiratory tract were considerably more severe in the naturally occurring than in the experimental cases of distemper. Only mild malaise and slight changes were seen

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

in the dogs suffering from experimental infection. These observations are in accord with those of previous workers.

The gross lesions encountered in the clinical cases were generally most prominent between the third and fifth weeks of illness. The abnormalities observed in the pulmonary tissue included (a) congestion, often focal in distribution; (b) oedema; and (c) occasional subpleural consolidation. No oedema of the lung was found in the experimental dogs.

Bacterial involvements of the lung were much more frequent in "street" distemper. Of the twenty experimental cases, only five yielded positive cultures, whereas most of the naturally infected dogs showed some degree of secondary infection. The strains of bacteria isolated from these cases included Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, staphylococci, streptococci and, in one dog, Brucella bronchiseptica.

One of the salient features of pneumonia was the interstitial cellular reaction. It manifested itself as a mononuclear infiltration of the bronchiolar walls, the adjacent interalveolar septa and, often, of the perivascular connective tissue. Other changes intimately associated with the disease process included hyperplasia of the alveolar epithelium, accumulation of a chiefly mononuclear exudate in the region of gaseous exchange, and oedema of alveolar and, not infrequently, of perivascular spaces.

The combined observations on the clinical and experimental cases of pneumonia revealed that the first tissue changes took place at the time of the initial rise in body temperature. The early signs of viral activity were shown not only by the cellular exudative phenomena but also by the appearance of cytoplasmic inclusion bodies in the bronchiolar epithelium. A few of these bodies were seen on the first day of illness.

Quite early in the disease process the oedema fluid started to collect in alveoli and around smaller vessels. Epithelial cells of some of the bronchioles showed a degree of glycogenic infiltration which in later stages resulted in partial denudation of the mucous membrane. With the progress of the disease, many mononuclear cells accumulated in the affected areas, and occasional polymorphonuclear leucocytes appeared in the alveolar spaces. Some of the intra-alveolar macrophages enlarged considerably and displayed multiple nuclei.

At the end of the first week, the lining of the alveoli showed signs of proliferative activity. As a result of this process, many peribronchiolar and subpleural alveoli became lined by a pavement of usually flat cells. Such "epithelialisation" was evidently due to hyperplasia of the alveolar epithelium. This observation is supported by the electron microscopic study of the normal lung which revealed that the pulmonary alveolar wall is lined by an uninterrupted although very tenuous epithelial membrane. The average thickness of this covering is in the range of 1,500-2,000 Å.

In some instances, when proliferation of epithelial elements was pronounced, bizarre and multinucleated cells could be seen. "Epithelialisation" of alveoli attained significant proportions with the third week of the disease, but began to disappear after the fifth week. At the height of infection, large areas of the lobes were sometimes involved by coalescence of the peribronchiolar and subpleural foci.

As the acute phase of pneumonia subsided, the elements of inflammatory and epithelial proliferative processes were removed from the alveolar spaces. A small number of mononuclear cells, however, often persisted in peribronchiolar and perivascular sites. After three months, minute accumulations of lymphocytes and macrophages were still present at an occasional bronchiole

or venule. In 50 per cent of the cases a degree of intrabronchiolar and intraductal organization was observed. The process evolved apparently in the second week of illness and, on occasion, was quite pronounced in the later phases. Sometimes it was accompanied by an appreciable degree of fibroplasia in the adjoining alveolar spaces and, more often, in the interalveolar septa.

Many of the pulmonary macrophages displayed a characteristic staining reaction with periodic acid-Schiff technique, which is thought to be indicative of some intracellular changes associated or concurrent with the phagocytic activity of these cells.

Cytoplasmic inclusion bodies appeared very early in the disease and often were quite numerous at the height of illness. After the seventh week they diminished rapidly in number and only a very few could be detected in the lung following the tenth week of infection. Their presence is regarded as pathognomonic for distemper. A recent demonstration of the antigenic specificity of these inclusions seems to indicate that they represent a phase in the intracellular growth of the virus itself.

STUDIES IN THE PATHOLOGY OF THE RESPIRATORY SYSTEM OF THE DOG IN VIRUS INFECTIONS

By

ADOLF MICHAEL WATRACH, M.R.C.V.S.

Department of Veterinary Pathology

THESIS

Submitted for the Degree of Doctor of Philosophy
in the Faculty of Medicine at the
University of Glasgow

October 1957

TABLE OF CONTENTS

PREFACE

The essential character of the tissue reaction to the invasion of viral agents is well manifested by the defensive, inflammatory processes in the respiratory system. The changes induced in the lung, particularly in its terminal airways, form a very distinct picture, considerably different from that presented by bacterial infections. The nature of these alterations has been a subject of much study, especially in the past three decades.

Among the viral infections of the respiratory tract, pneumonia of canine distemper holds a rare position. It not only displays the typical and prominent changes in the lung, but is also intrinsically associated with general, often severe, systemic illness; it constitutes an important phase of the highly complex disease rather than an independent condition. Of the known canine viral infections, distemper appears to be the only well recognized pathological entity which invariably shows involvement of the lung. The view held by some workers that there exists a separate although not entirely unrelated condition, termed "hard pad" disease, is not supported by many recent European and American observers.

Much has been written about distemper in the last fifty years. Many aspects of that disease have been described and new concepts have been put forward. And yet, despite the considerable volume of literature, there are surprisingly few communications dealing with the pathology of distemper pneumonia. Most of the existing reports offer but a fragmentary picture of the changes, or do not dwell comprehensively upon the subject. It was felt, therefore, that a detailed account of phenomena occurring in the respiratory

system of dogs suffering from distemper was needed to augment the available information.

The present study, undertaken with that intent, embodies the results of observations on morbid anatomy, histopathology, and bacteriology of the respiratory tract in distemper and is based on thirty clinical and twenty experimental cases of that disease.

In the course of observations, particular attention has been focused on histological changes in the lung. Some of these alterations, evidently common to many viral pneumonias, yield a remarkable and characteristic picture and involve long debated issues of the ultimate nature of the alveolar lining. Consequently, an exposition of the fundamental problems of structure of the pulmonary alveolar tissue was thought essential for the interpretation of changes encountered in the distemper-affected lung.

Electron microscopic studies, entered upon for that purpose, seem to have provided the answer to a controversial subject of histology: the structure of alveolar wall. They showed that the alveolar lining, so enigmatic in the normal lung but frequently conspicuous under pathological conditions, is formed by an apparently continuous layer of cells, attenuated to the extreme degree between the nucleus-bearing moieties. Identical observations have been made concurrently by other workers. The implications of this finding are of considerable significance. They would indicate that the process of alveolar "epithelialisation" is essentially that of hypertrophy and proliferation of the existing epithelium.

Another phase of this work revealed that mononuclear cells which constitute the predominating element of inflammatory reaction to the viral agent in the lung exhibit, in many instances, a characteristic histochemical reaction apparently closely associated with their enzymatic and phagocytic

activity. This distinctive tinctorial reaction is evidently common to many active elements of the macrophage system.

The results of the study pertaining to the histopathological observations indicate that distemper pneumonia is characterized by (1) a frequent occurrence of intra-alveolar and perivascular oedema, (2) often considerable and extensive hyperplastic alveolar epithelial changes, and (3), in common with many other viral pneumonias, by a preponderately mononuclear infiltration of the interstitium.

A histopathological survey of some animal and human viral pneumonias conducted for the purposes of comparative pathology reveals that tissue response to the viral agent in the examples of pneumonia studied is similar, if not often identical, in its nature and character. Only a few animal pulmonary disease entities, apparently of viral origin, show a somewhat different morphological picture.

While many observations presented in this work are not entirely novel in the field of histopathology of viral pneumonias in general, it is believed that some of the findings, especially those mentioned above, form a further contribution to the understanding of pulmonary changes not only in distemper, but also in other viral pneumonias.

These studies were initiated at the Glasgow University Veterinary School, Department of Veterinary Pathology, and by permission of the Senate, have been continued at the College of Veterinary Medicine, University of Illinois, United States of America. The author wishes to express his deep indebtedness to the supervisor of this study, Professor J. W. Emslie of the Department of Veterinary Pathology, University of Glasgow Veterinary School, for his invaluable guidance and much appreciated help extended throughout the course of these investigations. Sincere thanks are also due to

Professor Wishart of the Faculty of Medicine. Mr. R. S. F. Campbell, M.R.C.V.S., of the Department of Veterinary Pathology, University of Glasgow Veterinary School, by stimulating discussions of many problems and useful suggestions, assisted the author considerably in this work. An acknowledgment is also due to Mr. I. M. Lauder, M.R.C.V.S., of the same School, who furnished all cases for the preliminary observations. The Graduate Faculty of the College of Veterinary Medicine, University of Illinois, by providing the facilities in the Departments of Veterinary Pathology and Clinical Medicine, made the continuance of these studies possible. Dr. C. C. Morrill, acting as a representative of the Graduate Faculty in the capacity of an interim supervisor, helped the author greatly by his freely given advice and constructive criticism. Drs. R. E. Witter, A. G. Schiller, and D. Maksic of the Clinical Medicine Department supplied most of the clinical cases. Their cooperation is gratefully acknowledged. Special thanks are also due to Drs. J. O. Alberts, L. E. Boley, and P. D. Beamer for their help in various ways.

Electron microscopy of the normal lung of the rat was conducted in the Departments of Chemistry and Chemical Engineering, University of Illinois, with the invaluable cooperation and masterful technical assistance of Dr. A. E. Vatter. His contribution is much appreciated.

Finally, the author's thanks are extended with much pleasure and gratitude to his wife, who by her constant encouragement, unfailing sympathy, and advice in technical matters, helped greatly in getting this work accomplished.

HISTORICAL INTRODUCTION

Morbid Anatomy and Histopathology

Since the beginning of the nineteenth century, when first reports on distemper appeared in Britain, a body of information on the pathology of this canine contagion has accumulated, slowly at first but with increasing energy in later years.

Early writings dealt mostly with the clinical signs, treatment and control of the disease, and often carried little emphasis upon changes observed at autopsy. Some of these contributions, however, are still informative. It was Jenner (1809) who first described intelligently not only clinical but also morbid anatomical signs. Pathological changes were also recorded by Blaine (1817) in his "Canine Pathology," and by Youatt(1830; 1845). The latter writer mentioned such findings as marked inflammation of the mucous membranes of the nose, frontal sinus, pharynx, and trachea. "In the lung," he wrote in 1830, "we find inflammation of the bronchial passages, or, in a few instances inflammation of the substance of the lung"...occasionally with..."tubercles and vomicae".

In the latter part of the nineteenth century, when the science of bacteriology flourished, much attention was focused on the aetiology of distemper. Many communications of that period referred but briefly to morbid anatomical states and merely subscribed to what was already known.

More detailed descriptions of pathological changes appeared in a number of contributions which followed immediately the fundamental discovery of Carré (1905). Although many of these studies concerned primarily the bacteriological aspect of the disease, some contained usually comprehensive

notes on the morbid anatomy of the respiratory system. Most writers commented on the frequency of bronchopneumonia, often attended by suppuration, and noted the usual absence of pleurisy (Heuer, 1906; Friedberger and Fröhner, 1908; M'Gowan, 1912; Ferry, 1912; Torrey and Rahe, 1913).

It was then also that the pathological histology of distemper pneumonia made its first strides. M'Gowan (1911) illustrated his work with photomicrographs which showed a few bronchopneumonic patches with areas of collapse. "Within the lung alveoli," wrote Torrey and Rahe (1913), "the exudate consisted of serous fluid, many polymorphonuclear leucocytes, a few large mononuclear leucocytes and epithelial cells, but no fibrin".

Over a decade later, while conducting rigidly controlled studies on distemper, Dunkin and Laidlaw (1926a) found in about one third of inoculated ferrets "the lung lesions" of "a remarkable appearance which...have no exact parallel in other animals". The alveoli were filled with large, clear, vacuolated cells, some of which contained two nuclei. They were thought to represent epithelial cells "which have grown out from the tissue of the lung". Pneumonic changes which these authors observed in experimental dogs were usually very mild and consisted of bronchitis and small scattered patches of bronchopneumonia. "This comparative absence of lung involvement," wrote Dunkin and Laidlaw (1926b), "was quite unexpected" and not in agreement with findings made by workers in the field and by many previous observers.

Bronchopneumonia, patchy in distribution, and a considerable enlargement of the lymph nodes were briefly referred to by Perdrau and Pugh (1930) in their studies on disseminated encephalomyelitis of the dog.

Marinesco et al. (1933) contended that in their cases of distemper, pneumonia (only two lungs were examined!) was probably due to the action of bacteria. In a work of histopathology of natural and experimental distemper

DeMonbreun (1937) stated that pneumonia, which he found in twelve out of thirteen dogs, was interstitial in character and frequently peribronchial in distribution. Observations conducted on the thirteenth case which succumbed after a short illness, apparently to canine contagious hepatitis, led him to the erroneous evaluation of some of his findings.

In 1948, MacIntyre, Trevan, and Montgomerie presented a paper on canine encephalitides in which they made a distinction between "true" distemper of Laidlaw and Dunkin and other encephalitic conditions. One of these, termed "hard pad" disease, was claimed to be a separate although not entirely unrelated entity characterized by demyelinating encephalitis, histiocytic interstitial pneumonia, and proliferative changes in the pads. "Classical" distemper, according to the authors, did not produce inflammatory changes in the brain nor demyelination, but only destruction of nerve cells. The lung lesions in both conditions were thought to be "somewhat" similar except for congestion, haemorrhage, and oedema which usually accompanied "hard pad" disease.

The interest stimulated by this work was reflected in numerous communications from many countries. Some observers recognized the existence of "hard pad" disease as a separate entity (McIntyre, 1948; Pugh, 1949; Cohrs, 1951; Scheitlin et al., 1951; Furumoto, 1952; Nijland, 1952; Teunissen, 1952; Van Den Akker, 1952), while others did not support the original contention of MacIntyre and his co-workers (Weipers, 1948; Bateman, 1949; Edwards, 1949; Goret, 1950; Koprowski et al., 1950; Fankhauser, 1951; Mansi, 1948, 1951; Cabasso et al., 1954; Lauder et al., 1954; Gillespie et al., 1956; Haig, 1956).

Some of the morbid anatomical and histopathological features of distemper pneumonia in dogs were described at length by Cordy (1949) and Bienfet (1953), and in mink by Pinkerton et al. (1945). The changes regarded as

characteristic of distemper were essentially those of mononuclear infiltration of the interstitium, and proliferation and hypertrophy of the septal cells. Multinucleated giant cells containing inclusion bodies seen in a proportion of cases were compared to those observed in some forms of virus pneumonia of man.

In a comprehensive study on the histopathology of distemper, Potel (1951) noted that only five out of fifty-nine experimental dogs showed pneumonia, whereas all his field cases exhibited progressive changes in the lung. A frank bronchopneumonia was found in a few of the fourteen animals which suffered from a nervous form of the disease. No pneumonic lesions attributable to distemper were observed by Scheitlin et al. (1951) in a series of fifteen cases which showed clinical signs of so-called "hard pad" disease. Fankhauser (1951) indicated in his work based on clinical and pathological observations on 320 dogs that pneumonia was often attended by oedema and congestion. The hardening of pads in encephalitic cases was not regarded by him as specific to "hard pad" disease.

Extensive clinical and pathological studies of canine distemper were carried out by Lauder et al. (1954). The changes observed in the lung consisted of oedema, congestion, and occasional subpleural consolidation. Haemorrhage was less common. Distinct suppurative pneumonia was found in six dogs out of the fifty studied. Microscopically, the lesions were those of interstitial bronchopneumonia often accompanied by marked hyperplastic alveolar epithelial changes and multinucleated and bizarre cells. The conclusions drawn by the authors pointed to the unity of the distemper disease process.

Recently, Campbell et al. (1955) and Campbell (1956) reported that

a proportion of distemper cases (6 per cent) showed a coexistent infection with Toxoplasma organisms.

Inclusion Bodies

Distemper inclusion bodies were first observed by Lentz in 1907 during his studies on rabies and since then have been a subject of numerous communications. In seven out of ten dogs showing nervous symptoms, Lentz (1909) found them not only in the cytoplasm of ganglion cells but also in the nuclei.

The original discovery of Lentz was corroborated in 1908 by Standfuss. The presence of cytoplasmic inclusions in other organs, including the lung, was reported by Sinigaglia (1912; 1913), and Babes and Starcovici (1912). The bodies observed by these writers were strongly eosinophilic, and many of them possessed vacuoles.

Sanfelice (1915) extended the search for inclusions to many organs in his seven distemper cases and first described both cytoplasmic and intranuclear forms in the pulmonary alveolar epithelium. Cytoplasmic inclusion bodies in the alveolar macrophages were briefly referred to by Dunkin and Laidlaw (1926a).

Marinesco *et al.* (1933) maintained that the cellular bodies which they found in neurons and glial cells were predominantly intranuclear. Mostly cytoplasmic forms were described by Nicolau (1935) in many organs, including brain, in the four cases studies. Frauchiger and Walthard (1935) were unable to identify any inclusion bodies in the central nervous system of dogs suffering from distemper. A similar observation was made later by Fankhauser (1951), and Potel (1951; 1954).

Kriesel (1938) reported that intranuclear inclusions were present in 18 per cent, and cytoplasmic inclusions in 63 per cent, of his 55

experimental dogs. The nuclear type was seen in the reticulum cells of lymph nodes and spleen, in macrophages of the liver, and in alveolar cells of the lung. Both forms of distemper inclusions were also described by Green and Evans (1939a), Hurst et al. (1943), Sjolte (1947), MacIntyre et al. (1948), Innes (1949a), McGovern et al. (1950), Cohrs (1951), and Lauder et al. (1954), and specifically in the pneumonic lung by Pinkerton et al. (1945), Cordy (1949), and Bienfet (1953).

In their study on inclusion bodies in the nasal mucosa, Broadhurst et al. (1938) indicated that the presence of nigrosin-staining bodies in dogs recovered from distemper might have been associated with the state of immunity. Some of their observations were contested by Green and Evans (1939b). Recently, Lindgren (1951) found the cytoplasmic inclusions of distemper in 66 per cent of 60 dogs examined. The development of those bodies was studied in experimentally infected ferrets by Watson and Plummer (1942), who showed that the cytoplasmic type appeared on the first day of symptoms, i.e., 10 days after inoculation.

The nature and significance of cellular inclusions in distemper as well as in other virus diseases have long been a subject of dispute and speculation. Two opposing views have been put forward, viz., that inclusion bodies represent the products of cellular degeneration caused by the action of virus, or that they consist of virus particles. The latter view received some support from the experiments on the inclusions of fowlpox (Woodruff and Goodpasture, 1929) and molluscum contagiosum (Van Rooyen, 1938). Recently, a fluorescent antibody technique applied to cytoplasmic inclusion bodies of distemper (Moulton and Brown, 1954) and intranuclear inclusions of canine contagious hepatitis (Coffin et al., 1953) demonstrated their antigenicity and fortified considerably the opinion held by many observers.

While the diagnostic importance of cytoplasmic inclusions in distemper has been but rarely questioned (Fankhauser, 1951), the significance of intranuclear forms still awaits further clarification. The fact that it has been possible to produce in some animals certain forms of intranuclear inclusions by physical and chemical means (Lee, 1933; Blackman, 1936; Olitsky and Harford, 1937; Findlay, 1938) or to observe them in apparently normal tissues (Cowdry, 1934; Findlay, 1938), calls for some reservations regarding their diagnostic specificity.

Morphologically, the inclusion bodies of canine distemper resemble, often very closely, those found in some viral pneumonias of animals and man.

Cytoplasmic eosinophilic bodies, homogeneous in structure, of 1 to 6μ in their long axes were described by Jarrett (1954) in pneumonic lungs of calves. Adams (1941) reported eosinophilic inclusions, surrounded by a cytoplasmic halo, in primary virus pneumonitis of infants. The lung lesions encountered in measles are not infrequently characterized by the presence of cytoplasmic acidophilic bodies (Semsroth, 1939; Corbet, 1945). Intranuclear acidophilic inclusion bodies, often separated from the marginated chromatin by a clear zone, were observed in the lungs of infants in association with so-called "inclusion disease" (Faber and Wolbach, 1932), in pneumonias of infancy (Goodpasture et al., 1939), in interstitial pneumonitis of unknown origin in adults (McMillan, 1947), and in varicella (Cheatham et al., 1956). Both cytoplasmic and intranuclear forms were described in giant cell pneumonia by Pinkerton et al. (1945). Intranuclear inclusions of undetermined origin were found in the pneumonic lung in association with pertussis by McCordock (1932), and McCordock and Muckenfuss (1933).

Bacteriology of Distemper Pneumonia

The contagious nature of canine distemper was well recognized in the time of Jenner, but it was not until the latter part of the nineteenth century that first claims to the discovery of the causative agent were made.

According to Mathis (1887), Semmer (1875) was first to isolate a microorganism, a coccus, which he regarded as specific to distemper. A few years later similar bacteria were recovered by Laosson (1882) and Mathis (1887). Coccal forms, obtained from the lung in pure culture, were also described by Heuer (1906). Some workers believed that bacilli played the most important role (Millais, 1890; Galli- Valerio, 1895).

It was with the discovery of Carré (1905) that a new light was shed on the aetiology of canine distemper. The evidence produced by him indicated that a filterable agent was the essential cause of the disease. This finding was soon corroborated by Lignières (1906). Other investigators, however, maintained that distemper was caused by a pathogen, named Bacillus bronchiseptícus (Brucella bronchiseptica), which they had regularly isolated from the respiratory tract (M'Gowan, 1911; Ferry, 1911, 1912; Torrey and Rahe, 1913; Rhea, 1915). In addition to this organism, their cultures from affected lungs yielded streptococci, staphylococci, diphtheroid bacilli (Ferry), Escherichia coli, and Salmonella enteritidis (Torrey and Rahe). Comparable isolations were made by Schoichi (1923) and later by Schlingman (1932).

Hardenbergh (1925) contested these findings and voiced the opinion that Br. bronchiseptica was not the primary agent. The final confirmation of Carré's discovery came from the work of Laidlaw and Dunkin (1926) and, in consequence, Br. bronchiseptica which was never recovered from their experimental dogs was relegated to the group of secondary invaders.

In 1934, Shoetensack claimed to have isolated from dogs affected with distemper a filter-passing but culturable agent, resembling Asterococcus mycoides, which he regarded as a causative factor of the disease. Apparently an identical microorganism named pleuropneumonia-like organism (PPLO) was, however, soon recovered from many species of animals. Recently, Greig (1954) found it in the lung of a dog and described it as nonpathogenic.

Bacteriological observations on distemper pneumonia have been made in recent years by a number of workers (Cranshaw, 1949; Burkhart et al., 1950; Bindrich, 1950; Potel, 1951; Lauder et al., 1954). Their findings indicated that haemolytic streptococci, staphylococci (Staph. albus and aureus), E. coli, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, S. enteritidis, and occasionally Br. bronchiseptica were the most commonly isolated organisms. The fact that Br. bronchiseptica has been lately encountered only in a comparatively small proportion of cases attracted the attention of some observers (MacIntyre et al., 1948; Lauder et al., 1954).

Recently, in their investigations on a syndrome which resembled clinically some aspects of canine distemper, Groulade et al. (1954) claimed to have recovered from the lung a causative agent related serologically to Rickettsia psittaci.

Histopathology of Some Viral Pneumonias in Animals and Man

Since the reports of Bartels (1861), Delafield (1884), and Hecht (1910), an unusual form of pneumonia has been known to occur in man as the sequelae of measles and whooping cough. One of the most conspicuous changes encountered was a mononuclear infiltration of the pulmonary interstitium. In 1918, a term "interstitial bronchopneumonia" was introduced by MacCallum to define the character of similar changes observed during the epidemics of

pneumonia in the winter of 1917-18 in the United States Army Camps. These lesions, studied in great detail by MacCallum (1919), were shown to consist principally of the mononuclear infiltration of the bronchiolar walls and adjacent alveolar septa, proliferation and desquamation of cells lining the alveoli, oedema and, often, early organization of the exudate.

Pulmonary involvements of essentially the same morphological character have since been found in many viral pneumonias of animals and man.

During his studies on swine influence in 1931, Shope noted in the lung of affected animals an extensive peribronchial round-cell infiltration, frequent collapse of some groups of alveoli, and thickening of alveolar walls due to engorgement with mononuclear cells. Identical changes were also described by Schmidt (1936), and more recently by Hjärre et al. (1952).

Successful transmissions of swine and human influenza to mice and ferrets (Andrewes et al., 1934) facilitated greatly the experimental study of these two closely related diseases. Pulmonary lesions observed in the infected mice consisted chiefly of an early destruction and subsequent regeneration of the bronchiolar epithelium, and were often attended by considerable collapse of the lung tissue (Straub, 1937, 1940; Dubin, 1945; Hoyle and Orr, 1945; Loosli, 1949). The changes found in the epithelium of the respiratory tract in influenza of man showed essentially the same character, and were usually followed by a severe pulmonary oedema and intense congestion (Winternitz et al., 1920; Straub and Mulder, 1948; Mulder and Verdonk, 1949; Stuart - Harris, 1953). Parker et al. (1946) indicated that in their cases the epithelium was usually unaffected, and only the perivascular connective tissue and alveolar septa showed significant alterations.

Interstitial character of lesions in human influenza, measles, and pertussis were also described by McCordock and Muckenfuss (1933), who, while

reserving their opinion about the cause of conditions studied, suggested a term "interstitial virus pneumonia".

A somewhat different picture of interstitial involvement was seen by Jones and Maurer (1943) in equine influenza. The most prominent changes encountered were those of the accumulation of oedema fluid in connective tissue spaces and consolidation of the lung with haemorrhagic exudate. Organization and fibrosis were common in more advanced stages.

During the last three decades a new form of pneumonia has been recognized in man. Its clinical picture was found to be considerably different from the characteristic symptoms of bacterial lobar pneumonia. For this reason the terms "pneumonitis" (Gallagher, 1934), and "atypical pneumonia" (Cole, 1936) were introduced. Subsequent investigations soon revealed that this clinical syndrome could be produced by a number of viral and other related agents. The causative factors, so far isolated, include organisms of the psittacosis - lymphogranuloma group (Bedson et al., 1930), rickettsia of Q fever (Burnet and Freeman, 1937), and the virus of primary atypical pneumonia (Eaton et al., 1944). The lung lesions described in these conditions were characterized by the interstitial round-cell infiltration, accumulation of a predominantly mononuclear exudate in alveolar spaces, and, in primary atypical pneumonia, also by the marked swelling and proliferation of alveolar lining cells (Wilson, 1930; Rivers and Berry, 1931; Adams, 1941; Parker et al., 1947; Eaton, 1950; Whittick, 1950; Stuart - Harris, 1953).

The interstitial inflammatory reaction in the lung has also been encountered in a number of systemic viral diseases in man. McCordock and Muckenfuss (1933), Corbett (1945), and Pinkerton et al. (1945) described such changes in measles, and reported the presence of multinucleated giant cells in some of their cases. Interstitial pneumonia was also found in

meningo-pneumonitis (Gara and Furth, 1948), in smallpox (Pinkerton, 1953), varicella (Cheatham et al., 1956), and other viral conditions.

Many of the viral pneumonias known to occur in animals are primary in nature. Only a few are associated with the systemic diseases. In their studies on the pathology of pneumonia in food-producing animals, Langham et al. (1942) found an interstitial type of bronchopneumonia, often associated with hyperplastic alveolar epithelial changes, in many of the thirty-one calves, sheep, and pigs examined. More pronounced lesions of the same character and accompanied by the presence of multinucleated giant cells and a richly mononuclear exudate were reported by Thorp et al. (1942) in twenty-eight calves. Haemophilus-like bacteria were regularly isolated from all the cases examined, but the attempts to reproduce this condition by inoculations of the organisms failed to give consistent or conclusive results. Lamont and Kerr (1939) and Baker (1943) showed that a filter-passing agent obtained from the pneumonic lungs of calves was capable of producing pneumonia. Histologically, the changes observed in some of the field cases (Baker, 1943) consisted of varying degrees of bronchitis, some thickening of alveolar walls, and the accumulation of cellular exudate in alveolar spaces. Comparable changes were reported in so-called transmissible enzootic pneumonia in calves (Jennings and Glover, 1952). More advanced stages of this condition were characterized by the exudate rich in mononuclear cells, and also by some collapse and thickening of alveolar walls.

Two morphological varieties of a presumably viral pneumonia in calves were described by Jarrett (1954). Out of the fifty consecutive necropsies, six cases exhibited an extensive "epithelialisation" of alveoli and some giant cells. Cytoplasmic eosinophilic inclusion bodies were found in the bronchiolar and alveolar epithelium. Five other animals, from the

same group of fifty, showed an apparently different form of pneumonia. The conspicuous feature was a marked peribronchiolar lymphoid hyperplasia. Inclusion bodies and alveolar epithelial changes were not seen.

Extensive proliferations of lymphoid elements in peribronchiolar and perivascular regions were also found in "the grey lung disease" of mice (Andrewes and Glover, 1945; Niven, 1950), and in the viral pig pneumonia (V.P.P.) reported by Pattison (1956), and Schofield (1956). Other changes encountered in these conditions comprised the interstitial and intra-alveolar inflammatory reaction of the mononuclear type, some hyperplasia of the alveolar epithelial cells, and a moderate degree of oedema. In some cases giant cells were also seen giving the appearance of a "giant-cell pneumonia" (Andrewes and Glover, 1945; Schofield, 1956).

Lymphoid hyperplasia, usually peribronchiolar in distribution, was noted in a few instances of jaagsiekte by Dungal (1946). The essential character of this condition, however, centered around the centrifugal proliferation of the bronchiolar epithelium and the formation of an intra-alveolar mononuclear exudate. In the latter respect, jaagsiekte seemed to resemble maedi (Sigurdsson et al., 1953), an Icelandic infection of sheep. Both conditions were reported to be transmissible, and are apparently of viral origin. The changes identical to those in jaagsiekte were observed in the so-called progressive pneumonia of sheep (Cowdry and Marsh, 1927; Williган et al., 1954).

In 1942, Blake et al. described a viral pneumonia in cats, which presumably was related aetiologically to some human cases of primary atypical pneumonia. Experimental transmissions showed that a severe and fatal lung involvement could be regularly produced by intranasal instillation of the virus. Histologically, the pneumonia encountered displayed many features characteristic of the interstitial inflammatory reaction, with accompanying,

and often abundant, mononuclear alveolar exudate and hyperplastic epithelial changes. The latter presented themselves in a form of the alveolar "epithelialisation". No inclusions, nor elementary bodies were ever found in the affected tissues.

A different form of feline pneumonitis was reported by Baker (1944). The causative organism, identified as one of the psittacosis-lymphogranuloma group, produced basophilic elementary bodies. The lung lesions, as noted by the author, consisted chiefly of the alveolar mononuclear exudate and occasional necrotic changes.

Pneumonia of the acute interstitial type (interstitial pneumonitis) has been described not only in viral infections, but also in the conditions caused by other intracellular parasites.

In a study on the comparative pathology of scrub typhus (tsutsugamushi disease), Allen and Spitz (1945) showed that the lung lesions produced by Rickettsia tsutsugamushi (R. orientalis) were often indistinguishable from those provoked by viruses. A prominent feature in some of the cases studied was the "epithelialisation" of alveoli and early fibrosis. Similar changes, but milder in intensity, were also found by these workers in infections with R. prowazekii (epidemic typhus), and R. rickettsi (Rocky Mountain spotted fever).

Pneumonia encountered in Q fever (R. burneti) of man (Whittick, 1950; Stuart - Harris, 1953), and in cattle, sheep, and goats (Salisbury, 1953) exhibited all pathological and clinical manifestations characteristic of viral involvements.

The protozoan agents capable of producing an acute interstitial pneumonia comprise the organisms of Toxoplasma, and Pneumocystis carinii. Pinkerton and Henderson (1941) reported the instances of pulmonary

toxoplasmosis in man, and Olafson and Monlux (1942), and Watrach (1956) found the interstitial pneumonic changes in the cat. Concurrent infections with distemper virus and the organisms of Toxoplasma in dogs were studied by Campbell et al. (1955), and Campbell (1956). An interstitial plasma-cellular pneumonia due to a protozoan parasite, Pneumocystis carinii, was described in a twelve-weeks-old infant by Baar (1955). Many alveoli, alveolar ducts, and respiratory bronchioles were found to be lined by a continuous cuboidal lining. Similar observations were reported by Donohue (1956), but the organisms of Pneumocystis carinii were not identified in his cases.

The Pulmonary Alveolar Lining Under Normal and Pathological Conditions

The nature of the normal alveolar lining has been a subject of energetic and very productive dispute since 1843, when Addison put forward his views denying the presence of epithelium in the terminal airways. Although his concept was upheld by some workers (Rainey, 1855; Zenker, 1861; Munk 1862; Villemin, 1866), a great majority of authors in the latter part of the nineteenth century maintained that pulmonary alveoli were lined by a continuous cellular membrane. The alveolar epithelial lining was studied extensively, especially by means of silver nitrate technique, and was thought to consist either of flat nucleated cells (Chrzonszczewsky, 1863; Colberg, 1866), or of small nucleated polygonal elements and large non-nucleated squames (Eberth, 1862, 1864; Elenz, 1864; Kölliker, 1881). The latter view, supported by the authority of Kölliker, gained universal recognition and was not contested until the third decade of this century. The reports which appeared meanwhile merely reasserted the concept of continuity of the alveolar epithelium (Oppel, 1905; Briscoe, 1908; Lange, 1909; Ogawa, 1920; Stewart, 1923).

The entire problem of the nature of alveolar lining was reopened with communications of Lang (1926) and Policard (1926; 1929) who denied the existence of a continuous epithelium, although Lang (1926) admitted the presence of non-nucleated plates. Both authors also believed that the isolated nucleated cells, present in the alveolar walls, were of mesenchymal origin.

A few years later, Cappell (1929) in his extensive study on intravitam and supravital staining indicated that alveolar lining was continuous and consisted of three elements, viz., small cuboidal cells, "flattened nucleated cells closely applied to the capillary walls", and the large non-nucleated plates. The latter, according to him, formed a "relatively inconsiderable part of the alveolar lining". The continuity of the epithelium in alveoli was recognized by a number of workers (Miller, 1932; Orsós, 1933; Bensley and Bensley, 1935; Bensley and Groff, 1935; Bremer, 1935; Gazayerli, 1936; Macklin, 1936; Cooper, 1938; Ross, 1939; Grady and Stewart, 1940; Rosin, 1947; Tonelli, 1948; Stewart, 1953). Some of these investigators maintained that the lining cells varied significantly in morphology (Orsós, 1933; Gazayerli, 1936), while others thought that only one type of extremely attenuated elements formed the epithelial covering (Miller, 1932; Bensley and Bensley, 1935; Bensley and Groff, 1935; Bremer, 1935).

The existence of the continuous alveolar epithelial membrane was denied by Dogliotti (1931), Seemann (1931), Josselyn (1935), Loosli (1935), Bloom (1936), Clara (1936), Fried (1936), Palmer (1936), Barnard and Day (1937), Hom and Baldwin (1941), Geever et al. (1943), Hesse and Loosli (1949), Loosli et al. (1949), Waddell (1949), Hayek (1950), Horning (1950), Shimai and Tamaru (1951), Bertalanffy and Leblond (1953), and Policard (1955).

The essential nature of the cuboidal cells of the alveolar lining has been studied very extensively. A great majority of authors believed that

under conditions of stimulation many of these cells responded readily and exhibited a significant degree of phagocytic activity as free intra-alveolar macrophages. Tchistovitch (1889), Briscoe (1908), Sewell (1918), Cappell (1929), Seemann (1931), Carleton (1934), Bremer (1935), Macklin (1951; 1954), and others maintained that these cells were of entodermal origin.

A different view advocating the mesenchymal nature of alveolar phagocytes was put forward by Lang (1926) and Policard (1926) and received the support of Gardner and Smith (1927), Fried (1934), Josselyn (1935), Ungar and Wilson (1935), Clements (1940), Marshall (1946), Hesse and Loosli (1949), Taliaferro (1949), and Waddell (1949). The macrophages originating from the cuboidal cells of the alveolar walls and known under the term of "dust cells" were named by Lang (1926) as "septal cells". Only comparatively few authors believed that the alveolar phagocytes were derived entirely from the blood mononuclear cells (Slavjansky, 1869; Metchnikoff, 1901; Lewis, 1925; Foot, 1927), or from the endothelium of alveolar capillaries (Foot, 1920; Permar, 1920).

The century old diversity of opinion on the nature of the alveolar lining, despite the detailed and exhaustive studies of very many workers, has sufficiently demonstrated that conventional methods of observation utilizing the light microscope would not lead to the solution of this structural perplexity. An entirely new approach was essential. The decisive answer appears to have been provided by the application of the electron microscope to the studies of tissues.

The first report on the electron microscopic investigations of the lung was published in 1952 by Low. The author based his findings on the rat tissue fixed, after excision, in 1 per cent osmium tetroxide (Palade, 1952) and cut at the thickness of 1/10 - 3/50μ. In one case, the fixative was

injected slowly into the trachea and allowed to drain into the alveoli. The portions discoloured by osmium tetroxide were cut out and immersed in the fixative for four hours. No appreciable difference in the preservation of tissues with these two methods was noted. The conclusions drawn by this worker pointed to the existence of a continuous pulmonary epithelium "with cell bodies located chiefly on the thicker portions of alveolar walls" and attenuated "to form a complete covering of the alveolar walls". The thickness of the epithelial membrane was estimated at 0.1 to 0.7μ. In its very thin parts the pulmonary epithelium was thought to be "applied directly to the capillary endothelium". The ground substance, often containing fibrillar elements and interposed between capillary endothelium and surface epithelium, was seen by Low only in some segments of the alveolar wall. The non-nucleated plaques of Kölliker were not observed.

A year later, Low (1953) extended his studies of the alveolar epithelium on other laboratory animals (mice, guinea pig, rabbits, dogs) and man. His findings demonstrated the presence of a continuous epithelial lining in all species examined, and in addition, pointed to the existence of uninterrupted basement membranes "discretely localized along the basal surfaces of both epithelium and endothelium". Thin portions of epithelial cells were reported to contain "cytoplasmic inclusions" indistinguishable from those seen in the perinuclear moieties. Low admitted, however, that interruptions in the epithelial covering could be found at points where "free cells" were set against the wall of alveoli. In a subsequent communication, Low (1954) suggested that, in order to obtain a better preservation of cellular elements, the time of fixation with osmium tetroxide be reduced from four hours to one-half to one hour.

An early support of Low's observations came from Macklin (1954), in a paper on the pulmonary alveolar mucoid film and the pneumonocytes. Clemens (1954) at first appeared to have confirmed the findings of Low and described a continuous membrane separating the stroma of alveoli from the air spaces. In a later communication (1955), however, he denied the existence of an uninterrupted cellular layer and believed that alveolar epithelial cells covered only certain portions of the subjacent and continuous, structureless, basement membrane ("Alveolarmembran"). Swigart and Kane (1954) were first to oppose the view of Low (1952; 1953) on the nature of the alveolar lining. They recognized the presence of "some material intervening between the air space and the capillary", but were not able to demonstrate the uninterrupted cellular lining. The lung tissues studied by these authors were fixed for 8 hours in 1 per cent osmium tetroxide and examined only under low magnifications. Similar observations were made by Policard et al. (1954a; 1954b), who denied the presence of a continuous alveolar epithelium, and contended (1954c) that the endothelial cells of capillaries were either attenuated to less than 100 Å, or otherwise formed an interrupted membrane. Two years later Policard and Collet (1956) reversed their original opinion and recognized the existence of alveolar epithelium. They described it as consisting of two morphological elements, viz., small cells, poor in cytoplasm and extremely thin protoplasmic expansions. Policard et al. (1956) also changed their concept of the endothelial wall and admitted that it was continuous although thinned in some parts to 200 Å. Schlipköter (1954) studied the lung of rats and found that the alveolar lining was composed of two layers. He also showed that some of epithelial cells contained strongly osmiophilic bodies with a concentrically lamellated internal structure. According to Kisch (1955) many capillaries in the alveolar walls were bare and "in direct contact with the air".

In a preliminary report on the fine structure of pulmonary capillaries and alveoli of the mouse, Karrer (1956a) stated that alveolar walls were lined by a continuous epithelial layer of an average thickness of about 100 - 200 mμ. In some segments of the wall, both epithelial and endothelial cells were found to be as thin as 10 mμ. Interruptions in the epithelium, noted by Low (1953) and later by Breemen and Neustein (1956), were not seen by this author. Septal cells were regarded by him as "bulging nucleated parts of the epithelial cells". He also described the "basement membrane", and believed that it was essentially structureless although in some portions it seemed to contain the unit fibers of collagen. In a subsequent communication, Karrer (1956b) reasserted his original findings.

In extensive studies on the normal structure of lung capillaries (in the frog, rat, cat, and hedgehog), Bargmann and Knoop (1956) found that alveolar epithelium was continuous and rested on a homogenous basement membrane. Osmiophilic cytoplasmic inclusion bodies, first described by Schlipköter (1954), were regarded by the authors as a produce of a degenerative process possibly in mitochondria. Among other cellular structures observed by these workers were so-called microvilli located on the surface of some alveolar epithelial cells, and the endoplasmic reticulum which was thought to form a system of trabeculae and vacuoles. The average thickness of the cytoplasmic epithelial covering was estimated at 1000 Å.

Some of the earlier views on the nature and structure of the pulmonary alveolar lining stemmed from the study of pathological material. A number of workers have reported that, under certain ill-defined conditions of cellular stimulation, alveoli become lined by a layer of conspicuous spindle-shaped or cuboidal cells. Occasionally, this layer was seen to be separated and lifted from the parent wall.

According to Miller (1932), Colberg (1866) was one of the first workers who concluded from the study of pathological lung tissue that "in spite of constant negative statements, a continuous epithelium must exist in the alveoli of adult human lungs". His observations on the occurrence of alveolar cellular lining in certain pathological states were substantially similar to those made later by many authors. Dreschfeld (1876), Tchistovitch (1889), and Briscoe (1908), in their investigations on the response of pulmonary parenchyma to the introduction of various substances, noted that alveoli became covered by a complete cellular membrane. While Dreschfeld (1876) and Briscoe (1908) attributed this phenomenon to the active proliferation of alveolar epithelial cells, Tchistovitch (1889) believed that it resulted from an orderly arrangement and growth of emigrated lymphocytes along the wall.

In an extensive study on epithelial proliferations in the lung provoked by intrapleural injection of the solutions of electrolytes, Young (1930) observed that many alveoli subjacent to pleura exhibited a continuous cellular lining which occasionally gave rise to the formation of syncytial and giant elements. A particularly striking proliferation was elicited by lanthanum chloride. The author contended that "a precipitation of the colloids of the cell membrane was an essential phase in the sequence of changes which culminated in cell division". An appreciable degree of alveolar epithelial hyperplasia has also been found in pulmonary involvements associated with such noninfective agents as lipids (Pinkerton, 1928; Goodwin, 1934; Bell, 1943; Geever et al., 1943), iodized oil (Gowar and Gilmour, 1941), phosgene (Durlacher and Bunting, 1947), and X-ray radiation (Warren and Gates, 1940; Whitfield et al., 1954).

Most commonly, however, the "epithelialisation" of alveoli has been observed in pulmonary conditions precipitated by some specific infectious agents. The earlier communications which make mention of the alveolar epithelial proliferations are those of Colberg (1866), Delafield (1884), and Hecht (1910). The latter author also reported the presence of giant cells apparently formed from the epithelium of alveoli and bronchioles. Similar findings in the giant-cell pneumonia of children were recorded later by Karsner and Meyers (1913), Chown (1939), and Pinkerton et al. (1945). Although the nature of the aetiological factor or factors of giant-cell pneumonia of infancy has never been satisfactorily elucidated, the comparative pathological evidence was thought to suggest the viral origin (Pinkerton et al., 1945).

The formation of a characteristic cellular lining in alveoli has been observed in a variety of viral pneumonias in man and animals. A number of authors described it in measles (McCordock and Muckenfuss, 1933; Geever et al., 1943; Pinkerton et al., 1945), in influenza (MacCallum, 1919; Geever et al., 1943; Stuart - Harris, 1953), in atypical pneumonia of man (McMillan, 1947; Parker et al., 1947), and psittacosis (Rivers and Berry, 1931). In animals, these changes have been found in feline pneumonia (Blake et al., 1942), in pneumonia of calves (Jarrett, 1954) and other food-producing animals (Langham et al., 1942), in jaagsiekte (Cowdry, 1925; Dungal, 1946), and particularly in distemper (Pinkerton et al., 1945; Cordy, 1949; Bienfet, 1953; Lauder et al., 1954). Multinucleated giant cells derived apparently from the alveolar lining cells have been noted in some of these conditions, especially in measles (in the prodromal stage), in calf pneumonia, and distemper.

In addition to viral pneumonias, the "epithelialisation" of alveoli has been known to occur consistently in some other pulmonary infections.

Allen and Spitz (1945) believed that it was a characteristic feature of tsutsugamushi disease (scrub typhus). Rickettsia burnetti, an aetiologic agent of Q fever, was found to evoke similar epithelial proliferative changes (Whittick, 1950). Protozoan infections with the organisms of Toxoplasma in man (Pinkerton and Henderson, 1941) and in the cat (Olafson and Monlux, 1942; Watrach, 1956), and those with Pneumocystis carinii in man (Baar, 1955; Donohue, 1956; Reye and Ten Seldam, 1956) have also been connected with hyperplasia of the epithelial lining cells. In their paper on the pulmonary alveolar covering in various pathological conditions, Geever et al. (1943) indicated that the perifocal septal-cell proliferation was observed in animal pneumonias caused by the organisms of the Actinobacillus, Aspergillus, and Coccidioides groups.

Other pulmonary involvements which have been described as accompanied occasionally by localized areas of "epithelialisation" include fibrosis (Geever et al., 1943), focal scarring (Spencer and Raeburn, 1954), chronic passive congestion (Parker and Weiss, 1936; Bell, 1943), rheumatic pneumonia (Kuzma and Lustok, 1951), tuberculosis (Geever et al., 1943), and bronchiectasis (Watts and McDonald, 1948; Spencer and Raeburn, 1954). In 1953, Hjärre reported a new disease entity in piglets, the so-called verrucous dermatosis, which was accompanied by giant-cell pneumonia and "epithelialisation" of alveoli. The aetiological factor of this condition has not been established but was thought to be both of congenital and metabolic nature.

Comparatively recently, a form of pulmonary neoplasia--the "alveolar cell tumor"--has been recognized by a number of workers and believed to have its origin in the epithelial cells lining the alveoli (Grady and Stewart, 1940; Wells et al., 1941; Geever et al., 1943; Simon, 1947; Tonelli, 1948). This hypothesis, however, has not been accepted by all pathologists.

The origin of the alveolar cellular membrane and the mechanism of its formation in disease processes have been a subject of considerable dispute which stemmed from the controversy on the normal alveolar structure. Many of the more recent workers maintained that "epithelialisation" of alveoli attending certain pulmonary involvements was due to an active proliferation of the existing cells (Cowdry, 1925; Bremer, 1935; Ross, 1939; Pinkerton et al., 1945; Dungal, 1946; Rosin, 1947; Watts and McDonald, 1948; Waddell, 1949). Other authors believed that bronchiolar epithelial ingrowths were the only source of the epithelial pavementing of alveoli (Fried, 1934; Barnard and Day, 1937; Loosli, 1949; Horning, 1950; King, 1954; Spencer and Raeburn, 1954). Bell (1943) and Anderson (1952) adopted both views. Only a few modern investigators indicated that the mononuclear cells of inflammatory origin contribute significantly to the formation of the alveolar epithelial membrane seen in pathological conditions (Loosli, 1935; Bloom, 1936; Geever et al., 1943).

MATERIALS AND METHODS

The present study embodies the results of observations on the pathology of the respiratory system in thirty clinical (naturally infected) and twenty experimental cases of canine distemper. In addition, it presents a detailed account of electron microscopic findings on the normal structure of the pulmonary alveolar wall. The purpose and scope of these investigations have been outlined in the preface.

Materials

Clinical Cases

Most of the clinical cases (twenty-eight) were selected over a period of one year (September, 1952 - October, 1953) from a large number of dogs which were attended at the Small Animal Clinic of the College of Veterinary Medicine, Urbana, Illinois. The other two cases, hereinafter referred to as 1 and 2, were obtained through the courtesy of I. M. Lauder, M.R.C.V.S., from a clinic serving poor persons in Glasgow, Scotland.

The criteria on which the clinical diagnosis of distemper was based included the catarrhal discharge from eyes and/or nose, respiratory signs (cough, auscultatory sounds), gastrointestinal disturbances (vomition, diarrhoea), nervous signs (chorea, convulsions, or posterior paralysis), and hyperkeratosis of the pads. A majority of the cases studied (80 per cent) showed some catarrhal, respiratory, gastrointestinal, and nervous signs. There was no clinical evidence of the involvement of the central nervous system in 20 per cent of the dogs; all of them, however, showed either

respiratory, catarrhal or gastrointestinal signs. The hardening of digital and carpal pads was observed in nine cases (30 per cent), a proportion of which (5 of 9 affected) did not exhibit any discernible nervous symptoms.

Most of the dogs examined were under 8 months of age (83 per cent), and only one was over 2 years old.

Table 1. Age to the Nearest Month at Autopsy

	Months								Years		
	2	3	4	5	6	7	8	9	1	2	3
Number of dogs	1	8	2	3	6	5	3	1	0	1	0

The breed of the affected dogs included 11 varieties, with Cocker Spaniel, Collie, Alsatian and Dachshund as the most common, in that order. Mongrels and crosses formed approximately one-fifth of all pups. Female dogs predominated in a proportion of two to one.

A majority of the cases were either hospitalized, or received symptomatic treatment in the Clinic at frequent intervals. The progress of the disease and the development of symptoms were thus conveniently followed and could easily be evaluated. Only a small number of dogs was admitted at the advanced stage of illness and had to be put to death shortly thereafter due to pronounced nervous disturbances. In such cases the history of disease as related by the owner was considered reliable.

While many of the dogs studied had not been immunized actively against distemper, 8 pups had received a full dose of vaccine, some only a few days before the onset of symptoms. In the remaining 6 cases there was no history of vaccination available.

With the exception of one dog which died of the illness, all cases studied were put to death by euthanasia in an advanced stage of the disease at the request of owners.

Experimental Cases

Twenty dogs, eight to ten weeks old, were employed for the experimental investigations. A group of three litters comprising fifteen animals served as material for the study of the pathogenesis of pneumonia, and the remaining five (a litter of four and one additional dog) were sacrificed in the course of observations on the neurogenic form of pulmonary oedema.

Because of the lack of appropriate housing and isolation facilities for breeding of animals of known susceptibility to the virus of distemper, the supply of dogs was secured from neighbouring farms. Except one, all pups were obtained as litters of four to six dogs during the autumn and early winter of 1953. Litters I, II, and III comprised five, four, and six dogs, respectively, of four to six weeks of age at the time of accession. Group IV included five dogs, five weeks old, four of which were from one litter. Upon arrival, all dogs were immediately isolated in wooden housing units and, as far as could be ascertained, were not exposed to distemper-affected animals. The periods of observation, during which the body temperatures were taken daily, lasted from two weeks, in the case of Litter III, to over one month for Litters I, II, and IV. No sign of malaise nor significant rise in body temperature was noted in any of the dogs during that time. The animals were kept on a well-balanced diet of canned meal and milk, and each received a dose of anthelmintic.

Of twenty dogs, all of which were mongrels, twelve were female and eight male, a similar proportion to that encountered in clinical cases.

The observations on the group used for study of the pathogenesis of pneumonia were continued for a period of five months, at the end of which the last dog (2) was sacrificed, apparently at the stage of complete clinical recovery. The other fourteen animals of the same group were sacrificed on the 1st, 3rd, 5th, 7th, 8th, 9th, 11th, 15th, 18th, 25th, and 30th days of symptoms,

and on the 46th, 59th, and 79th days after the first signs of the disease. The experiments on pulmonary oedema were conducted in February, 1954.

The Source of Virus

The first two litters received the virus of distemper obtained through the courtesy of Dr. Gillespie from the Veterinary Virus Research Institute, New York State Veterinary College at Cornell University, Ithaca, New York (Batch No. SH. S.P.G.A. No. 1). The remaining eleven dogs were inoculated with a filtrate of a 10 per cent suspension of spleens collected from experimental dogs 3, 7, 22, and clinical case 33.

Electron Microscopic Observations

The studies on the structure of the pulmonary alveolar wall were initiated early in the spring of 1954. At first, lung tissue collected from a six-week-old dog was the only one used, but the quality of its fixation proved to be unsatisfactory for a critical morphological study. Since the known methods of preservation of tissues for electron microscopy did not seem to yield the required degree of perfection, it was anticipated that a number of animals would have to be used until a high quality of fixation was obtained by purely empirical methods. Under these circumstances, it was deemed more practical to employ the small laboratory mammal than to sacrifice a number of dogs. In consequence, the present observations are based solely on lung tissue collected from twelve albino rats, the ages of which ranged from six to twelve weeks. It would seem pertinent to recall that no structural differences in the pulmonary alveolar wall of various mammals have been reported in the existing papers on electron microscopy of the lung.

Methods

Clinical Cases

Post-mortem procedures. All dogs with the exception of one (Case 28) were put to death by an overdose of pentobarbital sodium (Nembutal, Abbot Laboratories, Chicago, Illinois) and examined by necropsy as soon as practicable, but in no case later than six hours after euthanasia.

In all cases necropsy consisted of a thorough examination not only of the respiratory system but also of other organs, including brain and skin. Particular attention, however, was paid to the respiratory tract. All its parts were exposed and subjected to careful inspection. The entire nasal cavity with its septum nasi and turbinate folds, the pharynx and associated lymphoid aggregates, the larynx, and trachea were examined in each case. Both lungs were inspected first in situ and then removed from the thorax. The amount of pleural fluid and the state of the parietal pleurae were also noted. All lobes of the lung were examined for evidence of gross alterations, and then incised along the main bronchial passages and through the parenchyma. The changes found were recorded on diagrams, and occasionally by means of colour photography. A considerable number of the lungs showed a degree of oedema which often was also evidenced by the presence of a frothy fluid in the bronchi and, occasionally, in the trachea. Whenever feasible, an attempt was made to estimate the specific gravity of the fluid present. This was done by placing a small drop of the collected material in two or three of the several jars containing graded solutions of cupric sulphate, the concentration of which ranged from 1.008 to 1.022.

Because the morbid-anatomical and bacteriological procedures necessitated the exposure of pulmonary parenchyma, the lung tissue was fixed in a

state of post-mortem collapse. Only in two cases (26 and 27) the unopened lungs were re-expanded to their normal capacity. This was accomplished, after tying the trachea, by the infusion with 10 per cent formal-sublimate under pressure of 12-15 cc. of water. A special device was used for that purpose. It consisted of a 1,000 cc. thick-walled filtering flask, into which a glass tube reaching the bottom of the vessel and a specially prepared manometer containing water were inserted through a tightly fitting rubber stopper. The air, introduced under gentle pressure by way of the side arm of the flask, forced the fixative through the glass tube and, via the attached rubber tubing with a needle (No. 18) inserted in the trachea, into the lungs. The pressure of 12-15 cc. of water was found completely adequate without causing rupture of alveoli. Following redistention with fixative, the entire lung was immersed in a suitable amount of 10 per cent formal-sublimate for a period of three to four days.

Histopathological methods. The tissues selected for histopathological studies included muzzle, alar and turbinate folds, respiratory and digestive parts of the pharynx, epiglottis, vocal cords, upper and lower segments of the trachea, bronchi, and lung. In the latter instance, the representative blocks of 5 to 8 mm. in thickness were excised with sharp scissors from the upper and lower parts of each lobe. In addition, some of the regional lymph nodes, viz., the parotid and bronchial, were generally included. Other tissues such as the cerebrum, cerebellum, pons, digital and carpal pads, liver, and tonsils were also collected in order to gain a basis for the correlation and interpretation of findings encountered in the distemper disease process.

The fixative employed was 10 per cent solution of formalin saturated with mercuric chloride (10 per cent formal-corrosive sublimate) as

recommended by Lendrum (1941). The time of fixation varied from two to four days.

Before embedding in paraffin (melting point, 56-58° C.), the tissues were dehydrated in two changes each of ascending grades of alcohols of 70, 80, 95, and 100 per cent, cleared in a mixture of absolute alcohol and benzene and in two changes of benezene and, finally, infiltrated with paraffin. The blocks of the lung tissue, prior to embedding, were infiltrated in a vacuum oven (60° C.) at a negative pressure of 22 to 23 ins. (204-178 mm. Hg) for a half to one hour.

All tissues were stained with haematoxylin and eosin (eosin bluish, C.I. No. 771, National Aniline Division, 0.5 per cent solution in 95 per cent ethyl alcohol) for the demonstration of histological changes in general, and by the haemalum-phloxine-tartrazine method of Lendrum (1947), or by the haematoxylin and eosin technique of Schleicher (1953) (eosin-bluish C.I. No. 771, 4 per cent solution in distilled water) for distemper inclusion bodies. The periodic acid-Schiff reagent method of McManus (1948) was employed frequently not only for the demonstration of polysaccharides, mucins, epithelial basement membranes, fibrin, and other related substances, but also as a very useful stain for the lung tissue in general. The differentiation of glycogen from other periodic acid-Schiff-positive materials was achieved by using a diastase digestion test. For that purpose paraffin sections were brought to water and digested with 1 per cent malt diastase for 60 minutes at room temperature prior to staining by the periodic acid-Schiff method. An untreated control section was always used along with the digested one.

Other tinctorial techniques employed from time to time included the Gordon and Sweets' method for reticular fibres (with brazilin as a counterstain), the method of Weigert for elastic elements, the picro-Mallory, Van Gieson's, or Mallory-Heidenhain's (as modified by Schleicher, 1943) techniques

for collagen, and Feulgen method for identification of desoxyribonucleic acid. The May-Grünwald-Giemsa stain (Coleman and Bell Co.) was used occasionally for mast cells. In a few cases, the method of Weigert-Pal was employed to demonstrate myelin sheaths.

Bacteriological procedures. Only the lungs were examined bacteriologically. The procedures employed for the isolation and identification of bacteria were based on those in use in the Department of Pathology, College of Veterinary Medicine, Urbana, Illinois.

Shortly after the lungs had been removed from the thorax, one or two small portions of the diaphragmatic lobes were excised with aseptic precautions and placed in a sterile Petri dish. On occasion, parts of other lobes, if significantly involved, were also included.

For the primary isolation two standard plating media were used, i.e., blood agar and MacConkey agar. The portions of tissue selected for culturing were first passed through the flame to prevent possible contamination and then cut with sterile scissors. The surface thus exposed was pressed gently against the medium. All cultures were examined in 24 hours and, if necessary, in 48 hours. Gram-positive microorganisms isolated from the blood agar were generally identified by their growth characteristics and morphological properties. Corynebacterium pyogenes required subculture in dextrose, lactose, maltose and sucrose in which it produced a slightly acid reaction.

With the exception of lactose-fermenting bacteria, all organisms recovered from MacConkey agar were subcultured on Kligler iron agar and in urea broth for further differentiation. Both media, together with tryptone broth, were used regularly for identification of the genus Proteus. In addition to Kligler iron agar, urea broth and tryptone broth, biochemical tests on a series of carbohydrates were employed for Br. bronchiseptica (H.

bronchisepticus) and Salmonella organisms. In the latter instance, the final identification was accomplished by serological typing. The genus Pseudomonas was usually recognized by the presence of a characteristic blue-green pigment.

All cultural examinations were conducted under aerobic conditions.

The author wishes to express his indebtedness to Mr. H. E. Rhoades, M.S., of the College of Veterinary Medicine, University of Illinois, for his help and advice in the course of bacteriological investigations.

Experimental Cases

Preparation of inoculum. The virus of distemper used for the inoculation of the first two groups of dogs was received in the form of a freeze-dried filtrate (Batch No. SH.S.P.G.A. No. 1) from the Veterinary Virus Research Institute, Ithaca, New York. Immediately before inoculation, the entire sample was diluted with sufficient sterile isotonic saline to make a 10 per cent suspension.

Because only a small amount of dried virus was obtained, the remaining dogs were inoculated with a filtrate prepared from the spleens of experimental cases 3, 7, 22 and clinical case 33. In each instance, the organ was removed at necropsy with aseptic precautions and placed immediately in a freezer at -20° C. Prior to inoculation a small portion was ground in a mortar with sterile sand and then diluted with appropriate amount of sterile saline to make a 10 per cent suspension. After centrifugation the supernatant fluid was filtered through "Selas" porcelain candles XF, 0.10 and 0.02. Dogs 16-22 received a pooled filtrate of spleens from cases 3, 7 and 33; and dogs 24-27 and 30 from cases 7, 22 and 33.

Routes of inoculation. All dogs employed for study of the pathogenesis of pneumonia were inoculated subcutaneously and some were also exposed intranasally. The amount of inoculum was 1 cc.

The intracerebral and intraspinal routes were used in experiments on the neurogenic form of pulmonary oedema. Two dogs (26 and 27) were given 0.1 cc. of the spleen filtrate intracerebrally, and the other three (24, 25, and 30) 0.2 cc. intraspinally. The site of the intracerebral inoculation was approximately 2.5 cm. from the parietal crest, halfway between the right supraorbital process and interparietal bone. The whole operation was performed under surgical conditions and the incision closed with a few sutures. Similar precautions were also observed at the intraspinal inoculation. The virus was introduced by means of a long needle into the spinal subarachnoid space, halfway between the atlas and occipital bone.

Clinical records. During the entire course of observations, the body temperature of each dog was taken daily, usually between 4 and 6 P.M. Clinical signs of the disease, such as the nasal and ocular discharges, gastrointestinal and respiratory symptoms, changes in demeanour, and the nervous signs were recorded in each case.

Post-morten procedures, histopathological methods, and bacteriological procedures were identical with those described under the clinical cases.

Electron Microscopic Techniques

As indicated previously, the animal employed for the study of the normal structure of the pulmonary alveolar wall was the albino rat (Wistar strain) of six to twelve weeks of age.

Collection of specimens. The first few animals were anaesthetized with ether. Following the opening of the thorax, small portions of the lung were excised and, after trimming to approximately mm.^{3}, were immersed immediately in 2-3 cc. of 1 per cent buffered solution of osmium tetroxide and left for four hours. This procedure, however, did not prove satisfactory. Many

components of the alveolar wall, with the exception of fibrillar elements, seemed to be either "washed out" or difficult to discern. Superior results were obtained after intratracheal introduction of fixative, and by shortening the time of fixation. The use of anaesthetic was discontinued. Three rats of approximately 100 gms. in weight were rendered unconscious by a sharp blow on the head. The trachea was exposed and tied securely with a thread. A hypodermic needle (No. 22, 1 inch long) was then inserted between the cartilaginous rings, and a sufficient amount of fixative (2-3 cc. of 1 per cent buffered osmium tetroxide at room temperature) was injected slowly into the lungs. A few minutes later the thorax was flayed open, and both lungs were removed. The parts well infused with osmium tetroxide (areas of dark grey colour) were excised with sharp scissors and placed on a cork with concave surface covered with a liberal amount of the same fixative. By using two sharp blades, scissors-wise, the portions of tissue were trimmed to blocks not exceeding mm^3.

Fixation. The selected blocks were transferred to 20 cc. stoppered glass vials containing 2-3 cc. of ice-cold 1 per cent osmium tetroxide buffered with acetate veronal to pH 7.6, and 8.2, and kept at 0° C. for one-half, one and one and a half hours. The tissues fixed in osmium tetroxide at pH 7.6 for 30 or 60 minutes showed the best preservation.

Processing. All tissues were washed for 30 minutes in three changes of tap water at 0° C. containing 0.1 per cent magnesium chloride. The dehydration took 3 hours, first in 70, then 95 per cent ethyl alcohol at 0° C., 30 minutes in each, and then for 75 minutes in absolute ethyl alcohol at 0° C. For the last 45 minutes the tissues were kept in absolute alcohol at room temperature. The infiltration was accomplished by immersion of the specimens in monomeric methacrylate (8:2 n-butyl and methyl methacrylate) for 2 hours.

Subsequently, the blocks were placed in separate plastic capsules No. 3 and flooded with a mixture of prepolymerized methacrylate. After polymerization for a period of 48-72 hours under ultra-violet rays at 30° C., the tissues were ready for sectioning.

Sectioning. The Sjöstrand ultramicrotome with mounts for steel or glass blades was used for sectioning. The glass blade (1/4 inch in width) proved to be much superior, as it was free from the objectionable vibrations generated during the sectioning. In general, good serial sections 150-300 °A thick were easily obtained with the glass edge.

Mounting. The segments of ribbons floating freely on the surface of a fluid (20 per cent ethyl alcohol), placed close to the edge of the knife, were picked up onto brass "Athene" grids (Smethaurst High-Light Ltd., England) held with fine forceps. All grids were previously coated with a film of collodion stabilized by a thin layer of pure carbon evaporated in a vacuum chamber.

Microscopy. The specimens thus mounted were placed in a microscope R.C.A. EMU 2e, with rear focal plane objective aperture, and examined under 1,000 to 18,000 magnification. Whenever a suitable field was found, series of photographs (5 x 5 cms.) were taken to be later enlarged 3 to 6 times for more detailed study.

OBSERVATIONS

Clinical

Clinical Cases

All data pertaining to the clinical picture of the disease were kindly supplied by the clinics in Glasgow, Scotland, and in Urbana, Illinois. No attempt is made to present the detailed histories of the cases studied, but rather to record the incidence of the more significant clinical features.

Duration of illness. This was estimated from the history obtained by questioning of the owner and from the period of illness observed in the clinic.

Most of the dogs (70 per cent) were ill from 2 to 7 weeks prior to euthanasia. Only in two cases (26 and 30) did the period of illness extend over 10 weeks. Seven dogs showed signs of the disease for less than 2 weeks.

Table 2. Duration of Illness

	Weeks			
	Less than 2	2-4	5-7	Over 10
Case	10, 17, 20, 21, 27, 28, 33	1, 2, 3, 4, 5, 7, 8, 12, 13, 15, 18, 19, 22, 29, 31, 32	11, 14, 16, 23, 25	26, 30

Cases 6, 9 and 24 were not used for the present study.

Temperature. With the exception of one dog (4) which at the time of examination showed a temperature of 102.8° F., all other cases often

exhibited prolonged fever (103° F. or above). In four dogs (13, 14, 22 and 28) pyrexia was very high and in one of them reached 105.8° F. The average temperature was slightly above 103° F.

Respiratory signs. Nasal discharge was recorded in 60 per cent of the cases. In some of the dogs (39 per cent), usually after the third week of illness, it took the form of greyish-yellow, thick, purulent exudate, often encrusted at the nostrils. A serous, watery discharge was seen in the remaining 21 per cent of the cases.

A detectable involvement of the lung was noted in 53 per cent of the dogs; it was manifested either by cough (Cases 4, 8, 13, 14, 15, 16, 19, 20, 23, 25, 26, 27, 29 and 33), or by increased vesicular sounds or moist rales (Cases 5 and 7).

Gastrointestinal signs. Vomition was observed in 30 per cent of the dogs, mostly in the initial stages of the disease (Cases 1, 5, 10, 11, 13, 15, 16, 25 and 26). Gastric disturbances were accompanied by diarrhoea of a moderate to severe character in 30 per cent of the cases (1, 2, 5, 7, 15, 22, 25, 26 and 28).

Nervous signs. Nervous disturbances occurred in a high proportion of the dogs (80 per cent). The most common manifestations were chorea and epileptiform convulsions (67 per cent). Posterior incoordination and paralysis appeared in 2 dogs. In 4 cases only fits of a hysterical type were encountered. Of 24 dogs affected, 4 showed nervous complication in the early stages of the disease (Cases 10, 18, 20 and 28).

Pads and nose. A moderate and only occasionally marked hyperkeratosis of the pads occurred in 9 cases (30 per cent) and was often attended by a thickening of the epidermis of the nose (6 dogs). This change became recognizable during the third or fourth week of illness. In 5 out of 9 cases

with hyperkeratosis of the digital and carpal pads, there was no evidence of nervous disturbance.

Treatment. Every case studied received some form of treatment. Often it included the use of distemper and "hard pad" antiserum, penicillin, streptomycin, bacitracin, aureomycin and sulphonamides. Occasionally, vitamins B_1 and B_{12} were also employed.

Experimental Cases

In contrast with the clinical cases, the symptoms encountered in the experimental dogs were usually very mild. It should be borne in mind, however, that the animals used for these studies were all mongrels of unknown susceptibility to the virus of distemper.

Incubation period. This varied appreciably between the groups. Litters I and II, comprising Cases 1, 2, 3,* 5, 6, 7, 8, 9 and 10, were very slow in response to the introduction of the virus of distemper. The first noticeable signs of illness appeared generally on the 7th day and were rather mild in degree. Only in one case from these two groups was the temperature above 103° F. (Case 3) on the first day of symptoms. The shortest incubation period (5 days) was shown by Dog 10.

Group IV, consisting of Cases 16, 17, 18,** 20, 21 and 22, displayed a much more vigorous reaction. The incubation period was considerably shorter and took 3 days in all cases of this group. The initial rise of temperature was quite marked, with one case (20) showing 104.4° F. on the first day of symptoms.

*Case 4 was not used for the present study.
**Experimental Cases 11, 12, 13, 14, 15, 19, 23, 28 and 29, although originally employed in the course of investigations, were not used for the present study.

In the remaining dogs, inoculated by intraspinal and intracerebral routes, the incubation period ranged from four (Cases 24, 25, 26 and 27) to six days (Case 30).

Duration of illness. All dogs employed for a study of the pathogenesis of pneumonia were sacrificed at different stages of the disease. Case 17 was destroyed on the first day of symptoms (3rd day after inoculation), 20 on the 3rd, 9 on the 5th, 3 on the 7th, 7 on the 8th, 22 on the 9th, 10 on the 11th, 8 on the 15th and 6 on the 30th days. Dogs 18, 1, 21 and 2 were sacrificed on the 46th, 59th, 79th and 157th days, respectively, after the first signs of illness.

Dogs 24, 25, 26 and 30 showed marked nervous disturbances and were put to death on the 11th, 13th, 16th and 12th days after inoculation. Case 27 did not display any nervous signs and was destroyed on the 60th day following intracerebral introduction of the virus.

Temperature. In the first two groups (I and II) the temperature was usually mild and only on one occasion reached 103.6° F. (Case 5) on the 15th day after inoculation. The diphasic nature of fever, although suggestive in a few cases, was not a clear-cut feature. Some of the dogs of these two groups displayed pyrexia indicative of a "plateau" type. A temperature of 99.9° F. was recorded an hour before euthanasia in Dog 7 showing violent epileptiform convulsions on the 17th day after inoculation.

The following are the daily recordings of the temperature for Case 3 as representative of Groups I and II.

Table 3. Daily Body Temperature of Case 3

Oct.	Day 23	24	25	26*	27	28	29	30	31
T°	101.8	101.4	101.8	101.5	102.1	101.6	102.1	101.5	101.5

Nov.	Day 1	2	3	4	5	6	7	8x
T°	101.8	103.4	101.4	102.0	101.5	101.6	102.5	102.0

* Day of inoculation.
x Euthanasia.

The initial pyrexia in Group IV was more marked. The average temperature on the first day of symptoms was 103.6° F., with one dog (20) showing 104.4° F. In 3 out of 7 dogs, the fever was evidently of diphasic character (Cases 18, 19 and 21).

Table 4. Daily Body Temperature of Representative Cases, Group IV

Nov. T°	Day 10	11	12	13*	14	15	16	17	18
Case 18	101.6	101.0	101.7	101.6	101.7	101.2	103.2	102.2	101.5
20	100.4	100.9	101.6	101.5	101.8	102.1	104.4	104.4	103.9x
22	100.3	100.6	101.5	102.0	100.9	102.4	103.9	102.9	102.2

Nov. T°	Day 19	20	21	22	23	24	25	26	Dec. 10
Case 18	102.1	103.2	102.6	102.1	104.3	103.5	101.8	100.9	Euthanasia
22	102.2	101.5	101.7	100.6	100.8	101.5x			

* Day of inoculation.
x Euthanasia.

The lowest temperature recorded in this group was 100.3 (Case 21) on the 13th day after inoculation.

Group V showed a comparable degree of pyrexia, with the highest recording of 104.6° F. (Case 25) on the 12th day following intraspinal

inoculation and the lowest (97.0° F.) in Dog 30 shortly before euthanasia. The latter case was in a state of coma during the last two days.

Respiratory signs. These in all experimental cases were usually mild and evidenced by a nasal discharge, first of a serous and in later stages of a muco-purulent character. Cough was observed only in one dog (18) and for a short period of time.

Gastrointestinal signs. Vomition and diarrhoea of a mild to moderate degree were quite frequent, particularly in Group IV and in the early stages of illness. They were usually accompanied by loss of appetite, listlessness, and some loss of weight. In a few cases flecks of blood could be found in the faeces.

Nervous signs. Nervous complications were observed in four out of five cases inoculate by intraspinal or intracerebral route. Case 24 showed signs of weakness in the hind legs on the seventh day and became partially paralyzed two days afterwards. Chorea and incoordination appeared on the ninth day in Case 25. Severe epileptiform convulsions were seen for the last three days in Dog 26 (13th to 15th day after inoculation) and a degree of posterior paralysis and, later, coma were noted in Case 30.

Nervous disturbances were much less common in Groups I, II, and IV. Only one dog (Case 7) showed very marked and prolonged epileptiform convulsions accompanied by coma and profuse salivation. Some whining was observed in Dogs 16, 19, and 22 on the fifth, sixth, and seventh days after inoculation, but more severe signs did not follow.

Pads and nose. Hyperkeratinization of pads and nose was not observed in any of the experimental cases.

Treatment. With the exception of one dose of 300,000 units of penicillin administered to Dog 18, which showed a mild cough, no treatment was

used in any of the 20 dogs studied.

Pathology

The study of the pathology of the respiratory system embraced the morbid anatomy and histopathology of the entire tract and bacteriology of the lung. Particular attention was paid to the microscopical changes observed in the pulmonary tissue. In addition, a detailed account of the structure of the normal alveolar wall is presented as essential to the understanding of the process of "epithelialisation".

Morbid Anatomy

Clinical cases

Upper respiratory tract. In the dogs which were ill for less than two weeks (Cases 10, 17, 20, 21, 27, 28 and 33), the changes observed were generally very mild. Only on three occasions (Cases 17, 20 and 28) did the nasal mucosa and, particularly, the turbinate folds show a moderate degree of hyperaemia. The digestive part of the pharynx and often the epiglottis were either not affected or exhibited only a slight congestion. Occasionally, the pharyngeal tonsil was found to be somewhat enlarged and granular in appearance, but not hyperaemic. With the exception of one case (20), which displayed an appreciable congestion of the lower part of the trachea, no significant changes were seen in the trachea or main bronchi. In 4 cases (20, 21, 27 and 28) both palatine tonsils showed a moderate degree of hyperaemia and slight enlargement.

The changes encountered in dogs ill from two to four weeks (Cases 1, 2, 3, 4, 5, 7, 8, 12, 13, 15, 18, 19, 22, 31 and 32) were generally more advanced and ranged from mild to very marked hyperaemia. The latter was commonly found in the nasal cavity and the respiratory part of the pharynx, and

in a few cases also in the trachea and main bronchi. The digestive part of the pharynx and larynx, except for a moderate congestion in one case (31), were either only slightly affected, or did not show any discernible alterations (Cases 4, 8 and 19). The changes seen in the pharyngeal and palatine tonsils were comparable to those observed in dogs of the first group, although hyperaemia of a mild degree was more common. Muco-purulent exudate was found in the nasal cavity in a few cases (7, 15, 22 and 31) and in the main bronchi of one dog (7). On two occasions, the lower trachea and main bronchi (Cases 7 and 18) exhibited a considerable amount of blood-tinged, frothy exudate. In both groups of dogs, there was usually a small to moderate quantity of thick mucus in the respiratory part of the pharynx, close to the pharyngeal tonsil.

Generally, mild changes were seen in dogs ill from five to seven weeks (Cases 11, 14, 16, 23 and 25). Three dogs (11, 16 and 23) showed significant congestion of the septum nasi and turbinate folds. The respiratory part of the pharynx and the main bronchi were also moderately congested in Case 11. In the remaining dogs, involvement of the upper respiratory tract was uniformly mild or evident to a slight degree only in the nasal cavity.

Except for very slight hyperaemia of the nasal septum, turbinate folds and the respiratory part of the pharynx in one case (26), no changes were found in 2 dogs examined after ten weeks of illness.

The foregoing observations indicate that the changes in the upper respiratory tract became increasingly more severe until they reached the peak between the 3rd and 4th weeks of illness. Beginning with the 5th week, there was a marked decrease in the intensity of lesions, and only minor residual alterations were present after the 7th week.

Lung. In a great majority of cases, the degree of pulmonary involvement was consistent with the duration of illness. Only a few dogs showed changes considerably milder than those observed in their respective groups.

Congestion of the lung parenchyma constituted the most common abnormality. With the exception of one case (26; illness of over 2 months), it was present to a varying degree in all dogs examined. Very seldom was the congestion of a uniform character. Most frequently it affected certain parts of the lung to a more marked degree than the remaining regions. This resulted either in a distinctly focal distribution of the more prominently hyperaemic areas, or in a massive confluent congestion of some portions of the lobes. The former usually presented itself, particularly in the earlier stages of the disease, as a diffuse "mottling", often accompanied by small haemorrhages (Figs. 1 and 2). The large areas of marked hyperaemia were frequently found in the dependent parts of the lungs but, on occasion, appeared only in the upper portions, with the lower regions showing a milder degree of congestion. In a small proportion of cases, the hyperaemia was of a diffuse type and moderate in degree but with large areas of marked congestion and haemorrhage. Only in 3 cases (25, 29 and 30) was the hyperaemia very mild.

Oedema often accompanied the pneumonia of distemper and could be recognized macroscopically in about 70 per cent of the cases. Generally, it was of a moderate degree, but sometimes it was so marked that the fluid issued freely from the cut surface of the lobes. The presence of oedema fluid combined with the intra-alveolar cellular exudate gave, in most cases, an increased consistency to the involved tissue. The lungs thus affected were turgid and did not collapse appreciably upon opening of the thorax. In the earlier stages of pneumonia, not complicated by secondary invasion, the lung

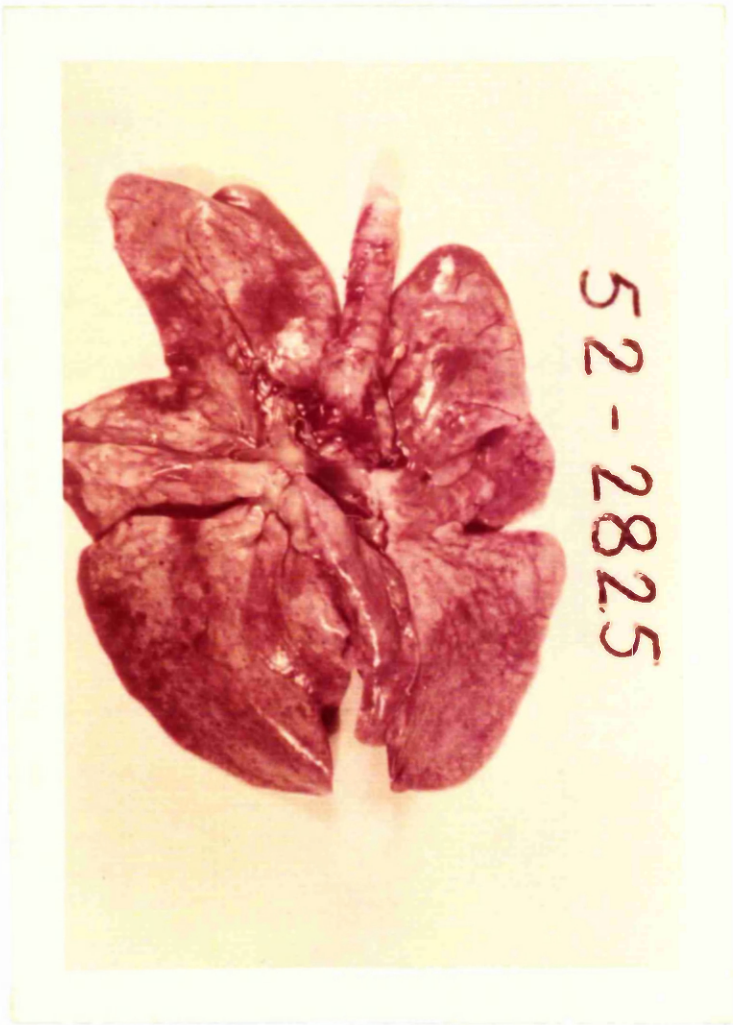

Fig. 1. Clinical Case 20. Mediastinal surface of lungs, showing patchy congestion and minute haemorrhages.

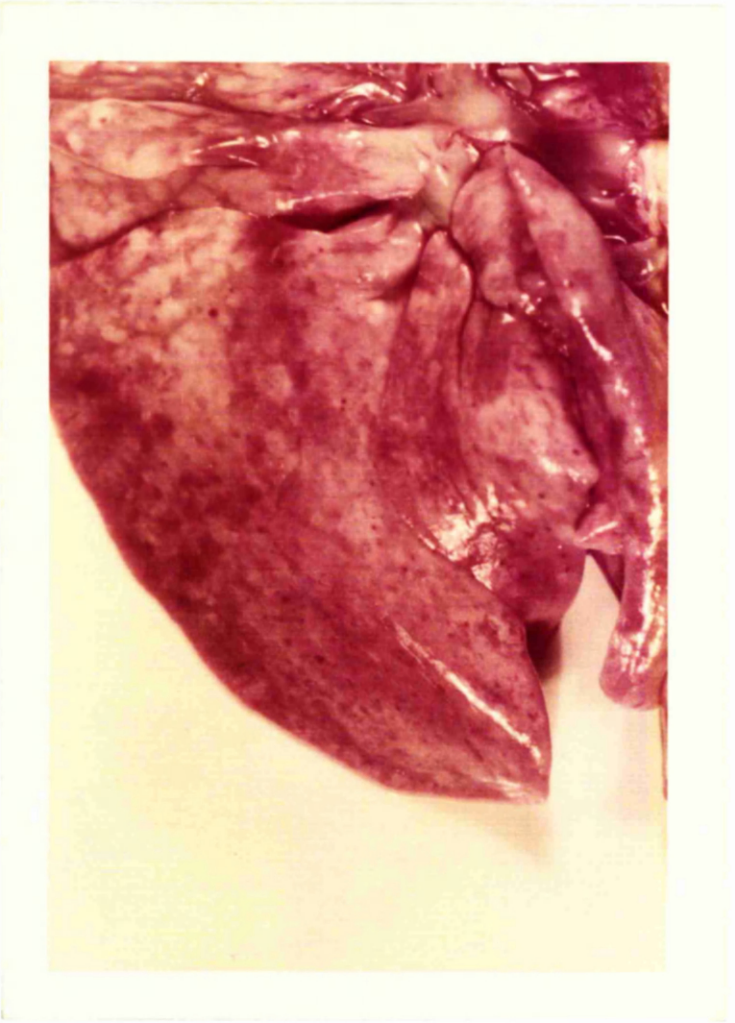

Fig. 2. Enlarged detail from Fig. 1. Right diaphragmatic and intermediate lobes. Note patchy congestion of a dull magenta colour and numerous minute haemorrhages.

often exhibited a characteristic dull magenta colour (Figs.1 and 2) which quickly turned to reddish brown on exposure to air. This colour reaction seemed to be connected with the presence of oedema, congestion and possibly cellular exudate.

A degree of consolidation, apparently due to concurrent bacterial infection, was found in 5 out of 30 cases. It was usually located in the lower parts of the apical or cardiac lobes. On one occasion, however, it was observed in the upper portion of the apical lobe (Dog 32). In several cases, a somewhat darkened, consolidated tissue was seen extending to a depth of a few millimeters under the pleura from the surface areas of the same colour. This change, apparently of viral origin, could be seen in a number of locations in the same lung.

A distinct, suppurative bronchopneumonia was not observed in the cases studied.

A very few of the lungs showed some emphysema. Whenever present, it was located either at the borders of the lung, or in the central portions of the apical lobes.

The larger bronchi were usually unaffected, but sometimes contained a small amount of catarrhal exudate. On many occasions, an oedematous fluid, often frothy in appearance, was seen in them.

The pleural sacs were free from the inflammatory process except for one case (17) which showed a fibrinous pleurisy with adhesions in the right lung. The amount of fluid present was sometimes slightly increased.

In the dogs which were ill for less than two weeks, the changes observed were generally moderate in degree. Some cases showed focal hyperaemia of a "mottling" type (Figs. 1 and 2), but in the remaining dogs, confluent congestion in some parts of the lungs predominated. Oedema was often

appreciable in degree with the exception of one case (21) in which it was quite marked. A small consolidated area was observed in the apical lobe of Dog 33.

The changes found in dogs ill from two to four weeks were more marked and sometimes showed intense congestion and oedema. The most common feature was a moderate to marked hyperaemia of the lower parts of the lobes with an appreciable, though sometimes mild, congestion in the upper regions. A few cases exhibited a disseminated focal involvement in all lobes. Consolidation was found in 3 cases (2, 19 and 32).

The dogs which showed signs of disease from five to seven weeks were affected to a milder degree. Only 2 cases displayed a marked hyperaemia in some parts of the lungs. This was found only in the upper portions of the lobes (Cases 11 and 14) with the remaining regions showing a moderate involvement. One case (25) was only slightly affected. The oedema in dogs of this group was usually moderate.

The remaining two cases (26 and 30) which showed signs of illness for over 10 weeks exhibited either no changes (26) or only a very mild congestion (30).

Regional lymph nodes. While all the regional lymph nodes reacted to invasion of the virus by some enlargement, the most prominent and sustained response was exhibited by the bronchial lymph nodes. This reaction, at first mild in degree, became quite marked between the 3rd and 4th weeks of illness. From the 6th week it seemed to subside. Only very mild alterations were evident after the 10th week of the disease.

Experimental cases

Upper respiratory tract. The first appreciable changes in the nasal cavity appeared on the first day of illness in the form of a very slight

patchy hyperaemia of the turbinate folds. Small amounts of thin, watery exudate were also present. No lesions of significance were seen in the pharynx, larynx, trachea or main bronchi. In the remaining dogs, sacrificed at various stages of the disease, the changes found in the upper respiratory tract were usually mild or sometimes absent from certain parts. Only 2 cases, examined on the 7th and 59th days of illness, showed moderate to marked congestion of the nasal mucous membrane, with some foci of suppuration in one dog (on the 59th day). A moderate amount of thin, clear fluid, the specific gravity of which ranged from 1.010 to 1.016, was observed in 3 dogs destroyed on the 3rd, 8th and 11th days of symptoms. Mild enlargement of the palatine tonsils occurred in most cases, and some degree of follicular hyperplasia was seen in the pharyngeal tonsil of a few dogs.

In contrast with the clinical cases, the changes found in the upper respiratory tract of all 20 experimental dogs were consistently mild, irrespective of the stage of illness.

Lung. The involvement of the lung in the first 5 days of illness was of mild degree (Cases 17, 20 and 9, destroyed on the 1st, 3rd and 5th days of illness, respectively). In all 3 cases the organ showed slight, often patchy, congestion and a number of minute haemorrhages. Although a small amount of watery fluid was seen in some larger bronchi, particularly in Dog 20, no oedema could be found in any part of the lung. On occasion, sharply circumscribed areas of emphysema were seen at the lower border of both apical and cardiac lobes.

A somewhat similar degree of involvement, with only minor variations, was exhibited by the remaining cases, with the exception of Dogs 3, 22 and 2. Dog 3 was sacrificed on the 7th day of symptoms, and its lungs displayed considerable hyperaemia, especially in the anterior parts of the apical and

cardiac lobes. Oedema, however, was not observed. A mild to moderate congestion of a fine "mottling" type, with a more pronounced hyperaemia at the lower border of both diaphragmatic lobes, was seen in Case 22 (9th day of illness). In addition, a number of haemorrhages and many areas of emphysema were present (Fig. 3). Dog 2, destroyed on the 157th day after the first signs of illness, did not show any discernible changes in the lung. At the time of euthanasia, this dog was apparently in a good state of health.

Only minor alterations were seen in the lungs of 5 cases used for a study of the neurogenic form of oedema (Cases 24, 25, 26, 27 and 30). On two occasions, very small areas of consolidation, apparently of bacterial origin, were noted in the cardiac (Case 24) and diaphragmatic lobes (Case 30). There was no macroscopical evidence of oedema in any of these cases.

Generally, the changes found in the lung of experimental cases were characteristically mild. The main features consisted of (1) slight hyperaemia, often with small areas of haemorrhage, (2) mild emphysema and (3) complete absence of oedema. No excess of pleural fluid was seen in any of the dogs. In most cases, the lung appeared to be comparatively "dry", flaccid and often more collapsed than would be expected from the usual post-mortem atelectasis. It seems reasonable to suspect that this state of collapse was associated, at least in part, with sudden constriction of bronchial passages elicited by an overdose of nembutal.

Regional lymph nodes. There was usually mild to moderate enlargement of the lymph nodes examined, i.e., parotid and bronchial. In addition, some oedema and, occasionally, a few small haemorrhages could be found in the affected nodes. Only one dog showed considerable enlargement of both nodes (Case 1, destroyed on the 59th day of illness). This occurrence was

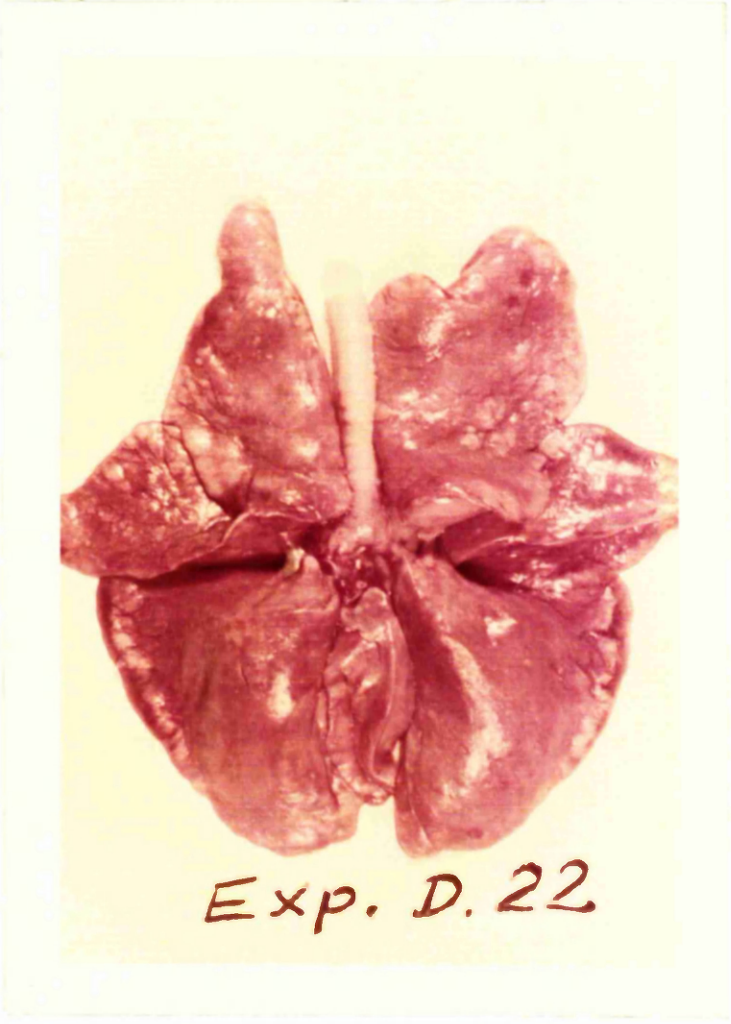

Fig. 3. Experimental Case 22. Costal surface of lungs, showing mild hyperaemia and areas of compensatory emphysema.

apparently associated with focal suppuration of the turbinate folds. Both lungs, however, showed only slight congestion.

Bacteriological Findings

Clinical cases

As indicated previously, bacteriological examination was conducted only on the lung tissue.

Of the 28 cases from which cultures were made, 14 per cent showed no bacterial growth on blood agar and MacConkey media. The lungs from the remaining dogs yielded Escherichia coli in 68 per cent, staphylococci in 46 per cent, and Proteus vulgaris in 21 per cent of the cases. Nonhaemolytic and α-haemolytic streptococci were isolated from five (17 per cent) and Pseudomonas aeruginosa from three dogs (10 per cent). Corynebacterium pyogenes was recovered from one puppy and Salmonella typhi-murium from a $2\frac{1}{2}$-year-old dog. Only one culture yielded Brucella bronchiseptica. This micro-organism was isolated from a four-month-old Beagle, the lungs of which showed moderate hyperaemia with a few foci of consolidation in the lower parts of the apical and cardiac lobes.

In most cases, the cultures yielded more than one species of micro-organism.

Experimental cases

The proportion of negative bacteriological results in the experimental dogs was considerably higher than in the clinical cases. Only 25 per cent of cultures yielded some bacteria. The most common was E. coli (10 per cent). Nonhaemolytic streptococci, staphylococci, and Ps. aeruginosa were isolated in 5 per cent of the cases. Br. bronchiseptica was recovered on two occasions from dogs inoculated intraspinally with the virus of distemper. In both cases the lungs were only mildly involved.

Histopathology

No attempt is made to describe in detail all histological changes found in the respiratory system and in its regional lymph nodes. Only the abnormalities which were encountered in the lung tissue are dealt with at length. The results of electron microscopic observations on the structure of the normal alveolar wall, on which the interpretation of certain pulmonary changes has been based, are also presented.

The structure of the alveolar wall.

Electron microscopic studies revealed that the pulmonary alveolar wall consists essentially of three elements, viz., (1) epithelium, (2) basement membrane, and (3) capillary endothelium. The supporting framework of fibres, although often abundant in some parts, was not discernible in the thin segments of the wall.

Alveolar epithelium. The surface of the alveolar wall was found to be lined by a continuous epithelium composed of widely strewn, nucleus-bearing cell bodies and their attenuated, remarkably long protoplasmic expansions (Figs. 4-14). The latter constituted the major portion of the alveolar epithelial lining.

The main body of the epithelial cell varied in its size and morphological properties. When found applied to the free segment of the wall, it was usually flat, elongated, and wedged between the loops of capillaries. In such instances, it could not be easily differentiated from the endothelial cell, if examined only under low magnification. The nucleus of this variant of the epithelial cell occupied usually a considerable part of the cytoplasmic body and was delineated by what appeared to be a double membrane. The perinuclear cytoplasm contained a few mitochondria, which could be identified by their characteristic structure (Sjöstrand and Rhodin, 1953) of internal

lamellae and a double delimiting external membrane (Fig. 6, M). The endoplasmic reticulum (Palade and Porter, 1954) consisting of fine tubules and vesicles could also be seen (Fig. 6, ER). The main body of the cell, as shown by Fig. 4, measured 8μ in length and 3.5μ in depth. The cytoplasmic processes attenuated gradually (Figs. 4, 5 and 6) and extended over the surface of the wall. At some points they became very thin, measuring approximately 180 Å (Fig. 6, AT). Generally, however, the thickness of the flattened part of the epithelium was greater and usually ranged from 500 Å to 2,000 Å. The thicker segments occasionally contained mitochondria and, more often, a rich network of the endoplasmic reticulum (Fig. 5, M; Figs, 10, 11 and 12, ER). The cytoplasm of the epithelial cell was enveloped by a membrane, represented in the electron-micrographs by a dense line, the average thickness of which was estimated as approximately 50-75 Å (Figs. 5 and 6, CM).

The other morphological variant of the epithelial cell was apparently identical with the "septal cell" of Lang. Its main body, ovoid in shape, measured approximately 4μ and was either located in the alveolar niche (Figs. 7 and 13) or stretched across the wall (Fig. 9). In addition to numerous mitochondria and abundant endoplasmic reticulum, the perinuclear cytoplasm contained a number of fairly large (200mμ - 500 mμ), markedly osmiophilic inclusions, many of which showed an irregularly laminated internal structure (Figs. 7, 8, 9, 13 and 14; OI). The origin and functional significance of these bodies have not been established. Bargman and Knoop (1956) thought that they represent a degenerate form of mitochondria. The surface of the main body of the cell was sometimes covered by small, fairly regular projections (microvilli), of an average length of 140mμ (Fig. 7).

The cytoplasmic extensions of these cells were identical in structure and character with those described previously, but their attenuation was

often very abrupt (Figs. 7-14).

Despite some morphological variations, the alveolar epithelial cells, as revealed by many other electron-micrographs, seemed to possess a unity of structure, the immediate forms of which appeared to be dependent upon the requirements of function and local stress.

Basement membrane. The alveolar epithelium and capillary endothelium were separated by a homogeneous membrane, the thickness of which varied from 270 Å in its thinnest parts (Fig. 6, B.M.) to approximately 1,000 Å (Fig. 12, BM). The average width was estimated as 500 Å. Areas of lighter density and somewhat cloudy in appearance could be discerned in many parts of the membrane. The electron-micrographs examined failed to reveal evidence of any fibrillar internal elements. The basement membrane seemed to possess a superior structural strength. This was well exhibited by imperfect sections (Figs. 4 and 5), in which the adjacent cytoplasmic elements were shown to be partly torn by mechanical stress (so-called "drag"), but the basement membrane remained intact. In parts of the alveolar wall which contained abundant collagen and elastic fibres (Figs. 7, 8, 13 and 14), the basement membrane appeared to follow the course of both the epithelium and endothelium, leaving the tissue space open.

Capillary endothelium. The endothelium of the alveolar capillaries was also continuous, and in some structural aspects resembled the epithelial lining. It consisted of prominent, elongated cell bodies and their cytoplasmic extensions. The latter were extremely long, and at some points showed a thickness of 90-100 Å (Figs. 5 and 12). The cytoplasm of both the cell body and the attenuated portions contained mitochondria and a rich network of endoplasmic reticulum (Figs. 5, 10 and 11). A thin (approximately 50 Å) cytoplasmic membrane was found to invest the endothelial cell. At the

ELECTRON MICROGRAPHS OF THE RAT LUNG

Abbreviations

ALV - alveolar space

BM - basement membrane

END - endothelium

EP - epithelium

NUC - nucleus

M - mitochondria

CAP - capillary

ER - endoplasmic reticulum

EL - elastic elements

COL - collagen fibrils

CP - coagulated plasma

OI - osmiophilic bodies

CM - cytoplasmic membrane

LEUC - leucocyte

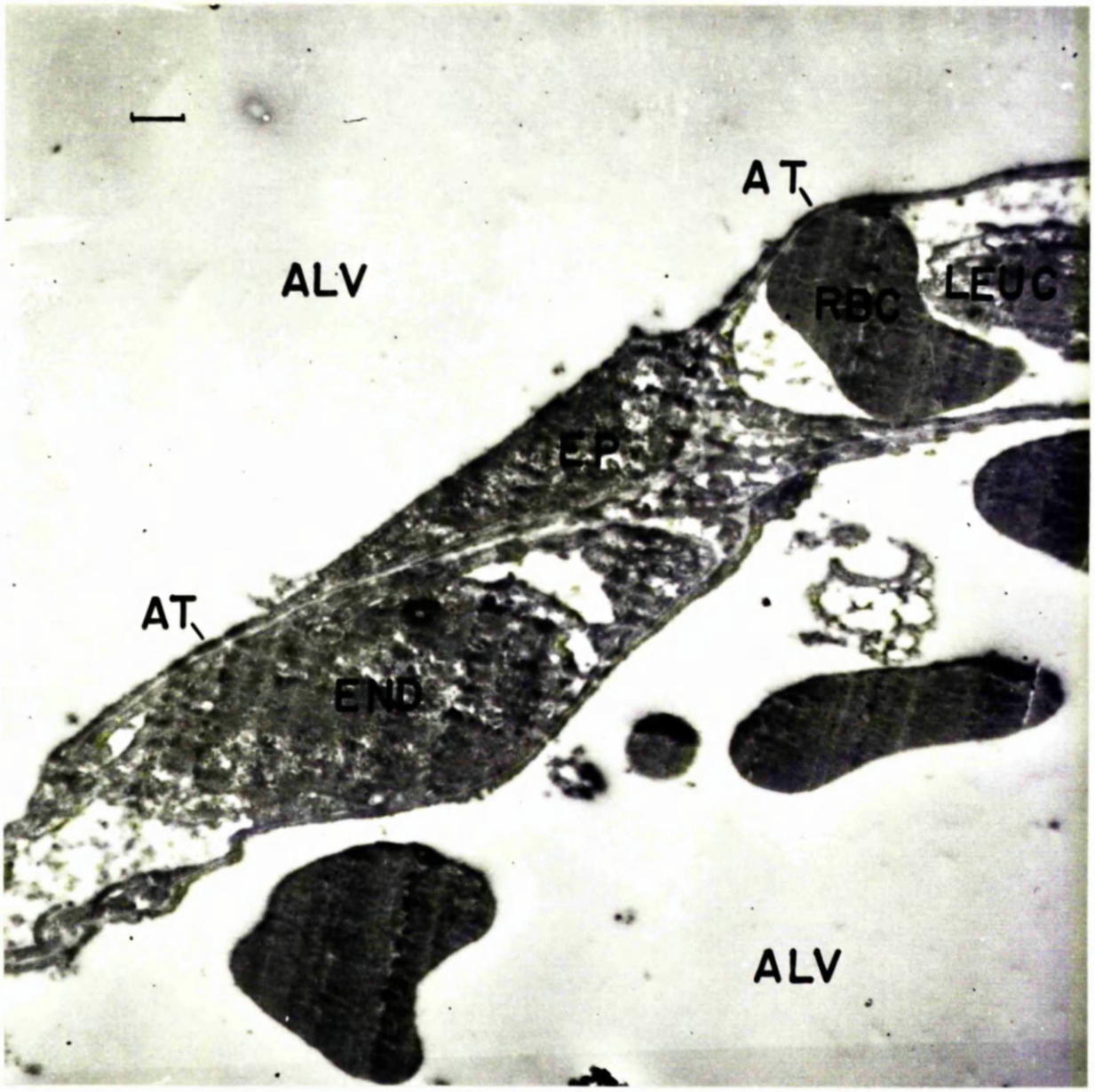

Fig. 4. Low power view of alveolar wall. Note two capillary loops and epithelial cell (EP) with cytoplasmic attenuations. x 7,500.

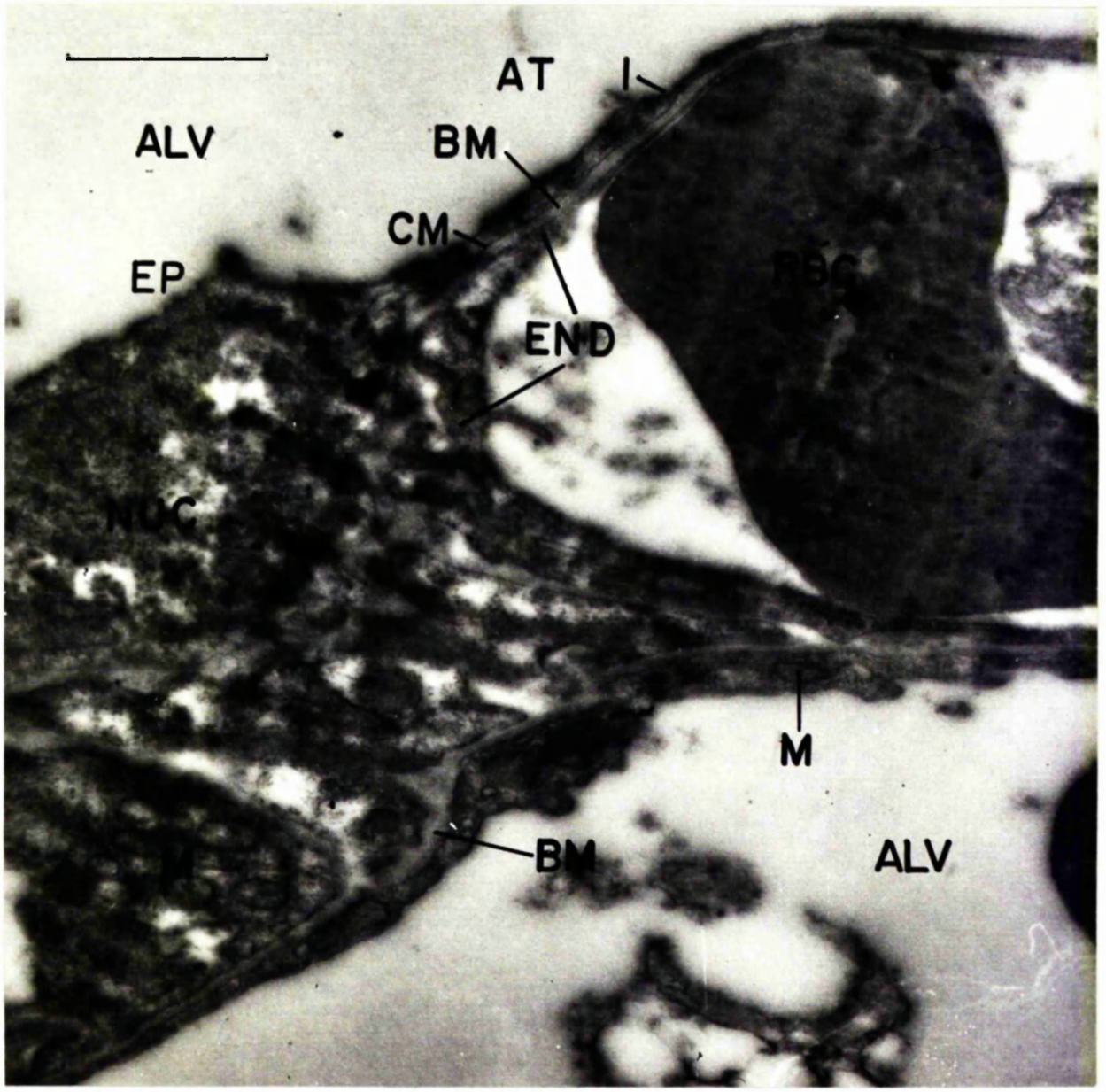

Fig. 5. Enlarged detail from Fig. 4. Epithelial cell (EP) attenuates to cover the alveolar wall. Epithelium and endothelium are separated by basement membrane (BM). At (1) the attenuated epithelial cell is 350 Å thick. x 28,000.

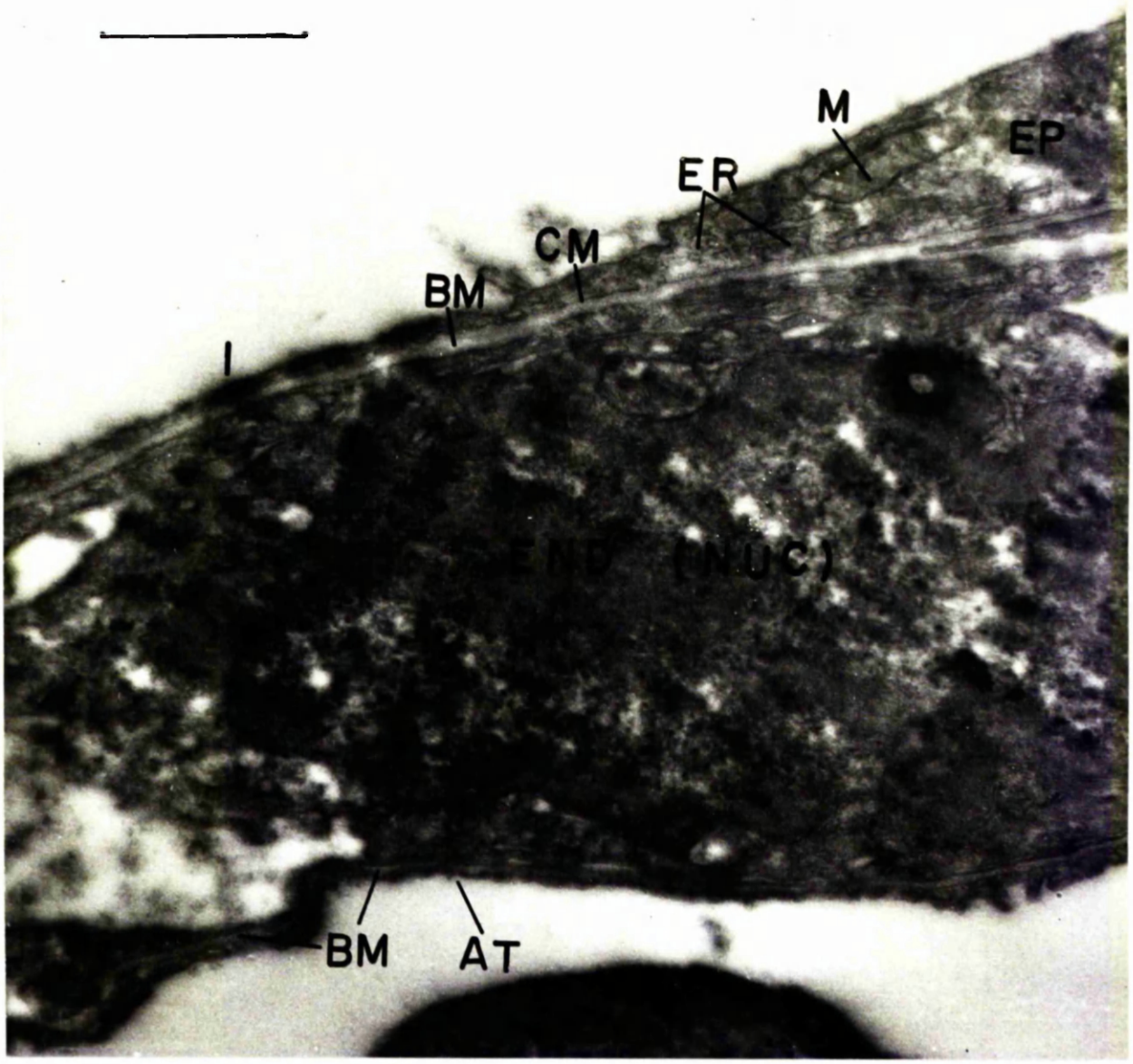

Fig. 6. Detail of Fig. 4. Epithelial cell (EP) attenuates to 550 Å at (1) and to 180 Å at (AT). Section was cut through the long axis of endothelial cell (END). x 28,000.

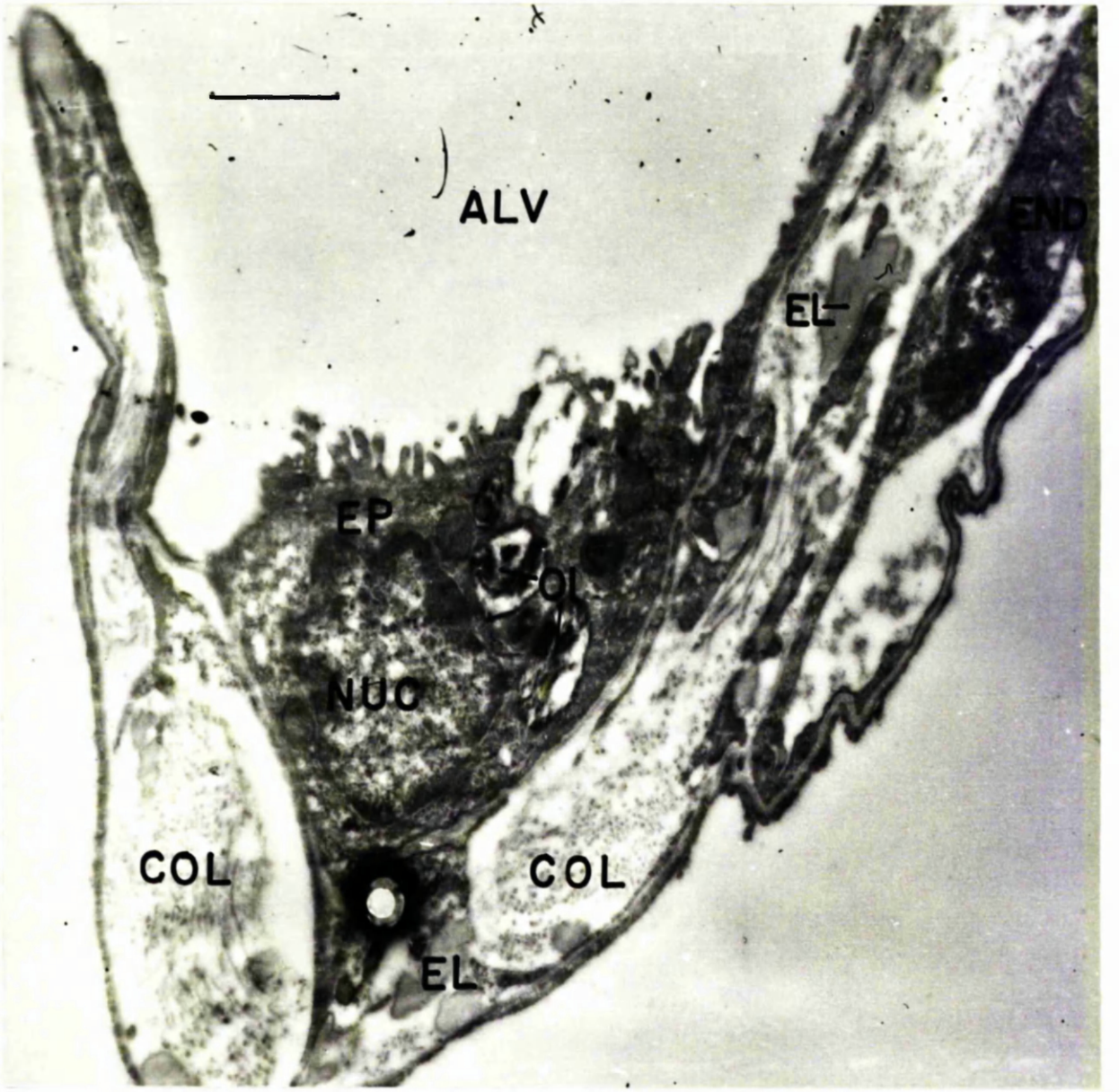

Fig. 7. Septal cell in alveolar niche. Note the presence of osmiophilic bodies (OI), microvilli and cytoplasmic attenuations. Tissue spaces are rich in fibrils of collagen (COL) and contain elastic elements (EL). A somewhat collapsed capillary with its endothelial cell is also seen. x 18,000.

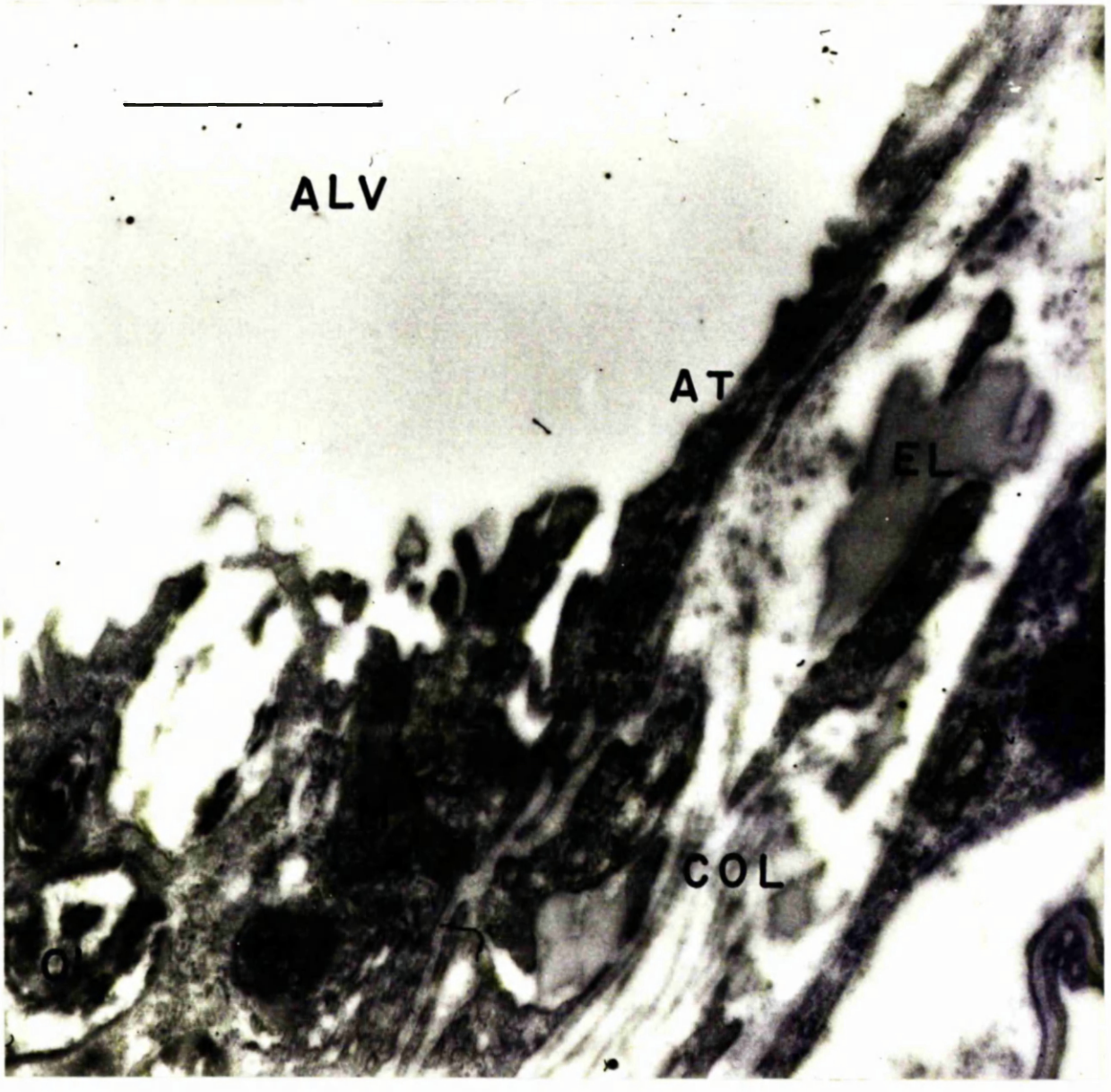

Fig. 8. High-power view of Fig. 7. Note abrupt attenuation of the cytoplasmic body, mitochondria and osmiophilic inclusions. Elastic elements and fibrils of collagen, a few of which show axial periodicity, are also seen. x 35,000.

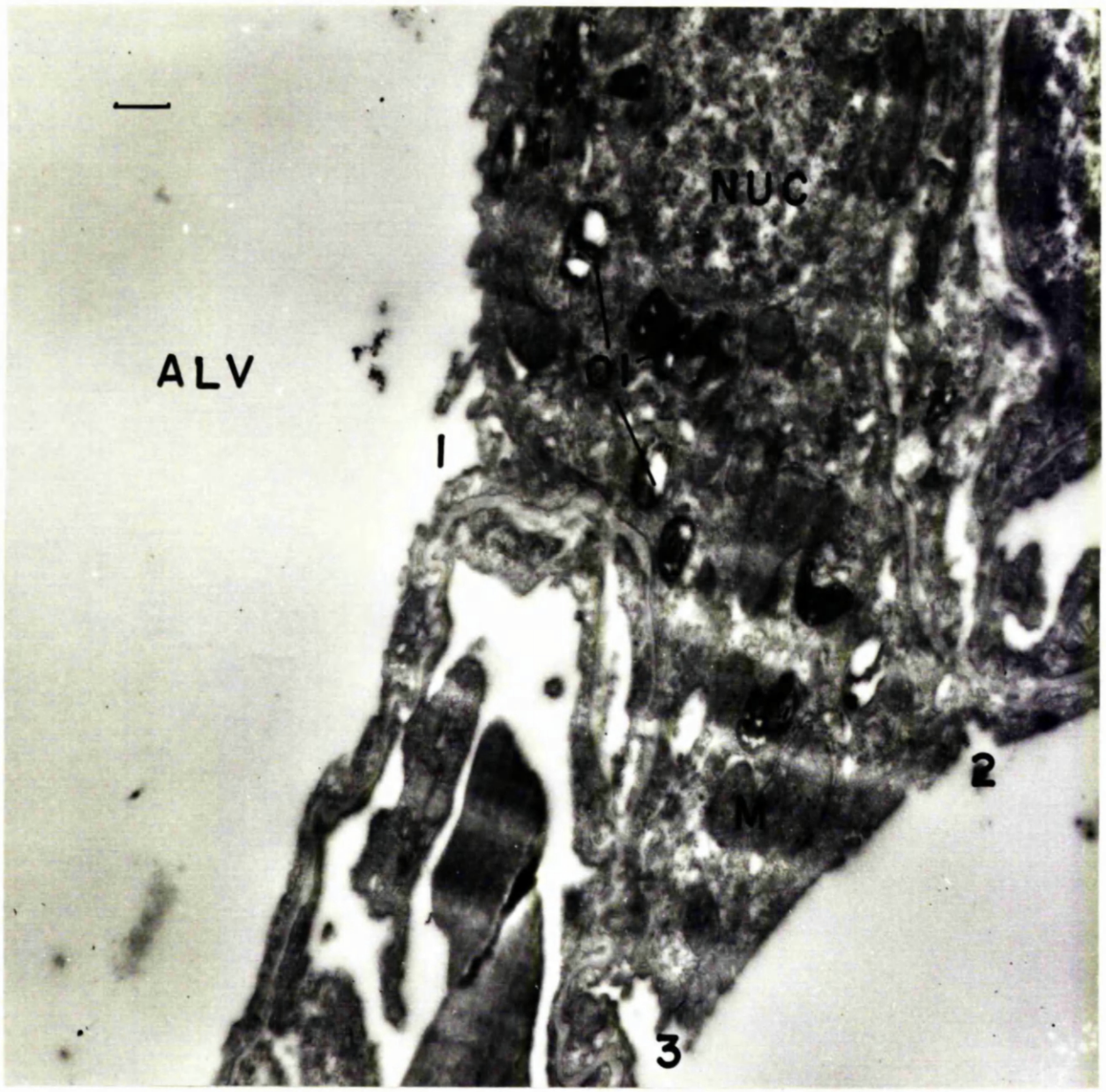

Fig. 9. Septal cell stretching across the wall. The cytoplasmic body shows attenuations at three points (1, 2 and 3). Osmiophilic inclusions and mitochondria are numerous. x 7,500.

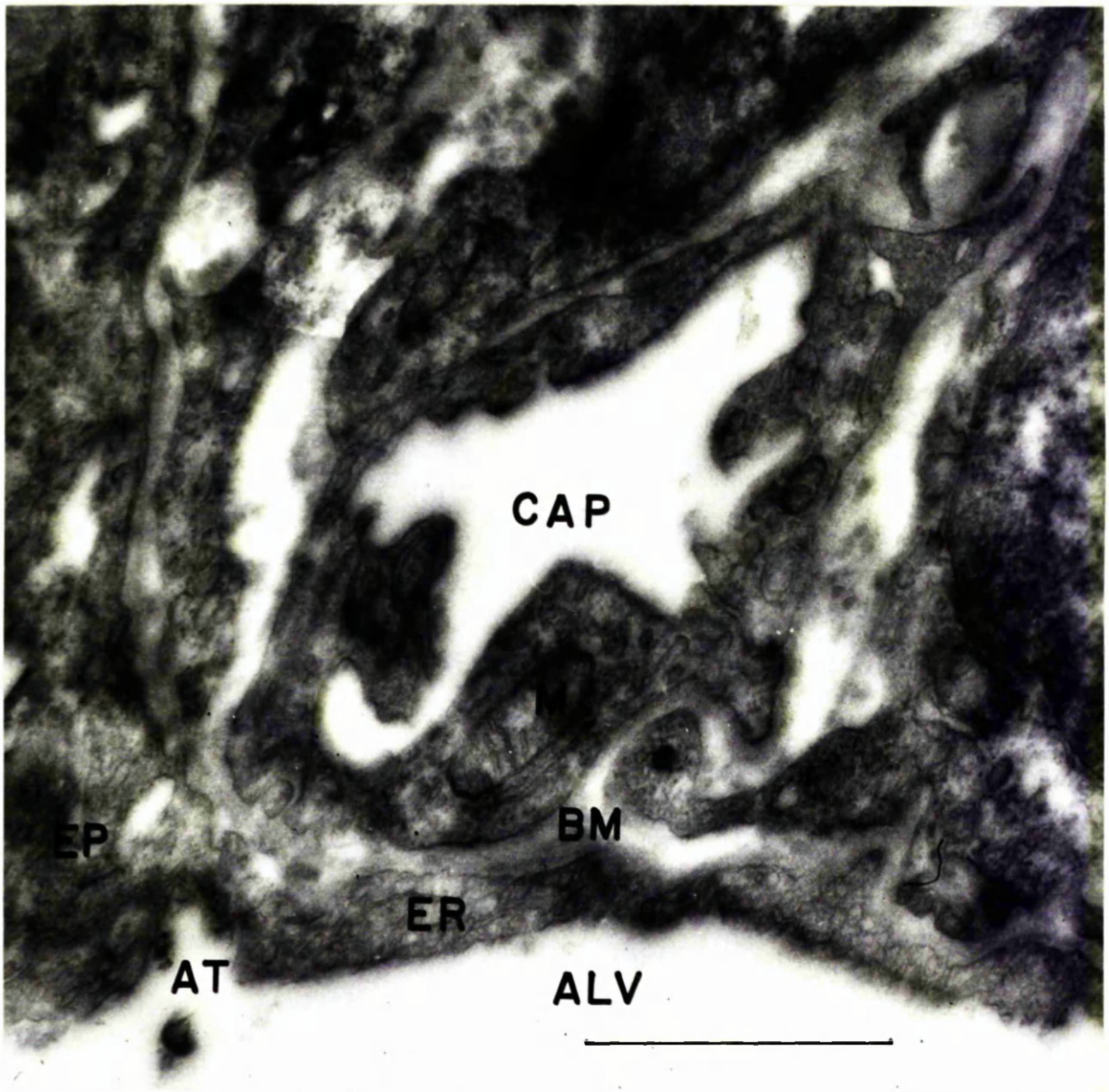

Fig. 10. Detail of Fig. 9 at (2). Note cytoplasmic attenuation (AT), mitochondria, endoplasmic reticulum and basement membrane. x 42,000.

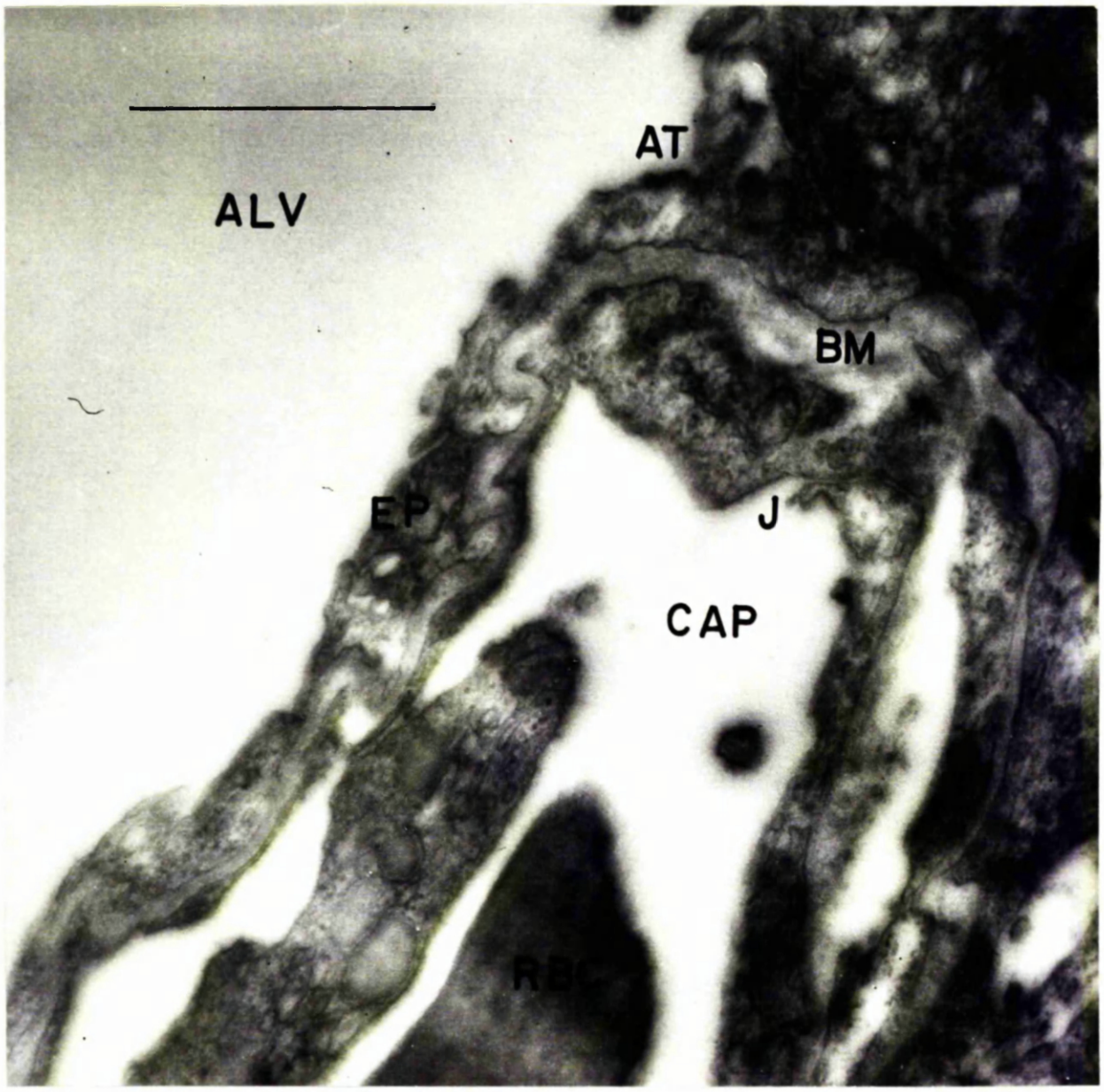

Fig. 11. Detail of Fig. 9 at (1). The point of attenuation is rich in endoplasmic reticulum. A junction of two endothelial cells could be seen at (J). x 42,000.

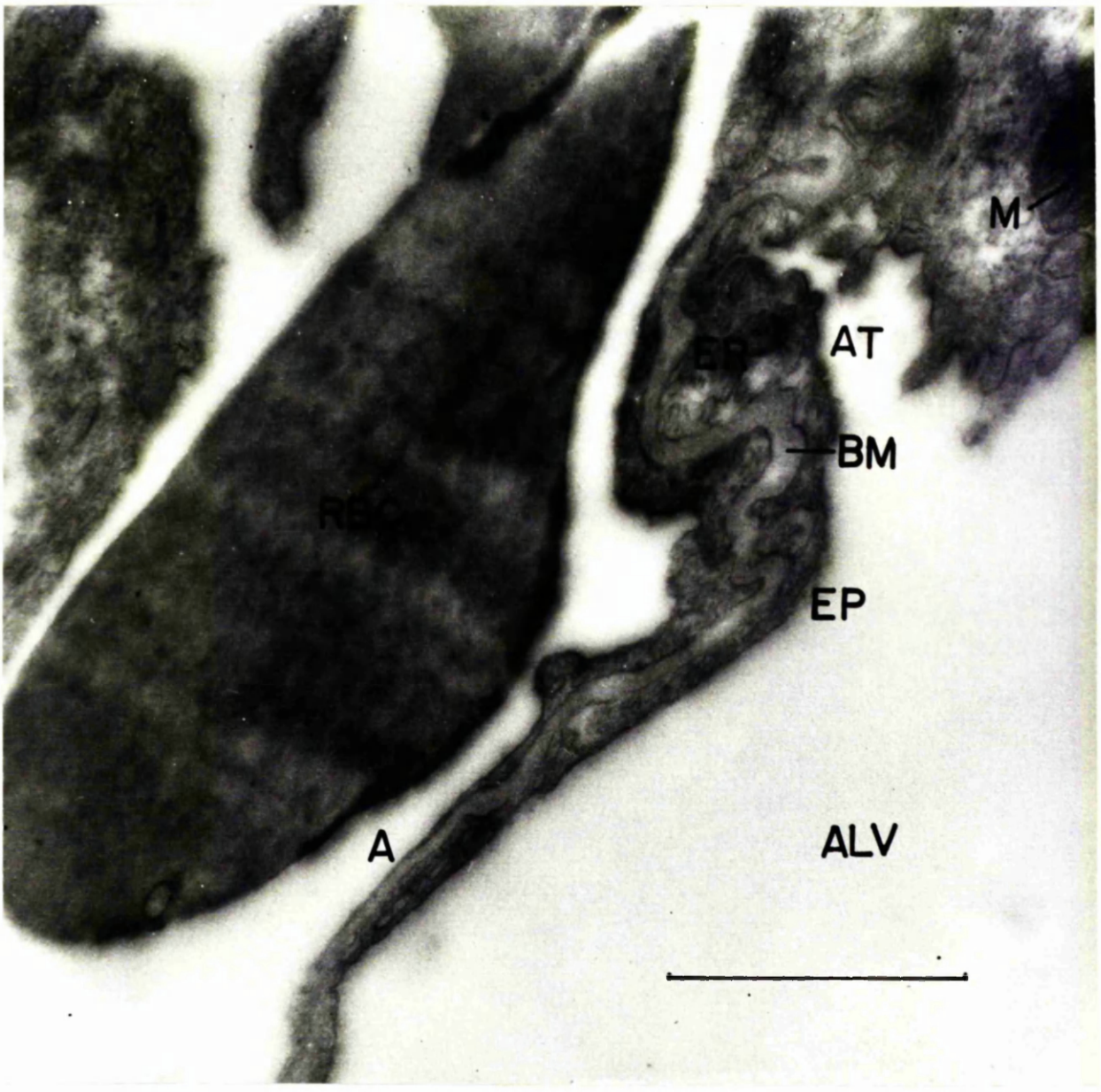

Fig. 12. Detail of Fig. 9 at (3). Note abrupt attenuation of the cytoplasm, endoplasmic reticulum and microvilli. At (A) the endothelium is approximately 150 Å thick. x 42,000.

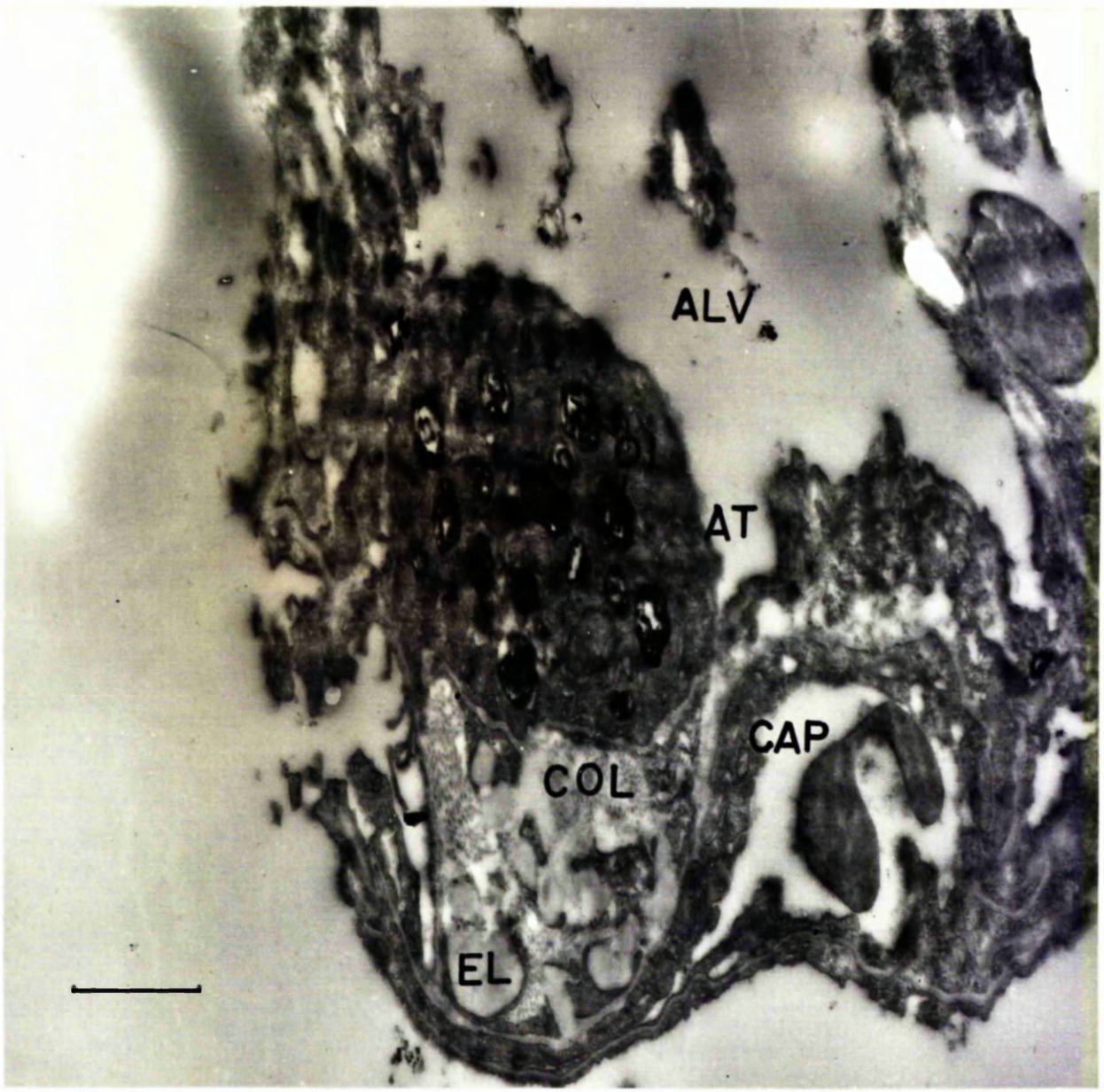

Fig. 13. Septal cell in alveolar niche. Osmiophilic inclusions are prominent. Note the abundance of collagenous and elastic elements in mural tissue space. x 18,000.

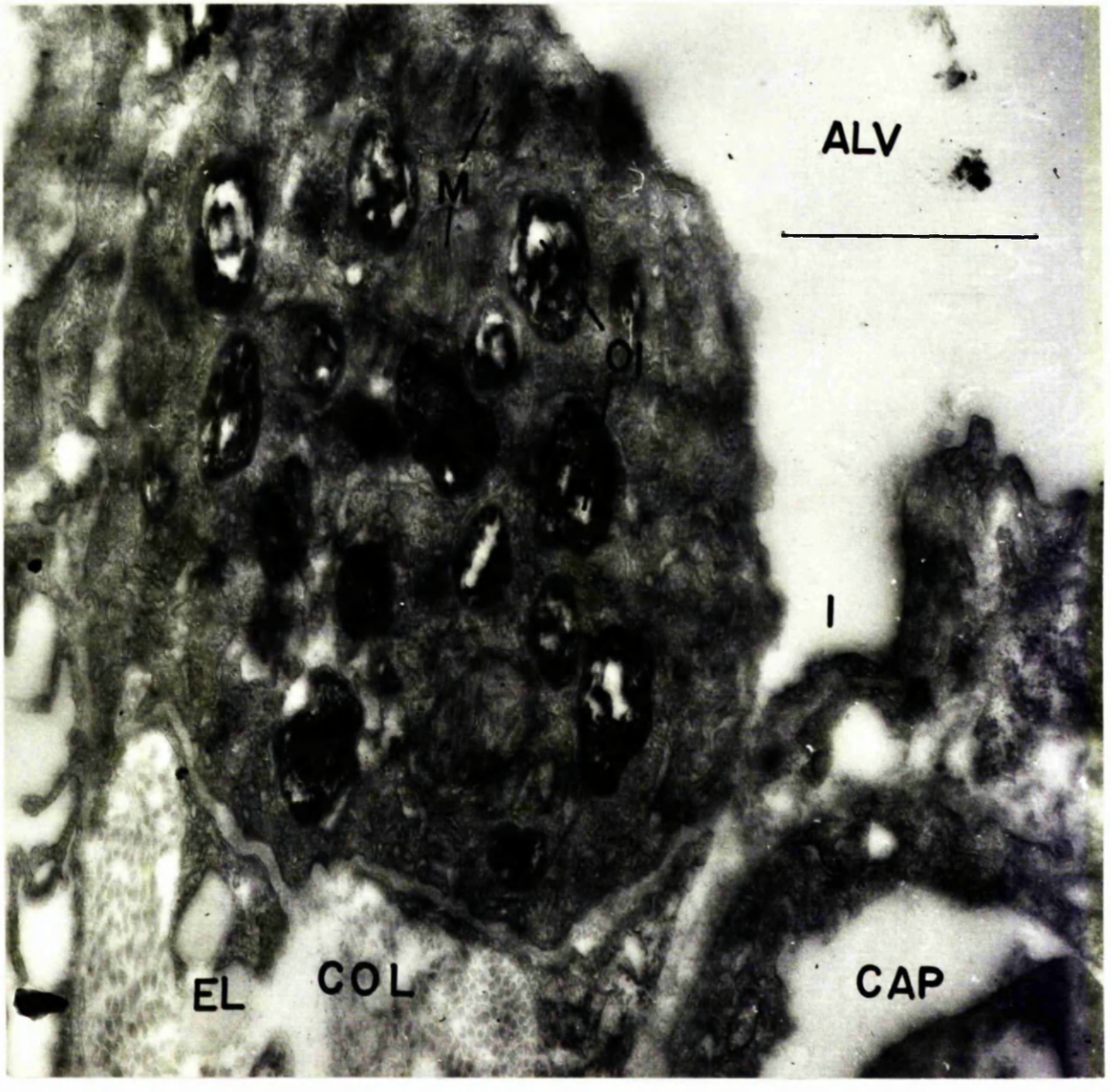

Fig. 14. Enlarged view from Fig. 13. Note the prominence of osmiophilic bodies and abundance of mitochondria. The cell attenuates abruptly at (1). Collagenous fibrils at cross section are approximately 140 Å thick. x 35,000.

junction of the two cells, the protoplasmic extensions were separated only by a narrow (200 Å) cleft (Fig. 11), which formed a direct connection between the lumen of the capillary and the basement membrane.

Collagenous and elastic elements. The supporting framework of the alveolar wall was particularly prominent in the vicinity of septal cells and in the alveolar angles (Figs. 7, 8, 13 and 14). It was represented by fibrils of collagen (unit fibres of collagen, Low, 1953) and by elastic elements. The former were very uniform in structure and had an average diameter of 150 Å. When sectioned longitudinally, they often showed a characteristic axial periodicity (Fig. 8). Their delimiting external membrane was very thin and did not exceed 60 Å. The elastic elements formed irregularly outlined masses and were apparently composed of fibrils, 70 Å in thickness, embedded in an amorphous cementing substance (Fig. 9).

Clinical cases

Upper respiratory tract. The changes found in the nasal cavity, pharynx, larynx and trachea were generally very mild in the first two weeks of illness. An occasional slight congestion of subepithelial blood vessels was observed, but deeper layers did not show any discernible abnormalities. Only one case (33) displayed a mild degree of catarrhal inflammation of the nasal mucous membrane, and a slight oedema of the larynx accompanied by an incipient acute inflammatory reaction were noted in another dog (Case 20). The cytoplasmic inclusion bodies manifested their presence in a majority of these cases (4 out of 7). They were most numerous in the epithelium of the nasal mucosa and its glands. One case only (33) displayed them abundantly in the tracheal epithelium.

The dogs which suffered from the disease for 2 to 4 weeks showed more prominent and advanced changes. The nasal passage in two-thirds of the

cases exhibited a mild to moderate catarrhal involvement (Fig. 15), which frequently was attended by some degree of hyperaemia and hyperactivity of the mucous glands. There was no appreciable lesion in the lamina propria. The cytoplasmic inclusion bodies were found in the majority of cases in the epithelium and in the mucous glands. The larynx and trachea revealed comparable catarrhal reaction and congestion. No changes of significance were generally noted in the pharynx except for a very slight and superficial inflammatory reaction and some--and on occasion pronounced--overproduction of mucin in the digestive part. One case, however, showed a significant degree of involvement. The mucous membrane was markedly disarranged and partly desquamated and revealed appreciable catarrhal changes (Fig. 16). A rather unusual occurrence was noted in Case 2, in which the tracheal mucosa presented a well-advanced and active subacute inflammatory process accompanied by considerable hyperaemia. Most of these cases exhibited cytoplasmic inclusion bodies in the epithelium of the larynx and trachea. Only in a few dogs were they found in the pharynx. Case 15 showed a small number of intranuclear inclusions in the epithelial cells of the tracheal mucous membrane.

The changes observed in the pharyngeal tonsil and the morphology of intranuclear inclusion bodies will be described in subsequent parts.

With the exception of a mild oedema of the lamina propria of the larynx (Case 16) and a slight catarrhal inflammation of the trachea (Case 11), no structural abnormalities were found in 5 dogs ill for 5 to 7 weeks. In three of these cases a moderate number of cytoplasmic inclusions was seen in the mucous membranes of the nasal cavity, pharynx, larynx and trachea.

The dogs which were examined after 10 weeks from the first signs of the disease (26 and 30) did not show any appreciable changes in the upper part of the respiratory tract.

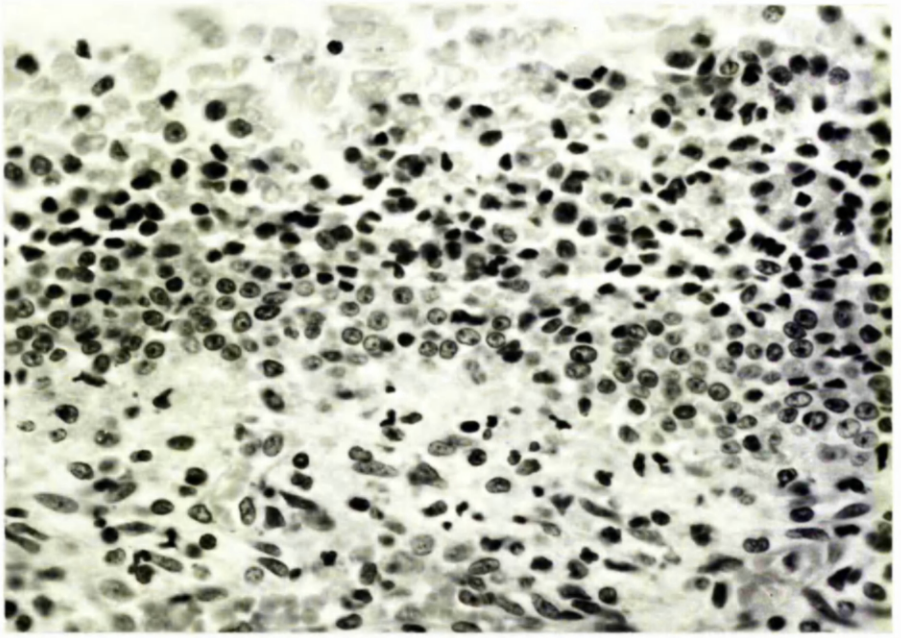

Fig. 15. Clinical Case 3. Nasal mucous membrane, showing desquamation of epithelial cells and mild catarrh. Haematoxylin and eosin. x 410.

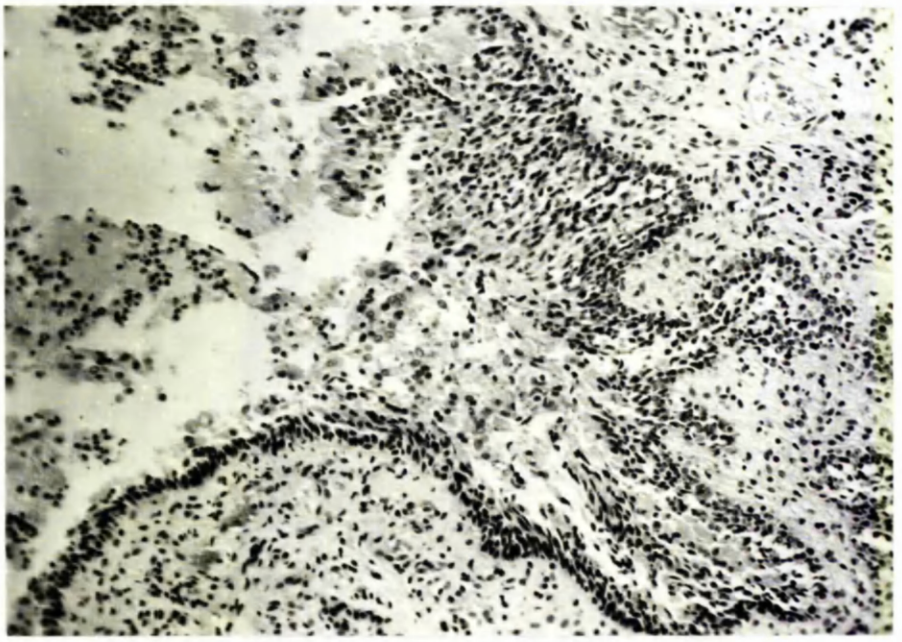

Fig. 16. Clinical Case 4. Pharyngeal mucosa. Moderate catarrh. Haematoxylin and eosin. x 100.

Lung. Hyperaemia constituted one of the earliest changes observed in the lung. Usually of a mild degree in the initial and late phases, it was often pronounced at the height of illness. On such occasions the affected capillaries were markedly tortuous and engorged with blood. Not infrequently, some extravasation of red blood cells accompanied the congestion, but areas of frank haemorrhage were seldom seen. Of 30 dogs examined, only three did not show any appreciable hyperaemia. One of these (26) was destroyed 10½ weeks after the first signs of the disease. The remaining two, 20 and 19, were ill for less than 2 and 4 weeks, respectively.

Oedema of alveoli and interstitium often attended the state of hyperaemia but was somewhat less common; it occurred to a varying extent in 77 per cent of cases. Seven dogs, including those which were ill for more than 10 weeks (26 and 30), did not show any discernible oedema. The outpouring of fluid into the alveoli was an early phenomenon, apparently concurrent with the evolement of congestion. In a number of cases, it was quite marked in the initial phases of the pulmonary reaction and often remained pronounced until the sixth week of illness. In later stages, the oedema, although present in some lungs, was only mild and focal in distribution.

Most commonly, the fluid accumulated in alveolar spaces, but in 53 per cent of cases it was also present, often to a considerable extent, in the perivascular interstitial tissue (Fig. 17). On a few occasions, peribronchiolar areas were similarly involved, apparently by extention of oedema from the adjacent perivascular regions (Fig. 18). In many instances, the fluid occupied the entire alveolar space, but in some appeared to be scalloped at the wall by the entrapped air. The protein content of oedema fluid, as judged by the intensity of staining reaction with periodic acid-Schiff technique, varied considerably not only from case to case but often also in the same microscopic

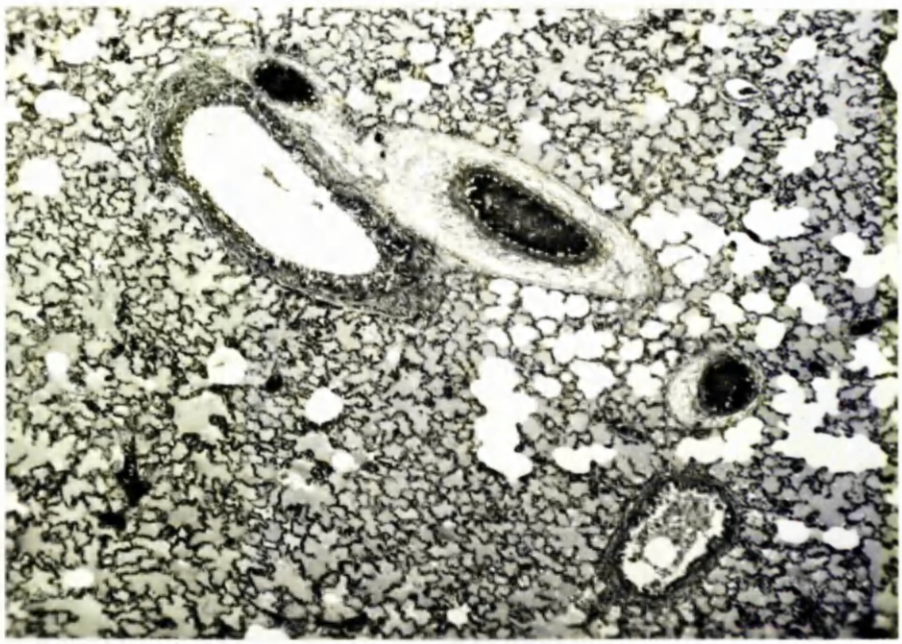

Fig. 17. Clinical Case 4. Lung. Intra-alveolar and perivascular oedema. Heidenhain's iron-haematoxylin and eosin. x 30.

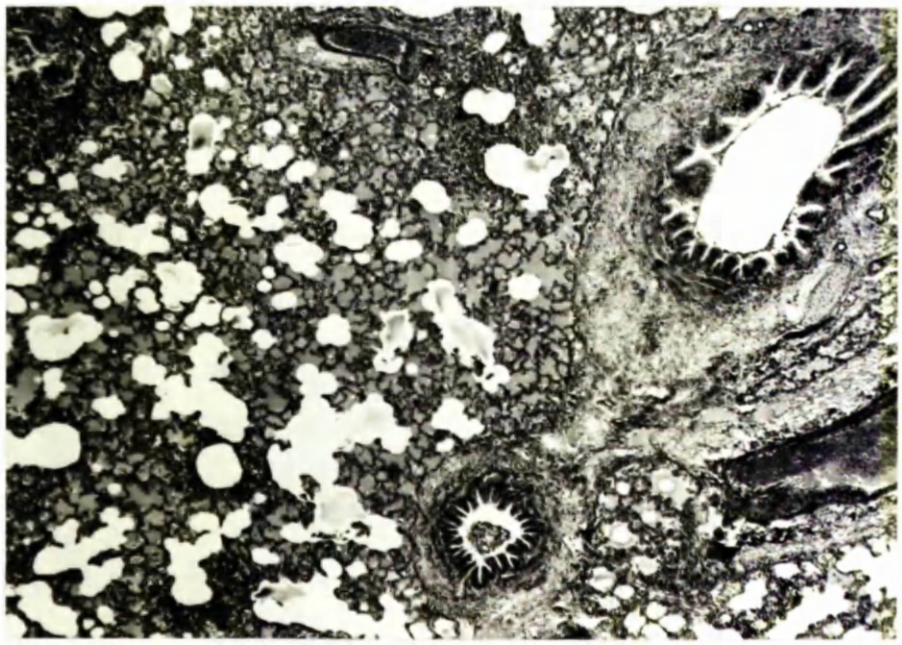

Fig. 18. Clinical Case 32. Lung. Intra-alveolar and peribronchiolar oedema. Haematoxylin and eosin. x 30.

field (Fig. 19). Occasional strands of fibrin were seen in many areas in most of the cases (70 per cent). This occurrence had some bearing on the formation of fibrinous "plugs", and apparently also on the process of intra-alveolar organization (v. infra).

The terminal air passage were usually free of oedema, but in some instances even the medium-sized bronchioles were flooded to a considerable extent. Only insignificant amounts of fluid could be found in the "epithelialised" areas.

A mild collapse of the pulmonary parenchyma occurred in 60 per cent of the cases. Most frequently, it was focal in distribution and entirely absent from the areas of oedema. On occasion, when it was more advanced (Cases 19 and 29), the involved parts showed a distinctly trabecular, lace-like arrangement of the collapsed groups of alveoli (Figs. 20 and 21). A degree of compensatory emphysema sometimes accompanied the subpleural areas of collapse.

No significant abnormalities were noted in the primary and secondary bronchi, with the exception of a few isolated cases which showed mild catarrh (2, 3, 4 and 15). The finer air passages, however, often exhibited characteristic, and sometimes pronounced, changes.

Early in the course of the disease, small accumulations of lymphocytes, macrophages and plasma cells appeared in and around bronchiolar walls (Fig. 22) and in perivascular tissue spaces, especially of venules (Fig. 23). The arterial channels did not appear to be involved. With the progress of illness, the accumulations of mononuclear cells not infrequently became more conspicuous but seldom reached considerable proportions. In the later stages only residual foci of infiltration remained in perivascular and peribronchiolar sites (Cases 26 and 30). Varying degrees of mononuclear infiltration

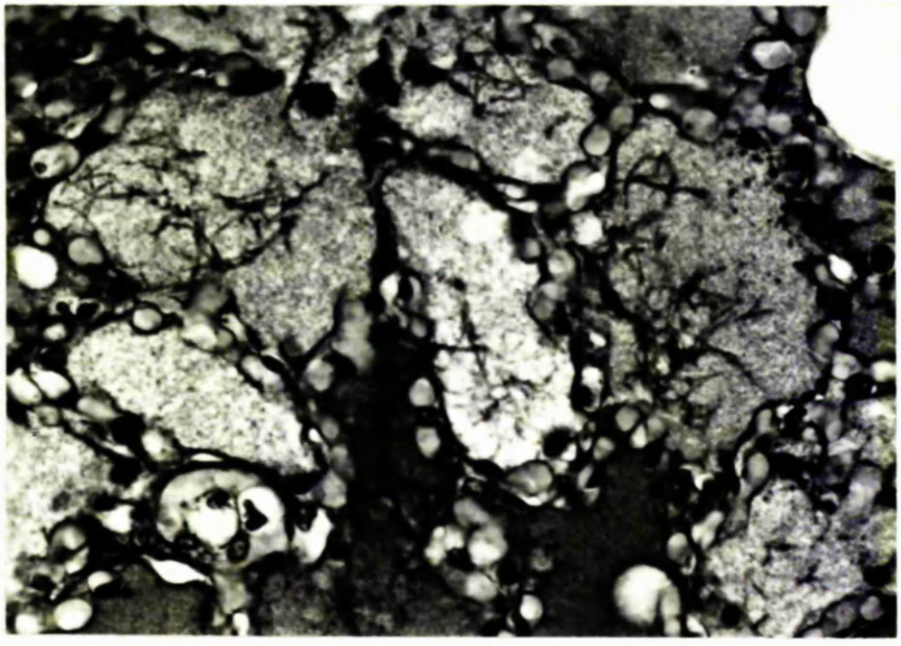

Fig. 19. Clinical Case 4. Lung. Strands of fibrin are seen in oedema fluid. Periodic acid-Schiff. x 530.

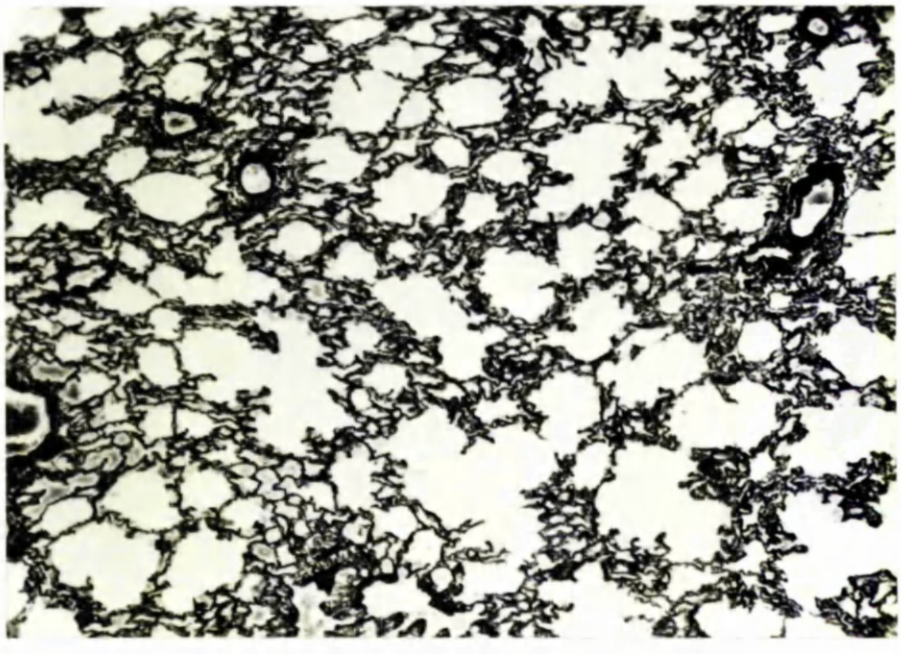

Fig. 20. Clinical Case 29. Lung, showing "trabecular" collapse of alveoli. Gordon and Sweets' silver method. x 28.

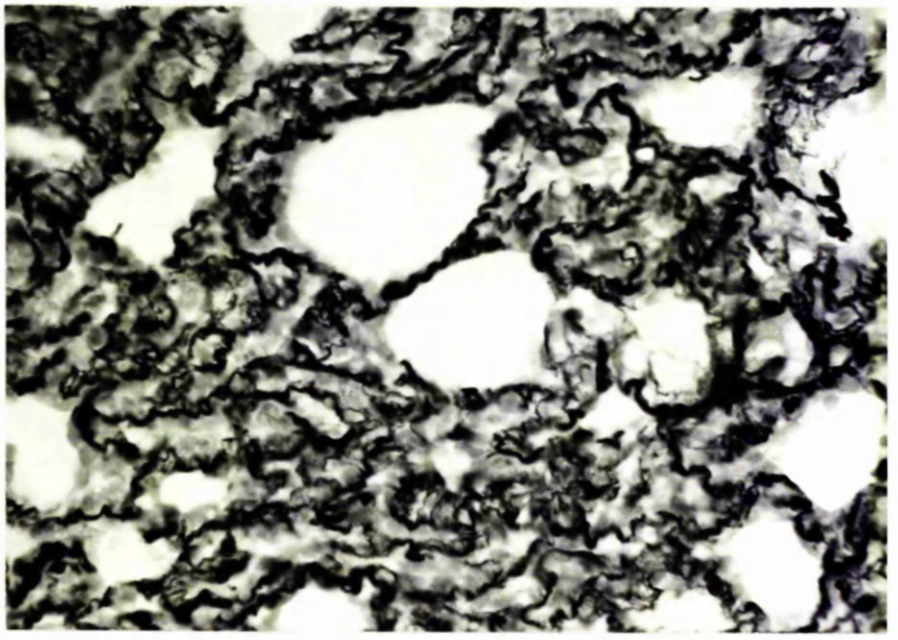

Fig. 21. Clinical Case 29. Lung. High-power view of collapsed alveoli. Gordon and Sweets' silver method. x 570.

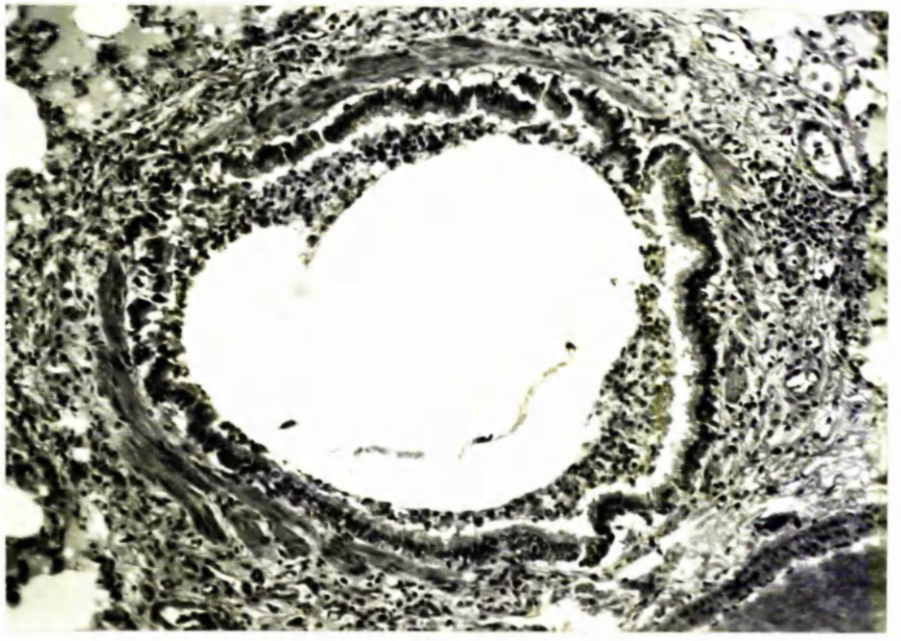

Fig. 22. Clinical Case 32. Early pneumonia, showing cellular reaction in and around bronchioles. Haematoxylin and eosin. x 110.

were observed in bronchiolar walls in 80 per cent and in perivascular areas in 53 per cent of the cases.

Often mononuclear elements invaded the adjacent alveolar walls and, together with the cells accumulated in the peribronchiolar connective tissue, constituted the interstitial inflammatory reaction, characteristic of many viral pneumonias.

Some of the cases (17 per cent) displayed degenerative changes in the bronchiolar epithelium. These were either of a foamy, vacuolar type accompanied by desquamation, or of glycogenic character as revealed by sections stained with periodic acid-Schiff (Fig. 24). In the latter instance, the involved epithelial cells often seemed to have shed most, if not all, of their cytoplasm, leaving the lamina propria partially denuded (Fig. 25). The healthy epithelial elements, remaining at the edge of the lesions, sometimes formed long, thin cytoplasmic processes which tended to cover the exposed areas (Fig. 26). In time these tenuous expansions gave rise to the continuous epithelial membrane. In some bronchioles, however, the bare segments of the wall were seen to be actively involved in the process of intrabronchiolar organization and fibrosis by supplying some of the collagenous elements (v. infra; Fig. 34).

On occasion,the lamina propria of the bronchioles displayed irregularly outlined, fuchsinophilic foci of hyaline changes (Fig. 27). These were seen usually at the height of illness, and only in a comparatively few cases.

A mild, rarely moderate, catarrhal bronchiolitis occurred in 33 per cent of the dogs, particularly between the 3rd and 5th weeks of the disease. Usually it was accompanied by an appreciable accumulation of exudate which sometimes tended to occlude the lumen. More often the bronchioles were not primarily involved but contained varying amounts of cellular, predominantly

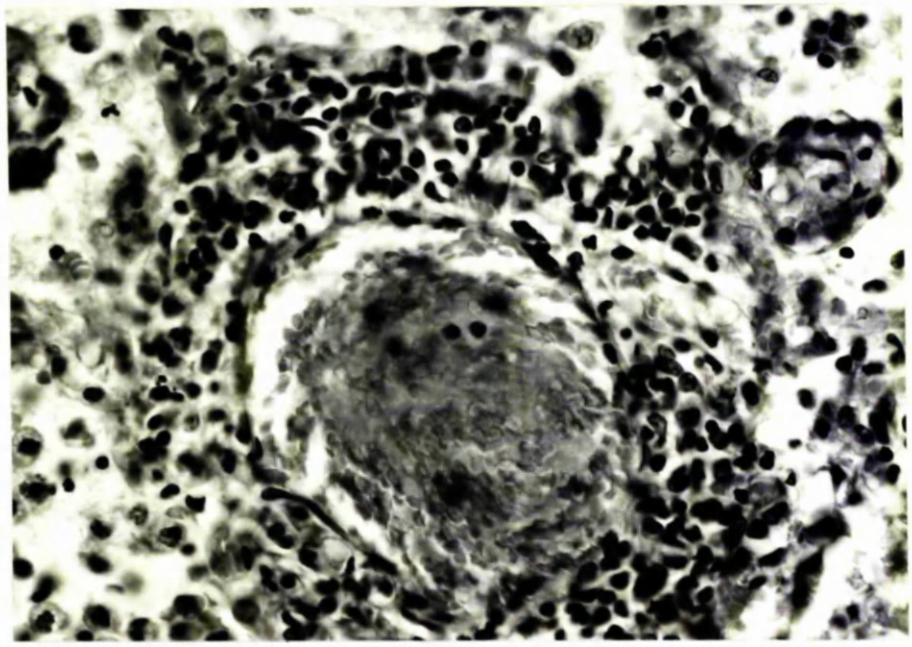

Fig. 23. Clinical Case 2. Lung. Perivascular mononuclear infiltration. Haemalum-phloxin-tartrazine. x 460.

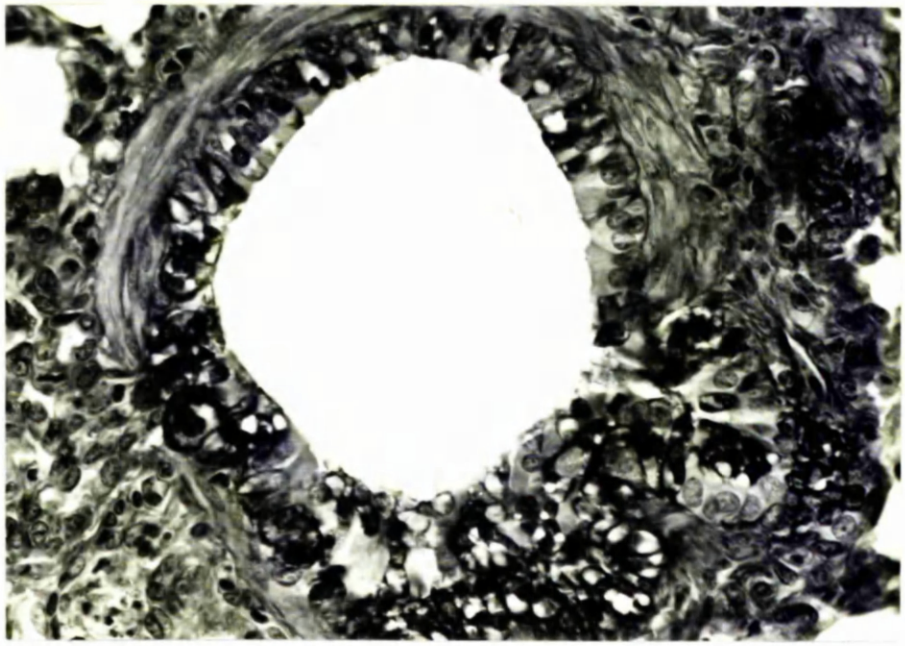

Fig. 24. Clinical Case 17. Bronchiolar mucosa, showing moderate glycogenic infiltration of epithelial cells. Periodic acid-Schiff. x 480.

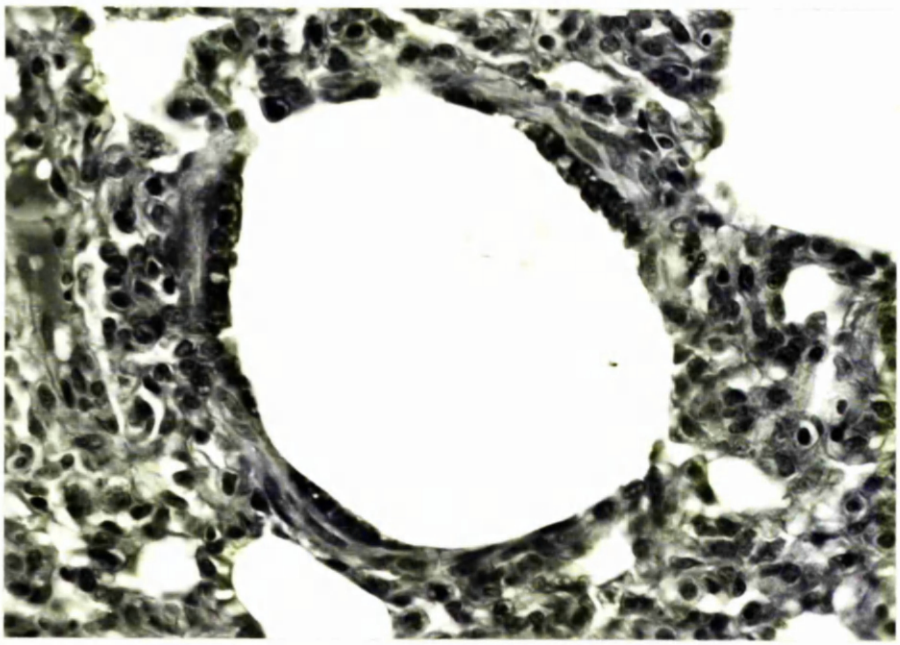

Fig. 25. Clinical Case 31. Bronchiolar mucosa. Advanced glycogenic infiltration of epithelium, with partial demudation of lamina propria. Periodic acid-Schiff. x 470.

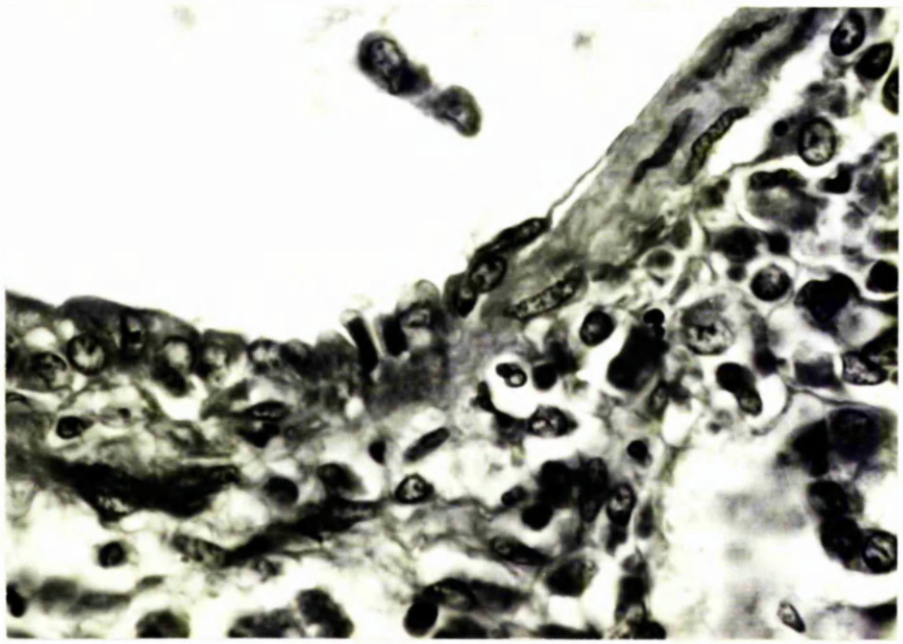

Fig. 26. Clinical Case 1. Bronchiolar wall. The remaining epithelial cells begin to cover the denuded bronchiolar wall with their cytoplasmic processes. Picro-Mallory. x 485.

mononuclear exudate originating from the alveoli. A frank suppurative bronchiolitis was noted only in one dog which showed a concurrent bacterial bronchopneumonia (Case 29).

In some cases, particularly 8 and 23, in which the pneumonia was characterized by marked proliferative changes, the bronchiolar epithelium displayed bizarre forms of cells. Many of these were considerably larger than normal epithelial cells, protruded into the lumen and often contained 2 or 3 pale staining, balooned nuclei with prominent solitary nucleoli (Fig. 28).

In a small proportion of cases showing signs of the disease for 2 or 3 weeks, some of the subpleural bronchioles, aveolar ducts and, occasionally, alveoli exhibited dense fibrinoid masses, with enmeshed erythrocytes and mononuclear cells (Figs. 29 and 30). At first amorphous and bare, the fibrinoid "plugs" became later infiltrated by fibroblasts and invested with a continuous epithelial covering. Ultimately, permanent, organized structures were formed. On occasion, the layer of fibrinoid material was seen to mould along the walls of alveolar ducts and alveoli (Fig. 31), producing amorphous, eosinophilic membranes comparable to those described by Hadfield (1938) and Epstein and Greenspan (1941) in rheumatic pneumonia of man.

Degrees of intrabronchiolar and intraductual fibrosis occurred in 50 per cent of the dogs. Most of the cases were in the 3rd to 5th week of illness (30 per cent), but some (10 per cent) showed the changes as early as in the second week (Cases 27, 28 and 33). Usually the process was focal in distribution and confined to subpleural areas. The initial changes appeared to be precipitated by the presence of fibrin-containing exudate and the paucity of polymorphonuclear leucocytes (Auerbach et al., 1952). The exudate in the affected terminal and respiratory bronchioles and alveolar ducts became invaded by, at first, thin and later by more prominent fibroblastic and

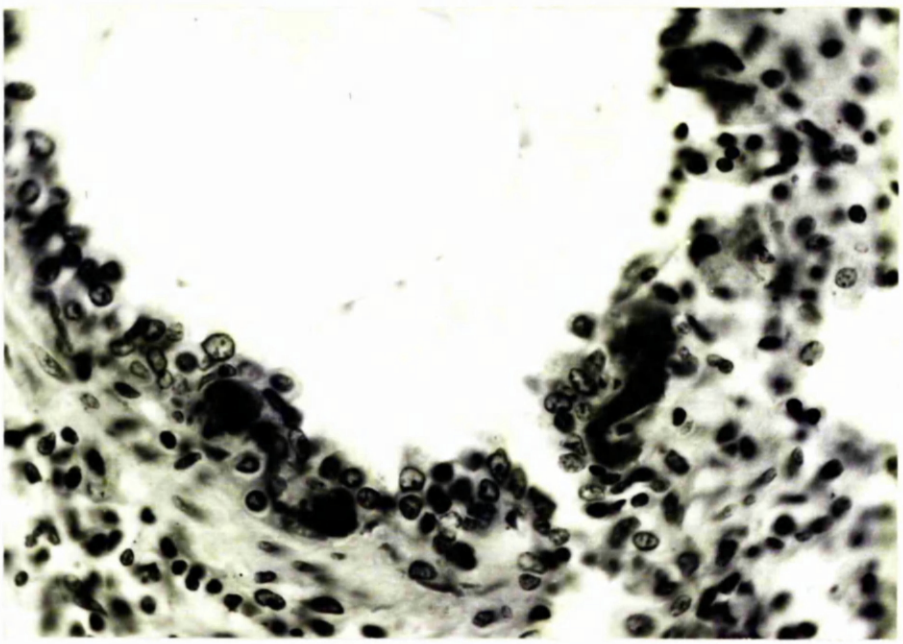

Fig. 27. Clinical Case 3. Bronchiolar wall. Hyaline changes in lamina propria. Haematoxylin and eosin. x 450.

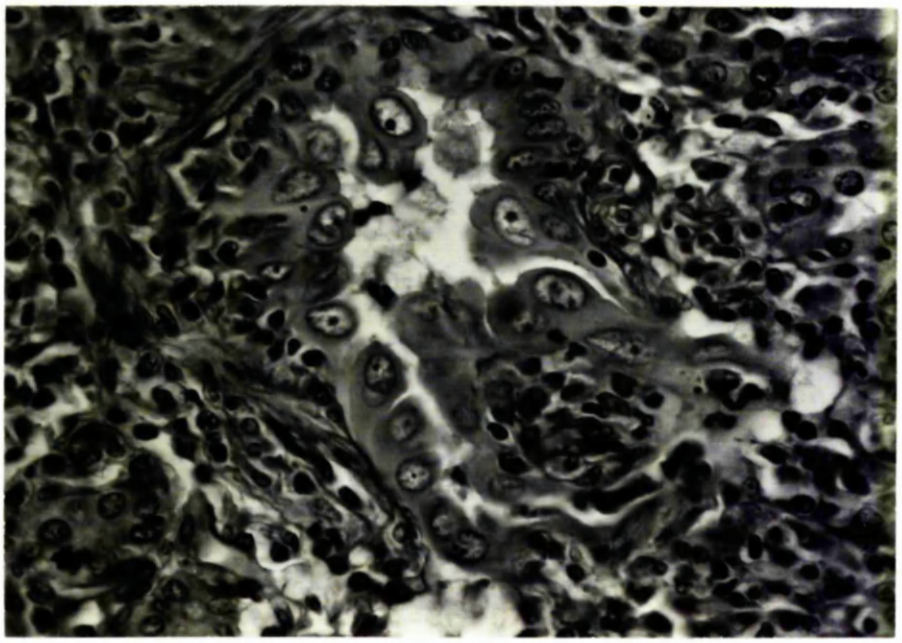

Fig. 28. Clinical Case 8. Bronchiole. Epithelial cells show bizarre and binucleated forms. Haematoxylin and eosin. x 515.

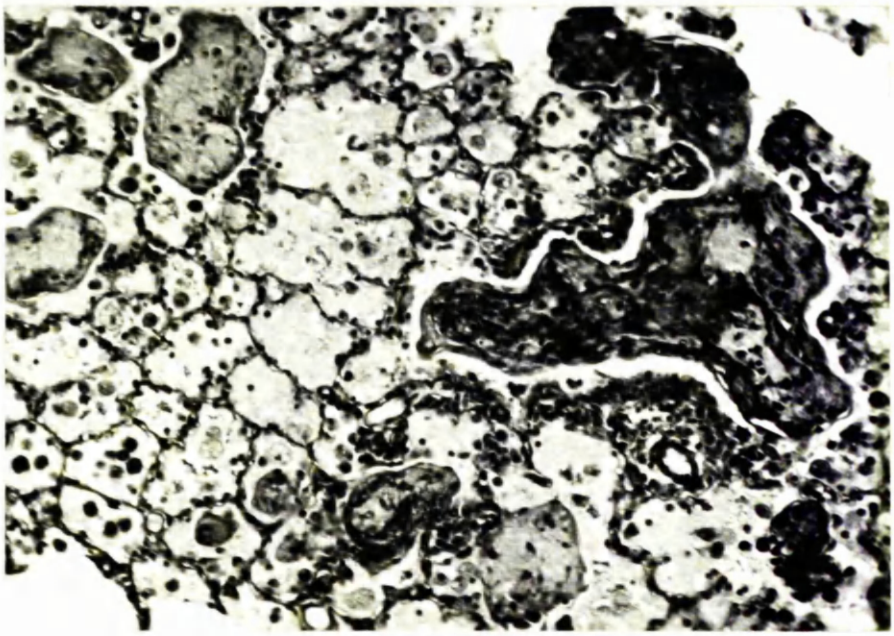

Fig. 29. Clinical Case 2. Lung. Respiratory bronchiole, alveolar ducts and alveoli contain dense fibrinous "plugs". Periodic acid-Schiff. x 125.

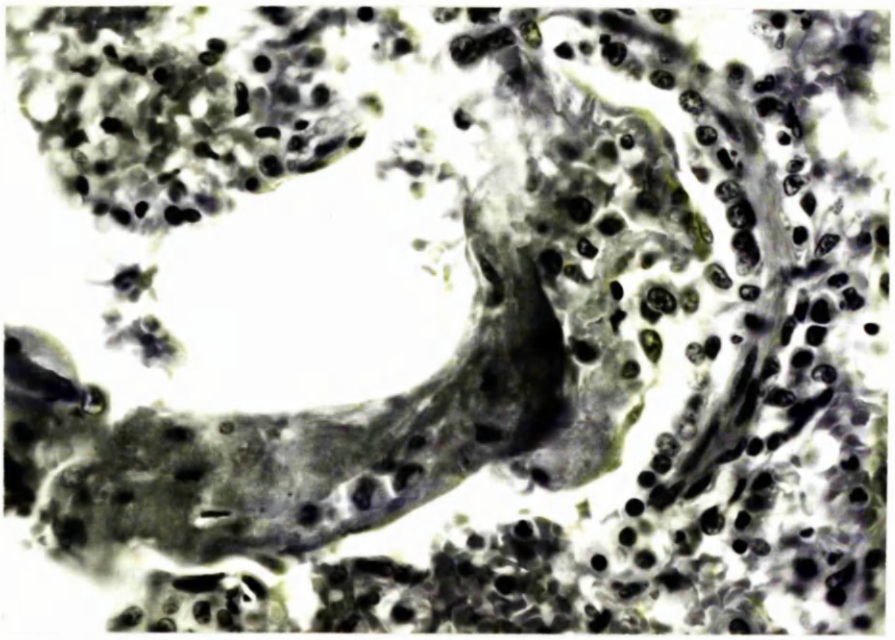

Fig. 30. Clinical Case 8. Respiratory bronchiole, showing fibrinous "plug" with enmeshed mononuclear cells. Haematoxylin and eosin. x 425.

collagenous elements, for which the strands of fibrin appeared to serve as a scaffolding (Figs. 32 and 33). A number of cells (predominantly mononuclear) was usually entrapped in the rich network of fibres. While many of the fibroblasts evidently migrated into the area from the adjacent connective tissue, it seemed plausible to assume that some of them could have had their origin from the transformed polyblasts (Maximow, 1927). In the later stages, the organized "plugs" became invested, partly or continuously, with a layer of cells derived from the remaining epithelium (Figs. 34 and 35).

The intrabronchiolar fibrosis was sometimes accompanied by intra-alveolar organization (17 per cent) and, more often, by an increase in number of reticular fibres within the walls of alveoli (24 per cent). The process appeared to be well advanced in some of the dogs ill from 2 to 4 weeks (8, 19, 22, 31 and 32). The evolution of fibrosis took a similar course to that observed in the bronchioles and alveolar ducts. Occasionally, the organized exudate became covered by an uneven layer of epithelial cells, evidently derived from the alveolar lining (Fig. 36). Intra-alveolar fibrosis was usually mild and peribronchiolar in distribution, but in a few cases involved larger subpleural areas (Fig. 37). In some instances, the walls of alveoli were appreciably thickened due to a moderate increase in the number of reticular and collagenous fibres and the accumulation of predominantly mononuclear cells (Figs. 38 and 39).

Except for sometimes marked engorgement with red blood cells and the presence of degenerating megakaryocytes (Fig. 40), the alveolar capillaries showed no significant abnormalities.

The lymphatic channels appeared to be distended not only in perivascular areas but also in the subpleural regions where they often carried a number of mononuclear phagocytic cells (Fig. 41).

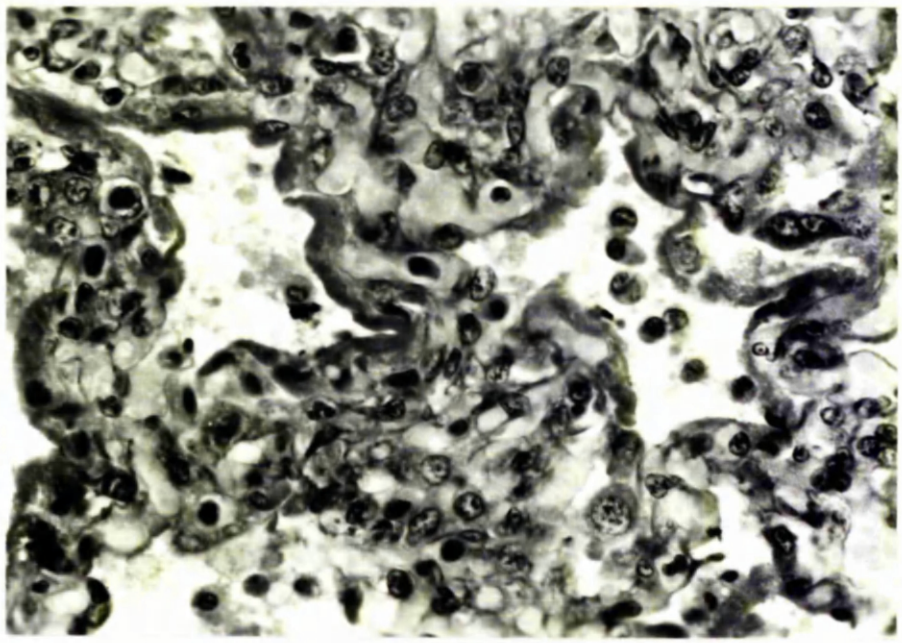

Fig. 31. Clinical Case 8. Lung, showing hyaline membranes lining the alveolar ducts. Periodic acid-Schiff. x 520.

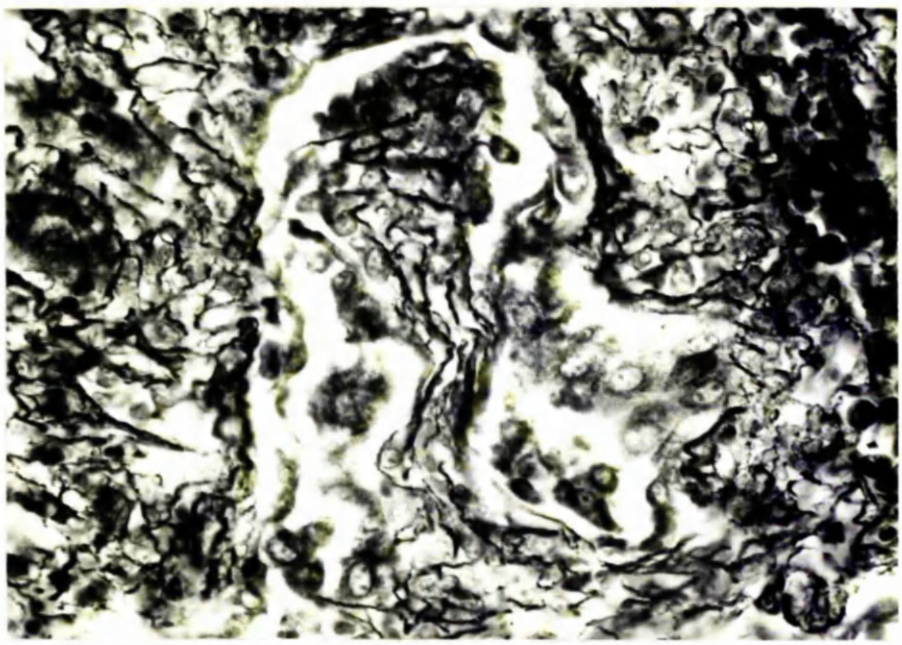

Fig. 32. Clinical Case 8. Respiratory bronchiole. Organization of the exudate. Gordon and Sweets' silver method. x 450.

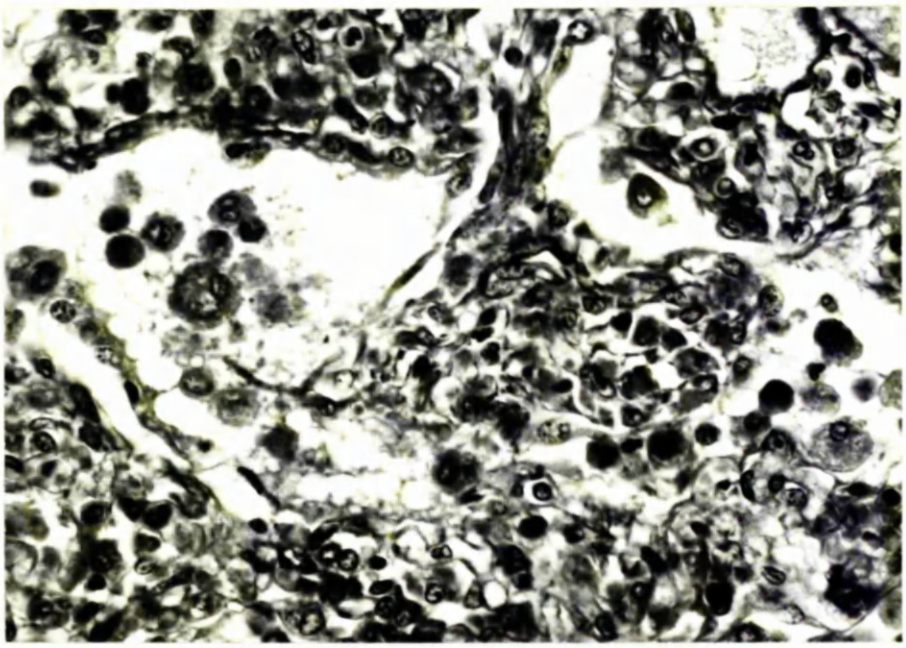

Fig. 33. Clinical Case 8. Respiratory bronchiole, showing invasion of exudate by fibroblasts. Picro-Mallory. x 540.

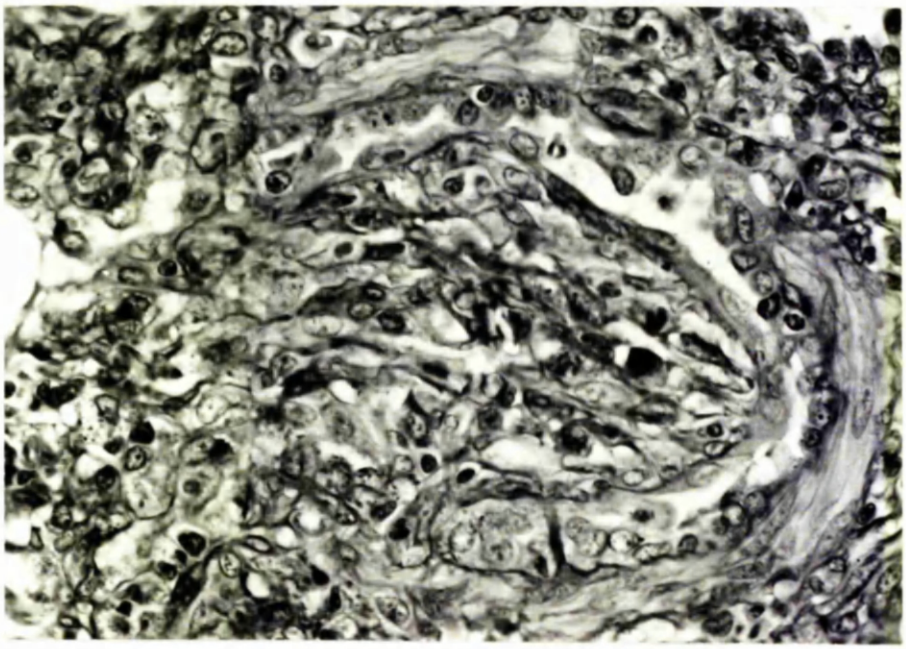

Fig. 34. Clinical Case 19. Respiratory bronchiole. Late stage of organization. The "plug" is covered by epithelial cells. Periodic acid-Schiff. x 480.

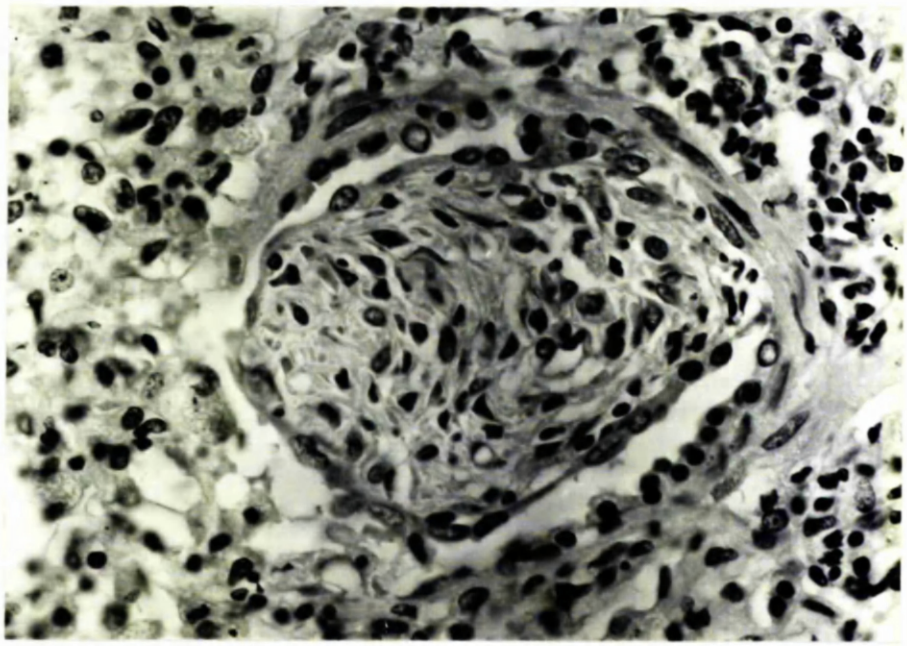

Fig. 35. Clinical Case 26. Respiratory bronchiole. Stage of fibrosis. Few capillaries could be seen in the fibrotic "plug" covered by irregular epithelial cells. Haematoxylin and eosin. x 400.

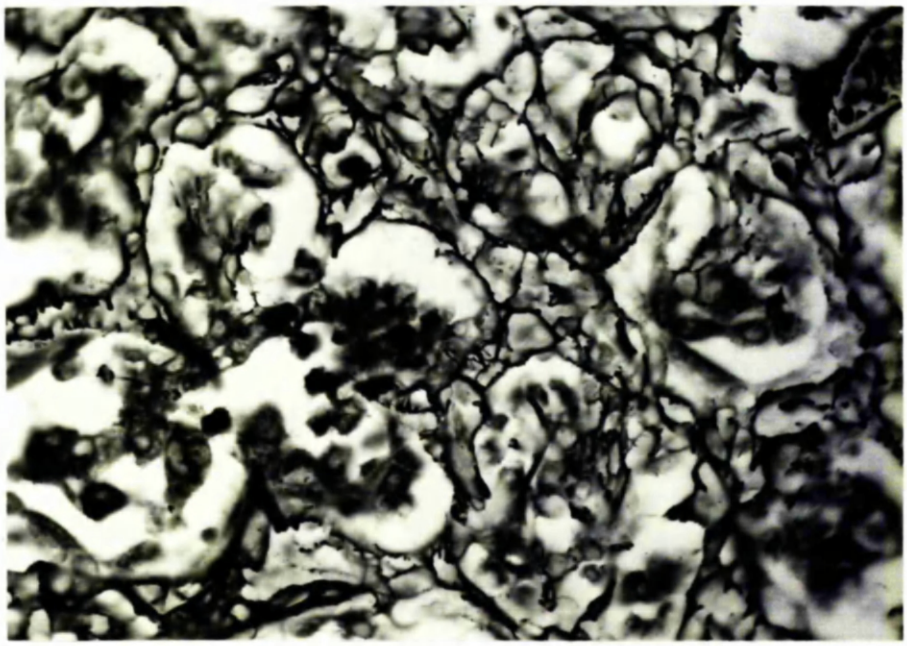

Fig. 36. Clinical Case 33. Lung. Organization of intra-alveolar exudate. Alveolar walls are thickened due to increase in reticular fibres. Gordon and Sweets' silver method. x 475.

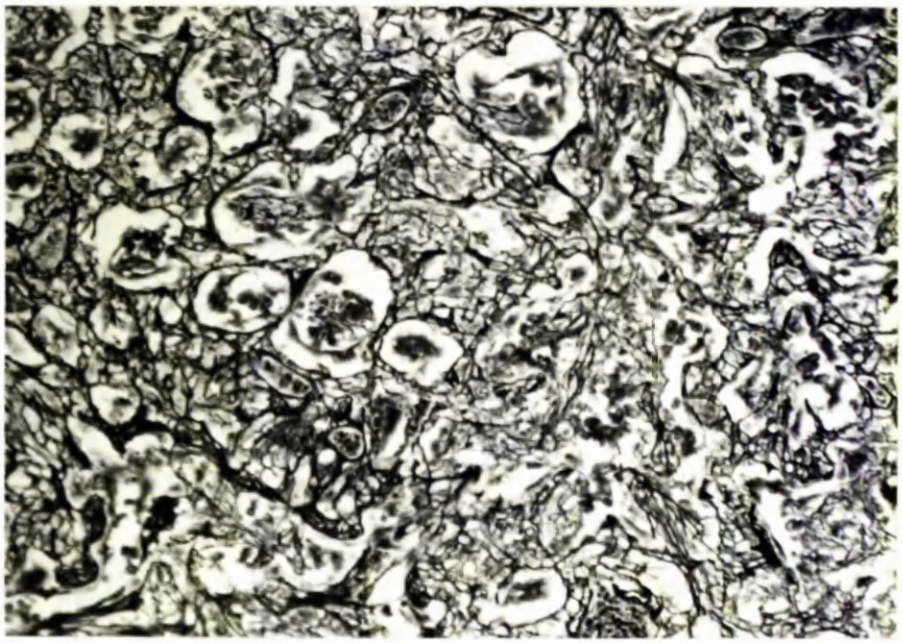

Fig. 37. Clinical Case 33. Lung, showing subpleural organization. Gordon and Sweets' silver method. x 130.

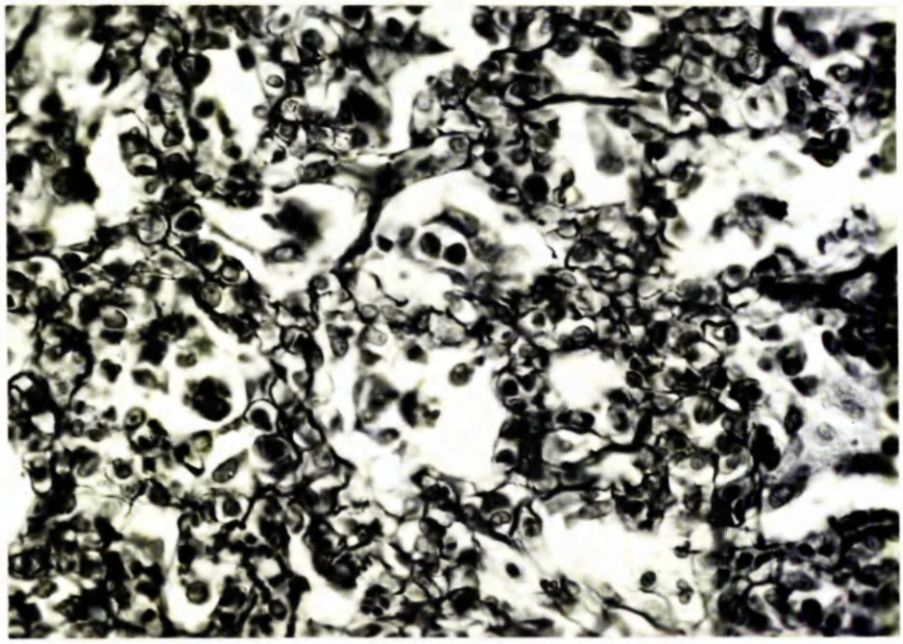

Fig. 38. Clinical Case 1. Lung. Alveolar walls are thickened due to increase in reticular fibres. Gordon and Sweets' silver method. x 425.

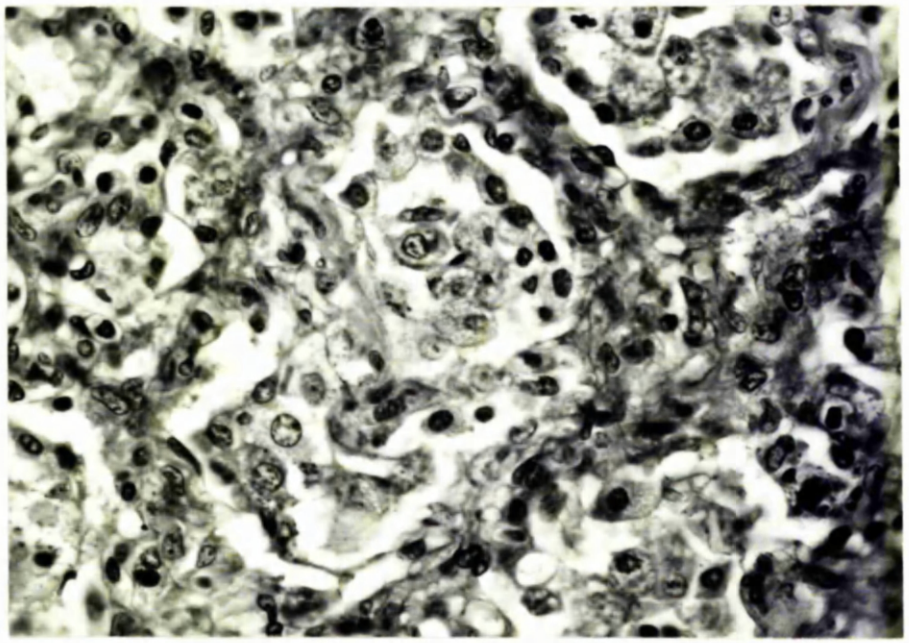

Fig. 39. Clinical Case 19. Lung, showing thickened alveolar walls, desquamated epithelial membranes and mononuclear exudate. Periodic acid-Schiff. x 520.

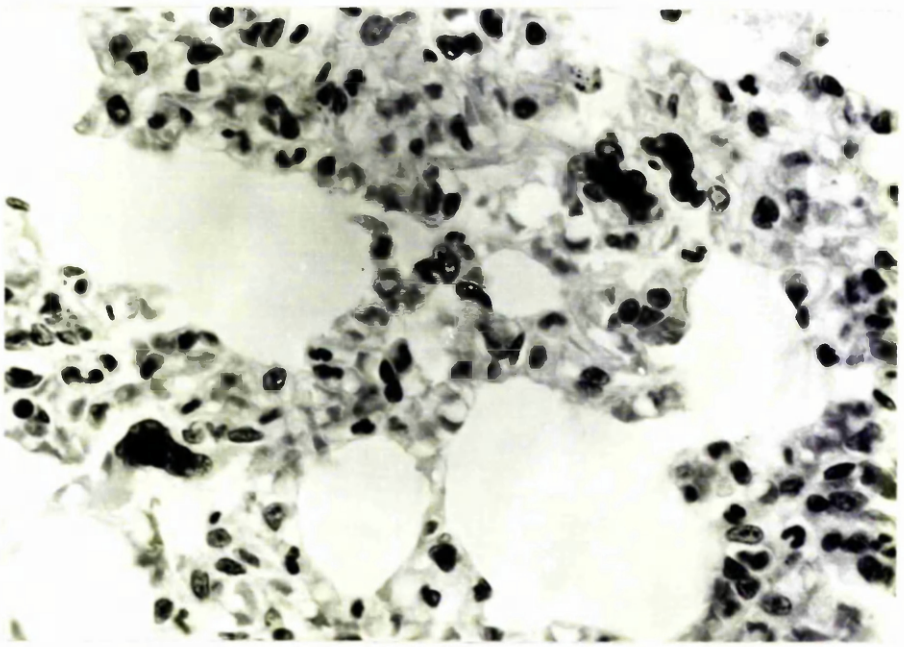

Fig. 40. Clinical Case 13. Lung. Capillaries contain degenerating megakaryocytes. Haematoxylin and eosin. x 430.

Early in the course of the disease, characteristic cellular changes had evolved in alveolar spaces and often remained manifest until the late stages. At the height of illness, they were sometimes pronounced, affecting larger segments of the lobes. These changes comprised (1) alveolar epithelial hyperplasia and (2) exudation of predominantly mononuclear cells.

Alveolar epithelial proliferation was an early process, developing evidently during the first two weeks of illness (Cases 10, 17, 20, 27, 28 and 33). In its initial phases, the hyperplasia was mild and peribronchiolar (Figs. 42 and 43) or subpleural in distribution. Sometimes such "epithelialisation" of alveoli remained localized throughout the entire course of the disease, involving only small areas of the subpleural or peribronchiolar tissue. In a small number of cases, the process diffused subpleurally to a considerable depth (Figs. 44 and 45) and, together with peribronchiolar "epithelialisation", involved large portions of the lobes.

In many instances, however, the changes found were either mild, particularly in the dogs ill for less than 2 weeks, or only moderately advanced, with only occasional foci of marked involvement.

Some degree of alveolar epithelial hyperplasia was observed in all but 5 of the 30 cases examined (5, 21, 26, 29 and 30). Two of the latter (26 and 30) showed signs of the disease for more than 10 weeks.

The process of alveolar "epithelialisation" was essentially that of hypertrophy and hyperplasia of a remarkably tenuous but uninterrupted epithelium which normally lines the wall of the pulmonary alveoli (v. pp. 58-73).

The initial changes observed in the epithelial cells included hypertrophy and, often, some vacuolation of the cytoplasm (Figs. 46 and 47). Generally, only the nucleus-bearing portion of the cell body became enlarged.

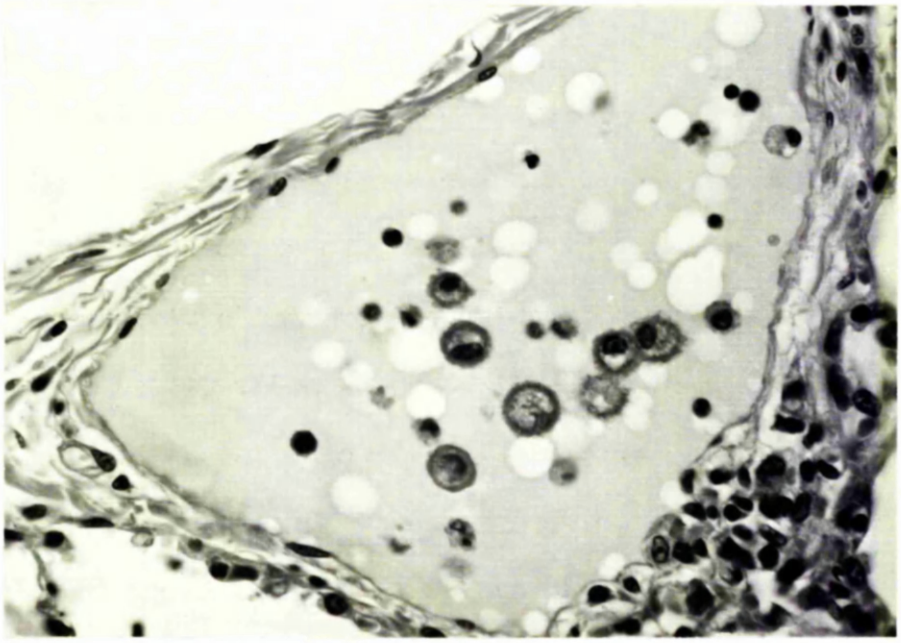

Fig. 41. Clinical Case 27. Lung. Distended subpleural lymphatic vessel with mononuclear cells. Haematoxylin and eosin. x 425.

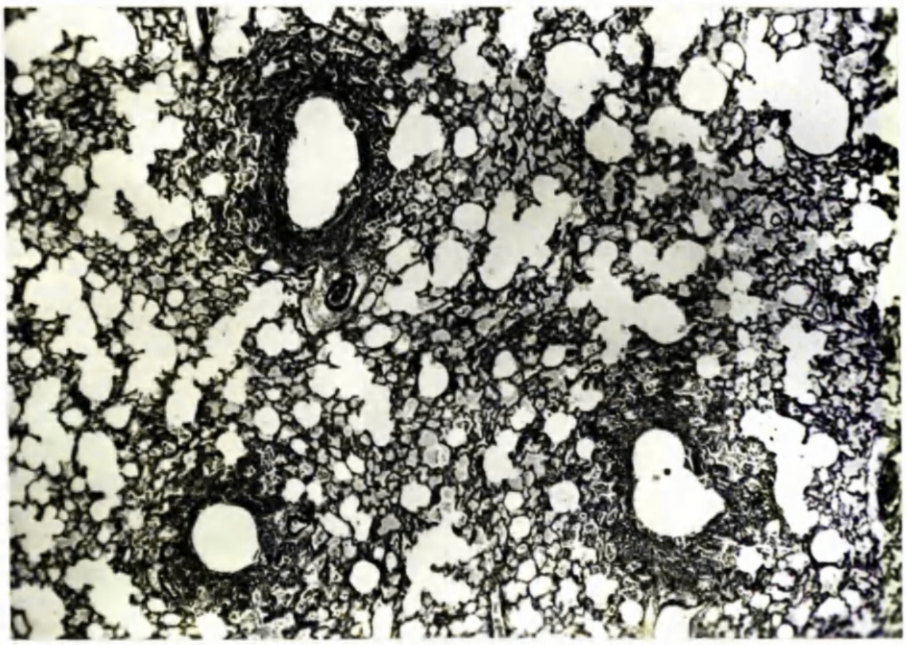

Fig. 42. Clinical Case 1. Lung. Early pneumonia, showing cellular reaction in and around bronchioles and adjacent alveoli. Slight alveolar and interstitial oedema is also present. Picro-Mallory. x 30.

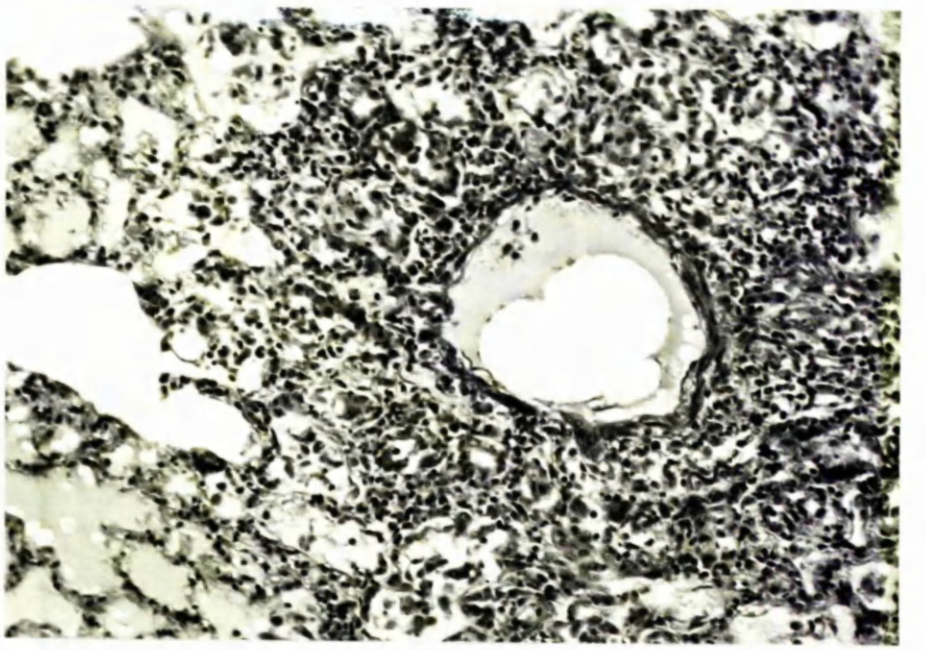

Fig. 43. Clinical Case 8. Lung. "Epithelialisation" of alveoli is present in the peribronchiolar area. Picro-Mallory. x 130.

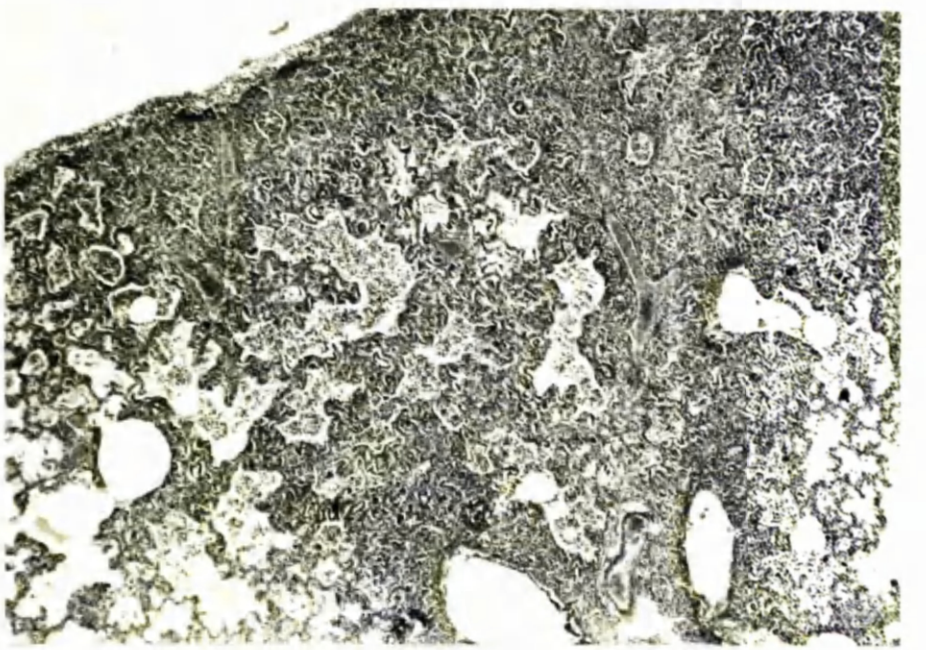

Fig. 44. Clinical Case 1. Lung, showing cellular changes in the subpleural area. Van Gieson. x 30.

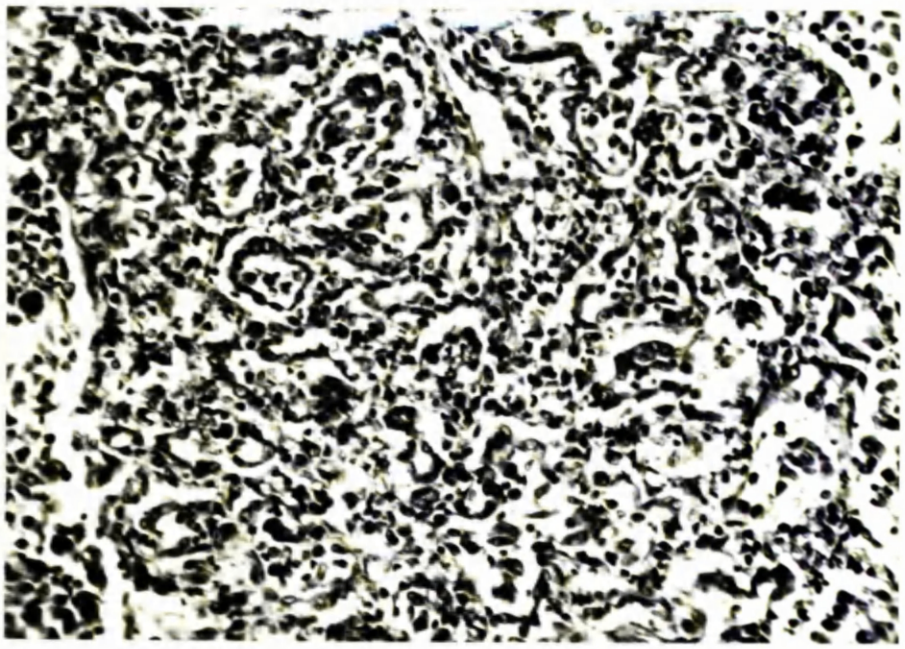

Fig. 45. Detail of Fig. 44. "Epithelialisation" of alveoli and cellular exudative changes are prominent. Picro-Mallory. x 150.

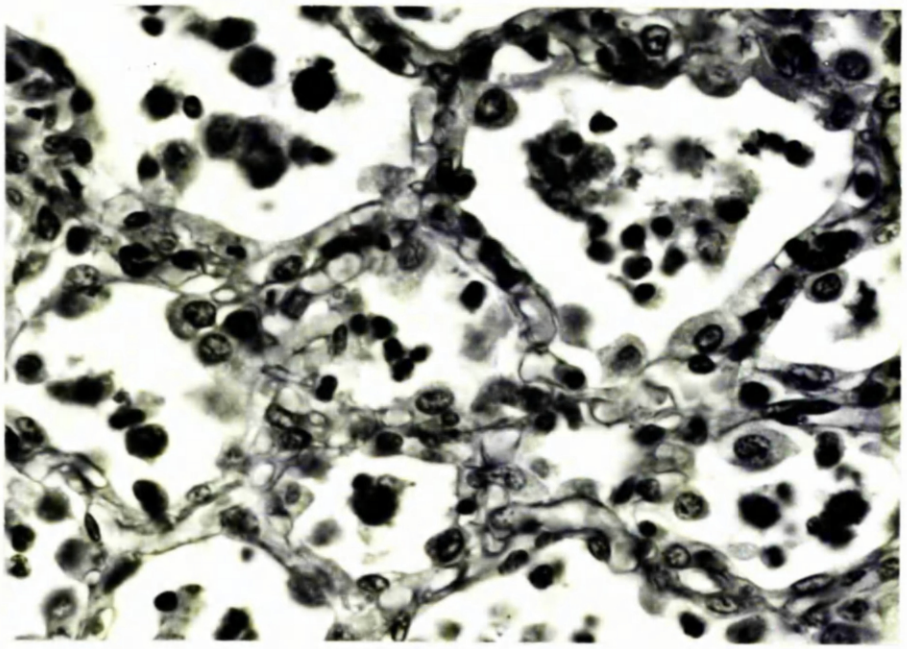

Fig. 46. Clinical Case 27. Lung. Hypertrophy of septal cells. There is also some intra-alveolar exudate. Periodic acid-Schiff. x 550.

The protoplasmic expansions remained relatively unaffected. On occasion, however, a considerable thickening of these segments of the epithelium could be observed (Fig. 48). The increase in size was usually moderate, two-or threefold, but sometimes it was seen to reach unusual proportions, with cells measuring up to 50μ in length and 20μ in width (Fig. 49).

Almost in every instance, the phase of hypertrophy was accompanied, or immediately followed, by a marked karyokinetic activity of the epithelial cells. Many daughter forms arose by mitotic (Fig. 47) and, possibly, by amitotic divisions, adding remarkably to the cellularity of the epithelial covering. As a result, a distinct pavement of somewhat irregularly shaped but usually flattened cells developed along the walls of the alveoli (Figs. 50 and 53). Not infrequently, the cytoplasm of these cells failed to segment in the process of nuclear division and, in an attempt to accommodate the increased number of nuclei, hypertrophied to a considerable degree, giving rise to binucleated (Figs. 48 and 50) and, sometimes, to multinucleated bizarre forms (Fig. 51).

Comparatively early in the process of "epithelialisation", some of the lining cells became detached from their mural berths and gradually pushed into the alveolar space, often dragging behind them filamentous attachments of their cytoplasm (Figs. 50 and 52). In time more cells were separated, at first in small groups (Fig. 52) and later in sheets (Fig. 53), until the entire membrane was free in the lumen. In more advanced phases, the desquamated lining underwent fragmentation and showed cellular degenerative changes. The epithelial elements set free in the alveolar space intermixed with the cells of inflammatory exudate, and were finally removed from the region of gaseous exchange by way of the bronchial tree. No traces of "epithelialisation"

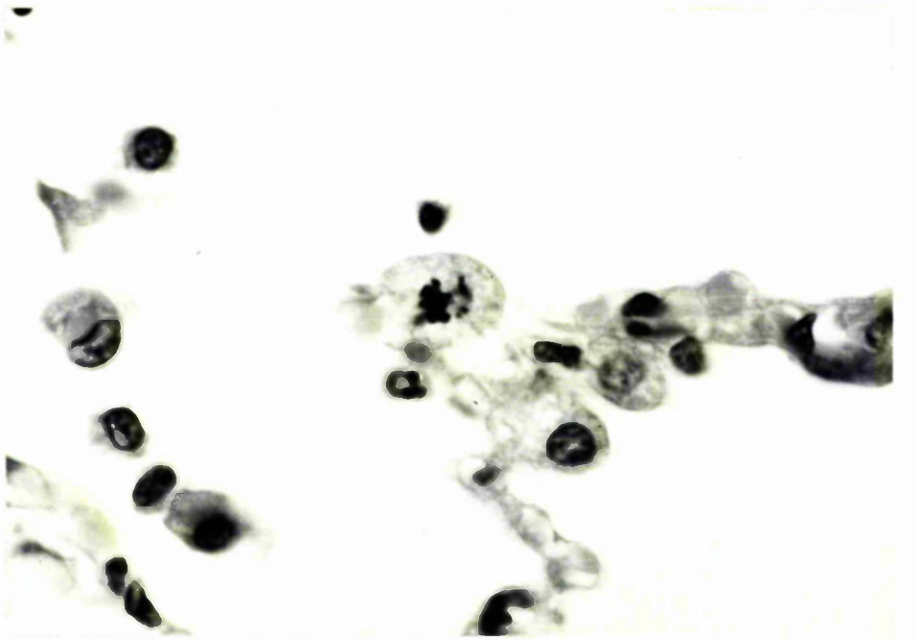

Fig. 47. Clinical Case 27. Alveolar wall. Mitotic figure in a septal cell. Note hypertrophy and vacuolation in other septal cells. Periodic acid-Schiff. x 710.

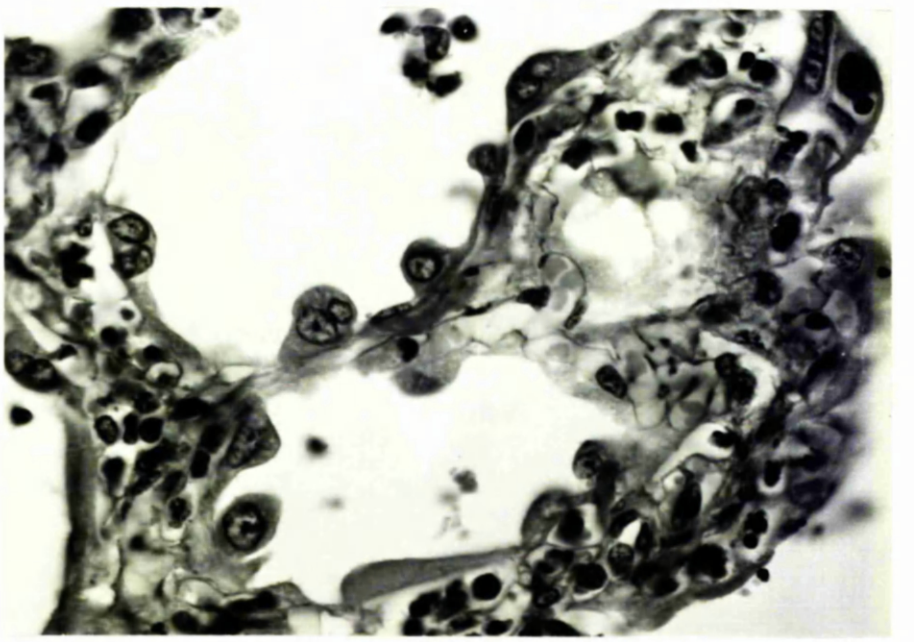

Fig. 48. From the same case. Binucleated and hypertrophied alveolar epithelial cells. Periodic acid-Schiff. x 800.

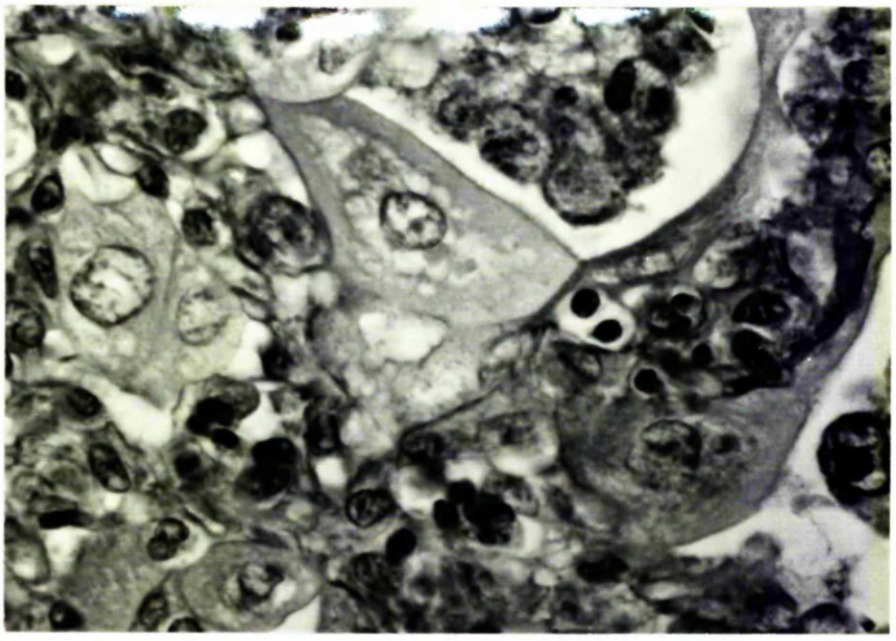

Fig. 49. Clinical Case 23. Lung. Bizarre alveolar epithelial cells. Periodic acid-Schiff. x 850.

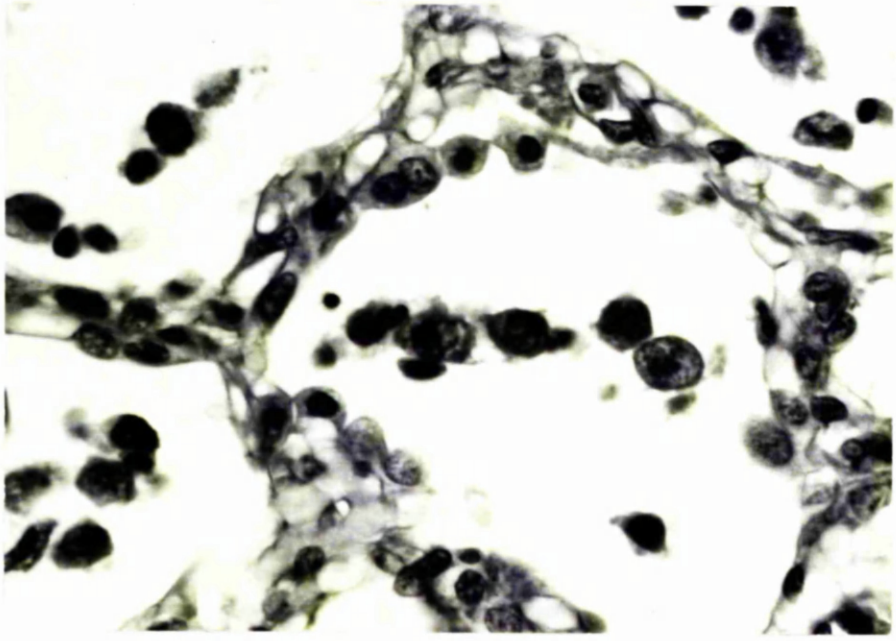

Fig. 50. Clinical Case 27. Lung. Early phase of alveolar "epithelialisation". Periodic acid-Schiff. x 760.

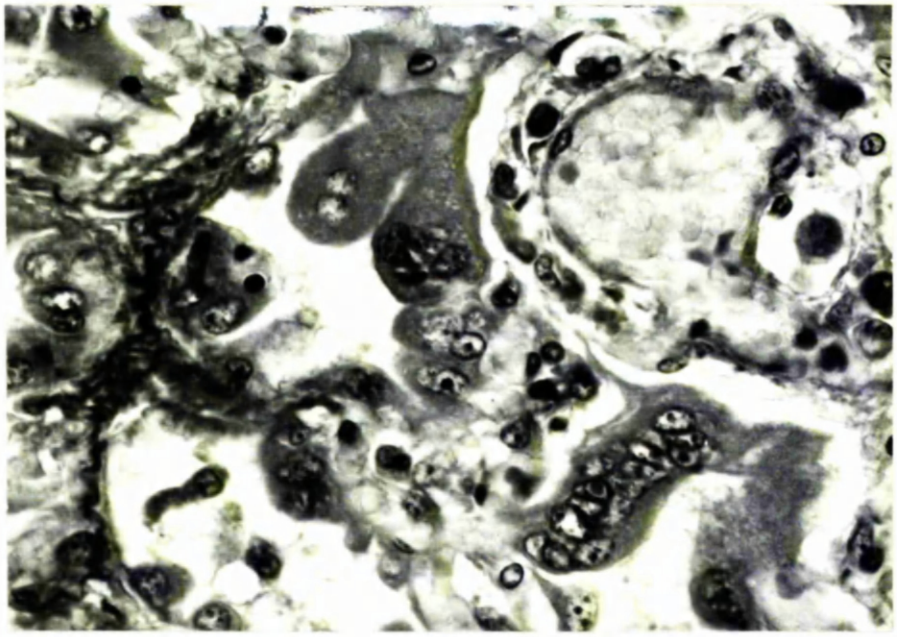

Fig. 51. Clinical Case 8. Lung. Bizarre multinucleated epithelial cells in alveoli and alveolar duct. Picro-Mallory. x 670.

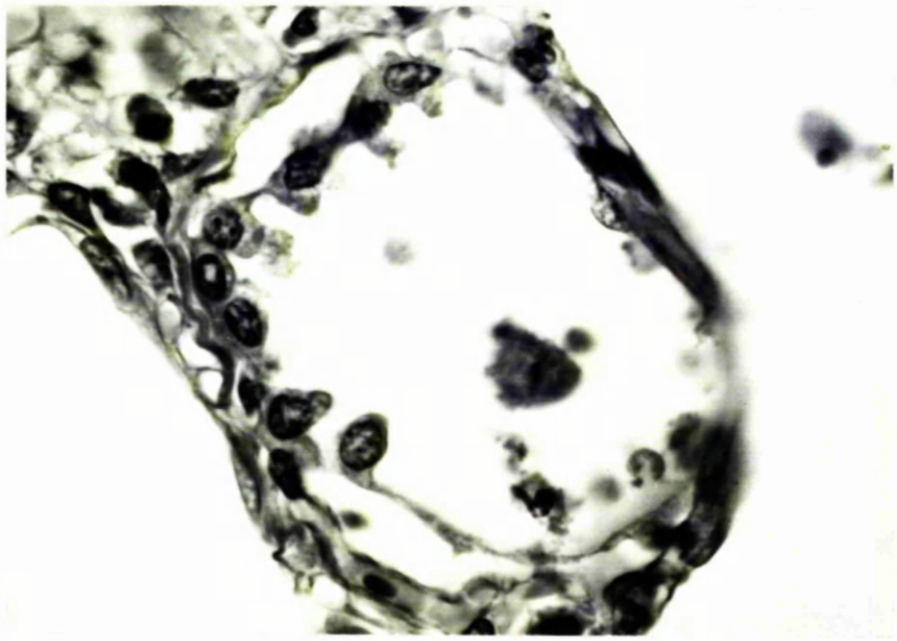

Fig. 52. Clinical Case 27. Lung. Early detachment of epithelial lining. Note length of cytoplasmic process. Periodic acid-Schiff. x 580.

were observed in the phase of resolution of the disease process.

The first cellular elements of the inflammatory exudate appeared in the alveolar spaces at an early stage of pneumonia. Appreciable numbers of them were present, especially in peribronchiolar and subpleural areas, at the time when alveolar epithelial cells were showing only incipient hypertrophic changes (Fig. 54). Many of the cells present comprised macrophages, lymphocytes and transitional hematogenous elements which apparently by further transformation gave rise to large phagocytes (Maximow, 1927; Rebuck and Crowley, 1955; Tompkins, 1955). Occasional polymorphonuclear leucocytes migrated into the alveoli but, in most cases, were scanty throughout the entire course of the disease. Septal cells were also seen to contribute appreciably to the exudate. Their bodies, at first lodged in alveolar angles, became swollen, protruded freely into the lumen (Figs. 55 and 56) and finally, having liberated themselves from attachments, joined other inflammatory cells in alveolar spaces. Sometimes a few plasma cells and an occasional eosinophil could be observed in alveoli immediately adjacent to the bronchioles.

Not infrequently, some of the macrophages gained considerably in size and in number of nuclei which became arranged usually at the periphery of the cell (Figs. 57 and 60). This was apparently due to nuclear divisions without accompanying segmentation of the cytoplasmic body. It seemed probable, however, that, on occasion, some of the mononuclear macrophages fused together to form multinucleated elements.

Many cells, some with two nuclei and evidently of septal origin, showed a moderate to marked but uniform vacuolation of the cytoplasm which gave them a distinct foamy appearance (Fig. 58). Such cells often showed a thick, homogenous cytoplasmic membrane. The contents of vacuoles, some of which were seen to indent the nuclear membrane, did not respond to any of the

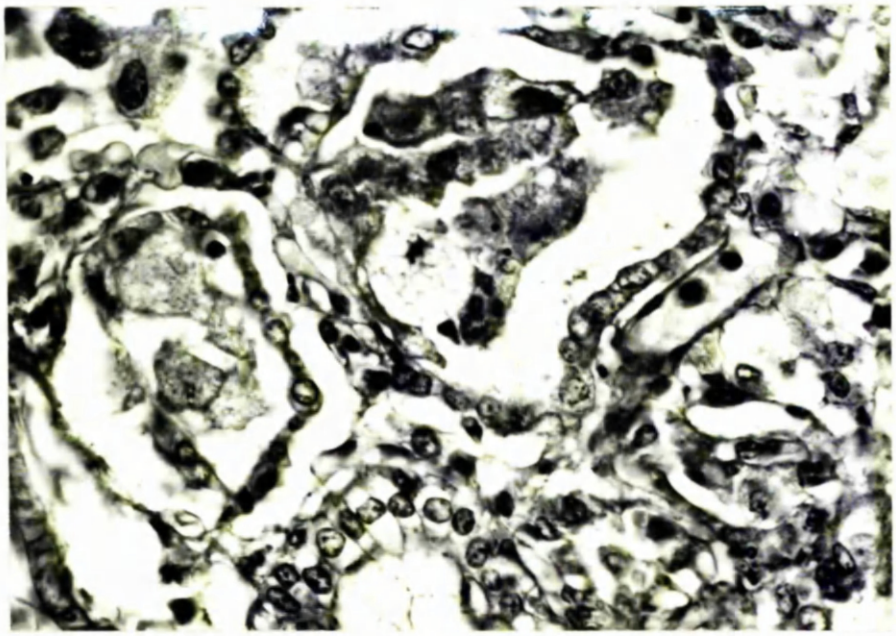

Fig. 53. Clinical Case 18. Lung. Alveolus at left shows complete detachment of lining. Note bronchiolar epithelium in lower centre. Periodic acid-Schiff. x 525.

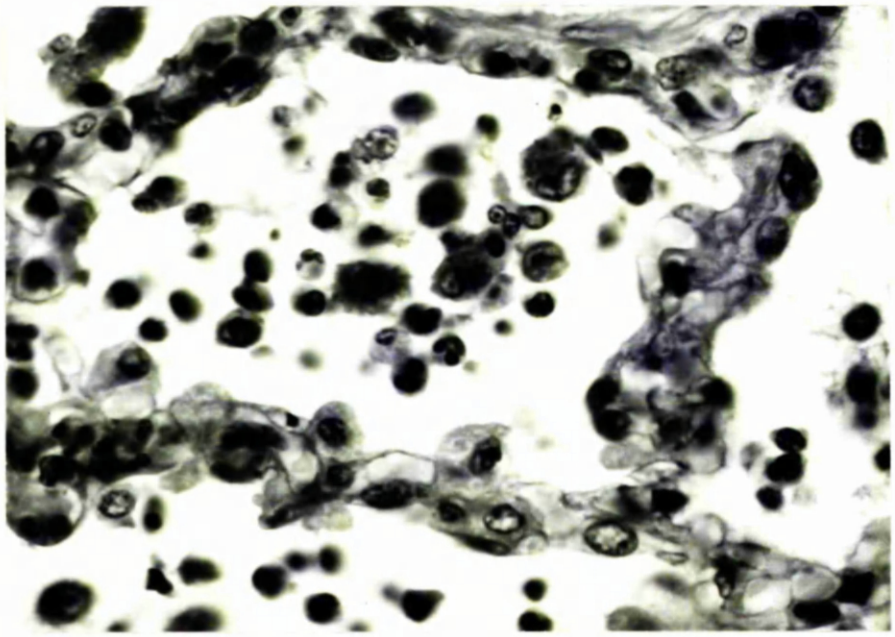

Fig. 54. Clinical Case 27. Lung. Intra-alveolar exudate, showing macrophages, lymphocytes, transitional forms and polymorphonuclear leucocytes. Periodic acid-Schiff. x 690.

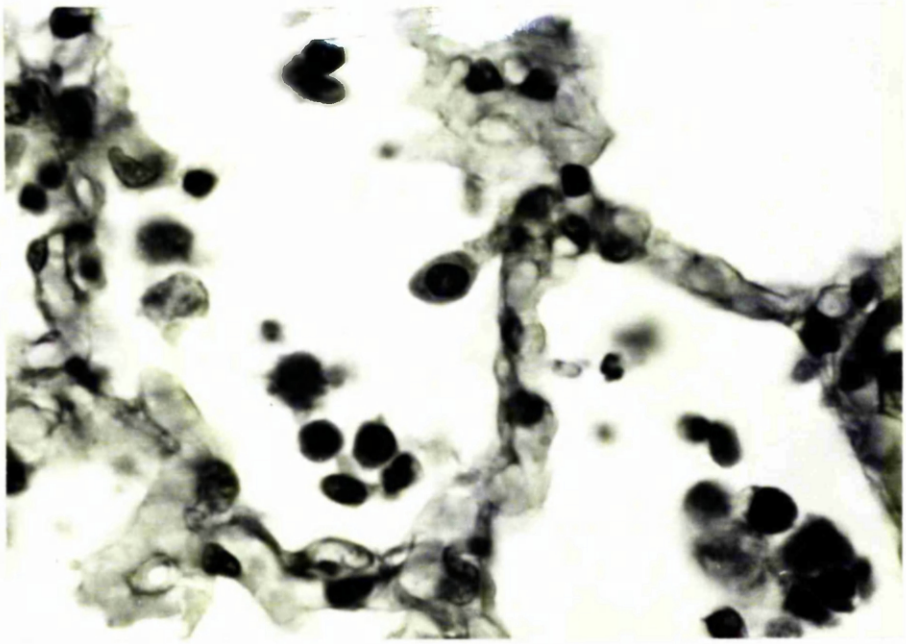

Fig. 55. Clinical Case 27. Septal cell approaching stage of detachment. Periodic acid-Schiff. x 810.

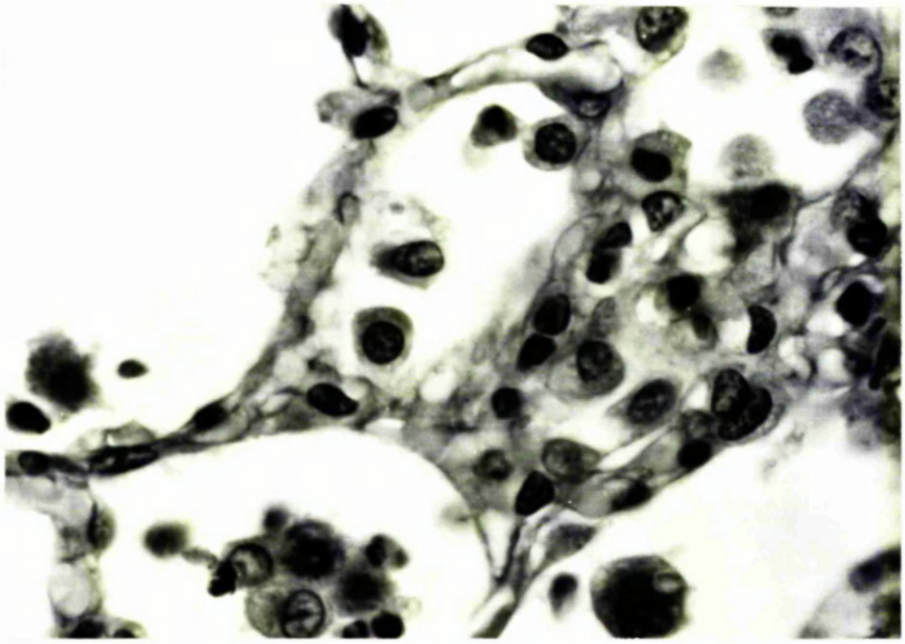

Fig. 56. From the same case. Septal cell is seen protruding into alveolar space. Periodic acid-Schiff. x 810.

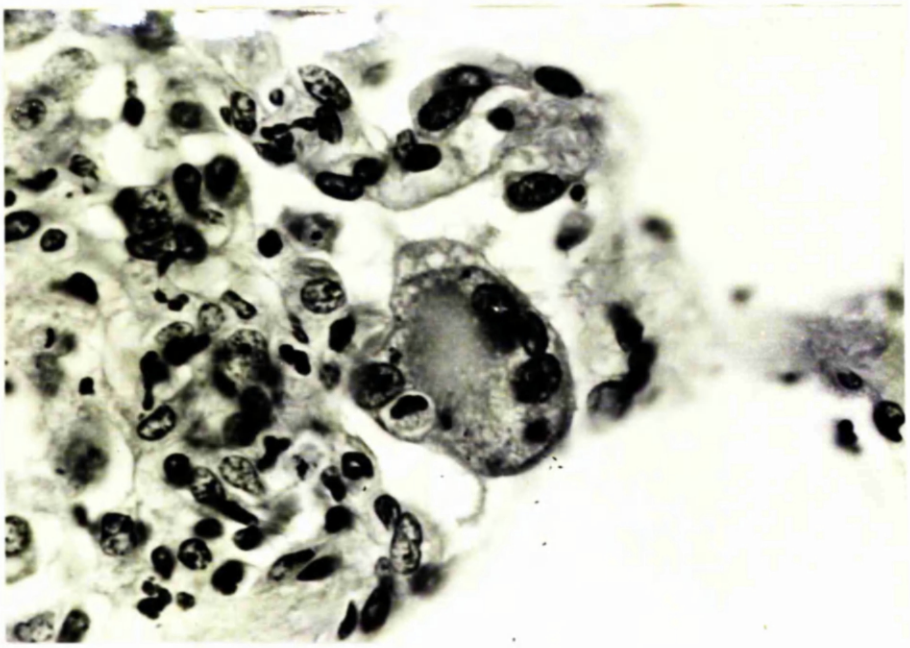

Fig. 57. Clinical Case 16. Lung. Multinucleated phagocytic cell. Haematoxylin and eosin. x 720.

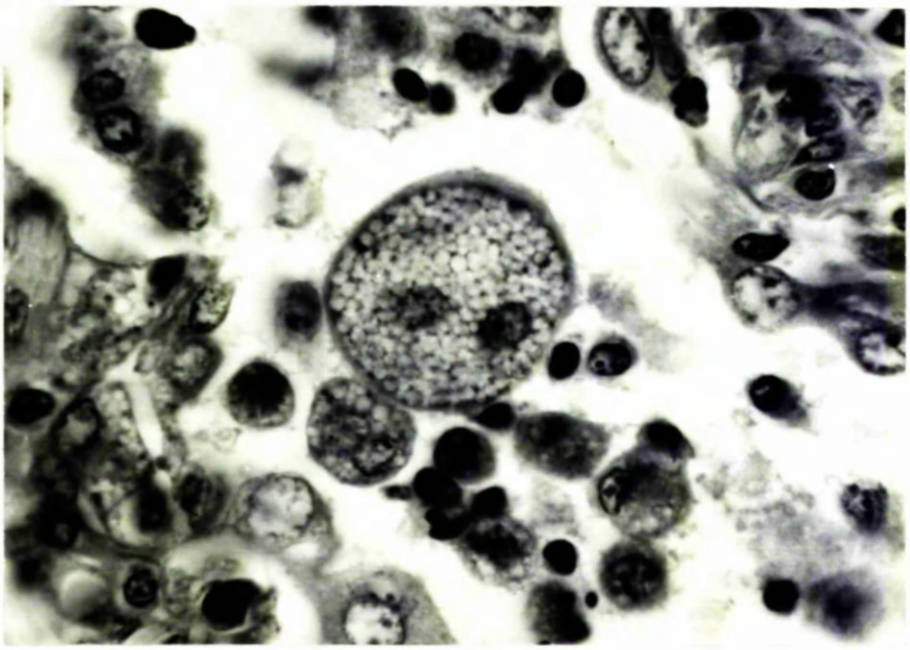

Fig. 58. Clinical Case 11. Lung. A giant "foamy" cell. Note regularity of vacuoles. Periodic acid-Schiff. x 850.

staining procedures used in this study. It would appear that the vacuolation represented a phase in cellular activity rather than a result of degeneration. Similar changes, but of considerably less magnitude, could be seen in some of the alveolar epithelial cells.

Many of the macrophages displayed a characteristic staining reaction with periodic acid-Schiff (P.A.S.) technique. The cytocentrum of these cells often showed an intense response to leucofuchsin of P.A.S., indicating the presence of a polysaccharide-containing substance (Figs. 59 and 60). The degree of reaction did not seem to be affected by prior treatment with diastase. It appeared very likely that the cytocentrum contained a substance, or substances, of mucoprotein or glycoprotein character which could in some way be associated with enzymatic, phagocytic processes of these cells. Identical staining reactions were seen in the active Kupffer cells, in many reticular cells of the lymphoid tissue, particularly in inflammation, and in macrophages of inflammatory reactions in various organs (Watrach and Watrach, 1955). Polymorphonuclear leucocytes and, occasionally, monocytes exhibited but a diffuse and moderately intense staining with P.A.S. In some instances, also the detaching septal cells were found to contain a number of P.A.S.-positive granules in their cytoplasm. The alveolar epithelium, in both the resting and proliferative phases, and the bizarre multinucleated forms seen in some cases of epithelial hyperplasia were evidently negative to P.A.S. (Fig. 61).

Usually after six to seven weeks of illness, as the acute phase subsided, mononuclear intra-alveolar cells diminished significantly in number. Some, however, still lingered in foci of residual inflammation, together with mononuclear elements in peribronchiolar and perivascular spaces (Fig. 62). Even after the eleventh week of the disease, small accumulations of mononuclear cells remained in the alveoli around bronchioles and venules.

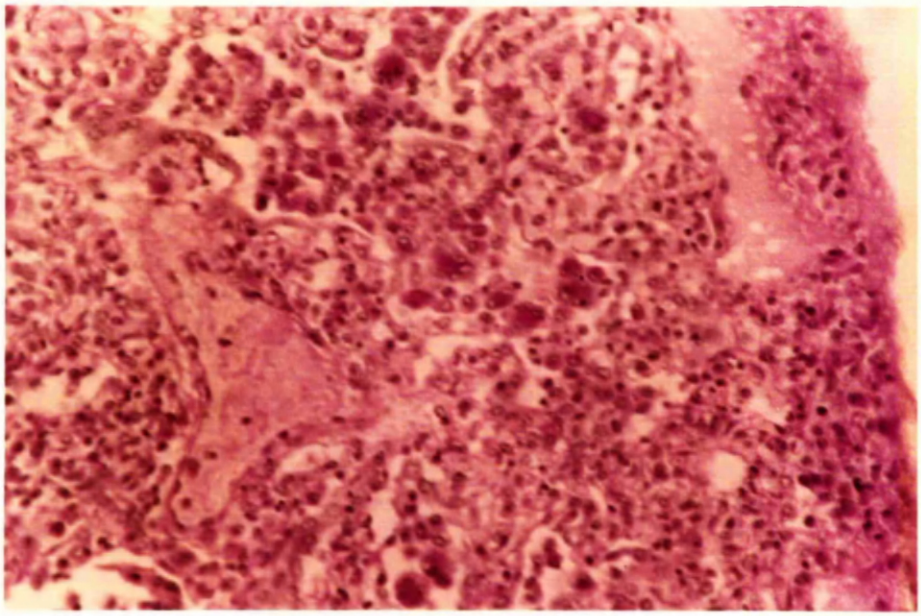

Fig. 59. Clinical Case 8. Lung. Macrophages, showing a positive reaction with periodic acid-Schiff. x 170.

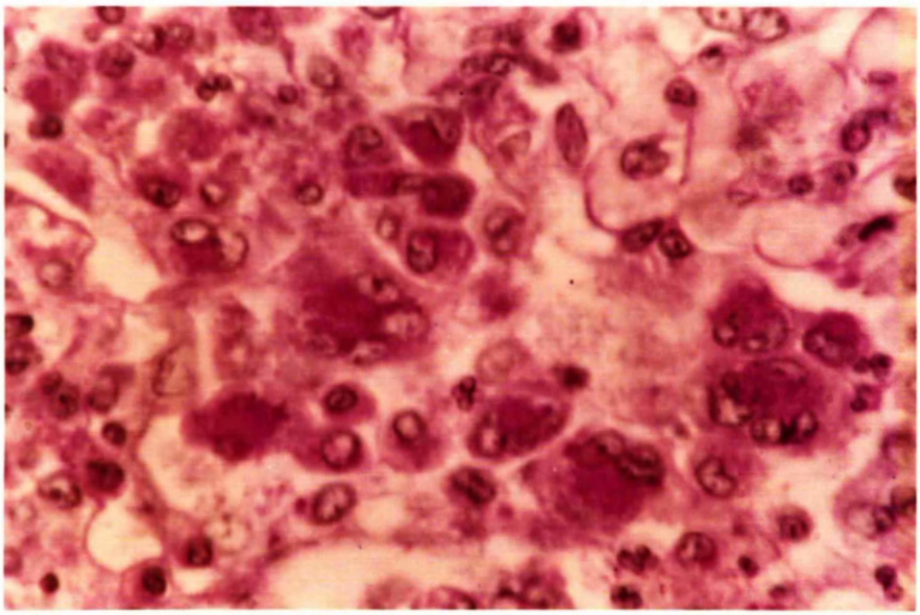

Fig. 60. Detail of Fig. 59. Macrophages, many of which are multinucleated, display a positive P.A.S. reaction in the cytoplasm. x 710.

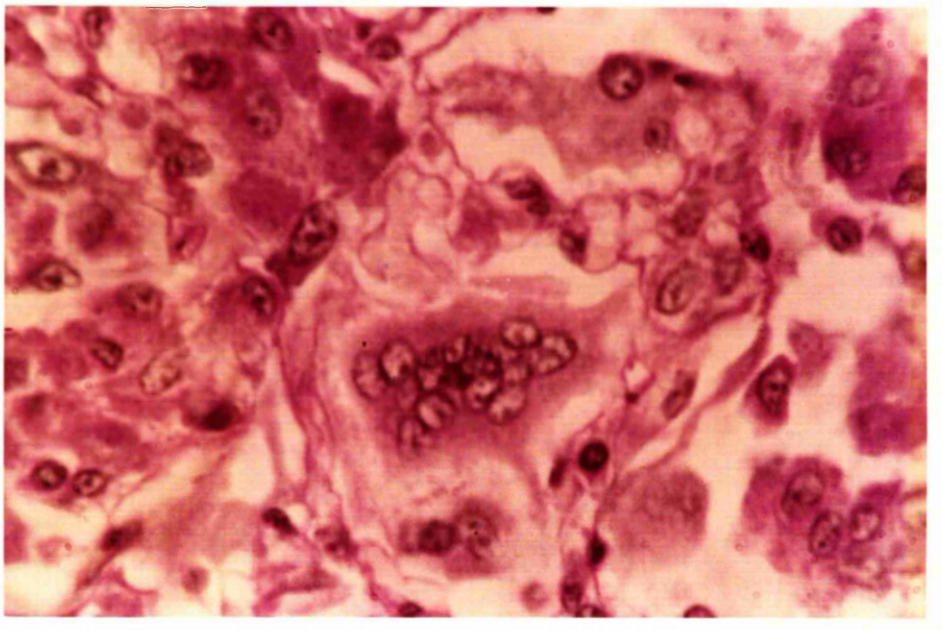

Fig. 61. Clinical Case 8. Lung. Multinucleated cell of alveolar epithelial origin. Note the absence of reaction with P.A.S. x 710.

In a few instances, especially in Case 29, when secondary bacterial infection did occur, polymorphonuclear leucocytes entered the alveoli and the bronchiolar lumina in considerable numbers. A frank suppurative softening of the lung parenchyma, however, was not observed.

Degrees of involvement of the visceral pleura occurred in 30 per cent of the dogs, most of which were ill from five to seven weeks. The changes observed were usually mild and characterized by some increase in subserosal collagenous elements. On a few occasions, however, the lesions were more marked, especially in Dog 19 (Fig. 63). Such cases often showed a cuboidal or columnar metaplasia of the mesothelium.

Cytoplasmic inclusion bodies were found in the lungs of all but two of the thirty clinical cases examined. One of the negative cases (10) was ill for five days and exhibited some muscular spasms in the hind legs. Its cerebellum, however, and some sweat glands of the pads showed a few cytoplasmic bodies of distemper type. In the other case (30), which displayed signs of illness for 11½ weeks, no inclusion bodies were seen, in spite of the prolonged search, in any of the organs examined (respiratory tract, cerebrum, cerebellum, pons, lymphatic tissues, liver, and pads).

The cytoplasmic inclusions of distemper were strongly acidophilic (Fig. 64) and varied in size from those just barely discernible to large bodies measuring 3-4μ in diameter. Most of them were surrounded by clear halos of the unstained cytoplasmic zone. In many instances the inclusions displayed a particulate or vacuolar internal structure. The latter, first referred to by Sinigaglia (1912) and Babes and Starcovici (1912), was well demonstrated in sections examined with a phase-contrast microscope (Fig. 65).

Most frequently, the cytoplasmic inclusion bodies were seen in the bronchial and bronchiolar epithelium (sometimes in great numbers), in alveolar

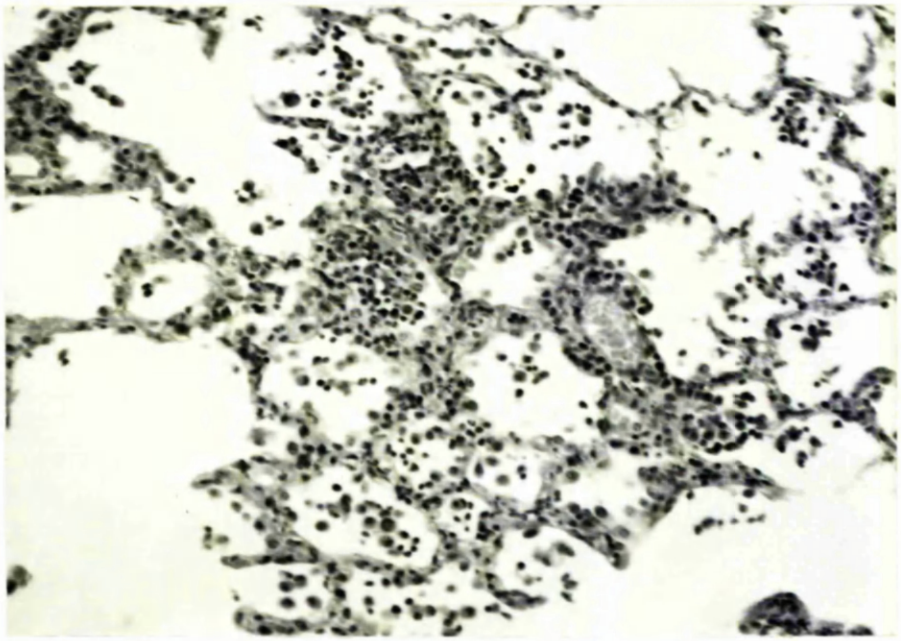

Fig. 62. Clinical Case 26. Lung. Intra-alveolar and perivascular interstitial reaction of late distemper. No evidence remains of "epithelialisation". Some alveolar walls are thickened. Haematoxylin and eosin. x 125.

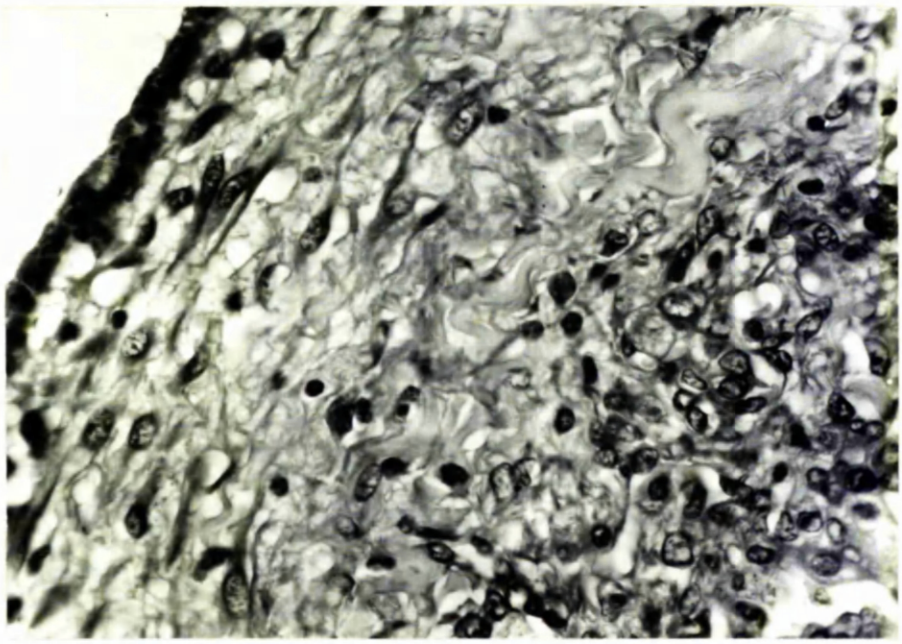

Fig. 63. Clinical Case 19. Lung. Advanced chronic pleurisy. Haematoxylin and eosin. x 450.

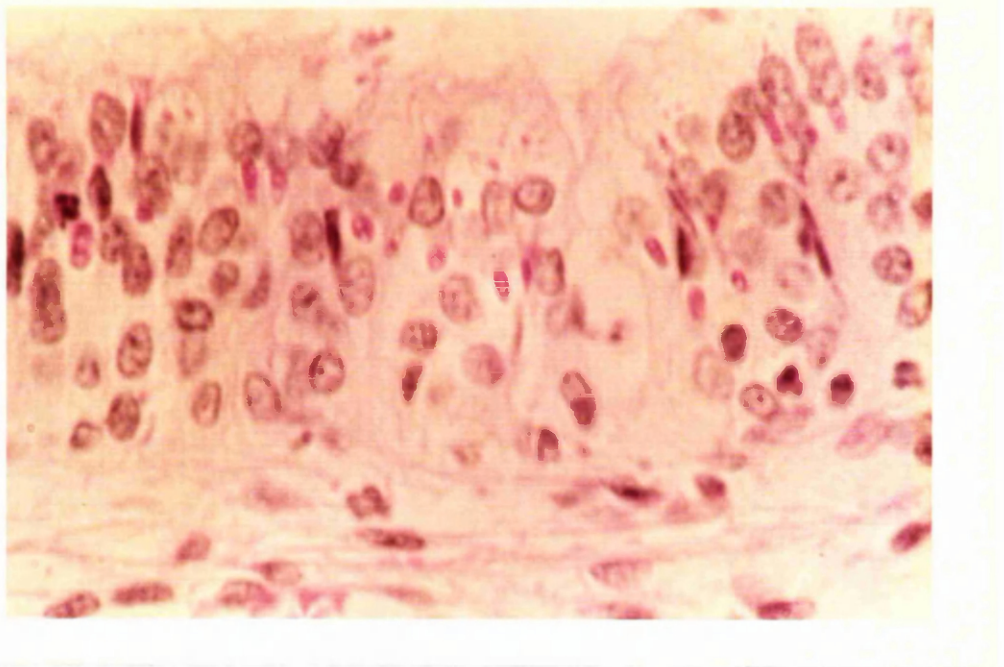

Fig. 64. Clinical Case 33. Bronchial mucosa. Many epithelial cells contain acidophilic cytoplasmic inclusion bodies, some of which show vacuolation. Haematoxylin and eosin. x 850.

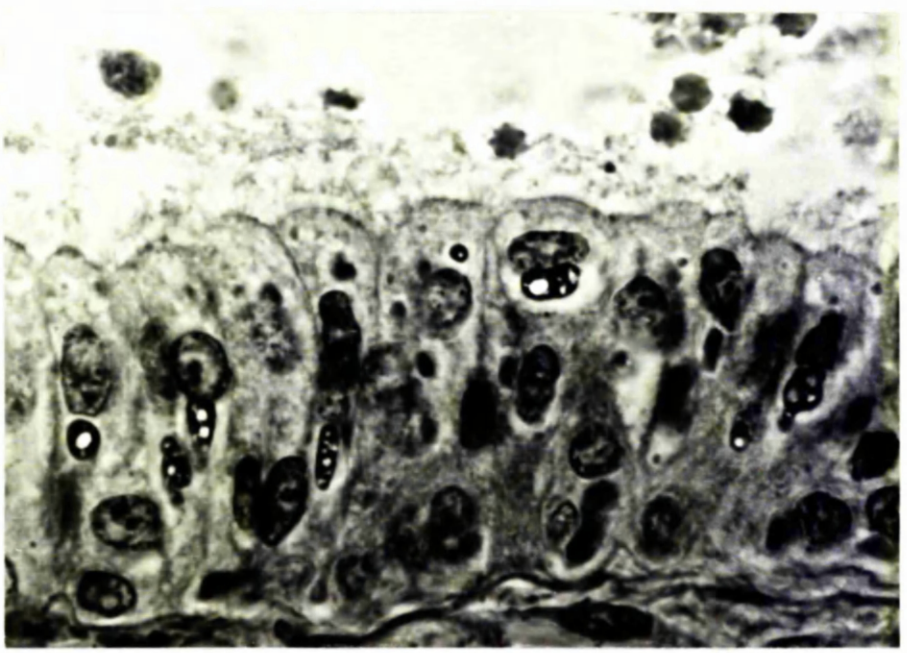

Fig. 65. From the same case. Bronchial mucosa. Note the prominence of vacuoles in cytoplasmic inclusions. Phase contrast. Haematoxylin and eosin. x 1,290.

epithelial cells and in the free macrophages. Occasionally, they could also be observed in vascular endothelial cells, lymphocytes, and polymorphonuclear leucocytes.

Intranuclear inclusion bodies, first described in the lung of distemper dogs by Sanfelice (1915), were found in fifteen of the thirty cases studied. Eight of these had clinical illnesses of two to four weeks, and the remaining were in their second, or from the fifth to seventh week, of the disease. No nuclear forms were encountered after the seventh week of observable signs.

The nuclear inclusion bodies usually conformed to the shape of the nucleus, and often displayed a somewhat hazy margin. In haematoxylin-eosin stained sections, they were dull pink in colour (Fig. 66) and showed a weakly basophilic affinity in haemalum-phloxin-tartrazine-treated preparations. With the Feulgen staining technique for desoxyribonucleic acid, these bodies invariably gave a negative reaction. The involved nuclei exhibited, in most instances, a distinct margination of the chromatin and displacement of their nucleoli. The nuclear inclusions occurred principally in the alveolar epithelial cells but, on occasion, could also be seen in the free macrophages and in the bronchial epithelium.

Regional lymph nodes. As indicated previously, only the parotid and bronchial lymph nodes were examined. In addition, the palatine tonsils were incorporated in the present study.

Quite early in the process of infection, significant changes evolved in the lymphoid tissue. In five of the seven cases showing signs of illness of less than two weeks, lymphocytes were found to be depleted, sometimes to a marked degree, and many of the remaining cellular elements showed karyorrhexis and pyknosis (Fig. 67). The decrease in number of lymphocytes resulted in a moderate or pronounced obliteration of the follicular architecture (Fig. 68).

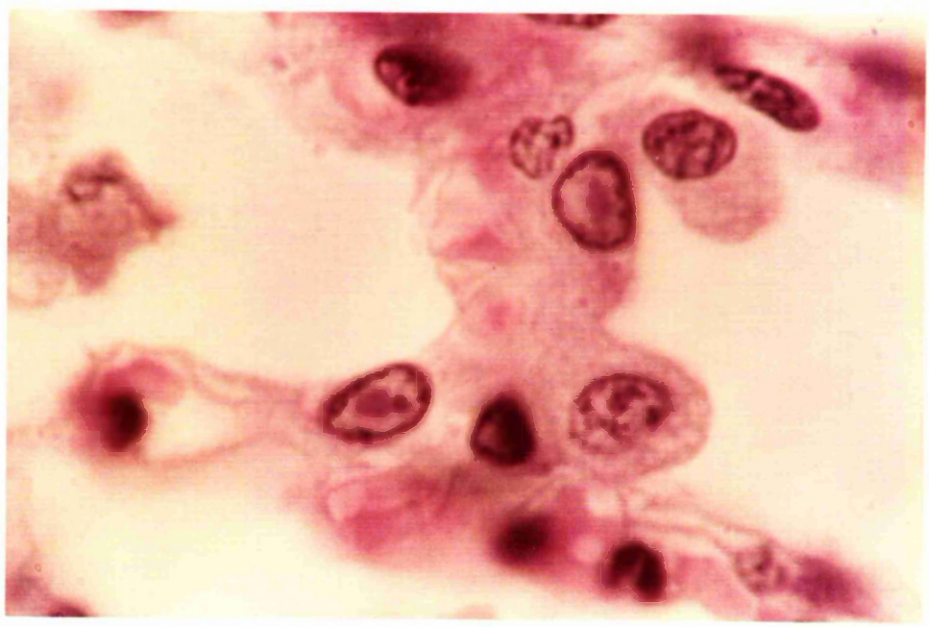

Fig. 66. Clinical Case 27. Lung. Nuclear inclusion bodies in alveolar epithelial cells. Haematoxylin and eosin. x 1,800.

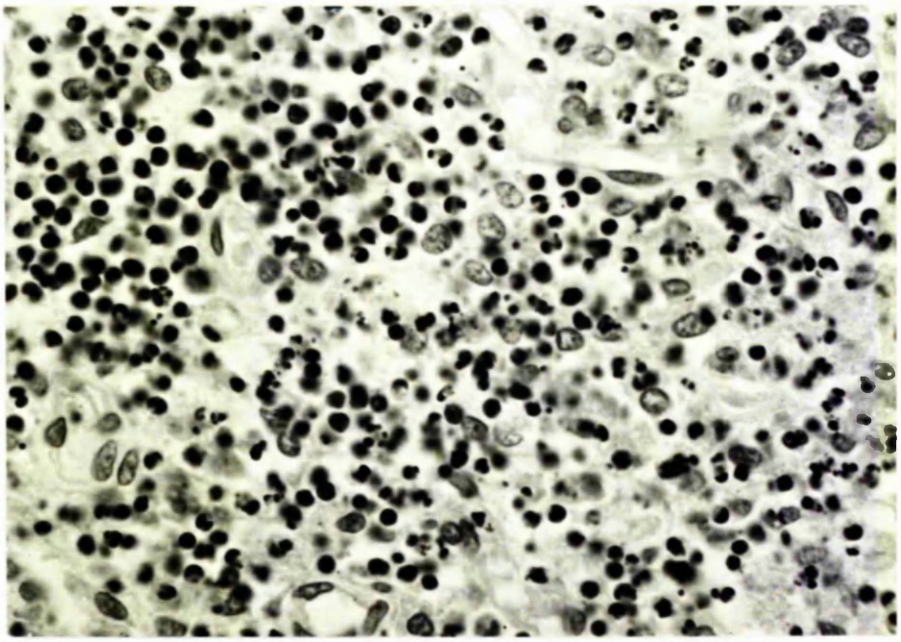

Fig. 67. Clinical Case 20. Bronchial lymph node, showing necrotic changes in lymphoid elements. Haematoxylin and eosin. x 540.

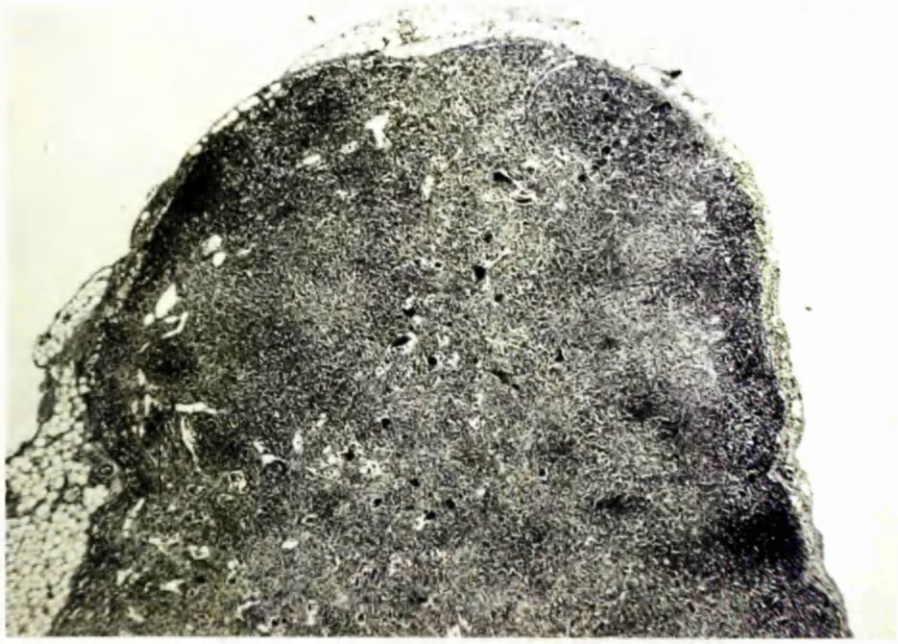

Fig. 68. Clinical Case 5. Bronchial lymph node. Note the complete obliteration of follicular architecture. Haematoxylin and eosin. x 25.

This was often accompanied by some oedema and congestion, particularly in the acute phase. Haemorrhages were less common. Not infrequently, reticular cells, especially of the cortex, showed nuclear inclusions identical in morphology and staining reactions with those observed in the lung (Fig. 69). Eosinophilic cytoplasmic bodies were seen quite often in the reticular cells and macrophages and occasionally in other elements, including lymphocytes and polymorphonuclear leucocytes. In the tonsil they were also present in the epithelium. Multinucleated giant cells occurred in a number of cases usually in the advanced stage of illness. Often they contained a great many nuclei and were situated chiefly in the cortical zone but could also be observed in deeper parts of the node (Fig. 70). Morphologically, they resembled giant cells seen in palatine tonsils in the prodromal stage of measles (Warthin, 1931).

A marked response to the infection was often shown by the local macrophages, many of which proliferated and appeared in increased numbers in the cortical and medullary sinuses.

After the sixth or seventh week of illness, when the acute phase subsided, lymphoid tissue showed regenerative changes, mild at first but clearly evident in the stage of recovery. Lymphoid follicles reappeared and mitotic figures were commonly seen in many cellular elements. Proliferative changes also occurred in the medulla and were sometimes accompanied by prominent accumulations of plasma cells. On occasion, a mild fibroblastic activity, as evidenced by increase in collagenous elements, could be found in some areas. There appeared to be also some increase in reticular fibres. After the tenth week from the onset, inclusion bodies, both nuclear and cytoplasmic, were no longer observed.

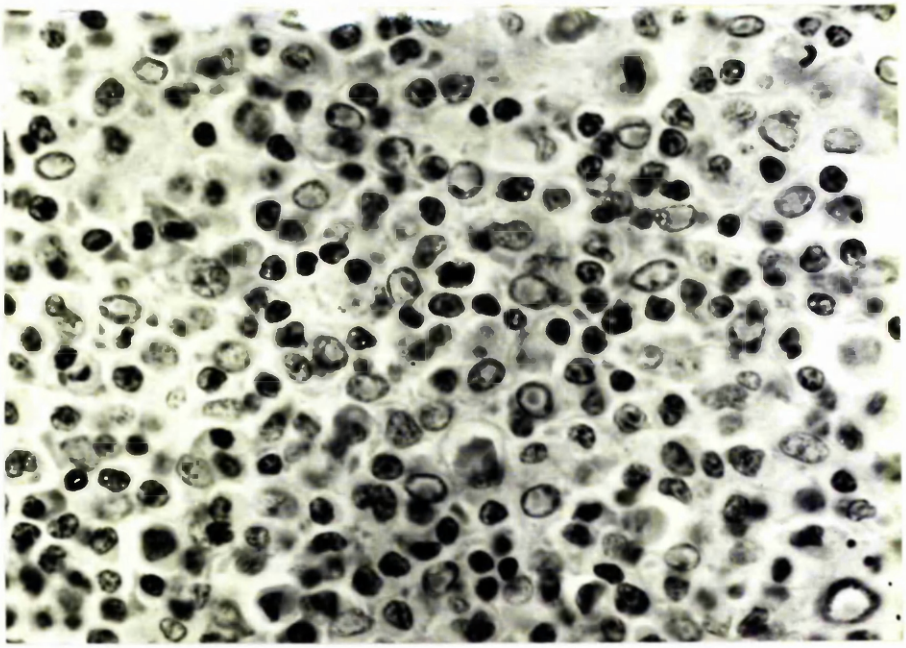

Fig. 69. Clinical Case 15. Palatine tonsil, showing numerous nuclear inclusion bodies in reticular cells. Haematoxylin and eosin. x 625.

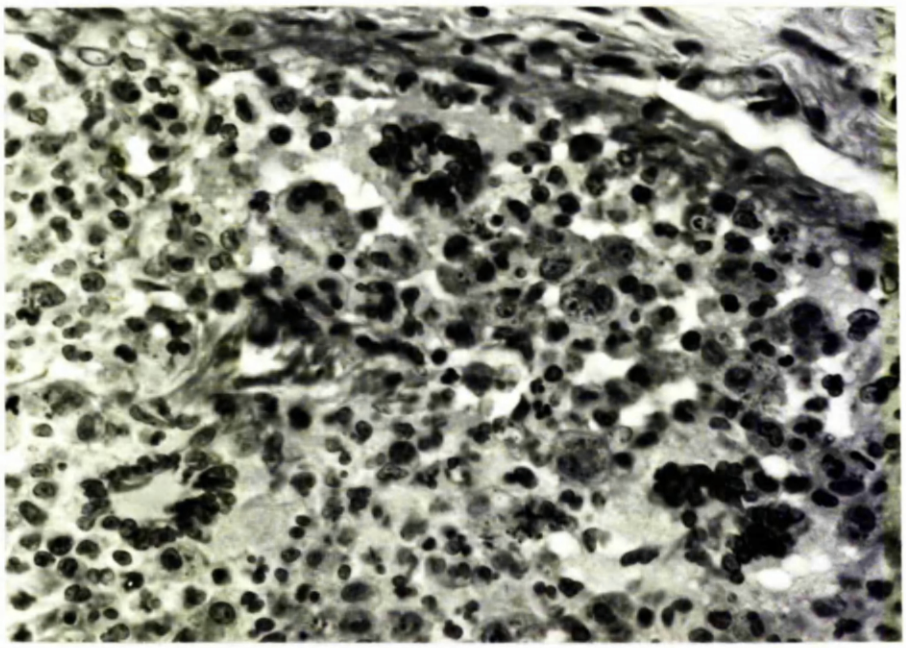

Fig. 70. Clinical Case 5. Bronchial lymph node. Multinucleated giant cells are present in the cortex. Haematoxylin and eosin. x 430.

Pads. Varying degrees of hyperplasia and obliteration of the normal epidermal structure of the pads occurred in nine of the thirty cases studied. Only one of these (Case 8) showed marked, epithelial proliferative changes in the lung, often attended by bizarre cellular forms. In the remaining eight, the pulmonary alveolar lesions were mild in degree and extent and were confined essentially to subpleural and peribronchiolar areas.

Experimental cases

Histological changes in the respiratory system of the experimental cases were studied in fifteen of the twenty dogs employed. The remaining five cases, which received the virus of distemper into the central nervous system, were examined only for the incidence and degree of oedema in the lung.

Eleven of the fifteen dogs were destroyed in the first thirty days of illness, viz., on the 1st, 3rd, 5th, 7th, 8th, 9th, 11th, 15th, 18th, 25th, and 30th days, and the remaining four on the 46th, 59th, 79th, and 157th days after the first signs of the disease.

Upper respiratory tract. Except for mild hyperaemia of subepithelial blood vessels and slight catarrh of the mucous membrane in a few isolated cases (1st, 7th, 8th, and 25th days), no significant changes were seen in the nasal cavities of all fifteen dogs. The first cytoplasmic inclusion bodies, few in number, occurred in the epithelium of the turbinate folds on the third day of illness (sixth day after inoculation). With more advanced stages, they became quite numerous but disappeared from the nasal epithelium after the 30th day of the disease.

Only minor abnormalities were observed in the mucous membrane of the respiratory and digestive parts of the pharynx. One case (9th day) showed an acute catarrh, and a slight hyperaemia accompanied by increased

activity of the mucous glands was noted in five other dogs in the first 30 days of illness. A small number of cytoplasmic inclusions could be observed, especially in the epithelium of the digestive part, during the first 11 days of infection. The changes found in the pharyngeal tonsil were more pronounced and conformed in character and extent to those observed in the parotid and bronchial lymph nodes (v. infra).

With the exception of a mild hyperactivity of the mucous glands in one case (3rd day) and occasional cytoplasmic inclusion bodies, no abnormalities were seen in the larynx and trachea.

Lung. As in the clinical cases, the pneumonia of the experimental dogs was ushered in with a state of hyperaemia. The degree of capillary injection, however, was mild and remained so during the first 5 weeks of illness. After the 46th day of infection, the signs of congestion could no longer be seen. Frank haemorrhages were not observed at any stage.

In sharp contrast with the clinical cases, oedema of the lung in experimental dogs was very uncommon. It occurred in two instances (8th and 18th days) and was confined chiefly to perivascular sites, with only negligible amounts of fluid present in some alveolar spaces.

Collapse of the lung parenchyma, however, was quite frequent and often pronounced, especially in the first 30 days of illness. In the later stages, it tended to subside but in some areas could still be seen even after two months (Figs. 71 and 72). Sometimes, especially in the early phases, the state of collapse was attended by a degree of compensatory emphysema, which on a few occasions was quite prominent in the lower parts of the lobes (Fig. 73).

No significant lesions were found in the larger bronchi, save a mild catarrh in one case (1st day).

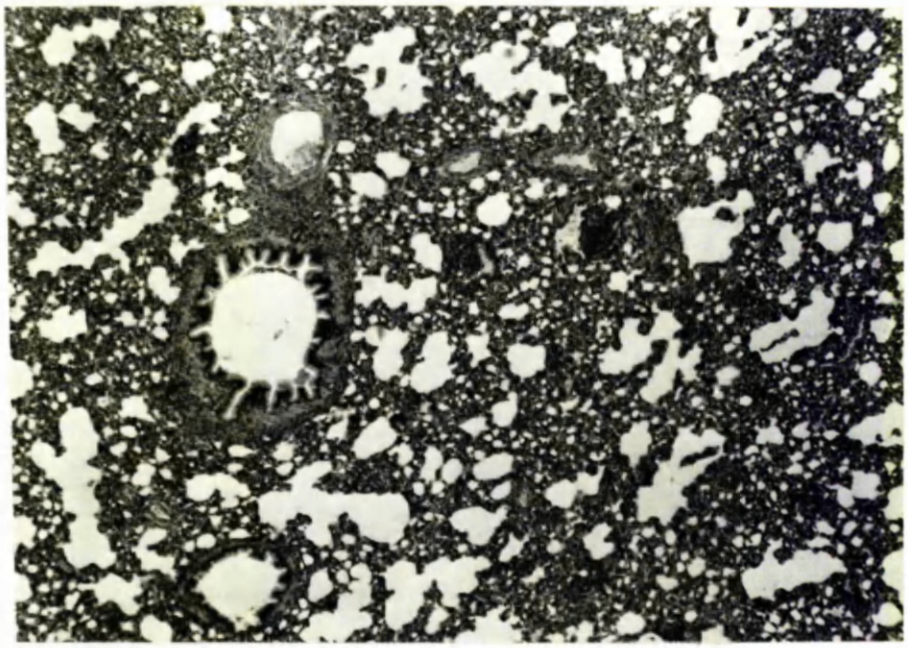

Fig. 71. Experimental Case 18. Left diaphragmatic lobe. There is a diffuse, mild "trabecular" collapse of alveoli (46th day of illness). Haematoxylin and eosin. x 35.

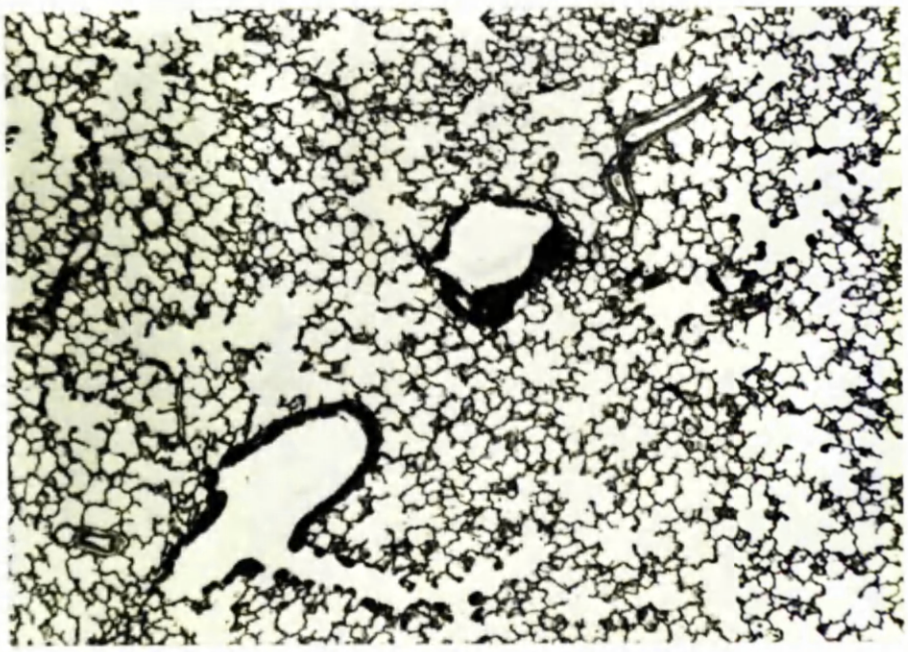

Fig. 72. From the same case. Right diaphragmatic lobe after intratracheal infusion with fixative. There is only a mild reaction at one bronchiole. Haemotoxylin and eosin. x 35.

Most of the changes encountered in the finer air passages were essentially similar in character, but not always in degree, to those observed in the clinical cases. Accumulations of mononuclear elements in and around bronchiolar walls occurred in 66 per cent of the cases and made their appearance on the first day of clinically detectable illness (3 days after inoculation). In the early phases, they were small but became quite prominent in the second and third weeks of infection. In such instances, adjacent alveolar walls were often seen to contain an increased number of mononuclear cells. With the process of resolution of pneumonia, after five or six weeks from the first signs of the disease, peribronchiolar accumulations became more dense, showed decrease in size and involved only small areas around the wall (Fig. 74). A few foci could still be seen even after five months from the onset of illness, when all other signs of inflammation had long since disappeared from the pulmonary tissue (Case 2).

Glycogenic infiltration of the bronchiolar epithelium, frequently resulting in partial denudation of the mucous membrane, was much more common than in the clinical cases. It occurred in all but two of the 15 dogs examined (87 per cent). Quite pronounced in the acute phase of illness, it tended to be very mild in the later stages and was followed by complete restitution of the epithelium.

Only one case (5th day) showed some hyaline degenerative changes in the lamina propria of medium-sized bronchioles.

On occasion, when marked proliferation of the epithelial elements took place, the bronchiolar mucous membrane was seen to be lined by bizarre mononuclear and multinucleated epithelial cells. This was well evident on the 11th and 18th days of illness.

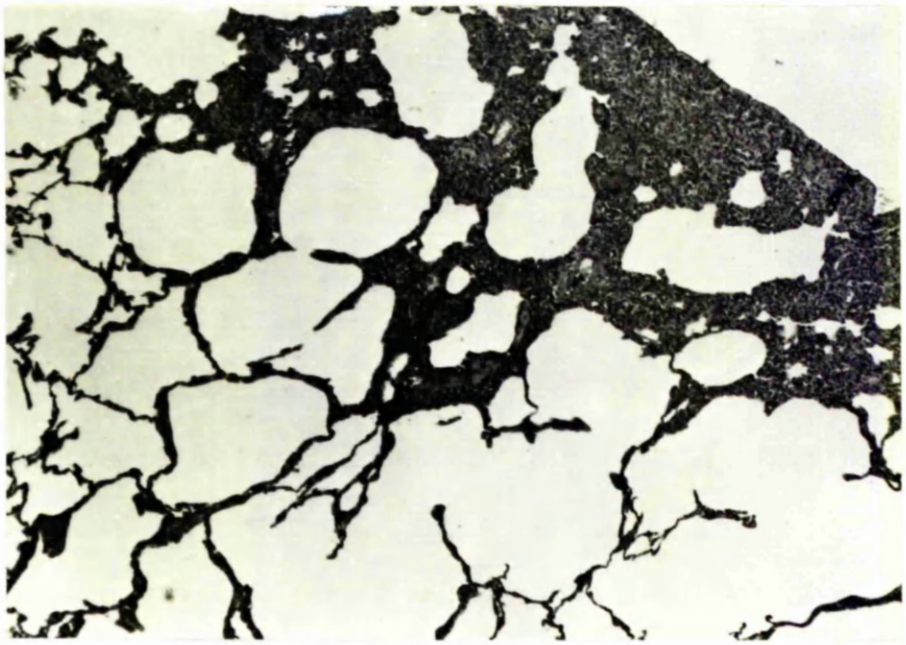

Fig. 73. Experimental Case 9. Lung, showing emphysema in the subpleural area. Haematoxylin and eosin. x 35.

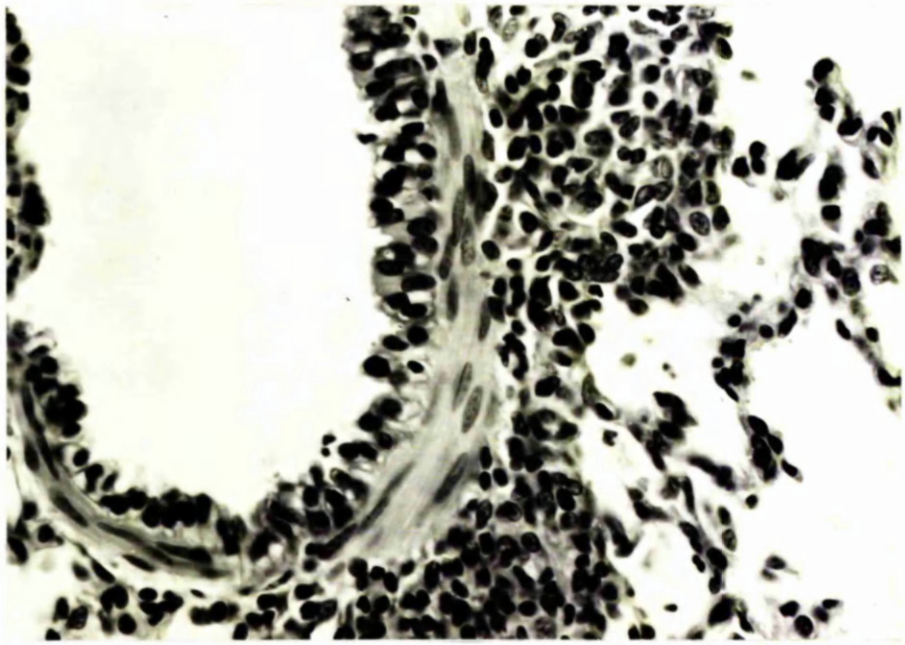

Fig. 74. Experimental Case 18. Bronchiole. Mononuclear aggregates in the peribronchiolar area. Late distemper (46th day of illness). Haematoxylin and eosin. x 420.

Catarrhal bronchiolitis of an appreciable degree was found only in two cases, in the earlier phases of the disease. More often the mucosa of some bronchioles showed mild infiltration with lymphocytes, plasma cells and occasional polymorphonuclear leucocytes.

Intrabronchiolar and, rarely, intra-alveolar formations of dense fibrinoid masses were sometimes observed during the third and fourth weeks of illness. An organizing process, however, did not follow. Occasionally, there appeared to be a moderate increase in collagenous and reticular fibres in the walls of some subpleural alveoli (9th, 11th and 18th days), but intra-alveolar and intrabronchiolar organization and fibrosis were not encountered in any of the experimental dogs.

Mononuclear infiltration of the perivascular connective tissue, especially in relation to venules, occurred in 66 per cent of the cases. It was clearly evident on the third day after inoculation and persisted in small accumulations around some venules for as long as five months from the first signs of the disease. Sometimes in the acute phases of infection, the adjacent alveolar walls were similarly involved and exhibited considerable thickening (Fig. 76).

The process of alveolar "epithelialisation" was identical in its evolution and character with that described in the clinical cases. It differed considerably, however, in the extent of involvement. At the height of illness only small peribronchiolar and subpleural areas were affected.

The first signs of alveolar "epithelialisation" appeared on the 7th day of illness in the form of an incomplete cellular pavement lining parts of the wall. Mitotic figures were often numerous among the proliferating septal cells in such areas. Within a short period of time, not more than four days, the entire walls of some alveoli became covered by an uninterrupted

layer of flattened and irregularly shaped cells. The process was well advanced on the 18th day from the onset of infection (Fig. 75) and was soon followed by the beginning of desquamation (Fig. 76). At the end of the fourth week, only small groups of peribronchiolar alveoli remained lined. Finally all traces of the alveolar epithelial hyperplasia disappeared after the sixth week of the disease. Sometimes when the process of "epithelialisation" was intense, bizarre forms of the epithelial cells could be seen in some alveoli and respiratory bronchioles. This occurrence, however, was considerably less frequent than in the clinical cases.

The character, intensity and morphological properties of the inflammatory cellular exudate were very often identical with those encountered in the clinical form of distemper. The first elements, predominantly mononuclear in type, were observed on the third day after inoculation. Initially, they were few in number and mostly subpleural in situation but, with the progress of illness, more cells entered alveolar spaces, not only in the peripheral regions but also around many bronchioles. Sometimes the accumulations of cells became very marked, especially along the lower borders of the lung, and presented all features of consolidation (Fig. 77). On many occasions, polymorphonuclear leucocytes migrated into the alveoli, but their numbers were usually very small. In two cases (9th and 18th days), however, they were seen in appreciable numbers in a few isolated foci.

In the late phases of illness, many of the cellular elements disappeared from the alveoli, and only small aggregates of mononuclear cells persisted for some time in a few peribronchiolar and perivascular areas.

The morphological and tinctorial features of the intra-alveolar phagocytes and the vacuolar cytoplasmic changes seen in some of the cells did not differ from those described in the clinical cases.

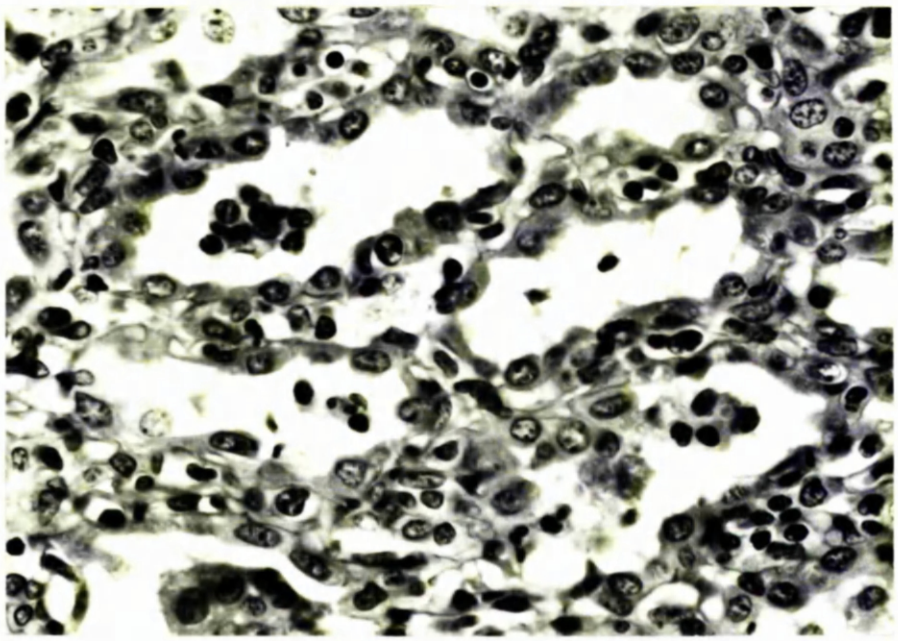

Fig. 75. Experimental Case 5. Eighteenth day of illness. Lung. "Epithelialisation" of alveoli attended by some thickening of their walls. Haematoxylin and eosin. x 580.

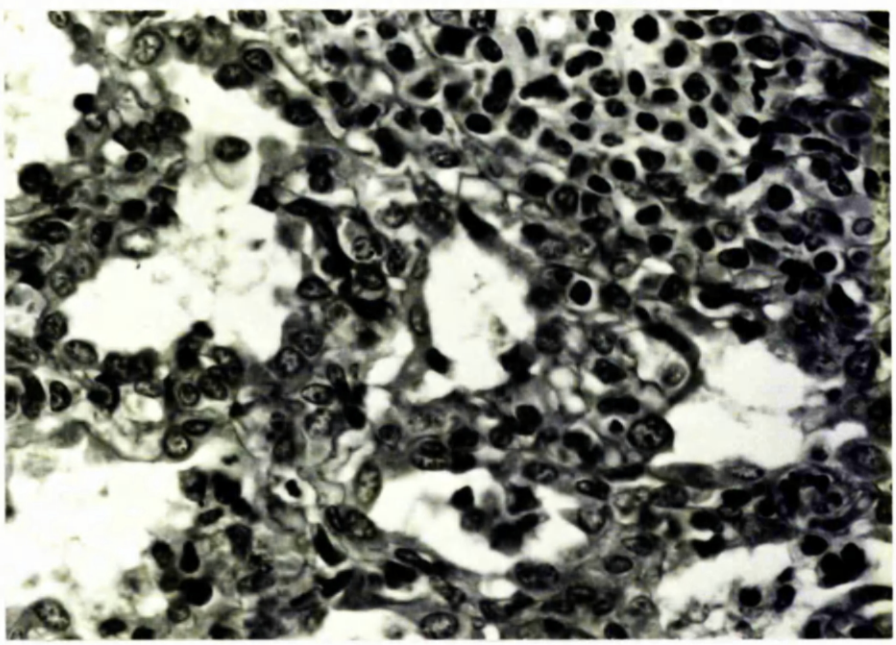

Fig. 76. From the same case. Lung. Note early detachment of the lining (in centre), thickening of alveolar walls and perivascular mononuclear infiltration (upper right). Haematoxylin and eosin. x 575.

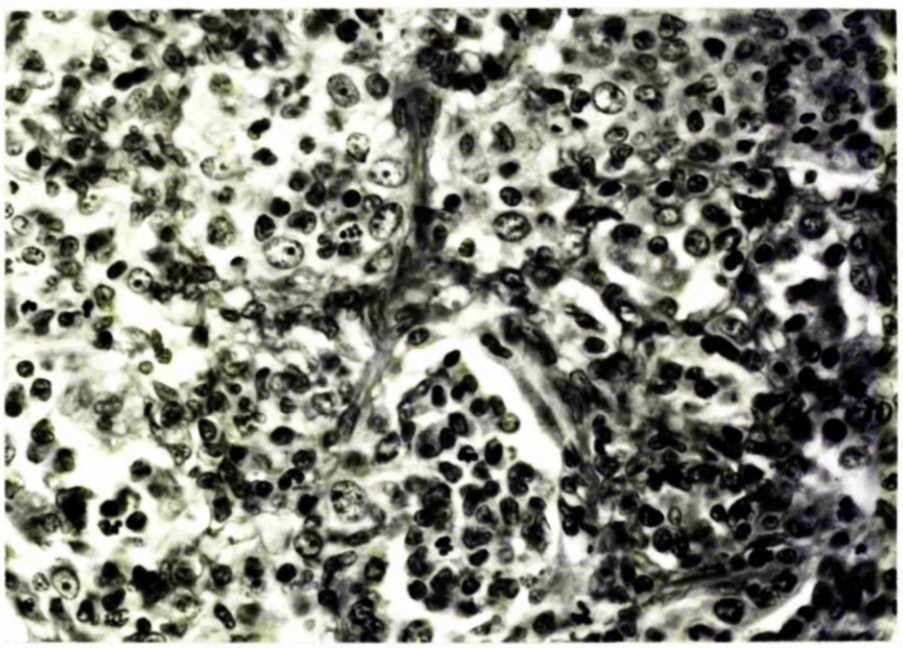

Fig. 77. Experimental Case 5. Lung, showing massive accumulation of mononuclear cells (18th day of illness). Haematoxylin and eosin. x 485.

No significant alterations were observed in the visceral and parietal pleurae of any of the experimental dogs.

Cytoplasmic inclusion bodies were first noted in the bronchiolar epithelium on the third day after inoculation (1st day of illness, Case 17). They varied in size from 0.5 to 1μ and were situated chiefly in the basal parts of the epithelial cells, often surrounded by a cytoplasmic "halo". On the seventh and eighth days of the disease, they became quite numerous, especially in the macrophages and alveolar epithelial cells. In time the cytoplasmic inclusions diminished in numbers, and only a few remained on the 30th day. After 6 weeks of illness, they were no longer seen in the pulmonary tissue.

The intranuclear inclusion bodies of distemper did not occur in the lung of experimental cases, but a few were found in the lymphoid tissue.

Regional lymph nodes. No appreciable differences in character and degree of changes in the lymphoid tissue were encountered between the clinical and experimental cases. A moderate obliteration of the follicular architecture, due to depletion of lymphocytes, developed on the fifth day of infection and reached considerable proportions after the seventh day. It remained marked for two weeks. The first signs of regeneration appeared on the 18th day and were quite pronounced after the fourth week of illness. Lymphatic nodules slowly reappeared in the cortical zone, and the lymph nodes gradually recovered their normal structural pattern. After the 59th day, from the onset of the disease, no significant abnormalities were observed in the lymphatic tissue, save some enhanced lymphocytic activity.

Multinucleated cells were present in the cortex as early as on the first day of illness but disappeared soon after the ninth day.

The first cytoplasmic inclusion bodies were noted on the fifth day of infection, mostly in the reticular cells and macrophages. They were quite numerous during the second week but diminished appreciably in number after the 18th day of illness.

Only two cases (7th and 18th days) showed a few intranuclear inclusion bodies. They were seen in reticular cells and displayed the same morphological and staining characteristics as those described in the clinical cases.

The changes found in the tonsillar tissue were identical with those seen in the lymph nodes.

Pads. No abnormalities were noted in the digital and carpal pads of any of the twenty experimental dogs examined.

Experiments on pulmonary oedema. Five dogs used for this study were inoculated with the virus of distemper, either intracerebrally (26 and 27) or intraspinally (24, 25, and 30). Although severe nervous disturbances appeared in four of these cases, no appreciable degree of intra-alveolar or perivascular oedema was found in any of the lungs examined.

The evidence obtained, though based admittedly on a small number of cases, seems to indicate that even a marked involvement of the central nervous system in distemper does not constitute the necessary factor in the development of pulmonary oedema.

Pathogenesis of distemper pneumonia

The result of observations on the clinical and experimental cases of pneumonia in distemper revealed that by the time the initial rise in temperature took place, the lung tissue had reacted to the invasion of virus by a degree of hyperaemia, mild intra-alveolar exudation of chiefly mononuclear cells, and by accumulation of macrophages and lymphocytes in and around bronchiolar

walls and in perivascular sites. The early signs of viral activity were also shown in the bronchiolar epithelium in the form of a small number of cytoplasmic inclusion bodies. They made their appearance on the fifth day of illness.

Soon, evidently during the first week, the oedema fluid started to collect in alveoli and around smaller vessels. Initially very mild and focal in distribution, it often affected large areas of the lung in more advanced stages of the disease. Quite early a proportion of the bronchioles exhibited glycogenic degenerative changes which, not infrequently, were seen to result in partial denudation of the mucous membrane. These changes, however, were seldom pronounced. Occasionally a mild degree of catarrhal bronchiolitis could be observed.

With the progress of infection, more cells entered the affected areas, particularly around bronchioles and in the subpleural regions. Some of the macrophages enlarged appreciably and showed more nuclei. A few polymorphonuclear leucocytes also appeared in the exudate. At the end of the first week, the epithelium lining the alveoli adjacent to the bronchioles and pleura exhibited signs of hypertrophy and proliferation and began covering the walls with a layer of cuboidal or flat cells. On occasion when hyperplasia was pronounced, multinucleated and bizarre epithelial cells were formed. The "epithelialisation" of alveoli was usually well advanced in the third week of the disease process, and by coalescence of the initial foci, sometimes involved considerable parts of the lobes. Consolidation of such "epithelialised" areas was not uncommon, particularly in the subpleural regions. The less involved, adjacent parts often showed a mild degree of focal collapse.

Beginning with the second week of infection, some of the consolidated areas displayed an appreciable degree of organization of the intrabronchiolar and intraductal exudate. The process became more pronounced in the later

phases and finally resulted in fibrosis and permanent occlusion of the affected spaces. Sometimes it was accompanied by a degree of fibroplasia in the alveoli and more often in their walls.

After six to seven weeks of illness, as the acute phase of pneumonia subsided, the elements which entered into the process of "epithelialisation" desquamated and were removed from the alveolar spaces. Also the cells of the exudate diminished significantly in number. Small accumulations of mononuclear cells, however, remained for some time in peribronchiolar and perivascular areas and in the adjacent alveoli. The cytoplasmic inclusion bodies, usually numerous at the height of infection, also became less frequent, but a few could still be seen as late as ten weeks after the onset.

After three months, only minute aggregates of lymphocytes and macrophages were found at an occasional bronchiole or venule.

Sometimes in the course of the disease, a mild secondary infection of the parenchym and, occasionally, of bronchioles did occur, but more advanced bacterial involvements were not encountered in any of the cases studied.

DISCUSSION

Degree of Pulmonary Involvement in the Experimental Cases

In their extensive studies on canine distemper, Dunkin and Laidlaw (1926b) encountered considerable differences in the intensity of clinical and pathological signs between the natural and experimental forms of this disease. Pneumonic lesions which these authors described in experimental dogs were very mild and consisted of small patches of bronchopneumonia and a degree of bronchitis. Similar observations have since been made by a number of workers. Recently Potel (1951) found pneumonia only in five of 59 experimental dogs which showed catarrhal form of distemper. The pulmonary changes developed and attained their climax during the first 10 days of infection. The author concluded that pneumonia, so often pronounced in clinical cases of distemper, is relatively infrequent and mild under experimental conditions.

The results of the present study are in accord with those observations. Not only were the pathological changes in the respiratory tract of all 15 experimental dogs considerably less advanced, but also the clinical signs were much milder and of short duration.

With few exceptions, the differences encountered were those of degree and not of character. The disease process in the experimental animals evolved essentially along the same path as in the clinical cases, though seldom reached significant proportions. Oedema of the lung and intrabronchiolar and intraductal fibrosis were not seen in this form of pneumonia.

The reasons for such differences in the pathological picture and symptomatology of clinical and experimental distemper are but little understood. Among factors suggested as possibly having some influence on the mode of host reaction to the invasion of virus are those of breed resistance (Hardenbergh, 1923) and concurrent bacterial infection. In the present investigations, the latter factor seemed to have but limited bearing upon the course and severity of pneumonia in spite of frequently positive bacteriological findings.

Relationship of Pulmonary Lesions to Duration of Illness

The investigations conducted on the clinical material and supplemented by findings in the experimental dogs revealed that the development of pulmonary lesions occurred in characteristic sequence. This is especially true of the more typical changes taking place in the first six weeks of the disease. In that period of infection, it appears possible to make an estimate of the duration of illness from a histopathological examination of representative portions of the lung tissue. After the seventh or eighth week from the onset, only a very approximate evaluation can be made.

Pneumonia

One of the salient features of pneumonia in distemper is the interstitial cellular reaction (DeMonbreun, 1937; Cordy, 1942; MacIntyre et al., 1948; Bienfet, 1953). It manifests itself as a mononuclear infiltration of the bronchiolar walls, the adjacent alveolar septa and, often, also of the perivascular connective tissue. Other changes intrinsically associated with the disease process include (1) hyperplasia of the alveolar epithelium, (2) oedema and (3) accumulation of a predominantly mononuclear exudate in alveolar spaces.

Pneumonia of similar morphological character has been described in many viral infections of animals and man, and also in some rickettsial (Allen and Spitz, 1945) and protozoan involvements (Pinkerton and Henderson, 1941; Baar, 1945).

While the interstitial and mononuclear reaction in all of these pneumonic conditions appears to be a prominent and constant feature, the attending alveolar "epithelialisation" rarely reaches so pronounced a degree as in distemper or in certain forms of atypical pneumonia of man (Parker et al., 1947). In some viral infections, particularly in those of the influenza type, the alveolar epithelial proliferation is frequently absent (Shope, 1931; Jones and Maurer, 1943; Parker et al., 1946; Stuart-Harris, 1953).

A considerable proportion of the clinical cases used in the present study showed evidence of early organization and fibrosis (hitherto unreported in distemper) in the finer air passages and in neighbouring alveolar spaces.

An identical process, but often greater in intensity and extent, has been described on a number of occasions in viral pneumonias of man (MacCallum, 1919; Parker et al., 1947; Auerbach et al., 1952). Organization of the exudate in those cases was evident in the early stages of infection, usually in the first two weeks (MacCallum). Sometimes, however, it was seen as early as on the 7th, 8th or 9th day after the onset of illness (Auerbach et al.). As a result of fibroblastic invasion of the exudate, dense fibrous masses were formed in bronchioles and alveolar ducts. Quite often these "plugs" became invested with a single layer of cells apparently derived from the bronchiolar epithelium. Alveolar walls adjacent to the affected bronchioles and ducts showed a significant widening due to accumulation of collagenous elements. Later in the process, many of the adjoining alveolar spaces became completely occluded by fibrous formations (Auerbach et al., 1952).

A similar fibroblastic organization of the exudate was reported by Ehrich and McIntosh (1932) in "uraemic" pneumonia (bronchiolitis obliterans) and in the rheumatic lung by Hadfield (1938) and Harris (1954).

The factors which precipitate such early organization and fibrosis in distemper and other viral pneumonias are not clearly understood. There is, however, a growing body of opinion that the common use of antibiotics which suppress the activity of secondary organisms and thus lower the demand for polymorphonuclear leucocytes, with resultant retention of fibrin in the exudate, contributes significantly to the evolution of this process (Auerbach et al., 1952).

Pulmonary Oedema

Oedema of the lung is a very characteristic feature of pneumonia in distemper. Flooding of the alveolar spaces occurs at an early stage of the disease and often involves large areas of the organ, contributing significantly to the severity of respiratory signs.

Many aspects of the pathogenesis of this highly complex process are not fully understood. This is particularly true of some of the indirect causative factors. In recent years there has accumulated a considerable amount of experimental evidence indicating that indirect determinants of pulmonary oedema in many instances are of nervous origin (Cameron and De, 1949; Campbell and Visscher, 1949; Cameron and Sheikh, 1951; Naim, 1951; Sarnoff, 1952; Tennekoon, 1954; Visscher et al., 1956). Alterations in the central, peripheral and autonomic nervous systems have been found to be associated with accumulation of fluid in the area of gaseous exchange in the lung. The exact mechanism of this process is still a matter of conjecture. Cameron and De (1949) were of the opinion that medullary excitation, particularly in the region of the dorsal nucleus of the vagus nerve, was the prime cause.

The frequent occurrence of oedema in distemper, with no evidence of cardiac failure to account for it, led MacIntyre et al. (1948) to the assumption that neurogenic factors may be involved. These seem to be associated with the autonomic nervous system, chiefly its cranial part with its preganglionic and postganglionic neurons, as the changes in the brain substance alone are apparently not sufficient to cause the oedema of the lung.

This view seems to be borne out by the present study. In all four dogs inoculated with the virus of distemper, either intracerebrally or intraspinally, there was no evidence of oedema in spite of severe involvement of the central nervous system.

The experiment showed also that introduction of the virus into the brain tissue or the spinal subarachnoid space is capable of producing the nervous form of the disease. This observation is at variance with that of MacIntyre et al., (1948) and Innes (1949b).

More comprehensive studies on the causation and mechanism of pulmonary oedema in distemper were beyond the scope of the present work. Any future attempts at elucidation of the process should follow a more fundamental approach and be based, in part at least, on the use of sympathetic and parasympathetic stimulants and inhibitors.

Alveolar Lining and the Process of Alveolar "Epithelialisation"

The results of the electron microscopic studies on the structure of the pulmonary alveolar wall revealed that the lining of alveoli is formed by a continuous and, in parts, extremely thin cellular membrane. Similar observations were made in recent years by Low (1952, 1953), Bargmann and Knoop (1956), Breemen and Neustein (1956), Karrer (1956a, 1956b) and Policard and Collet (1956).

The implications of this finding are of considerable pathological significance. The process of alveolar "epithelialisation" so characteristic of distemper and many other viral pneumonias becomes satifactorily explained on the basis of hypertrophy and proliferation of the existing alveolar epithelium. The identity of the factor or factors which precipitate such changes is still a matter of conjecture, but it seems highly probable that the viral agent alone may in many instances stimulate cells to pronounced hyperplastic activity. Of other influences, deficiency of vitamin A has been claimed to be associated with epithelial hyperplasia and formation of multinucleated cells in some pneumonias (Chown, 1939; Hjärre, 1953).

Alveolar epithelial proliferation often shows considerable variation in degree and extent among cases with illness of the same duration. Such occurrences suggest as possible factors either changes in the "epitheliotropism" of the virus or individual differences in the susceptibility of the host.

The occurrence and pathogenesis of alveolar "epithelialisation" have been the subject of numerous communications in the last four decades. The process has been encountered not only in viral conditions, but also in some protozoan infections (Fig. 78), in such entities as progressive pneumonia of sheep (Fig. 79) and "atypical" pneumonia of calves (Fig. 80), and in many other pulmonary diseases associated with bacterial invasions or with non-infectious agents.

In most instances, particularly in acute conditions, alveolar "epithelialisation" is evidently the result of hypertrophy and hyperplasia of the local epithelial cells. Cowdry (1925) attributed many of the changes seen in such a chronic lung disease as jaagsiekte to this process.

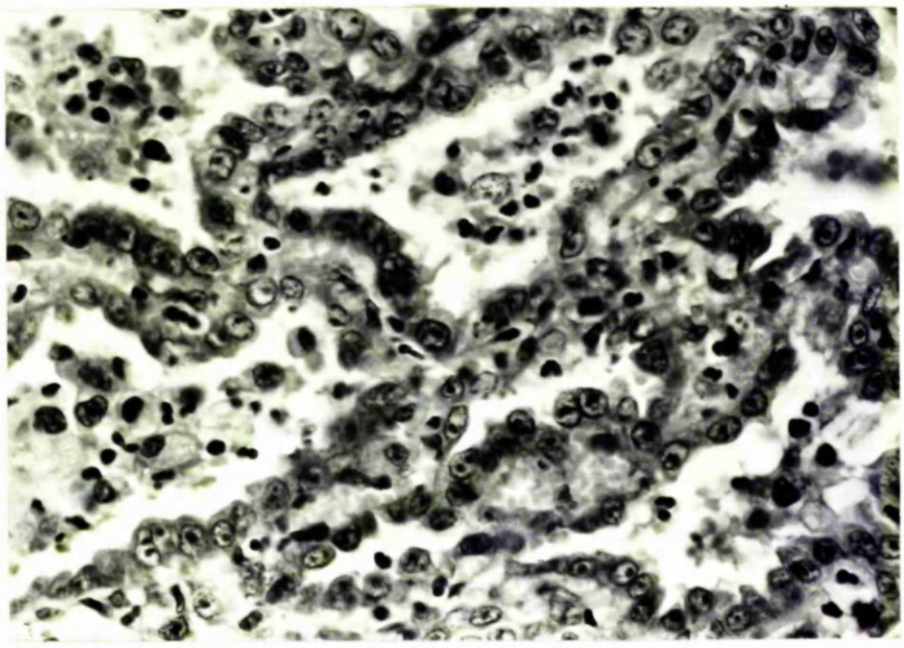

Fig. 78. Lung. Alveolar "epithelialisation". From a case of feline toxoplasmosis. Haematoxylin and eosin. x 460.

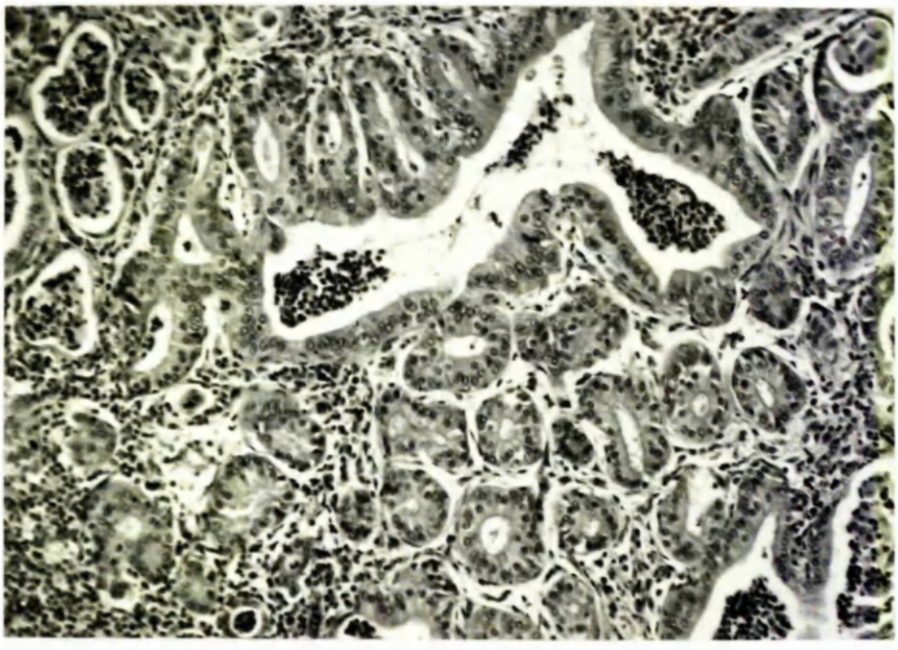

Fig. 79. Lung. "Epithelialisation" of alveoli from a case of progressive pneumonia of sheep. Haemtoxylin and eosin. x 410.

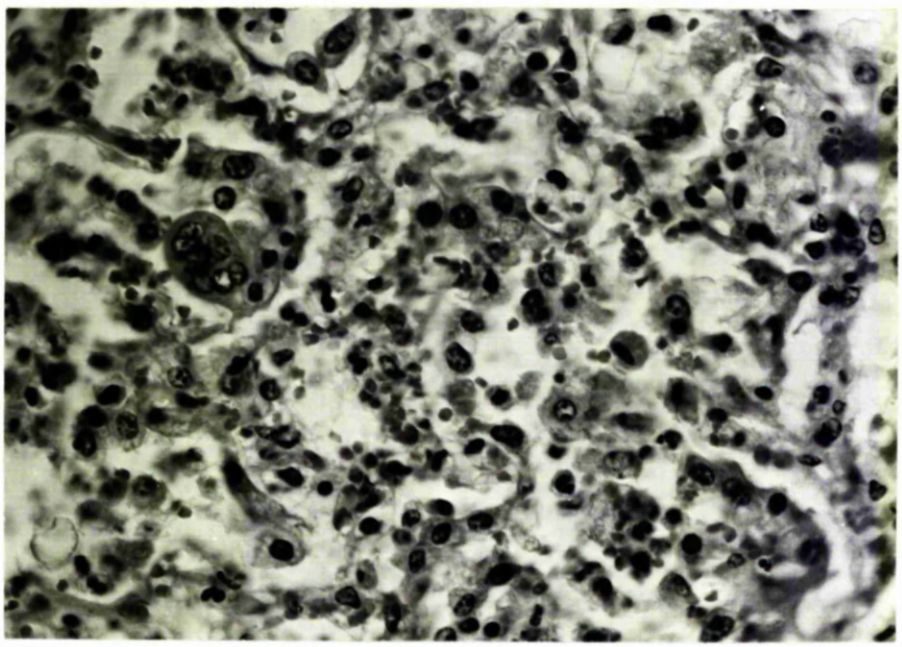

Fig. 80. Lung. "Atypical" pneumonia of calves. Note "epithelialisation" of alveoli (late stage) and a giant multinucleated cell. Haemotoxylin and eosin. x 430.

There is little doubt that on occasion proliferation of the bronchiolar epithelium, with subsequent invasion of the adjoining alveoli, does occur; it has been described by a number of authors (Geever et al., 1943; Watts and McDonald, 1948; King, 1954; Spencer and Raeburn, 1954). It appears, however, that such changes are usually limited to small peribronchiolar areas, and that they take place chiefly in the more chronic conditions.

On those occasions when acute infection of viral origin is accompanied by degeneration and desquamation of bronchiolar epithelium, "epithelialisation," usually concurrent with those changes, is obviously the result of hyperplasia of the alveolar lining.

Some Tinctorial Aspects of Pulmonary Macrophages

The staining reaction exhibited by many pulmonary macrophages with periodic acid-Schiff technique, after preliminary treatment with diastase, seems to indicate that the substance contained in the cytocentrum is of mucoprotein or glycoprotein nature (Teilum, 1956). Identical tinctorial properties are displayed by phagocytic cells of the reticulo-endothelial system and by macrophages of various inflammatory conditions (Weiss and Fawcett, 1953; Theilum, 1956). Potential phagocytic cells show only a mild and usually diffuse reaction. Under stimulation, however, many of them acquire appreciable amounts of the stainable material. This is evidently concurrent with accumulation of such enzymes as oxidases, dehydrogenases, nonspecific alkaline phosphatases (Wachstein, 1955) and esterases (Chessick, 1953), all of which appear to play important roles in the phagocytic process.

It seems plausible to assume that the concurrent mobilization of these enzymes and of mucoprotein or glycoprotein may be linked in some way, and that the demonstration of the P.A.S.-stainable material (after treatment

with diastase) in cells of the inflammatory exudate indicates a high level of phagocytic activity.

Inclusion Bodies

The pathognomonic significance of cytoplasmic inclusion bodies in distemper has rarely been questioned in recent years (Fankhauser, 1951), especially since the demonstration of their antigenic specificity (Moulton and Brown, 1954). Their diagnostic value, however, would be more fully appreciated if critical methods of staining were commonly employed for their detection. This is particularly true of the initial and late stages of illness when the inclusion bodies are few in number and difficult to recognize. Lendrum's phloxin-tartrazine technique with cellosolve as a differentiator has proved to be invaluable in that respect.

The nuclear inclusion bodies, encountered mostly in the acute stages of illness, not only in the lung but also in other tissues, appear to be intrinsically associated with the distemper disease process. Their exact relation to the activity and phases of development of the virus, however, has not as yet been established. The application of fluorescent antibody technique would undoubtedly shed light on their nature and significance.

SUMMARY

1. This study was entered upon with the purpose of presenting a comprehensive account of the pathological phenomena occurring in the respiratory system of dogs suffering from distemper. It was designed to supplement and enlarge upon the available but scanty information.

2. Morbid-anatomical, histopathological, and bacteriological observations form the main body of this work. Special attention has been focused on histological changes in the lung. The basis for the interpretation of some of the pulmonary lesions was attained by an additional, electron microscopic study on the structure of the normal alveolar lining.

3. A review of previous works relevant to this study is presented. It embraces the following themes: (a) morbid anatomy and histopathology of distemper, (b) inclusion bodies, (c) bacteriology of distemper pneumonia, (d) histopathology of some viral pneumonias in animals and man, and (e) pulmonary alveolar lining under normal and pathological conditions.

4. Thirty clinical and 20 experimental cases constitute the material on which this study is based. Twenty-eight dogs with naturally acquired distemper were selected over a period of one year from a number of dogs which were attended at the Small Animal Clinic of the College of Veterinary Medicine in Urbana, Illinois, U.S.A. The remaining two cases were from a larger group surveyed by the author in Glasgow, Scotland. Of the 20 experimental cases, 15 were employed for observations on the development of lung lesions, and five served as material for investigations on the pathogenesis of pulmonary oedema.

5. Considerable differences in the degree of the clinical and pathological signs were observed between the experimental and field cases of the disease. Only relatively mild abnormalities were shown by the experimental dogs.

6. The gross changes observed in the clinical cases varied in severity and extent with the duration of illness. Generally, they were most prominent between the third and fifth weeks of the disease. Beginning with the sixth or seventh week, there was a marked decrease in the intensity of lesions. The changes found in the lung comprised (a) congestion, usually focal in distribution, (b) oedema, and (c) occasional subpleural consolidation. Oedema of the pulmonary parenchyma was not observed in the experimental dogs.

7. Bacteriological findings in the lungs of the clinical cases were essentially similar to those recorded by other workers. The most common were Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, staphylococci, and streptococci. One culture yielded Brucella bronchiseptica. Of the twenty experimental dogs, only five showed the presence of bacteria in the pulmonary tissue.

8. Electron microscopic studies of the structure of the pulmonary alveolar wall were conducted on albino rats of Wistar strain. The results obtained indicate that the alveolar wall consists essentially of three elements: viz., (1) epithelium, (2) basement membrane, and (3) capillary endothelium. The epithelium lining the surface of the wall is formed by a tenuous but uninterrupted cellular membrane composed of the nucleus-bearing cell bodies and their attenuated protoplasmic extensions.

9. The implications of electron microscopic findings are of considerable morphological interest. The process of alveolar "epithelialisation", characteristic not only of distemper, but also of other viral pneumonias, becomes satisfactorily explained on the basis of hypertrophy and proliferation

of the existing epithelium. It seems very likely that the influence of the virus alone may precipitate such hyperplastic changes.

10. The inflammation of the lung was a constant finding in the clinical cases. One of the oustanding features of pneumonia was the interstitial cellular reaction, especially in the bronchiolar walls and the adjacent interalveolar septa. Other salient characteristics comprised (1) hyperplasia of the alveolar epithelium, (2) accumulation of a chiefly mononuclear exudate in alveolar spaces, and (3) oedema of the region of gaseous exchange and, often, also of the perivascular interstitium. Pulmonary changes evolved early in the disease and attained their height between the third and fifth weeks. After the eighth week of illness, only small aggregations of mononuclear cells remained in some of the peribronchiolar areas.

11. Intracerebral and intraspinal inoculation of distemper virus produced severe nervous disturbances in four out of five dogs but did not provoke pulmonary oedema. The pathogenesis of this process is still largely a matter of conjecture and awaits further, more fundamental studies.

12. A considerable proportion of the clinical cases displayed varying degrees of early organization of the exudate in the finer air passages and, on occasion, in the alveolar spaces. There appears to be some evidence suggesting that the use of antibiotics, which suppress the activity of secondary organisms, may be a contributing factor.

13. Many of the pulmonary macrophages exhibited a characteristic tinctorial reaction with P.A.S. which was thought to be indicative of some intracellular processes evidently concurrent with the phagocytic activity.

14. Cytoplasmic inclusion bodies were observed as early as on the first day of detectable illness (three days after inoculation). During the first three days, they were seen only in the bronchiolar epithelium but later

in the disease many appeared in a wide variety of cells. A small number of these inclusions persisted in the lung for 10-11 weeks from the onset of infection. The cytoplasmic inclusion bodies are regarded as pathognomonic for distemper.

15. The combined evidence gathered from the present study and from the preliminary observations on pneumonia in distemper conducted by the author in Glasgow, Scotland, points to the identity of the pneumonic process encountered in the United States and in Europe.

REFERENCES

Adams, J. M. (1941.) Primary virus pneumonitis with cytoplasmic inclusion bodies. J. Amer. med. Ass. 116. 925-933.

Addison, T. (1843.) Observations on pneumonia and its consequences. Guy's Hosp. Rep. I. (ser. 2.) 365. (Quoted by Miller, 1932.)

Allen, A. C., and Spitz, S. (1945.) A comparative study of the pathology of scrub typhus (tsutsugamushi disease) and other rickettsial diseases. Amer. J. Path. 21. 603-681.

Anderson, W. A. D. (1952.) "Synopsis of Pathology". Hyperplasia of the pulmonary alveolar lining. p. 386.

Andrewes, C. H., Laidlaw, P. P., and Smith, W. (1934.) The susceptibility of mice to the viruses and human and swine influenza. Lancet. 227. (2) 859-862.

_____, Glover, R. E. (1945.) Grey lung virus: an agent pathogenic for mice and other rodents. Brit. J. exp. Path. 26. 379-387.

Auerbach, S. H., Mims, O. M., and Goodpasture, E. W. (1952.) Pulmonary fibrosis secondary to pneumonia. Amer. J. Path. 28. 69-87.

Baar, H. S. (1955.) Interstitial plasma-cellular pneumonia due to Pneumocystis carinii. J. clin. Path. 8. 19-24.

Babes, V., and Starcovici, C. (1912.) Sur des corpuscules particuliers trouvés dans la maladie des jeunes chiens. C. R. Soc. Biol. (Paris) 73. 229-232.

Baker, J. A. (1943.) A filterable virus causing enteritis and pneumonia in calves. J. exp. Med. 78. 435-446.

_____. (1944.) A virus causing pneumonia in cats and producing elementary bodies. J. exp. Med. 79. 159-171.

Bargmann, W., and Knoop, A. (1956.) Vergleichende elektronenmikroskopische Untersuchungen der Lungenkapillaren. Z. Zellforsch. 44. 263-281.

Barnard, W. G., and Day, T. D. (1937.) The development of the terminal air passages of the human lung. J. Path. Bact. 45. 67-73.

Bartels (1861.) Bemerkungen über eine im Frühjahre 1860 in der Poliklinik in Kiel beobachtete Masern epidemie, mit besonderer Berücksichtingungen der dabei vorgekommenen Lungen-affectionen. Virchows Arch. path. Anat. 21. 65-84.

Bateman, J. K. (1949.) Recent experiences in post-inoculation behavior in the greyhound, following the use of Field Distemper method I, anti-vaccination. Vet. Rec. 61. 278-283.

Bedson, S. P., Western, G. I., and Levy Simpson, S. (1930.) Observations on the aetiology of psittacosis. Lancet. 218. (1). 235-236.

Bell, E. T. (1943.) Hyperplasia of the pulmonary alveolar epithelium in disease. Amer. J. Path. 19. 901-913.

Bensley, R. D., and Bensley, S. H. (1935.) Studies of the lining of the pulmonary alveolus of normal lungs of adult animals. Anat. Rec. 64. 41-50.

Bensley, S. H., and Groff, M. B. (1935.) Changes in the alveolar epithelium of the rat at birth. Anat. Rec. 64. 27-39.

Bertalanffy, F. D., and Leblond, C. P. (1953.) The continuous renewal of the two types of alveolar cells in the lung of the rat. Anat. Rec. 115. 515-541.

Bienfet, V. (1953.) Les pneumonies interstitielles à cellules géantes. Ann. Méd. vét. 97. 51-62.

Bindrich, H. (1950.) Beobachtungen bei Staupe-Virus-Passagen an Hunden. Exp. Vet.-Med. I. 69-81.

Blackman, S. S., Jr. (1936.) Intranuclear inclusion bodies in the kidney and liver caused by lead poisoning. Bull. Johns Hopk. Hosp. 58. 384-403.

Blaine, V. (1817.) "Canine Pathology". London. (Quoted by Lauder, I. M. et al., 1954).

Blake, F. G., Howard, M. E., and Tatlock, H. (1942.) Feline virus pneumonia and its possible relation to some cases of primary atypical pneumonia in man. Yale J. Biol. Med. 15. 139-166.

Bloom, W. (1936.) In "Pulmonic alveolar epithelium" by Macklin. J. thorac. Surg. 6. 82-88.

Breemen, V. L., and Neustein, H. B. (1956.) Electron microscopic studies of lung tissue from guinea pigs and premature infants. Anat. Rec. 124. 472-473.

Bremer, J. L. (1935.) Contribution to embryology. Carnegie Institution Publication. 25. 85-110.

Briscoe, J. C. (1908.) An experimental investigation of the phagocytic action of the alveolar cells of the lung. J. Path. Bact. 12. 66-100.

Broadhurst, J., MacLean, M. E., and Saurino, V. (1938.) Nasal inclusion bodies in dog distemper. Cornell Vet. 28. 9-15.

Burkhart, R. L., Poppensiek, G. C., and Zink, A. (1950.) A study of canine encephalitis, with special reference to clinical, bacteriological and post-mortem findings. Vet. Med. 45. 157-162.

Burnet, F. M., and Freeman, M. (1937.) Experimental study on virus of "Q" fever. Med. J. Aust. 7. 299-305.

Cabasso, V. J., Stebbins, M. R., and Cox, H. R. (1954.) Experimental canine distemper encephalitis and immunization of puppies against it. Cornell Vet. 44. 153-167.

Cameron, G. R., and De, S. N. (1949.) Experimental pulmonary oedema of nervous origin. J. Path. Bact. 61. 375-387.

_____, and Sheikh, A. H. (1951.) The experimental production of pulmonary oedema with ammonium salts together with a classification of lung oedemas. J. Path. Bact. 63. 609-617.

Campbell, G. S., and Visscher, M. B. (1949.) Pulmonary lesions in guinea pigs with increased intracranial pressure and effect of bilateral cervical vagotomy. Amer. J. Physiol. 157. 130-134.

Campbell, R. S. F., Martin, W. B., and Gordon, E. D. (1955.) Toxoplasmosis as a complication of canine distemper. Vet. Rec. 67. 708-712.

_____. (1956.) Canine toxoplasmosis. Vet. Rec. 68. 591-592.

Cappell, D. F. (1929.) Intravitam and supravital staining. III. The nature of the normal lining of the pulmonary alveoli and the origin of the alveolar phagocytes in the light of vital and supravital staining. J. Path. Bact. 32. 675-707.

Carleton, H. M. (1934.) Studies on epithelial phagocytosis. II. A method for demonstrating the origin of dust cells. Proc. Roy. Soc. London. Biol. 114. 513-522.

Carré, H. (1905.) Sur la maladie des jeunes chiens. C. R. Acad. Sci. (Paris). 140. 689-690.

Cheatham, W. J., Weller, T. H., Dolan, T. F., Jr., and Dower, J. C. (1956.) Varicella: Report of two fatal cases with necropsy, virus isolation, and serologic studies. Amer. J. Path. 32. 1015-1035.

Chessick, R. D. (1953.) Histochemical study of the distribution of esterases. J. Histochem. Cytochem. I. 471-485.

Chown, B. (1939.) Giant cell pneumonia in infancy as a manifestation of vitamin A deficiency. Amer. J. Dis. Child. 57. 489-505.

Chrzonszczewsky, N. (1863.) Ueber das Epithel der Lungenbläschen der Säugetiere. Würzburger med. Z. 4. 206. (Quoted by Miller, W. S., 1932.)

Clara, M. (1936.) Vergleichende Histobiologie des Nierenglomerulus und der Lungenalveole. Z. mikr.-anat. Forsch. 40. 147-280.

Clemens, H. J. (1954.) Elektronenoptische Untersuchungen über den Bau der Alveolenwand in der Rattenlunge. Z. Zellforsch. 40. 1-7.

_____. (1955.) Elektronenmikroskopische Beobachtungen an der Lungenalveole. Gegenbaurs morph. Jb. 94. No. 3-4. 471-492.

Clements, L. P. (1940.) On the origin and relations of the pulmonary macrophages. Anat. Rec. 78. 429-448.

Coffin, D. L., Coons, A. H., and Cabasso, V. J. (1953.) A histological study of infectious canine hepatitis by means of fluorescent antibody. J. exp. Med. 98. 13-20.

Cohrs, P. (1951.) Die Entmarkungs-Enzephalitis (hard pad disease) des Hundes. Dtsch. Tierärztl. Wschr. 68. 129-134.

Colberg, A. (1866.) Beiträge zur normalen und patologischen Anatomie der Lungen. Dtsch. Arch. klin. Med. 2. 453. (Quoted by Miller, W. S., 1932).

Cole, R., and MacCallum, W. G. (1918.) Pneumonia at a Base Hospital. Part II. Pathology. J. Amer. med. Ass. 70. 1153-1156.

_____. (1936.) Treatment of pneumonia. Ann. intern. Med. 10. 1-12.

Cooper, E. R. A. (1938.) A histological investigation of the development and structure of the human lung. J. Path. Bact. 47. 105-114.

Corbett, E. U. (1945.) The visceral lesions in measles with a report of Koplik spots in the colon. Amer. J. Path. 21. 905-919.

Cordy, D. R. (1949.) Interstitial pneumonia with giant-cells and inclusions. J. Amer. vet med. Ass. 114. 21-26.

Cowdry, E. V. (1925.) Studies on etiology of jaagsiekte. II. Origin of the epithelial proliferations. J. exp. Med. 42. 334-345.

_____, and Marsh, H. (1927.) Comparative pathology of South African jagziekte and Montana progressive pneumonia of sheep. J. exp. Med. 45. 571-586.

_____. (1934.) The problem of intranuclear inclusions in virus diseases. Arch. Path. 18. 527-542.

Crawshaw, H. A. (1949.) Discussion. So-called "hard pad" disease. Vet. Rec. 61. 364-365.

Delafield, F. (1884.) The pathology of broncho-pneumonia. Med. News. 45. 534-537.

DeMonbreun, W. A. (1937.) The histopathology of natural and experimental canine distemper. Amer. J. Path. 13. 187-212.

Dogliotti, G. C. (1931.) La struttura dell' alveolo polmonare dell' uomo nel periodo antecedente alla nascita. Boll. Soc. ital. Biol. sper. 6. 31-33.

Donohue, W. L. (1956.) Interstitial plasma cell pneumonia. Lab. Invest. 5. 97-105.

Dreschfeld, J. (1876.) Experimental researches on the pathology of vagus pneumonia. Lancet. (London). I. 47-49.

Dubin, I. N. (1945.) A pathological study of mice infected with the virus of swine influenza. Amer. J. Path. 21. 1121-1141.

Dungal, N. (1946.) Experiments with jaagsiekte. Amer. J. Path. 22. 737-759.

Dunkin, G. W., and Laidlaw, P. P. (1926a.) Studies in dog-distemper: I. Dog-distemper in the ferret. J. comp. Path. 39. 201-212.

_____. (1926b.) Studies in dog-distemper: II. Experimental distemper in the dog. J. comp. Path. 39. 213-221.

Durlacher, S. H., and Bunting, H. (1947.) Pulmonary changes following exposure to phosgene. Amer. J. Path. 23. 679-693.

Eaton, M. D., Meiklejohn, G., and Van Herick, W. (1944.) Studies on etiology of primary atypical pneumonia; filterable agent transmissible to cotton rats, hamsters, and chick embryos. J. exp. Med. 79. 649-668.

_____. (1950.) Virus pneumonia and pneumonitis viruses of man and animals, in "Handbuch der Virusforschung." Eds: Doerr, R., and Hallauer, C. II. Ergänzungsband. Springer-Verlag. Vienna.

Eberth, C. J. (1862.) Der Streit über das Epithel der Lungenbläschen. Arch. path. Anat. Physiol. 24. 503. (Quoted by Miller, W. S., 1932.)

_____. (1864.) Zu den Kontroversen über das Lungenepithel. Würzburger naturwiss. Z. 5. 84. (Quoted by Miller, W. S., 1932.)

Edwards, J. T. (1949.) Central Veterinary Society Discussion. Distemper and hard pad disease in dogs. Vet. Rec. 61. 175-178.

Ehrich, W., and McIntosh, J. F. (1932.) The pathogenesis of bronchiolitis obliterans. Arch. Path. 13. 69-79.

Elenz, E. (1864.) Ueber das Lungenepithel. Würzburger naturwiss. Z. 5. 66-97. (Quoted by Josselyn, L. E., 1935.)

Epstein, E. Z., and Greenspan, E. B. (1941.) Rheumatic pneumonia. Arch. intern. Med. 68. 1074-1094.

Fankhauser, R. (1951.) Encephalitis und Hard-pad-Symptom beim Hunde. Schweiz. Arch. Tierheilk. 93. 715-730.

Farber, S., and Wolbach, S. B. (1932.) Intranuclear and cytoplasmic inclusions ("protozoan-like bodies") in the salivary glands and other organs of infants. Amer. J. Path. 8. 123-135.

Ferry, N. S. (1911.) Etiology of canine distemper. J. infect. Dis. 8. 399-420.

_____. (1912.) Bacillus bronchisepticus (bronchicanis): The cause of distemper in dogs and a similar disease in other animals. Vet. J. 68. (new series 19). 376-391.

Findlay, G. M. (1938.) Inclusion bodies and their relationship to viruses. "Handbuch der Virusforschung", I. Eds.: Doerr, R., and Hallauer, C. Julius Springer. Wien. pp. 292-368.

Foot, N. C. (1920.) The endothelium in experimental pulmonary tuberculosis. J. exp. Med. 32. 533-546.

_____. (1927.) Studies on endothelial reactions. X. On the origin of the pulmonary "dust cell". Amer. J. Path. 3. 413-443.

Frauchiger, E., and Walthard, K. M. (1935.) Zur Histopathologie der nervösen Staupe. Arch. wiss. prakt. Thierheilk. 69. 231-249.

Fried, B. M. (1934.) The lungs and the macrophage system. Arch. Path. 17. 76-103.

_____. (1936.) In "Pulmonic alveolar epithelium" by Macklin. J. thorac. Surg. 6. 82-88.

Friedberger, F., and Fröhner, E. (1908.) "Veterinary Pathology". Translated by Hayes, M. H., 6th ed. Hurst and Blackett, Ltd., London.

Furumoto, H. H. (1952.) Hard pad disease. A review of clinical cases. N. Amer. Vet. 33. 550-552.

Gallagher, J. R. (1934.) Bronchopneumonia in adolescence. Yale J. Biol. Med. 7. 23-40.

Galli-Valerio. (1895.) Le microbe de la maladie des jeunes chiens. Zbl. Bakt., I. Abt. Ref. 19. 694-698.

Gara, P. F., de., and Furth, J. (1948.) Pneumonia produced by a meningo-pneumotropic virus. Arch. Path. 45. 474-493.

Gardner, L. U., and Smith, D. T. (1927.) The origin of the alveolar phagocyte studied in paraffin sections of tissue stained supravitally with neutral red. Amer. J. Path. 3. 445-460.

Gazayerli, M., El. (1936.) On the nature of the pulmonary alveolar lining and the origin of the alveolar phagocyte. J. Path. Bact. 43. 357-366.

Geever, E. F., Neuburger, K. T., and Davis, C. L. (1943.) The pulmonary alveolar lining under various pathologic conditions in man and animals. Amer. J. Path. 19. 913-937.

Gillespie, J. H., and Rickard, C. G. (1956.) Encephalitis in dogs produced by distemper virus. Amer. J. vet Res. 17. 103-108.

Goodpasture, E. W., Auerbach, S. H., Swanson, H. S., and Cotter, F. F. (1939.) Virus pneumonia of infants secondary to epidemic infections. Amer. J. Dis. Child. 57. 997-1011.

Goodwin, T. C. (1934.) Lipoid cell pneumonia. Amer. J. Dis. Child. 48. 309-326.

Goret, P. (1950.) Experimental studies upon the canine distemper virus: variations in virulence and their relation to the symptomatology and prophylaxis of the disease. Vet. Rec. 62. 67-71.

Gowar, F. J. S., and Gilmour, J. R. (1941.) Changes in the lung following injections of iodized oil into the trachea. Brit. J. exp. Path. 22. 262-273.

Grady, H. G., and Stewart, H. L. (1940.) Histogenesis of induced pulmonary tumors in strain A mice. Amer. J. Path. 16. 417-432.

Green, R. G., and Evans, C. A. (1939a.) A comparative study of distemper inclusions. Amer. J. Hyg. 29. Sec. B. 73-87.

_____. (1939b.) Rapid diagnosis of canine distemper. Cornell Vet. 29. 35-40.

Greig, A. S. (1954.) The significance of a pleuropneumonia like organism. Canad. J. comp. Med. 18. 275-278.

Groulade, P., Roger, F., and Dartois, N. (1954.) Contribution a l'étude d'on syndrome infectieux du chien répondant serologiquement à une souche de Rickettsia psittaci. Rev. Path. gen. 54. 1426-1434.

Hadfield, G. (1938.) The rheumatic lung. Lancet. 235. (2). 710-712.

Haig, D. A. (1956.) Canine distemper-immunization with avianized virus. Onderstepoort. J. vet.Res. 27. 19-53.

Ham, A. W., and Baldwin, K. W. (1941.) A histological study of the development of the lung with particular reference to the nature of alveoli. Anat. Rec. 81. 363-379.

Hardenbergh, J. G. (1923.) Practical observations on certain disease of dog and other laboratory animals. J. Amer. vet.med. Ass. 64. 193-203.

_____. (1925.) The significance of Bacillus bronchisepticus in cases of canine distemper. J. Amer. vet. med. Ass. 68. 309-320.

Harris, L. H. (1954.) Pulmonary manifestations of "rheumatoid disease". Lancet. 266. 119-120.

Hayek, von, H. (1950.) Physiologische Formanderung der Alveolarepithelzellen. Anat. Nachr. I. 82-83.

Hecht, V. (1910.) Die Riesenzellen pneumonie im Kindesalter. Beitr. path. Anat. 48. 263-310.

Hesse, F. E., and Loosli, C. G. (1949.) The lining of the alveoli in mice, rats, dogs, and frogs following acute pulmonary oedema produced by A.N.T.U. poisoning. Anat. Rec. 105. 299-323.

Heuer, G. J. (1906.) Observations on distemper. Bull. Johns Hopk. Hosp. 17. 385-393.

Hjärre, A., Dinter, Z., and Bakos, K. (1952.) Recherches comparées sur les grippes porcines suédoise et americaine. Nord. VetMed. 4. 1025-1045.

_____. (1953.) Vegetierende Dermatosen mit Riesenzellenpneumonien bei Schweinen. Dtsch. Tierärztl. Wschr. 60. 105-110.

Horning, E. S. (1950.) Studies on the introduction of lung cancer in mice. Brit. J. Cancer. 4. 235-244.

Hoyle, L., and Orr, J. W. (1945.) The histogenesis of experimental pneumonia in mice. J. Path. Bact. 57. 441-449.

Hurst, W., Cooke, B. T., and Melvin, P. (1943.) "Nervous distemper" in dogs. A pathological and experimental study, with some reference to demyelinating diseases in general. Aust. J. exp. Biol. med. Sci. 21. 115-126.

Innes, J. R. M. (1949a.) Inclusion and crystalline bodies in distemper, fox encephalitis, contagious canine hepatitis and in normal dogs. Brit. Vet. J. 105. 99-102.

_____. (1949b.) The relation of distemper infection to the aetiology of canine encephalopathies. Vet. Rec. 61. 73-77.

Jarrett, W. F. H. (1954.) Atypical pneumonia in calves. J. Path. Bact. 67. 441-454.

Jenner, E. (1809.) Med. chir. Trans. I. 263. (Quoted by Lauder et al., 1954.)

Jennings, A. R., and Glover, R. E. (1952.) Enzootic pneumonia in calves. II. The experimental disease. J. comp. Path. 62. 6-22.

Jones, T. C., and Maurer, F. D. (1943.) The pathology of equine influenza. Amer. J. vet.Res. 4. 15-31.

Josselyn, L. E. (1935.) The nature of the pulmonary alveolar lining. Anat. Rec. 62. 147-177.

Karrer, H. E. (1956a.) An electron microscopic study of the fine structure of pulmonary capillaries and alveoli of the mouse. Bull. Johns Hopk. Hosp. 98. (No. 2). 65-83.

_____. (1956b.) The ultra-structure of mouse lung. J. biophys. biochem. Cytol. 2. 241-252.

Karsner, H. T., and Meyers, A. E. (1913.) Giant-cell pneumonia. Arch. intern. Med. 11. 534-541.

King, L. S. (1954.) Atypical proliferation of bronchiolar epithelium. Arch. Path. 58. 59-70.

Kisch, B. (1955.) Electron microscopic investigation of the lungs. Exp. Med. Surg. 13. 101-117.

Kölliker, A. (1881.) Zur Kenntnis des Baues der Lunge des Menschen. Verh. phys.-med. Ges. Würzburg. 16. 1-24.

Koprowski, H., Jervis, G. A., James, T. R., Burkhart, R. L., and Poppensiek, G. C. (1950.) A study of canine encephalitis. Amer. J. Hyg. 51. 63-75.

Kriesel, H. R. (1938.) A comparative study of the manifestations and histopathology of canine distemper and experimental fox encephalitis infection in dogs. Cornell Vet. 28. 324-330.

Kuzma, J. F., and Lustok, M. J. (1951.) Rheumatic fever pneumonitis (twenty six necropsy cases). Amer. J. Path. 27. 696-697.

Laidlaw, P. P., and Dunkin, G. W. (1926.) Studies in dog-distemper: III. The nature of the virus. J. comp. Path. 39. 222-230.

Lamont, J. K., and Kerr, W. R. (1939.) Infectious pneumonia of calves or calf influenzal pneumonia. Vet. Rec. 51. 672-674.

Lang, F. J. (1926.) Über Gewebskulturen der Lunge. Ein Beitrag zur Histologie des respiratorischen Epithels und zur Histogenese der Alveolarphagozyten. Arch. exp. Zellforsch. 2. 93-122.

Lange, F. (1909.) Untersuchungen über das Epithel der Lungenalveolen. Frankfurt. Z. Path. 3. 170-179.

Langham, R. F., Thorp, F., Jr., Ingle, R. T., and Sholl, L. B. (1942.) Some observations on the pathology of pneumonia in the food-producing animals. Amer. J. vet.Res. 3. 139-145.

Laosson. (1882.) Ueber Geschichte und Contagiosität der Staupe. Thesis. Dorpat. (Quoted by Mathis, M., 1887.)

Lauder, I. M., Martin, W. B., Gordon, E. B., Lawson, D. D., Campbell, R. S. F., and Watrach, A. M. (1954.) A survey of canine distemper. Vet. Rec. 66. 607-611; 623-631.

Lee, J. (1933.) Nuclear changes following intravenous injection of various solutions. Proc. Soc. exp. Biol. (N.Y.). 31. 383-385.

Lendrum, A. C. (1941.) Fixation of post-mortem material. J. Path. Bact. 52. 132-137.

_____. (1947.) The phloxin-tartrazine method as a general histological stain and for the demonstration of inclusion bodies. J. Path. Bact. 59. 399-404.

Lentz, O. (1907.) Ein Beitrag zur Färbung der Negrischen Körperchen. Zbl. Bakt. I. Abt. Orig. 44. 374-378.

_____. (1909.) Über spezifische Veränderungen an den ganglienzellen wut-staupekranker Tiere. Z. Hyg. 62. 63-94.

Lewis, Margaret R. (1925.) Origin of the phagocytic cells of the lung of the frog. Bull. Johns Hopk. Hosp. 36. 361-375.

Lignières, J. (1906.) Sur la maladie des chiens et le virus filtrant de Carré. Bull. Soc. cent. Méd. vét. 60. 622-630.

Lindgren, N. O. (1951.) Studier över cytoplasmatiska inklusioner vid valpsjuka nos hund. (Cytoplasmic inclusions in dog distemper.) VetMed. 3. 403-424. (From English summary.)

Loosli, C. G. (1935.) The rabbits' lung after phrenicotomy and pneumothorax. Anat. Rec. 62. 381-407.

_____. (1949.) The pathogenesis and pathology of experimental air-borne influenza virus A infections in mice. J. infect. Dis. 84. 153-168.

Low, F. N. (1952.) Electron microscopy of the rat lung. Anat. Rec. 113. 437-450.

_____. (1953.) The pulmonary alveolar epithelium of laboratory mammals and men. Anat. Rec. 117. 241-264.

_____. (1954.) The electron microscopy of sectioned lung tissue after varied duration of fixation in buffered osmium tetroxide. Anat. Rec. 120. 827-851.

MacCallum, W. G. (1919.) The pathology of the pneumonia in the United States Army camps during the winter of 1917-18. Monogr. Rockefeller Inst. med. Res. 10.

MacIntyre, A. B., Trevan, D. J., and Montgomerie, R. F. (1948.) Observations on canine encephalitis. Vet. Rec. 60. 635-639.

Macklin, C. C. (1936.) A round table conference. J. thorac. Surg. 6. 82-88.

_____. (1951.) The dust cells in the lungs of the albino mouse. Lancet. 260. 432-435.

_____. (1954.) The pulmonary alveolar mucoid film and the pneumonocytes. Lancet. 266. 1099-1104.

Mansi, W. (1948.) Discussion. Observations on canine encephalitis. Vet. Rec. 60. 642-643.

_____. (1951.) The isolation of a neurotropic virus from a dog suffering from the so-called nervous distemper. Brit. Vet. J. 107. 214-229.

Marinesco, G., Draganesco, S., and Stroesco, G. (1933.) Reserches histo-pathologiques sur la maladie des jeunes chiens (maladie de Carré). Ann. Inst. Pasteur. 51. 215-236.

Marshall, A. H. E. (1946.) Observations on the pulmonary macrophage system. J. Path. Bact. 58. 729-738.

Mathis, M. (1887.) De la nature microbienne de la maladie des jeunes chiens. Rec. Méd. Vét. 4. 229-241.

Maximow, A. A. (1927.) Morphology of the mesenchymal reactions. Arch. Path. 4. 557-606.

McCordock, H. A. (1932.) Intranuclear inclusions in pertussis. Proc. Soc. exp. Biol. (N.Y.). 29. 1288-1291.

_____, and Muckenfuss, R. S. (1933.) The similarity of virus pneumonia in animals to epidemic influenza and interstitial bronchopneumonia in man. Amer. J. Path. 9. 221-251.

McGovern, V. J., Steel, J. D., Wyke, B. D., and Dodson, M. E. (1950.) Canine encephalitis causing a syndrome characterized by tremor. Aust. J. exp. Biol. med. Sci. 28. 433-447.

McIntyre, I. (1948.) Discussion. Observations on canine encephalitis. Vet. Rec. 60. 635-648.

McManus, J. F. H. (1948.) Periodic acid routine applied to kidney. Amer. J. Path. 24. 643-648.

McMillan, G. C. (1947.) Fatal inclusion-disease pneumonitis in an adult. Amer. J. Path. 23. 995-1003.

Metchnikoff, E. (1901.) L'immunité dans les maladies infectieuses. Masson & Cie. Paris. (Quoted by Fried, B. M., 1934.)

M'Gowan, J. P. (1911.) Some observations on a laboratory epidemic, principally among dogs and cats, in which the animals affected presented the symptoms of the disease called "distemper". J. Path. Bact. 15. 372-425.

_____. (1912.) Some observations on the clinical symptoms, prophylaxis and treatment of distemper. Vet. J. 68. (new series 19). 7-17.

Millais, E. (1890.) The pathogenic microbe of distemper in dogs and its use for protective inoculation. Vet J. Ann. comp. Path. 30. 313-321.

Miller, W. S. (1932.) The epithelium of the lower respiratory tract. "Special Cytology". ed. by Cowdry, E. V. 2nd ed., Paul B. Hoeber, inc., N. Y. pp. 133-150.

Moulton, J. E., and Brown, C. H. (1954.) Antigenicity of canine distemper inclusion bodies as demonstrated by fluorescent antibody technique. Proc. Soc. exp. Biol. (N. Y.). 86. 99-102.

Mulder, J., and Verdonk, G. J. (1949.) Studies on the pathogenesis of a case of influenza--A pneumonia of three days' duration. J. Path. Bact. 61. 55-61.

Munk, P. (1862.) Ueber das Epithel der Lungenalveolen. Virch. Arch. 24. 603-606.

Nairn, R. C. (1951.) Oedema and capillary anoxia. J. Path. Bact. 63. 213-234.

Nicolau, S. (1935.) Etude sur les inclusions qui caractérisent la maladie de Carré. C. R. Soc. Biol. (Paris). 119. 269-272.

Nijland, G. J. (1952.) "Hard pad disease". T. Diergeneesk. 77. 501-506.

Niven, Janette, S. F. (1950.) The histology of "grey lung virus" lesion in mice and cotton-rats. Brit. J. exp. Path. 31. 759-766.

Ogawa, C. (1920.) Contributions to the histology of the respiratory spaces of the vertebrate lungs. Amer. J. Anat. 27. 333-393.

Olafson, P., and Monlux, W. S. (1942.) Toxoplasma infection in animals. Cornell Vet. 32. 176-190.

Olitsky, P. K., and Harford, C. G. (1937.) Intranuclear inclusion bodies in the tissue reactions produced by injections of certain foreign substances. Amer. J. Path. 13. 729-747.

Oppel, A. (1905.) "Lehrbuch der Vergleichenden Mikroskopischen Anatomie." Atmungsapparat (Respiratory system). Pt. 6. Jena. (Quoted by Miller, 1932.)

Orsós, F. (1933.) Das Epithel der Lungenalveolen. Zbl. allg. Path. path. Anat. 57. 81-88.

Palade, G. E. (1952.) A study of fixation for electron microscopy. J. exp. Med. 95. 285-298.

_____, and Porter, K. E. (1954.) Studies on the endoplasmic reticulum. I. Its identification in cells in situ. J. exp. Med. 100. 641-656.

Palmer, D. M. (1936.) In "Pulmonic alveolar epithelium" by Macklin. J. thorac. Surg. 6. 82-88.

Parker, F., Jr., and Weiss, S. (1936.) The nature and significance of the structural changes in the lungs in mitral stenosis. Amer. J. Path. 12. 573-598.

_____, Jolliffe, L. S., Barnes, M. W., and Finland, M. (1946.) Pathologic findings in the lungs of five cases from which influenza virus was isolated. Amer. J. Path. 22. 797-819.

_____, and Finland, M. (1947.) Primary atypical pneumonia. Arch. Path. 44. 581-608.

Pattison, I. H. (1956.) A histological study of a transmissible pneumonia of pigs characterized by extensive lymphoid hyperplasia. Vet. Rec. 68. 490-494.

Perdrau, J. R., and Pugh, L. P. (1930.) The pathology of disseminated encephalomyelitis of the dog (the "nervous form of canine distemper"). J. Path. Bact. 33. 79-91.

Permar, H. H. (1920.) An experimental study of the mononuclear phagocytes of the lung. J. med. Res. 42. 9-32.

Pinkerton, H. (1928.) Reaction to oils and fats in lung. Arch. Path. 5. 380-401.

_____, and Henderson, R. G. (1941.) Adult toxoplasmosis. A previously unrecognized disease entity simulating the typhus-spotted fever group. J. Amer. med. Ass. 116. 807-814.

_____, Smiley, W. L., and Anderson, W. A. D. (1945.) Giant cell pneumonia with inclusions. A lesion common to Hecht's disease, distemper and measles. Amer. J. Path. 21. 1-23.

_____. (1953.) Rickettsial and viral diseases. "Pathology". 2nd ed. Ed. by Anderson, W. A. D., C. V. Mosby Company, St. Louis.

Policard, A. (1926.) Sur la nature du revêtement des alvéoles pulmonaires des mammifères. Bull. Hist. appl. 3. 236. (Quoted by Policard, 1955.)

_____. (1929.) Les nouvelles idées sur la disposition de la surface respiratoire pulmonaire. Presse med. 37. 1293-1295.

Policard, A., Collet, A., and Giltaire-Ralyte, L. (1954a.) Étude au microscope électronique de la cellule alvéolaire de mammifère. C. R. Acad. Sci. (Paris) 239. No. 8. 573-575.

_____. (1954b.) L'alvéole pulmonaire au microscope électronique. La Press. Med. 62. No. 86. 1775-1777.

_____. (1954c.) Étude au microscope électronique des capillaires pulmonaires chez les mammifères. C. R. Acad. Sci. (Paris) 239. No. 12. 687-689.

_____. (1955.) "Le Poumon. Structures et Mécanismes a l'État Normal et patholgique". 2nd ed. Masson et Cie, Paris.

_____, and Collet, A. (1956.) Apports de la microscopie électronique à la connaissance histo-physiologique de la paroi alvéolaire. J. Physiologie. 48. 687-690.

_____, and Prégermain, S. (1956.) Sur quelques dispositions inframicroscopiques de l'endothélium des capillaires pulmonaires chez les mammifères. C. R. Acad. Sci. 243. 8-11.

Potel, K. (1951.) Histopathologie der Hundestaupe mit besonderer Berücksichtigung der nervösen Form. Exp. Vet.-Med. 4. 44-97.

_____. (1954.) Der gegenwärtige Stand der Staupefarschung am Friedrich-Löffler-Institut auf der Insel Riems bei Greifswald. Schweiz. Arch. Tierheilk. 96. 260-271.

Pugh, L. P. (1949.) So-called "hard pad" disease. Vet. Rec. 61. 363-364.

Rainey, G. (1855.) Critical examination of the evidence for and against the presence of epithelium in the air cells of the human lung. Brit. med. chirurg. Rev. 16. 491. (Quoted by Miller, 1932.)

Rebuck, J. W., and Crowley, J. H. (1955.) A method of studying leukocytic functions in vivo. Ann. N. Y. Acad. Sci. 59. art. 5. 757-805.

Reye, D. K., and Ten Seldam, R. E. J. (1956.) Pneumocystis pneumonia. J. Path. Bact. 72. 451-458.

Rhea, L. J. (1915.) The comparative pathology of the tracheal and bronchial lesions produced in man by B. pertussis (whooping cough) and those produced in dogs by B. bronchisepticus (canine distemper). J. med. Res. 32. 471-474.

Rivers, T. M., and Berry, G. P. (1931.) Psittacosis. IV. Experimentally induced infections in monkeys. J. exp. Med. 54. 129-143.

Rooyen van, C. E. (1938.) The micromanipulation and microdissection of the molluscum contagiosum inclusion body. J. Path. Bact. 46. 425-436.

Rosin, A. (1947.) Studies on lung tissue in vitro with special reference to the nature of the lining cells of the alveolus. Anat. Rec. 57. 447-469.

Ross, I. S. (1939.) Pulmonary epithelium and proliferative reaction in lungs; study of cellular response in lung after intratracheal injection of toxic and nontoxic foreign substance. Arch. Path. 27. 478-496.

Salisbury, R. M. (1953.) "Q" fever and its human and animal associations. N. Z. med. J. 52. 260-264. (Abstr.: Vet. Rec. 66. 389.)

Sanfelice, F. (1915.) Ueber die bei der Staupe vorkommenden Einschlusskörperchen. Zbl. Bakt., I. Abt. Orig. 76. 495-511.

Sarnoff, S. J. (1952.) Neurohemodynamics of pulmonary edema. Amer. J. Physiol. 170. 588-600.

Scheitlin, M., Seiferle, E., and Stünzi, H. (1951.) Klinische und pathologisch-anatomische Beobachtungen über die sog. "Hard Pad Disease" beim Hund. Schweiz. Arch. Tierheilk. 93. 91-129.

Schleicher, E. M. (1943.) Mallory-Heidenhain's connective tissue stain employing domestic azocarmine B,analine blue and orange G in balanced concentrations. Amer. J. clin. Path. 7. 35-39.

_____. (1953.) Personal communication.

Schlingman, A. S. (1932.) Studies on canine distemper: I. The bacteriology of one hundred naturally infected cases. J. Amer. vet. med. Ass. 80. 729-742.

Schlipköter, H. W. (1954.) Elektronenoptische Untersuchungen ultradünner Lungenschnitte. Dtsch. med. Wschr. 79. No. 45. 1658-1659.

Schmidt, W. (1936.) Vergleichende Untersuchungen zur Pathologie pneumotroper Virusarten. Arch. wiss. prakt. Tierheilk. 70. 1-22.

Schofield, F. W. (1956.) Virus pneumonia-like (VPP) lesions in the lungs of Canadian swine. Canad. J. comp. Med. 20. 252-266.

Schoichi, K. (1923.) Bacteriological studies on distemper. J. Soc. vet. de Tokio. (Abstr. in Vet. J. 80. 327.)

Seemann, G. (1931.) Histobiologie der Lungenalveole. G. Fischer. Jena.

Semmer. (1875.) Die Staupe der Hunde. Dtsch. Z. Tiermed. I. 204. (Quoted by Mathis, 1887.)

Semsroth, K. H. (1939.) Multinucleate epithelial giant cells with inclusion bodies in prodromal measles. Arch. Path. 28. 386-389.

Sewell, W. T. (1918-19.) The phagocytic properties of the alveolar cells of the lung. J. Path. Bact. 22. 40-55.

Shimai, K., and Tamaru, K. (1951.) A contribution to the histological study of the alveoli of the human lung. Okayama Folia Anat. Jap. 23. No. 6. 351-355.

Shoetensack, M. (1934.) Pure cultivation of the filterable virus isolated from canine distemper. Kitasato Arch. exp. Med. 11. 277-290.

Shope, R. E. (1931) Swine influenza. I. Experimental transmission and pathology. J. exp. Med. 54. 349-358.

Sigurdsson, B., Pálsson, P. A., and Tryggvadóttir, A. (1953.) Transmission experiments with maedi. J. infect. Dis. 93. 166-175.

Simon, M. A. (1947.) So-called pulmonary adenomatosis and "alveolar cell tumors". Amer. J. Path. 23. 413-427.

Sinigaglia, G. (1912.) Osservazioni sul cimurro. Clin. Vet. 35. 421-446.

_____. (1913.) Ulteriori osservazioni sul cimurro. Pathologica 5. 107. (Quoted by Lauder et al., 1954.)

Sjolte, I. P. (1947.) Om cytoplasmatiske og nucleare inclusioners forekomst ved spontant forkommende hundesyge. (Occurrence of cytoplasmic and nuclear inclusion bodies in dogs with distemper.) Skand. VetTidskr. 37. 350-364. (From English summary.)

Sjöstrand, F. S., and Rhodin, J. (1953.) The ultrastructure of the proximal convoluted tubules of the mouse kidney as revealed by high resolution electron microscopy. Exp. Cell Res. 4. 426-456.

Slavjansky, K. (1869.) Experimentelle Beiträge zur Pneumokoniosis-Lehre. Virchows Arch. 48. 326-332.

Spencer, H., and Raeburn, C. (1954.) Atypical proliferation of bronchiolar epithelium. J. Path. Bact. 67. 187-193.

Standfuss, R. (1908.) Ueber die ätiologische und diagnostische Bedeutung der Negrischen Tollwutkörperchen. Arch. wiss. prakt. Tierheilk. 34. 109-132.

Stewart, F. W. (1923.) A histogenetic study of the respiratory epithelium. Anat. Rec. 25. 181-199.

Stewart, H. L. (1953.) In "Physiopathology of Cancer". (F. Homburger and W. Fishman, eds.) pp. 93-112. Paul B. Hoeber, Inc., N. Y.

Straub, M. (1937.) The microscopical changes in the lungs of mice infested with influenza virus. J. Path. Bact. 45. 75-78.

_____. (1940.) The histology of catarrhal influenzal bronchitis and collapse of the lung in mice infected with influenza virus. J. Path. Bact. 50. 31-36.

Straub, M., and Mulder, J. (1948.) Epithelial lesions in the respiratory tract in human influenzal pneumonia. J. Path. Bact. 60. 429-434.

Stuart-Harris, C. H. (1953.) "Influenza and other virus infections of the respiratory tract". Edward Arnold and Co., London.

Swigart, R. H., and Kane, D. J. (1954.) Electron microscopic observations of pulmonary alveoli. Anat. Rec. 118. 57-71.

Taliaferro, W. H. (1949.) The cellular basis of immunity. Ann. Rev. Microbiol. 3. 159-194.

Tchistovitch. (1889.) Des phenomènes de phagocytose dans les poumons. Ann. Inst. Pasteur (Paris) 3. 337-361.

Teilum, G. (1956.) Periodic acid-Schiff positive reticulo-endothelial cells producing glycoprotein. Functional significance during formation of amyloid. Amer. J. Path. 32. 945-959.

Tennekoon, G. E. (1954.) Pulmonary oedema due to thiosemicarbazide. J. Path. Bact. 67. 341-347.

Teunissen, G. H. B. (1952.) "Hard pad disease". T. Diergeneesk. 77. 507-510.

Thorp, W. T. S., Shigley, J. F., and Farrell, M. A. (1942.) Studies on the etiology and pathology of calf pneumonia. Amer. J. vet.Res. 3. 342-349.

Tompkins, E. H. (1955.) The monocyte. Ann. N. Y. Acad. Sci. 59. art. 5. 732-745.

Tonelli, L. (1948.) Clinical aspects, anatomical picture and histogenetic problem of alveolar carcinoma of the lung. Arch. Chir. Torace. 3. 261-302. (Abstr. in Excerpta Med. 3. 116. 1950.)

Torrey, J. C., and Rahe, A. H. (1913.) Studies in canine distemper. J. med. Res. 27. 291-364.

Ungar, J., and Wilson, G. R. (1935.) Monocytes as a source of alveolar phagocytes. Amer. J. Path. 11. 681-692.

Van Den Akker, S. (1952.) "Hard pad disease". T. Diergeneesk. 77. 511-514.

Villemin, J. (1866.) Recherches sur la vésicule pulmonaire et l'emphyseme. Arch. gén. Méd. 8. 385. (Quoted by Josselyn, L. E., 1935.)

Visscher, M. B., Haddy, F. J., and Stephens, G. (1956.) The physiology and pharmacology of lung edema. Pharmacol. Rev. 8. 389-434.

Wachstein, M. (1955.) Histochemistry of leukocytes. Ann. N. Y. Acad. Sci. 59. art. 5. 1052-1065.

Waddell, W. R. (1949.) Organoid differentiation of the foetal lung; histological study of differentiation of mammalian fetal lung in utero and in transplants. Arch. Path. 47. 227-247.

Warren, S., and Gates, O. (1940.) Radiation pneumonitis. Experimental and pathologic observations. Arch. Path. 30. 440-460.

Warthin, A. S. (1931.) Occurrence of numerous large giant cells in the tonsils and pharyngeal mucosa in the prodromal stage of measles. Arch. Path. 11. 864-874.

Watrach, A. M. (1956.) Unpublished data.

Watrach, Marian A., and Watrach, A. M. (1955.) Unpublished data.

Watson, E. A., and Plummer, P. J. G. (1942.) Distemper inclusion bodies. Amer. J. vet.Res. 3. 350-357.

Watts, C. F., and McDonald, J. R. (1948.) Pulmonary alveolar lining in bronchiectasis. Arch. Path. 45. 742-751.

Weipers, W. L. (1948.) Discussion. Observations on canine encephalitis. Vet. Rec. 60. 643-644.

Weiss, L. P., and Fawcett, D. W. (1953.) Cytochemical observations on chicken monocytes, macrophages and giant cells in tissue culture. J. Histochem. Cytochem. I. 47-65.

Wells, H. G., Slye, M., and Holmes, H. F. (1941.) Occurrence and pathology of spontaneous carcinoma of lung in mice. Cancer Res. I. 259-261.

Whitfield, A. G. W., Lannigan, R., and Bond, W. H. (1954.) Fatal post-radiation pneumonitis. Lancet. 266. 117-119.

Whittick, J. W. (1950.) Necropsy findings in a case of Q fever in Britain. Brit. med. J. I. 979-980.

Willigan, D. A., Watrach, A. M., and Morrill, C. C. (1954.) Unpublished data.

Wilson, G. H. (1930.) The pathological changes found in a fatal case of psittacosis. J. Path. Bact. 33. 957-961.

Winternitz, M. C., Wason, I. M., and McNamara, F. P. (1920.) "Pathology of Influenza". Yale Univ. Press. New Haven, Conn.

Woodruff, C. E., and Goodpasture, E. W. (1929.) The infectivity of isolated inclusion bodies of fowl-pox. Amer. J. Path. 5. 1-9.

Youatt, W. (1830.) The Veterinarian. 3. 75-88.

_____. (1845.) "The Dog". London.

Young, J. S. (1930.) Epithelial proliferation in the lung of the rabbit, brought about by intrapleural injection of solutions of electrolytes--a physico-chemical interpretation of the phenomenon. J. Path. Bact. 33. 363-381.

Zenker, K. (1861.) Beiträge zur Anatomie und Physiologie der Lunge. Dresden. (Quoted by Munk, 1862.)