STUDIES IN PNEUMONIA OF ANIMALS

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CONTENTS

SUMMARY

Four groups of sheep were selected and examined post mortem for evidence of pneumonia. The conditions observed were atypical pneumonia, acute necrotising pneumonia (so-called "enzootic pneumonia"), pulmonary adenomatosis, pulmonary abscesses, pulmonary aspergillosis, aspiration pneumonia, pneumonia of haematogenous origin and pneumonia characterised by exudative and proliferative lesions. The latter has been described previously in sheep introduced into pens at the Moredun Institute (Stamp and Nisbet, 1963; Gilmour and Brotherston, 1963) but the histopathological lesions described were of a non-specific nature.

Two outbreaks of respiratory disease in recently introduced sheep were investigated. In the first outbreak, acidophilic cytoplasmic inclusions in bronchial and bronchiolar epithelial cells and in alveolar lining cells were observed, providing affected sheep were killed early in the course of the disease. Other lesions such as syncytium formation, pseudo-epithelialisation of alveoli and hyperplasia of the bronchiolar epithelium were also present. Similar acidophilic cytoplasmic inclusions and proliferative lesions were seen in young lambs inoculated via the respiratory tract with an ovine strain of parainfluenza 3 (PI3) virus. The inclusions, which were shown to represent aggregates of virus using immunofluorescence and electron microscopy, are of transient duration. The fact that these inclusions persist for only eight days after inoculation indicates the necessity of obtaining material within that time if PI3 virus is to be implicated on histopathological grounds in

natural outbreaks of respiratory disease. Qualitatively similar lesions were observed in lambs inoculated with a bovine strain of PI3 virus and in calves inoculated with an ovine strain of PI3 virus.

If slaughter of affected sheep, in natural outbreaks of respiratory disease, was delayed, acidophilic cytoplasmic inclusions were absent and the histological lesions resembled those described for atypical pneumonia (Stamp and Nisbet, 1963).

Inoculation of lambs with Bedsonia organisms resulted in a marked clinical response and macroscopic lesions of pneumonia. Bedsonia elementary bodies were numerous in cryostat sections of lung from lambs killed three and five days after inoculation but were difficult to detect in the lungs of lambs killed on days nine and twelve. The histological lesions in the lungs of lambs killed three and five days after inoculation were characterised by foci of necrosis and a sero-cellular exudate in alveoli and bronchioles, whereas, in lambs killed nine and twelve days after inoculation the principal lung lesions were hyperplasia of the bronchiolar epithelium, pseudo-epithelialisation and epithelialisation of alveoli and infiltration of the interalveolar septa by macrophages and lymphocytes.

In the later stages of infection in lambs inoculated with either PI3 virus or Bedsonia organisms, histological lesions which resembled those of atypical pneumonia were observed. It is considered therefore, that atypical pneumonia of sheep (Stamp and Nisbet, 1963) is not a specific entity.

In the second outbreak of respiratory disease in sheep at the Moredun Institute a pneumonia morphologically distinct from those hitherto described was studied. The salient histological features were hyperplasia of the

bronchiolar epithelium, marked alveolar epithelialisation, macrophage infiltration of alveoli and hyperplasia of the reticulin network around affected bronchioles and alveoli. The possible relationship of this condition to other epithelialising pneumonias is discussed.

In a histological study of respiratory disease in intensively-reared calves, two types of pneumonia were encountered. One was characterised by bronchitis, bronchiolitis, a cellular exudate in alveoli, syncytium formation, alveolar epithelialisation and acidophilic cytoplasmic inclusions. It was concluded that the lesions were due to concomitant PI3 virus and bacterial infections. The histological lesions of the second type consisted of peribronchial and peribronchiolar lymphocytic hyperplasia, atelectasis and mild interstitial pneumonia. A comparison of the amount of intrapulmonary lymphoid tissue in apparently normal calves and in calves which had experienced a respiratory infection showed that in either group, the degree of peribronchial or peribronchiolar lymphocytic hyperplasia varied.

GENERAL INTRODUCTION

Cattle, sheep and pigs are the species of major economic significance in Britain. Although respiratory disease of pigs is a problem studies were confined to cattle and sheep because of their availability and an interest in the comparative pathology of respiratory conditions in these species.

An indication of the importance of respiratory disease in sheep and cattle may be obtained from the results of a survey carried out over a two year period at Veterinary Investigation Centres in England and Wales which showed that 8.2 per cent. of 9,950 sheep (Ministry of Agriculture, Fisheries and Food, 1964a) and 9.5 per cent. of 3,667 calves under six months of age (Ministry of Agriculture, Fisheries and Food, 1964b) submitted for post-mortem examination died of pneumonia. The economic losses from fatal pulmonary infections are therefore substantial but of even greater importance are those due to unthriftiness or the cost of treatment or preventive measures in non-fatal cases. Also, with some of these diseases (e.g. ovine pulmonary adenomatosis) the threat to the export market must also be kept in mind (Stevens, 1957). Since 1961 there has been a trend towards more intensified methods of rearing cattle with a resulting increase in the incidence of pneumonia. Similarly where sheep have been intensively-reared there has been an increase particularly in sheep that have been badly housed (Farm Building Centre, 1967).

Recently there are indications that pulmonary adenomatosis of sheep is a transmissible neoplasm (Markson and Terlecki, 1964; Mackay and Nisbet, 1966). Nevertheless, it has been included in the scope of this

thesis because it has been previously described as a pneumonia (Stamp and Nisbet, 1963) and a viral aetiology has been postulated.

Viruses have often been postulated as being causes of both acute and chronic respiratory disease in sheep but the only condition from which a virus has been isolated, grown in tissue culture and shown to be capable of causing the disease experimentally is maedi. When this investigation was begun respiratory viruses had not been isolated from sheep in Britain but there was serological evidence to suggest that infections with parainfluenza 3 (PI3) virus and adenovirus occurred (Hore, 1965). Similar viruses have been isolated from cattle and experimentally, it was shown that pathognomonic lesions were produced (Betts, Jennings, Omar, Page, Spence and Walker, 1964; Darbyshire, Jennings, Dawson, Lamont and Omar, 1965; 1966). Since similar lesions might also occur in naturally infected sheep and cattle, histological examination of the respiratory diseases occurring in sheep maintained by the Moredun Institute and in intensively-reared calves was undertaken.

Six months later a strain of PI3 virus was isolated from the upper respiratory tract of an intensively-reared lamb (Hore, 1966). The pathology of the lesions produced in susceptible lambs and calves after inoculation with this strain of PI3 virus and those observed in lambs after inoculation with a strain of bovine origin are described in detail.

Young lambs were also inoculated with Bedsoniae as these organisms have been isolated from sheep in Britain (Foggie, 1967) but their pathogenicity in young lambs had not been tested.

REVIEW OF LITERATURE

SHEEP

"Enzootic Pneumonia"

The name "enzootic pneumonia" was first used by Montgomerie, Bosworth and Glover (1938) to describe an outbreak of acute pneumonia, primarily in adult sheep, in Wales. Since then it has been used by other workers to describe commonly occurring types of pneumonia in many parts of the world (Shirlaw, 1959). It is not a specific term in that by definition it means only a pneumonia which has a low incidence and is constantly present in a given community. Therefore in addition to acute necrotising pneumonia (i.e. "enzootic pneumonia") the name could also refer to pulmonary adenomatosis as it occurs in this particular area of Scotland. Details of the distribution, incidence, bacteriology, serology and pathology of "enzootic pneumonia" as reviewed by Shirlaw (1959) indicate that it is probably not a specific entity. Because Pasteurella haemolytica is often isolated from cases of "enzootic pneumonia", many regard it as being the sole cause of the condition. However, experimental evidence has shown that P. haemolytica is capable of causing an acute necrotising pneumonia in sheep similar to that seen in natural cases, only if special inoculation techniques and large doses of the organism are employed (Smith, 1964; Biberstein, Nisbet and Thompson, 1967).

Stamp and Nisbet (1963) described the lesions of so-called "enzootic pneumonia" as varying in degree but not in type. The anterior lobes, particularly the right apical lobe, and often the anterior portions of the

diaphragmatic lobes were generally consolidated. The lobar and lobular septae were very distinct and opalescent. The thorax contained a serous exudate and, in addition to numerous haemorrhages on the heart and serous surface, there might be a fibrinous pleurisy or thickening of the pleura. The histological appearance varied from area to area according to the stage of the inflammatory process. There was intense hyperaemia of the capillaries and serous effusion and haemorrhage into the alveoli. Some areas contained alveoli densely packed with elongated cells situated within a structureless exudate while others were undergoing necrosis. Surrounding these areas were alveoli filled with large macrophages. Fibrin and/or neutrophils were occasionally present but were never conspicuous. Gram-negative cocco-bacillary bacteria were usually numerous in all affected areas.

Genus Chlamydia (Psittacosis-lymphogranuloma venereum - trachoma group of organisms)

Organisms of the psittacosis-lymphogranuloma venereum-trachoma group, presently considered to be bacteria and not viruses, have been placed in the genus Chlamydia, replacing Miyagawanella, Bedsonia and Rakeia by Page (1966). This classification has not been universally accepted so in keeping with the prevailing nomenclature in this country, the term Bedsonia has been used in this review.

Ovine strains of Bedsonia organisms were first isolated from the lungs of sheep with pneumonia by McKercher (1952). Since then there have been many reports on the isolation of Bedsonia agents from the lungs of sheep (Boidin, Cordy and Adler, 1958; Hamdy, Pounden and Ferguson, 1959; Hamdy

and Sanger, 1959; Romvary, 1962; Sarateanu, Popescu, Gheorghiu, Demetrescu and Petrescu, 1965). Bedsoniae have also been recovered from the faeces of normal sheep (Kawakami, Kaji, Sugimura, Omori and Matumoto, 1958; Dungworth and Cordy, 1962b; Wilson and Dungworth, 1963; Storz, 1963) and serological evidence (Dane and Clapp, 1956) has indicated that they are more widespread in sheep than is generally thought.

The relationship of strains of Bedsonia organisms found in sheep and those found in other species requires further investigation but it would appear from the experimental evidence available that the difference between certain members of the Bedsonia group lies more in degree of pathogenicity than in host or tissue tropism (Dungworth, 1963). Experimentally it has been shown that the organism causing enzootic abortion of ewes is capable of causing pneumonia in sheep (Dungworth and Cordy, 1962b; Romvary, 1962; Popovici, 1966) while abortion (Storz, 1963) and pneumonia (Dungworth and Cordy, 1962b) in sheep can be produced by the inoculation of Bedsonia organisms recovered from the faeces of normal sheep. Also, pneumonia in sheep could result from infection with a cattle strain (Romvary, 1964), a goat strain (Omori, Ishii, Harada, Ichikawa, Murase, Katada and Araumi, 1953) and a turkey strain (Pierce, Moore, Carroll and Bridges, 1963) while the intratracheal inoculation of an ovine pneumonic strain into pigs resulted in a characteristic pneumonia and clinical signs identical to those of sheep (Pan and Cordy, 1962). Frazer and Berman (1965), stating that the grouping of strains on epizootiological grounds is of questionable validity, placed fourteen strains into seven sub-groups in which an ovine pneumonitis strain and a feline pneumonitis strain were placed in sub-group VI. Using a serum

neutralisation test, sheep faecal and pneumonitis strains have been shown to be antigenically similar (Wilson, 1966).

The lung lesions produced after the intratracheal inoculation of sheep with either an ovine faecal strain, an ovine pneumonitis strain or the agent of enzootic abortion of ewes were identical, varying only in the duration of the response (Dungworth and Cordy, 1962b). In the majority of studies the intratracheal inoculation of sheep with ovine pulmonary strains of Bedsonia resulted in a marked clinical reaction (McKercher, 1952; Boidin et al., 1958; Dungworth and Cordy, 1962a). Within twenty-four hours after inoculation there was a rapid rise in temperature which lasted for four or five days. At the same time the lambs were moderately depressed and there was a jerky abdominal lift during expiration without any significant increase in the respiratory rate. Occasionally there was a dry cough.

Dungworth and Cordy (1962a, b) have described the pathology of the lung lesions in detail. The macroscopic lesions began as irregular dark red streaks and bands, increasing in extent until by the fourth or fifth day after inoculation the right apical and to a lesser extent, the cardiac and antero-ventral portions of the diaphragmatic lobes were involved. Resolution began on the ninth day and seemed to be almost complete by the thirtieth day after inoculation.

Histologically, the consolidation arose by confluence of foci with bronchiolar distribution. An early acute inflammatory response was followed by proliferation of the alveolar cells which resulted in large numbers of free alveolar macrophages and partial epithelialisation of alveoli. Often the attached alveolar cells were binucleate or had formed small syncytial

cell masses. Perivascular and peribronchiolar accumulations of lymphocytes, plasma cells and reticulo-endothelial cells were first seen on the second day after inoculation, and continued to increase in size as long as active cellular consolidation was present. Unfortunately none of the accompanying photomicrographs illustrated this lesion. Boidin et al., (1958) also noted early evidence of lymphoid hyperplasia around bronchioles which was well developed by the twentieth day after inoculation.

Elementary bodies, the pathognomonic feature of pneumonia caused by organisms of the Bedsonia group, were seen occasionally in lung sections on the second day of infection but their demonstration was of little practical value (Dungworth and Cordy, 1962a).

Parainfluenza Virus

Limited serological surveys, using bovine strains of parainfluenza 3 (PI3) virus as antigen, have demonstrated that a high proportion of sheep in the United States of America have antibodies against PI3 virus (Fischman, 1965; Woods, Sibinovic and Marquis, 1965a).

Parainfluenza 1 and parainfluenza 2 viruses do not appear to play an important role in sheep (Fischman, 1965).

Woods et al., (1965a) reported that the inoculation of a lamb with a bovine strain of PI3 virus resulted in infection. Another lamb held in contact with the inoculated lamb also became infected.

Reports relating to PI3 virus infection of sheep which have been published since 1965 will be discussed later in relation to part of the work described in this thesis.

Fungi

Pulmonary infections due to fungi are relatively rare. Whether this is due to an inherent resistance of sheep to fungal infections or is only a reflection of our ignorance is unknown. References to only three pulmonary fungal infections in sheep (aspergillosis, cryptococcosis and nocardiosis) were found and of these only aspergillosis had been diagnosed in Britain.

Aspergillosis

The earlier literature relating to aspergillosis in sheep, which consists of four reports, has been reviewed by Ainsworth and Austwick (1959). Since then three more accounts of the disease in sheep have been published.

Austwick, Gitter and Watkins (1960) described seven cases of pulmonary aspergillosis in one to three-week-old lambs in south and south-west England while Gracey and Baxter (1961) reported a case of generalised Aspergillus fumigatus infection in a two-month-old lamb in Northern Ireland in which the lungs, mediastinal lymph nodes, myocardium and both kidneys were involved. The fungus has also been isolated from the lungs of two adult sheep and a six-month-old lamb in West Pakistan (Ayaz, Ilahi and Afzal, 1966).

Macroscopically, greyish-white nodules varying in size from 0.4 to three millimetres (Austwick et al., 1960) to approximately three centimetres (Gracey and Baxter, 1961) in diameter were scattered throughout the lungs. The lesions in the mediastinal lymph nodes, myocardium and both kidneys varied in size but generally were similar in appearance to the nodules found in the lungs (Gracey and Baxter, 1961). A. fumigatus was recovered from

the nodules.

Various stages of purulent nodular pneumonitis were observed histopathologically. In the early lesions a central fungal colony, surrounded by neutrophils, cellular debris and macrophages was observed which resulted in compression of the alveoli at the periphery of the granulomata. In older lesions macrophages, epithelioid cells and fibrous tissue proliferation were the dominant features (Austwick et al., 1960). Giant cells were not observed by Austwick et al., (1960) nor by Gracey and Baxter (1961) but have been described in cases of aspergillosis in mature sheep by van Hellens (1902-03). The appearance of the granulomatous lesions in the mediastinal lymph nodes, myocardium and kidneys was similar to those seen in the lungs (Gracey and Baxter, 1961).

Three types of hyphal growth were observed by Austwick et al., (1960). Within the granulomata swollen hyphal cells were seen in the acute stage of the disease while the closely branched "actinomycetoid" form was present at the periphery of the necrotic areas in the more chronic stages. The third type resembled the rapid vegetative growth produced in submerged culture and occurred in hyperaemic areas away from the nodular lesions. In the first type the hyphae stained well with the periodic acid - Schiff and Gomori-Gridley techniques but failed to stain with either haematoxylin or eosin whereas, the hyphae in the second type were basophilic and stained well with the haematoxylin and eosin technique but not by the other two methods.

Jaagsiekte or Pulmonary Adenomatosis

Since the aetiology of either condition is not yet known it can not be definitely stated that jaagsiekte and pulmonary adenomatosis are identical but because they are similar in every respect apart from mortality rate (Dungal, Gislason and Taylor, 1938) they will be discussed together.

Jaagsiekte refers to chronic pulmonary conditions of sheep in South Africa characterised by progressive dyspnoea, emaciation and eventual death, whereas pulmonary adenomatosis is the term generally used for a similar condition occurring elsewhere. In some countries (e.g. Great Britain) either term is used.

The Afrikaans spelling of jaagsiekte (from jaagen, to drive, siekte, sickness) is the term more commonly used at present but the Dutch version, jagsiekte, and another alternative, jaagziekte (Nieberle and Cohrs, 1967) have been used as well. The term originated because severely affected sheep appeared as if they had been rapidly driven (Hutcheon, cited by Mitchell, 1915). However, it is not a specific term since similar signs may be seen in other chronic respiratory diseases of sheep (e.g. maedi, Montana progressive pneumonia). Indeed the report by Mitchell (1915) clearly illustrated that other respiratory conditions (in Mitchell's case, Graaff-Reinet disease) could be mistaken for jaagsiekte. For these reasons the more descriptive and more commonly used name, pulmonary adenomatosis, will be used in this review.

Pulmonary adenomatosis was first described in South Africa (Hutcheon, cited by Mitchell, 1915; Robertson, 1904; Mitchell, 1915; Cowdry, 1925a, b;

Cowdry and Marsh, 1927; De Kock, 1929a, b) and has since been reported from many other countries, viz. in Great Britain (M'Fadyean, 1938; Blakemore and Bosworth, 1941; Harbour and Jamieson, 1946; Stevens, 1957; Stamp and Nisbet, 1963; Markson and Terlecki, 1964; Mackay and Nisbet, 1966); France (Aynaud, 1926); Italy (Romboli, 1959); Greece (Christodoulou and Tarlatzis, 1952); Spain (Perez, 1963; Lugue, 1963); Germany (Jakob and Krause, 1965; Nieberle and Cohrs, 1967); Yugoslavia (Cvjetanovic and Martincic, 1962); Bulgaria (Enchev, 1963); Russia (Mitrofanov, 1964; Kostenko, 1964); Turkey (Sevki, 1956); Israel (Pattison, 1946; Nobel, 1958); Kenya (Shirlaw, 1959; Wandera, 1967); India (Rajya and Singh, 1964); and Peru (Cuba-Caparo, de la Vega and Capaira, 1961). It appeared as an epizootic in Iceland (Dungal et al., 1938) but has since been eradicated (Sigurdsson, 1958).

Pulmonary adenomatosis is primarily a disease of sheep but has been recorded in goats in Peru (Cuba-Caparo et al., 1961) and in India (Rajya and Singh, 1964). All breeds of sheep are susceptible (Dungal et al., 1938; Cuba-Caparo et al., 1961) and there is no evidence to suggest that there is an age predisposition (Cuba-Caparo et al., 1961). Other predisposing factors, mainly climatic conditions, have been suggested (Cowdry, 1925b; Cuba-Caparo et al., 1961) but as the disease is almost world-wide in its distribution it would be very difficult to assign a primary role to any one of these factors other than prolonged close contact. Accurate figures concerning the incidence of pulmonary adenomatosis are difficult to obtain since most of the reported cases refer to clinical

cases and do not include subclinically infected sheep (Mackay and Nisbet, 1966). Epizootics, with a flock mortality rate of fifty to eighty per cent. have occurred (Dungal et al., 1938) but the enzootic form, with an annual flock mortality rate varying from 1.5 per cent. (Mitchell, 1915) to thirty per cent. (Cuba-Caparo et al., 1961) is more common. Stevens (1957) and Mackay and Nisbet (1966) have stated that the incidence of pulmonary adenomatosis is likely to increase following the introduction of more intensive methods of sheep husbandry.

The aetiology has not yet been determined but there is ample evidence to show that the disease is contagious (Dungal et al., 1938; Dungal, 1946; Sigurdsson, 1958; Markson and Terlecki, 1964). Lungworms and their larvae were at one time thought to be the cause of the disease but Dungal et al., (1938) showed conclusively that they were of no significance. Bacteria (Dungal et al., 1938), including Mycoplasma spp. (Nobel, 1958; Mackay, 1966) have been isolated from cases of pulmonary adenomatosis but it is doubtful that they have a primary aetiological role. Mackay (1966), Markson and Terlecki (1964), Dungal (1946) and Dungal et al., (1938) support the suggestion by Theiler (cited by Cowdry and Marsh, 1927) that a virus might be the cause of the disease. The only report on the isolation of a virus is that by Shirlaw (1959). However, Markson and Terlecki (1964), who examined sections of lung from lambs inoculated with this virus by Shirlaw, were unable to find any evidence of pulmonary adenomatosis.

Pulmonary adenomatosis is most common in adult sheep (Cowdry, 1925a) but has been observed in lambs three to four months old (Cuba-Caparo et al.,

1961). There is a long incubation period, usually six to eight months or more (Dungal et al., 1938). Early signs of the disease include an occasional cough, a shortness in depth and an increase in the rate of respiration (Robertson, 1904) particularly after exercise (Mitchell, 1915). Dyspnoea, moist rales and emaciation are seen in the later stages (Mitchell, 1915; Cowdry, 1925a; Dungal et al., 1938). The most characteristic sign, when present, is a profuse watery discharge which runs from the nasal passages when affected sheep are held up by the hind legs (Mitchell, 1915; Dungal et al., 1938; Blakemore and Bosworth, 1941). However, in early cases or in the later stages of the disease this sign may be absent. In uncomplicated cases there is no elevation of body temperature and the appetite is normal (Dungal et al., 1938). Affected sheep usually die in about two or three months after the onset of clinical signs but the course of the disease in any one sheep is very variable (Dungal et al., 1938).

Macroscopically, specific lesions of pulmonary adenomatosis are confined to the thoracic cavity (Robertson, 1904; Cowdry and Marsh, 1927; Dungal et al., 1938). Early lesions appearing as smooth rounded swellings two to three centimetres in diameter and projecting slightly from the surrounding normal lung tissue, have been observed in affected sheep at this Institute. In more advanced cases the lungs are usually increased in size and weight (Dungal et al., 1938; Stevens, 1957) and are light grey or light purplish with more or less widespread greyish areas of consolidation (Dungal et al., 1938). The transparent, greyish-white circumscribed nodules

increase in number and size until about two-thirds of the ventral aspect of the lung is consolidated (De Kock, 1929a). Necrosis, abscess formation, chronic pleuritis with adhesions or verminous pneumonitis may be present in the consolidated areas in advanced cases (De Kock, 1929a; Cuba-Caparo et al., 1961; Markson and Terlecki, 1964). Histological confirmation is required in these cases. The pulmonary lymph nodes are enlarged, pale and oedematous (Robertson, 1904).

Diverse opinions as to the fundamental lesion in pulmonary adenomatosis still exist and are likely to continue until susceptible sheep, known to be free from the disease and kept in strict isolation, are inoculated with a purified strain of the causal agent. Cowdry (1925a) and Wandera (1967) consider that the initial histological changes are congestion of the alveolar capillaries and thickening of the interalveolar septa due to the infiltration of macrophages, lymphocytes and a few neutrophils. According to Cowdry (1925b) the epithelial proliferations, which are so characteristic of pulmonary adenomatosis, arise only in portions of the lung so modified by the primary interstitial reaction. De Kock (1929a) unable to verify the above conclusions, states that the primary lesion is a proliferation of the cuboidal cells lining the alveoli which results in papillary ingrowths projecting into the alveolar spaces. Cuba-Caparo et al., (1961) describe thickening of the interalveolar septa but believe it to be a non-specific lesion of secondary nature. The epithelial proliferations soon become confluent so that large areas of pulmonary tissue assume an adenomatous appearance (De Kock, 1929a). Histochemically, the ribonucleic acid (RNA) content of adenomatous tissue is higher than normal (Aliev, 1967). Cowdry (1925b) maintains that

the initial interstitial reaction progresses and persists whereas De Kock (1929a) and Stamp and Nisbet (1963) state that the papillomatous formations invade apparently normal alveoli. The surrounding alveoli may contain large mononuclear cells (Dungal et al., 1938) and giant cells (Cowdry, 1925a; Stamp and Nisbet, 1963). Interalveolar fibrosis and infiltration of alveolar septa with lymphocytes and macrophages are seen in older lesions (Cowdry, 1925b; De Kock, 1929a). In a small proportion of cases myxomatous nodules are present in advanced cases (Cowdry, 1925b; Cuba-Caparo et al., 1961; Stamp and Nisbet, 1963). Proliferation of the bronchiolar epithelium occurs in some cases (Cowdry, 1925b; De Kock, 1929a). Although peribronchial lymphocytic hyperplasia may be present (Dungal et al., 1938; Stamp and Nisbet, 1963), extensive peribronchial lymphocytic infiltration as described by Mitchell (1915) is not a constant feature of pulmonary adenomatosis. De Kock (1929a) found an almost complete absence of lymphoid tissue in the majority of lungs examined from sheep with pulmonary adenomatosis and suggested that Mitchell might have confused pulmonary adenomatosis with a second specific respiratory disease of sheep in South Africa (Graaff-Reinet disease) in which extensive lymphoid hyperplasia is a prominent lesion. Similarly, lesions of acute catarrhal bronchopneumonia which are sometimes present are the result of a terminal bacterial infection, usually caused by P. haemolytica (Dungal et al., 1938).

Metastases to the bronchial or mediastinal lymph nodes have been recorded in various countries e.g. in France (Aynaud, 1926); Peru (Paredes, 1953; Cuba-Caparo et al., 1961); Bulgaria (Enchev, 1963);

Spain (Lugue, 1963); Britain (Stamp and Nisbet, 1963; Markson and Terlecki, 1964) and Russia (Mitrofanov, 1964); but not, apparently, in Iceland or South Africa. Affected lymph nodes are enlarged but histological examination is necessary to exclude enlargement due to lymphoid hyperplasia (Cuba-Caparo et al., 1961). The only report in which lesions of pulmonary adenomatosis have been found outside the thoracic cavity is that by Enchev (1963) who recorded lesions in the liver, spleen, kidney and heart. In view of these reports it would appear that, contrary to the opinion of M'Fadyean (1938), pulmonary adenomatosis is essentially a transmissible neoplasm (Markson and Terlecki, 1964; Stamp and Nisbet, 1963; De Kock, 1929a).

Atypical Pneumonia

Atypical pneumonia was the name suggested by Stamp and Nisbet (1963) for a type of pneumonia occurring commonly in Scotland. A similar condition exists in lambs in California (McGowan, Moulton and Shultz, 1957). There is some doubt as to its economic importance. Stamp and Nisbet (1963), stated that it appeared to be a common cause of economic loss, whereas, Gilmour and Brotherston (1963) maintained that in the absence of secondary bacterial infection (usually P. haemolytica) there was no appreciable change in the general condition of the sheep. In many ways it is similar to Graaff-Reinet disease (De Kock, 1929a) and Montana progressive pneumonia (Marsh, 1923).

The aetiology of atypical pneumonia is unknown. Stamp and Nisbet (1963), unable to associate the lesions of atypical pneumonia with any specific agent, compared it with somewhat similar diseases of cattle (Jarrett, McIntyre and

Urquhart, 1953; Jarrett, 1954), pigs (Pattison, 1956) and mice (Andrewes and Glover, 1945; Niven, 1950). They also stated that the lesions resembled those found in sheep inoculated with an organism of the Bedsonia group (Dungworth and Cordy, 1962a). Biberstein et al., (1967) claimed to have produced a microscopic lesion which "differed in no important detail from that described for atypical pneumonia" by the inoculation of an organism of the Bedsonia group obtained from a naturally-occurring case of atypical pneumonia. However, they qualified this by stating that "while the existence of such lung changes prior to the experiments cannot be ruled out, it would be a strange coincidence if all these were strictly confined to the animals exposed in the course of the investigation to an agent known to be capable of causing such lesions". The fact that three out of six animals, inoculated tracheobronchially with P. haemolytica alone, had lesions of atypical pneumonia would tend to suggest that the lesions were present prior to their experiments. Unfortunately, attempts to isolate organisms of the Bedsonia group were not made.

The clinical signs of atypical pneumonia are mild and can be easily missed unless a detailed examination of the thorax is carried out (Gilmour and Brotherston, 1963). There are high temperatures and increased respiratory rates but dullness, inappetence and dyspnoea are not observed. Dark red or grey areas of consolidation or narrow branching bands of collapse are confined mainly to the anterior lobes.

Stamp and Nisbet (1963) divide the histological lesions into two types: those cases in which lymphoid hyperplasia is the most prominent lesion and those in which there is interstitial pneumonia, with or without lymphoid

hyperplasia. Cases of atypical pneumonia in which only lymphoid hyperplasia is found are comparatively rare. The lesions are usually confined to the bronchi or bronchioles of the apical lobes and consist of accumulations of lymphocytes, plasma cells and reticulo-endothelial cells which may either form a "cuff" around airways or only partially surround them. In either case they compress the involved air passages and the adjacent alveoli. In the second, more common type peribronchial and peribronchiolar lymphocytic "cuffing" may be present especially in advanced cases. In early cases there is infiltration of the interalveolar septa with lymphocytes and macrophages and the surrounding alveoli contain large macrophages. Fibrous tissue ingrowths, or in more advanced cases hyaline or myxomatous scars are often associated with chronically inflamed bronchi and bronchioles. Stamp and Nisbet (1963) state that these ingrowths are a characteristic finding in all of their cases but such an incidence has not been observed in the past three years by the present author. There is focal alveolar epithelialisation in the vicinity of affected bronchi and bronchioles which may resemble adenomatosis but papillary ingrowths, as seen in pulmonary adenomatosis, are not seen.

Therefore on pathological and aetiological grounds it seems unlikely that atypical pneumonia of sheep as described by Stamp and Nisbet (1963) represents a single entity.

CATTLE

A comprehensive review of the respiratory diseases of cattle will not be included in this thesis as the subject has been discussed by Abinanti (1963), Omar (1966) and more recently by Phillip (1968). Only recent publications or those relating directly to the work to be described will be discussed. Many of the recent papers, particularly those from the United States of America, describe results of experimental or field trials testing the efficacy of PI3 virus vaccines and, although interesting and often contradictory, they do not have a direct bearing on the subject matter of this thesis and are, therefore, excluded.

Parainfluenza 3 Virus

Reisinger, Heddleston and Manthei (1959) are usually credited with the primary isolation of PI3 virus from cattle. However, four years earlier a similar virus, though not identified as PI3 virus at the time, was isolated from cattle with acute respiratory disease by Gillespie (1958). Since then it has been isolated from cattle in a number of countries and all strains appear to be serologically identical to the original SF4 strain (Omar, 1966), which has been shown to be antigenically related to a human strain of PI3 virus (Abinanti and Huebner, 1959). Parainfluenza 3 virus has also been isolated from water buffaloes (Singh and Baz, 1966) and recently strains have been isolated from the intestinal contents of a thirty-six hour-old calf that died of "pneumo-enteritis" (Hamdy, 1966) and from an aborted foetus (Sattar, 1966).

Ample evidence of its widespread distribution among cattle is evident from serological studies carried out in a number of countries (Omar, 1966). Using "several" thousand paired serum samples taken from cattle in outbreaks of respiratory disease, significant rises in antibody titre to PI3 virus were found in nineteen per cent. of the samples (Phillip, 1968). Similarly, Omar (1966) in a histological survey of naturally occurring pneumonias in calves in Britain, found lesions characteristic of PI3 virus infection in the lungs of sixteen per cent. of 125 outbreaks.

In naturally infected calves it is not possible to make a diagnosis of PI3 virus infection on clinical grounds as similar signs may be observed in calves infected with other respiratory viruses (Phillip, 1968). Clinical signs may or may not be observed in calves experimentally infected with PI3 virus (Abinanti, 1963). When present they are generally mild (Reisinger et al., 1959; Woods, Sibinovic and Starkey, 1965b; Dawson, Darbyshire and Lamont, 1965) although on occasion a more severe reaction, with temperatures as high as 41.7 degrees centigrade, coughing and nasal discharge have been described (Abinanti, 1963; Betts et al., 1964). Reinfection, without observable signs, as has been described in children infected with PI3 virus (Chanock, Bell and Parrott, 1961), has also been recorded in calves (Abinanti, 1963). A more severe disease resembling that seen in field outbreaks, has been produced in calves inoculated with PI3 virus and Pasteurella species, with or without physical stress (Heddleston, Reisinger and Watko, 1962; Hetrick, Chang, Byrne and Hansen, 1963; Baldwin, Marshall and Wessman, 1967).

In naturally infected calves the macroscopic lesions were distributed in the anterior and anterior ventral parts of the lungs and were grey or

deep red (Betts et al., 1964). The interlobular septa were prominent and cut bronchioles exuded a catarrhal exudate. Necrosis and focal abscess formation were also evident. Somewhat similar lesions were produced in hysterectomy-derived, colostrum-deprived calves inoculated with the J121 strain of PI3 virus (Betts et al., 1964; Omar, Jennings and Betts, 1966) and in colostrum-deprived calves inoculated with the bovine T1 strain (Dawson, Darbyshire, Lamont and Paterson, 1964). Woods et al., (1965b), on the other hand, state that the only macroscopic lesion in two hysterectomy-derived, colostrum-deprived calves inoculated with the Illinois 811 strain of PI3 virus was congestion. Other macroscopic lesions seen by the latter authors were congestion of the kidney cortices and tracheal mucosae in both calves. Such lesions were not observed by Dawson et al., (1965) nor by Omar et al., (1966).

Although colostrum-deprived calves were used by Woods et al., (1965b), Dawson et al., (1965) and Omar et al., (1966), the results of histopathological examination varied. Dawson et al., (1965) stated that lesions were confined to the lungs and Omar et al., (1966) found that they were confined to the respiratory tract, including the pulmonary lymph nodes. However, Woods et al., (1965b), in addition to lesions in the respiratory tract, describe a variety of changes affecting the kidneys, liver and mesenteric lymph nodes in one calf and the spleen, liver, kidneys and colic lymph node in the second calf. Woods et al., (1965a) also described lesions in the liver, kidneys and spleen, in addition to lung lesions, in two calves, one of which had been inoculated intranasally with

the Illinois 811 strain of PI3 virus while the other was held as a contact control.

A comprehensive description of the histological lesions resulting from the intratracheal and intranasal inoculation of the J121 strain of PI3 virus has been reported by Omar et al., (1966). Although much briefer the description by Dawson et al., (1965) was similar. Little can be said about the histological results of Woods et al., (1965a, b) as the only descriptions given for the lung were "pneumonitis" or "interlobular oedema" and diffuse thickening of the alveolar 'septums' with accumulation of erythrocytes and mononuclear leucocytes.

Mild tracheitis and turbinitis were present in calves inoculated with the J121 strain of virus (Omar et al., 1966). Lung lesions four days after inoculation of the T1 strain (Dawson et al., 1965) consisted of proliferation and necrosis of bronchiolar epithelial cells, many of which contained eosinophilic cytoplasmic inclusions; accumulation of cellular debris in bronchiolar lumina, neutrophil and mononuclear cell infiltration of alveoli; scattered foci of necrotic cells and eosinophilic cytoplasmic inclusions in alveolar lining cells. In calves killed five days after inoculation with the J121 strain (Omar et al., 1966) both exudative and proliferative reactions were present. The exudate which was composed of acidophilic fluid, mononuclear cells and a few neutrophils, partially masked the normal structure of the lung in places. There was hypertrophy and hyperplasia of bronchiolar and alveolar epithelial cells and giant cell formation in small bronchioles. Septal cell proliferation was also seen

resulting in pseudo-epithelialisation (Omar, 1964) of alveoli. Mitotic figures were observed among epithelial cells of bronchioles and alveoli. Occasionally there was necrosis and desquamation of the hyperplastic bronchiolar epithelium. Eosinophilic cytoplasmic and less frequently, nuclear inclusions, were seen in bronchiolar and alveolar epithelial cells as well as in alveolar septal cells and in macrophage-type giant cells in alveoli. There was slight peribronchiolar lympho-reticular hyperplasia.

In calves killed seven days after inoculation the cellular infiltration was less marked (Dawson et al., 1965; Omar et al., 1966) but oedema was still extensive (Omar et al., 1966). There was thickening of the inter-alveolar septa, due to cellular infiltration and an increase in the number and thickness of reticulin fibres, and alveolar epithelialisation was more extensive (Omar et al., 1966). The hyperplastic epithelial cells of the bronchioles had been replaced by hyperchromatic squamous cells and many bronchioles were filled with necrotic debris causing collapse of the surrounding alveoli (Omar et al., 1966). Syncytia were occasionally observed in alveoli (Dawson et al., 1965) but epithelial giant cells were not seen (Omar et al., 1966). Eosinophilic nuclear and less frequently, cytoplasmic, inclusions were observed by Dawson et al., (1965) but according to Omar et al., (1966) the inclusions had largely disappeared by the seventh day; only occasionally were nuclear inclusions seen in epithelial and septal cells. Peribronchiolar lympho-reticular hyperplasia was more marked than at five days after inoculation (Omar et al., 1966).

Similar histopathological lesions have been described in naturally infected calves by Betts et al., (1964) and by Jolly and Ditchfield (1965). Betts et al., (1964) confirmed their diagnosis by isolating two strains of PI3 virus from two calves whereas Jolly and Ditchfield (1965) were able to implicate PI3 virus because of a positive complement fixation test and marked fluorescence with specific labelled antisera using antigen which had been fixed in formalin. There was no mention of control preparations used in the fluorescence procedures by the latter authors.

In retrospect it would appear that the pneumonia described by Jarrett (1954) and later called inclusion body pneumonia (Jarrett, 1956) was caused by a PI3 virus infection. The salient histopathological features were cuboidal epithelialisation of alveoli, syncytium formation and acidophilic cytoplasmic inclusions (Jarrett, 1954, 1956), lesions which are considered to be pathognomonic of PI3 virus infections (Omar, 1966). Also of interest, as pointed out by Dawson et al., (1965), Jarrett (1954) commented on the similarity of the histopathological lesions with those seen in the lungs of patients with measles and in canine distemper, both of which are caused by myxoviruses.

"Cuffing Pneumonia"

"Cuffing pneumonia" was the name suggested by Jarrett et al., (1953) for a type of pneumonia occurring in calves in Britain characterised by peribronchial lymphoid hyperplasia. A similar condition has been described in Canada (Carter and Rowsell, 1958).

The clinical signs have been described in detail by Jarrett et al., (1953) and Jarrett, McIntyre and Urquhart, (1954). Briefly they consist of tachypnoea, or dyspnoea in advanced cases and chronic, intermittent coughing. Affected calves usually recover in several months but a proportion of them develop chronic pulmonary lesions such as bronchiectasis (McIntyre, 1963).

Macroscopic lung lesions were usually confined to the apical, cardiac and anterior ventral parts of the diaphragmatic lobes (Jarrett, 1954). The affected areas were usually deep red and collapsed. The bronchi and bronchioles were surrounded by greyish-white lymphoid "cuffs" and appeared to pout from the cut surface (Jarrett et al., 1953; Jarrett, 1954). A mucopurulent exudate could often be expressed from cut air passages. Marked hyperplasia of the pulmonary lymph nodes was also observed.

Peribronchial lymphoid tissue is normally present only in a very small amount in calves (Jarrett et al., 1953). In "cuffing pneumonia", however, the amount of lymphoid tissue is greatly increased. The first indication of the disease is the formation of a lymphoid germ centre in the septal tissues which gradually enlarges and extends around affected airways to form a "cuff" (Jarrett et al., 1953; Jarrett, 1954). As the lymphoid "cuffs" increase in size there is compression collapse of adjacent alveoli and the bronchial lumina are often reduced to an irregular slit. In very advanced cases lymphoid cells invade the bronchial wall replacing muscle and elastic tissue (Jarrett, 1956). In advanced cases affected bronchi often contain inflammatory debris, necrotic cells and clumps of bacteria (Jarrett, 1954). Occasionally, adjacent blood vessels are surrounded by lymphoid tissue. Acute inflammatory changes in alveoli are usually absent but macrophages

may be present (Jarrett, 1954). In some, the lesions persist and may result in severe subacute pneumonia at a later date (McIntyre, 1963).

The aetiology of this condition, if in fact it is a single entity, is not known. Jarrett et al., (1953) and Jarrett (1954, 1956) have commented on the similarities of the bovine disease with those of "known virus aetiology" such as grey lung disease of mice, spontaneous pneumonitis of guinea pigs, cotton rat virus pneumonia and virus pneumonia of pigs. However, they qualified this statement by saying that they were not attempting to postulate aetiology on purely anatomical grounds. The causes of the above conditions are not definitely known. Both viruses and Mycoplasma spp. have been incriminated in one or more of them. The possibility that "cuffing pneumonia" of calves may be the end result of a mycoplasmosis has been discussed by Omar (1966).

GENERAL MATERIALS AND METHODS

Materials and techniques that are common to more than one section of the thesis are detailed below. Other procedures relating only to a particular section or exceptions from the general materials and methods are described in that section.

Experimental Animals

Lambs

Generally, an attempt was made to secure lambs which had not been allowed a colostral feed. Healthy ewes from a variety of sources were obtained and kept in pens at this Institute. In order to get colostrum-deprived lambs without having to be in continual attendance the following procedure was adopted. The udder of each ewe was thoroughly washed, dried and cleansed with cotton-wool moistened with ether. Strips of zinc oxide adhesive were then applied until the udder was completely covered. The ewes did not appear to be distressed by this procedure and mastitis was not a problem. Some of the ewes were used for this purpose for two successive years (1967 and 1968) without ill-effect.

The lambs were removed from the ewes as soon as possible and placed in small heated pens. They were bottle-fed three times daily on ten ounces of warmed cow's milk which was usually supplemented twice daily for the first three or four days with five cubic centimetres of a commercial oral solution

of streptomycin sulphate and neomycin sulphate (Streptovex-N, Glaxo Laboratories Ltd.). Between feeds the plastic bottles and rubber teats were thoroughly washed and immersed in a 1:80 solution of sodium hypochlorite and sodium chloride (Milton, Vick International Ltd.). Clinical signs, including rectal temperatures, were recorded at 9.30 a.m. and 4.30 p.m. daily. Blood samples were taken from each lamb prior to inoculation and at autopsy.

Calves

Colostrum-deprived calves were obtained from local farms and housed in pens at this Institute. Each calf was fed thirty ounces of warmed cow's milk twice daily to which was added a commercial antibiotic - vitamin A supplement (Streptovex-N tablets, Glaxo Laboratories Ltd.). Clinical examination and collection of blood samples were carried out as described for lambs.

Methods of Inoculation

The procedures used were similar for both calves and lambs.

Intranasal inoculation: The intranasal inoculum was slowly deposited in the air passages by means of a polythene catheter attached to a syringe. The catheter was inserted into the nasopharyngeal area and particular care was taken to distribute the relatively small dose of virus along the length of the nasal passages as the catheter was withdrawn.

Intratracheal inoculation: The skin and subcutaneous tissues were

anaesthetised. A sixteen gauge record hypodermic needle was inserted into the mid-cervical section of the trachea and a polythene catheter (twenty centimetres long, one millimetre external diameter) was then passed into the trachea through the needle. This procedure ensured that the inoculum was sprayed into the thoracic part of the trachea while the lamb or calf was being held in its normal standing position and obviated the risk of peri-tracheal inoculation of virus due to movement of the animal.

Pathology

Autopsy Procedure

Young lambs were killed by first anaesthetising them with an overdose of pentobarbitone sodium (Abbott Laboratories Ltd.) followed by exsanguination, care being taken to avoid cutting the trachea or inhalation of blood or ruminal contents. Similar procedures were used to kill older lambs, adult sheep and calves except that stunning by a captive bolt pistol (Accles and Shelvoke Ltd.) using one and one quarter grain cartridges was used instead of anaesthesia.

After the skin was reflected the trachea was dissected away from the oesophagus and a metal clamp was applied so that the lungs would not collapse when the thorax was opened. The surface of the thorax was sterilised by flame before opening along the costochondral junction to obtain specimens for cultural purposes. The lungs and pleural surfaces were examined for evidence of pleurisy and macroscopic lesions were photographed.

The lungs were then carefully removed and the distribution of lesions, if any, was recorded on a diagram of the lung outline. The remaining organs were examined and if abnormalities were observed, pieces of affected tissues were taken for histopathological examination.

Histological Procedures

Pieces of lung, liver, kidney, spleen, mediastinal and left and right bronchial lymph nodes were routinely collected. When collecting samples from the lungs, pieces of tissue were obtained from consolidated areas, from areas containing both consolidated and normal areas and from apparently normal areas. The specimens were usually fixed in ten per cent. formol-saline but in some instances Zenker-acetic and Clarke's (Culling, 1963) fixatives were also used. Post-fixation in formol-sublimate (ninety millilitres saturated aqueous mercuric chloride and ten millilitres of formalin) for twenty-four to forty-eight hours was routinely carried out unless contra-indicated for specific staining procedures. All samples were dehydrated through alcohols, cleared in chloroform and embedded in paraffin with a melting point of fifty-six to fifty-eight degrees centigrade. Sections five to seven microns thick were cut and mounted on albuminised slides.

Small pieces of lung (five to ten by five by seven millimetres) from selected cases were cut at a thickness of six to eight microns in a cryostat (Bright's Refrigerator Service Ltd.). Sections were mounted on clean, dry

slides and were either immersed directly into fixative or allowed to dry in air before being fixed in either methyl alcohol, acetone or Clarke's fluid.

For the demonstration of Bedsonia elementary bodies small pieces of consolidated lung (approximately five cubic millimetres) were rapidly frozen in a mixture of carbon dioxide (CO_2) and ethanol, cut as thinly as possible (usually two microns thick), air dried on clean dry slides and fixed by heat.

Standard reference texts by Culling (1963) and Lillie (1954) were used for the majority of the histological techniques. Sections were deparaffinised in xylol and hydrated with graded alcohols. Those which had been in a mercuric fixative were passed through iodine and sodium thiosulphate prior to staining.

One section from each block was routinely stained with celestin blue-haematoxylin and eosin (H & E). In addition, selected sections were stained by a number of other methods, notably; azure A-eosin B (Lillie, 1954), van Gieson, Gram, MacNeal (Conn, 1929), Heidenhain's iron haematoxylin, Mallory's phosphotungstic acid haematoxylin, fuchsin Miller (Slidders, 1961), picro-Mallory V (Lendrum, Fraser, Slidders and Henderson, 1962), Martius-Scarlet-Blue (Lendrum et al., 1962), periodic acid - Schiff, Feulgen, and Gridley (1953). Sections were stained for reticulin by the methods of Slidders, Fraser and Lendrum (1958) and Gordon and Sweets (1936).

For the demonstration of cellular inclusions the following stains were employed: the trichrome methods of MacFarlane (1944), Pollak (1944), Sellers (1927), Page and Green (1942) and Zlotnik (1953); phloxine-tartrazine (Lendrum, 1947) and pyronin-methyl green (Kurnick, 1955; Trevan and Sharrock,

1951; and the method of Jordan and Baker as described by Culling, 1963).

Bedsonia elementary bodies were stained with a variation of the Giemsa method (Wolbach, 1919) and with a modification of the Ziehl-Neelsen method. The technique for the latter stain varied with the method used in processing the material. In cryostat sections and lung impression smears the procedure was as follows: Flood the section with dilute carbol fuchsin (1:10) and leave for three minutes. Rinse in tap water and decolourise in five per cent. acetic acid for ten seconds. Rinse in tap water and then counter stain with 1:10 Loeffler's methylene blue for thirty seconds. Rinse again in tap water, blot and dry in air. For paraffin sections the method was altered by staining in dilute carbol fuchsin for five minutes, rinsing in tap water and decolourising in five per cent. acetic acid for thirty seconds or longer (depending on the thickness of the section). They were then stained for sixty seconds with methylene blue, rinsed in tap water, blotted, dehydrated and mounted in DPX (Kirkpatrick and Lendrum, 1941).

Virology, Serology and Bacteriology

Parallel studies of the virological and serological aspects of PI3 virus infections in lambs and calves were carried out by Dr. D. E. Hore and some of his results have been included in this thesis when deemed necessary. The techniques employed in the above investigations have been described previously (Hore, 1968).

Specimens from lambs and calves were submitted to the Department of Microbiology, Moredun Institute and the bacteriological findings are recorded with the permission of Mr. D. Thompson.

Abbreviations

The following abbreviations have been used:

CO_2 = carbon dioxide

H & E = celestine blue - haematoxylin and eosin

HI = haemagglutination inhibition

MEP = "moderate epithelialising pneumonia"

PAS = periodic acid-Schiff

PBS = phosphate buffered saline

PI2 = parainfluenza 2

PI3 = parainfluenza 3

RNA = ribonucleic acid

$TCID_{50}$ = fifty per cent. tissue culture infectious doses

SECTION 1

INVESTIGATION OF THE MORPHOLOGICAL TYPES OF RESPIRATORY DISEASE IN SHEEP MAINTAINED AT THE MOREDUN INSTITUTE

INTRODUCTION

The last description of the morphological types of respiratory disease in sheep in Britain was by Stamp and Nisbet in 1963. Antibodies against parainfluenza 3 (PI3) virus and adenovirus have been demonstrated in the sera of sheep in Britain (Hore, 1965). Similar serological results have been recorded for cattle (Dawson and Darbyshire, 1964; Phillip, 1968) and since pathognomonic lesions are produced by each of these viruses in experimental and natural infections in cattle (Omar, 1966), it was considered that sheep might also exhibit such lesions and for these reasons an investigation of the respiratory diseases of sheep was undertaken.

MATERIALS AND METHODS

The sheep at the Moredun Institute were chosen. The number of sheep varies from year to year but on average there are about 1200. After lambing the number increases to about 1800 or 1900. The most common breeds are Scottish Blackface, Suffolk, South Country Cheviot and their various crosses. Other breeds are also kept and of these the Dorset Horn is the most common. The ages of the sheep vary and are somewhat dependent

on the experimental programme (e.g. in the scrapie experiments some sheep may be eight or nine years old). Some of the lambs are born at the Institute but the majority are born on two farms where grazing is rented. In the autumn, lambs are brought into the Institute either from these two farms or, less often, are purchased in the open market. A substantial number of these develop respiratory disease soon after they are housed in covered pens (Gilmour and Brotherston, 1963) and, as a result, it is possible to select lambs showing early signs of respiratory illness for post mortem examination. Apart from the lambs which are brought into the Institute in the autumn there is little contact with other sheep. Occasionally sheep are sent to the Institute either for research or diagnostic purposes (e.g. pulmonary adenomatosis) but these animals are kept isolated until they are killed.

The accommodation varies from outdoor grazing, supplemented by hay and concentrates as required, to indoor housing the year round. The majority of the housed sheep are kept in a large covered building in which the size of the pens can be adjusted to meet individual requirements. Three sides of the building have walls which are approximately five feet high so that ventilation is adequate. The remaining buildings are completely enclosed and are divided into pens which can accommodate from one to thirty sheep. The type of feeding depends somewhat on the experimental programme but generally consists of hay, concentrates and water ad lib.

The lungs which were examined came from four groups of sheep.

Group 1

The first group was made up of sixty-four apparently healthy sheep

which were killed during the course of other experiments and were examined for evidence of atypical pneumonia.

Group 2

Routine post mortem examination of seventy sheep presented to the Department of Pathology for diagnosis made up the second group. Of these sheep, fifty-four were found dead; seven were killed to terminate primary research programmes and were found to have pulmonary lesions which differed from those of atypical pneumonia, and the remaining nine were clinically ill sheep which had not responded to treatment and had been killed.

Group 3

In previous years pneumonia has been a problem in lambs recently introduced into covered pens at this Institute. For a study of the problem ten lambs were selected from the 1966 intake. Four of the ten lambs were killed between the twelfth and sixteenth days after introduction and form the basis of the third group.

Group 4

The fourth group consisted of thirty-four lambs which were introduced into covered pens at this Institute the following year. Four of these lambs developed a respiratory disease soon after their arrival and were killed, either in extremis or after having failed to respond to antibiotic treatment. The remaining thirty were killed two to three months later in connection with

the primary research programme (Johne's disease).

Lesions caused by lungworm infections were frequently observed in the lungs of all four groups of sheep but are not included as they have recently been described by Poynter and Selway (1966).

Pathology

The procedures used have been described under General Materials and Methods.

RESULTS

Group 1

Atypical Pneumonia

Thirty-five of the apparently healthy sheep had lesions of atypical pneumonia. Histologically, the lesions were in agreement with those described by Stamp and Nisbet (1963) except that bronchial ingrowths were less frequently observed.

Group 2

Acute Necrotising Pneumonia Associated with Pasteurella haemolytica

Unusual lesions were seen in two instances, the remaining twenty-three were typical of the disease as described in the review of literature. The anterior lobes and often the anterior ventral portions of the diaphragmatic

lobes are generally consolidated but in two sheep which were found dead, there were raised, red, circular, consolidated areas in the mid-dorsal part of the right diaphragmatic lobes. Histologically there was a sero-fibrinous exudate in many alveoli and foci of alveolar necrosis as is seen in typical cases of so-called "enzootic pneumonia".

Pulmonary Adenomatosis

Sixteen of the twenty-nine lungs with pulmonary adenomatosis were complicated by a P. haemolytica infection. In most of these sheep it was possible to diagnose pulmonary adenomatosis on macroscopic findings (Figure 1) but in some, histological examination was required (Figure 2). In those cases the most common histological lesions were neutrophil infiltration of both bronchiolar epithelium and adenomatous foci and a serous exudate in the surrounding alveoli but occasionally verminous pneumonia or small abscesses were encountered.

Histologically there were several interesting variations. Adenomatous-like proliferations arising from the bronchiolar epithelium were observed in paraffin sections (Figure 3). Serial cryostat sections of bronchioles showing outgrowths of epithelium extending down these air passages were examined but it was not possible to follow them into alveoli. The atypical proliferations were lined by columnar cells, had a connective tissue core and were apparently growing down the bronchioles in the form of several finger-like projections which sometimes branched (Figure 5). Adenomatous foci, apparently originating in alveoli, were occasionally observed in small bronchioles and alveolar ducts (Figure 4). In sections of affected lung

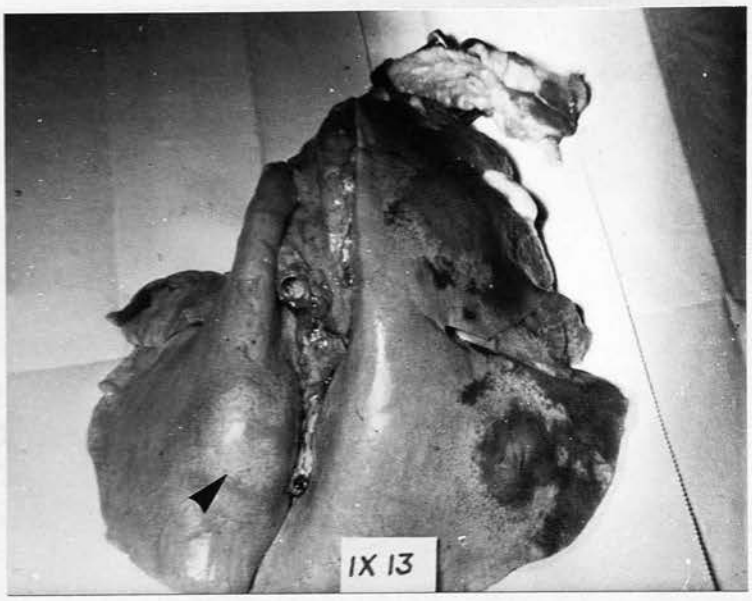


Figure 1. Pulmonary adenomatosis. Note the large raised lesion (arrow) in the dorsal part of the left diaphragmatic lobe.

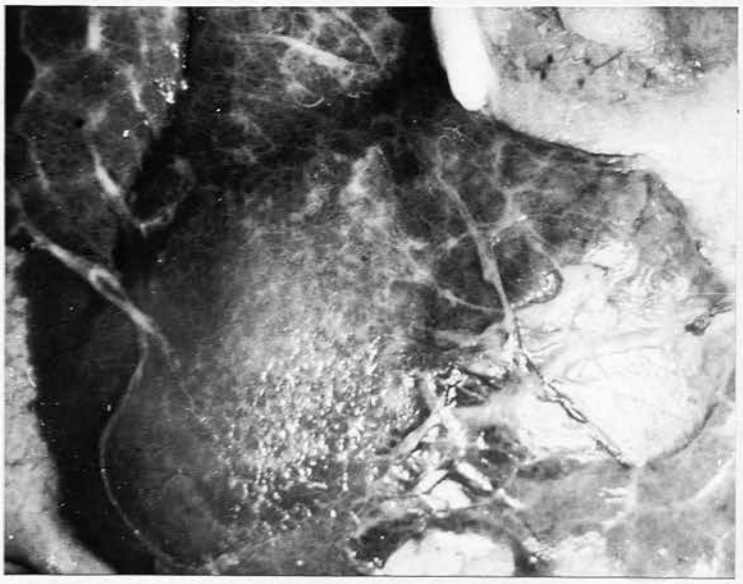


Figure 2. Pulmonary adenomatosis complicated by a fibrinous pleuritis.

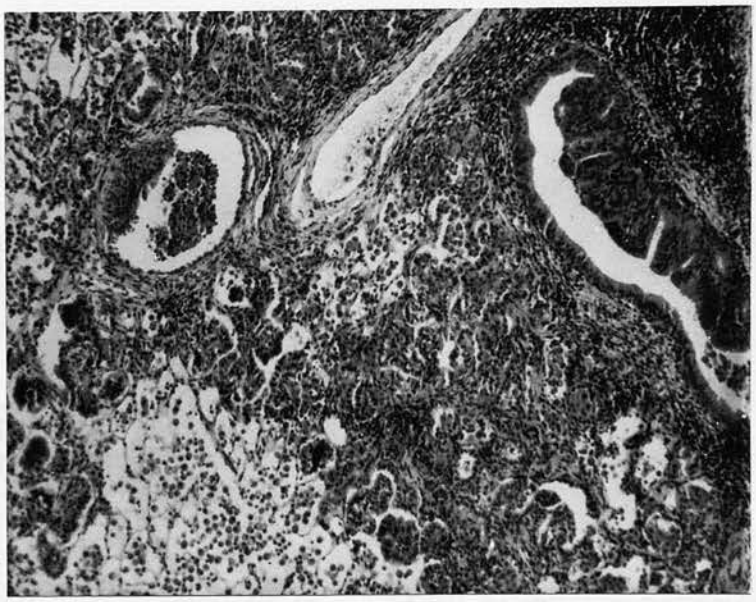


Figure 3. Paraffin section of lung from a case of pulmonary adenomatosis showing proliferation of the bronchiolar epithelium. Note adenomatous foci and free alveolar macrophages in the surrounding alveoli.
H & E X90

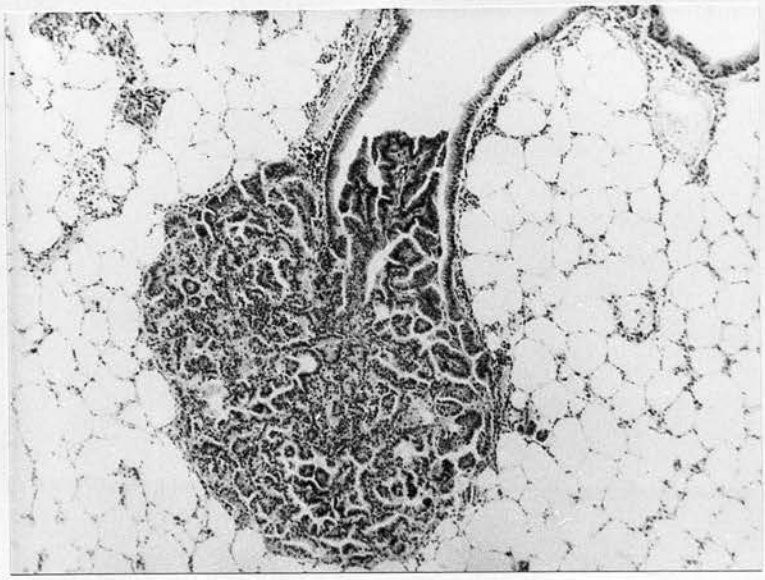


Figure 4. Isolated focus of adenomatous tissue extending into an alveolar duct. The majority of the surrounding alveoli are normal.
H & E X70

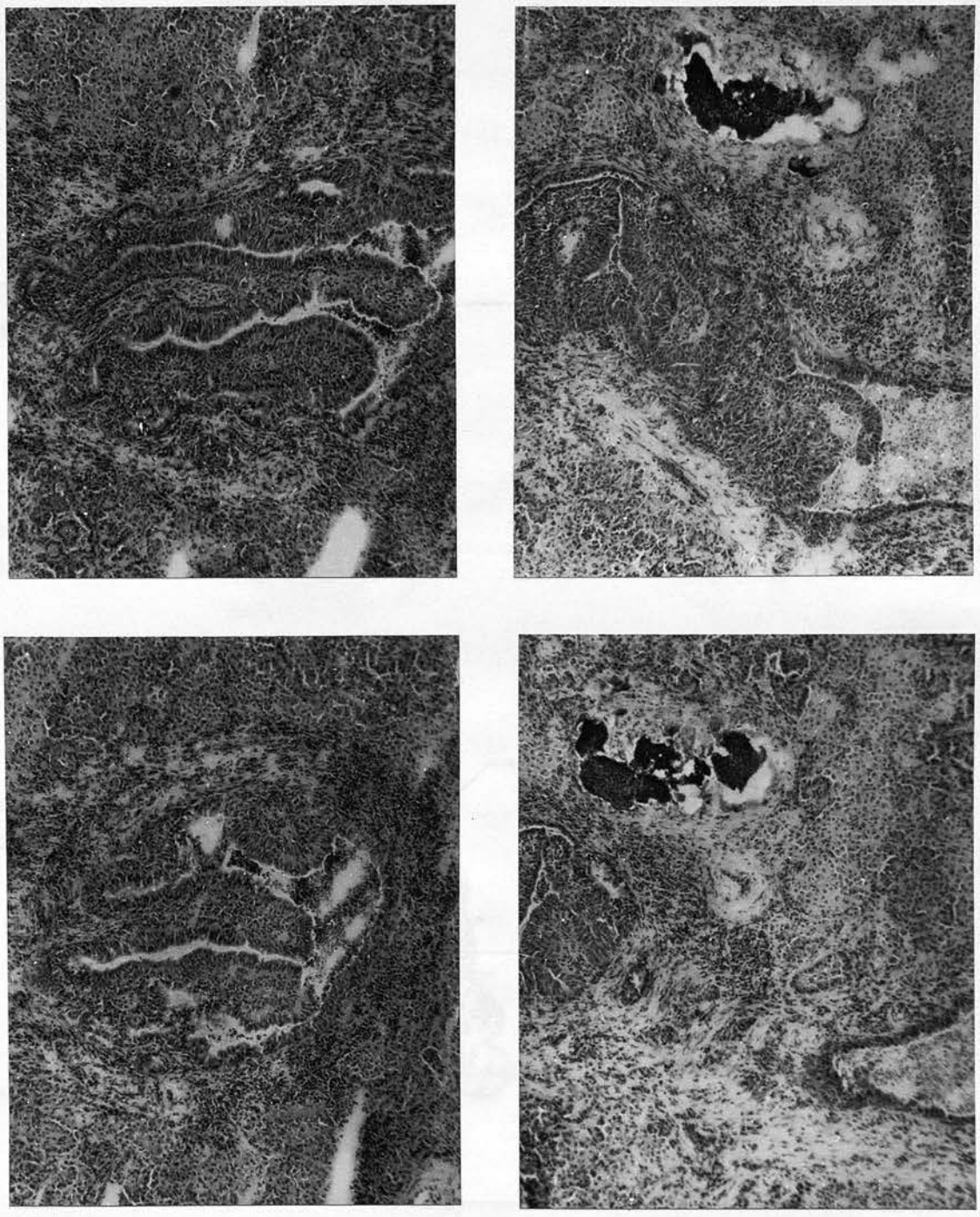


Figure 5. Two fields from a cryostat section of lung from a case of pulmonary adenomatosis are shown above illustrating proliferation of the bronchiolar epithelium. The bottom two photomicrographs are of the same bronchioles ten sections later.

H & E X75

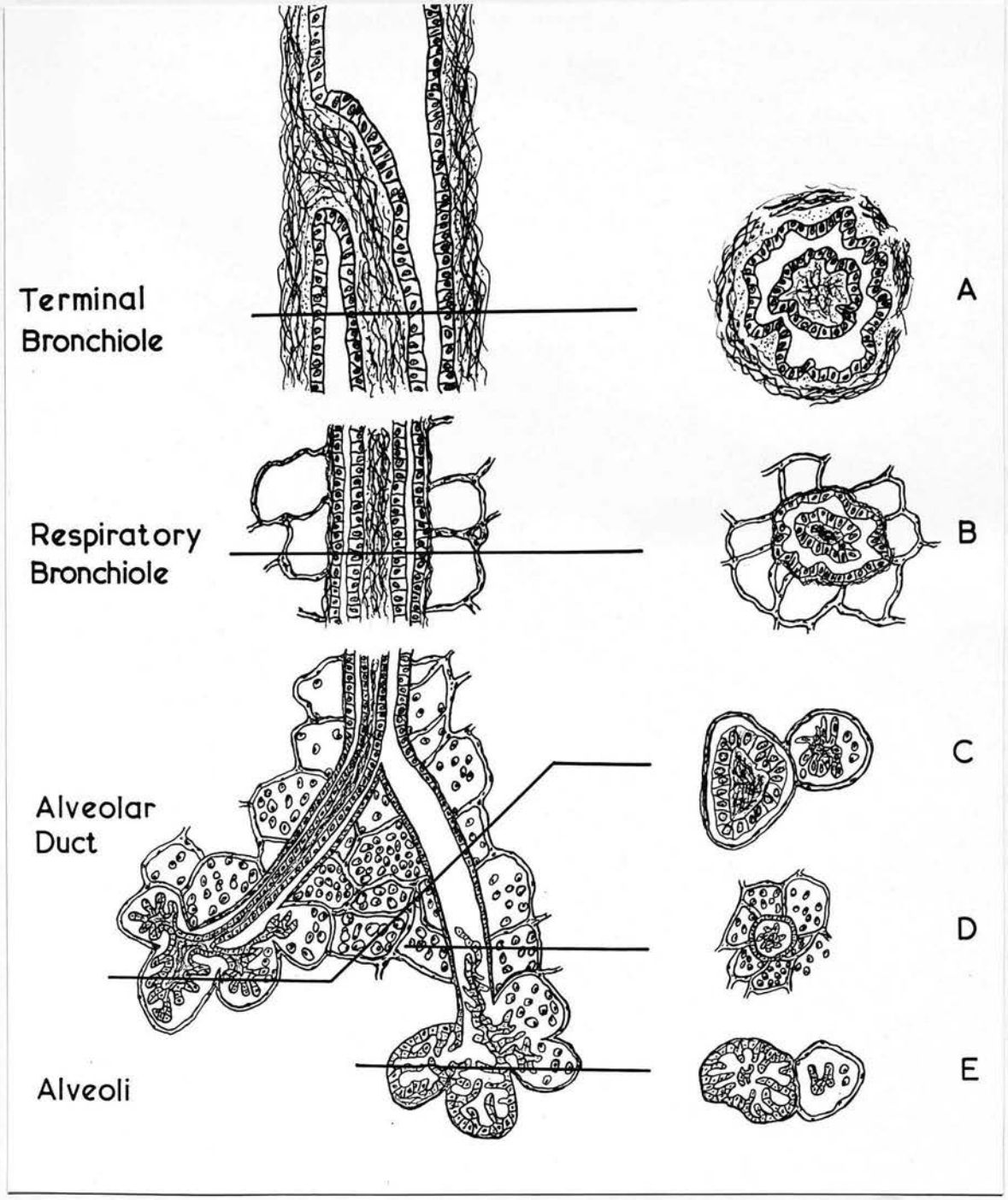


Figure 6. Diagrammatic representation of the lung lesions in pulmonary adenomatosis.

there were differences in the appearance of the adenomatous tissue in alveoli which have been illustrated diagrammatically in Figure 6. In the one type the epithelial proliferations, which often appeared primarily at the junction between alveolar ducts and alveoli, were attached to the alveolar wall and, although they assumed a papillomatous appearance, it was still possible to recognise the lumen of an affected alveolus (Figure 6, E). In the other type, the lining of the alveolus was normal but the lumen contained adenomatous tissue in which the apices of the cells were directed towards the alveolar wall (Figure 6, C). Figure 6 also illustrates how the bronchiolar epithelium can contribute in the formation of adenomatous tissue and why it would not be possible to determine where the adenomatous tissue originated if it was seen in cross sections of affected bronchioles (Figure 6, B, D) or alveoli (Figure 6, C, E). In several lungs, clumps of columnar cells were seen in large blood vessels (Figure 7).

In three sheep which were killed, solitary greyish-red, firm, raised nodules each about one centimetre in diameter were present in the left cardiac lobe of one sheep and in the right and left diaphragmatic lobes of the others (Figure 8). Microscopically, there were well-developed adenomatous foci and macrophage infiltration of the surrounding alveoli. In addition, large giant cells were seen in the surrounding alveoli and dilated bronchioles in one case.

Pulmonary Abscesses

Eight sheep with lung abscesses were examined. Except for an occasional cough the affected sheep did not exhibit specific signs of pneumonia.

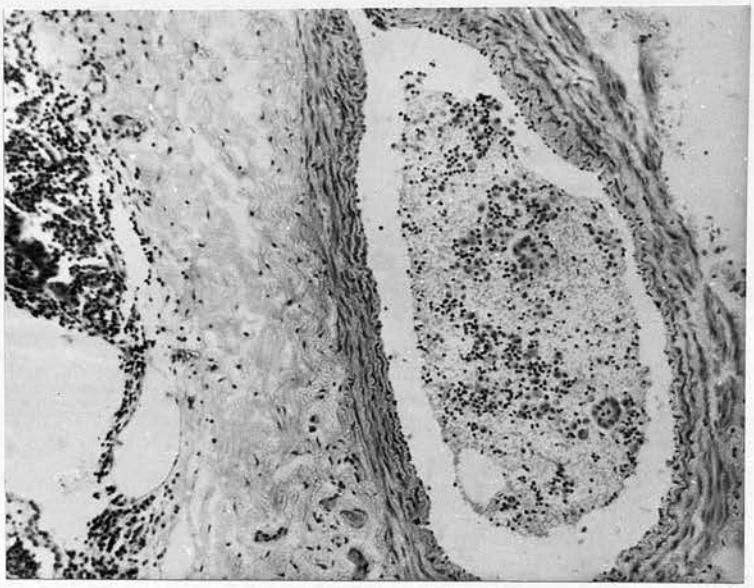


Figure 7. Several adenomato us -like foci in a medium sized artery.

H & E X90

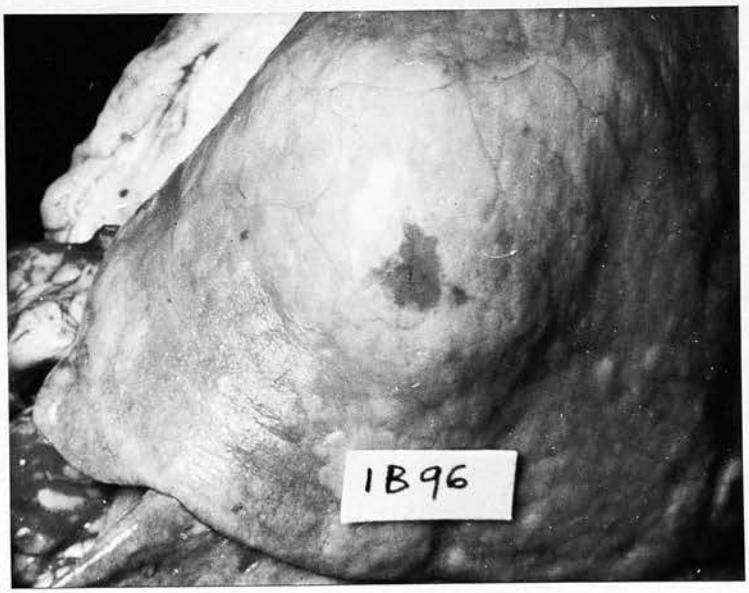


Figure 8. Solitary raised nodule in the posterior dorsal part of the right diaphragmatic lobe.

Usually the history was one of "ill-thrift" and failure to respond to antibiotic treatment. Post mortem, multiple thick-walled abscesses containing greenish pus were scattered mainly throughout the anterior lobes but were sometimes present in the diaphragmatic lobes. Corynebacterium pyogenes was recovered from seven cases while a Group D streptococcus was isolated from the remaining one. Histologically, the abscesses consisted of a central necrotic core surrounded by numerous degenerate and normal-looking neutrophils, with fibrous tissue proliferation at the periphery causing collapse of adjacent alveoli.

Pulmonary aspergillosis

Pulmonary aspergillosis was diagnosed in an eighteen-day-old lamb. Pneumonia was not suspected ante mortem. Clinically the lamb was weak and depressed and had a large subcutaneous abscess near the thoracic inlet, distension of the abdomen and diarrhoea. It failed to respond to antibiotic treatment and was killed one week later. In addition to the large subcutaneous abscess, numerous abscesses were seen in the lungs, liver and spleen. Sphaerophorus (Fusiformis) necrophorus was isolated from one of the hepatic abscesses. Also present in the lungs were numerous, raised, greenish, subpleural foci about two to five millimetres in diameter. Histologically, the larger abscesses in the lungs were composed of a number of smaller ones in the centre of which were large, septate, branching fungal hyphae surrounded by cellular debris, neutrophils and macrophages which in turn were surrounded by epithelioid cells. Between these micro-abscesses there was compression collapse and macrophage infiltration of alveoli. The small subpleural foci

were fungal granulomata as well. The hyphae stained well with the periodic acid-Schiff and Gridley (1953) techniques but not with haematoxylin or eosin.

Aspiration Pneumonia

One lamb was presented for autopsy with the history that it had been recently dosed with liquid paraffin. A major portion of the lungs was consolidated and there was a small amount of fibrinous exudate on the visceral pleura. On section, droplets of oil could be expressed from the lung tissue and after fixation overnight, a thin layer of oil was present on the surface of the formol-saline. Microscopically, there was hyperaemia of the alveolar capillaries and accumulations of a sero-fibrinous exudate mixed with neutrophils and macrophages in the alveoli. Some alveoli also contained micro-colonies of bacteria surrounded by neutrophils while in other areas, there were foci of alveolar necrosis. Circular, unstained spaces, presumably droplets of liquid paraffin, were seen in alveoli and in the lumina of bronchioles surrounded by an exudate composed mainly of neutrophils.

Haematogenous Pneumonia

Pneumonia of haematogenous origin was suspected in two, three-day-old lambs. The lungs were hyperaemic and there were several small foci of red consolidation in the apical, cardiac and diaphragmatic lobes. In one lamb there was interlobular oedema, particularly in the right apical lobe. The liver, spleen, kidneys and small intestine were hyperaemic also. Histologically, the lung lesions were confined to the alveoli and alveolar capillaries. The latter were hyperaemic and some contained bacterial emboli. There was slight

thickening of the interalveolar septa due to macrophage infiltration. A few alveoli contained either free alveolar macrophages or neutrophils. Partial collapse of alveoli was also present.

Because of the absence of inflammatory changes in bronchi, bronchioles and alveolar ducts and since lesions were confined to alveoli and alveolar capillaries, these cases were classified as pneumonia of haematogenous origin.

Pneumonia Characterised by Exudative and Proliferative Lesions

The lung lesions in four sheep were characterised by exudative and proliferative reactions. The pathology of the lesions resembled those caused by PI3 virus or organisms of the Bedsonia group which are described in detail in Sections 2 and 4 respectively. Of the four sheep, two had temperatures over 40.6 degrees centigrade when they were killed and two were found dead. Pasteurella haemolytica was isolated from the lungs of one sheep which was killed. Macroscopically, the apical, cardiac, intermediate and anterior ventral portions of the diaphragmatic lobes were affected. The degree of involvement varied but generally one half to two thirds of the right apical lobe was affected, whereas in the other lobes, the degree of involvement was much less. The affected areas were firm and dark red with a distinct border between affected and normal areas. In one lung there were strands of fibrin overlying the pneumonic areas. In two lungs there were dark red branching bands of collapse of varying thickness in the cardiac lobes. The consolidated areas appeared to surround air passages from which a greyish mucopurulent exudate could be expressed. The pulmonary lymph nodes were enlarged and

congested.

Histologically, two types of lesions were present. The more obvious was an exudative reaction while the other was of a proliferative nature. Except for the presence of a neutrophil exudate in their lumina, the bronchi were normal. A similar exudate was present in many of the bronchioles and in the surrounding alveoli. The bronchiolar epithelium was hyperplastic and, in places, had been invaded by neutrophils (Figure 9). Varying numbers of lymphocytes, macrophages and plasma cells had infiltrated the peribronchial and peribronchiolar tissues but marked lymphocytic "cuffing" was not observed. The alveolar reaction varied in different areas and in different sheep. Most alveoli contained a mixture of serous exudate, neutrophils and macrophages. Occasionally, strands of fibrin were also present. Pseudo-epithelialisation of alveoli was quite common as was syncytium formation, whereas, alveolar epithelialisation was seen less frequently. A thorough search, using a variety of stains for cytoplasmic or nuclear inclusions yielded negative results. Lesions of acute lymphadenitis were present in the pulmonary lymph nodes.

Group 3

Pneumonia Associated with Parainfluenza 3 Virus and Pasteurella haemolytica

For some years outbreaks of respiratory disease have occurred in young sheep soon after their introduction into pens in a covered yard at this Institute. A proportion of the 1966 intake of lambs which developed pneumonia soon after their arrival in the Institute, was selected for a

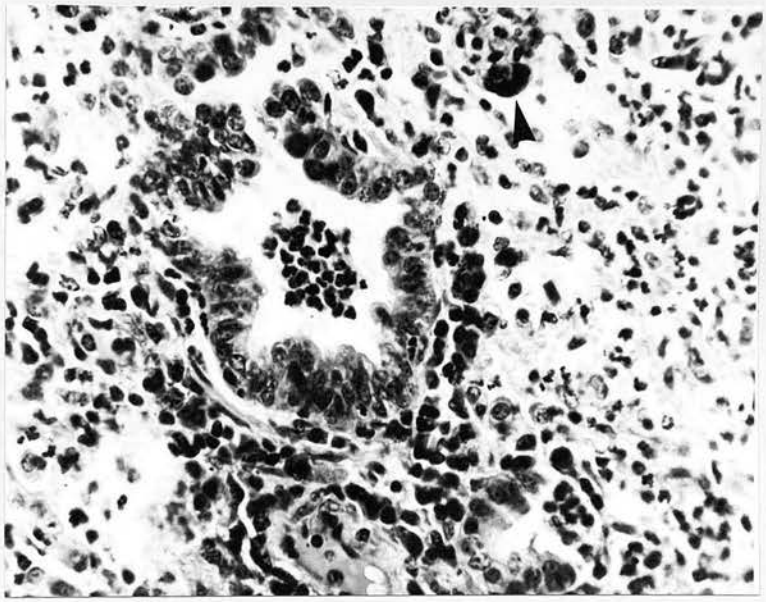


Figure 9. Hyperplasia of the bronchiolar epithelium and syncytium formation (arrow) in an alveolus.
H & E X360

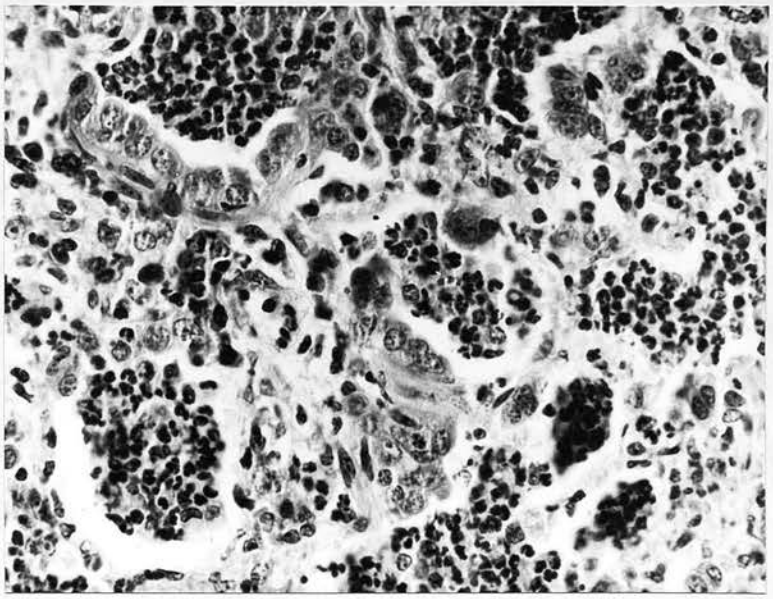


Figure 10. Hyperplasia of the bronchiolar epithelium, syncytium formation and pseudo-epithelialisation of alveoli.
H & E X400

study of the problem, the results of which have been published in the Journal of Comparative Pathology under the title "Isolation of Parainfluenza Virus from the Lungs and Nasal Passages of Sheep Showing Respiratory Disease". The results of virological, serological and bacteriological examinations are summarised in Table 1, and a reprint of the publication is included in the Appendix. (Although there were a number of authors the pathology was done solely by the present writer).

Ten lambs were selected for close examination and of these, four (13X90, 14X00, 14X06, 13X93) were killed on the twelfth, fifteenth and sixteenth days after introduction when temperatures of 41.1 degrees centigrade or greater were recorded. Pasteurella haemolytica was isolated from the lungs of all four, Bedsonia organisms from lambs 14X00 and 14X06, Mycoplasma spp. from lambs 13X90 and 14X00 and PI3 virus from the lungs of the two lambs (13X90 and 14X00) killed on the twelfth day after introduction (Table 1). The pathology of lambs 14X06 and 13X93, killed on the fifteenth and sixteenth days after introduction, were similar to the lesions described previously under the heading "Pneumonia Characterised by Exudative and Proliferative Lesions".

In the two lambs (13X90, 14X00) killed on the twelfth day following their introduction there were macroscopic lesions of pneumonia. In lamb 13X90 all of the right apical lobe and approximately one-third of the cardiac lobes were consolidated. The affected areas were dark red, firm and slightly collapsed. The lesions in the apical and cardiac lobes of the second lamb (14X00) had a similar appearance but the degree of involvement was much less. Also present in lamb 14X00 were dark red branching bands

TABLE 1

PI3 VIRUS, HI ANTIBODY TITRES AND BACTERIOLOGY IN RECENTLY INTRODUCED LAMBS$^{\phi}$

Day after introduction	0		12	14		15	16	28		48	
Lamb No.	HI* titre	Nasal swab	Lung	HI titre	Nasal swab	Lung	Lung	HI titre	Nasal swab	HI titre	Bacteriology (lungs)
13X90	<10	-	V	..	..	..	..	..	..	..	P. haemolytica $A^{\frac{1}{1}}$, $2^{\frac{11}{11}}$ and Mycoplasma sp.
14X00	<10	-	V	..	..	..	..	..	..	..	P. haemolytica A, 2 Bedsoniae and Mycoplasma sp.
14X06	<10	-	..	<10	V	-	..	..	..	..	P. haemolytica A, 6 and Bedsoniae
13X93	<10	-	..	20	-	..	-	..	..	..	P. haemolytica A, 6
14X02	<10	-	..	<10	V	..	..	40	-	40	..
13X98	<10	-	..	<10	V	..	..	80	-	40	..
13X81	<10	-	..	160	-	..	..	640	-	640	..
13X99	<10	-	..	<10	V	..	..	320	-	160	..
14X04	<10	-	..	10	-	..	..	10	-	<10	..
14X01	<10	-	..	<10	-	..	..	40	-	640	..

ϕ A complete account of the results obtained in this investigation will be found in the reprint entitled "Isolation of Parainfluenza Virus from the Lungs and Nasal Passages of Sheep Showing Respiratory Disease" by Hore, Stevenson, Gilmour, Vantsis and Thompson (1968) in the Appendix.

V = PI3 virus isolated
- = No virus isolated

$^{\frac{1}{1}}$ = Biotype
$^{\frac{11}{11}}$ = Serotype

HI* = Haemagglutination titre against PI3 virus expressed as reciprocal of serum dilution.

of alveolar collapse several millimetres in thickness in the cardiac lobes and marked interlobular oedema in the right apical lobe. In both lambs there were areas of red consolidation two to four centimetres in diameter in the posterior parts of the diaphragmatic lobes which on section, revealed large numbers of adult Dictyocaulus filaria in the smaller bronchi.

The histopathological appearance of the dark red areas of consolidation were similar in both lambs except for the presence of interlobular and perivascular oedema in lamb 14X00. The epithelium of the bronchi and bronchioles was hyperplastic and sometimes inflamed or less commonly, necrotic (Figure 10). Within the lumina of the air passages there was a cellular exudate consisting mainly of neutrophils whereas in the surrounding tissues there were moderate numbers of lymphocytes, macrophages and plasma cells. Most alveoli contained either serous fluid or a cellular exudate composed of neutrophils and/or macrophages. In alveoli filled with neutrophils, the interalveolar septa were normal except for hyperaemia of the capillaries, whereas in others the septa were thickened due to increased numbers of macrophages and lymphocytes. Syncytium formation and pseudo-epithelialisation but not alveolar epithelialisation, were common in the latter areas (Figure 10). In one lamb (13X90) giant cell bronchiolitis, acidophilic cytoplasmic inclusions in bronchiolar epithelial cells and occasionally in alveolar syncytia were observed (Figure 11). The shape, size and staining affinity of the inclusions were the same as observed in experimental PI3 virus infections in lambs to be described later (Section 2, Subsection 1).

The dark red sunken areas seen in the cardiac lobes of lamb 14X00 consisted of atelectasis and moderate neutrophil infiltration of the bronchiolar epithelium and alveoli.

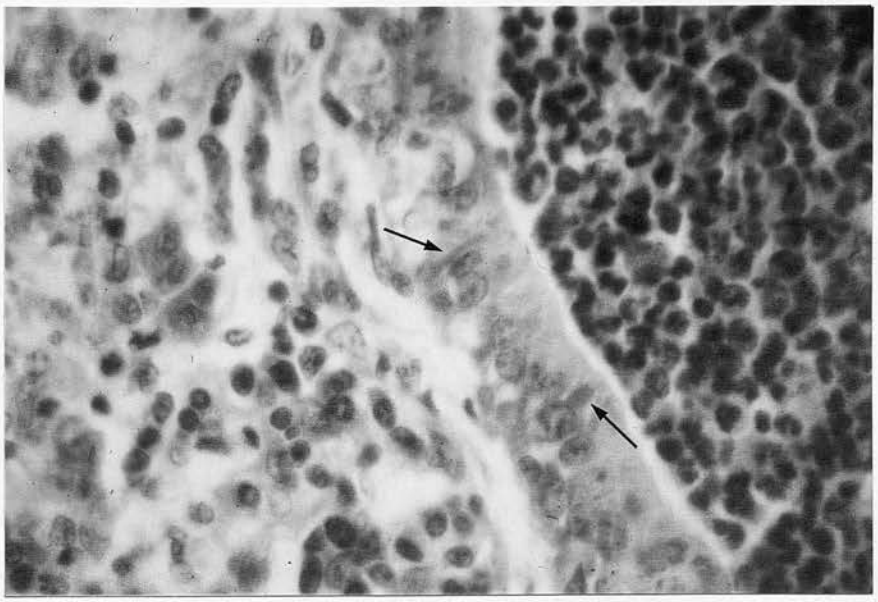


Figure 11. Bronchiole showing numerous acidophilic cytoplasmic inclusions (arrows) in epithelial cells, neutrophil exudate in lumen and mononuclear infiltration of surrounding alveoli.
Pollak X730

Figure 12. Macroscopic appearance of the lung lesions in lambs with "moderate epithelialising pneumonia".

In sections taken from the consolidated areas in the posterior parts of the diaphragmatic lobes the lesions were those of verminous pneumonia.

Group 4

"Moderate Epithelialising Pneumonia"

A morphological type of pneumonia in which alveolar epithelialisation and macrophage infiltration of alveoli were prominent features, was encountered in lambs brought into this Institute in July, 1967 and housed in covered pens.

On arrival, the lambs were in poor condition and many had a dry hacking cough. Seven weeks later one lamb was treated for pneumonia. The following day it and one other lamb were found recumbent and both were killed. Macroscopic lesions of pneumonia were present in both lambs which histologically resembled the lesions of atypical pneumonia and D. filaria infection. Two weeks later another lamb (1H21) was noticed ill and on examination had a temperature of 41.4 degrees centigrade and an increased respiratory rate. It improved following antibiotic treatment but relapsed eight days later. It was killed after having failed to respond to a second treatment. One week later another lamb (1H16) had clinical signs of pneumonia and was also killed. The remaining thirty lambs were killed in October and November, 1967 in connection with the primary research programme (Johne's disease).

Direct tissue cultures from lamb 1H21 and five others (1H11, 9F95, 9F99, 1H07, 1H08) were set up and nuclear inclusions were observed in the cultures from lambs 1H21, 1H11, 9F95 and 9F99 (Hore, 1967). The agent

responsible for these effects has not been further characterised (Hore, 1967).

Since the gross and microscopic lesions in lamb 1H21 differed from the other three lambs they will be described separately, followed by a general description of the lesions in the remaining three lambs.

Macroscopic lesions were confined to the thoracic cavity in all of the lambs. In lamb 1H21 about sixty per cent. of the lungs were affected. The consolidated areas involved primarily the ventral parts of all lobes and were greyish-brown, very firm and slightly sunken. There was a distinct border between affected and normal lung tissue (Figure 12). On section the pneumonic areas had a granular appearance and a greyish exudate could be expressed from the air passages. Several small, subpleural, circular yellowish-green foci were present while in the substance of the affected lung there were small foci of necrosis. No lungworms were seen. The pulmonary lymph nodes were moderately enlarged.

In the other lambs (1H11, 9F95 and 9F99) the degree of involvement was considerably less, the affected areas being confined mainly to the anterior lobes, particularly the right apical lobe. The pneumonic areas were reddish-grey, firm, slightly collapsed and in some, the interlobular septa were very prominent. Linear areas of alveolar collapse were occasionally seen in the cardiac lobes. Lungworm lesions in the posterior parts of the diaphragmatic lobes were common but adult D. filaria were found in the bronchi in only one lamb (9F95).

Histologically, there were two types of lesions in lamb 1H21. One was characterised by proliferation and the other by exudative changes. There was epithelial hyperplasia in some bronchi and the majority contained an

exudate composed of neutrophils and macrophages. In the larger bronchioles the reaction resembled that in the bronchi whereas, in the smaller bronchioles and alveolar ducts, there was marked epithelial hyperplasia and many of these air passages were filled with a cellular exudate (Figure 13). The hyperplastic epithelial cells had large, pale-staining oval nuclei containing one or more large pink nucleoli and pale pink vacuolated cytoplasm. When stained by the periodic acid-Schiff (PAS) technique many of these cells contained PAS-positive granules. Occasionally there were epithelial cells in varying stages of degeneration ranging from vacuolation of the cytoplasm and pink-staining of nuclei (H & E) to complete necrosis. Peribronchial, peribronchiolar and perivascular lymphocytic and plasma cell infiltration varied from none to moderate.

The alveolar reaction was unique. Many alveoli contained neutrophils and a few necrotic cells but the major change was of a proliferative nature. The majority of alveoli were packed with large pale-staining mononuclear cells, the appearance of which resembled tissue culture monolayers (Figure 13). Some of the cells were lying free in alveoli while most appeared to be attached to the alveolar walls. Individual alveoli were difficult to discern in sections stained with H & E but in reticulin-stained sections it was possible to see that many alveoli were lined by a continuous row of cells, with macrophage-like cells and neutrophils lying free in the alveolar spaces (Figure 14).

Some of the free mononuclear cells were larger than the attached cells and had an eccentrically placed oval nucleus, pink-staining cytoplasm and sometimes contained ingested neutrophils or cellular debris. However, other cells were also present in the alveoli which could not be differentiated

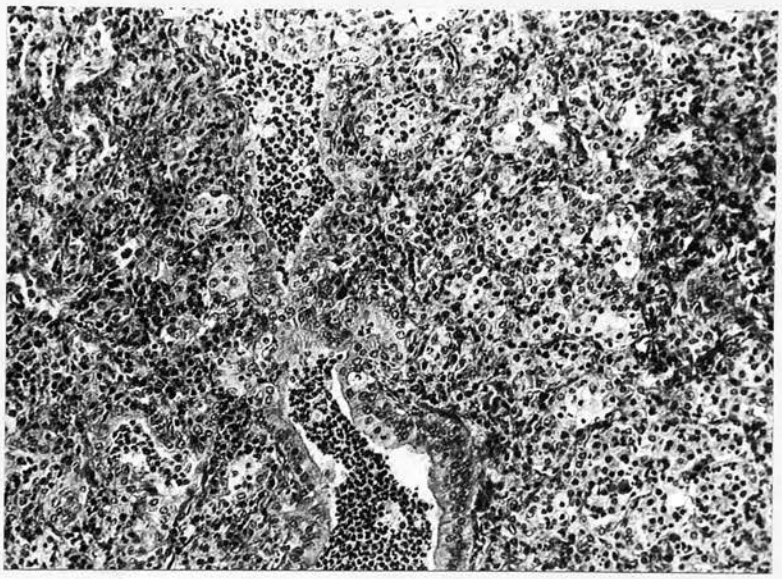


Figure 13. Neutrophil exudate within a bronchiole showing epithelial hyperplasia. There is epithelialisation of the surrounding alveoli.
Pollak X155

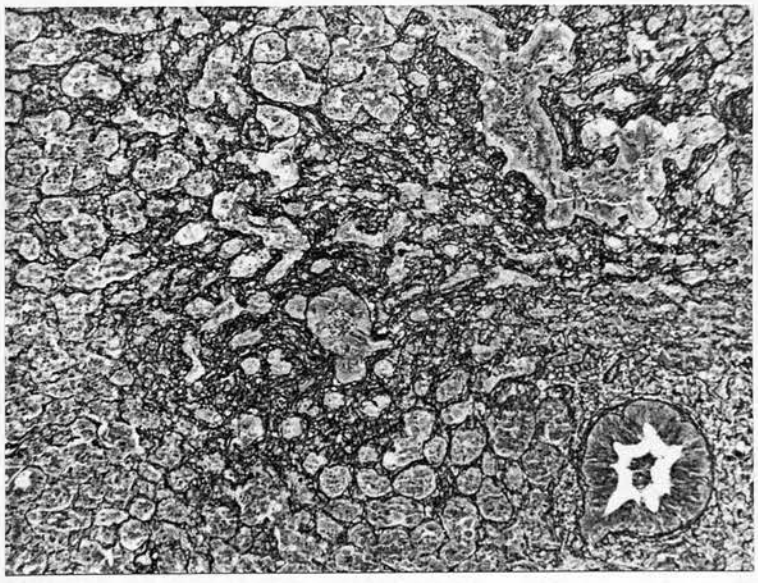


Figure 14. Hyperplasia of the reticulin network surrounding small bronchioles. Note that the majority of the cells are on the lumen side of the alveolar reticulin fibres.
Silver reticulin method of Slidders et al., (1958) X75

from the attached mononuclear cells and which resembled the bronchiolar epithelial cells. In PAS preparations, a proportion of each type of cell contained PAS-positive material in their cytoplasm. In other areas where the interalveolar septa appeared to be normal, the alveoli contained numerous free alveolar macrophages (Figure 15) such as are seen in cases of pulmonary adenomatosis (Figure 3). Around bronchioles and alveoli showing obvious epithelialisation there was a marked increase in the number of reticulin fibres (Figure 14). Mononuclear cells both on the lumen side of alveoli and within the interalveolar septa were numerous. In other areas, particularly where there were recognisable alveoli, there was little or no hyperplasia of the reticulin network. The small subpleural yellowish foci seen macroscopically were areas of necrosis containing small colonies of bacteria surrounded by a dense zone of neutrophils. Some of the alveoli adjacent to these necrotic foci had small syncytia (three to four cells) sprouting from their walls or small (two to three nuclei) free alveolar giant cells while others contained a bright pink serous exudate (H & E).

The reaction in the pulmonary lymph nodes was characterised by lympho-follicular hyperplasia, large numbers of plasma cells and moderate neutrophil and eosinophil cell infiltration.

Quantitative rather than qualitative differences were seen in the remaining three lambs. In these lambs bronchiolar epithelial hyperplasia was less obvious whereas peribronchiolar lymphocytic hyperplasia was more marked. The number of alveoli showing epithelialisation was fewer and tended to involve alveoli adjacent to bronchioles (Figure 16). Many alveoli, often showing only slight thickening of the interalveolar septa,

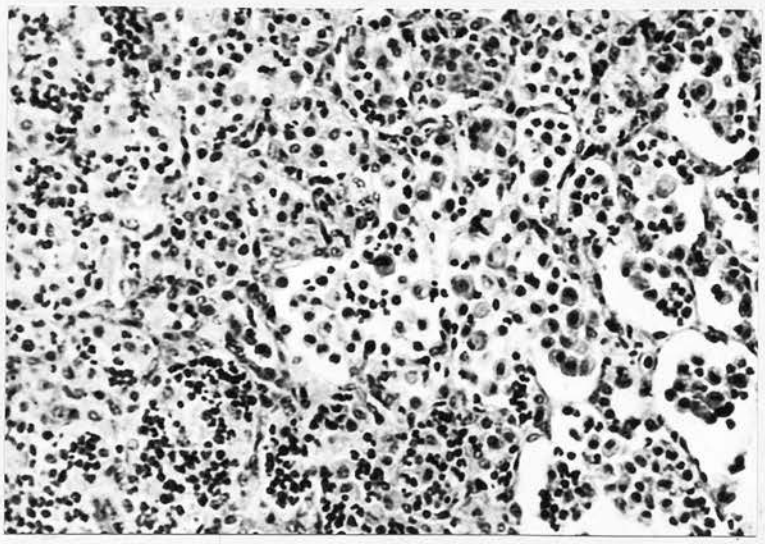


Figure 15. Free alveolar macrophages of the type commonly seen in pulmonary adenomatosis as shown in Figure 3.
H & E X280

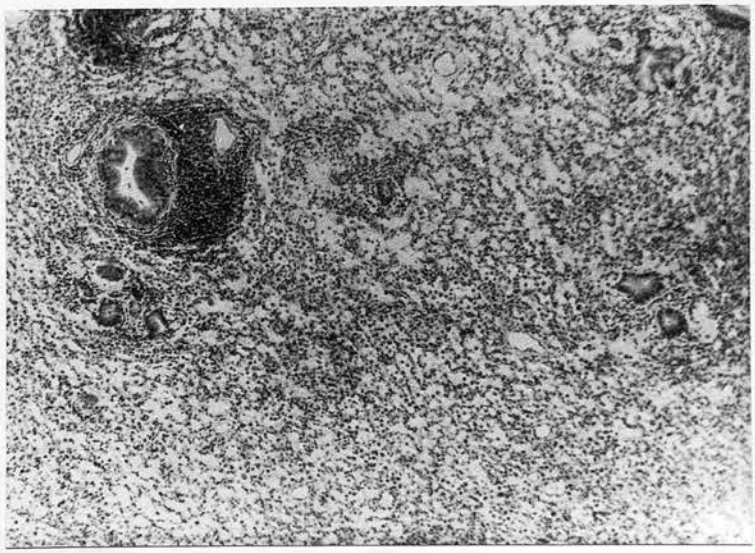


Figure 16. Focal alveolar epithelialisation. The surrounding alveoli contain serous fluid and macrophages. Peribronchiolar and perivascular lymphocytic hyperplasia is also evident.
H & E X70

contained large numbers of mononuclear cells and a serous exudate (Figure 16). The majority of the mononuclear cells were lying free in the alveoli but some were attached to the alveolar walls.

DISCUSSION

The sheep at the Moredun Institute were chosen for several reasons. Both conventional and intensive methods of sheep husbandry are employed and pneumonia is a common cause of death in these sheep. Also, experience with acute respiratory disease in calves has shown that many of the specific features associated with the various agents are of transient duration so that affected animals must be killed soon after the first signs of illness are observed if pathognomonic lesions are to be found post mortem. Assuming that a similar situation existed in sheep, then access to sheep where economics was a secondary consideration was required.

A system of classifying the different types of respiratory disease encountered in the present investigation was required. A classification based on aetiological agents would be ideal but as yet, there are still a number of types of pneumonia whose aetiology remains unknown. If the pneumonias are classified on an anatomical basis there is the possibility that similar lesions might be caused by more than one agent. Therefore, a classification based on both morphology and aetiology was used in this survey, a fact made possible by co-operation between pathologist and microbiologist.

Atypical pneumonia was observed in thirty-five of sixty-four randomly

killed sheep. The macroscopic and histological lesions were in accordance with those of the original descriptions (Stamp and Nisbet, 1963; Gilmour and Brotherston, 1963). Such a high incidence was not anticipated. However, when it is considered that the histological lesions range from peribronchiolar lymphocytic hyperplasia alone, to marked interstitial pneumonia and the fact that the aetiology of ovine atypical pneumonia is not known (thus it may represent the end result of one or more agents), such an occurrence is not unreasonable.

Most histopathological descriptions of so-called "enzootic-pneumonia" associated with a Pasteurella infection state that alveolar macrophages are the predominant cell type and that neutrophils are less frequently seen (Montgomerie et al., 1938; Stamp and Nisbet, 1963). This finding is not confined to sheep but also includes the fibrinous pneumonias of other ruminants and pigs caused by Pasteurella species (Jubb and Kennedy, 1963). In other respiratory diseases complicated by a P. haemolytica infection, the histological lesions associated with such an infection are characterised by an acute exudative reaction in which neutrophils are the predominant cell type (Hore et al., 1968). The reason for this discrepancy is obscure. Similar serotypes of P. haemolytica can be recovered from either condition so the cause of the different cellular reaction is unlikely to be due to the organism, unless it is associated with the number of organisms initiating the infection. If the number of organisms is large and the onset sudden then it is possible that the mononuclear cells seen are those which are normally present in the interalveolar septa (i.e. septal cells). If so, then this would be the primary defensive mechanism and other cells of haematogenous origin would represent the second line of defence.

Methods by which the adenomatous tissue spreads within the lung were observed in several sheep. The initial lesion begins in one or numerous foci (Stamp, 1967) and multicentric spread occurs throughout the lung (Stamp and Nisbet, 1963). It is assumed that the isolated foci spread by increasing in size, affecting adjacent alveoli, until the lesions become confluent and that on occasion, fragments of adenomatous tissue break away and lodge in other sites. Another method of spread which was demonstrated in this investigation was by the intrabronchiolar route. Cowdry (1925b) and De Kock (1929a) state that the bronchiolar epithelium also contributes to the adenomatous formations and several of De Kock's photomicrographs illustrate this lesion. Cuba-Caparo et al., (1961) were of the opinion that the epithelial component in pulmonary adenomatosis was of bronchiolar origin since cilia and PAS-positive material, assumed to be mucus, could be demonstrated in adenomatous foci.

The history, clinical signs and pathology of the lung lesions in the lamb with pulmonary aspergillosis were similar to those reported by Austwick et al., (1960) and Gracey and Baxter (1961). Unfortunately, sections of liver and spleen were not taken for histological examination since at the time it was thought that the lesions in those organs were caused by S. necrophorus. On reflection however, it is possible that some of the abscesses may have been caused by an aspergillus infection since they have been recorded in similar organs in cattle (Bendixen, 1926).

Two cases of ovine pulmonary aspergillosis, diagnosed solely on histological grounds, have been encountered in lambs at this Institute previously. They are noteworthy in that contrary to the findings of

Austwick et al., (1960), Gracey and Baxter (1961) and in the case in this survey, giant cells were present in both cases. One of the lambs was five weeks old (sections kindly supplied by D. I. Nisbet) and the lesions were similar to those described by Austwick et al., (1960) but for the presence of an occasional giant cell at the periphery of the nodules. In the other lamb, which was seven months old, the granulomata were large and tended to coalesce. They were composed of neutrophils, macrophages, plasma cells, epithelioid cells and numerous giant cells, many of which contained engulfed hyphae. No acid-fast bacteria or helminth larvae were seen.

It has been suggested that pulmonary aspergillosis is more common than is generally realised (Austwick et al., 1960). Subclinical infections are difficult to diagnose macroscopically because of the similarity of the lung nodules caused by the fungus and those caused by infection with Muellerius capillaris (Andersen, 1927). More information is required regarding the incidence and epizootiology of aspergillosis in lambs since under intensive husbandry conditions it could become an important economic problem.

The macroscopic lesions of the pneumonia characterised by exudative and proliferative reactions resembled those of ovine atypical pneumonia (Gilmour and Brotherston, 1963; Stamp and Nisbet, 1963), the aetiology of which is unknown. It was not possible to incriminate a particular agent since somewhat similar changes were observed in lambs experimentally infected with organisms of the Bedsonia group (Dungworth and Cordy, 1962a; Section 4), PI3 virus (Section 2, Subsections 1 and 2) and in a natural outbreak of pneumonia in recently introduced lambs associated with PI3 virus and P. haemolytica.

The respiratory disease encountered in recently introduced lambs was similar to that recorded by Gilmour and Brotherston (1963). These authors described the clinical and pathological findings in an outbreak of pneumonia in sheep which had also been recently introduced into this Institute and suggested that the sporadic cases of acute pneumonia may have been superimposed on subclinical pneumonias of interstitial or "cuffing" types. The aetiology of the pneumonia was not determined. In the present outbreak five agents (namely, P. haemolytica, PI3 virus, Bedsonia organisms, Mycoplasma spp. and D. filaria) were isolated from the lungs of four sheep which presented difficulties in determining the cause or causes of the respiratory disease. Before the results of the cultural and serological examinations were known it was postulated, using pathological criteria only, that the most likely causes of the outbreak were PI3 virus and P. haemolytica infections. (This assumption was later confirmed by Hore et al., (1968) on the basis of their clinical, bacteriological, virological and pathological findings (see Appendix)).

Lungworms were eliminated as they would not be expected to initiate pneumonia in lambs which had been housed for a minimum of twelve days and in any case the lesions, except in the posterior diaphragmatic lobes, were not typical of a lungworm infection, either macroscopically or histologically.

Mycoplasma spp. have yet to be shown to be capable of producing pneumonia in sheep (Greig, 1955; Boidin et al., 1958; Hamdy and Pounden, 1959) and for this reason they were excluded. Therefore, three known lung pathogens of sheep remained.

The microscopic lung lesions bore some resemblance to those produced by organisms of the Bedsonia group (Dungworth and Cordy, 1962a, b) but as there

are no pathognomonic features in Bedsonia infections in lambs other than demonstration of the organisms (Dungworth and Cordy, 1962a) and since similar lesions may be seen in experimental PI3 virus infections in young lambs (Section 2, Subsections 1 and 2) and in older lambs (unpublished observation), Bedsonia organisms could not be definitely incriminated.

In addition to the non-specific lesions such as bronchiolar epithelial hyperplasia, pseudo-epithelialisation and alveolar epithelialisation which were seen in all of the lungs and also occur in experimental PI3 virus infections (Section 2, Subsections 1 and 2), acidophilic cytoplasmic inclusions within bronchiolar epithelial cells and occasionally in alveolar syncytia were observed in one lamb (13X90). These inclusions are pathognomonic but transient features of experimental PI3 virus infection in lambs (Section 2, Subsection 1). Inclusions and giant cell bronchiolitis were not seen in the remaining three lambs which were killed. This suggests a PI3 virus infection of more than eight days duration since in experimentally infected lambs (Section 2, Subsections 1 and 2) acidophilic cytoplasmic inclusions are not observed after this time. Therefore, the proliferative reaction seen in the lungs of these lambs was most likely the result of a PI3 virus infection.

The acute exudative lesion characterised by hyperaemia of the alveolar capillaries, serous exudation and neutrophil infiltration of the air passages and alveoli are pathological changes usually associated with a bacterial agent. The organism most commonly isolated from cases of ovine pneumonia is P. haemolytica (Ministry of Agriculture, Fisheries and Food, 1964a). It is unlikely that P. haemolytica was the sole cause of this outbreak because of the two different reactions seen histologically and also because it has yet to

be conclusively proven that P. haemolytica is a primary lung pathogen of sheep. Smith (1964) and Biberstein et al., (1967) were able to produce pneumonia in sheep inoculated experimentally with P. haemolytica only when the intrabronchial route and enormous numbers of bacteria were used. Other workers (Montgomerie et al., 1938; Salisbury, 1957; Downey, 1957) have suggested that P. haemolytica is secondary to a primary virus infection. Analagous to this is shipping fever of cattle, a name long used in the United States of America and Canada to denote a clinical condition usually, but not necessarily, affecting recently transported cattle. Prior to the isolation of a bovine strain of PI3 virus (Reisinger et al., 1959) it was generally thought that shipping fever was primarily a pasteurellosis. It has now been shown that the severe clinical disease is the result of an interaction between Pasteurella spp. and PI3 virus (Heddleston et al., 1962; Hetrick et al., 1963; Baldwin et al., 1967). Similar experimental evidence for sheep is lacking but field observations and the fact that experimentally it was shown that PI3 virus was capable of causing damage which would interfere with the defence mechanisms of the lungs of lambs (Section 2, Subsections 1 and 2), suggest that the same might apply to sheep. This hypothesis has not been proven as it would require a large number of hysterectomy-derived, colostrum-deprived lambs and strict isolation facilities which, at the time, were not available.

While this investigation was in progress several portions of consolidated lung from an extensively managed lamb were submitted for examination. Histologically, the lesions consisted of an acute exudative reaction characterised by serous effusion and neutrophil infiltration of alveoli, as well as increased numbers of macrophages, pseudo-epithelialisation of

alveoli, discrete adenomatous foci and the presence of acidophilic cytoplasmic inclusions in bronchiolar and alveolar epithelial cells and in the adenomatous cells (Figure 17). The histological diagnosis was pulmonary adenomatosis complicated by a PI3 virus infection and a bacterial infection, probably due to P. haemolytica. Parainfluenza 3 virus was subsequently isolated from the lung (Hore, 1968) but prior antibiotic treatment precluded the recovery of bacteria.

The type of pneumonia tentatively called moderate epithelialising pneumonia (MEP) was seen in a group of sheep of approximately the same age and which had a similar history except that some of them were being used in a Johne's disease experiment. The poor condition and dry hacking cough seen initially was attributed to a heavy D. filaria infection. When two of these lambs died, lesions of atypical pneumonia and a lungworm infection were present.

The significance of the nuclear inclusions seen in direct tissue cultures from the four lambs is not known. No nuclear inclusions were seen in histological preparations of these lungs but similar inclusions are found in pulmonary macrophage cultures from the lungs of sheep with pulmonary adenomatosis (Mackay, 1968).

The macroscopic and histological lung lesions in the three lambs killed after lamb 1H21 were similar to those seen in some cases of atypical pneumonia. Although the aetiology of atypical pneumonia is not definitely known lesions resembling those of atypical pneumonia have been produced experimentally in lambs by the inoculation of organisms of the Bedsonia group (Dungworth and Cordy, 1962a). Also, Bedsonia agents have been isolated from the lungs of

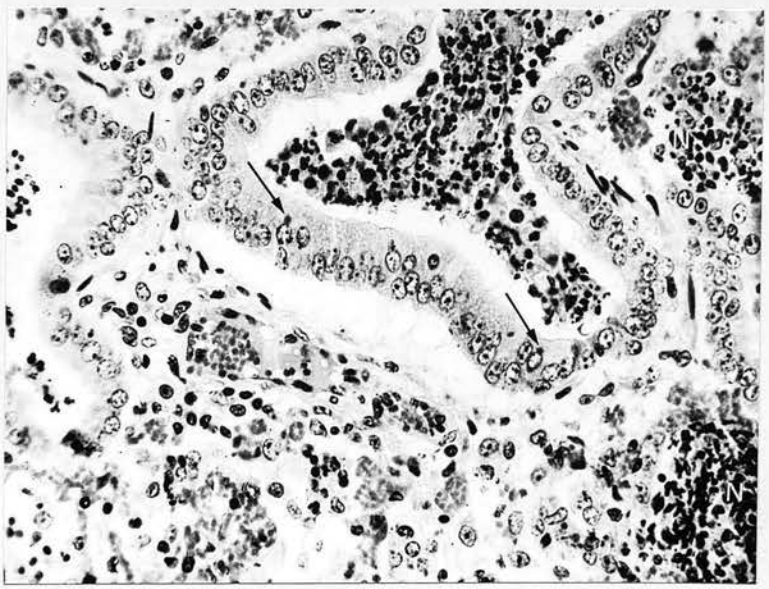


Figure 17. Acidophilic cytoplasmic inclusions (arrows) in the epithelial cells of a bronchiole which is filled with a cellular exudate. Surrounding the bronchiole there are necrotic foci (N) and hyperaemia of the alveolar capillaries.
Pollak X330

sheep with atypical pneumonia (Foggie, 1967). In lamb 1H21 the macroscopic and histological lesions were unlike those observed in cases of atypical pneumonia. Somewhat similar macroscopic lesions have occasionally been seen in cases of pulmonary adenomatosis and the proliferative reaction in the lungs of lamb 1H21 resembled more the adenomatous lesions seen in pulmonary adenomatosis than the hyperplastic changes as described for either atypical pneumonia (Stamp and Nisbet, 1963) or experimental Bedsonia infection (Dungworth and Cordy, 1962a). Since the hyperplastic reaction was more marked than that commonly seen in atypical pneumonia but not as pronounced as that seen in pulmonary adenomatosis the possibility exists that the lesions in these lambs (particularly lamb 1H21) represent an earlier stage of pulmonary adenomatosis.

SECTION 2

INOCULATION OF LAMBS WITH AN OVINE STRAIN OF PARAINFLUENZA 3 VIRUS

Subsection 1. The Acute Phase

INTRODUCTION

Parainfluenza 3 (PI3) virus has been isolated from the upper respiratory tract of lambs in Scotland (Hore, 1966) and in Canada (Ditchfield, 1966). In both instances the isolations were associated with respiratory disease. Serological evidence from limited surveys in America (Fischman, 1965, 1967; Woods et al., 1965a; Howe, Woods and Marquis, 1966), Australia (St. George and French, 1966) and Egypt (Singh and Ata, 1967) indicate that PI3 virus is widespread in sheep and therefore may be of importance as a disease entity in this species.

In Britain, Dawson et al., (1965) and Omar et al., (1966) have shown that PI3 virus is capable of causing lower respiratory tract disease in calves in the absence of other known respiratory pathogens. Woods et al., (1965a) infected one lamb with the bovine Illinois 811 strain of PI3 virus and recovered virus from lung tissue of this and a second lamb held in contact.

With the isolation of an ovine strain (G2) of PI3 virus at Moredun Institute (Hore, 1966) the role of this agent as a possible lung pathogen in sheep was of interest. This section describes the experimental disease produced in lambs inoculated with the ovine G2 strain of PI3 virus. Some aspects of the present work were the subject of a

preliminary communication entitled "Experimental Virus Pneumonia in Lambs"; a reprint of which is included in the Appendix.

MATERIALS AND METHODS

Lambs

Lambs were obtained at birth (colostrum-deprived) or in six cases (98, 97, 572, 945, 944 and 482) immediately following a colostral feed. They were housed and fed as described under General Materials and Methods.

Lambs were inoculated twenty-four to forty-eight hours after birth. Fifteen lambs (including four fed colostrum) were each inoculated with two millilitres of virus-infected tissue culture harvest fluid intranasally and a further three millilitres of virus was injected into the lower part of the trachea as described under General Materials and Methods.

Seven lambs (two fed colostrum) were inoculated with virus-free tissue culture harvest fluid in a similar manner for control purposes. The control group was housed separately.

Virus-inoculated lambs were killed daily from the third to tenth days after inoculation and those in the control group were killed on the fifth, sixth, seventh and ninth days.

Virus

The G2 strain of PI3 virus, used in this and subsequent experiments where an ovine strain was required, was originally isolated from the

nasal passages of an intensively-reared lamb (Hore, 1966). Its physico-chemical, cultural, haemagglutinating and structural characteristics as well as its serological relationship to human and bovine strains of PI3 virus have been examined (Hore, 1968).

The inoculum for thirteen lambs was in the form of harvest fluid from the fourth passage of virus in ovine embryo kidney cultures; the infectivity titre was $10^{5.0}$ $TCID_{50}$ per millilitre. The experiment was later repeated when two lambs (71 and 72) in a second group were given fourth passage G2 strain virus which had an infectivity titre of $10^{6.2}$ $TCID_{50}$ per millilitre. Two control lambs were given virus-free ovine embryo kidney culture harvest fluid at the same time.

The inoculum for the first group of thirteen lambs was at a dilution of at least 10^{-7} of the nasal swab fluid from which virus was originally isolated. In the second group the inoculum was at a dilution of 10^{-14} of the original material.

Before use, both virus-infected and virus-free inocula were centrifuged at 2000 g for fifteen minutes to remove cellular material.

Recovery of Virus

The recovery of virus from the nasal passages and lungs was carried out by Dr. D. E. Hore and the results are summarised in Table 2.

Pathology

The procedures used have been described under General Materials

TABLE 2

RECOVERY OF VIRUS AND DEMONSTRATION OF CYTOPLASMIC INCLUSIONS IN THE LUNGS OF LAMBS INFECTED WITH THE OVINE G2 STRAIN OF PI3 VIRUS†

Lamb No.	Colostral Status	Nasal Swabs Days PI††	Days after inoculation	LUNG	
				Virus	Cytoplasmic Inclusions
413	D	+ (3)	3	+	*
881	D	+ (3,4)	4	+	*
877	D	+ (3,4)	5	+	*
426	D	+ (3,4)	5	+	*
572	F	+ (3,4)	6	+	*
412	D	+ (3,4)	6	+	*
71	D	+ (3,4)	6	+	*
72	D	+ (3,4)	6	+	*
878	D	+ (3,4)	7	+	. .
945	F	+ (3,4)	7	+	. .
876	D	+ (3,4)	8	-	. .
425	D	+ (3,4)	8	+	*
944	F	+ (3,4)	8	+	*
482	F	+ (3,4)	9	+	. .
415	D	+ (3,4)	10	-	. .

† = Modified from Hore and Stevenson (1968).

PI†† = Post Inoculation.

D = Colostrum-deprived

F = colostrum-fed

\+ = virus recovered

* = acidophilic cytoplasmic inclusions observed

\- = no virus recovered

. . = no acidophilic cytoplasmic inclusions observed

and Methods.

RESULTS

Clinical Observations

Appetite and respiration remained normal in each of the lambs killed before the fifth day. A mild mucoid nasal discharge appeared in all the lambs after forty-eight to seventy-two hours and generally persisted for the period during which virus was recovered. The nasal mucus obtained during most of this period contained more cellular material than that from normal lambs.

A slight transient rise in temperature (0.5 to 0.8 degree centigrade) occurred at the time of onset of nasal discharge in nine lambs. There was a further more pronounced rise (0.8 to 1.7 degrees centigrade) on the fourth to sixth days in eight lambs (Figure 18). At about the same time they developed a cough and showed respiratory distress particularly during feeding. One lamb (945) developed a bilateral ocular discharge on the fifth and sixth days.

Macroscopic Pathology

Upper Respiratory Tract

In both the control and infected groups the nasal and turbinate mucosae were hyperaemic and a mucoid or mucopurulent exudate frequently

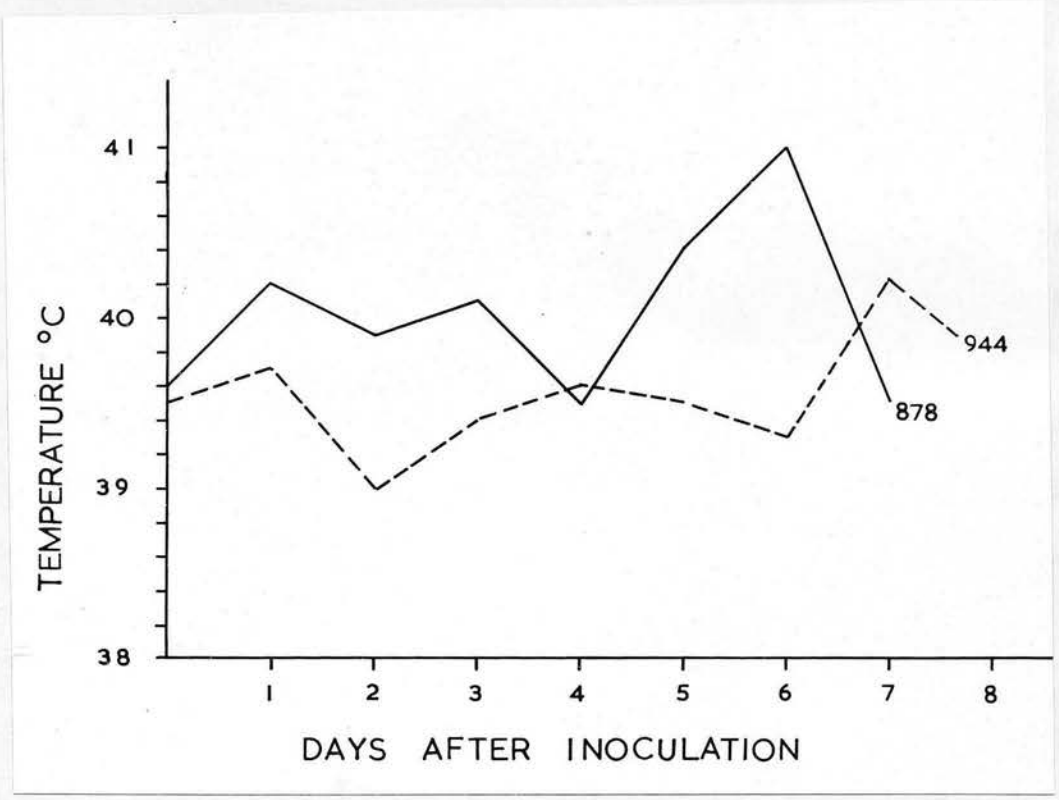


Figure 18. Temperature response in two lambs inoculated with the ovine G2 strain of PI3 virus.

containing small amounts of blood, was present in the nasal passages of the majority of lambs. There were no significant tracheal lesions in any of the lambs.

Lungs

No lesions, apart from slight thickening of the pleura over the posterior dorsal aspect of the diaphragmatic lobes in one lamb, were observed in lungs from the control group (Figure 19).

Lambs inoculated with PI3 virus had macroscopic lesions of pneumonia in all lobes of the lung, but only the right apical lobe was constantly involved. The distribution of areas of consolidation in the virus-inoculated lambs is shown in Figure 20. Initially the affected areas, which were dull red, atelectatic and consolidated, consisted of small linear areas gradually increasing in size (Figures 21, 22). On cross section the consolidated areas were quite extensive and appeared to follow the smaller bronchi and bronchioles. By the eighth day after inoculation maximum lesions were present (Figures 23, 24). The consolidated areas were now reddish-brown, with small greyish, slightly raised foci being evident on their cut surface. In two lambs killed on the ninth and tenth days the macroscopic lesions were quite small, resembling those seen in the lamb killed three days after inoculation.

Pulmonary Lymph Nodes

The mediastinal, left and right bronchial lymph nodes were normal

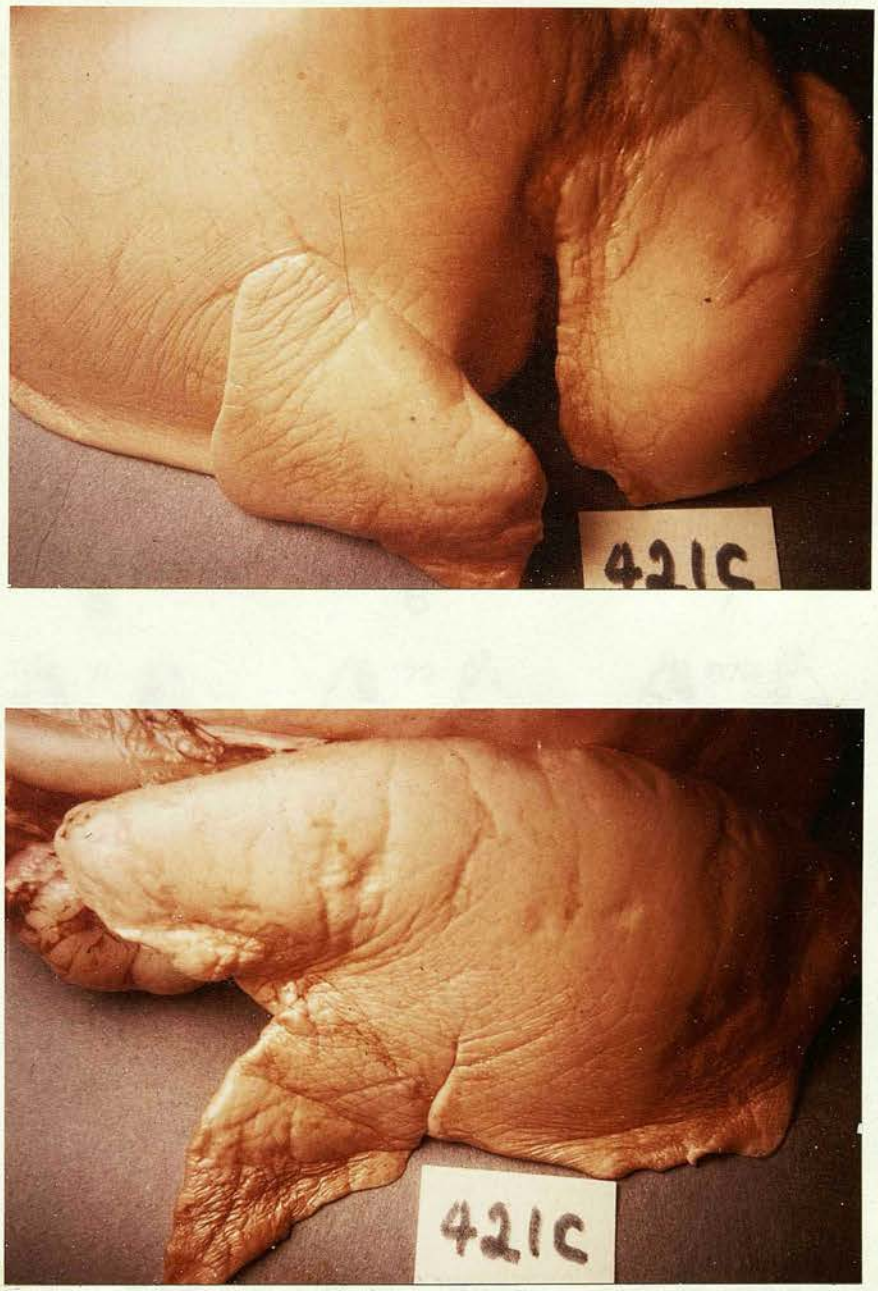


Figure 19. Macroscopic appearance of the lungs of a lamb inoculated with virus-free tissue culture harvest fluid.

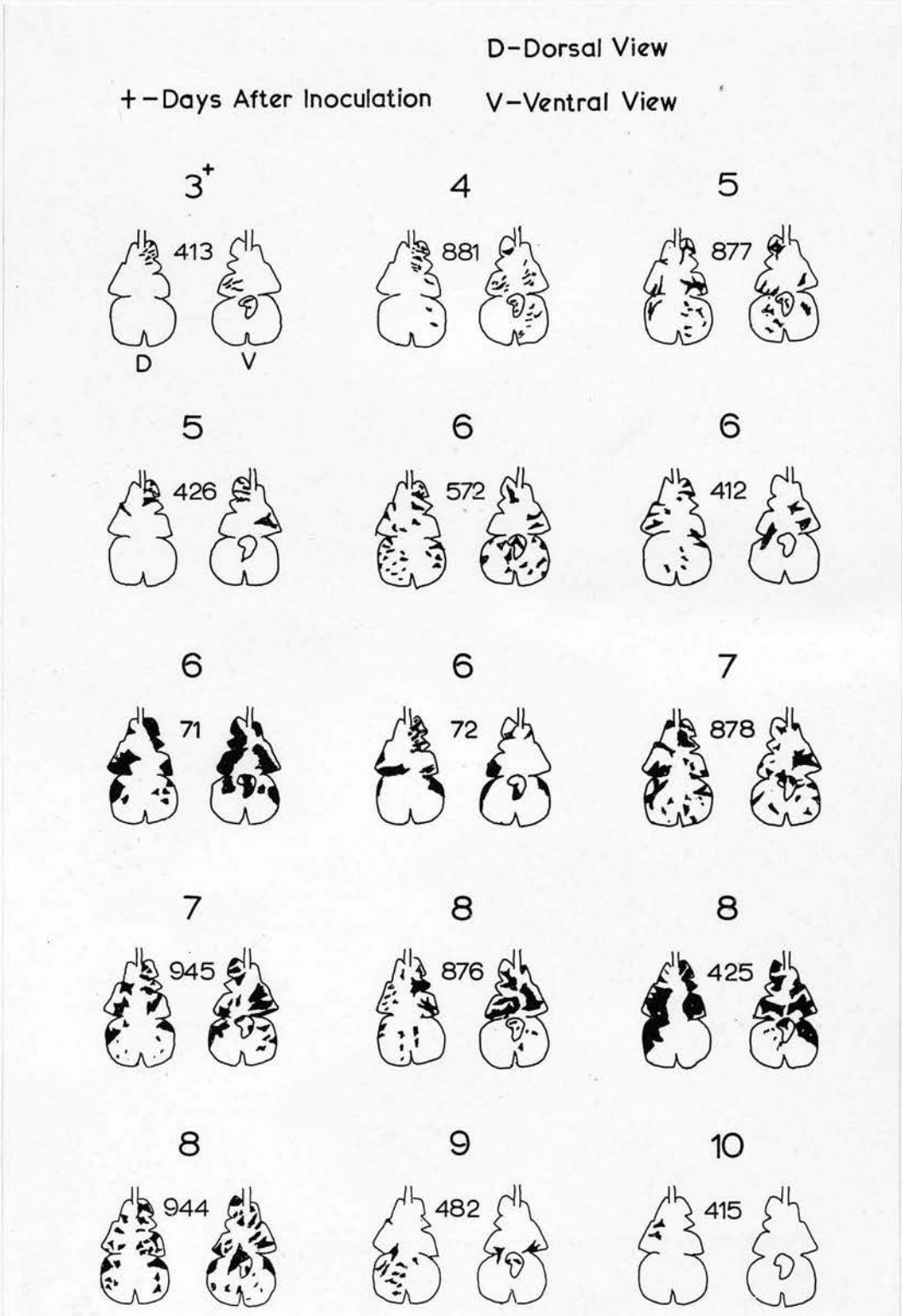


Figure 20. Distribution of the lung lesions in lambs inoculated with the ovine G2 strain of PI3 virus.

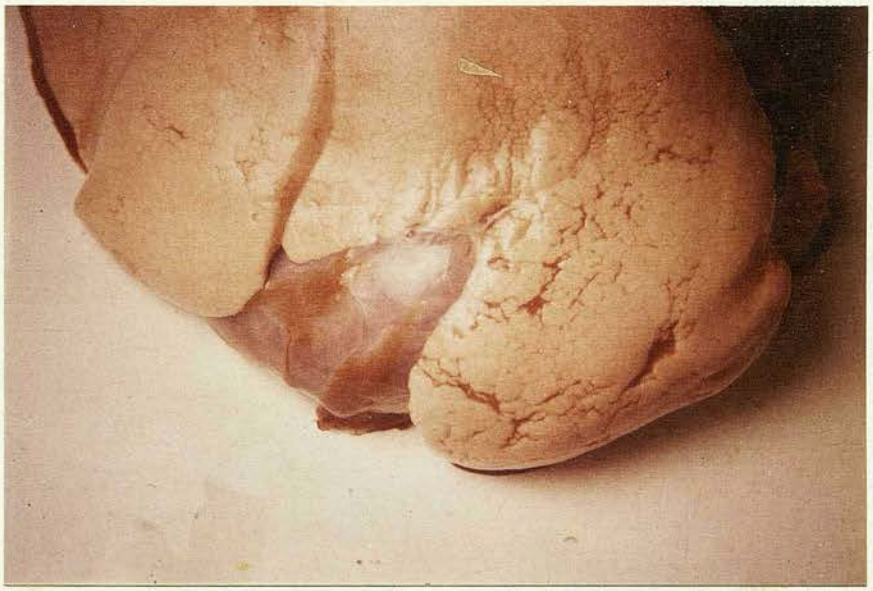


Figure 21. Macroscopic appearance of the lung lesions in lambs killed three days after inoculation with the ovine G2 strain of PI3 virus.

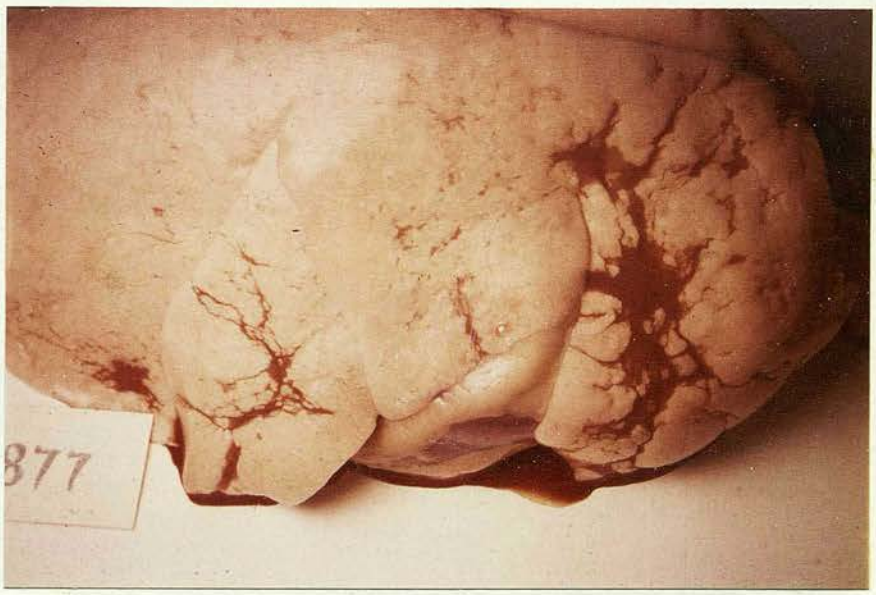


Figure 22. Macroscopic appearance of the lung lesions in lambs killed five days after inoculation with the ovine G2 strain of PI3 virus.

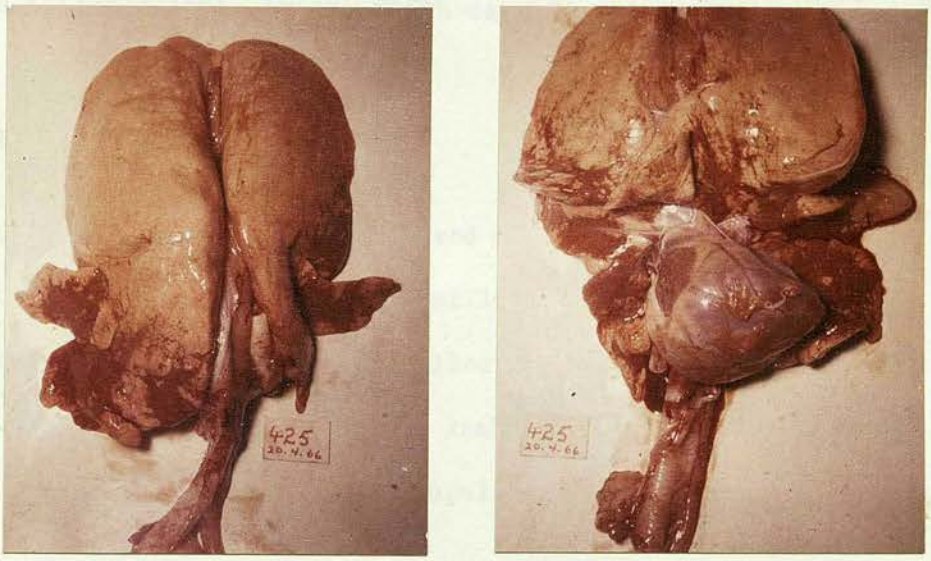


Figure 23. Dorsal and ventral views of the lung lesions in lambs killed eight days after inoculation with the ovine G2 strain of PI3 virus.

Figure 24. Close-up of Figure 23 showing the lesions in the left lobes of the lung.

in the control lambs but were noticeably enlarged without being hyperaemic in the infected lambs by the fifth day after inoculation.

Other Organs

No significant lesions were observed in the kidneys, liver or spleen. Thin-walled cysts, up to five millimetres in diameter, were present in the pancreas of two lambs killed on the third and fourth days after inoculation. In another two lambs killed on the fifth and eighth days after inoculation the prescapular lymph nodes were enlarged.

Histopathology

Upper Respiratory Tract

There were focal areas of ulceration as well as erosion and neutrophil infiltration of the nasal and turbinate mucosae in lambs of both the control and infected groups. An exudate composed of neutrophils, erythrocytes, epithelial cells and mucus was present on the surface of the nasal and turbinate mucosae in varying amounts. No significant lesions were found in the tracheas of either the control or infected lambs.

Lungs

The bronchi and bronchioles in the control lambs were normal, as

were the alveoli except for some focal areas of atelectasis.

In the virus-inoculated lambs the bronchi were normal on the third day after inoculation. The bronchioles contained a small amount of exudate and there was slight hyperplasia of the epithelium in some of the bronchioles. Small numbers of lymphocytes and macrophages and occasionally neutrophils were present in the peribronchiolar tissues. Scattered throughout the section and usually around or near small bronchioles were foci of interstitial pneumonia (Figure 25). The thickening of the interalveolar septa was due to infiltrations of lymphocytes and macrophages and septal cell proliferation. Some of the macrophages were attached to the alveolar walls while others were found lying free in the alveolar spaces. The macrophages had a pale staining, oval or indented nucleus and abundant cytoplasm which showed varying degrees of vacuolation.

In lambs killed on the fourth and fifth days after inoculation the exudate in the bronchioles was more abundant and was now present in some of the bronchi. In many of the smaller bronchioles the epithelium was often two to three cells thick and in some this hyperplasia was focal, resulting in giant cell bronchiolitis (Figure 26). Mitotic figures were occasionally observed in the bronchiolar epithelium. The areas of interstitial pneumonia were larger but similar in composition to those found in the lamb killed on the third day after inoculation. Pseudo-epithelialisation of alveoli and small syncytia attached to alveolar walls were present (Figure 27). Compensatory vesicular emphysema was seen in some lobules while in others there was partial

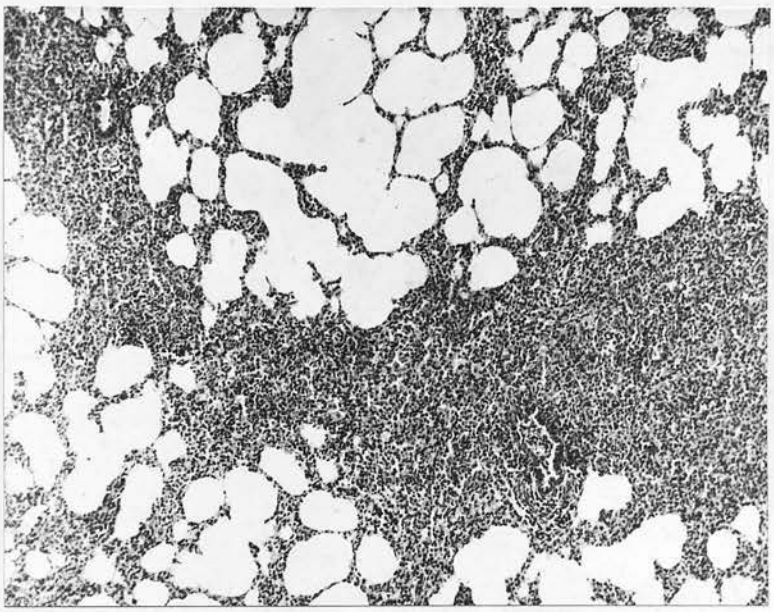


Figure 25. Peribronchiolar distribution of areas of consolidation.
H & E X125

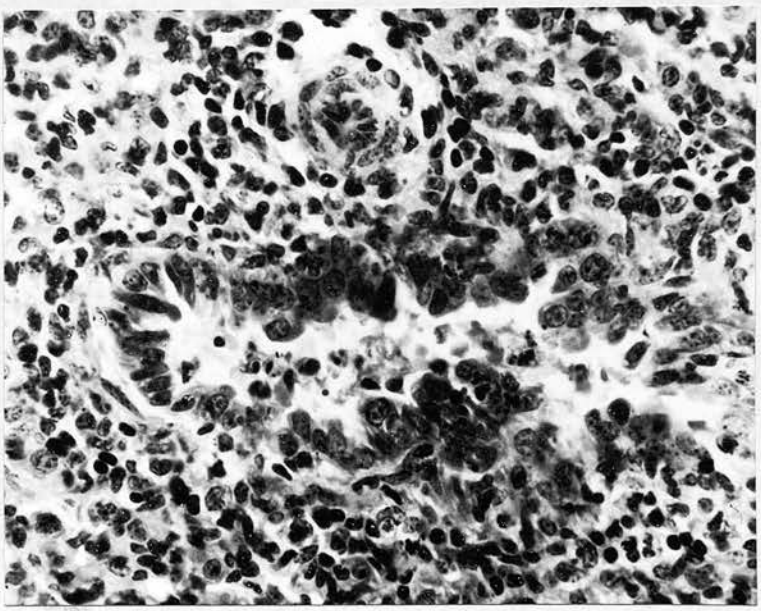


Figure 26. Hyperplasia of the epithelium of a bronchiole within an area of interstitial pneumonia.
H & E X450

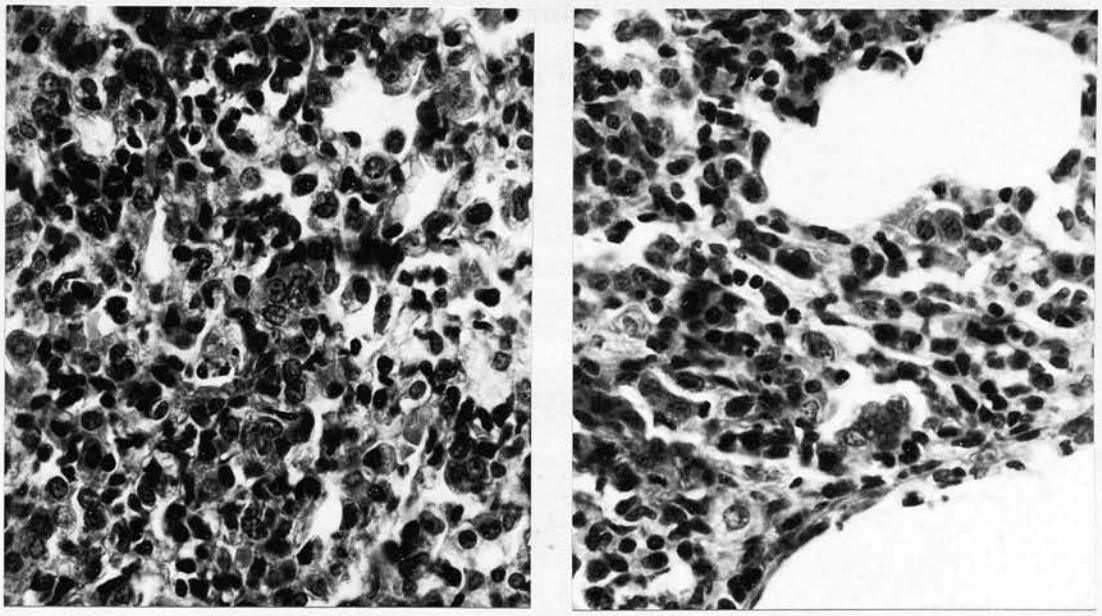


Figure 27. Two areas illustrating pseudo-epithelialisation of alveoli and syncytium formation.
Left - Pollak X480 Right - H & E X460

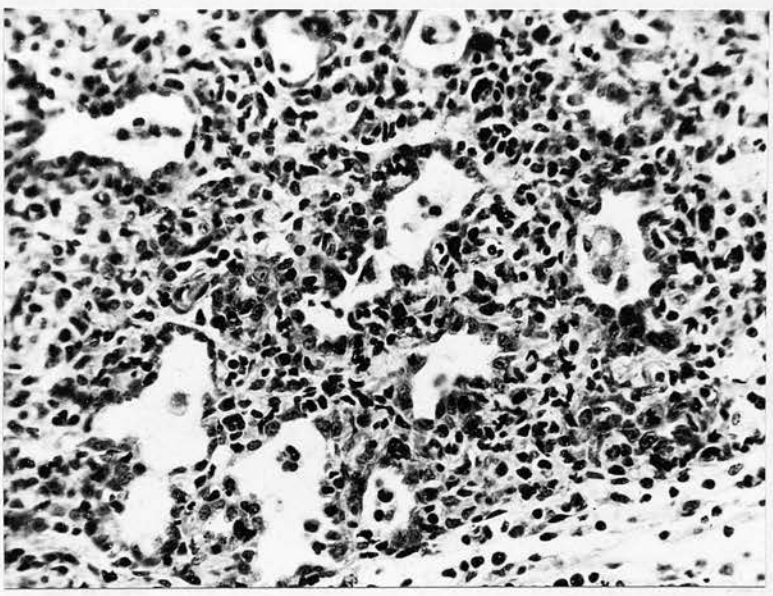


Figure 28. Interstitial reaction and focal alveolar epithelialisation.
H & E X330

atelectasis. There was slight perivascular oedema and, in one out of three lambs, scattered foci of necrosis and neutrophil infiltration of alveoli were observed.

By the sixth day after inoculation there was an increased amount of exudate in the bronchi and bronchioles and degeneration of the hyperplastic bronchiolar epithelium. The interstitial reaction was now lobular in distribution and in addition to syncytia and pseudo-epithelialisation, small areas of alveolar epithelialisation were seen (Figure 28). Scattered foci of alveolar necrosis and neutrophil infiltration persisted as did the limited peribronchiolar lymphocytic infiltration and areas of atelectasis.

The lesions in lambs killed on the seventh and eighth days after inoculation were similar. The exudate was still present and mitotic figures were common in the hyperplastic epithelium of the airways. Bronchiolar lesions varied considerably. The epithelial cells of some bronchioles were greatly enlarged and had large pale nuclei with prominent nucleoli, while in others the epithelium had been replaced by a layer of flat cells with hyperchromatic nuclei (Figure 29). There were also signs of necrosis and desquamation of the epithelium in bronchioles showing hyperplastic changes (Figure 30). Individual alveoli were now more obvious in the areas showing interstitial pneumonia as were mitotic figures. Many alveoli were lined by a continuous row of cells. In some, the cells were flat with bulging hyperchromatic nuclei while in others they were much bigger with large pale-staining nuclei and prominent nucleoli. The cellular exudate consisted of macrophages and neutrophils, the latter

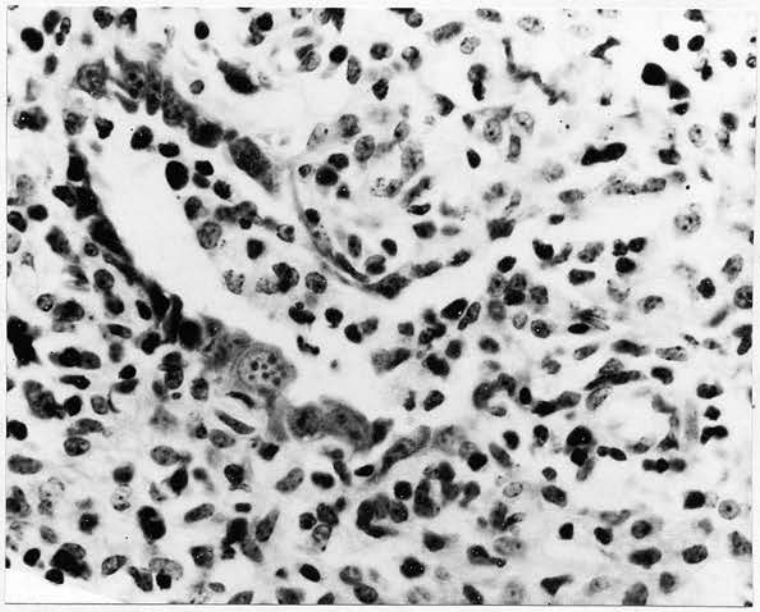


Figure 29. Bronchiole illustrating the differences in the epithelial cells. Some are hypertrophied while others are much smaller and have hyperchromatic nuclei.

H & E X590

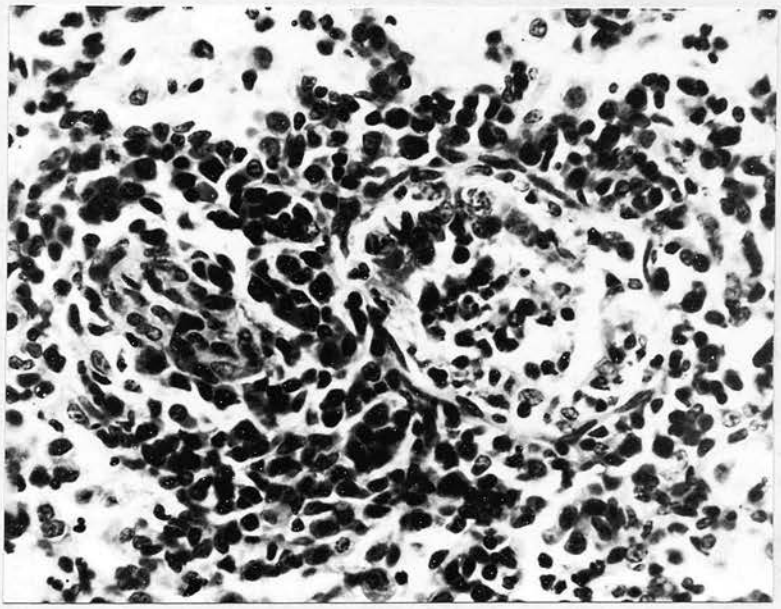


Figure 30. Necrosis of bronchiolar epithelium. The lumen is partially occluded by desquamated epithelial cells and there is peribronchiolar and perivascular lymphocytic infiltration.

H & E X480

being associated with focal areas of alveolar necrosis. In one of these lambs necrotic debris, which was present in the peribronchial and perivascular lymphatics, was also found in the subcapsular sinuses of the bronchial and mediastinal lymph nodes. Moderate perivascular oedema and peribronchiolar lymphocytic infiltration were observed. Areas of interstitial pneumonia stained to demonstrate reticulin showed that while some alveoli were partially collapsed, the majority of them were patent. Mononuclear cells were commonly seen attached to the alveolar walls and within the reticulin network (Figure 31).

In the lungs of lambs killed nine and ten days after inoculation, a small quantity of exudate was present in the smaller air passages. They appeared relatively normal except for vacuolation of some bronchiolar epithelial cells and small foci of epithelial hyperplasia. A mild to marked interstitial reaction and pseudo-epithelialisation of alveoli were still present. Syncytia and mitotic figures were rarely encountered.

Inclusions

The earliest lesion observed in lambs inoculated with PI3 virus was the presence of acidophilic inclusions within the cytoplasm of bronchiolar epithelial cells (Figure 32). Usually the inclusions occurred singly but occasionally they were situated at either pole of the nucleus (Figure 33). They were common in the bronchiolar epithelium three days after inoculation and reached their maximum number on the sixth day after inoculation when it was not uncommon to find more than fifty per cent. of the epithelial cells affected (Figure 34). At this time inclusions were also seen in epithelial

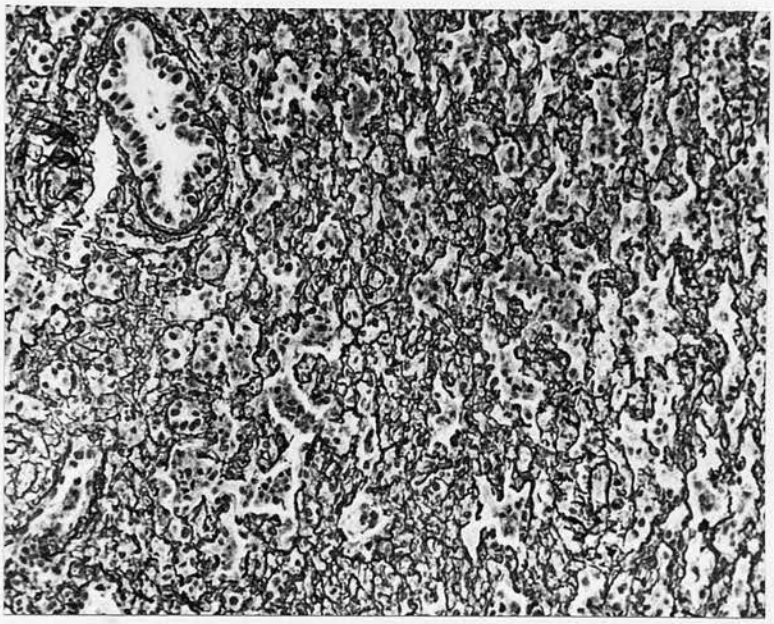


Figure 31. Hyperplasia of the reticulin fibres. Note that the majority of the cells are on the lumen side of the reticulin network.
Gordon and Sweets X200

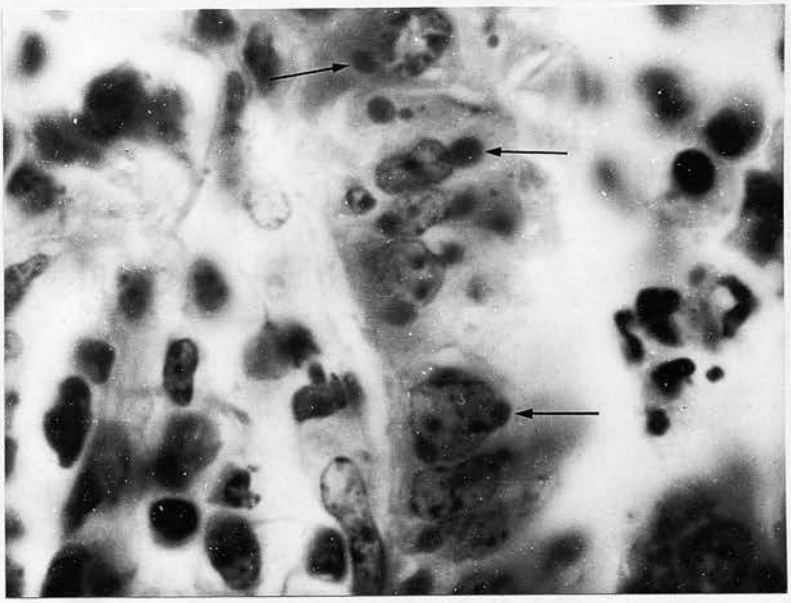


Figure 32. Bronchiole showing numerous cytoplasmic inclusions (arrows) in the epithelial cells.
picro-Mallory X1800

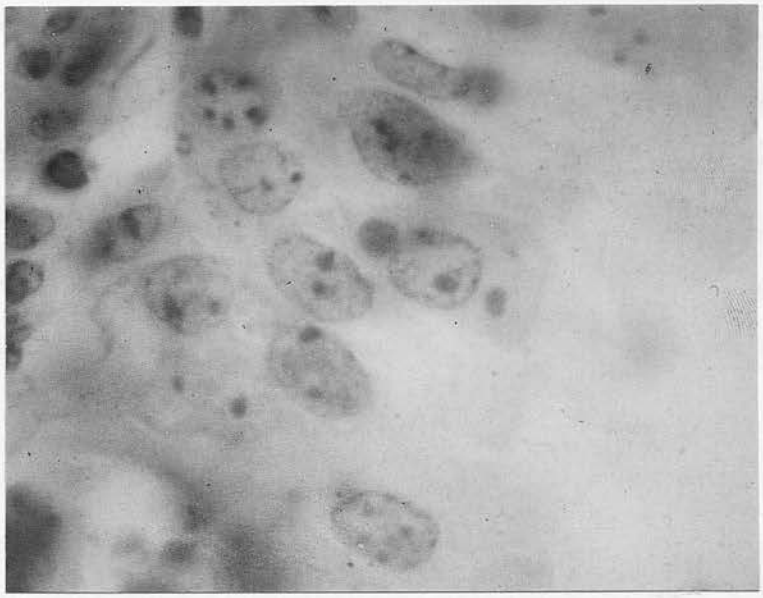


Figure 33. Acidophilic cytoplasmic inclusions in a bronchial epithelial cell.
picro-Mallory X1900

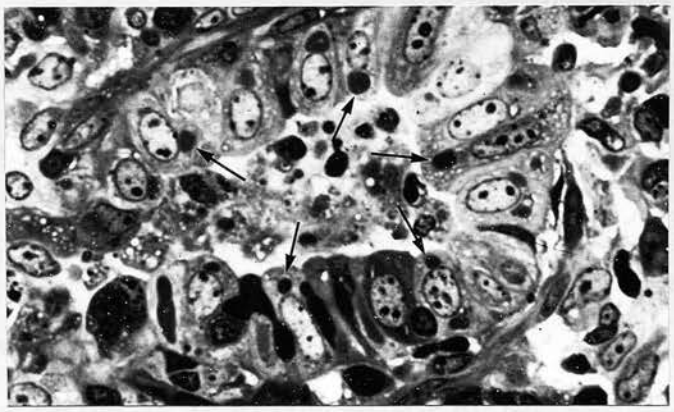


Figure 34. Numerous acidophilic, cytoplasmic inclusions (arrows) in bronchiolar epithelial cells six days after inoculation with the ovine G2 strain of PI3 virus. Section embedded in Araldite and cut at a thickness of one micron.
Giemsa X700

cells in the bronchi and alveoli and occasionally in syncytia and alveolar macrophages. Cytoplasmic inclusions were not observed in the lungs of lambs killed on the seventh, ninth or tenth days after inoculation but were seen in free alveolar macrophages and in bronchiolar epithelial cells in two lambs (425 and 944) killed on the eighth day. The presence of inclusions was closely associated with isolation of virus from the lungs (Table 2).

The inclusions varied considerably in shape and size (which was probably largely due to the plane of section in which they were cut) from small round bodies approximately two microns in diameter to long cylindrical shapes up to twenty-five microns in diameter. The majority however were oval and approximately eight to ten microns in diameter. The inclusions were difficult to detect in sections stained with haematoxylin and eosin particularly if the sections were overstained with eosin, and for this reason a variety of other staining techniques were tried. The inclusions were pale pink when stained by Giemsa and could not be readily distinguished from erythrocytes. Phloxine tartrazine gave variable results; sometimes the inclusions stained strongly with phloxine while at other times they stained a dull red or yellowish colour. The methods of Sellers (1927), Page and Green (1942) and Zlotnik (1953) were satisfactory but the best results were obtained using McFarlane's (1944) modification of the picro-Mallory technique and the more simple trichrome method of Pollak (1944). With the latter two stains the cytoplasmic inclusions stained bright red in contrast to the yellowish or orange colour of erythrocytes.

The results obtained with methyl green-pyronin were extremely variable.

Using the methods of Trevan and Sharrock (1951) and Jordan and Baker (Culling, 1963) the cytoplasmic inclusions were stained by the pyronin and corresponded in size and position with those seen in Pollak-stained sections. When examined under oil immersion, the inclusions were seen to contain numerous refractile granules of uniform size and even distribution. However, the inclusions remained pyroninophilic after treatment with perchloric acid (Culling, 1963). If the pyronin was first extracted with chloroform, as recommended by Kurnick (1955), the results varied with the source of the pyronin used. In one sample (Hopkins and Williams Ltd.) all of the pyronin was extracted by the chloroform. Using pyronin obtained from a different source (G. T. Gurr Ltd.) and extracted with chloroform, the inclusions did not stain. Negative results were obtained with the PAS and Feulgen techniques. Nuclear inclusions were not observed.

Pulmonary Lymph Nodes

There were no significant differences in the composition of the lymph nodes except that in the virus-inoculated lambs, plasma cells were commonly seen by the seventh day after inoculation. Occasionally, very small (one to two microns) phloxinophilic inclusions were seen in the cytoplasm of macrophages in the mediastinal lymph node in one lamb (72).

Other Organs

Histopathological lesions were not observed in the kidneys, liver or spleen. The pancreatic cysts had an inner layer composed of very low

elongated cells with rounded ends while the outer layer consisted of fibrous tissue of variable thickness. The cysts contained pale eosinophilic material and mononuclear cells resembling macrophages, many of which were necrotic. Increased numbers of macrophages, neutrophils and eosinophils in the medulla of one and neutrophil infiltration of the cortex in the other accounted for the enlargement of the prescapular lymph nodes seen in two lambs.

DISCUSSION

In the absence of hysterectomy-derived and specially reared stock neonatal lambs were used in the present and subsequent studies as this was the only age at which a low incidence of naturally occurring pneumonic lesions could be assumed (Section 1). The six colostrum-fed lambs were collected within four hours of birth. The PI3 virus antibody content of the maternal sera was unknown. Since no HI antibodies were detected at a dilution of 1:5 in the pre-inoculation serum samples these lambs were included in the experiment. The observations in relation to antibody response, virus recoveries (Hore, 1968) and pathological lesions did not distinguish lambs in this group from those deprived of colostrum.

The absence of a marked clinical reaction is noteworthy in that the clinical signs seen in lambs inoculated with PI3 virus were mild and in commercial flocks would probably be missed by the casual observer. This may be one of the reasons why respiratory disease due to PI3 virus

has not previously been recognised in the field even though there is serological evidence that the infection is widespread in sheep in this country (Hore, 1968).

The similarity of the lesions in the upper respiratory tracts of the control and infected groups suggests that they were the direct result of the swabbing procedure although lesions in the upper respiratory tract of animals inoculated with human (Craighead, 1966) and bovine (Omar et al., 1966) strains of PI3 virus have been described.

The extent of gross lesions were minimal for the first three to four days after infection and the most severely affected lungs were found in lambs killed on the sixth to eighth days. The macroscopic appearance of the lesions also varied with the infection-slaughter interval but none of the gross changes was pathognomonic of PI3 virus infection.

Except for the absence of nuclear inclusions and marked serous exudation, the histopathological lesions were similar to those which have been described in experimental PI3 virus infections in calves (Dawson et al., 1965; Omar et al., 1966). They also bore some resemblance to those produced by the intratracheal inoculation of four to five month-old lambs with organisms of the Bedsonia group (Dungworth and Cordy, 1962a). Thickening of the interalveolar septa, pseudo-epithelialisation of alveoli and alveolar epithelialisation were features of both Bedsonia and PI3 infections. Peribronchial, peribronchiolar and perivascular accumulations of lymphocytes were more pronounced in Bedsonia infections but this might be a reflection of the age difference in the experimental animals as it has been shown that in some species there is an increase

of intra-pulmonary lymphoid tissue with increasing age (Jericho, 1966). Hyperplasia of the bronchiolar epithelium was also a feature of Bedsonia infections in sheep but giant cell bronchiolitis, as seen in a few lambs in this series, was not described. The only pathognomonic lesion associated with PI3 virus infection in lambs is the presence of acidophilic cytoplasmic inclusions which are of transient duration. The inclusions resembled the cytoplasmic inclusions observed in calves inoculated with bovine strains of PI3 virus (Dawson et al., 1965; Omar et al., 1966) and in organ cultures inoculated with human (Craighead and Brennan, 1968) and bovine (Campbell and Martin, 1968) strains of PI3 virus. Maximum numbers were seen in bronchiolar epithelial cells on the sixth day after inoculation and persisted until the eighth day after inoculation. Their presence was closely associated with isolation of virus but it was apparent that virus might be isolated from lung tissue in the later stages of infection when inclusions could not be readily demonstrated.

The mild clinical signs and the presence of areas of consolidation primarily in the anterior lobes of the lungs in lambs inoculated with the ovine G2 strain of PI3 virus are reminiscent of those described by Gilmour and Brotherston (1963) and those observed in lambs recently introduced into pens at this Institute (Section 1). Gilmour and Brotherston (1963) suggest that the acute pneumonias they encountered may have been superimposed on subclinical interstitial or "cuffing" pneumonia of unknown aetiology. The inoculation of lambs with an ovine strain of PI3 virus results in an interstitial pneumonia so that it is possible that PI3 virus is one of the agents that may have been responsible for the apparently symptomless, apical subacute

pneumonia reported by Gilmour and Brotherston (1963). The sporadic cases of acute pneumonia they describe may represent superinfection with another agent (e.g. P. haemolytica) as was suggested for the outbreak of respiratory disease in recently introduced lambs described in Section 1. This hypothesis has yet to be tested but since infection of lambs with the ovine G2 strain of PI3 virus destroys the integrity of the lower respiratory tract, it is conceivable that the resulting lesions may predispose affected lambs to a more severe disease (e.g. infection with P. haemolytica).

Further evidence incriminating PI3 virus as a cause of atypical pneumonia of sheep is presented in the following subsection.

SECTION 2

INOCULATION OF LAMBS WITH AN OVINE STRAIN OF PARAINFLUENZA 3 VIRUS

Subsection 2. The Subacute and Resolving Phases

INTRODUCTION

The results of the previous experiment showed that the G2 strain of PI3 virus was pathogenic for neonatal lambs and that pathognomonic lesions were produced. The present experiment was designed to confirm the earlier results and to extend the work to include lambs which had been allowed to live for twenty-eight days after inoculation with the same strain of PI3 virus. Also, the role of this virus in the production of ovine atypical pneumonia (Stamp and Nisbet, 1963) was investigated.

MATERIALS AND METHODS

Lambs

Unless otherwise specified the procedures were similar to those used in the previous experiment.

Eight pregnant Border Leicester ewes were purchased from a local farm, divided into two lots, and placed in separate pens at the Moredun Institute. Except in two instances the ewes lambed without assistance.

Sixteen lambs were born and all but two were removed before they were able to obtain colostrum. Twin lambs 487 and 488 had at least one colostral feed before being removed. The lambs were placed in isolation and fed as described under General Materials and Methods.

Each of fourteen lambs was inoculated with three millilitres of infected tissue culture harvest fluid intratracheally and two millilitres intranasally. The two control lambs were each inoculated with the same volume of uninfected tissue culture harvest fluid by the same routes and were housed together in a separate building.

Virology, Serology and Bacteriology

The virological and serological examinations were done by Dr. D. E. Hore. Mr. D. Thompson carried out the routine bacteriological examination. The results are summarised in Table 3.

RESULTS

Clinical Observations

No clinical signs of disease were seen in the control lambs. No deaths were recorded and, except for lamb 477, none of the lambs which had been given virus showed signs of acute illness. A mild mucoid nasal discharge, which first appeared two or three days after inoculation and persisted for four or five days, was the first abnormality seen in the

TABLE 3

RESULTS OF VIROLOGICAL, SEROLOGICAL AND BACTERIOLOGICAL EXAMINATIONS IN LAMBS INOCULATED WITH THE OVINE G2 STRAIN OF PI3 VIRUS$^{\phi}$

Lamb No.	Colostral Status	Recovery of Virus: Nasal Swabs Days PI† 3	4	Recovery of Virus: Lungs Days PI	HI Titre*: Pre-inoculation	HI Titre*: Post-inoculation	Bacteriology
485	D	-	-	- (6)	<5	<5	S. marcescens
486	D	-	-	- (6)	<5	<5	S. marcescens
480	D	+	ND	+ (4)	<5	<5	. .
482	D	+	+	+ (6)	<5	20	. .
483	D	+	+	+ (6)	<5	40	. .
477	D	+	+	- (9)	<5	+160	. .
487	F	+	-	- (9)	+160	+160	. .
488	F	+	+	- (12)	+160	+160	. .
489	D	+	+	- (12)	<5	+160	. .
484	D	+	+	- (15)	<5	+160	. .
481	D	+	+	- (15)	<5	+160	. .
478	D	+	+	- (18)	<5	+160	. .
479	D	+	+	- (18)	<5	+160	. .
475	D	+	+	- (21)	<5	+160	. .
476	D	+	+	- (21)	<5	+160	. .
490	D	+	+	- (28)	<5	+160	. .

ϕ = Results supplied by Dr. D. E. Hore.

* = Haemagglutination inhibition titre expressed as reciprocal of serum dilution.

† = Days after inoculation.

ND = Not Done.

F = Colostrum-fed

D = Colostrum-deprived

\+ = virus recovered

\- = no virus recovered

. . = no bacteria recovered

lambs. This was soon followed by an increased respiratory rate and hyperpnoea but rarely did this progress to dyspnoea. Appetite was normal until respiratory signs developed. The majority of the lambs would begin to drink but because the act of suckling interfered with respiration they would frequently consume only one third to one half of their normal quota. Coughing and a rise in body temperature of one to two degrees centigrade was recorded in the lambs at this time. Except for lamb 477 which was depressed and dyspnoeic, the lambs rapidly improved from the eighth day onwards. The only abnormality seen in any of the lambs after the ninth day was diarrhoea which persisted for three days in three lambs.

Bacteriology

No bacteria were isolated from the lungs of the virus-inoculated lambs but Serratia marcescens, which was resistant to penicillin, was recovered from the lungs of the two control lambs (Table 3).

Pathology

Except for the presence of pancreatic cysts in seven of the virus-inoculated lambs macroscopic lesions were confined to the respiratory tract.

Upper Respiratory Tract

Hyperaemia of the nasal and turbinate mucosae and occasionally, a mucoid exudate, were seen in the control lambs and in virus-inoculated

lambs killed on the fourth, sixth and ninth days after inoculation. No macroscopic lesions were seen in lambs killed after the ninth day of infection. Tracheal lesions were not observed.

Virus-inoculated Lambs

Lungs

The distribution of areas of consolidation in all virus-inoculated lambs is shown in Figure 35. In lamb 480 killed on the fourth day after inoculation the only abnormalities were small, reddish, partially collapsed areas between the left apical and cardiac lobes and interlobular oedema. Oedematous interlobular septa were also seen in the two lambs (482 and 483) killed on the sixth day after inoculation. The consolidated areas, which involved all lobes of the lung, were dark red, firm and level with or slightly raised above the surface of the surrounding lung tissue (Figure 36). Although there was considerable variation in the extent of consolidation in the lambs killed on the ninth (Figure 37), twelfth (Figure 38), and fifteenth (Figure 39) days following infection, the appearance of the lesions was similar, the affected areas being dark red, firm and slightly collapsed. This variation was most obvious in the two lambs which had pre-inoculation HI titres of 1:160 or greater (Table 3) and were killed nine (487) and twelve (488) days after inoculation. In the remaining lambs the lesions were less extensive and tended to affect the apical, cardiac and anterior ventral parts of the diaphragmatic lobes (Figure 40). In one lamb (475) there was a small, raised greyish subpleural nodule, which had a yellowish-pinpoint centre, in the right diaphragmatic lobe.

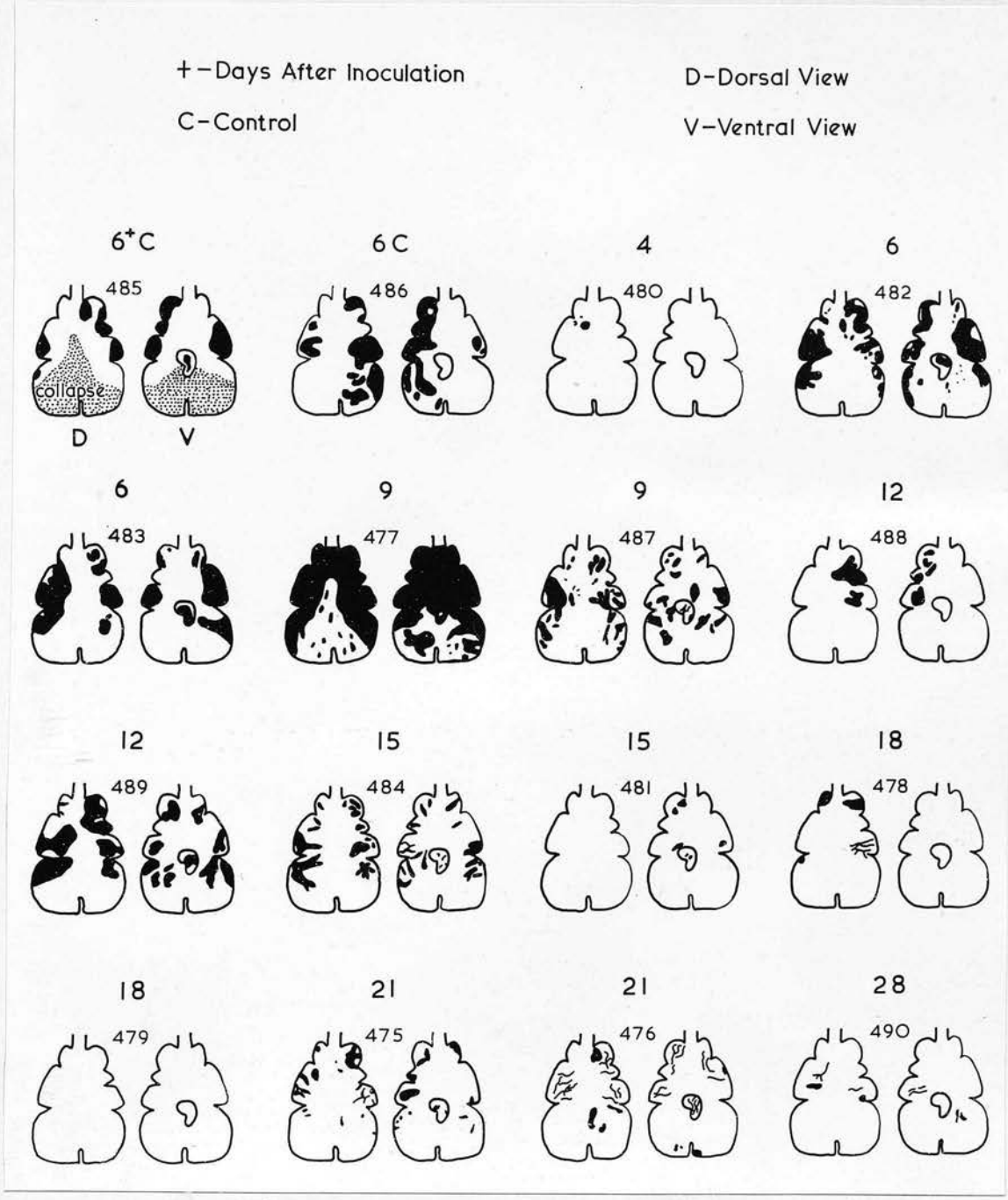


Figure 35. Distribution of the lung lesions in lambs inoculated with either virus-free tissue culture fluid or the ovine G2 strain of PI3 virus.

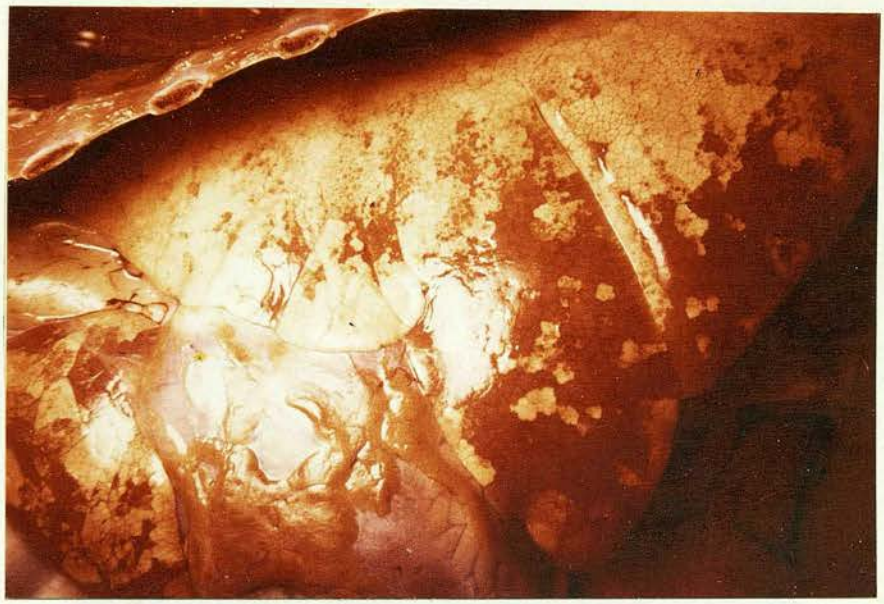


Figure 36. Macroscopic appearance of the lesions in the lungs of a lamb killed six days after inoculation with the ovine G2 strain of PI3 virus.

Figure 37. Macroscopic appearance of the lung lesions in lambs killed nine days after inoculation with the ovine G2 strain of PI3 virus.

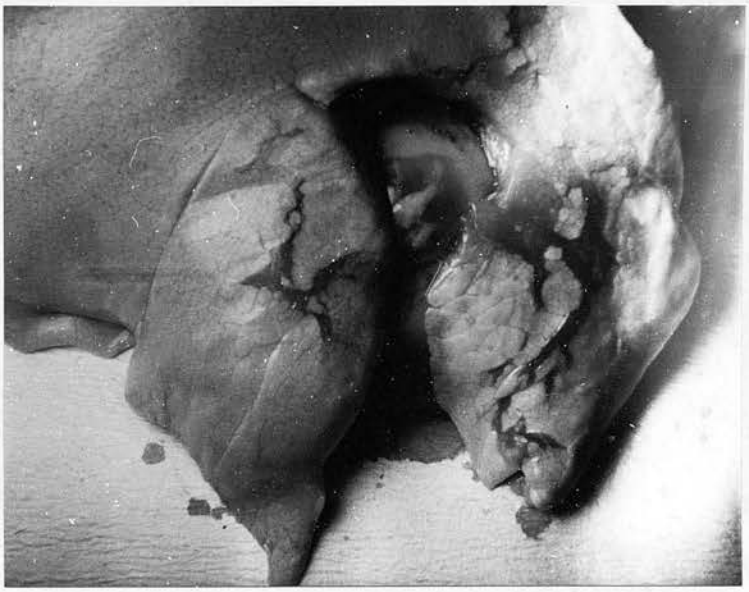


Figure 38. Macroscopic appearance of the lung lesions in lambs killed twelve days after inoculation with the ovine G2 strain of PI3 virus.

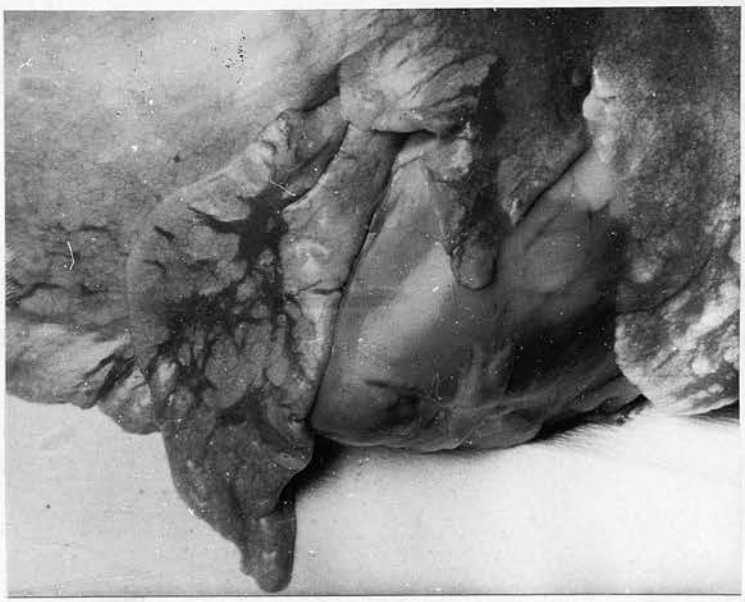


Figure 39. Macroscopic appearance of the lung lesions in lambs killed fifteen days after inoculation with the ovine G2 strain of PI3 virus.

Pulmonary Lymph Nodes

The pulmonary lymph nodes in the virus-inoculated lambs killed after the fourth day of inoculation showed only enlargement.

Histopathology

Upper Respiratory Tract

The lesions in the upper respiratory tract of the two control lambs and in the virus-inoculated lambs killed on or before the fifteenth day of inoculation were similar to those described previously (Section 2, Subsection 1). In lambs killed on the eighteenth, twenty-first and twenty-eighth days after inoculation there was no ulceration or erosion of the nasal or turbinate mucosae but focal neutrophil infiltration persisted. Tracheal lesions were absent.

Virus-inoculated Lambs

Lungs

The histological lung lesions in lambs killed on the fourth and sixth days after inoculation were similar to those described in the previous subsection.

Resolution had begun by the ninth day as evidenced by the re-appearance of a recognisable alveolar pattern, numerous mitotic figures in the bronchiolar epithelium and by the absence of cytoplasmic inclusions. In most areas the bronchiolar epithelium was normal but in places it was either hyperplastic or consisted of a single layer of low cuboidal or squamous cells (Figure 41).

Figure 40. Macroscopic appearance of the lung lesions in lambs killed twenty-one days after inoculation with the ovine G2 strain of PI3 virus.

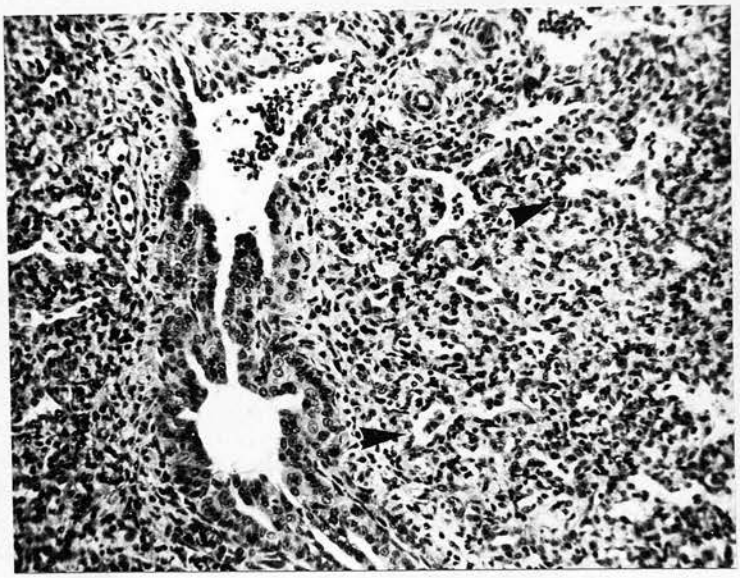


Figure 41. Bronchiolar changes in the lungs of a lamb killed nine days after inoculation with the ovine G2 strain of PI3 virus. Note foci of alveolar epithelialisation (arrows).
H & E X200

The amount of exudate in the bronchioles had decreased. There was minimal or no peribronchiolar or perivascular lymphocytic hyperplasia but the lymphatic vessels were somewhat dilated with oedematous fluid. The interstitial reaction was still present and there were macrophages attached to the alveolar walls but the lumina of alveoli contained fewer cells. Mitotic figures were occasionally seen in the attached alveolar cells. Syncytia and areas of epithelialisation were present. The cells lining the alveoli were of low cuboidal type and widely spaced so that their oval nuclei tended to bulge into the alveolar lumen. Cytoplasmic inclusions were not observed in these nor in any of the other lambs which were subsequently killed.

The histological lung lesions seen in lambs killed on the twelfth day after inoculation were essentially similar to those in lambs killed at nine days. The interalveolar septa were still more cellular than normal but individual alveoli, many of which were partially collapsed, could be recognised. Mitotic figures in the bronchiolar epithelium were quite common, particularly in lamb 488.

Areas of interstitial pneumonia were still present in lambs killed fifteen and eighteen days after inoculation. The affected areas were smaller and the degree of cellular infiltration of the interalveolar septa was less marked. Apart from slight peribronchiolar lymphocytic hyperplasia and the presence of small numbers of free alveolar macrophages the only other significant change was alveolar atelectasis. In places, seen macroscopically as the dark red, sunken, branching areas, there was complete alveolar collapse, whereas in the majority of alveoli only partial

atelectasis was evident (Figure 42).

Of the two lambs killed on the twenty-first day, the lung lesions in one (lamb 476) were minimal and consisted of areas of mild interstitial pneumonia, partial to complete atelectasis and slight peribronchiolar lymphocytic hyperplasia (Figure 43). In lamb 475 areas of interstitial pneumonia were more extensive and in places there were foci of alveolar epithelialisation (Figure 44). In a few areas the alveolar exudate appeared to be undergoing organisation. The subpleural nodule seen in the right diaphragmatic lobe consisted of a central basophilic necrotic core surrounded by an inner zone of macrophages and epithelioid cells and an outer zone of macrophages, lymphocytes and a few fibroblasts. Dispersed throughout the granuloma were structures which resembled fungal hyphae or spores. A similar lesion was observed in one of the mesenteric lymph nodes. When stained by the periodic acid-Schiff (PAS) technique the bodies were PAS positive and had a definite cell wall enclosing a tear-shaped body within some of them. Cut longitudinally, they resembled septate fungal hyphae or budding forms of a yeast. Cultural examination was not carried out as, at the time, a fungal infection was not suspected.

In the lamb (490) killed on the twenty-eighth day after inoculation the lesions were minimal and consisted of foci of interstitial pneumonia, linear areas of atelectasis and small accumulations of lymphocytes around the bronchi, bronchioles and less frequently, blood vessels.

A representative section from the lungs of at least one lamb killed on each of the days and from both control lambs was stained for reticulin by the method of Slidders et al., (1958). In the aerated alveoli in the

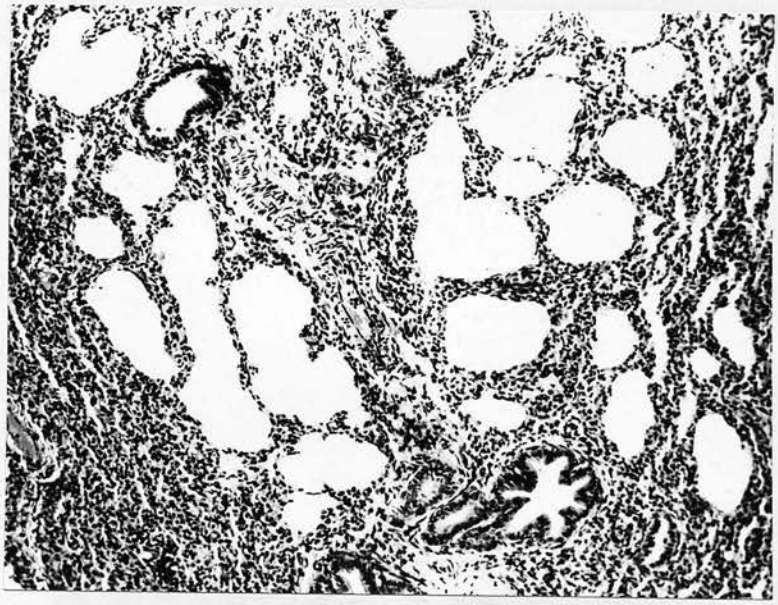


Figure 42. Varying degrees of alveolar atelectasis in the lungs of lambs killed fifteen days after inoculation with the ovine G2 strain of PI3 virus.

H & E X130

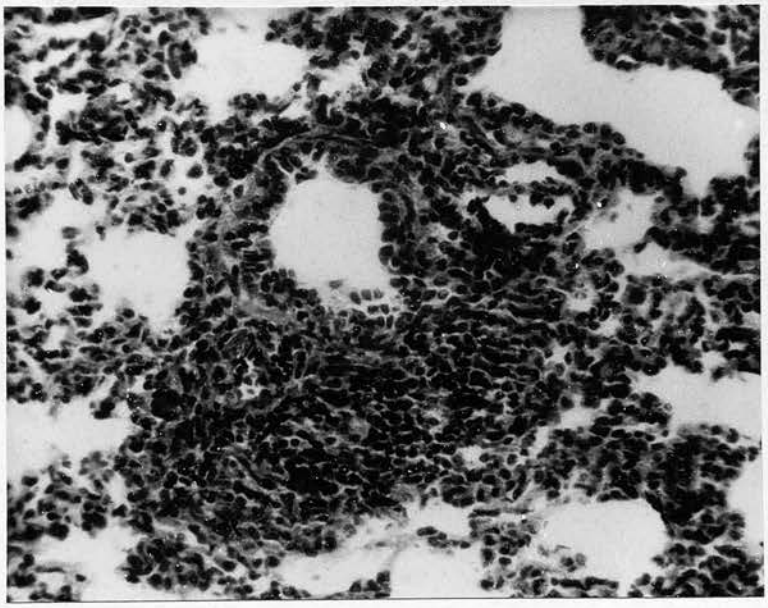


Figure 43. Slight peribronchiolar and perivascular lymphocytic hyperplasia in the lungs of a lamb killed twenty-one days after inoculation with the ovine G2 strain of PI3 virus.

H & E X260

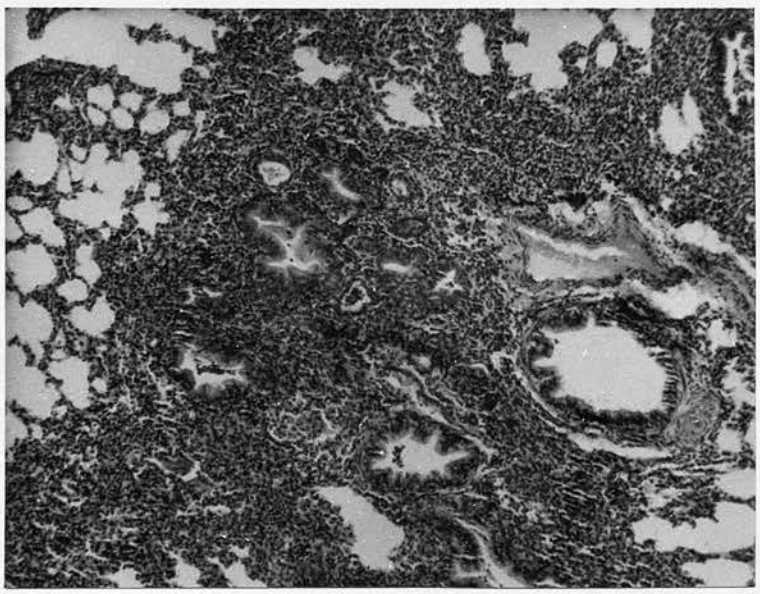


Figure 44. Focal alveolar epithelialisation in an area of interstitial pneumonia in the lungs of a lamb killed twenty-one days after inoculation with the ovine G2 strain of PI3 virus.
H & E X90

lungs of the control lambs killed six days after inoculation the reticulin fibres were very fine and there were a few free and attached alveolar cells, whereas in areas showing atelectasis the reticulin fibres were slightly thicker and the free and attached alveolar cells appeared more numerous.

In lambs killed four days after inoculation most of the alveoli were normal but in the small foci of interstitial pneumonia there was partial atelectasis and numerous cells, usually on the lumen side of the reticulin fibres, were present. Some of the reticulin fibres were slightly thickened.

Sections of lung from lambs killed on the sixth and ninth days after inoculation were similar. The appearance of the lesions was very regular. Within consolidated areas there was partial atelectasis, thickening of the reticulin fibres and alveoli filled with cells. The majority of the cells were attached to the lumen side of the reticulum but occasionally there were a few cells within the interalveolar septa and some lying free in the lumina of alveoli.

Some alveoli were normal while others were collapsed and contained numerous cells on the lumen side of the reticulin network in lambs killed on the twelfth and fifteenth days after inoculation.

There was alveolar collapse and affected alveoli in lambs killed on the eighteenth day contained only a few cells; the majority of which were free alveolar macrophages. Of the two lambs killed twenty-one days after inoculation the lesions in lamb 476 were similar to those seen at eighteen days while in lamb 475 the lesions resembled those seen at twelve days.

Except for a few small linear areas of collapse, the lungs of lamb 490 killed on the twenty-eighth day after inoculation were normal.

Pulmonary Lymph Nodes

In lambs killed on the fourth and sixth days after inoculation the lesions were minimal. No lymphoid follicles were seen. Macrophages and a few neutrophils were present in the medullary sinuses and on several occasions small eosinophilic, cytoplasmic inclusions were seen in macrophages in the lamb killed on the fourth day. By the ninth day lymphoid follicles were beginning to form and in addition to macrophages and neutrophils, necrotic debris was seen in the medullary sinuses along with small numbers of eosinophils and plasma cells.

The lesions in the pulmonary lymph nodes of the remaining lambs were essentially similar. Within the cortex mature follicles, with or without active germinal centres, were constantly seen while numerous macrophages, lymphocytes and fewer plasma cells, neutrophils and occasionally eosinophils, were present in the medullary sinuses.

Other Organs

Pancreatic cysts were observed in seven lambs inoculated with virus. No apparent predisposition for a particular part of the pancreas was noted. The number of cysts in each of the pancreas varied from one or two (lambs 480, 488, 478) to six or more (lambs 483, 479, 475 and 476) and ranged in size from approximately one millimetre to twelve millimetres in diameter (Figure 45). Two or three cysts about five millimetres in diameter were most common. In all but one lamb (480) the cysts were visible on the surface of the pancreas. The smaller cysts were level with or slightly raised above the surrounding normal tissue whereas the larger ones always

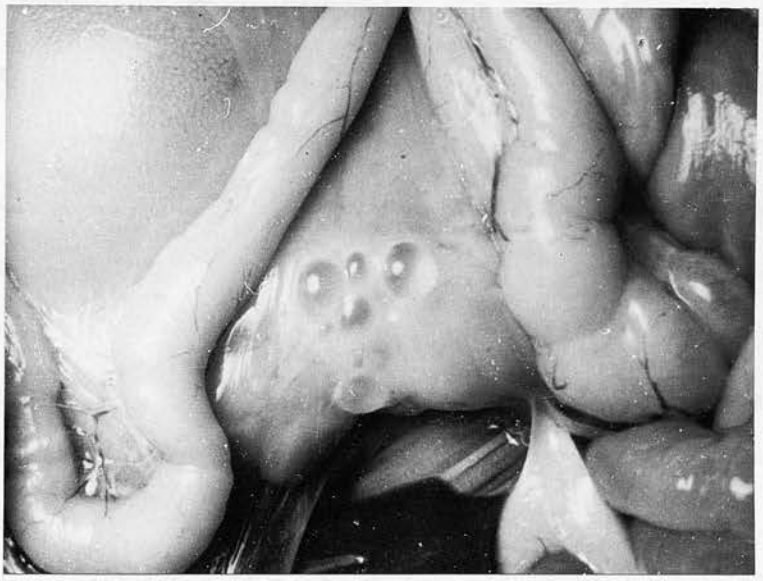


Figure 45. Numerous cysts protruding from the ventral surface of the pancreas in a lamb killed twenty-one days after inoculation with the ovine G2 strain of PI3 virus.

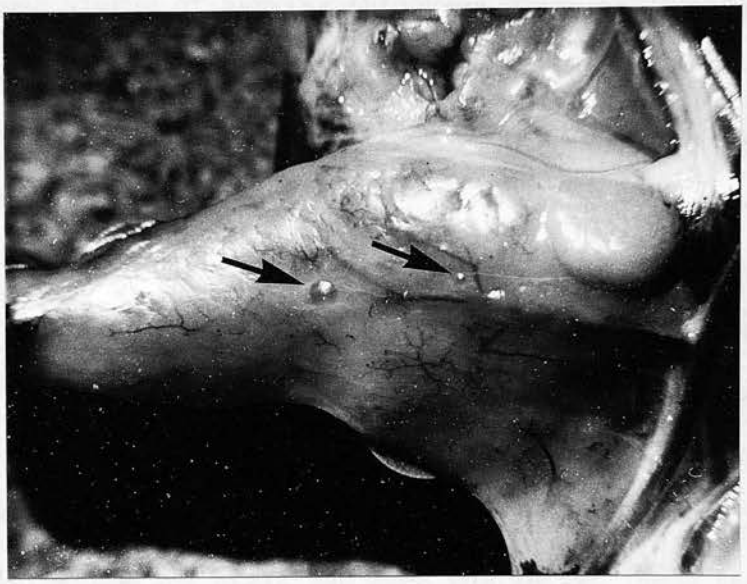


Figure 46. Ventral surface of the pancreas of a lamb killed five days after inoculation with the ovine G2 strain of PI3 virus. Toluidine blue is present in the pancreatic ducts but not in the cysts (arrows).

protruded from the pancreas. The cysts had a thin glistening translucent capsule and contained a colourless, slightly opalescent liquid. They were usually unilocular but occasionally were divided by thin septa into two or more loculi. No communication between the cyst cavities and the pancreatic ducts could be demonstrated after injecting the main pancreatic duct with toluidine blue (Figure 46).

There were a number of variations in the histological appearance of the pancreatic cysts but as these differences occurred in the same pancreas as well as in pancreas from different lambs only a composite description will be given. The cysts contained a pinkish-staining (H & E) fluid and varying numbers of cells, some of which were neutrophils but the majority resembled macrophages. In every instance the cysts had an epithelial lining which consisted of a row of squamous or low cuboidal epithelial cells usually one but occasionally two or more cells thick. The epithelial layer was surrounded by fibrous tissue of varying thickness which in some areas had been infiltrated by macrophages and lymphocytes. Where the cysts protruded above the surface of the pancreas the fibrous tissue capsule was quite thin, whereas, within the substance of the pancreas the amount of fibrous tissue was relatively more abundant. Around some of the cysts, particularly the larger ones, there was pressure atrophy of acinar cells and replacement of acini by fibrous tissue. In one lamb (480) islets of Langerhans had been partially replaced by fibrous tissue and in another lamb (476) myxomatous nodules were observed in the acinar tissue between cysts. No inflammatory reaction directly involving the cysts was seen but in two lambs (483 and 478) necrosis and in places, sloughing of the mucosa were seen in a large collecting duct. The connective tissue surrounding these ducts had been

infiltrated by lymphocytes, macrophages and numerous plasma cells. No bacteria were seen in Gram-stained preparations nor were cytoplasmic inclusions observed in sections stained by Pollak's (1944) trichrome method.

Control Lambs

Lesions were observed in the lungs of the control lambs, from which S. marcescens was isolated, but were quite different from those observed in the virus-inoculated lambs. Macroscopically, they consisted of firm, dull reddish-grey areas in the anterior lobes and large areas of atelectasis in the diaphragmatic lobes (Figure 47). The interlobular septa in areas of consolidation were oedematous. The pulmonary lymph nodes were enlarged and oedematous.

Histologically, the reaction was largely exudative in type (Figure 48). The interlobular septa were thickened due to the accumulation of neutrophils, macrophages, serous fluid and, to a lesser extent, by focal haemorrhage. The bronchioles were often filled with an exudate, consisting mainly of neutrophils. The epithelium of the bronchioles had been infiltrated by neutrophils. The lymphatics in the region of affected bronchioles were distended with exudate. A serous exudate, bacteria, neutrophils and, to a lesser degree, macrophages, were present in alveoli.

In sections from the diaphragmatic lobes partial collapse of alveoli was the only abnormality. Proliferative lesions and acidophilic cytoplasmic inclusions, as seen in the virus-inoculated lambs killed on the same day after inoculation were not observed.

There were no appreciable differences in the lesions observed in the mediastinal and right and left bronchial lymph nodes. All were oedematous and

Figure 47. Macroscopic appearance of the lesions in the lungs of a lamb killed six days after inoculation with virus-free tissue culture harvest fluid. Serratia marcescens was isolated from the consolidated areas in the cardiac lobes.

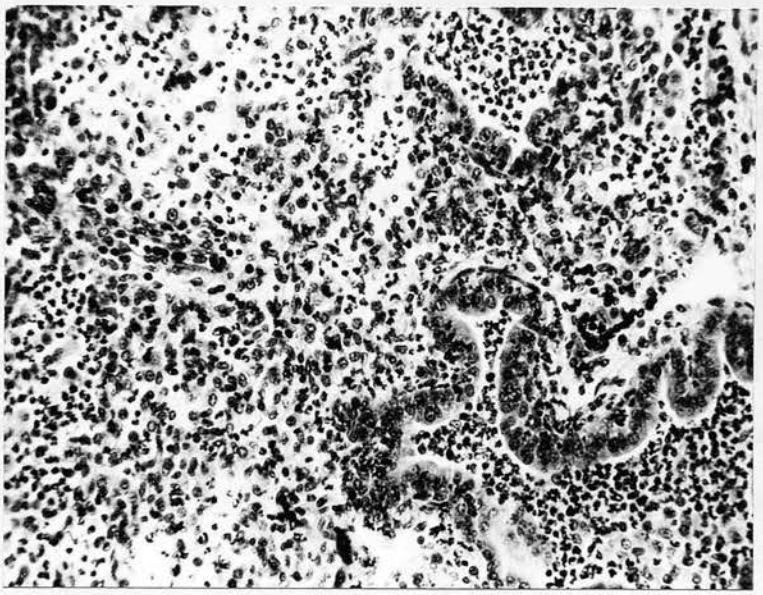


Figure 48. Acute inflammatory reaction in the lungs of a control lamb from which Serratia marcescens was isolated.
H & E X230

contained large numbers of neutrophils. Mature lymphoid follicles were not present.

DISCUSSION

Generally the clinical signs in the virus-inoculated lambs were mild and except in one lamb (477), would be difficult to detect in most flocks. The signs seen in lamb 477 were probably the result of anoxic anoxia since at least two thirds of the lung tissue was consolidated. In sheep naturally infected with PI3 virus (Hore et al., 1968) and in older lambs inoculated intranasally with the ovine G2 strain of PI3 virus, clinical signs attributable to this virus were minimal unless complicated by a secondary bacterial infection (Hore, 1968). The isolation of S. marcescens from the lungs of both control lambs was unexpected. Both lambs were housed together in a separate building and were fed by a separate attendant. Rectal temperatures were not recorded but daily inspection revealed no signs of respiratory disease and the lambs continued to feed normally. The source of this organism was not discovered. The original inocula were shown to be bacteriologically and virologically sterile. The feeding utensils were a possible but unlikely source of infection since they were routinely disinfected with a solution of chlorine (Milton, Vick International). Also, in a subsequent experiment using a bovine strain of PI3 virus, the same organism was isolated from the lungs of a lamb which had been left on its dam. Serratia marcescens is normally regarded as a saprophyte but the finding of this organism in the lungs of both control lambs indicates that such a statement is not necessarily correct

and that the role of this organism as a potential pathogen of young lambs should be investigated.

The macroscopic lesions in lambs killed on the fourth and sixth days after inoculation were similar in appearance and extent to those killed on the same days in the previous experiment. The lesions reached their maximum size on the ninth day and resembled those seen in lambs killed on the eighth but not on the ninth day in the previous experiment. In lambs 487 and 488 which had pre-inoculation HI titres of 1:160 or greater and were killed on the ninth and twelfth days after inoculation respectively, the lesions were less extensive than in lambs 477 and 489 which had pre-inoculation HI titres of less than 1:5 and were killed on the same days. The differences may have been due to the colostral status of the lambs. The question of whether there is a correlation between the level of circulating antibody and the degree of protection is by no means settled. Dawson et al., (1965) were unable to produce a clinical response in two calves, which had pre-inoculation serum HI antibody levels of 1:32 or greater, after the intratracheal or intranasal inoculation of the Umeå strain of PI3 virus. Others have also found that if calves, which had HI antibody titres of 1:20 or greater (Byrne, Abinanti and Huebner, 1961) or 1:40 or greater (Mohanty and Lillie, 1964), were challenged with PI3 virus, attempts to reisolate virus from the nasal passages were unsuccessful. On the other hand it has been shown that even in the presence of high titres of circulating antibody against PI3 virus, calves (Hamparian, Washko, Ketler and Hilleman, 1961; Abinanti, 1963; Hamdy and Trapp, 1964; Hamdy, King and Trapp, 1965), humans (Chanock et al., 1961; Tyrrell, Bynoe, Petersen, Sutton and Pereira, 1959; Kapikian, Chanock, Reichelderfer, Ward, Huebner and Bell, 1961) and sheep (Hore, 1968; Gilmour, Drysdale, Stevenson

Hore and Brotherston, 1968) are susceptible to reinfection but that clinical signs during the second infection are usually milder (Chanock et al., 1961) or absent (Abinanti, 1963).

It has been suggested that the multiplication of virus in the nasal passages of calves depends on the antibody titre against PI3 virus in the nasal secretions (Hamdy and Trapp, 1964). Experimental evidence for the above hypothesis in relation to PI3 virus infections in cattle and sheep is lacking but in a series of experiments using mice infected with strains of influenza virus, Fazekas de St. Groth and his associates (Fazekas de St. Groth and Donnelley, 1950a, b; Fazekas de St. Groth and Graham, 1954) were able to show that "the bronchial antibody content, and hence the degree of protection, varies independently from the serum antibody level, as it is governed not only by mechanisms at work in the production of antibody but also by factors which influence its distribution". Also the concentration of passively acquired antibody in the respiratory tract varies not only with the time interval between administration and the acquisition of a serum antibody level but also on factors which influence its distribution (e.g. selective permeability and "pathotropic" adjuvants - i.e. agents which are mild irritants but which themselves are incapable of affording any protection, (Fazekas de St. Groth and Donnelley, 1950c)). If the same response occurs in lambs infected with PI3 virus as in mice infected with influenza virus, then this might explain the less extensive macroscopic lesions or earlier resolution observed in lambs 487 and 488.

In the majority of lambs killed after the twelfth day of inoculation the macroscopic and histological lesions were similar to those seen in some

cases of ovine atypical pneumonia (Stamp and Nisbet, 1963) and in cases of so-called slaughter-house pneumonia (Hamdy et al., 1959; Boidin et al., 1958; Pounden, Bell, Edginton and Thomas, 1956; McGowan et al., 1957). Similar lesions have also been produced experimentally in lambs inoculated with a Bedsonia agent (Boidin et al., 1958; Dungworth and Cordy, 1962a). Dungworth and Cordy (1962a) state that the lesions elicited by their strain of Bedsonia were not pathognomonic and that undoubtedly there were other agents capable of causing the lesions found in the lungs of slaughtered lambs. The absence of marked peribronchiolar or perivascular lymphoid hyperplasia as is seen in atypical pneumonia (Stamp and Nisbet, 1963) and in experimental Bedsonia infections (Boidin et al., 1958; Dungworth and Cordy, 1962a), might have been due to the age of the lambs in the present experiment. Whether infection of very young, colostrum-deprived lambs with Bedsonia would elicit such a response was not known at this time.

The presence of a co-existing or superimposed infection by an unidentified fungus probably accounted for the more extensive lesions found in lamb 475 killed on the twenty-first day after inoculation.

It had been expected that resolution would have been completed within three weeks after inoculation. In every lamb but one (479) residual lesions were still present. Had the lambs not been reared in isolation, it is possible that a proportion of them would have become secondarily infected with other agents (e.g. Pasteurella or Mycoplasma spp.) resulting in either an acute infection (Hore et al., 1968) or further reduction in the rate of resolution (Dungworth and Cordy, 1962a).

The histological lesions in the control lambs and in the virus-inoculated lambs were quite different. Histologically, the most conspicuous lesions

in the control lambs were the presence of a serous exudate, neutrophils and bacteria in affected alveoli and atelectasis, whereas in the virus-inoculated group a proliferative reaction involving the interalveolar septa and epithelial layers, together with the presence of acidophilic cytoplasmic inclusions were the characteristic features. The lesions in the former are those usually associated with a bacterial agent and the latter have been stated as being characteristic of a viral pneumonia (Blood and Henderson, 1960) although this is not necessarily correct unless specific lesions (e.g. inclusions) are also present (Omar, 1966).

These experiments have demonstrated that the ovine G2 strain of PI3 virus is a potential lung pathogen in sheep and that the experimental disease is characterised by histopathological changes which occur in natural outbreaks of respiratory disease in sheep in Britain (Hore et al., 1968; Stamp and Nisbet, 1963).

SECTION 2

INOCULATION OF LAMBS WITH AN OVINE STRAIN OF PARAINFLUENZA 3 VIRUS

Subsection 3. Detection of Parainfluenza 3 Virus in the Lungs of Lambs by Immunofluorescence

INTRODUCTION

A review of the literature relating to the fluorescent antibody technique is beyond the scope of this thesis. The monograph by Nairn (1962) and the recent reviews by Hers (1963) and Mims (1964) provide sufficient evidence of its widespread application for the visualisation and identification of viral and other antigens. A number of myxoviruses have been examined by immunofluorescent methods in tissue culture (e.g. Watson, 1952; Traver, Northrop and Walker, 1960; Liu, Chu, Sharp and Detert, 1961; Kisch, Johnson and Chanock, 1962; Maassab and Loh, 1962; Omar, 1965; Valicek and Smid, 1967) but no published accounts of fluorescent antibody studies of parainfluenza 3 (PI3) virus infections in their natural hosts were found.

This subsection describes the results of an experiment designed to show the sites of virus replication in the lungs of lambs inoculated with an ovine strain of PI3 virus, and to determine whether these sites correspond with the acidophilic cytoplasmic inclusions seen in cryostat and paraffin sections.

MATERIALS AND METHODS

Five colostrum-deprived lambs were obtained and reared as described previously (General Materials and Methods). They were inoculated intranasally (two millilitres) and intratracheally (three millilitres) as described in Section 2, Subsection 1 and killed on the fourth, fifth and sixth days after inoculation.

Controls

Control specimens were obtained from an adult sheep with pulmonary adenomatosis and from a colostrum-deprived lamb killed nine days after inoculation with an ovine strain of Bedsonia as described in Section 4.

Antigen

The inoculum was the ovine G2 strain of PI3 virus at the fourth passage in ovine embryo kidney cultures and had an infectivity titre of $10^{6.2}$ $TCID_{50}$ per millilitre.

Sera

Antisera prepared in sheep and rabbits against the ovine G2 strain of PI3 virus were provided by Dr. D. E. Hore. The sheep antisera had an haemagglutination-inhibition (HI) titre of 1:1024 and a neutralising titre of greater than 1:350. The rabbit antisera had a neutralising

titre or greater than 1:600.

Preparation of Conjugates

Conjugation of sheep anti-PI3 sera with fluorescein isothiocyanate was carried out by Mr. C. Gardiner, according to the method of Gilmour and Gardiner (1968). Commercially prepared conjugated rabbit globulin antiglobulin prepared in goats (Difco Laboratories) was used in the indirect method.

Before use both the conjugated sheep anti-PI3 sera and the conjugated rabbit globulin antiglobulin were absorbed twice with foetal lamb tissue powders. The tissue powders were prepared from the livers, kidneys, and lungs of foetal lambs since they were likely to be the most reliable source of antigen-free tissues. The methods of preparing the tissue powders and absorption of the conjugates were similar to those described by Nairn (1962). Following absorption the conjugates were used immediately or after storage at four degrees centigrade for up to five days.

Preparation of Specimens

Small pieces of lung from lambs infected with the ovine G2 strain of PI3 virus and from control lambs were taken and either cut immediately in a cryostat or put in wide-necked universal containers and placed in a mixture of solid carbon dioxide and ethanol. When the tissues were thoroughly frozen the universal containers were removed and stored at minus twenty degrees centigrade. Sections cut in a cryostat were air

dried. Some were stored unfixed while a proportion of them were fixed in acetone for ten minutes. Both fixed and unfixed sections were stored at minus twenty degrees centigrade. Lung impression smears from infected and control lambs were treated in a similar manner.

On the day the test was to be carried out fresh cryostat sections of infected and control lung were cut and, along with stored unfixed preparations, were fixed in acetone for ten minutes.

Direct Staining Technique

Cryostat sections of lung and impression smears from infected lambs were rinsed for ten minutes in three changes of phosphate buffered saline (PBS) (pH 7.1) then covered with fluorescein isothiocyanate-conjugated sheep anti-PI3 serum, placed in a moist chamber and incubated at thirty-seven degrees centigrade for either thirty or sixty minutes. Following a rinse for ten minutes in at least three changes of PBS the excess fluid was removed and the preparations were mounted in equal volumes of glycerol and PBS (pH 7.1).

Tests for Specificity of Staining

1. Cryostat sections of lung and impression smears from the control sheep were treated as described above.
2. Similar preparations from virus-infected lambs were fixed in acetone and rinsed in PBS as described above. Unconjugated sheep anti-PI3 serum was applied and the sections incubated in a moist

chamber at thirty-seven degrees centigrade for thirty minutes. After rinsing for ten minutes in three changes of PBS, they were stained with fluorescein isothiocyanate-conjugated sheep anti-PI3 sera and treated as described for infected lambs.

Indirect Staining Technique

Cryostat sections of lung and impression smears from virus-infected lambs were fixed as described for the direct test and rinsed for ten minutes in three changes of PBS (pH 7.1).

Sections from infected lambs were covered with rabbit anti-PI3 sera and incubated in a moist chamber for thirty minutes at thirty-seven degrees centigrade. They were rinsed again for ten minutes in PBS and then stained with fluorescein isothiocyanate-conjugated goat anti-rabbit sera. After incubating for ten minutes at eighteen degrees centigrade, followed by another rinse in PBS for ten minutes, they were mounted in equal volumes of glycerol and PBS (pH 7.1).

Tests for Specificity of Staining

1. Cryostat sections of lung and impression smears from lambs which had been inoculated with virus-free material were treated as described above.
2. After fixation and rinsing as previously described, cryostat sections and lung impression smears from virus-infected lambs were covered with unconjugated sheep anti-PI3 sera, incubated in

a moist chamber for thirty minutes at thirty-seven degrees centigrade and rinsed in three changes of PBS for ten minutes. They were then stained with fluorescein isothiocyanate-conjugated goat anti-rabbit sera and treated as described for infected lambs.

3. Infected specimens were stained with normal rabbit sera and incubated in a moist chamber for thirty minutes at thirty-seven degrees centigrade and then rinsed in three changes of PBS for ten minutes. They were then stained with fluorescein isothiocyanate-conjugated goat anti-rabbit sera. After an incubation period of ten minutes at eighteen degrees centigrade, they were rinsed in PBS for ten minutes and mounted in equal volumes of glycerol and PBS (pH 7.1).

4. Another set of infected tissues was first stained with unconjugated sheep anti-PI3 sera, incubated in a moist chamber for thirty minutes at thirty-seven degrees centigrade and then rinsed in three changes of PBS for ten minutes. Rabbit anti-PI3 sera was then added and the sections treated as for sheep anti-PI3 sera. After rinsing in PBS, fluorescein isothiocyanate-conjugated goat anti-rabbit sera was added and the sections treated as described for infected lambs.

Once experience with the techniques had been gained, coding of both control and test preparations was employed along with the tests for specificity to ensure objective evaluations.

Cytopathology

Suitable areas of specific fluorescence in the test specimens were photographed and their location determined using an England Finder (Graticules Ltd.). The test specimens were then rinsed in PBS and stained either with H & E or by the method of Pollak (1944), Sellers (1927) or Foot (Lillie, 1954). The same areas were again located and re-photographed. Because of the poor results usually obtained in this procedure, other cryostat sections from the same pieces of lung and paraffin sections from adjacent areas of lung were prepared and stained by one or more of the above methods to ensure that cytoplasmic inclusions were present.

Microscopy

The slides were examined using a Reichert Binolux microscope and H.B.O. 200 Mercury vapour lamp with a dark ground condenser. The exciter filter was UG1/1.5 mm. (E2) and the absorption filter was GG 13/3 - 1 mm. Wratten 2B (Sp 2). A X8 eyepiece was used with X10, X25, X40, X63 and X100 objectives.

Photography

Using high speed black and white 35 millimetre film (Tri-x, Kodak Ltd.), exposure times of five minutes gave satisfactory results. Developing and printing times were standardised for all exposed films.

RESULTS

Tests for Specificity

Lung tissue from sheep exhibit a dull green fluorescence when examined with ultra-violet (UV) light. This autofluorescence is usually confined to the cytoplasm but occasionally nucleoli autofluoresce.

Specific "apple green" fluorescence, characteristic of fluorescein isothiocyanate was not observed in any of the control specimens treated with labelled antibody (Figure 49); nor was specific fluorescence observed in PI3 virus infected-tissues pretreated with normal rabbit sera or unlabelled sheep anti-PI3 sera followed by labelled goat anti-rabbit sera (Figure 50). Similar results were also observed when virus-infected specimens were treated with unlabelled sheep anti-PI3 sera, followed by unlabelled rabbit anti-PI3 sera, and then labelled goat anti-rabbit sera.

Direct Test

Cryostat Sections

Specific "apple green" fluorescence was seen in sections of lung from each of the virus-inoculated lambs killed on the fourth, fifth and sixth days after inoculation. The intensity of the fluorescence was greater in specimens incubated for sixty minutes than in those incubated for thirty minutes at thirty-seven degrees centigrade. No appreciable difference in the total amount or distribution of fluorescence in the

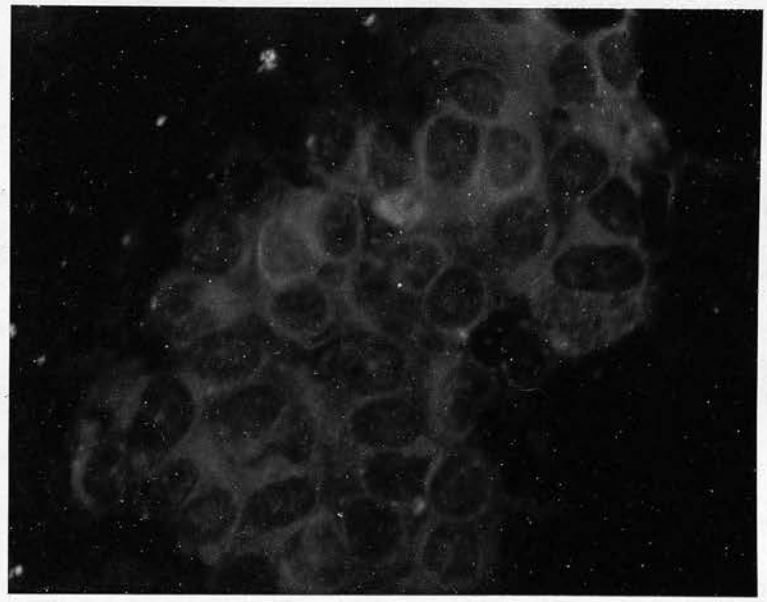


Figure 49. Lung impression smear from a control lamb treated with labelled sheep anti-PI3 sera. There is no specific fluorescence.
Control, Direct Test X1100

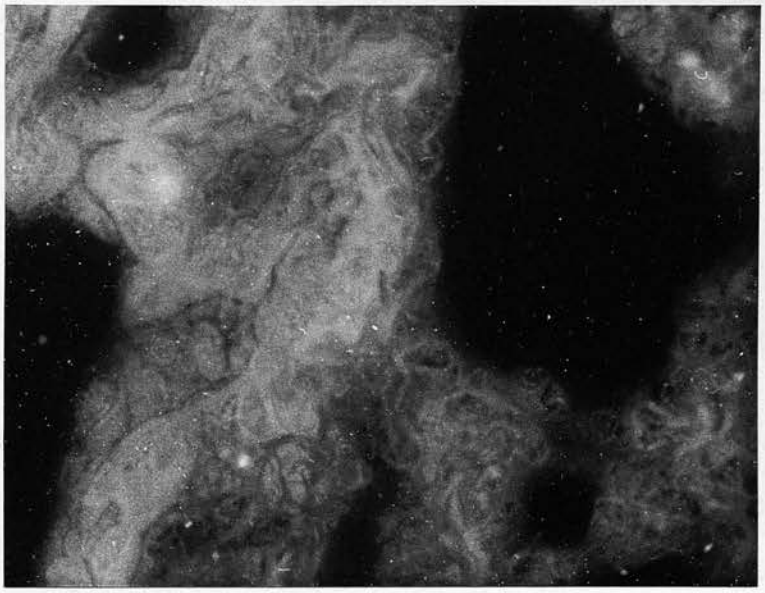


Figure 50. Cryostat section of lung from a PI3 virus-infected lamb treated with normal rabbit sera followed by labelled goat anti-rabbit sera. Autofluorescence but no specific "apple green" fluorescence is present.
Control, Indirect Test X510

lungs of lambs killed on any of the three days was noted.

The majority of the fluorescent areas in the bronchi stained intensely and varied in size from small granules to larger, apparently homogeneous masses (Figure 51). Other less intensely stained foci were also seen. Although difficult to determine because of the thickness of the cryostat sections, fluorescence seemed to occur only in the cytoplasm. Occasionally fluorescence was seen in the cytoplasm at either pole of the nucleus (Figure 52).

The distribution of the fluorescent areas in bronchioles and alveoli was more diffuse than in bronchi. Not all bronchioles were affected but in those that were, the brightly fluorescing areas contrasted markedly with the background autofluorescence (Figure 53). The fluorescent areas ranged in size from small circular foci to large irregularly defined masses. In some of the bronchioles showing specific fluorescence there was hyperplasia of the epithelium or a cellular exudate in their lumina but in many, there was no apparent abnormality. Similar fluorescent masses were observed in alveoli (Figure 54). They were present in mononuclear cells attached to the alveolar walls and in similar cells lying free in the alveolar lumina.

Impression Smears

Location of antigen was much more precise in impression smears than in cryostat sections of lung. Specific fluorescence was confined to the cytoplasm. In mononuclear cells containing one or two areas of

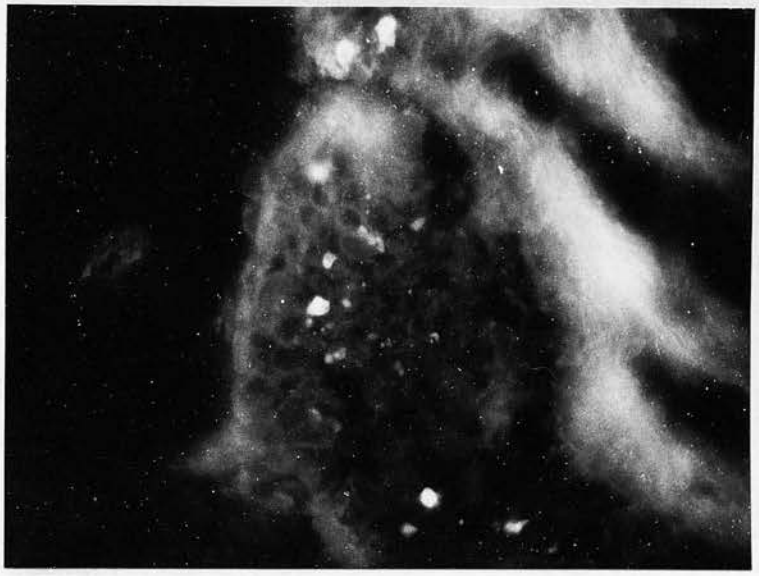


Figure 51. Cryostat section of lung showing foci of specific fluorescence in a bronchus.
Direct Test X430

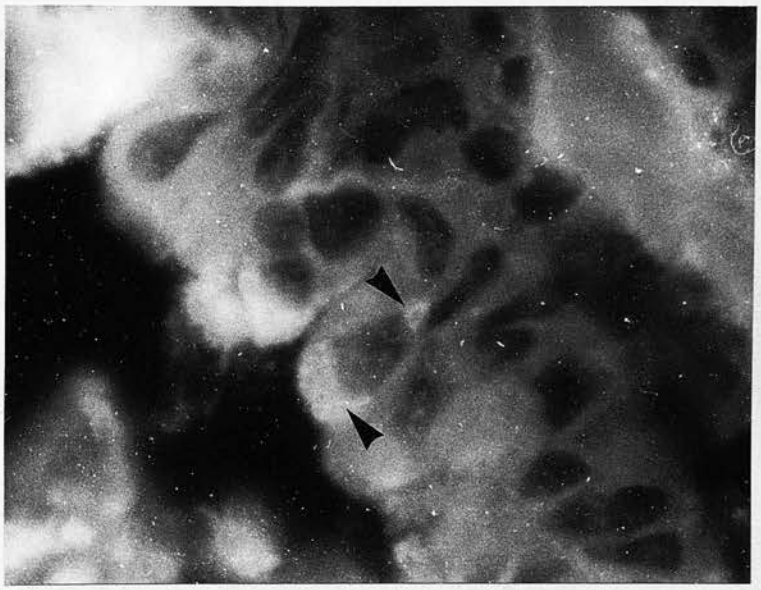


Figure 52. Cryostat section of lung. Areas of specific fluorescence in the cytoplasm of a bronchial epithelial cell at either pole of the nucleus (arrows).
Direct Test X1100

Figure 53. Cryostat section of lung showing numerous foci of fluorescence in bronchioles.
Direct Test X125

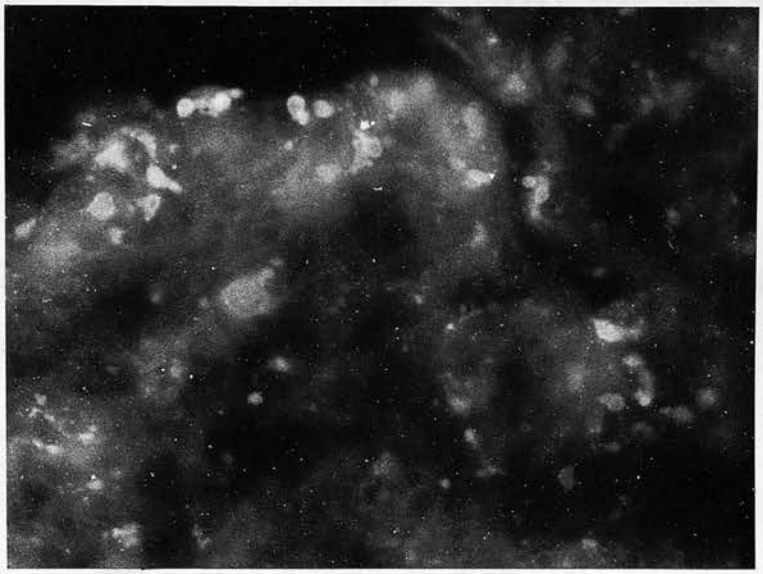


Figure 54. Cryostat section of lung showing areas of fluorescence scattered throughout alveoli.
Direct Test X670

fluorescence, specific staining was seen in the perinuclear region. As the amount of fluorescent material increased, specific fluorescence was seen surrounding the nucleus and in the more peripheral areas of the cytoplasm.

Figure 55 illustrates the progression of specific fluorescence. In cell A there is autofluoresce of the cytoplasm but no specific fluorescence. Two fluorescent globular inclusions and several pinpoint areas are present in the perinuclear region of cell B. In cell C the number of fluorescent particles has increased and almost completely surround the nucleus whereas in cell D the perinuclear region is completely involved and there are fluorescent foci in the peripheral cytoplasm.

Some of the fluorescent particles appeared to be uniform in consistency whereas others were composed of fluorescent granules of varying size (Figure 56). Both types of inclusions have been observed in cryostat and paraffin sections although the homogeneous type was more common.

Indirect Test

The results obtained using the indirect test were similar to those recorded for the direct test.

Cytopathology

Cytoplasmic inclusions were present in bronchial, bronchiolar and

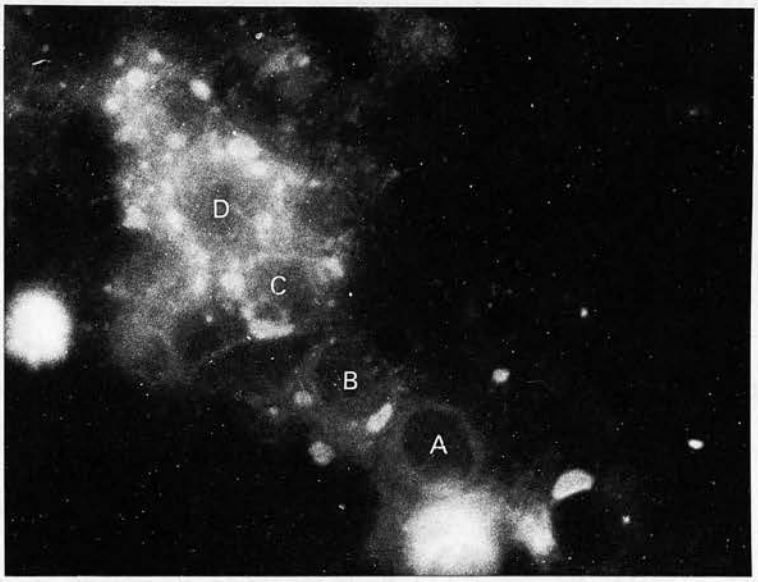


Figure 55. Lung impression smear showing varying degrees of fluorescence in the cytoplasm of infected cells.
Direct Test X1300

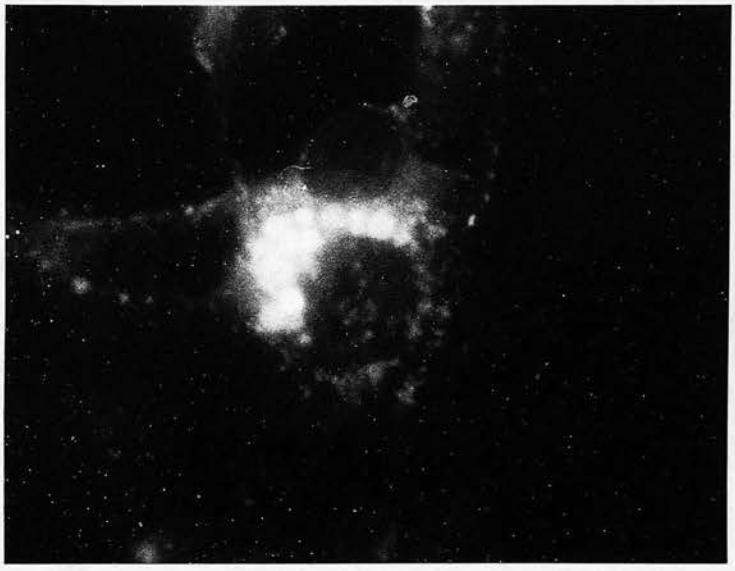


Figure 56. Lung impression smear illustrating granular foci of fluorescence.
Direct Test X1650

alveolar epithelial cells in cryostat (Figure 57) and paraffin (Figure 58) sections of infected lung. Generally, specimens which had first been used in the fluorescent antibody tests were unsuitable for further examination. In H & E stained preparations individual cells, as well as being distorted, showed little detail and the cytoplasmic inclusions were difficult to demonstrate photographically. The results obtained after staining by the methods of Sellers (1927) or Foot (Lillie, 1954) were less satisfactory. Occasionally cryostat sections were stained satisfactorily with H & E and in these it was possible to compare the eosinophilic cytoplasmic inclusion with foci of specific fluorescence (Figures 59, 60) in the same cells. Other lesions such as hyperplasia or necrosis of the bronchiolar epithelium and desquamation of epithelial cells were also observed.

DISCUSSION

Specific fluorescence, indicating the presence of PI3 virus antigen, was observed in the bronchiolar epithelium, in the cells lining alveoli and in the bronchial epithelium. Foci of fluorescence were most commonly observed in the bronchioles and in the surrounding alveoli and it is in these sites that the initial histopathological changes are seen (Section 2, Subsections 1 and 2). The primary specific lesions in paraffin sections are inclusion body formation and epithelial hyperplasia followed by necrosis and desquamation of affected cells. Fluorescent masses, morphologically similar to the acidophilic inclusions seen histologically, were seen in bronchiolar epithelial cells in cryostat sections of lung examined with UV light and in the same cells later stained with H & E. However, it was

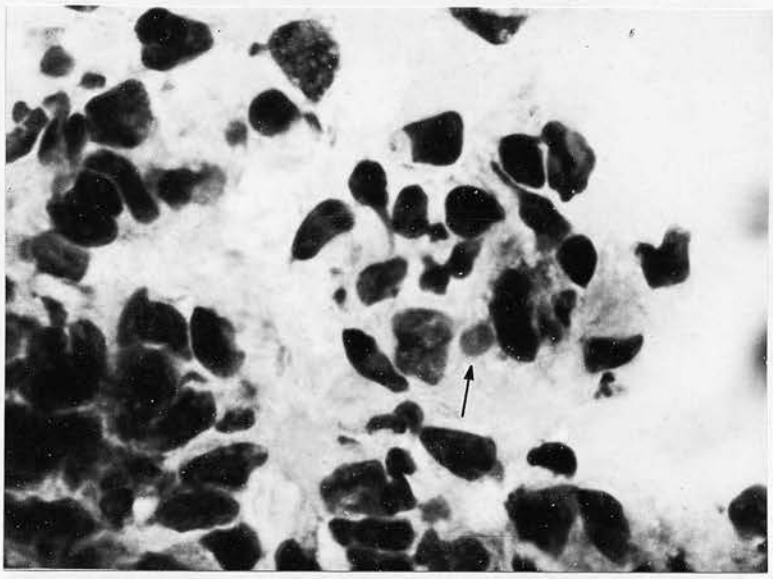


Figure 57. Cryostat section of lung showing an inclusion (arrow) in the cytoplasm of an alveolar epithelial cell.
H & E X1200

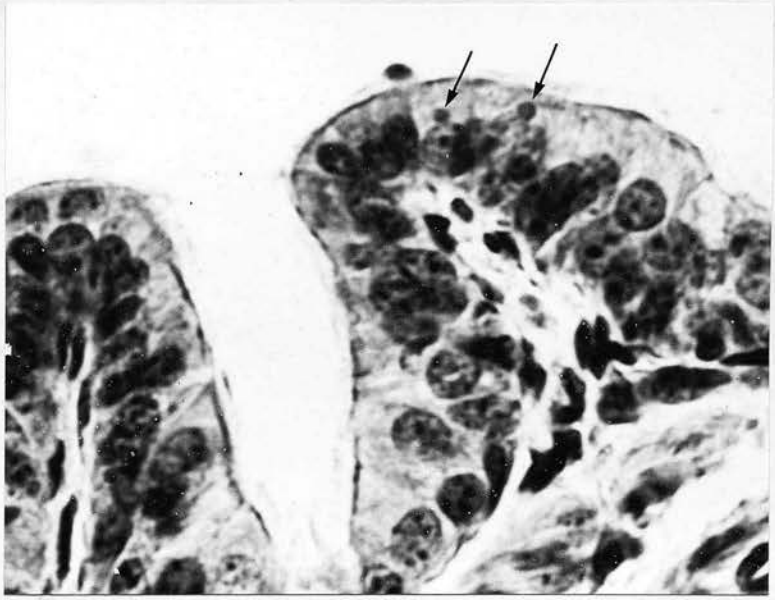


Figure 58. Paraffin section of lung showing cytoplasmic inclusions (arrows) in bronchial epithelial cells.
Pollak X1000

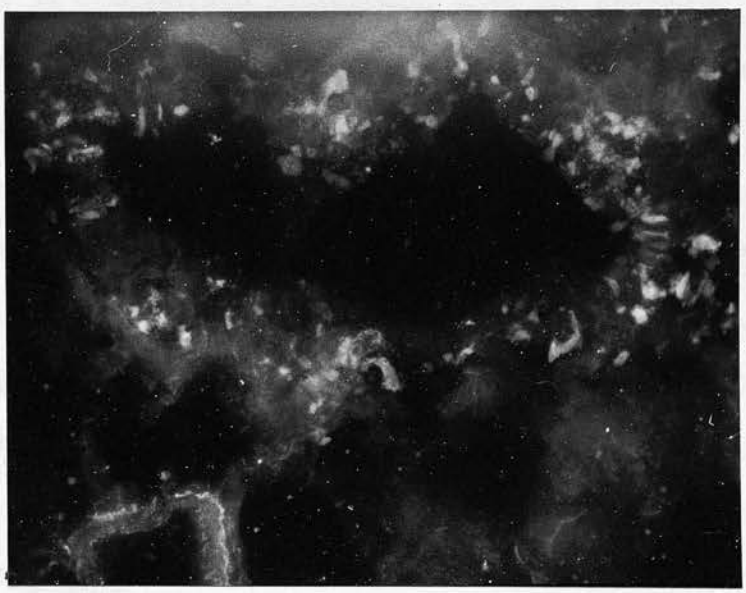


Figure 59. Cryostat section of lung showing numerous foci of specific fluorescence.
Direct Test X300

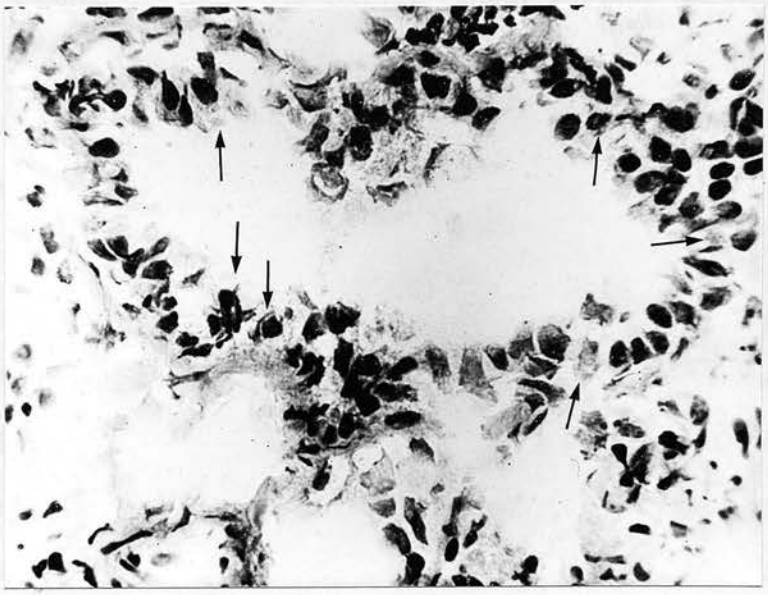


Figure 60. The same field as in Figure 59 after staining with H & E showing cytoplasmic inclusions (arrows).
H & E X300

not always possible to demonstrate inclusions, with any degree of certainty, where there were foci of fluorescence. As stated previously, this may have been due to the poor quality of the stained sections. Coffin and Liu (1957) also found that frozen sections, after treatment with fluorescent antibody, were lacking in detail. Another explanation might be that some of the fluorescent foci represent earlier or later stages of inclusion body formation and do not stain with H & E. The inclusions of canine distemper, another member of the myxovirus group, may or may not contain viral antigen, depending on the staining method used (Coffin and Liu, 1957). Omar (1965), in tissue culture studies with bovine strains of PI3 virus, was unable to correlate the presence of cytoplasmic inclusions with specific immuno-fluorescence, nor with acridine orange staining, even though it has been shown by other investigators (Kasten and Churchill, 1966) that the cytoplasmic inclusions produced by bovine strains of PI3 virus contain ribonucleic acid (RNA) in the later stages of their formation. Howe, Morgan, de Vaux St. Cyr, Hsu and Rose (1967), on the other hand, found that the eosinophilic inclusions produced by parainfluenza 2 (PI2) virus in HeLa cells corresponded in their distribution to the areas of specific cytoplasmic fluorescence seen in infected cells treated with labelled antibody.

The cellular localisation of antigen has not been reported previously in PI3 virus infections in their natural hosts but in tissue culture systems infected with human (Liu et al., 1961; Maassab and Loh, 1962) and bovine (Omar, 1965; Valicek and Smid, 1967) strains of PI3 virus, the results were similar in that specific fluorescence was limited to the cytoplasm. The distribution of the antigen within the cytoplasm as shown

in Figure 55 may represent different stages in the production of infective virus and haemagglutinin as has been shown by Howe et al., (1967) in stable amnion cells and HeLa cells infected with PI2 virus. In some of the cells in the present study there was diffuse specific cytoplasmic fluorescence while in others the fluorescent foci were more condensed. These differences may be related to the development of virus or to the nature of the antigen being demonstrated as has been shown by Howe et al., (1967).

The results obtained using the indirect ("sandwich") fluorescent antibody method were equal to, but not superior than, those obtained with the direct test. This was probably due to the fact that the serum neutralising titre of the antisera used in the direct test was quite high (equal to or greater than 1:350). If an antiserum with a low titre had to be used or if the fluorescent antibody technique were to be employed in the diagnosis of ovine respiratory diseases then the indirect test would be the method of choice because of its greater sensitivity and also to the fact that fewer labelled antisera would be required.

No specific fluorescence was observed in the lungs of the lamb infected with Bedsonia organisms nor in the lungs of an adult ewe which died of pulmonary adenomatosis. This suggests that the fluorescent antibody technique would be suitable for diagnosing PI3 virus infections using either the direct or indirect staining techniques.

SECTION 2

Subsection 4. Electron Microscopic Examination of Cytoplasmic Inclusions in Experimental Parainfluenza 3 Virus Infection in Lambs

INTRODUCTION

The development of paramyxoviruses or the inclusions produced by these viruses have been examined by a number of authors (e.g. Tawara, Goodman, Imagawa and Adams, 1961; Reczko and Bögel, 1962; Kuhn and Harford, 1963; Prose, Balk, Liebhaber and Krugman, 1965; Compans, Holmes, Dales and Choppin, 1966; Howe et al., 1967) using electron microscopy. Except for a brief account of the cytoplasmic inclusions seen in bladder epithelial cells of ferrets infected with distemper virus (Tawara et al., 1961) the above studies were carried out in a variety of tissue culture systems. In this subsection the results of electron microscopic examination of the cytoplasmic inclusions in lung tissue from lambs infected with the ovine G2 strain of PI3 virus are described.

MATERIALS AND METHODS

Lambs

Lung tissues were obtained from three colostrum-deprived lambs inoculated with the ovine G2 strain of PI3 virus as described in Section 2, Subsection 1

and killed on the fifth (one lamb) and sixth (two lambs) days after inoculation. Lung material from two of these lambs killed on the fifth and sixth days after inoculation was also used for the detection of PI3 viral antigen using immunofluorescence as described in the previous subsection. The other lamb (482) had been used in the experiment described in Section 2, Subsection 2.

Assistance in preparing specimens for electron microscopy was provided by Mr. W. Smith and Mr. E. Gray.

Small pieces (one cubic millimetre) of lung were taken immediately after death and fixed in gluteraldehyde. They were then post-fixed in osmium tetroxide and embedded in Araldite. Sections were cut on a LKB Ultrotome 1 using glass knives.

One micron thick locating sections were cut from the complete block face and stained with Giemsa (1:10 solution at sixty degrees centigrade). From these, suitable areas were selected and a series of thin sections for examination in the electron microscope were cut and stained with lead citrate. The contiguous section was cut at a thickness of one micron and stained with Giemsa and the area to be examined was photographed using a light microscope. The thin sections were examined in a Siemens Elmiskop 1 electron microscope using double condenser illumination.

RESULTS

Examination in the electron microscope revealed that the composition of the inclusions in bronchiolar epithelial cells of lambs killed five or six

days after inoculation of PI3 virus was similar. For this reason only one cell has been selected and described.

The inclusions were frequently found in the perinuclear region and appeared homogeneous when examined with the light microscope (Figures 34, 61). In Figure 61 a bronchiolar epithelial cell containing an inclusion is shown in relation to other infected cells. A higher magnification of the same cell is shown in Figure 62. Mitochondria and other cytoplasmic components are not associated with the inclusion; instead they have been displaced peripherally by it. No limiting membrane surrounds the inclusion. Several vacuoles are present in the inclusion. Similar vacuoles were often seen in inclusions in other cells but their size and numbers varied. The inclusion now appears granular. At the opposite pole of the nucleus there is a small membrane-less structure (Figure 62) which may represent an earlier stage in inclusion formation or it may be a mature inclusion in a different plane of section. At higher magnifications (Figures 63, 64) the inclusion is seen to be composed of thread-like structures which are so tortuous and interwoven that measurements of them are difficult. The average diameter of the fibrils is approximately fifteen millimicrons. In one of the vacuoles in the inclusion several particles resembling ribosomes are present.

DISCUSSION

Numerous acidophilic cytoplasmic inclusions were present in paraffin sections from each of the lambs, and in the two lambs examined with the fluorescent

Figure 61. Cytoplasmic inclusions within bronchiolar epithelial cells. The cell chosen for examination in the electron microscope is indicated by an arrow.
Giemsa X660

Figure 62. Low power electron micrograph illustrating the position of the inclusion in relation to other cytoplasmic components. The small granular structure at the opposite pole of the nucleus (see text) is indicated by an arrow.
Lead citrate X6400

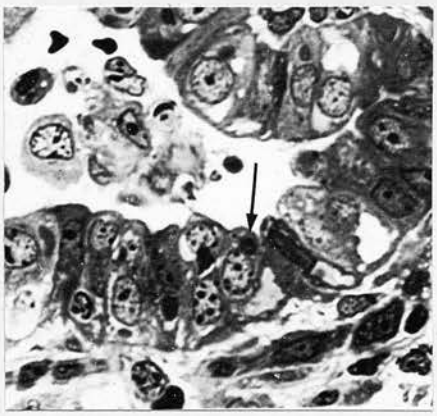


Figure 61.

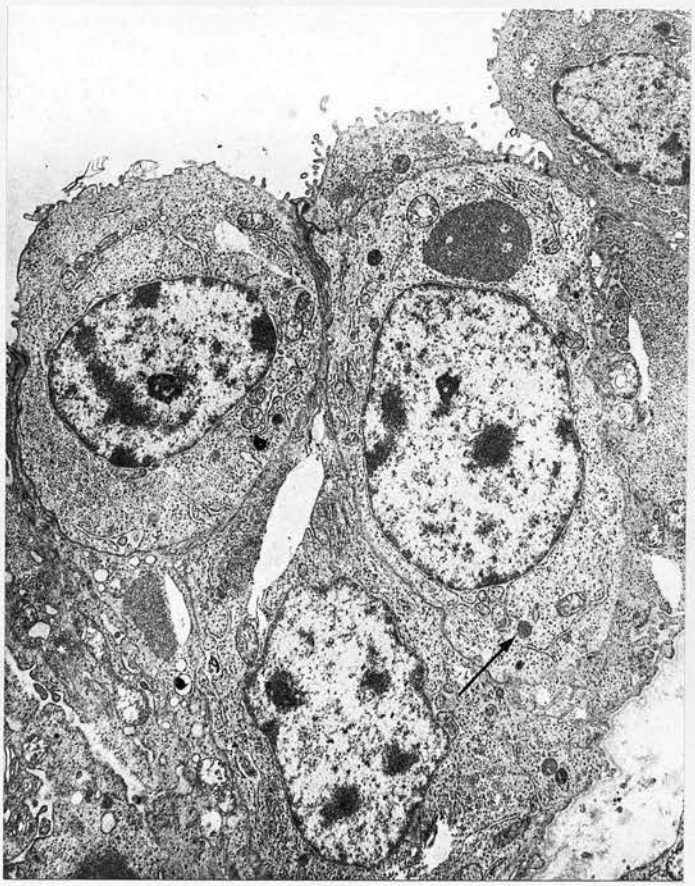


Figure 62.

Figure 63. At a higher magnification the inclusion is seen to be composed of numerous granules and lacks a surrounding membrane.

Lead citrate X16000

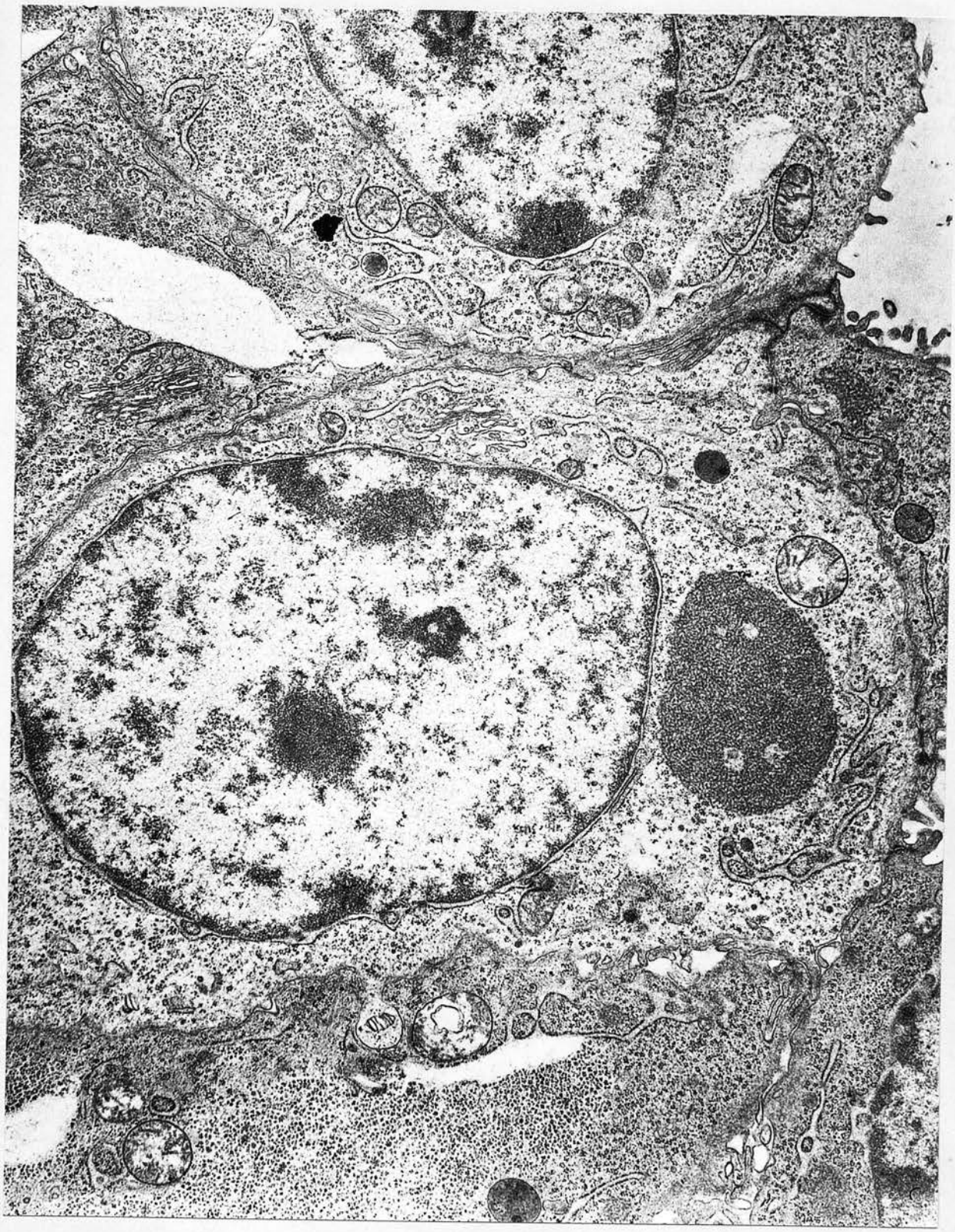


Figure 63.

Figure 64. At this magnification the granules in the inclusion appear as intertwining thread-like structures. The average diameter of the fibrils (arrows) is approximately fifteen millimicrons. Particles resembling ribosomes are present in one of the vacuoles within the inclusion.
Lead citrate X64000

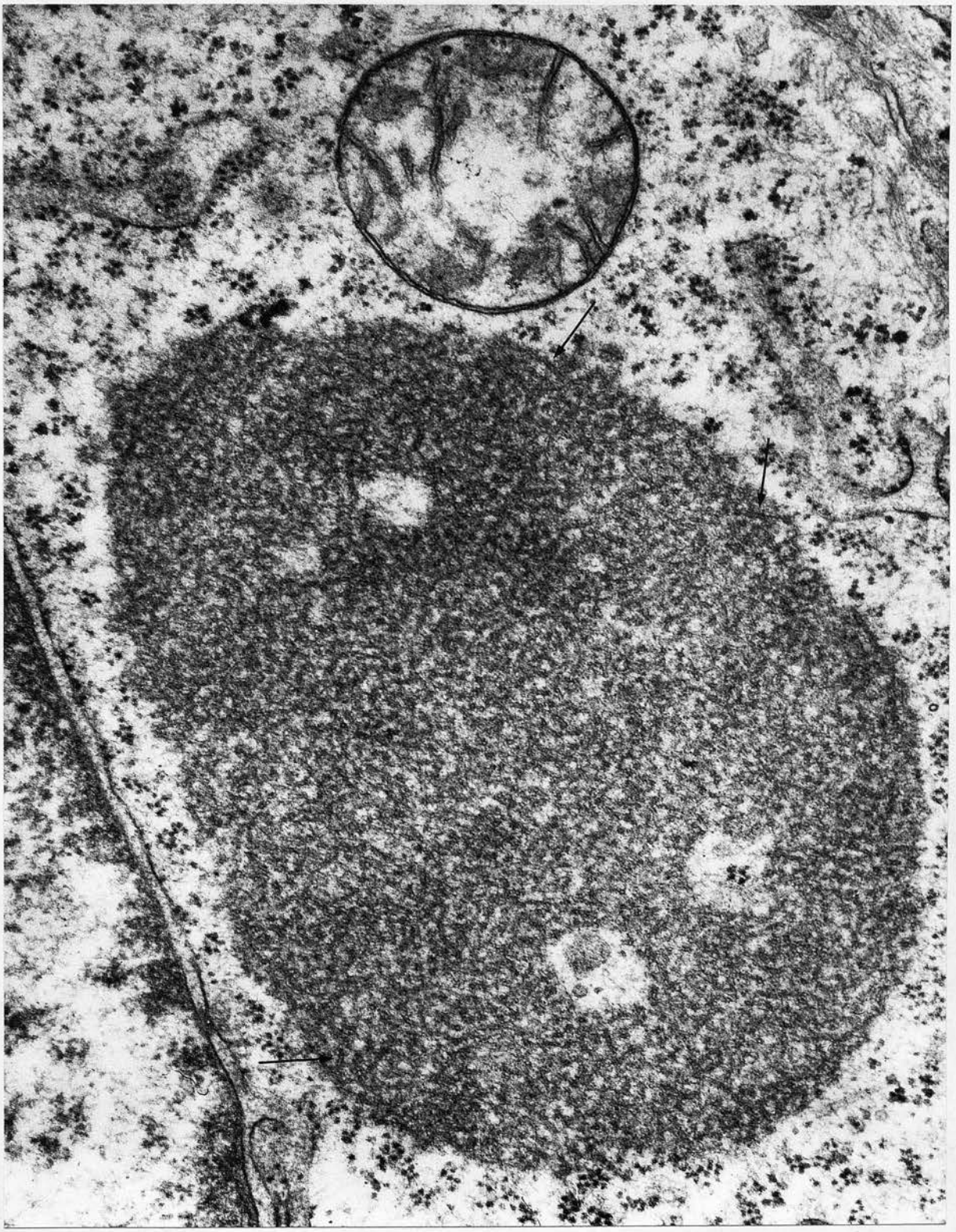


Figure 64.

antibody technique, specific cytoplasmic fluorescence was demonstrated (Figure 55). The inclusions as seen in the light microscope have been described previously (Section 2, Subsection 1).

The inclusions seen in the lungs of lambs infected with an ovine strain of PI3 virus were morphologically similar to those described in tissue cultures infected with PI2 virus (Kuhn and Harford, 1963; Howe et al., 1967); SV5 virus (Compans et al., 1966); a bovine strain of PI3 virus (Reczko and Bögel, 1962) and WB virus (Prose et al., 1965). In each of these reports and in the present study, the inclusions were composed of intertwining thread-like fibrils or granules. The diameter of the fibrils in the cytoplasm of bronchiolar epithelial cells in lambs infected with the G2 strain of PI3 virus, as in WB virus infection in tissue culture (Prose et al., 1965), was fifteen millimicrons, whereas, in PI2 infections a diameter of ten millimicrons was recorded (Kuhn and Harford, 1963). The morphology of the ovine G2 strain of PI3 virus has been described by Hore (1968) who stated that the diameter of the nucleocapsid, in preparations of the ovine G2 strain of PI3 virus stained for negative contrast, was approximately fifteen millimicrons (Figure 65). This suggests that the fibrils seen in the inclusions in the present study represent parainfluenza 3 virus nucleocapsid. In studies with WB virus the thread-like structures found in the cytoplasm of infected cells were clearly shown to be continuous with, and identical in appearance to, the loosely coiled internal component seen within virus filaments at the cell surface (Prose et al., 1965). Using ferritin conjugated antisera Reczko and Bögel (1962) and Howe et al., (1967) also considered the thread-like fibrils to be composed of viral nucleoprotein.

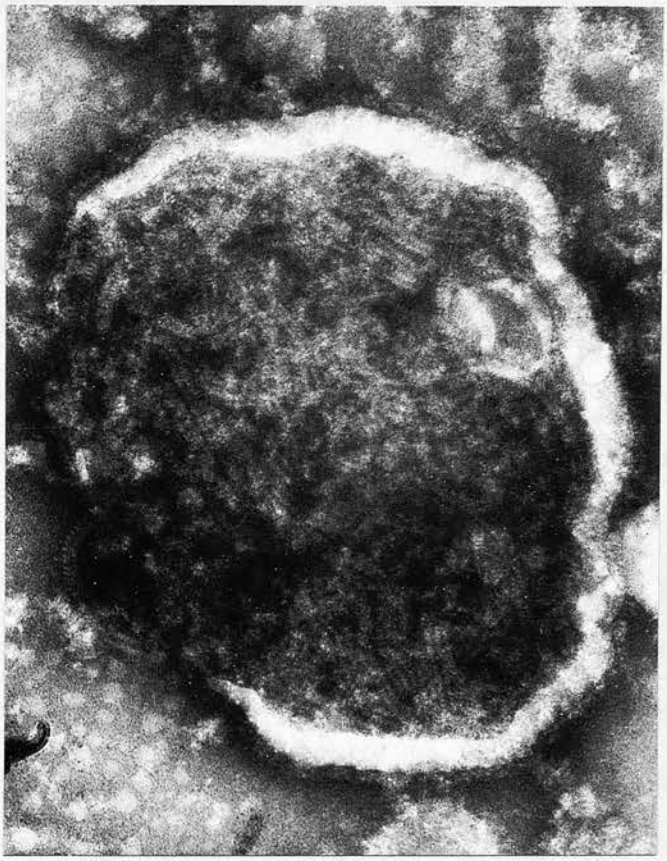


Figure 65. Partially disrupted virus particle showing internal helical component (diameter approximately fifteen millimicrons) and external projections. Sodium phosphotungstic acid X128000

SECTION 3

INOCULATION OF LAMBS WITH A STRAIN OF PARAINFLUENZA 3 VIRUS ISOLATED FROM CALVES

INTRODUCTION

The pathology of infection in lambs inoculated with an ovine strain of parainfluenza 3 (PI3) virus was described in the previous section. Since ovine and bovine strains are serologically related (Hore, 1968), and human strains, which are also serologically related to bovine strains (Abinanti, Chanock, Cook, Wong and Warfield, 1961), can produce lesions in the respiratory tract of suckling mice (Craighead, 1966), hamsters (Buthala and Soret, 1964) and hysterectomy-produced, colostrum-deprived piglets (Betts and Jennings, 1966), the possibility exists that either strain may be capable of causing infection in both species of animal. This is of more than academic interest since under either normal or intensive methods of husbandry, cattle and sheep are often kept together. Woods et al., (1965a) reported infection in two lambs caused by a bovine strain but did not consider the resulting histopathological lesions to be significant. In this section the pathology of the lesions in lambs inoculated with a bovine strain of PI3 virus is described. The results obtained in calves inoculated with an ovine strain of PI3 virus are given in Section 6.

MATERIALS AND METHODS

Lambs

Seven colostrum-deprived lambs and one lamb (494) which was left with its dam in a separate pen, were used. The colostrum-deprived lambs were fed as described under General Materials and Methods. The five colostrum-deprived lambs were inoculated with a bovine strain of PI3 virus within forty-eight hours of birth. The colostrum-fed lamb (494) was six days old at the time of inoculation. The inoculum consisted of infected bovine kidney tissue culture harvest fluid and was given intranasally (two millilitres) and intratracheally (three millilitres) as described under General Materials and Methods. The control lambs (495 and 498) were inoculated similarly within forty-eight hours of birth with uninfected tissue culture harvest fluid and were housed in a separate building. The virus-inoculated lambs were killed four, six, seven and eight days after inoculation and the control lambs on days four and seven.

Virus

A bovine strain of PI3 virus isolated from an intensively-reared calf by Mr. S. Mahalingham was used. It was serologically related to the bovine T1 strain (Dawson and Cruickshank, 1963) of PI3 virus and produced both cytoplasmic and nuclear inclusions in bovine kidney tissue cultures. It was at the second passage level in bovine kidney cultures and had an infectivity

titre of $10^{7.0}$ $TCID_{50}$ per millilitre.

Virological, Serological and Bacteriological Examinations

The virological and serological aspects of infection of lambs with a bovine strain of PI3 virus were examined by Dr. D. E. Hore.

Mr. D. Thompson carried out the routine bacteriological examination. The results are summarised in Table 4.

Pathology

The procedures carried out were as described under General Materials and Methods.

RESULTS

Clinical Observations

Clinical signs of a respiratory infection were absent in the control lambs. In the virus-inoculated group clinical signs were confined to the respiratory tract. Rapid, shallow respiration was first noted on the second day after inoculation. These signs progressed until there was obvious dyspnoea by the fifth day. Appetite appeared to be normal but, because of the degree of respiratory distress, lambs had difficulty in drinking. Quite often paroxysmal coughing occurred while the lambs were drinking and continued for a short time afterwards. If left undisturbed, however, coughing was

TABLE 4

RESULTS OF VIROLOGICAL, SEROLOGICAL AND BACTERIOLOGICAL EXAMINATIONS OF LAMBS INOCULATED WITH A BOVINE STRAIN OF PI3 VIRUS+

Lamb No.	Recovery of Virus‡		HI Test⊖		Bacteriology
	Nasal Swabs Days PI	Lungs Days PI	Preinoculation	Post inoculation	
498	- (3,4)	- (4)	<5	<5	-
495	- (3,4)	- (7)	<5	<5	-
491	+ (3,4)	+ (4)	<5	<5	-
497	+ (3,4)	+ (6)	<5	10	-
493	+ (3,4)	+ (6)	<5	20	-
494*	+ (3,4)	+ (6)	10	20	S. marcescens
496	+ (3,4)	+ (7)	<5	20	-
492	+ (3,4)	- (8)	<5	40	-

\+ = Supplied by Dr. D. E. Hore and D. Thompson.

‡ = Serologically identified as the bovine strain of PI3 virus.

* = Left on dam. Dam had an HI titre of 1:10 when lamb inoculated.

PI = Post Inoculation.

⊖ = Haemagglutination-inhibition titre expressed as reciprocal of serum dilution.

infrequently heard.

Pathology

Hyperaemia of the nasal and turbinate mucosae and a small amount of exudate in the nasal passages were seen in the control and infected lambs. No other lesions were seen in the control lambs (Figure 66). In the infected group lesions were confined to the respiratory tract in all but two cases. Numerous cysts of varying size were present in the pancreas of lamb 494, and, in the liver of lamb 492 there were two small raised foci which, histologically, consisted of accumulations of neutrophils.

The distribution of the lung lesions is shown in Figure 67. Four days after inoculation the macroscopic lesions consisted of streaky areas of dark red consolidation affecting all lobes (Figure 68). In two of the three lambs (493 and 497) killed on the sixth day after inoculation (Figure 69) and in the lambs killed on the seventh (496) and eighth (492) days after inoculation (Figure 70), one half to two thirds of the lungs were consolidated. The affected areas were greyish-red and slightly raised or level with the surrounding normal lung tissue. In the colostrum-fed lamb (494) from which Serratia marcescens was isolated, the lesions resembled those seen four days after inoculation, consisting mainly of streaky, dark red areas of consolidation affecting all lobes of the lung (Figure 71).

Histopathology

In every lamb there was focal neutrophil infiltration and erosion of

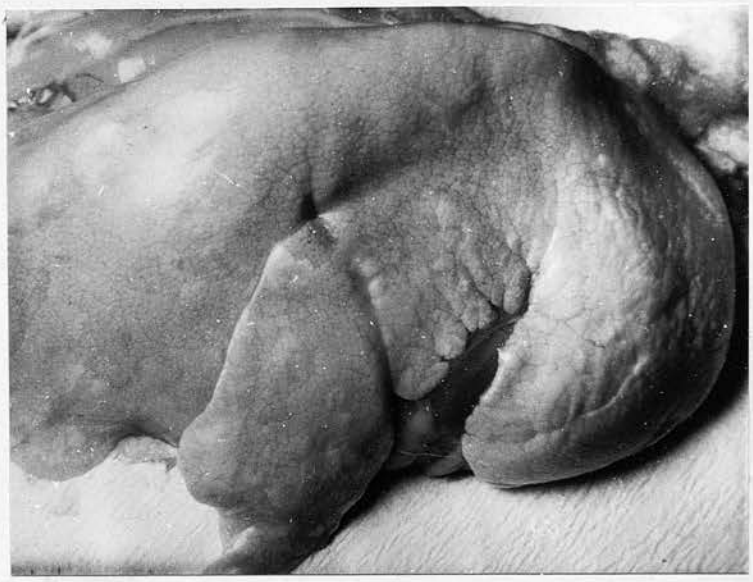


Figure 66. Macroscopic appearance of the right lung of a lamb inoculated with virus-free tissue culture harvest fluid.

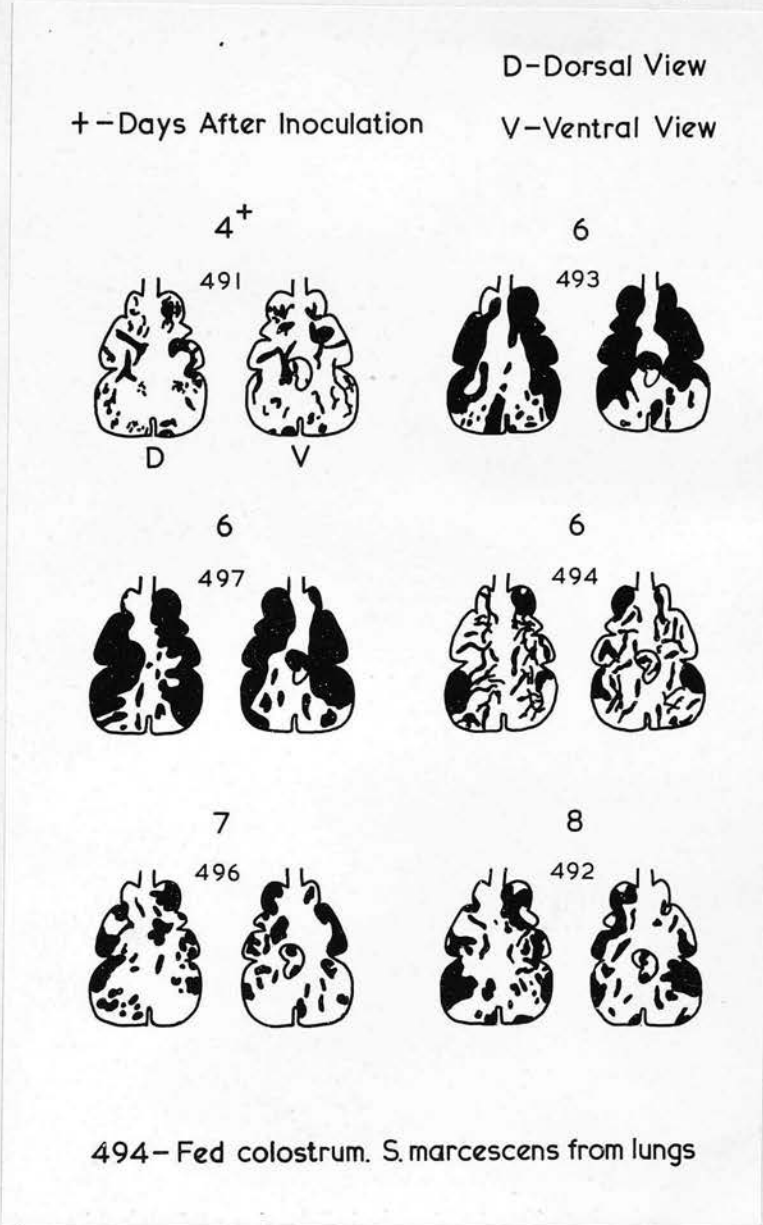


Figure 67. Distribution of the lung lesions in lambs inoculated with a bovine strain of PI3 virus.

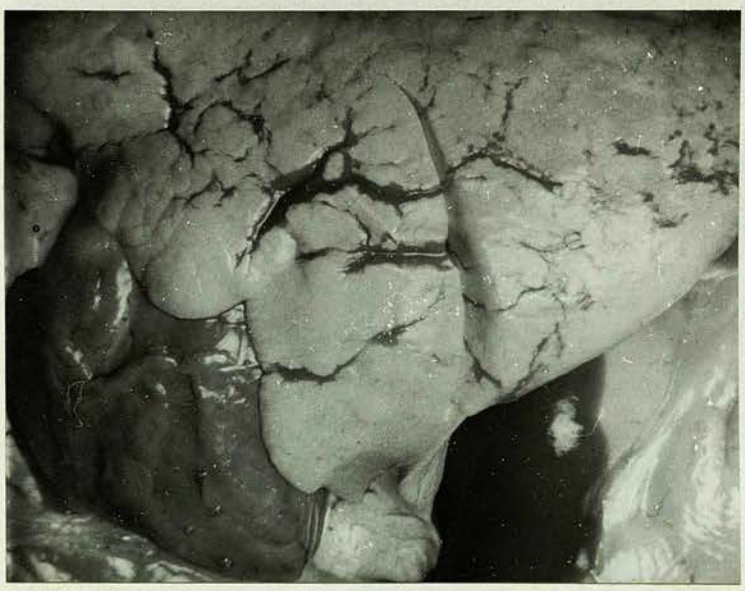


Figure 68. Macroscopic appearance of the lung lesions four days after inoculation with a bovine strain of PI3 virus.

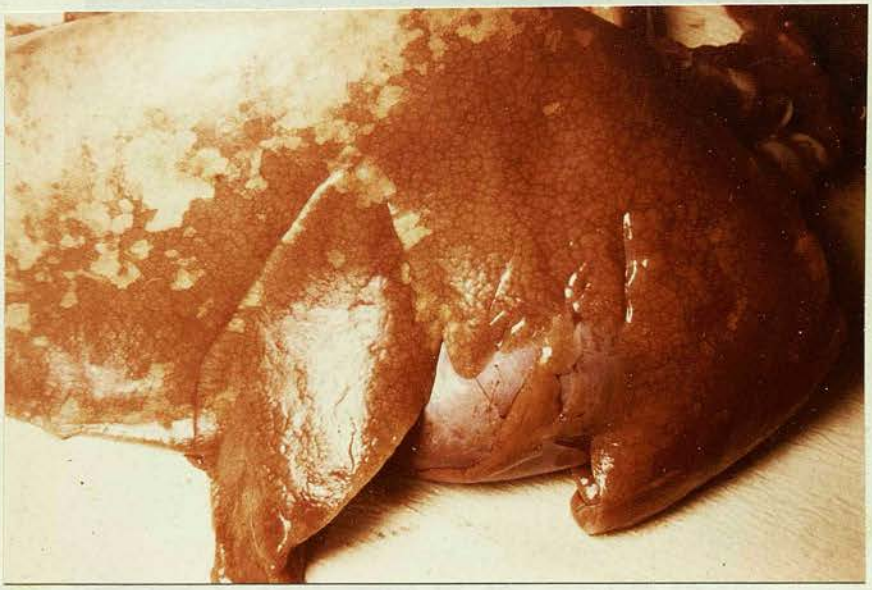


Figure 69. Macroscopic appearance of the lung lesions six days after inoculation with a bovine strain of PI3 virus.

Figure 70. Lobular pattern of consolidation in the right lung of a lamb killed eight days after inoculation with a bovine strain of PI3 virus.

Figure 71. Contrasting appearance of the lesions in the lungs of two lambs killed six days after inoculation with a bovine strain of PI3 virus. Serratia marcescens was isolated from the lung on the right.

the turbinate mucosa and a small amount of exudate on the affected surfaces. Histologically, the remaining organs in the control lambs were normal.

In the lungs of the infected lamb (491) killed four days after inoculation the bronchi were normal except for the presence of a few acidophilic cytoplasmic inclusions. In the bronchioles, the epithelium was often hyperplastic and in some instances giant cell bronchiolitis was observed, while in others the epithelial cells were undergoing degenerative changes. Acidophilic, cytoplasmic inclusions were very common and were similar in morphology and staining affinity to those caused by an ovine strain (Section 2, Subsection 1) in lambs. The inclusions, which varied in shape and size, usually occurred singly but sometimes two were present in the same cell at either pole of the nucleus. Affected alveoli were partially collapsed. Macrophages and lymphocytes had infiltrated the interalveolar septa and large mononuclear cells were often seen attached to the alveolar walls, sometimes forming small syncytia. Alveolar epithelialisation was occasionally seen. The majority of affected alveoli contained alveolar macrophages and on occasion, neutrophils and necrotic debris. Mitotic figures were at times seen in the bronchiolar epithelium and in the interalveolar septa. By the sixth day after inoculation the lesions in two of the lambs (493 and 497) had increased in size and severity. Hyperplasia and, in places, necrosis of the bronchiolar epithelium were more pronounced but in general the lesions were similar morphologically to those seen four days after inoculation (Figure 72). The number of inclusions had increased and they were seen in alveolar epithelial cells and macrophages as well as in bronchiolar and bronchial epithelial cells (Figure 73). Moderate numbers of lymphocytes and macrophages were

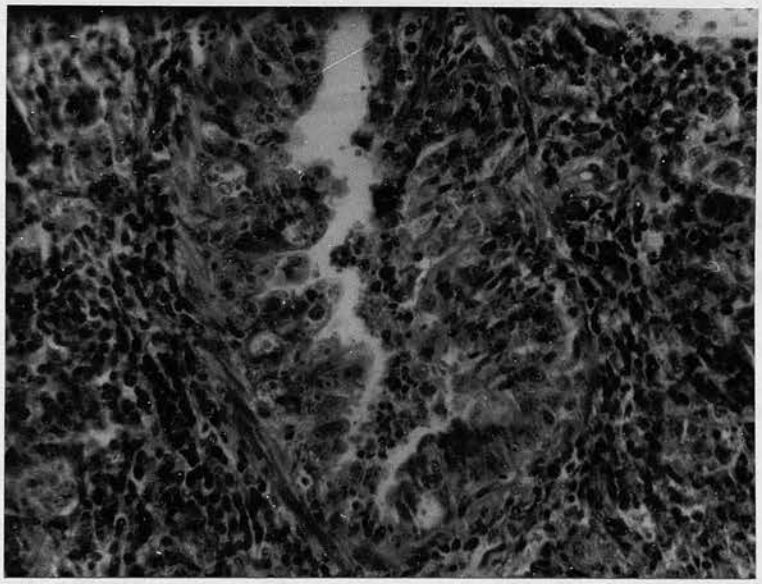


Figure 72. Hyperplasia and focal necrosis of the bronchiolar epithelium and moderate peribronchiolar lymphocytic infiltration.
Pollak X270

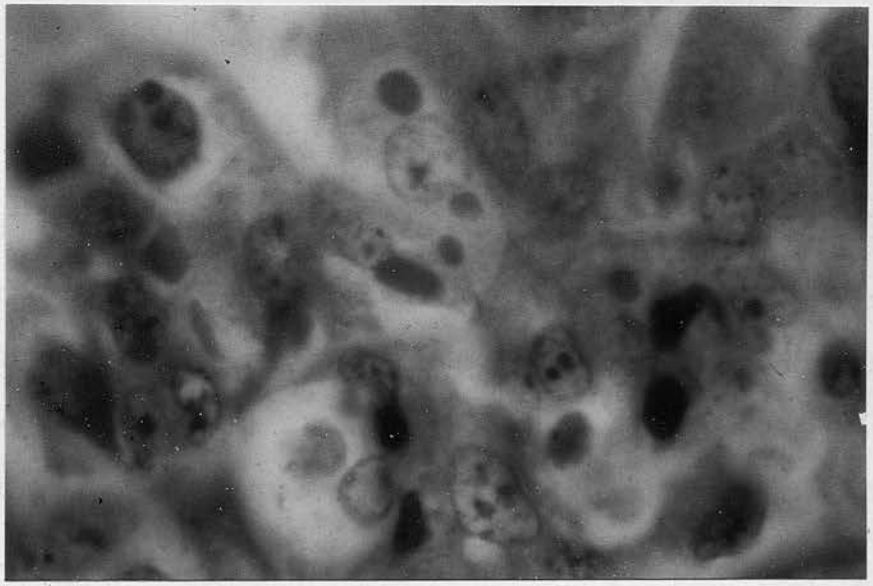


Figure 73. A higher magnification of Figure 72 illustrating the variations in shape, size and position of the cytoplasmic inclusions.
Pollak X1800

occasionally seen in the peribronchiolar tissues. In lamb 494 the inclusions were fewer. The cellular reaction in some parts of the lung of lamb 494 was essentially the same as in lambs 493 and 497 while in other areas the majority of the air passages, from medium sized bronchi downwards, were filled with an exudate composed of bacteria, desquamated epithelial cells and degenerating neutrophils and macrophages. Cytoplasmic inclusions were not seen in the lungs of the lambs killed on the seventh and eighth days after inoculation. Although bronchioles showing epithelial hyperplasia or necrosis were present (Figure 74), most had an epithelial lining consisting of a single layer of cells with bulging nuclei (Figure 75). Mitotic figures in the bronchiolar epithelium and in the interalveolar septa were more common than at four days. Within affected areas the amount of exudate had diminished and individual alveoli could now be discerned. These changes suggested that resolution was commencing.

At no time were nuclear inclusions observed.

DISCUSSION

In lambs inoculated with the bovine strain of PI3 virus the degree of respiratory distress was more pronounced than in lambs inoculated with an ovine strain of the same virus (Section 2, Subsection 1) but otherwise the clinical signs were similar. Whether the same response would be elicited by a different bovine strain or, for that matter, a different ovine strain is not known.

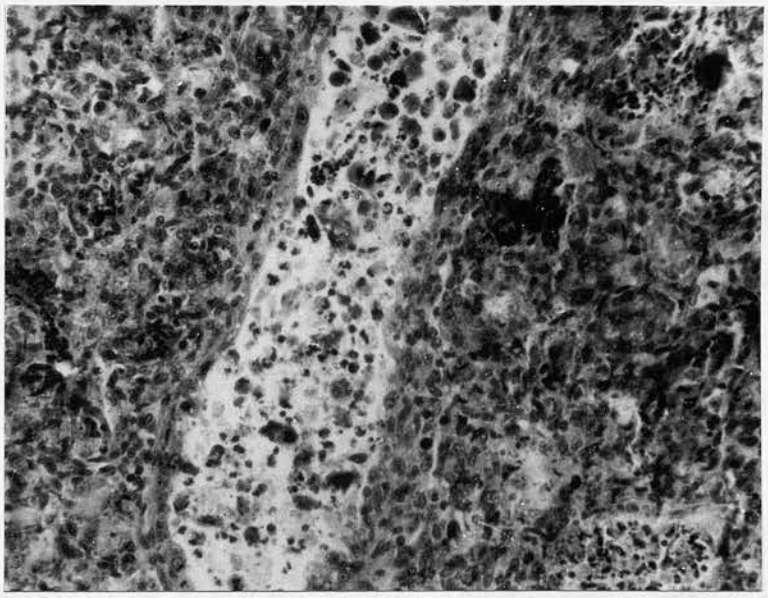


Figure 74. Necrosis and desquamation of bronchiolar epithelium in an area of marked interstitial pneumonia.
H & E X240

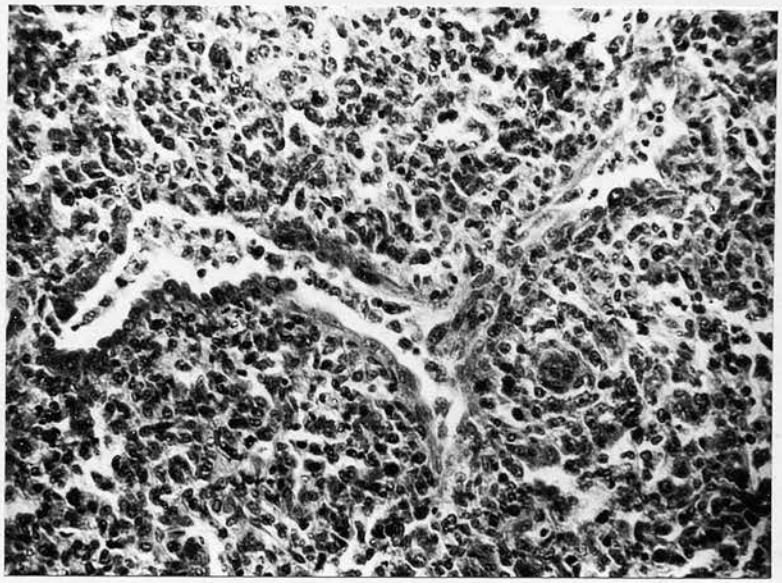


Figure 75. A bronchiole lined by a single layer of low cuboidal epithelial cells. Re-appearance of a recognisable alveolar pattern is also evident.
H & E X240

Changes in the upper respiratory tract of lambs resulting from infection with either ovine or bovine strains of PI3 virus have not been observed in the present studies but in calves inoculated with a bovine strain, lesions have been reported (Omar et al., 1966). Similar results have also been obtained after inoculation of suckling mice (Craighead, 1966) with a human strain of PI3 virus.

The lung lesions in lamb 494 differed from those seen in the two other lambs (493 and 497) killed on the same day after inoculation. The differences were probably due to the co-existing infection with S. marcescens and not to the presence of a positive HI titre of 1:10 at the time of inoculation. Somewhat similar histological lesions have been seen in the lungs of colostrum-deprived lambs inoculated with uninfected tissue culture fluid from which S. marcescens was isolated, but not in lambs having high HI antibody titres when inoculated with an ovine strain of PI3 virus (Section 2, Subsection 2).

More extensive macroscopic and histological lesions were produced in the lambs inoculated with the bovine strain than in lambs inoculated with an ovine strain (Section 2, Subsections 1 and 2) of PI3 virus but qualitatively the lesions were similar. Acidophilic, cytoplasmic inclusions in bronchial, bronchiolar and alveolar epithelial cells, round cell infiltration of the interalveolar septa, syncytium formation and pseudo-epithelialisation of alveoli were common to both groups.

Contrary to these observations, Woods et al., (1965a) reported that, although the lungs of two lambs (one inoculated with a bovine strain of PI3 virus and the other held in contact with the inoculated lamb) were congested, and areas of consolidation were present along the ventral border of the

apical and cardiac lobes in the lamb inoculated intranasally with the bovine Illinois 811 strain of PI3 virus, histologically "significant lesions were not observed in the lungs, spleen and kidneys from the lambs in the infected group". Their negative histopathological results may have been due to the interval of fourteen days between inoculation and death, since in lambs inoculated with the ovine G2 strain of PI3 virus resolution usually commenced at about nine days after inoculation, and in some instances was almost complete by the fifteenth day (Section 2, Subsection 2). Also, in the present experiment, the histological appearance of the lesions in the lambs killed seven and eight days after inoculation suggested that resolution was commencing. Acidophilic, nuclear inclusions, a feature which has been described in calves inoculated with bovine strains of PI3 virus (Dawson et al., 1965; Omar et al., 1966) were not observed in the present investigation even though the bovine strain used regularly produced nuclear inclusions in tissue culture (Mahalingham, 1968).

SECTION 4

INOCULATION OF LAMBS WITH *BEDSONIAE*

INTRODUCTION

The intranasal or intratracheal inoculation of lambs with Bedsonia organisms isolated from the respiratory tract of sheep has not been recorded in Britain. Studies carried out in the United States of America, which have been discussed in the review of literature, showed that the lesions produced were either primarily exudative or proliferative, the type of reaction depending on the interval between inoculation and slaughter. During the stage of resolution the appearance of some of the lungs resembled that seen in cases of subclinical pneumonia (Dungworth and Cordy, 1962a). Somewhat similar lesions were observed during the stage of resolution in parainfluenza 3 (PI3) virus infections (Section 2, Subsection 2). However, one feature described for Bedsonia infections in older lambs (Boidin *et al.*, 1958; Dungworth and Cordy, 1962a) but not for PI3 virus infections in young colostrum-deprived lambs (Section 2, Subsections 1 and 2) is marked peribronchiolar lymphocytic hyperplasia. The presence or absence of this lesion could be of importance in the differential diagnosis of these infections.

MATERIALS AND METHODS

The technical procedures involved in producing the inoculum and in

re-isolation of Bedsonia organisms from the lungs of the lambs were carried out in the Department of Microbiology, Moredun Institute by Mr. G. Robinson.

Bedsonia Organism

The organism used was a strain isolated in the yolk sacs of embryonating hens' eggs from a case of ovine atypical pneumonia by Dr. A. Foggie of the Moredun Institute. At no time had it been passaged through laboratory animals. It was received from the Microbiology Department as a 1:10 saline suspension of Bedsonia infected yolk sac and had an infectivity titre of $10^{3.4}$ pneumonia producing $doses_{50}$ per 0.05 millilitres.

Lambs

The procedures for obtaining and rearing colostrum-deprived lambs have been described under General Materials and Methods and were the same in this experiment except that antibiotics were omitted. Four lambs were inoculated intratracheally by the method described under General Materials and Methods with three millilitres of a 1:10 saline suspension of Bedsonia infected yolk sac and were autopsied at three, five, nine and twelve days after inoculation. Two control lambs were inoculated with three millilitres of a 10^{-3} saline suspension of normal yolk sac and were housed separately. They were killed four and nine days after inoculation.

Pathology

Pieces of lung, turbinate mucosa, trachea, liver, kidney, spleen and

pulmonary lymph nodes were routinely taken and treated as described under General Materials and Methods. For the detection of elementary bodies three types of preparations were used, namely paraffin sections, cryostat sections and lung impression smears. They were stained with a modification of the Ziehl-Neelsen technique and with a modification by Wolbach (1919) of the Giemsa method. For cryostat sections small (approximately five cubic millimetres) pieces of consolidated lung were rapidly frozen in a CO_2-ethanol mixture and cut at a thickness of one to two microns. The sections were then air dried and fixed by heat. Impression smears were made from the cut surface of consolidated areas and treated in a similar manner.

RESULTS

Clinical Examination

The two lambs (499 and 500) which received normal yolk sac material remained healthy.

In the four lambs which received infected yolk sac suspension the earliest sign was a marked rise in body temperature (Figure 76). In lamb 300 the body temperature had risen to 40.7 degrees centigrade thirty hours after inoculation, whereas, in lambs 296, 297 and 120 significant rises in temperature were not recorded until forty-eight hours after inoculation. Along with pyrexia there was dyspnoea and the lambs refused to take their normal quota of milk. Weakness, anorexia, marked dyspnoea and a lowered body temperature were seen in lamb 300 two days after inoculation.

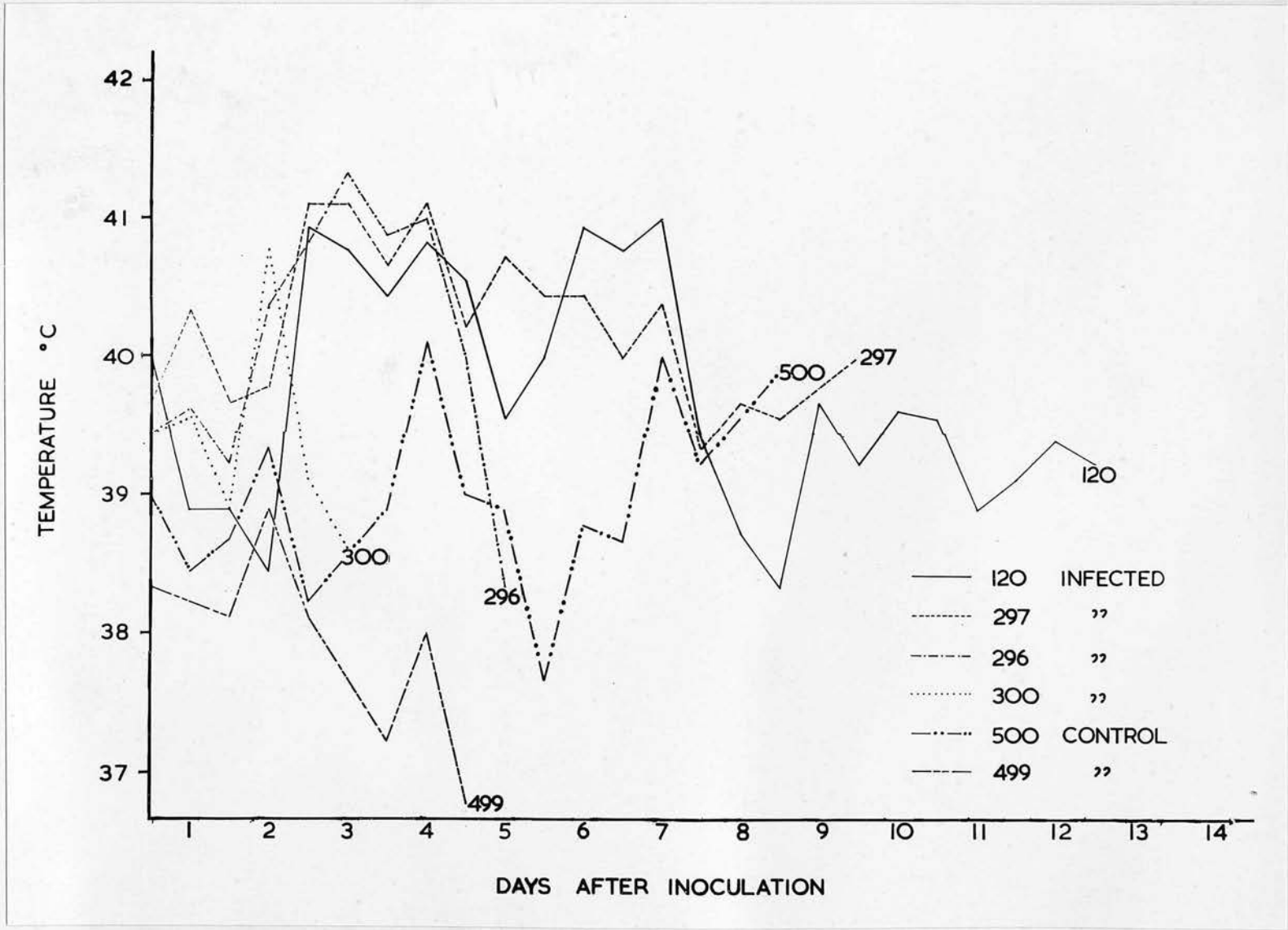


Figure 76. Temperature response in Bedsonia-inoculated lambs and lambs inoculated with normal yolk sac suspension.

Lamb 300 was killed the following morning when it was found recumbent with a dry crusty exudate around its nostrils and showing signs of marked dyspnoea and anorexia. Lamb 296 showed similar signs and was found dead on the fifth day after inoculation. Elevated temperatures, dyspnoea and anorexia persisted in lambs 297 and 120 for three and five days respectively. After the body temperature returned to normal the other clinical signs gradually subsided and by the eighth or ninth day after inoculation these lambs appeared clinically normal.

Recovery of Bedsonia Organisms

Bedsonia organisms were not isolated from the control lambs but were recovered from each of the Bedsonia-inoculated lambs.

Demonstration of Bedsoniae in Lung Impression Smears and in Tissue Sections

Using either Wolbach's (1919) modification of the Giemsa method or a modified Ziehl-Neelsen technique it was possible to demonstrate intracellular and extracellular elementary bodies in cryostat sections of all Bedsonia-inoculated lambs and in lung impression smears of lambs 300, 296 and 297 (Figures 77, 78). Coccoid elementary bodies in the cytoplasm of macrophages and epithelial cells in bronchioles and alveoli were most numerous in lambs 300 and 296 killed on the third and fifth days after inoculation. In lamb 297, killed on the ninth day, elementary bodies were less frequently seen and in the lamb (120) killed on the twelfth day a thorough search of cryostat sections was required before typical clusters of elementary bodies were found.

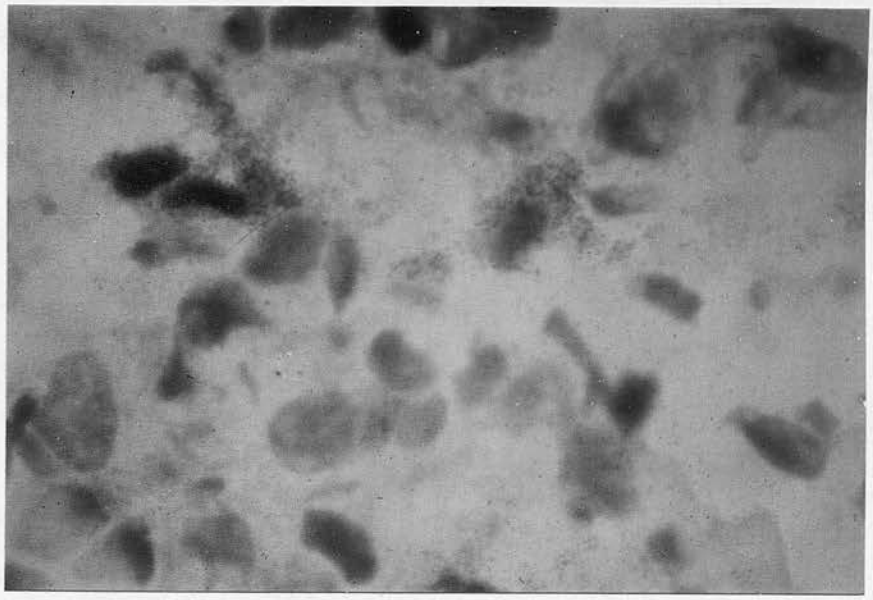


Figure 77. Cryostat Section. Intracellular and extracellular Bedsonia elementary bodies.
Modified Ziehl-Neelsen X1850

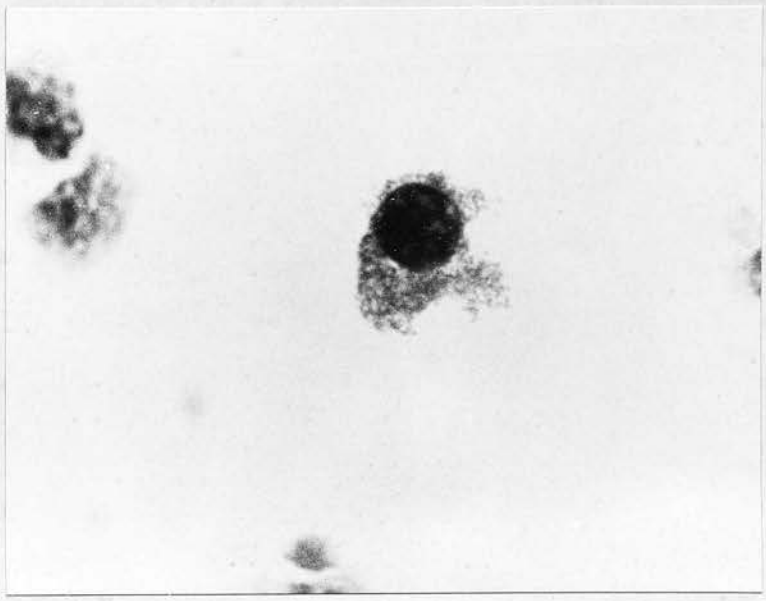


Figure 78. Lung Impression Smear. Numerous intracellular Bedsonia elementary bodies in a mononuclear cell.
Wolbach's Giemsa X1750

The detection of elementary bodies in paraffin sections was more difficult but positive identification was made in one instance (Figure 79). Demonstration of the organisms was achieved with greater ease using the modified Ziehl-Neelsen technique for impression smears and cryostat sections but for paraffin sections Wolbach's (1919) modification of the Giemsa method was superior.

Pathology

Gross Lesions

No macroscopic lesions were seen in any of the organs in the control lambs.

Except for the presence of pancreatic cysts in lambs 300, 296 and 297 macroscopic lesions were confined to the respiratory tract and pulmonary lymph nodes in the Bedsonia-inoculated group. The appearance, size and distribution of the pancreatic cysts were similar to those seen previously (Section 2, Subsections 1 and 2). A single cyst, approximately seven millimetres in diameter, on the ventral surface of the pancreas near where the pancreatic duct joins the common bile duct was chosen for serial sectioning to determine the relationship of the cyst to the duct system. The pancreas, liver and duodenum along with the intact pancreatic and common bile ducts were carefully removed from another lamb (297) and placed in warm normal saline. A ten per cent. solution of gelatine, to which was added sufficient toluidine blue to provide colour, was injected into the

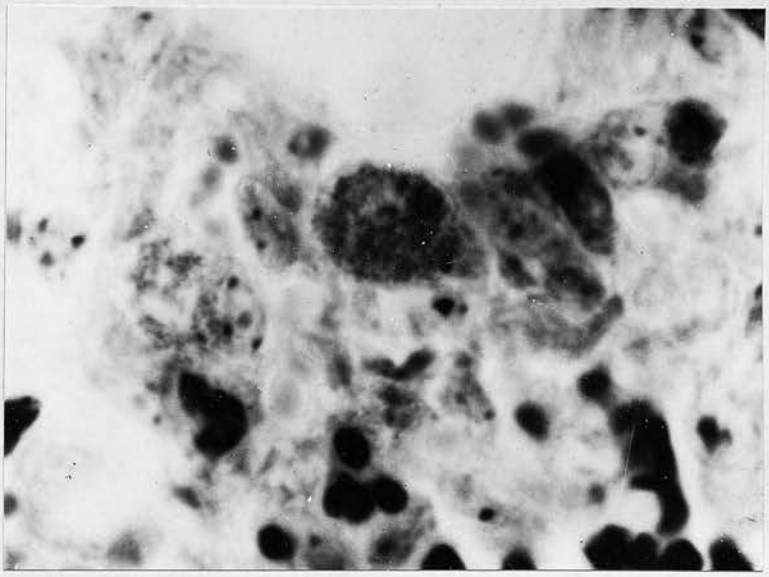


Figure 79. Paraffin Section. Bedsonia elementary bodies in an alveolar lining cell.
Wolbach's Giemsa X1800

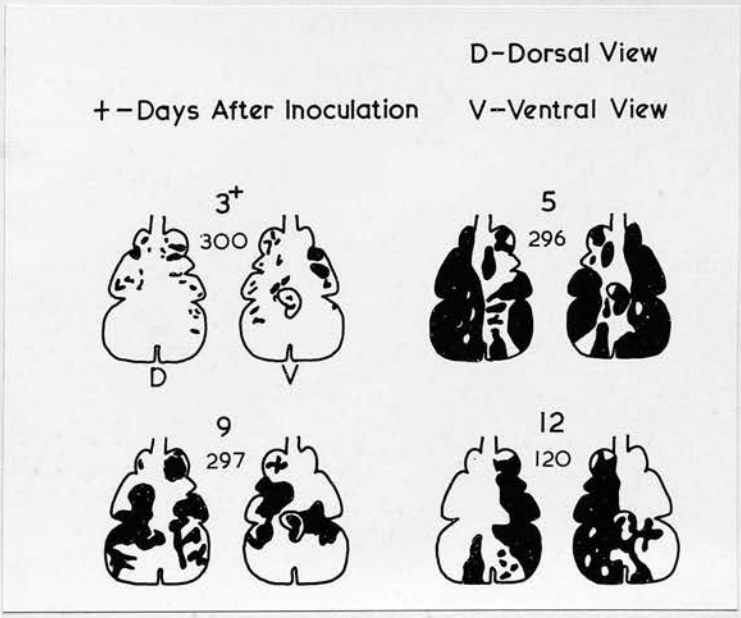


Figure 80. Distribution of the lung lesions in lambs inoculated with Bedsonia organisms.

common bile duct after it had been ligated above and below where the pancreatic duct enters. The gelatine-dye mixture remained in the pancreatic ducts and in no instance was the mixture seen in the cysts. No abnormalities were detected in the tissues of the upper respiratory tract.

The distribution of the lung lesions in the Bedsonia-inoculated lambs is shown in Figure 80. In lamb 300, killed on the third day after inoculation, there were patchy and streaky areas of reddish-brown consolidation scattered throughout the apical and cardiac lobes and anterior ventral portions of the diaphragmatic lobes (Figure 81). The affected areas were level with the surrounding normal lung tissue. The cut surface was moist, dark red and a greyish exudate could be expressed from the airways. Similar areas of consolidation were present within the substance of the lung surrounding small bronchi and bronchioles.

In lamb 296, which was found dead five days after inoculation, the lesions were very extensive; approximately eighty per cent. of the lungs were involved. The affected areas were very firm, dark red and level with or slightly raised above the small amount of normal lung tissue remaining. The interlobular septa were oedematous and there was a frothy exudate in the bronchi. In lambs 297 and 120, killed nine and twelve days after inoculation respectively, the lesions varied from area to area. Some were raised and dark red while others were greyish and slightly sunken (Figures 82, 83). In some lobes the interlobular septa were oedematous.

When compared with the pulmonary lymph nodes of the two control lambs those of the Bedsonia-inoculated group were slightly larger.

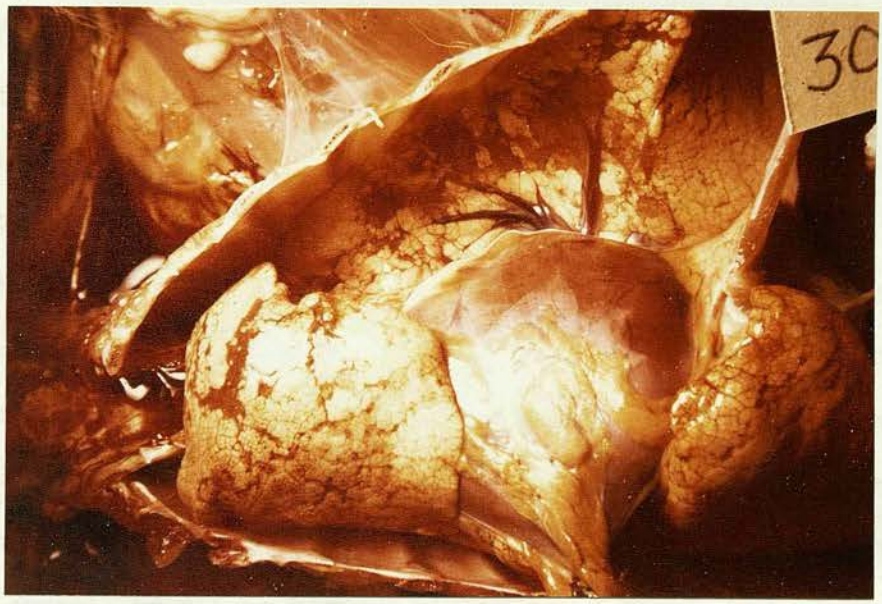


Figure 81. Macroscopic appearance of the lung lesions in a lamb killed three days after inoculation with Bedsonia organisms.

Figure 82. Macroscopic appearance of the lung lesions in a lamb killed nine days after inoculation with Bedsonia organisms.

Histopathology

In randomly cut sections the appearance of the pancreatic cysts was similar to those described previously (Section 2, Subsections 1 and 2). Serial sectioning of one of the cysts proved that there was no direct connection between the cyst and the collecting ducts. Figures 84, 85 and 86 illustrate the relationship of the cyst to the ducts and also show the multi-locular structure observed in some of the cysts. Contrary to previous observations inflammatory changes associated with the cysts were seen in serial sections. Neutrophils had infiltrated the epithelial lining and sub-epithelial tissues of the cyst (Figure 87) and were occasionally seen in the connective tissue between the cyst and the pancreatic duct. No bacteria were observed in these sites.

Lymphoid follicles were not seen in the pulmonary lymph nodes of the control lambs nor in Bedsonia-inoculated lambs 300 and 296. By the ninth day follicle formation had commenced and there were well-developed follicles in the pulmonary lymph nodes of lamb 120 killed on the twelfth day. In lambs 300 and 296 the medullary sinuses contained neutrophils, macrophages and necrotic debris, whereas, in lambs 297 and 120 macrophages, lymphocytes and plasma cells predominated.

The only other organ examined that consistently showed abnormalities was the liver. Here the lesions ranged from early degeneration of hepatic cells and a few neutrophils in the central veins and sinusoids to diffuse centrilobular fatty degeneration. Lesions tended to be more marked in the lambs killed three and five days after inoculation.

Lesions in the upper respiratory tract were minimal and consisted of a small amount of exudate in the tracheal lumen or scattered neutrophils within

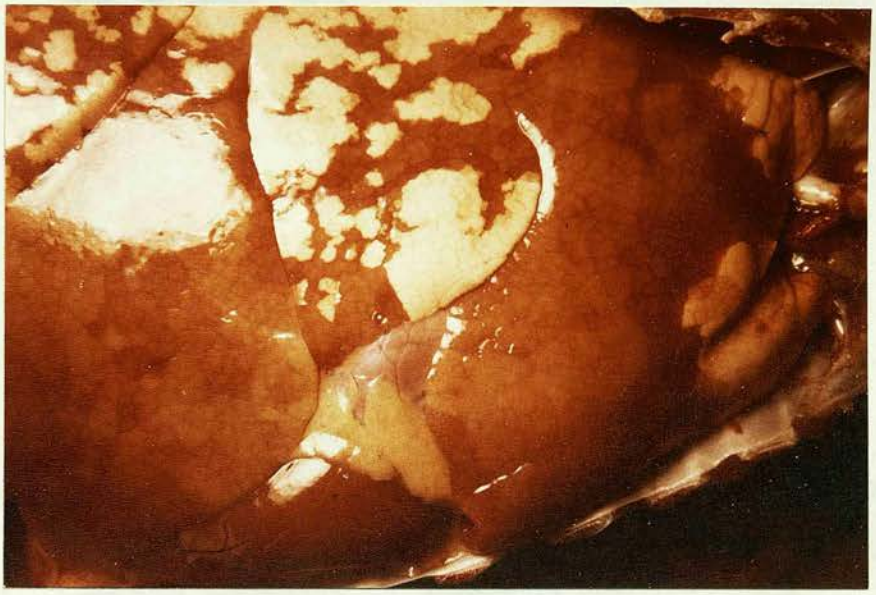


Figure 83. Macroscopic appearance of the lung lesions in a lamb killed twelve days after inoculation with Bedsonia organisms.

Figure 84. A pancreatic cyst (c) surrounded on three sides by acinar tissue. (A). There is no communication between the pancreatic ducts (D) and the cyst.
H & E X14

Figure 85. The same cyst as in Figure 84 after cutting into the cyst for a distance of 0.6 millimetres. The cyst is partially divided by a septum. There is no communication between the ducts and the cyst.
H & E X14

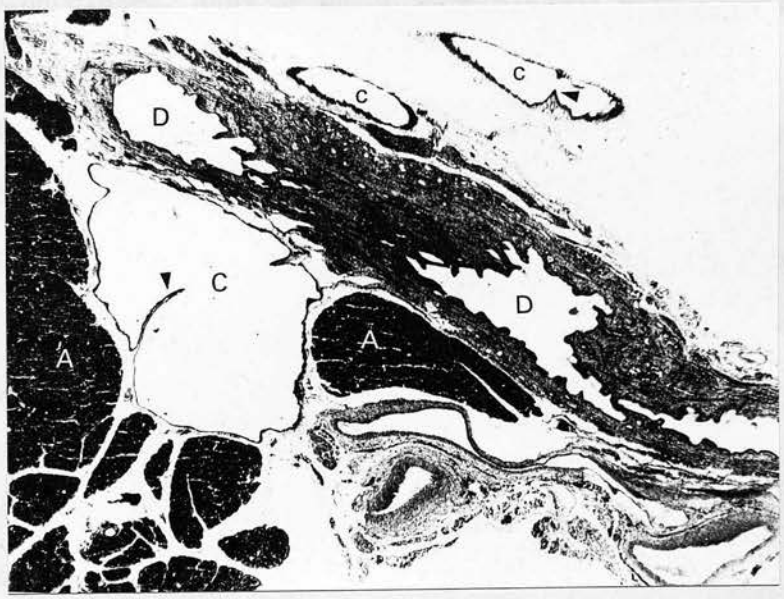


Figure 86. The same cyst as in Figure 84 after cutting into the cyst for a distance of one millimetre. The pancreatic duct by-passes the cyst. The cyst is being further divided by thin septa (arrows).
H & E X14

the turbinate mucosa and submucosa. The latter were also seen in one of the control lambs.

The histological lung lesions consisted of both proliferative and exudative changes. In the earlier stages the reaction was characterised mainly by serous exudation and neutrophil infiltration, whereas, in lambs killed on the ninth and twelfth days after inoculation, hyperplasia of epithelial and mesenchymal elements predominated.

In the acute stage (lambs 300 and 296) the bronchi usually contained only a small amount of exudate but occasionally they were completely occluded. The bronchial epithelium was intact and had been infiltrated by small numbers of neutrophils. The appearance of the lesions in the larger bronchioles was similar but in the smaller air passages the reaction was more severe. Within the smaller bronchioles, many of which were plugged with necrotic debris and degenerate neutrophils, the epithelial changes varied. In some, small numbers of neutrophils had invaded the epithelium while in others neutrophils were more numerous and there were varying degrees of cellular degeneration ranging from vacuolation of the cytoplasm to complete necrosis (Figure 88). The necrotic foci were never widespread; only a few cells being involved in any one bronchiole in a section. The affected alveoli contained a serous exudate and large numbers of neutrophils and were usually associated with small bronchioles. Some alveoli contained macrophages which were either lying free in alveoli or attached to the alveolar walls either singly or in groups of two or three protruding into the alveolar lumen. Hyperaemia of the alveolar capillaries, serous

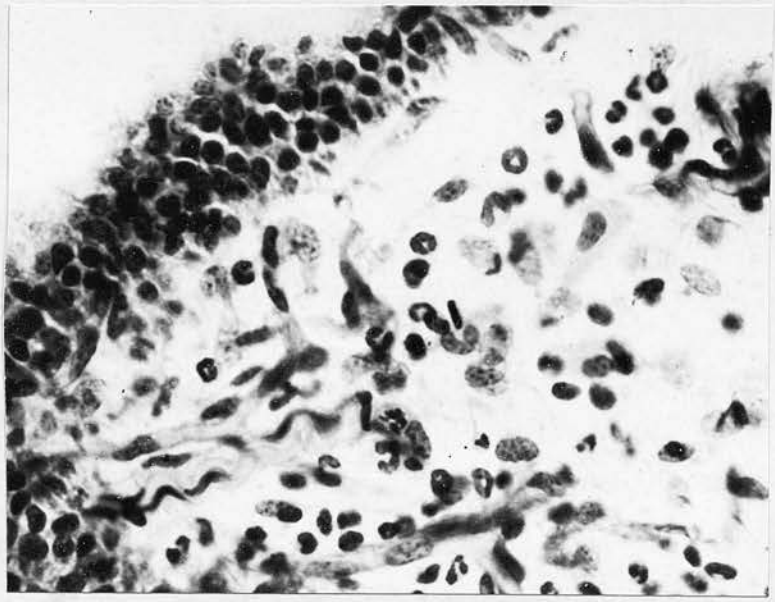


Figure 87. Pancreatic cyst showing neutrophil infiltration of the sub-epithelial tissues.
H & E X600

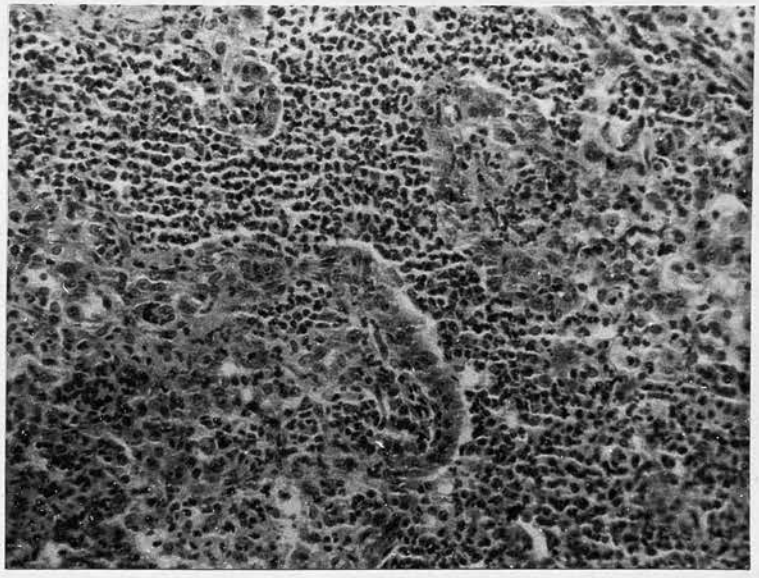


Figure 88. Neutrophil infiltration of the epithelium and occlusion of the lumen of a bronchiole. The surrounding alveoli contain serous fluid, neutrophils and macrophages.
H & E X245

exudation and proliferation and infiltration of cellular elements had resulted in thickening of the interalveolar septa. In some places the reaction appeared to be more acute in that the serous exudate stained a deeper shade of pink (H & E) and there were increased numbers of neutrophils and degenerate cells. Examination of reticulin stained sections of these areas showed that there was necrosis with fragmentation and separation of the reticulin fibres (Figure 89). The interstitial tissues were oedematous and contained small numbers of inflammatory cells and erythrocytes. The peribronchial lymphatics were distended and the walls of arterioles were oedematous but not inflamed. Small numbers of lymphocytes and macrophages were present in the peribronchial, peribronchiolar and perivascular tissues.

By the ninth day after inoculation the exudative reaction had subsided. Moderate epithelial hyperplasia and small amounts of exudate were occasionally seen in bronchi and larger bronchioles. The changes in the smaller bronchioles were similar except that the degree of epithelial hyperplasia and the amount of exudate were greater. The alveolar lesions varied but generally proliferative changes dominated (Figure 90). At the periphery of the lesions, where individual alveoli could be discerned, there was cellular thickening of the interalveolar septa and many of the alveoli contained both free and attached mononuclear cells*. Towards the centre

* Cells that were attached or lying free in alveoli which could not be positively identified as epithelial or mesenchymal in origin were called mononuclear cells.

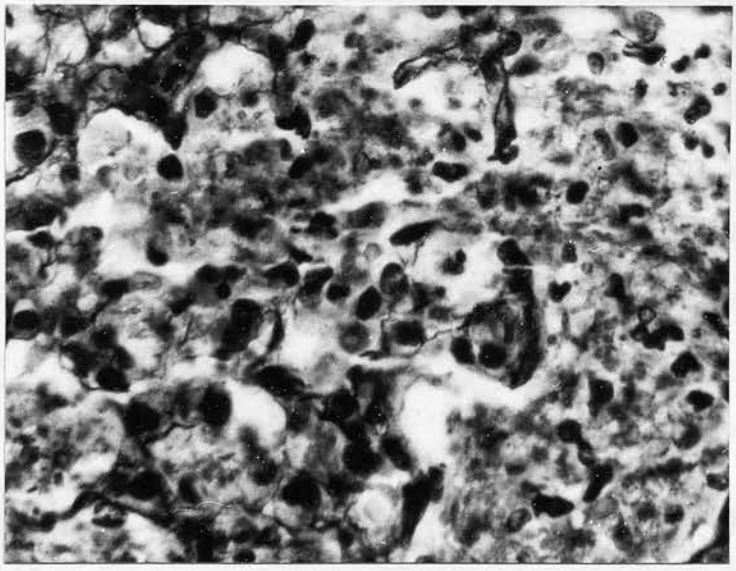


Figure 89. Separation and fragmentation of the alveolar reticulin network.
Silver reticulin method of Slidders et al., (1958) X660

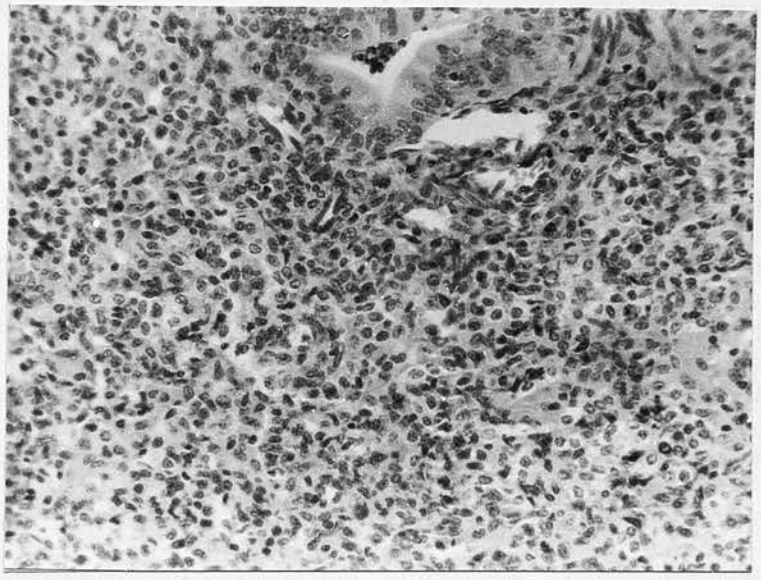


Figure 90. Hyperplasia of the bronchiolar epithelium and mononuclear cell infiltration of the surrounding alveoli. Note the absence of neutrophils in alveoli.
H & E X230

of the lesion it was difficult and sometimes impossible to make out individual alveoli as they were either atelectatic or had been infiltrated by numerous leucocytes. Reticulin stained sections of these areas showed that the majority of the cells were on the lumen side of the thickened reticulin fibres with smaller numbers within the septa. Partial to complete vacuolation of epithelial cells was seen in bronchioles and alveoli. Variable numbers of neutrophils were still present. Generally they tended to be scattered throughout the section but in places focal accumulations were seen. Small numbers of macrophages, lymphocytes and occasionally plasma cells were present in the peribronchial and perivascular tissues.

The lung lesions on the twelfth day, except for a small amount of exudate in the larger air passages, were confined to the bronchioles, alveoli and interstitial tissues. Hyperplasia of the bronchiolar epithelium was less frequently seen. There was no obvious change in the amount of exudate. Many of the bronchioles were occluded, resulting in partial to complete collapse of alveoli. The numbers of lymphocytes, plasma cells and macrophages in the peribronchiolar and perivascular tissues had increased slightly. The alveolar reaction varied. In some of the more peripheral parts patchy areas of mild interstitial pneumonia were the only lesions seen, while in other areas the alveolar reaction was similar to that seen at nine days. Resolution had commenced as indicated by the amount and character of the exudate and by the fact that individual alveoli could be seen. Within these alveoli, which were partially collapsed, there were attached and free mononuclear cells. Some alveoli had a continuous row of low cuboidal cells resembling the epithelialisation seen in cases of atypical pneumonia, while

others were only partially lined by cells (Figure 91). In some of the sections hyperplasia of the epithelium in the terminal bronchioles and alveolar ducts was quite marked. The cells were large with an oval nucleus and abundant pale pink staining (H & E) cytoplasm. Usually there was only slight thickening of the interalveolar septa in these areas. Alveoli adjacent to these areas often contained macrophages of the type commonly seen in cases of pulmonary adenomatosis (Figure 92). Reticulin stained sections revealed that there was often an increase in the number and thickness of reticulin fibres, particularly around alveoli showing epithelialisation (Figure 93).

DISCUSSION

The clinical disease observed after the intranasal and intratracheal inoculation of young colostrum-deprived lambs with Bedsonia organisms was more severe than that produced either in older, conventionally reared lambs inoculated intratracheally with similar agents (Boidin et al., 1958; Dungworth and Cordy, 1962a, b) or in young, colostrum-deprived lambs inoculated with ovine or bovine strains of PI3 virus (Section 2, Subsections 1 and 2, Section 3). The acute reaction in these lambs may have been due to their age, or colostral status, or to the number of organisms inoculated. Contrary to these findings the inoculation of colostrum-deprived calves with a strain of Bedsonia, isolated from cattle with a history of respiratory disease, resulted in a very mild clinical response (Phillip, Omar, Popovici, Lamont and Darbyshire, 1968).

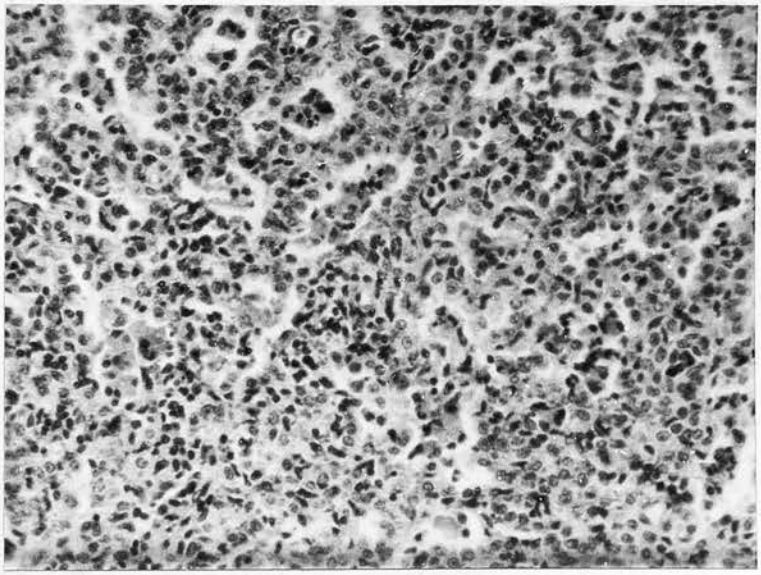


Figure 91. Epithelialisation and pseudo-epithelialisation of alveoli. The alveoli contain macrophages and neutrophils.

H & E X240

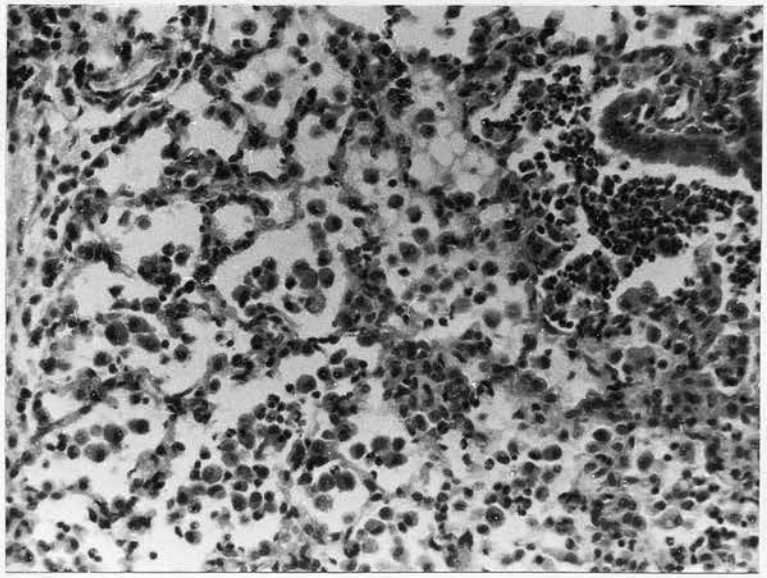


Figure 92. Macrophage infiltration of alveoli and an exudate composed of neutrophils and macrophages in an alveolar duct.

H & E X260

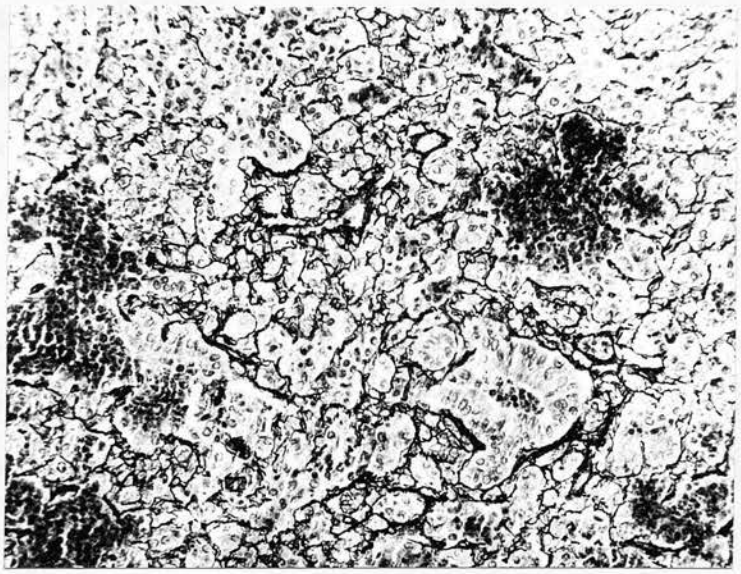


Figure 93. Hyperplasia of the reticulin fibres in an area of interstitial pneumonia.
Silver reticulin method of Slidders et al., (1958). X165

Apart from being more extensive, the macroscopic lung lesions were similar in distribution and appearance to those seen in lambs inoculated with ovine or bovine strains of PI3 virus (Section 2, Subsections 1 and 2, Section 3). However, in the early stages of infection it would be possible to differentiate the two conditions histologically. In experimental Bedsonia infections in lambs killed three and five days after inoculation, the histological lesions were characterised by serous exudation, neutrophil infiltration and fragmentation and separation of the alveolar reticulin network, whereas, in experimental PI3 virus pneumonia (Section 2, Subsections 1 and 2, Section 3) acidophilic cytoplasmic inclusions and a proliferative reaction were the salient features. In lambs killed on or after the ninth day of inoculation it was not possible to diagnose either condition with any degree of certainty as the acute exudative changes in Bedsonia infections were replaced by a proliferative reaction which did not differ qualitatively to any extent from the reaction seen in lambs inoculated with PI3 virus and killed at similar times. Separation and fragmentation of the alveolar reticulin network has been stated as being of importance in differentiating Bedsonia pneumonia from viral pneumonias in calves (Phillip et al., 1968). In calves and lambs killed prior to the ninth day after inoculation this statement appears to be correct but in the present study it was observed that in lambs killed on or after the ninth day of inoculation this did not apply since in both Bedsonia and PI3 virus infections there was an increase in number and size of reticulin fibres. This has also been observed in older lambs inoculated with Bedsonia organisms (Dungworth

and Cordy, 1962a).

Elementary bodies in older lambs infected with Bedsonia organisms could not be demonstrated unequivocally in tissue sections after the second day (Dungworth and Cordy, 1962a) nor in impression smears from lung lesions after the fourth day of inoculation (Dungworth and Cordy, 1962b). In the present experiment elementary bodies could be demonstrated unequivocally in paraffin sections of lung only in the lamb killed three days after inoculation and in lung impression smears of the three lambs killed three, five and nine days after inoculation. Elementary bodies could be identified in thin cryostat sections of lung stained with modified Ziehl-Neelsen in each of the Bedsonia inoculated lambs although by the twelfth day an extensive search was required.

In previous reports of the histological lesions of experimental Bedsonia infections in lambs (Boidin et al., 1958; Dungworth and Cordy, 1962a, b) and in calves (Omori, Ishii and Matumoto, 1960; Phillip et al., 1968) peribronchiolar accumulations of lympho-reticular cells have been described. They were seen in young, colostrum-deprived lambs in this experiment but did not achieve the dimensions described by Dungworth and Cordy (1962a). Possible explanations are the age of the lambs and their source. In young colostrum-deprived lambs maintained in isolated conditions, peribronchiolar lympho-reticular tissue is lacking or present in very small amounts, whereas, in older conventionally-reared sheep, the lympho-reticular elements in the lung have undoubtedly been stimulated by a variety of antigens since birth and when presented with a large amount of antigen would react faster and to

a greater degree than in "inexperienced" very young lambs kept in isolation. Therefore, hyperplasia of lymphoid tissue in the lungs of sheep should not be considered pathognomonic of infection due to Bedsonia organisms as undoubtedly there are other agents capable of causing a similar reaction.

Dungworth and Cordy (1962a), commenting on the similarity of the lesions observed in lambs inoculated with Bedsonia with those of subclinical infections in sheep in the United States of America, state that Bedsonia organisms might be one of the causes of such lesions but that undoubtedly there were other agents capable of causing similar lesions. Likewise, in the present experiment, lesions were observed which resembled those described for atypical pneumonia of sheep in Britain (Stamp and Nisbet, 1963). As discussed previously (Section 2, Subsection 2), somewhat similar histopathological findings were occasionally produced by an ovine strain of PI3 virus. It would appear, therefore, that two causes of the subclinical pneumonia frequently observed in slaughtered sheep (i.e. atypical pneumonia) are Bedsonia organisms and PI3 virus.

Pancreatic cysts in lambs are rare. Del Bono, Emdin and Pierotti (1959) described the occurrence of pancreatic cysts in seventy-six of 2,535 lambs killed between twenty and thirty days of age but were unable to determine the aetiology or pathogenesis of the disorder. In the present investigation PI3 virus was initially considered as a cause but the failure to isolate PI3 virus from affected pancreas (Hore, 1967) together with the finding of cysts in the pancreas of lambs inoculated with Bedsonia organisms and subsequently from a seven-day-old lamb with Border disease, indicate that other unknown factors are involved.

SECTION 5

INVESTIGATION OF THE MORPHOLOGICAL TYPES OF PNEUMONIA OCCURRING IN INTENSIVELY-REARED CALVES

INTRODUCTION

One of the major causes of economic loss in intensive beef production is death or unthriftiness resulting from pneumonia (Preston, 1963; Anon, 1964; Omar, 1966), a problem which should have been anticipated from experience with the intensification of other species (Goodwin, 1963). In calves, as in other species, the aetiology of pneumonia is complex and is likely to remain so until the causal agents can be related to specific lesions.

The purpose of the investigation was to describe the types of pneumonia which occurred in intensively-reared calves on two farms and to attempt to relate the lesions to possible aetiological factors. The lesions were classified on a morphological basis.

The lungs of another group of calves were examined at a later date and the results are included in this section for comparative purposes.

Part of this work has been published in the Journal of Comparative Pathology and Therapeutics under the title "Pathology of Pneumonia in Intensively-reared Calves", a reprint of which is included in the Appendix.

MATERIALS AND METHODS

Two farms (S and R) in East Lothian which had a history of pneumonia in

recently introduced calves were chosen. The lungs to be used for comparative purposes were obtained from eight calves which had been purchased from one farm and were approximately one-week-old when acquired. They were reared under the direct supervision of a veterinary surgeon at the Rowett Research Institute. The ages at which the calves were killed are given in Table 5 .

Farm S

Two groups of calves were observed. The first group consisted of fourteen, twelve-week-old Friesian calves and the second group was made up of thirty, fourteen-week-old calves of the same breed. Two weeks after the arrival of the second lot the two groups were housed together in an old stone building which comprised the east side of a quadrangle. Within the building there were five pens each approximately twenty feet by fifty feet, with slatted floors and containing from thirty to forty-five calves, the number varying with the size of the calves. Ventilation was provided by a half door leading into an uncovered exercise yard and by sliding partitions, at floor level, which opened onto a closed passageway. During warm weather an extractor fan in an outside wall was also used. Barley and water were fed ad lib. with no supplementary roughage. The calves were observed at least once a day.

Farm R

One group of twenty-one, twelve-week-old Friesian calves was examined

on this farm. On arrival the calves were isolated in a pen in an old stone building. The pen was approximately twelve feet by forty feet and was well bedded with straw. A large window, above the level of the calves and facing onto a covered courtyard, provided the only means of ventilation. A small fan heater was used during very cold weather.

After two or more weeks, depending on their health, the calves were moved to a large newly-erected building containing four large pens. Straw was used as bedding and barley and water were fed ad lib. Ventilation was good and there was no overcrowding at any time. The calves were inspected daily and any calves noted ill were promptly treated by a veterinary surgeon.

On farms S and R clinical examination of the calves was carried out weekly at first and then at fortnightly intervals. Post mortem examination was carried out on eight animals which died when about twenty weeks old and a further twenty-eight were examined when slaughtered at the end of the feeding period, when they were approximately one year of age.

Pieces of lung, pulmonary lymph nodes, trachea and other tissues when available, were taken and treated as described under General Materials and Methods.

The lungs of nine animals comprising Group A were submitted for bacteriological examination.

RESULTS

Clinical Signs

The clinical signs in the calves on farms S and R were similar.

On arrival some of the calves were coughing and several had a serous nasal discharge. Within seven to ten days the majority were coughing and had a serous or mucoid nasal and sometimes ocular discharge, but within two weeks most of them were clinically normal except for a persistent cough. In the few calves which developed a secondary bronchopneumonia the serous or mucoid nasal discharge became purulent, the temperature rose to 40.6 degrees centigrade or higher and there was dyspnoea with rales. Partial to complete anorexia and depression preceded death.

Diarrhoea was observed in two cases, one had a concurrent pneumonia while the other was clinically normal except for an occasional cough.

Clinical evidence of a respiratory illness was observed in only one instance (423) in the calves reared at the Rowett Institute.

Morphological Types of Pneumonia Encountered on Farm S and Farm R

The lesions in the lungs can be divided into two distinct types on gross and histopathological findings. Group A consists of nine animals, eight of which died on Farm S. Pneumonia was the cause of death in seven, while the other death was due to primary ruminal tympany. The remaining calf was from Farm R and was clinically normal when slaughtered.

Group B is made up of twenty-seven animals, thirteen from Farm S and fourteen from Farm R. Except for an occasional cough these animals when slaughtered, were clinically normal.

Group A

Gross Pathology

In seven calves the only normal areas were scattered lobules in the

dorsal part of the diaphragmatic lobes (Figure 94). The firm, dark red areas were lobular in distribution and failed to collapse when the thorax was opened. Numerous small greyish foci were seen beneath the pleura and on the cut surface of consolidated lobules in five of the seven cases. A mucopurulent exudate could be expressed from cut bronchi and bronchioles. In the remaining two cases the gross lesions were similar, but were confined to either the right apical or cardiac lobes and necrotic foci were not present. Interstitial emphysema, sometimes with the formation of large bullae and thickening of the pleura, was present in the diaphragmatic lobes of three animals. The pulmonary lymph nodes were congested and oedematous.

Escherichia coli was isolated from four cases, Pasteurella multocida from one case and P. haemolytica type A serotype 1 and Streptococcus viridans from another case. The remaining three lungs were bacteriologically sterile. Two animals from one farm had recently been treated by a veterinary surgeon while the third animal was clinically normal and there was no history of previous antibiotic therapy.

Histopathology

The histopathological findings in this group were bronchitis, bronchiolitis, a cellular exudate in alveoli, focal areas of necrosis, syncytium formation and epithelialisation of alveoli. Small eosinophilic, cytoplasmic inclusions were occasionally seen in bronchial and bronchiolar epithelial cells.

The trachea was examined in three cases. The changes were similar and

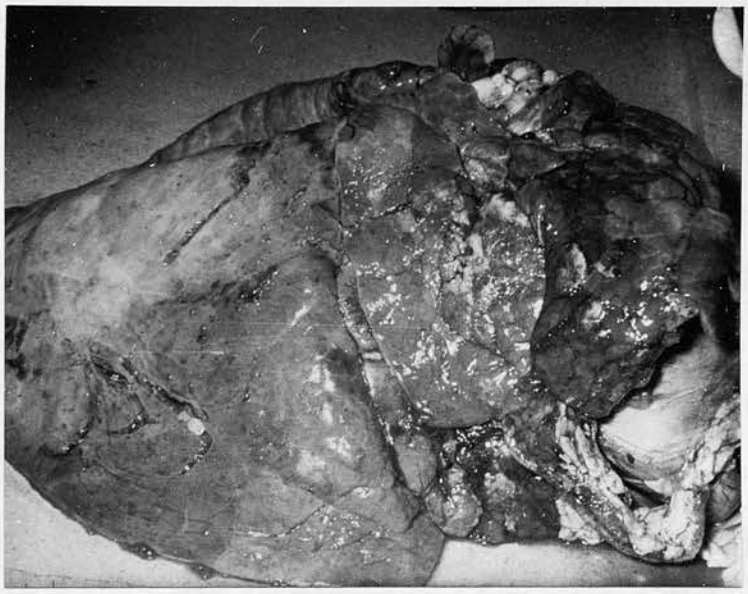


Figure 94. Consolidation of the apical, cardiac and anterior ventral parts of the diaphragmatic lobes in the lungs of an intensively-reared calf.

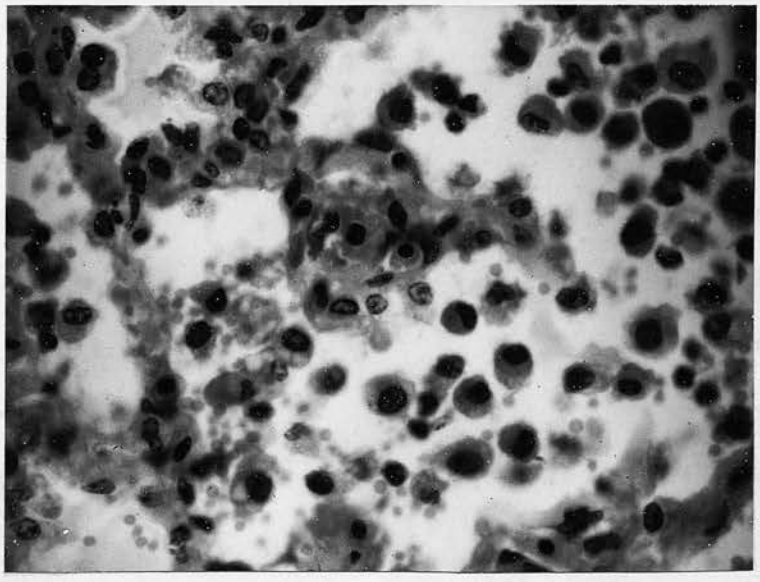


Figure 95. Lung, group A. Hyperaemia of the alveolar capillaries and macrophage infiltration of alveoli.
H & E X650

consisted of neutrophil and lymphocyte infiltration of the mucosa. The epithelium was often tattered and in one case there were small areas devoid of epithelium while in another case there were small, acidophilic, cytoplasmic inclusions. Lymphocytes, plasma cells and small numbers of macrophages were present in the submucosa.

The bronchi and particularly the bronchioles contained a necrotic exudate composed of dead or dying neutrophils, macrophages, epithelial cells and lymphocytes. Occasionally erythrocytes, fibrin and small colonies of bacteria made up part of the exudate. The epithelium was infiltrated with neutrophils and lymphocytes and had undergone degenerative changes ranging from vacuolation of epithelial cells to complete necrosis of the epithelium and, in two cases, of the mural structures. There was epithelial metaplasia in three cases; very tall columnar cells were seen in one of the cases while in the other two cases the epithelium consisted of a single layer of low cuboidal cells. In the peribronchial and peribronchiolar tissues there was congestion of the blood vessels and accumulations of round cells (lymphocytes, plasma cells and occasionally macrophages) and fibrous tissue proliferation in older lesions.

Within the alveoli the reaction was exudative but the type of exudate varied considerably. In all of the cases there was a serous exudate, while in eight out of nine there was haemorrhage and necrosis of alveoli and in seven there was a fibrinous exudate which in places was beginning to undergo organisation. Macrophages were the predominant cell type with variable numbers of neutrophils. Two types of macrophages were observed (Figure 95). The majority had a large pale-staining oval or indented nucleus with abundant, often foamy, cytoplasm which stained faintly with eosin. These cells often

contained ingested material. The others were smaller and had a more densely staining oval or round nucleus and deep-pink homogeneous cytoplasm (H & E). These cells did not usually contain phagocytised material. In seven out of nine cases multinucleated macrophages and large syncytial giant cells were present, usually lying free in alveoli (Figure 96). In six of the cases the large syncytial giant cells appeared to be of epithelial origin while in the remaining case the majority were, according to the classification of Omar (1964), alveolar giant cells. In one of the cases a small, acidophilic, cytoplasmic inclusion was seen in a syncytium. Phagocytised material was rarely seen in the syncytial giant cells. In four of the seven cases containing syncytia there were focal areas of alveolar epithelialisation and the cells lining the alveoli were low cuboidal in type (Figure 97). In three cases there were groups of alveoli packed with alveolar macrophages, many of which were elongated, situated in an amorphous exudate which stained densely basophilic with haematoxylin (Figure 98). The cells and the exudate were seen in the pores of Kohn, involving adjacent alveoli and were occasionally seen in bronchioles. While it was possible to demonstrate fibrin in adjacent alveoli no strands of fibrin could be seen within the basophilic amorphous exudate. However, small, strongly acidophilic masses were seen in the cytoplasm of the alveolar macrophages. There was fibrin in the perivascular, peribronchial, interlobular and pleural lymphatics. Within or near these areas there were usually foci of necrosis. The capillaries were congested and there were increased numbers of macrophages and lymphocytes in the interalveolar septa. In three of the cases there were small focal areas of alveolar collapse. The interlobular septa were thickened

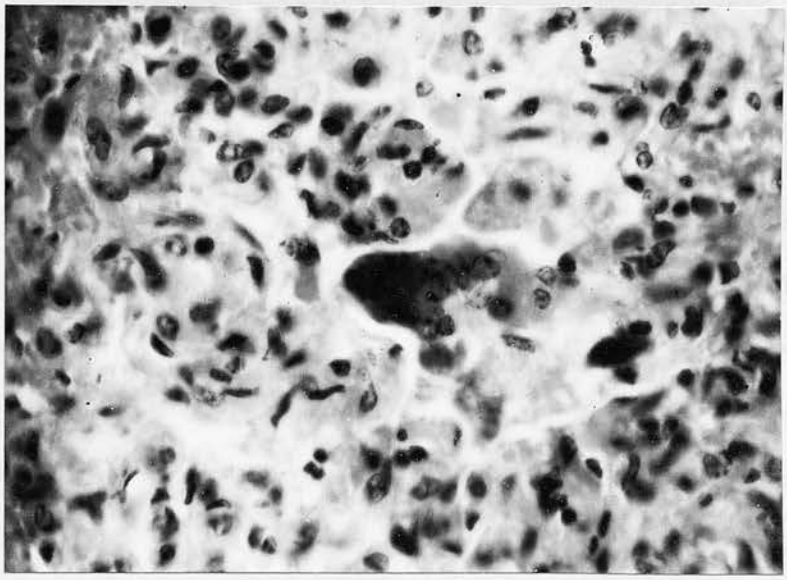


Figure 96. Lung, group A, showing a syncytium within an alveolus.
H & E X600

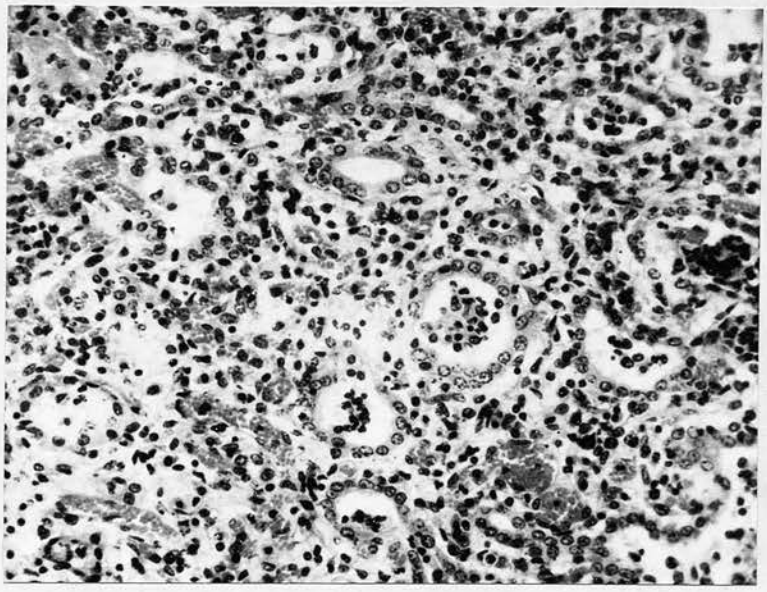


Figure 97. Lung, group A, showing cuboidal epithelialisation of alveoli.
H & E X250

due to distended lymphatics, congestion of the blood vessels and leucocytic infiltration.

In four of the nine cases there were a few small (three to five microns) phloxinophilic, cytoplasmic inclusions in bronchial and bronchiolar epithelial cells and occasionally, in alveolar epithelial cells (Figure 99). The inclusions were usually situated in the cytoplasm near the apex of the cell. Although small and few in number, they were easily recognised in sections stained with phloxine-tartrazine but less so with haematoxylin and eosin. In one case cytoplasmic inclusions were also present in the nasal mucosa, tracheal mucosa and in the liver. The inclusions in the liver were always situated in or near areas of centrilobular degeneration.

The pulmonary lymph nodes were oedematous and the blood vessels were congested. The peripheral sinuses contained fibrin, neutrophils, macrophages and lymphocytes. Within the cortex there were increased numbers of plasma cells. The medullary sinuses contained large numbers of neutrophils, lymphocytes, macrophages and plasma cells.

Group B

Gross Pathology

In this group the lesions were very uniform and varied only in their distribution and size. The affected areas were greyish-blue, depressed below the level of the surrounding alveoli and did not crepitate when squeezed. The cut surface was dull red and many bronchi and larger bronchioles appeared

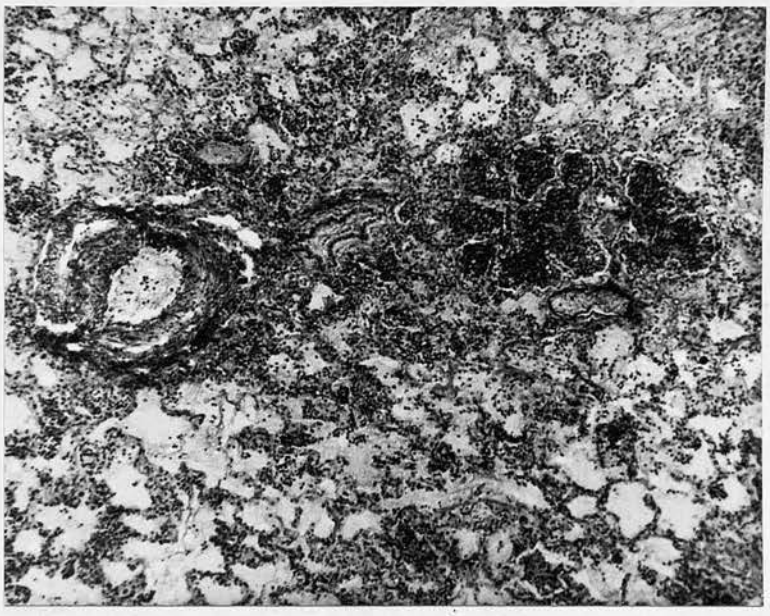


Figure 98. Lung, group A, showing oedema and fibrin strands in alveoli. Some alveoli are filled with an amorphous exudate.
picro-Mallory X70

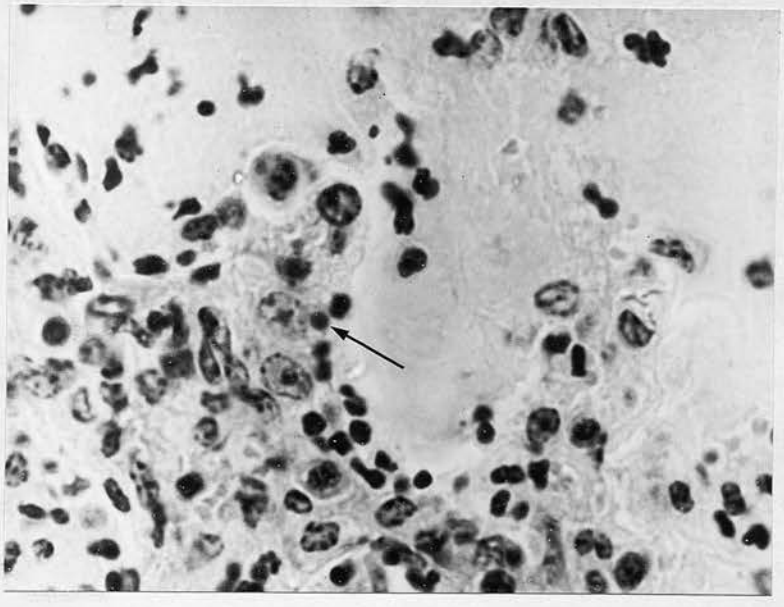


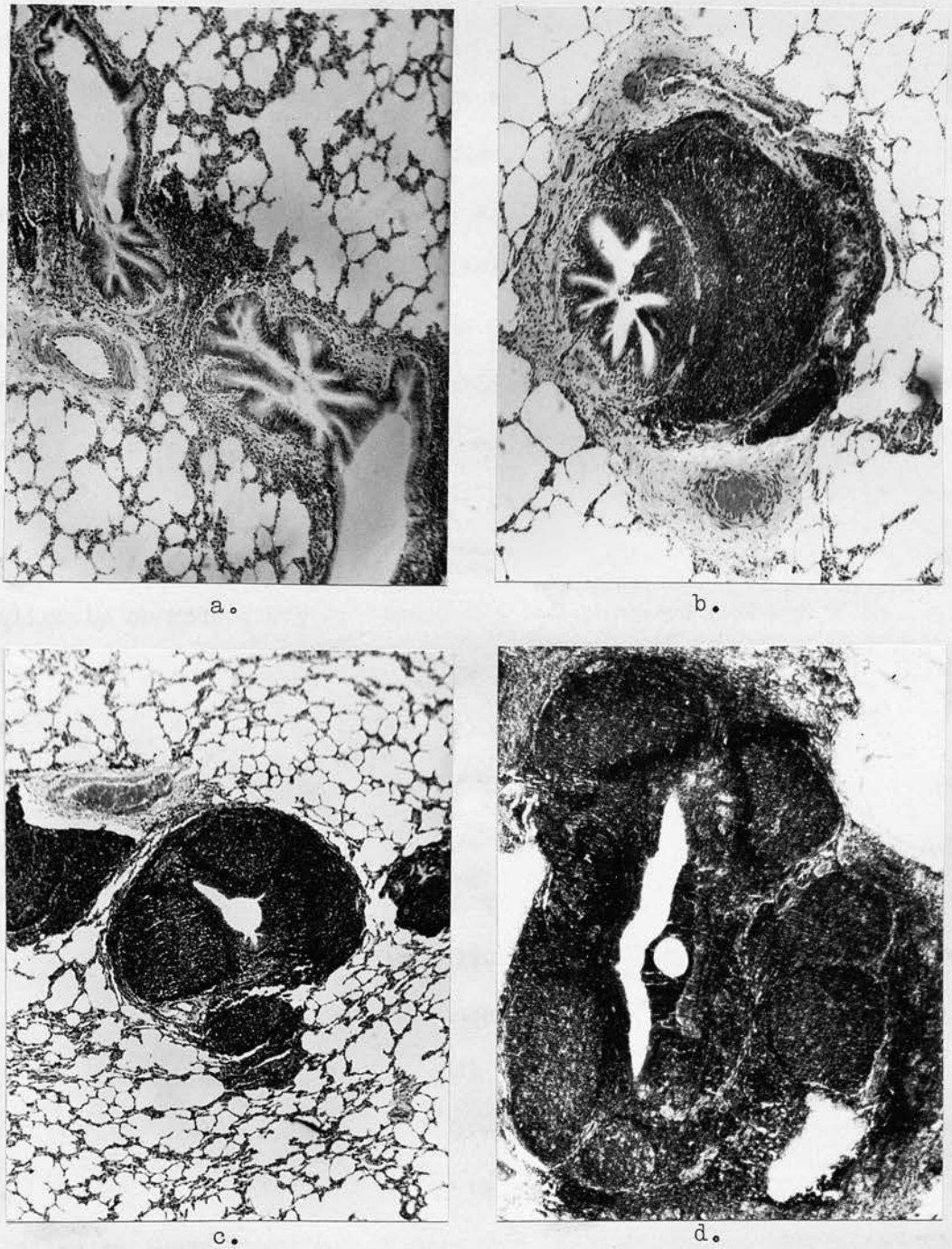
Figure 99. Lung, group A, showing an inclusion (arrow) within an alveolar lining cell.
Phloxine-tartrazine X860

to be dilated and often contained a mucoid or mucopurulent exudate. In twenty-one animals these atelectatic areas were confined to the anterior lobes; mainly the right apical lobe. Of the remaining six animals, three had lesions in the anterior lobes and either the diaphragmatic or intermediate lobes; three appeared normal. The pulmonary lymph nodes were enlarged in the majority of animals.

Histopathology

The pathology of the lungs of this group was characterised by peribronchial and peribronchiolar lymphocytic hyperplasia, atelectasis, focal vesicular emphysema and mild interstitial pneumonia.

The peribronchial and peribronchiolar lymphocytic hyperplasia varied in degree from very slight to very marked, with the formation of lymphocytic "cuffs" (Figure100) which appeared to start as peribronchial and peribronchiolar infiltrations of mature lymphocytes. As the infiltrations enlarged, blast cells could be recognised among the mature lymphocytes and increased in number until a pale germinal or reactive centre was formed. At this stage there was usually a solid ring or "cuff" of mature lymphocytes surrounding the affected airway. As shown by serial sections these "cuffs" extended for some distance down a particular bronchus or bronchiole. In the final stage of "cuff" formation plasma cells appeared around the periphery and between the "cuff" and the affected bronchus or bronchiole. In those cases where lymphoid hyperplasia was considerable, there was complete peribronchial, peribronchiolar and, in four out of twenty-seven lungs, perivascular "cuffing", in addition to partial or complete collapse of affected bronchioles and



a.

b.

c.

d.

Figure 100. Lungs, group B, showing the varying degrees of peribronchial and peribronchiolar lymphocytic "cuffing".

H & E a. X60 b. X60 c. X35 d. X30

the surrounding alveoli.

The alveolar changes were not striking except for the extent of atelectasis. Where there was slight or moderate "cuffing" there were focal areas of atelectasis, whereas, in areas where the"cuffs" were considerable the atelectasis was lobular in distribution (Figure 101). In four lungs atelectasis was not observed. In fourteen lungs there was no cellular reaction, nine contained small numbers of macrophages in collapsed alveoli and in the remaining four the alveoli contained small numbers of macrophages and either neutrophils or giant cells. In nineteen lungs there were focal areas of vesicular emphysema. The interalveolar septa in all cases were slightly or moderately thickened due to increased numbers of macrophages and lymphocytes, and in fourteen lungs there were small numbers of neutrophils. Eosinophils were present in four lungs.

The inflammatory response of the bronchi and bronchioles varied with the degree of "cuffing". Where there was little or no inflammatory response, "cuffing" was minimal. As the degree of "cuffing" increased so did the inflammatory response (Figure 102). In one lung, in which there was a marked inflammatory response in the bronchi, there were acidophilic, cytoplasmic inclusions in bronchial epithelial cells (Figure 103).

In six lungs there were lesions affecting medium-sized arteries consisting of either intimal fibrosis (four cases) or medial hypertrophy (two cases). In twelve lungs there was an apparent increase in the number and size of arterioles especially in the areas near the anterior border of the right apical lobe. In addition, there was usually perivascular, peribronchial

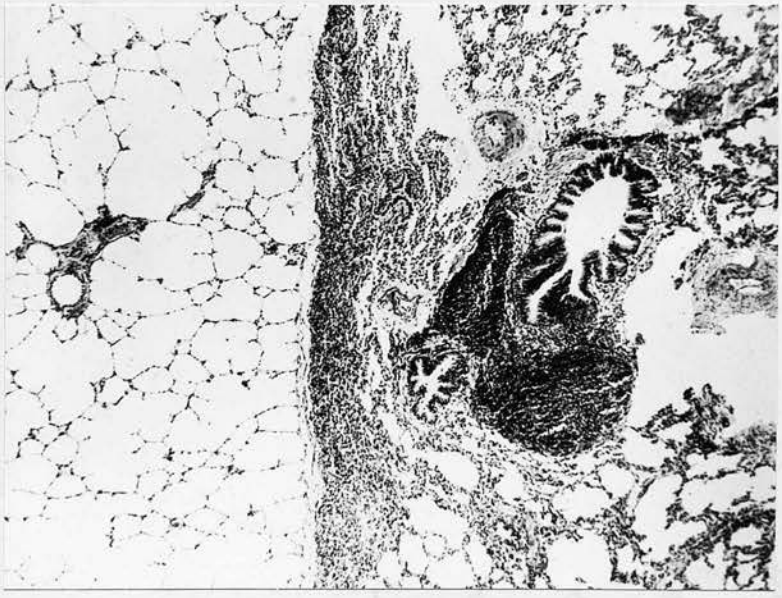


Figure 101. Lung, group B, showing peribronchiolar lymphoid hyperplasia, focal atelectasis and mild interstitial pneumonia. Note the lobular distribution of the lesions.
H & E X50

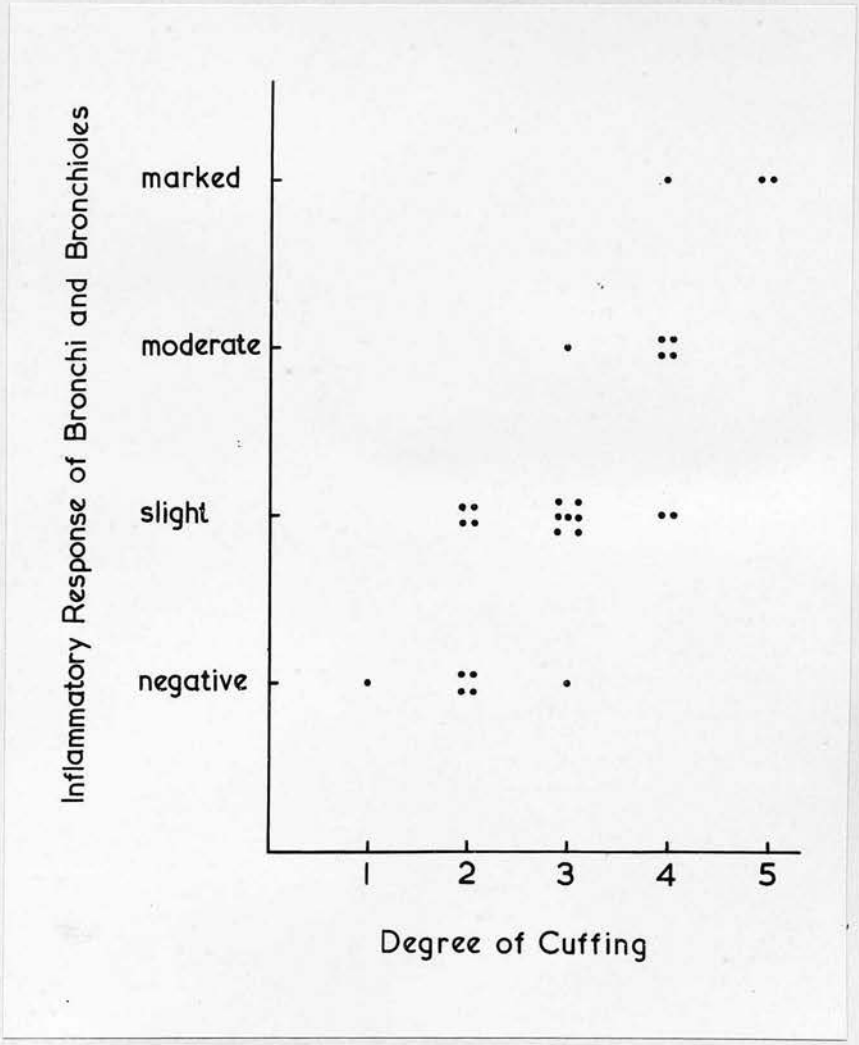


Figure 102. Relationship between the degree of lymphocytic "cuffing" and the inflammatory response of bronchi and bronchioles.

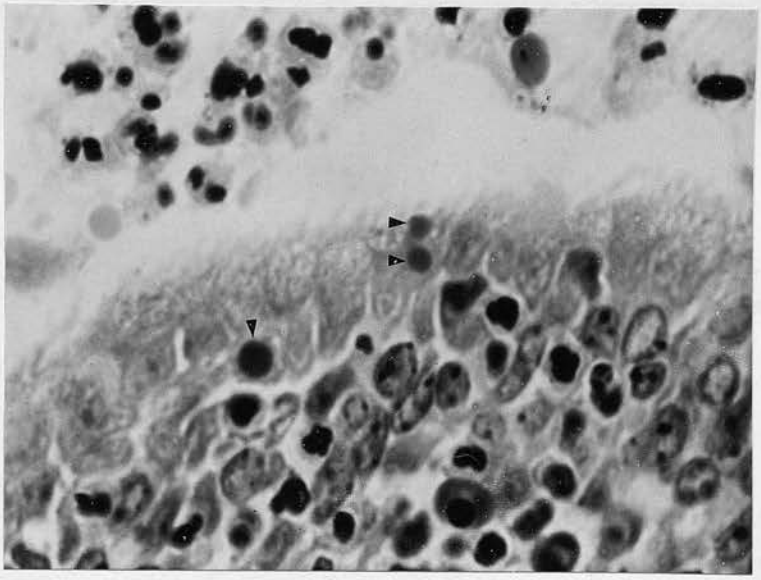


Figure 103. Lung, group B. Cytoplasmic inclusions within bronchial epithelial cells. Note the neutrophil and plasma cell infiltration of the mucosa.
Phloxine-tartrazine X1000

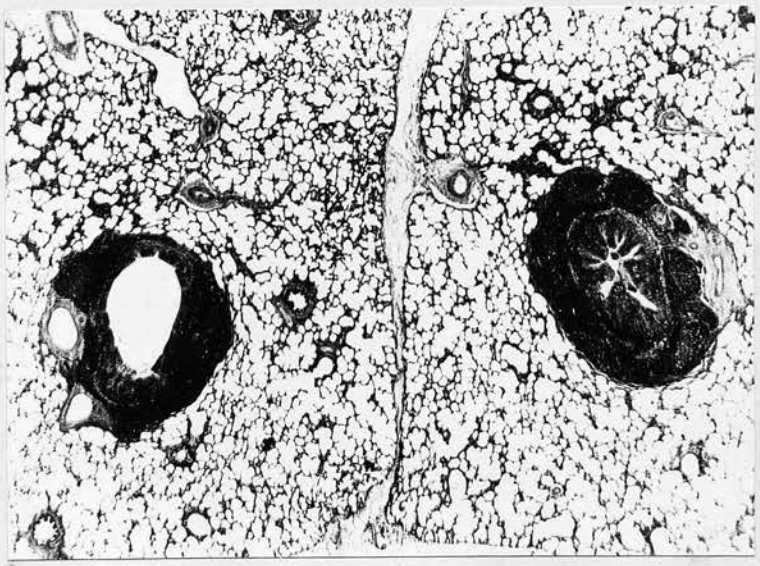


Figure 104. Marked peribronchiolar lymphocytic "cuffing" and collapse of the surrounding alveoli in the lungs of an apparently normal calf.
H & E X14

and peribronchiolar fibrosis and lobular collapse of alveoli. Bronchiectasis was also a feature in these areas.

Inflammatory changes in the tracheal mucosa were very slight or absent. In fourteen lungs there was slight lymphocytic infiltration of the submucosa but this was considered to be normal (Trautmann and Fiebiger, 1952).

There were no significant differences between the mediastinal and left and right bronchial lymph nodes. Lympho-follicular hyperplasia was present and in fifteen there were small numbers of neutrophils within the medullary sinuses. Increased numbers of macrophages and plasma cells within the medullary sinuses were seen in over fifty per cent. of the lymph nodes while in two there was eosinophil cell infiltration of the cortex, particularly in and around the trabeculae.

Lesions Encountered in the Lungs of Calves from the Rowett Research Institute

Gross Pathology

The lungs of two calves were macroscopically normal, in five there was partial atelectasis of some lobules in the right apical lobe and in the remaining calf (423), which showed clinical signs of a respiratory illness, there were small dark red areas of consolidation in the right apical lobe.

Histopathology

In the right apical lobe of each of the eight lungs there were microscopic lesions of intra-pulmonary lymphoid hyperplasia ranging in severity from very

mild peribronchiolar lymphoid hyperplasia to marked lymphocytic "cuffing" of bronchi, bronchioles and, to a lesser degree, blood vessels (Figure 104). In four calves there was neutrophil infiltration of the bronchial or bronchiolar epithelium, the degree of inflammation being more marked in airways in which there was pronounced lymphoid hyperplasia (Table 5).

DISCUSSION

The two types of pneumonia described in this paper are very similar to atypical pneumonia of calves described by Jarrett (1954), a name given to two morphologically distinct types of pneumonia. Atypical pneumonia, group A or inclusion body pneumonia (Jarrett, 1956), is characterised mainly by cuboidal epithelialisation of alveoli. Acidophilic, cytoplasmic inclusions and syncytium formation may also be present. Group B, atypical pneumonia or "cuffing" pneumonia, first described by Jarrett et al., (1953) is characterised by peribronchial and peribronchiolar lymphocytic hyperplasia and, in the later stages, by atelectasis and mononuclear cell infiltration of alveoli.

The histological findings in group A of this investigation are those of either acute or subacute bronchopneumonia. The variations in the alveolar exudate may be due to any one or a combination of three factors: the stage of inflammation, the virulence of the organism(s) involved or the effects of treatment.

Macrophages are usually seen in the later stages of pneumonia, after the polymorphonuclear leucocytic infiltration, but may be present in the

TABLE 5

INTRA-PULMONARY LYMPHOID HYPERPLASIA IN INTENSIVELY REARED CALVES

Calf No.	Age (weeks)	Clinical Signs	Macroscopic Lung Lesions	Intra-pulmonary lymphoid tissue			Bronchitis and/or Bronchiolitis
				PB	Pb	Pv	
422	21	-	Foci of atelectasis	3+	3+	+	3+
423	24	+	Foci of red consolidation	3+	2+	+	2+
425	24	-	-	-	+	-	-
427	25	-	Foci of atelectasis	-	2+	+	+
429	26	-	"	-	+	-	-
430	26	-	"	-	2+	-	-
426	29	-	"	-	3+	-	+
428	32	-	-	+	+	-	-

- negative
+ slight
2+ moderate
3+ marked

PB Peribronchial
Pb Peribronchiolar
Pv Perivascular

early stages as well. According to the classification of Omar (1964) the larger pale staining cells containing ingested material are probably true macrophages or septal cells while the smaller cells may be either recently 'discharged' septal cells or epithelial lining cells which have desquamated. The smaller mononuclear cells and some of the giant cells are similar to those described by Jarrett (1956) in cases of acute purulent and acute necrotising bronchopneumonia. The blue staining elongated cells which were seen in alveoli in three cases resemble those described by Downey (1957) in cases of enzootic sheep pneumonia and by Jubb and Kennedy (1963). The latter authors consider these cells to be monocytes, derived from the alveolar lining and which undergo changes to become elongate or oat-shaped, forming whorls in the alveoli and streamers in the alveolar ducts and respiratory bronchioles. The same authors believe that these monocytes or alveolar macrophages are derived from the alveolar epithelial cells of the discontinuous lining of the alveoli. On the other hand, Omar (1964) states that the alveolar lining is continuous and is composed of entodermal epithelial cells and mesodermal septal cells in which the cytoplasm of both cells attenuates to form the alveolar lining. According to this author the alveolar macrophages are derived from the mesodermal septal cells. Recent work has indicated that in mice two-thirds of the alveolar macrophages arise from the haemopoietic system while only one-third are of pulmonary origin (Pinkett, Cowdry and Nowell, 1966). Why these macrophages should become elongate or oat-shaped is not known.

Attempts to demonstrate fibrin in the basophilic amorphous exudate were unsuccessful but the small strongly acidophilic masses seen in the

cytoplasm of macrophages within the exudate could have been fibrin (Lendrum et al., 1962). It has been stated that fibrin resembles a sponge, in that when cells undergo karyolysis the fibrin absorbs the released chromatin and is therefore stained basophilic (Smith and Jones, 1957). This could account for the failure to stain free fibrin and the ability to stain ingested fibrin.

Lesions suggestive of a viral pneumonia, although partly obscured by the bacterial infection, were present as well. Syncytium formation was present in seven out of nine cases. In one, only syncytia were seen while in four there was also cuboidal epithelialisation of alveoli: in the remaining two there was syncytium formation, cuboidal epithelialisation of alveoli and acidophilic cytoplasmic inclusions within bronchial and bronchiolar epithelial cells and within a syncytium.

Jarrett (1954, 1956) postulated a viral aetiology for his cases of inclusion body pneumonia because of the histological similarities between them and canine distemper (including canine infectious demyelinating encephalitis), giant cell pneumonia of young children and human measles, all proven viral infections. Eosinophilic, cytoplasmic inclusions similar to those described by Jarrett (1954) were found in bronchiolar and alveolar epithelial cells in colostrum-deprived calves and in hysterectomy-derived, colostrum-deprived calves inoculated with the T1 strain (Dawson et al., 1964; Dawson et al., 1965) and the J121 strain (Betts et al., 1964; Omar et al., 1966) of PI3 virus respectively. According to Omar et al., (1966), as cited by Darbyshire et al., (1966), the principal lesions in calves infected with PI3 virus are epithelialisation, syncytium

formation and specific cytoplasmic and nuclear acidophilic inclusions which are of transient duration. Thus it would appear that in six out of nine cases of pneumonia in group A of this survey, PI3 virus infection may have been an underlying factor, death being due to a secondary bacterial infection. Jolly and Ditchfield (1965) by histopathology, and by positive fluorescent antibody and complement fixation tests were able to diagnose bronchopneumonia due to PI3 virus in two clinical cases of calf pneumonia.

The gross and histopathological findings in group B resemble the "cuffing" pneumonia of calves first reported by Jarrett et al., (1953; 1954) and described in more detail by Jarrett (1954). Since that time a few reports of this condition have been published (Carter and Rowsell, 1958; Martin, 1963; McIntyre, 1963). Whether the lesions in group B are due to a specific aetiological agent or are the end result of a previous bronchopneumonia is not known.

Although large numbers of lobules may not be functioning properly due to collapse or interalveolar septal thickening, the clinical signs are usually very mild. This has been noted by McIntyre (1963) in "cuffing pneumonia" and is probably due to the large reserve of lung tissue left and to the absence of toxaemia.

In many respects the lesions in group B are similar to those of bronchiectasis in man in which there is dilatation with fibrous thickening of bronchiolar walls along with nodular and diffuse peribronchiolar lymphocytic infiltration (Gunn, 1961). As cited by Gunn (1961), Ogilvie (1941) states that about eighty per cent. of cases of bronchiectasis have their beginning in childhood, while Boyd (1931) found bronchopneumonia to be the

commonest form of disease which provided the conditions for bronchial dilatation in childhood. Bronchopneumonia is a common condition in young calves; moreover, according to Jubb and Kennedy (1963) bronchiectasis is principally a disease of young bovines. Chronic peribronchial, peribronchiolar and perivascular fibrosis, arterial lesions and bronchiectasis were observed in twelve lungs in this survey indicating that the initial pneumonia does not resolve but becomes chronic in a large number of animals. Two cases of bronchiectasis associated with "cuffing pneumonia" have been reported by Jarrett (1956).

In this investigation it is interesting to note the correlation between the degree of lymphoid hyperplasia ("cuffing") and the extent of atelectasis and inflammatory response of the bronchi and bronchioles. It is possible that as the lymphoid accumulations increase in size and compress the airways, either collapse or vesicular emphysema, (depending on the degree of interference with the flow of air) results. Where the lumina of bronchioles are markedly constricted the epithelial lining often consists of a layer of low cuboidal cells. Because of the obstruction to the flow of air and the interference with normal ciliary action, any bacteria reaching these sites remain and may elicit an inflammatory reaction. An exudate then accumulates in the affected airways, further contributing to the degree and extent of collapse. Niven (1950) has suggested that peribronchiolar lymphocytic infiltration, particularly of the terminal bronchioles, may be an indirect factor favouring secondary bacterial invasion in grey lung virus disease of mice.

It has been suggested by Jarrett (1954) that a virus may be involved

in "cuffing pneumonia" because of the histological similarities between "cuffing pneumonia" of calves (Jarrett et al., 1953), grey-lung virus disease of mice (Niven, 1950), a pneumonia of cotton rats (Andrewes and Niven, 1950) and a transmissable non-bacterial pneumonitis of guinea-pigs (Jarrett, 1954). However, it has yet to be proven that these infections are caused by viruses.

Marked peribronchial, peribronchiolar and perivascular lymphocytic hyperplasia is commonly observed in pigs with "enzootic pneumonia". The aetiology of this condition is also unsettled but Mycoplasma spp. have been incriminated (Goodwin, Pomeroy and Whittlestone, 1965; Mare and Switzer, 1965). Mycoplasma spp. have been isolated from cattle on numerous occasions (Carter, 1954; Carter and McSherry, 1955; Hamdy, Gale and King, 1958; Olson, Seymour, Bootheand Dozsa, 1960; Hudson and Etheridge, 1963; Chinn, 1963; Harbourne, Hunter and Leach, 1965) but only a few of these reports describe the histological lesions. Acute bronchopneumonia has been described by Harbourne et al., (1965) whereas Carter (1954) found that in his case, from which only a Mycoplasma was isolated, the lesions were of a proliferative nature. Carter (1954) and Hamdy et al., (1958) were unable to produce pneumonia in calves exposed to their strains of Mycoplasma. The presence of Mycoplasma spp. in the lungs of calves with acute pneumonia and the failure to infect calves experimentally with these organisms raises the question as to their significance in the aetiology of calf pneumonia. Further experimental work is required before this question can be answered, but at present it would appear that the Mycoplasmata commonly isolated from calves are of low virulence and

proliferate only because of the existing conditions in affected lungs.

It has been stated previously (Jarrett et al., 1953) that there is normally only a very small amount of lymphoid tissue present in the bronchial wall of the calf. That such is not necessarily the case was demonstrated in the lungs of calves reared at the Rowett Research Institute. Seven of the eight calves showed no clinical signs of respiratory disease, yet in each of them there were varying degrees of lymphoid hyperplasia in either the peribronchial, peribronchiolar or perivascular tissues. It is possible that the seven apparently-healthy calves were exposed to the same agent as calf 423 but failed to exhibit clinical signs of infection. However, exposure to such an agent could have resulted in stimulation of the intra-pulmonary lymphoid tissue. Jericho (1966), in a review of the literature of intra-pulmonary lymphoid tissue in pigs, discusses the variables (e.g. age, environment, etc.) which must be considered in interpreting these lesions and concludes that standardisation of experimental procedure (e.g. gnotobiotic animals produced and maintained in known and constant environmental conditions) and method of fixation are necessary before such lesions can be accurately assessed. The same may be said for cattle but the problems and cost of such an undertaking do not, at present, make it a practical proposition.

SECTION 6

INOCULATION OF CALVES WITH A STRAIN OF PARAINFLUENZA 3 VIRUS ISOLATED FROM SHEEP

INTRODUCTION

Human (Tyrrell et al., 1959; Kapikian et al., 1961), bovine (Dawson et al., 1965; Omar et al., 1966) and ovine (Section 2, Subsections 1 and 2) strains of parainfluenza 3 (PI3) virus have been shown to be pathogenic for their respective hosts but little is known about their pathological effects in other species. Tyrrell (1965) states that human strains, which are serologically related to bovine strains (Abinanti et al., 1961), produce no overt disease when given intranasally to a variety of laboratory animals and calves and that the lungs of the inoculated animals appear to be normal. Other workers have found that human strains of PI3 virus are capable of producing lesions in the nasal mucosa of suckling mice (Craighead, 1966), in the bronchi of hamsters (Buthala and Soret, 1964) and in the lungs of hysterectomy-produced, colostrum-deprived piglets (Betts and Jennings, 1966).

Since bovine and ovine strains of PI3 virus are serologically related (Hore, 1968) and a bovine strain has been shown to be capable of infecting lambs (Section 3), the infectivity of an ovine strain for calves was investigated. As already mentioned this could be of economic and epizootiological importance where sheep and cattle are run together.

This experiment permitted a study of the pathology of the lung lesions in calves inoculated intratracheally with an ovine strain of PI3 virus and their comparison with those resulting from infection of calves with a bovine strain of PI3 virus.

MATERIALS AND METHODS

Calves

Four colostrum-deprived calves were obtained from local farms. Calves 8F19 and 8F20 were twins and were housed together. Calf 8F23 was placed in a separate pen adjacent to the twin calves. Calf 8F21, which was to be inoculated with control fluids, was placed in another pen and kept isolated from the others. Within forty-eight hours of birth, calves 8F19, 8F20 and 8F23 were each inoculated intratracheally with the ovine G2 strain of PI3 virus. The inocula consisted of ten millilitres of infected ovine embryo kidney tissue culture harvest fluid and had an infectivity titre of $10^{6.2}$ $TCID_{50}$ per millilitre. The control calf (8F21) was inoculated similarly within forty-eight hours of birth with virus-free ovine embryo kidney tissue culture fluid. Post mortem examinations were carried out three, four and six days after inoculation for the virus-inoculated calves and on day four for the control calf.

To ensure that the virus-inoculated calves were infected, nasal swabs were collected on the third and fourth days after inoculation along with portions

of lung at autopsy and, as in previous sections, were submitted for virological examination. Lung specimens from each of the calves were also submitted for bacteriological examination. The results are summarised in Table 6.

Pathology

Pieces of lung and any other tissues showing macroscopic abnormalities were taken and treated as described under General Materials and Methods.

Sections of bovine PI3 virus-infected lungs, to be used in the comparison of the histological lung lesions produced by the ovine G2 strain of PI3 virus in calves, were kindly supplied by Dr. P. S. Dawson, Central Veterinary Laboratory, Weybridge, Surrey. Since the macroscopic and histological lung lesions produced by this strain of virus have been described previously (Dawson et al., 1965), only those features which are of diagnostic significance will be given here.

RESULTS

Clinical Examination

The control calf remained healthy. No respiratory signs were seen in any of the calves inoculated with the ovine strain of PI3 virus. On the third day after inoculation calf 8F20 was recumbent and very depressed. Clinical signs included opisthotonus, rigidity of the back and neck and 'paddling' movements of the limbs. The calf died shortly afterwards. On the following day its

TABLE 6

RESULTS OF VIROLOGICAL AND BACTERIOLOGICAL EXAMINATIONS OF CALVES INOCULATED WITH AN OVINE STRAIN OF PI3 VIRUS†

Calf No.	Recovery of Virus*		Bacteriology
	Nasal Swabs Days PI	Lung Days PI	
8F21	- (3)	- (4)	E. coli
8F20	+ (3)	+ (3)	Haemolytic E. coli
8F19	+ (3)	+ (4)	Proteus sp. P. haemolytica
8F23	+ (3,4)	+ (6)	Negative

† = Supplied by Dr. D. E. Hore and D. Thompson

* = Serologically identified as the ovine G2 strain of PI3 virus

PI = Post Inoculation

twin (8F19) was showing similar signs and was killed. Calf 8F23 remained healthy throughout the period of observation.

Pathology

Gross

No macroscopic abnormalities were seen in the control calf (8F21) and in calf 8F23 lesions were confined to the respiratory tract. However, the twin calves (8F19 and 8F20), in addition to pulmonary changes, had lesions of an acute septicaemia. A histological diagnosis of purulent meningo-encephalitis was made in both of these calves. The lung lesions in calf 8F20 were partially obscured by the intense hyperaemia due to the septicaemic changes and hypostatic congestion but small foci of consolidation were seen in the right cardiac lobe and in the anterior ventral part of the right diaphragmatic lobe. On the fourth day after inoculation several small areas of red consolidation were present in the right apical lobe and in the intermediate lobe of calf 8F19 (Figure 105). Similar but more numerous areas of red consolidation affecting all lobes of the lung were seen in calf 8F23 killed two days later.

Histological Lesions Produced by the Ovine G2 Strain of PI3 Virus in Calves

Lesions in the turbinate mucosa were common to all four calves and consisted of neutrophil infiltration, erosion and, in places, ulceration of the epithelium.

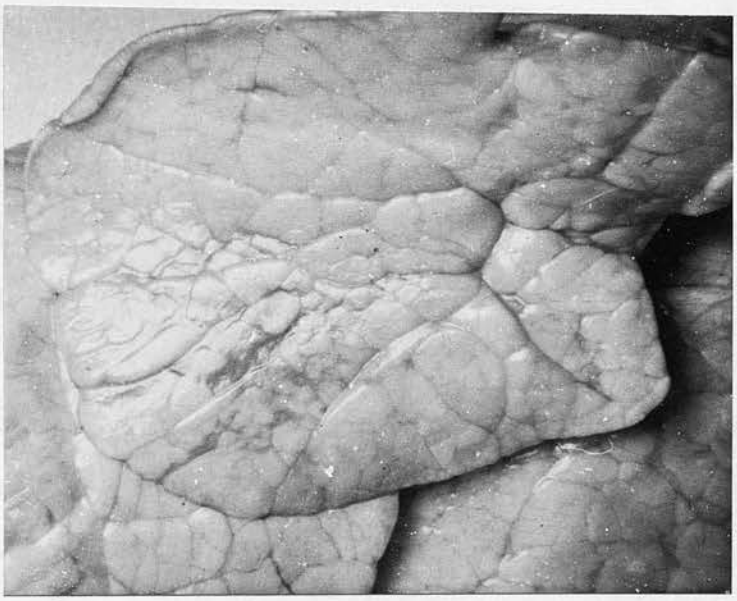


Figure 105. Small areas of consolidation in the intermediate lobe of the lungs of a calf killed four days after inoculation with an ovine strain of PI3 virus.

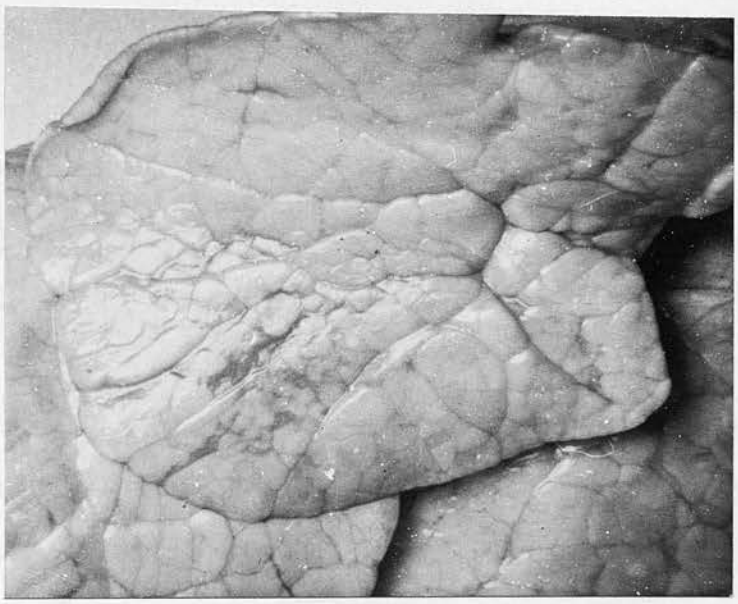


Figure 105. Small areas of consolidation in the intermediate lobe of the lungs of a calf killed four days after inoculation with an ovine strain of PI3 virus.

No lesions were seen in the lungs of the control calf (8F21). In calf 8F20, which died on the third day after inoculation, the only lesion attributable to PI3 virus infection was the presence of a few acidophilic, cytoplasmic inclusions in bronchiolar epithelial cells. Other lesions seen were hyperaemia of the alveolar capillaries, neutrophil infiltration of alveoli, occasional bacterial emboli in capillaries and alveoli, and distension of the sub-pleural lymphatics. Histological lesions of a PI3 virus infection were more evident in the calf (8F19) killed on the fourth day after inoculation. The bronchi, except for the presence of occasional acidophilic, cytoplasmic inclusions in epithelial cells, were normal. Many of the smaller air passages, however, contained varying amounts of an exudate composed of mucus, neutrophils and a few macrophages. Hyperplasia or, less frequently, foci of degeneration of the bronchiolar epithelium were occasionally seen. Acidophilic, cytoplasmic inclusions were more numerous but still not to the same extent as in lambs inoculated with the same strain of virus. There were scattered small foci of interstitial pneumonia usually surrounding, or in close proximity to, affected bronchioles. Within these areas it was difficult to discern individual alveoli but in less consolidated areas macrophages and neutrophils were seen in the thickened interalveolar septa and in the alveolar lumina. In other areas some of the alveoli were partially collapsed and the hyperaemic alveolar capillaries often contained increased numbers of neutrophils.

In the calf killed on the sixth day after inoculation the lesions in the bronchi and bronchioles were similar to those at four days except that cytoplasmic inclusions were more numerous and there were small numbers of lymphocytes and macrophages in the peribronchial tissues (Figure 10).

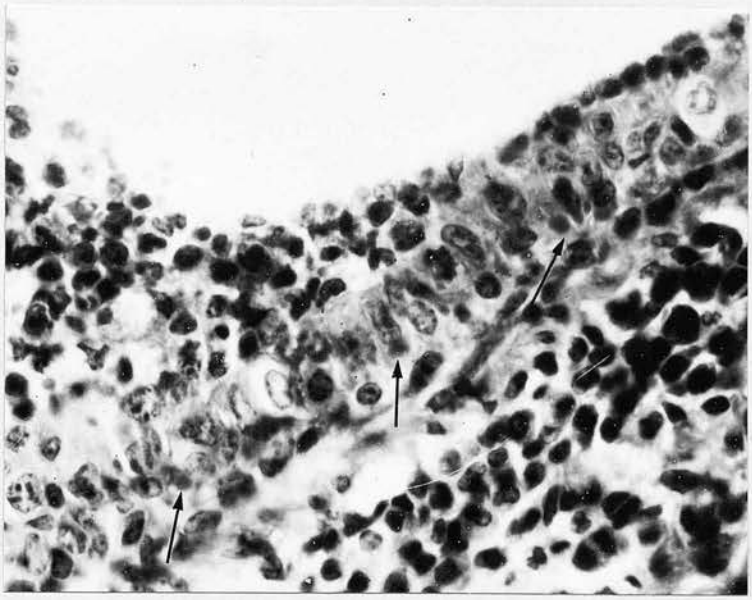


Figure 106. Acidophilic cytoplasmic inclusions (arrows) in bronchiolar epithelial cells. Note the cellular exudate in the bronchiolar lumen and moderate infiltration of the peribronchiolar tissues by lympho-reticular cells.
Pollak X640

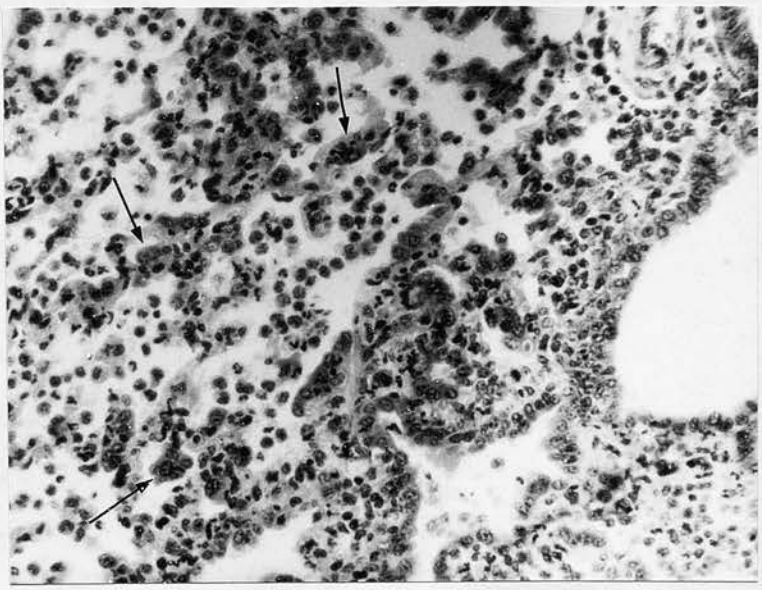


Figure 107. Syncytium formation (arrows), macrophage and neutrophil infiltration of alveoli adjacent to a bronchiole.
H & E X250

Inclusions were also seen in alveolar epithelial cells and in macrophages but not to the same degree as in lambs infected with this strain of virus and killed six days after inoculation. The areas of interstitial pneumonia were larger and consisted of cellular infiltration of the interalveolar septa, small syncytia attached to alveolar walls, pseudo-epithelialisation of alveoli and macrophage infiltration of alveoli (Figure 107). Neutrophils were less frequently seen.

Nuclear inclusions were not observed in the lungs of any of the calves.

Moderate numbers of neutrophils were observed in the medullary sinuses of the pulmonary lymph nodes of the control calf (8F21). The reaction in the pulmonary lymph nodes of calves 8F19 and 8F20 was characterised by oedema and neutrophil infiltration, whereas, in calf 8F23 the only abnormality was the infrequent finding of small, acidophilic, cytoplasmic inclusions in macrophages within the medullary sinuses.

Lesions in the remaining organs were seen only in calves 8F19 and 8F20 and were considered to be due to the septicaemia.

Histological Lesions Produced by the Bovine T1 Strain of PI3 Virus in Calves

Sections of infected lung from calves killed four and seven days after experimental inoculation of the T1 strain of bovine PI3 virus were examined and the results are summarised below.

In sections of lung from a calf killed four days after inoculation there was a small amount of exudate in some bronchi and a few acidophilic, cytoplasmic inclusions in bronchial epithelial cells. Similar but more extensive lesions

were present in the bronchioles. In addition there were focal areas of hyperplasia or necrosis of the bronchiolar epithelium. The interalveolar septa were thickened and, in places, the normal alveolar pattern had been lost. Macrophages and neutrophils were present in the alveolar walls and in alveolar lumina, along with necrotic debris. Acidophilic, cytoplasmic inclusions were seen in free alveolar macrophages and in cells attached to the alveolar walls. Occasionally acidophilic, nuclear inclusions were seen in bronchiolar and alveolar epithelial cells.

In sections from the lung of the calf killed on the seventh day there was still a considerable amount of exudate in the bronchioles. Hyperplastic and degenerative lesions of the epithelium were also present (Figure 108). Acidophilic, nuclear inclusions were now more common than cytoplasmic inclusions (Figure 109). Cellular infiltration of alveoli and interalveolar septa was less marked. In some areas syncytia were seen lying free in alveolar spaces while in others they were attached to the alveolar walls. Nuclear and cytoplasmic inclusions were sometimes seen in the syncytia and in alveolar macrophages.

DISCUSSION

Virus was re-isolated from the nasal passages and lungs of the virus-inoculated calves which proved that they were infected even though respiratory signs were not observed. The absence of clinical signs was not unusual since even after the inoculation of calves with bovine strains, clinical signs may be

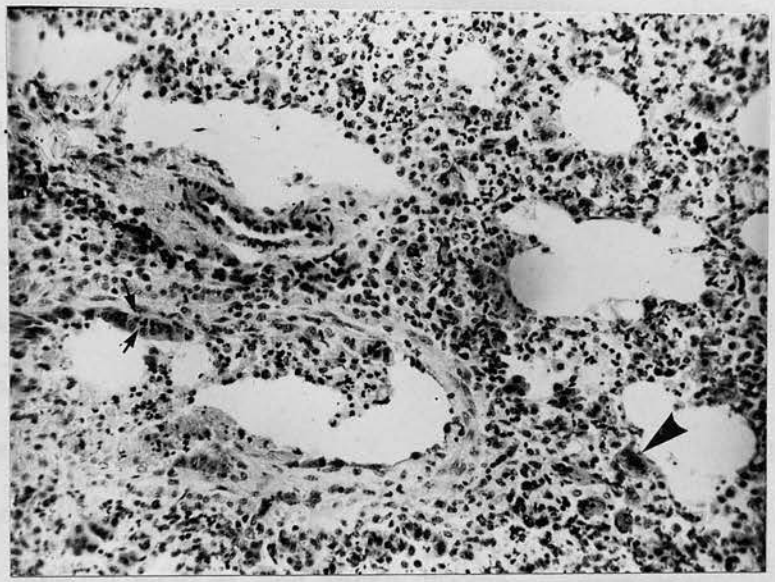


Figure 108. Necrosis and desquamation of the bronchiolar epithelium with acidophilic cytoplasmic inclusions in the remaining epithelial cells (arrows). The surrounding alveoli contain macrophages, lymphocytes, neutrophils and syncytia (►).
Phloxine-tartrazine X165

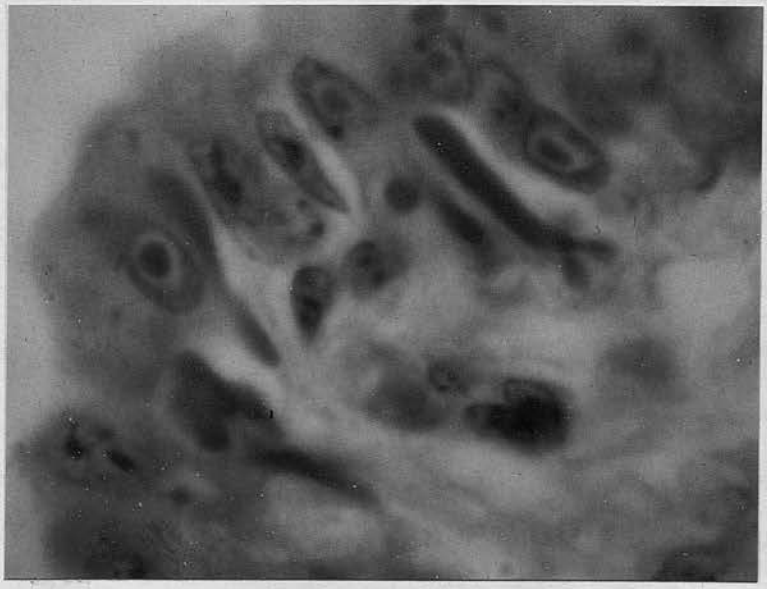


Figure 109. Numerous acidophilic, nuclear and cytoplasmic inclusions in bronchiolar epithelial cells.
Phloxine-tartrazine X1800

either marked (Woods, Sibinovic, Segre and Thurmon, 1964; Omar et al., 1966), mild (Dawson et al., 1965) or absent (Hetrick et al., 1963). These variations have been suggested as being related to the virulence of the strain of virus used (Woods et al., 1965a; Omar et al., 1966).

In the two infected calves (8F19 and 8F20) from which bacteria were isolated, the histological lung lesions differed from those seen in calf 8F23. In addition to specific lesions of a PI3 virus infection (such as the presence of acidophilic, cytoplasmic inclusions in bronchial, bronchiolar and alveolar epithelial cells) seen in each of the three calves, serous exudation and neutrophil infiltration of alveoli were present to a marked degree in calves 8F19 and 8F20. Similar lesions have been seen in field outbreaks of PI3 virus infections complicated by a bacterial infection in sheep (Section 1) and in calves (Section 5).

The lesions in the turbinate mucosae were probably caused by the swabbing procedure since they were present in both the control and infected calves. Similar results were obtained in lambs inoculated with the ovine G2 strain of PI3 virus (Section 2, Subsections 1 and 2, Section 3).

A comparison of the histological lung lesions produced by the ovine and bovine strains of PI3 virus showed that the differences, generally, were quantitative rather than qualitative. Acidophilic, cytoplasmic inclusions in bronchiolar and alveolar epithelial cells, round cell infiltration of the interalveolar septa, syncytium formation and pseudo-epithelialisation of alveoli were observed in calves and lambs (Section 2, Subsections 1 and 2; Section 3) inoculated with either strain of virus. The only feature of diagnostic value, which has previously been described by Dawson et al., (1965),

was the finding of nuclear inclusions in bronchial, bronchiolar and alveolar epithelial cells in the lungs of calves inoculated with the bovine strain of virus. Nuclear inclusions were not observed in experimentally or naturally infected sheep in this study, nor have they been seen in tissue cultures infected with ovine strains of PI3 virus (Hore, 1968). Although they have been described in the lungs of calves and in a variety of tissue culture systems infected with bovine strains of PI3 virus (Reisinger et al., 1959; Churchill, 1963; Dawson et al., 1965; Omar, 1965; Omar et al., 1966), they should not be regarded as a characteristic feature of the cytopathic effect in cell cultures, as Omar (1965) advocates, since not all bovine strains produce nuclear inclusions (Dinter, Hermodsson and Bakos, 1960; Schiøtt and Jensen, 1963; Dawson, 1964; Drzeniek, Bögel and Rott, 1967). Also in strains which do produce them, the nuclear inclusions tend to appear later in the course of infection than do cytoplasmic inclusions (Dawson et al., 1965).

GENERAL DISCUSSION

The importance and complex nature of respiratory disease of cattle in Britain has been well documented (Omar, 1966; Phillip, 1968). Similarly, in sheep, respiratory disease is a major cause of economic loss (Ministry of Agriculture, Fisheries and Food, 1964a) which will probably increase with the development, in the United Kingdom, of intensified methods of management. The present studies have shown that, as in cattle, there are potentially a number of aetiological agents associated with respiratory disease of sheep. The histopathology of the lesions produced by two such organisms has been described in detail.

Differential diagnosis of respiratory disease in sheep and cattle whether on clinical, aetiological or pathological grounds, is difficult in either species. The clinical signs associated with respiratory infections in intensively-reared calves are generally similar (Omar, 1966). Features of diagnostic significance in PI3 virus, adenovirus, reovirus and Bedsonia infections in cattle have been reviewed by Omar (1966). The specific lesions described, however, have been shown to be only characteristic in calves killed within seven days after inoculation with any of these agents. It is not yet known for how long the characteristic lesions (e.g. the inclusions in PI3 virus and adenovirus infections) persist. Such information is important as calves are frequently presented for post mortem examination after they have been ill for more than one week.

The clinical reaction observed in lambs inoculated with Bedsonia organisms was more marked than in lambs inoculated with PI3 virus but a severe clinical illness can also result from infection with organisms other than Bedsonia,

as shown in Section 1. The macroscopic lung lesions in Bedsonia or PI3 virus infections are of little use in differential diagnosis. However, in the acute stages of infection it is possible to differentiate the two infections histologically. The early lesions observed in Bedsonia infections are primarily exudative and it is possible to demonstrate the organism in affected portions of lung. For the latter purpose, cryostat sections were found to be most suitable. On the other hand, the early lesions of PI3 virus infections are characterised by a proliferative reaction and acidophilic cytoplasmic inclusions in bronchial or bronchiolar epithelial cells or in alveolar lining cells. Nine days after inoculation it was more difficult to demonstrate Bedsonia elementary bodies and the acidophilic cytoplasmic inclusions observed in PI3 virus infection had disappeared. Furthermore, the cellular response in the lungs in either infection is characterised by a non-specific proliferative reaction (i.e. infiltration of the interalveolar septa by macrophages and lymphocytes, pseudo-epithelialisation and epithelialisation of alveoli, macrophage infiltration of alveoli and hyperplasia of the alveolar reticulin network). Thus, it is not possible to incriminate a specific agent when only these lesions are observed.

Respiratory diseases in calves (Reisinger et al., 1959; Dawson et al., 1965) and lambs (Section 2, Subsections 2 and 3, Section 3) experimentally infected with PI3 virus are usually mild. However, PI3 virus is capable of destroying the integrity of the respiratory tract (Section 2, Subsections 1 and 2, Section 3) and thereby "may open the way to attack by invading bacteria" (Andrewes, 1967). In calves inoculated with PI3 virus and

Pasteurella spp. a more severe disease is produced (Heddleston et al., 1962; Hetrick et al., 1963; Baldwin et al., 1967). It appears likely that the respiratory disease observed in the intensively-reared calves described in Section 5 was due to PI3 virus complicated by a secondary bacterial infection. Experimentally, it has yet to be shown that there is a synergistic action between PI3 virus and bacteria in sheep. However, since the lesions in lambs inoculated with PI3 virus are comparable with those occurring in calves and since it has been shown that, using large numbers of organisms, P. haemolytica is a lung pathogen of sheep (Smith, 1964; Biberstein et al., 1967), it would appear that the respiratory disease in the recently introduced lambs described in Section 1 was due to a primary PI3 virus infection followed by infection with P. haemolytica.

A similar outbreak of respiratory disease in sheep introduced into covered pens at the Moredun Institute was described by Gilmour and Brotherston (1963) and classified as atypical pneumonia by Stamp and Nisbet (1963). The cause or causes of the pneumonia were not determined but, in retrospect, it seems probable that PI3 virus might have been the inciting agent. Sections of lung from lambs in the initial outbreak described by Gilmour and Brotherston (1963), were re-examined and except for the absence of acidophilic cytoplasmic inclusions, the lesions were similar to those seen in this investigation. The absence of inclusions could have been due to the interval between initial infection and the time of slaughter as was observed in three of the lambs (14X00, 14X06, 13X93) killed in the outbreak described in Section 1.

In the present investigations, lesions resembling those of atypical

pneumonia were produced in young lambs inoculated with PI3 virus (Section 2, Subsection 2) or Bedsonia organisms (Section 4). Stamp and Nisbet (1963) suggested that lungworms might also be responsible for the lesions they observed and it is interesting to note that recently, an epithelialising pneumonia of calves was described from which D. viviparus and PI3 virus were recovered (Campbell and Martin, 1968). The role of D. filaria either alone or in combination with other agents, requires further study.

While there are difficulties in classifying the respiratory diseases of sheep, the present studies have shown that PI3 virus and Bedsonia organisms are respiratory pathogens of sheep that can be histologically recognised as such in the acute disease.

The description of a type of pneumonia morphologically distinct from those hitherto described ("moderate epithelialising pneumonia", Section 1) indicates that other respiratory agents remain to be identified.

Although classification of sheep pneumonias, based on histopathology may be of limited value (because similar morphological changes can be produced by more than one agent), pathological methods in the present investigation (Section 1), as previously (Jarrett, 1956; Stamp and Nisbet, 1963), have defined conditions in which infectious agents may be implicated.

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APPENDIX

Reprinted from THE VETERINARY RECORD, January 7th, 1967. Vol. 80. No. 1. Pp. 26-7.

Experimental Virus Pneumonia in Lambs

Sir,—The isolation of an ovine strain of parainfluenza virus, serologically related to parainfluenza 3 (P13) virus, was reported recently (Hore, 1966). This strain was designated G2 and was isolated from a lamb in an intensively run flock in which there had been an outbreak of mild respiratory disease.

This preliminary communication records the experimental production of upper respiratory tract infection and pneumonia in lambs by the G2 strain of parainfluenza virus.

Lambs were obtained at birth (colostrum-deprived) or immediately following a colostral feed. They were bottle-fed three times daily on ½ pint of heated cow's milk which was supplemented with antibiotics for the first three days.

Nine colostrum-deprived and four colostrum-fed lambs were inoculated with virus by injection of 2 ml. of infected foetal lamb kidney (FLK) tissue culture fluid into the lower trachea and 1 ml. intranasally. The inoculum was at the fourth passage level in FLK cultures and had a titre of $10^{5.0}$ $TCID_{50}$ per ml.

In the control group of five lambs (two colostrum-fed) which was housed separately, each lamb received 3 ml. of virus-free tissue culture fluid intratracheally and 1 ml. intranasally. All lambs were inoculated when 24 to 48 hours old.

Nasal swabs were taken each day from all lambs and immediately placed into 3 ml. of Hanks's balanced salt solution containing 2 per cent. bovalbumin and antibiotics. This was lightly centrifuged for tissue culture inoculation. Recovery of virus was similarly attempted from the conjunctiva of two lambs.

After inoculation, lambs were killed at daily intervals from the third to the tenth day.

At autopsy the pharynx and trachea were swabbed and suspensions of tonsillar, retropharyngeal lymph node, bronchial lymph node and lung tissue were prepared for tissue culture inoculation. All virus recoveries were made on FLK monolayers in stationary tubes which were examined by the haemadsorption technique on the fifth day post-inoculation.

Virus was obtained from the nasal passages of three lambs 24 hours after infection, then daily from each surviving lamb up to the sixth day, from five of six lambs on the seventh day and three of four on the eighth day. No recoveries were made from nasal swabs taken on the ninth and tenth day. Virus was also obtained from a conjunctival swab taken from one lamb on the fifth day.

At autopsy, virus was recovered from two to five of each of the tissues or positions examined in 12 lambs but not from the lamb killed on the tenth day.

Virus recovered from each of the infected lambs was serologically identified as G2 strain. No virus was isolated from swabs or tissues taken from the lambs in the control group.

The clinical signs following infection were minimal in the lambs killed before the fifth day. A mild mucoid nasal discharge appeared after 48 to 72 hours and generally persisted for the period during which virus was recovered. A slight transient rise in temperature (1°F to 1.5°F) occurred at the time of onset of the nasal discharge in nine lambs. There was a further more pronounced rise (1.5°F to 3°F) on the fourth to sixth day in seven of the remaining lambs which developed a cough and a moderate degree of respiratory distress at this stage. One lamb, from which the positive conjunctival swab was taken, showed a bilateral ocular discharge on fifth day.

Haemagglutination inhibition (HI) tests on sera from the infected group showed antibodies after the fifth day in eight of nine lambs. Serum samples taken from all the lambs between 24 and 48 hours after birth were negative to the HI test at a dilution of 1 in 5. There was no difference in the response to infection between the colostrum-fed and colostrum-deprived groups.

All the infected lambs developed lesions of pneumonia. In those killed on or before the seventh day after inoculation the macroscopic lesions were similar in appearance and distribution but varied in their extent. The pneumonic areas began as small dull red sunken bands gradually increasing in amount. By the seventh day the lesions involved the apical, cardiac and anterior ventral portions of the diaphragmatic lobes (Fig. 1). In lambs killed after the 8th day the consolidated areas were reddish-brown, partially

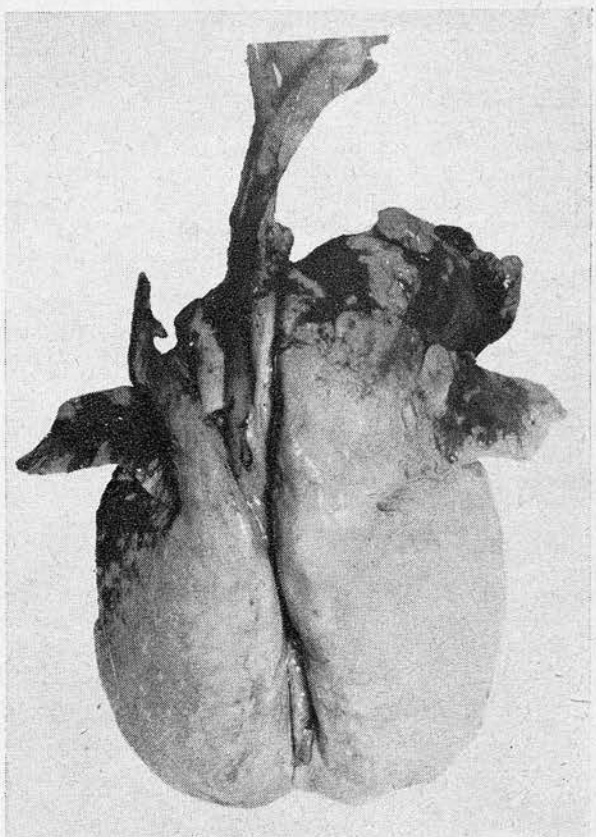

FIG. 1.—Dorsal view of lung showing the extent and distribution of the consolidated areas.

collapsed and contained small greyish, slightly raised foci on their cut surface.

The histological lesions consisted of an infiltration of the interalveolar septa with macrophages and lymphocytes and hyperplasia of the bronchiolar epithelium in early cases. By the eighth day epithelial giant cells and pseudo-epithelialisation of alveoli were present and there was necrosis and desquamation of the hyperplastic bronchiolar epithelium. Eosinophilic cytoplasmic inclusions were seen most often in the bronchiolar epithelium but were also present in bronchial and alveolar epithelial cells.

The lungs of the control lambs were macroscopically normal and some small areas of atelectasis were the only histological lesions seen.

November 18*th*, 1966. Yours faithfully,

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ISOLATION OF PARAINFLUENZA VIRUS FROM THE LUNGS AND NASAL PASSAGES OF SHEEP SHOWING RESPIRATORY DISEASE

By

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INTRODUCTION

For some years outbreaks of respiratory disease have occurred in young sheep soon after their introduction to pens in a covered yard at the Moredun Institute. The clinical and pathological findings in one such outbreak affecting a group of sheep approximately 6 months of age were described by Gilmour and Brotherston (1963). An outbreak of pneumonia occurred in a sample of the 1966 intake of lambs which had been selected for a study of the problem. This paper reports the isolation of parainfluenza 3 (PI3) virus from the lungs and upper respiratory tract of lambs during the course of the outbreak and describes the clinical and pathological observations made at the same time.

MATERIALS AND METHODS

Sheep. Ten South Country Cheviot ram lambs of approximately 7 months of age were brought into the Institute. They had been reared on a farm on which there had been no recent outbreak of respiratory disease. On the day of introduction each lamb was clinically examined, a serum sample was collected and a nasal swab taken. The 10 ram lambs were then penned with 19 lambs which had been housed in the covered yard since weaning 5 months previously. There was no obvious respiratory condition in the group of 19 already in the pen, although on introduction to the yard coughing and slight ocular and nasal discharges had been noted in a proportion of them. There had been no deaths from pneumonia in this particular group, but one lamb from the same source in the next pen had died from pneumonia shortly after housing. The 10 introduced lambs were inspected twice daily. Temperatures were taken and a clinical examination made at the same time each day for 23 days following introduction. Temperatures of 40·6°C. or greater were considered abnormal. Further nasal swabs and serum samples were collected from the ram lambs on the 14th and 28th days after introduction. A final serum sample was obtained from the surviving lambs on the 48th day.

Virus isolation procedures. Nasal swabs were placed into Hank's salt solution with 0·5 per cent. (w/v) bovine albumin and antibiotics (600 units penicillin and 300 μg. streptomycin/ml.) and held overnight at 4°C. before inoculation. Lung material was stored at −20°C. Lung tissue was prepared for inoculation by grinding in a mortar without abrasive and a 10 per cent. (w/v) suspension was made in M/15 Sörensen's phosphate buffer with 0·5 per cent. bovine albumin (pH 7·1). Nasal swab media and suspensions of lung material were centrifuged at 2,000 g. for 50 minutes at 4°C. and 0·2 ml. volumes of the supernatant fluid were inoculated into secondary ovine kidney (OK) cultures in tubes and in tubes with flying coverslips. After adsorption at 34°C. for 1 hour, 1 ml. maintenance medium was added

to each tube. This consisted of medium 199 with 2 per cent. inactivated horse serum, penicillin (600 units/ml.), streptomycin (300 μg./ml.), kanamycin (100 μg./ml.) and mycostatin (100 units/ml.). Cultures were incubated at 37°C. in stationary racks and examined by the haemadsorption technique of Vogel and Shelokov (1957) on the 5th or 6th day after inoculation using a 0·5 per cent. suspension of guinea pig erythrocytes at 18°C. for 20 minutes. Coverslip preparations were examined every second day after fixation in Bouin's fluid and staining with haematoxylin and eosin. Cultures which showed neither cytopathic effect (CPE) nor haemadsorption were passaged once in OK cultures and if after a further 15 days' incubation there was no evidence of CPE or haemadsorption the cultures were discarded. Infectivity titrations were carried out in secondary OK cultures. Cultures were examined on the 4th day after inoculation by the haemadsorption technique and $TCID_{50}$ titres were calculated by the method of Kärber (1931).

Viruses and antisera. The 3 strains of virus used were (a) a strain of haemadsorbing agent isolated from a lamb in the present investigation (designated M1), (b) the G2 strain of PI3 virus, a strain previously isolated from the nasal passages of a lamb (Hore, 1966), and (c) a reference strain of human PI3 virus obtained from the Central Public Health Laboratory, Colindale. Antisera against each of the 3 strains were prepared in rabbits by intravenous inoculation of 2 ml. infective tissue culture fluid followed 14 days later by 2 ml. intravenously and 3 ml. intraperitoneally. Sera were collected 10 days after the 2nd inoculation.

Bacteriological examination of lung material. Material from lung specimens was inoculated into 5 ml. amounts of infusion broth and on to 7 per cent. sheep blood agar plates and incubated at 37°C. overnight. Broth cultures were sub-cultured to blood agar the following day and suspected colonies of *Pasteurella haemolytica* were sub-cultured and examined further. The strains of *P. haemolytica* were serologically typed according to the method of Biberstein and Thompson (1966).

Tests for PI3 virus antibodies. All sera were inactivated at 56°C. for 30 minutes. Serum neutralisation (SN) tests were carried out by mixing serial 2-fold dilutions of serum with an equal volume of virus at a dilution calculated to contain 100 $TCID_{50}$. The mixtures were incubated at 18°C. for 1 hour before inoculation of 4 OK cultures per dilution. End-points were determined by the haemadsorption technique on the 4th day of incubation. The titre was expressed as the highest initial dilution of serum preventing growth of virus in 50 per cent. of tubes. Haemadsorption inhibition tests were performed as described by Grist, Ross, Bell and Stott (1966). Sera for use in haemagglutination inhibition (HI) tests were absorbed with guinea pig erythrocytes and titrated as described by Dawson (1963). The HI antibody titre was expressed as the highest initial dilution of serum which completely inhibited agglutination by 4 units of virus. The source of haemagglutinin was tissue culture fluid harvested from OK cultures 5 days after infection with the M1 haemadsorbing agent.

Pathology. Lungs were removed from the thorax and the distribution of lesions recorded. Representative pieces of lung and pulmonary lymph nodes were fixed in 10 per cent. formol-saline with secondary fixation in formol-sublimate. Paraffin sections were cut at 5 μ and stained by a variety of methods including celestin blue-haematoxylin and eosin, azure-eosin, picro-Mallory, Pollak's trichrome and phloxine tartrazine.

RESULTS

Clinical Observations

The temperature reactions of 6 lambs between the 8th and 24th days after introduction and of 4 lambs on the day of slaughter are shown in Fig. 1. No temperatures exceeded 40·6°C. until the 11th day after introduction when two lambs had temperatures of 40·6°C. (14X00) and 40·9°C. (13X90). These lambs

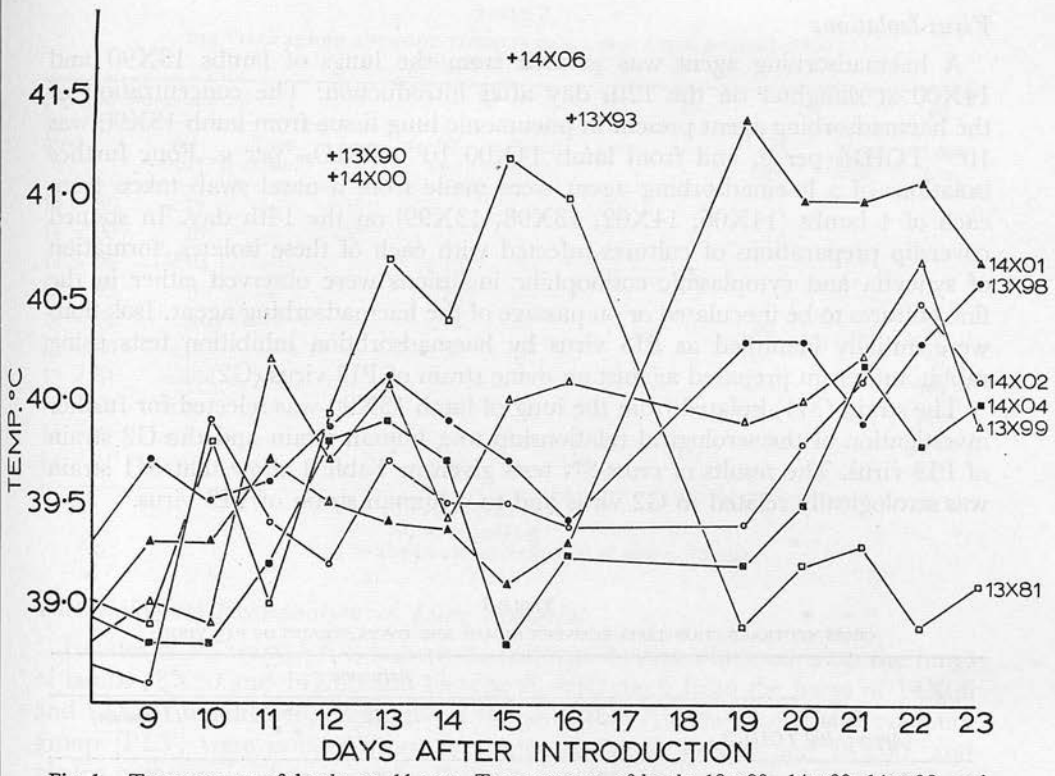


Fig. 1. Temperatures of lambs at 11 a.m. Temperatures of lambs 13×90, 14×00, 14 ×06 and 13×93 are those recorded on the day of slaughter.

were killed for post-mortem examination on the 12th day when their temperatures were 41·2°C. (13X90) and 41·1°C. (14X00). Apart from elevated temperature, neither lamb showed clinical disturbance and appetites were normal. On the 15th day lamb 14X06 was killed when a temperature of 41·8°C. was recorded. Except for injection of the conjunctival mucosa no abnormality was evident and its temperature had shown only a slight rise (39·8°C. to 40·1°C.) between the 13th and 14th days. A 4th lamb (13X93) was killed on the 16th day after developing a temperature of 41·4°C. This lamb also appeared clinically normal prior to the time of slaughter with a temperature of 39·4°C. on the 14th and 40·4°C. on the 15th day. Over a period from the 13th to 16th days a fifth lamb (13X81) showed more obvious signs of respiratory disease. It had a temperature of 40·7°C. on the 13th day and between day 13 and day 17 was dull, anoretic and slightly dyspnoeic. This lamb was retained for observation and subsequently recovered. Elevated temperatures were observed in lamb 14X01 from the 19th to 23rd days, in 14X02 and 13X99 on the 22nd day and 13X98 on the 23rd day. No apparent clinical abnormalities were observed in any lamb at these times, but when the nasal swabs were taken on the 28th day, each of the 6 lambs which had not been killed had a slight purulent nasal discharge. The temperature of lamb 14X04 remained below 40·6°C. throughout the period of observation.

Virus Isolations

A haemadsorbing agent was isolated from the lungs of lambs 13X90 and 14X00 at slaughter on the 12th day after introduction. The concentration of the haemadsorbing agent present in pneumonic lung tissue from lamb 13X90 was $10^{2.7}$ $TCID_{50}$ per g. and from lamb 14X00 $10^{3.2}$ $TCID_{50}$ per g. Four further isolations of a haemadsorbing agent were made from a nasal swab taken from each of 4 lambs (14X06, 14X02, 13X98, 13X99) on the 14th day. In stained coverslip preparations of cultures infected with each of these isolates, formation of syncytia and cytoplasmic eosinophilic inclusions were observed either in the first cultures to be inoculated or on passage of the haemadsorbing agent. Isolations were initially identified as PI3 virus by haemadsorbtion inhibition tests using rabbit antiserum prepared against an ovine strain of PI3 virus (G2).

The strain (M1) isolated from the lung of lamb 13X90 was selected for further investigation of the serological relationship to a human strain and the G2 strain of PI3 virus. The results of cross SN tests given in Table 1 show that M1 strain was serologically related to G2 virus and to a human strain of PI3 virus.

TABLE 1

CROSS NEUTRALISATION TESTS BETWEEN HUMAN AND OVINE STRAINS OF PI3 VIRUS

	Antiserum		
Virus (Approx. 100 $TCID_{50}$)	*M1*	*G2*	*PI3 (human)*
M1 (ovine)	750*	890	160
G2 (ovine)	640	890	70
PI3 (human)	80	130	2160

*Titre expressed as reciprocal of serum dilution
Pre-immunisation sera titred < 4 for neutralising antibody.

Serological Response (PI3 Virus Antibodies)

The serum HI antibody response in relation to virus isolation is shown in Table 2. Sera collected from the 10 lambs at the time of introduction were all negative for HI antibodies when tested at a dilution of 1 : 10. On the 14th day after introduction lambs 13X81 and 13X93 had HI antibody titres of 1 : 160 and 1 : 20 respectively, 14X04 was positive at a dilution of 1 : 10 and the remainder were still negative to the HI test at this level. By the 28th day after introduction the surviving lambs (14X02, 13X98, 13X99 and 14X01) which were negative on the 14th day had HI antibody titres ranging from 1 : 40 to 1 : 320. In this group the antibody titre of lamb 14X01 rose between the 28th and 48th days while the titres of the other 3 remained at approximately the same level. The antibody titre of 14X04 remained at 1 : 10 on the 28th day and was negative to the HI test at this dilution on the 48th day.

TABLE 2

PI3 VIRUS AND HI ANTIBODY TITRES IN RAM LAMBS AFTER INTRODUCTION TO INSTITUTE FLOCK

Day after introduction	*0*		*12*	*14*		*15*	*16*	*28*		*48*
Sheep No.	*HI titre**	*Nasal swab*	*Lung*	*HI titre*	*Nasal swab*	*Lung*	*Lung*	*HI titre*	*Nasal swab*	*HI titre*
13 × 90	<10	—	V	..	..	..	..	..	..	..
14 × 00	<10	—	V	..	..	..	..	..	..	..
14 × 06	<10	—	..	<10	V	—	..	..	..	..
13 × 93	<10	—	..	20	—	..	—	..	..	..
14 × 02	<10	—	..	<10	V	..	..	40	—	40
13 × 98	<10	—	..	<10	V	..	..	80	—	40
13 × 81	<10	—	..	160	—	..	..	640	—	640
13 × 99	<10	—	..	<10	V	..	..	320	—	160
14 × 04	<10	—	..	10	—	..	..	10	—	<10
14 × 01	<10	—	..	<10	—	..	..	40	—	640

V = PI3 virus isolated
— = No virus isolated
* = Expressed as reciprocal of serum dilution

Bacteriological Examination of Lung Material

Isolates of *P. haemolytica* biotype A, serotype 2, were obtained from the lungs of lambs 13X90 and 14X00 and biotype A, serotype 6 from the lungs of 14X06 and 13X93. In addition, organisms of the psittacosis-lymphogranuloma venereum group (PLV) were isolated from the lungs of lambs 14X06 and 14X00, and pleuro pneumonia-like organisms (PPLO) from the lungs of lambs 14X00 and 13X90. The significance of these organisms is discussed later.

Pathology

In each of the 4 lambs which were killed, macroscopic lung lesions were present in the apical and cardiac lobes. Approximately two-thirds of the lung tissue in the apical lobes was consolidated. The affected areas were dark red, firm and slightly depressed. The apical lobes of 3 lungs (13X93, 14X00 and 14X06) showed marked interlobular oedema and in 2 of these (13X93 and 14X06) small greyish foci were seen beneath the pleura and on the cut surface. Dark red branching bands of alveolar collapse several mm. in thickness were consistently present in the cardiac lobes. There were areas of red consolidation 2 to 4 cm. in diameter in the posterior parts of the diaphragmatic lobes in 3 lungs (13X90, 13X93 and 14X00) and section revealed large numbers of adult *Dictyocaulus filaria* in the bronchi in 2 of them (13X90 and 14X00). In one of the lungs (14X06) linear areas of collapse similar to those seen in the cardiac lobes were also present in the intermediate lobe and the anterior borders of the diapragmatic lobes.

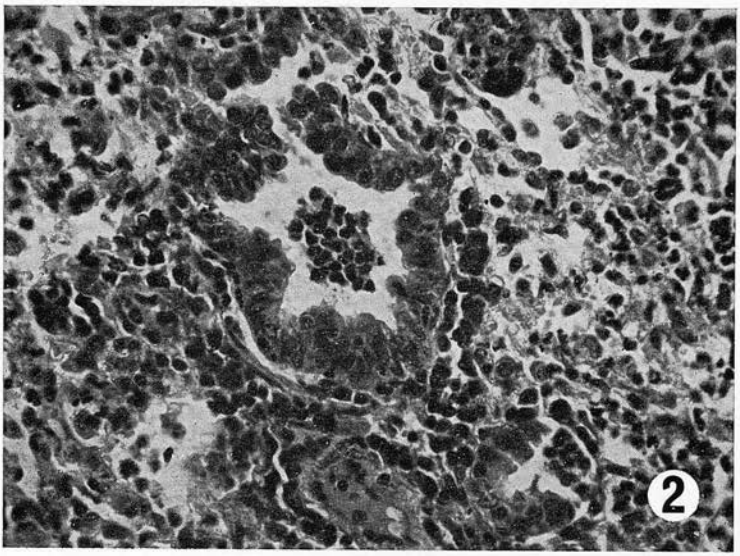
The histological lesions varied from lobe to lobe, but within each lobe were essentially similar in all 4 lambs. In the apical lobes the epithelium of the bronchi and bronchioles was hyperplastic and sometimes inflamed or, less commonly, necrotic (Fig. 2). Within the lumen of the air passages there was a cellular exudate

consisting mainly of neutrophils whereas in the surrounding tissues there were moderate numbers of lymphocytes, macrophages and plasma cells. Most of the alveoli contained either serous fluid or a cellular exudate composed of neutrophils and/or macrophages. In alveoli filled with neutrophils the interalveolar septa were normal except for hyperaemia of the capillaries whereas in others the septa were thickened due to increased numbers of macrophages and lymphocytes. Syncytia and pseudo-epithelialisation, but not alveolar epithelialisation, were common in the latter areas. In one lamb (13X90) giant cell bronchiolitis and acidophilic cytoplasmic inclusions in bronchiolar epithelial cells and occasionally in alveolar syncytia were observed (Fig. 3). The shape, size and staining affinity of the inclusions were the same as observed in experimental PI3 virus infections in lambs (Hore and Stevenson, in preparation). The lesions in all the cardiac lobes, in the anterior borders of the diaphragmatic lobes and the intermediate lobe in one lamb (14X06) consisted of atelectasis, mild bronchiolitis and alveolitis. Parasitic granulomata were seen in the posterior parts of the diaphragmatic lobes in all but one lamb (14X06). Hyperplasia and acute lymphadenitis constituted the histological lesions in the pulmonary lymph nodes.

DISCUSSION

As it had been decided that virus isolation should be attempted as early as possible in any animal suffering from respiratory disease, 4 of the 10 lambs were killed when they showed a sudden rise in temperature which was the first recognisable sign of the possible development of a pneumonic condition. It is doubtful whether temperatures of 41·1°C. and 41·7°C. were caused by PI3 virus alone since experimental infections with this agent have not in our experience produced temperatures of this order in sheep of comparable age except on 2 occasions when *P. haemolytica* was also isolated from lungs showing acute pneumonitis (Hore, unpublished observations). The rapid and marked rise in temperature in lambs 13X90, 14X00, 14X06 and 13X93 could have been due to the combined effect of several or all of the agents known to be present. As mentioned earlier, organisms of the PLV group were isolated from the lungs of 2 lambs (14X06 and 14X00) and PPLO from the lungs of lambs 14X00 and 13X90. The pathogenic significance of these isolates is not clear. The only agent common to all 4 lungs was *P. haemolytica* and it may have been the main contributing factor. This is supported by the histological appearances since 2 distinct types of lesion were evident in each lung. The more obvious lesion was that of an acute exudative inflammation usually associated with bacterial infections, the other being of a proliferative nature generally regarded as viral in aetiology. Acidophilic cytoplasmic inclusions, which are a pathognomonic feature of experimental PI3 virus infection in lambs (Hore and Stevenson, 1967), were seen in one (13X90) of the 2 animals (13X90 and 14X00) in which virus was isolated from lungs.

In all except one (14X04) of the lambs which were not killed, virus isolations and serological data showed a close temporal relationship between PI3 virus infection and febrile illness.



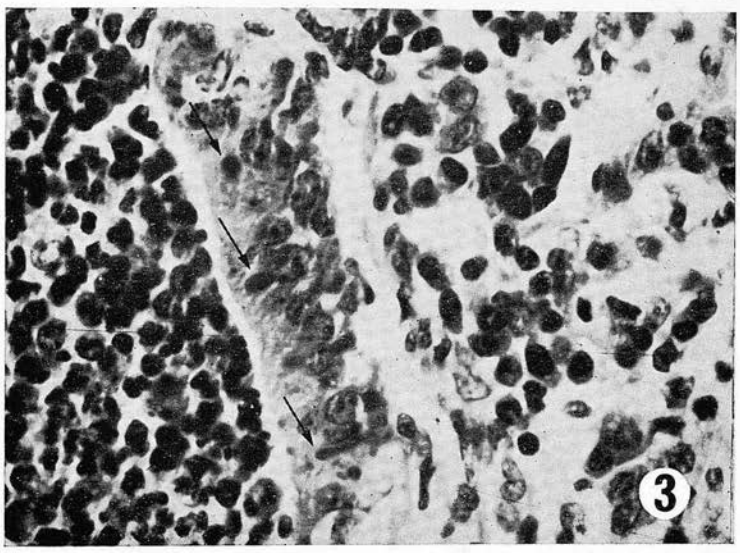


Fig. 2. Lamb 14×00. Irregular hyperplasia of bronchiolar epithelium, cellular infiltration of alveoli and partial epithelialisation of an alveolus. H & E × 360.

Fig. 3. Lamb 13×90. Acidophilic cytoplasmic inclusions (arrows) in bronchiolar epithelial cells and macrophage infiltration of the surrounding alveoli. Pollak's trichrome × 720.

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In this study, histopathological evidence together with serological findings suggest that PI3 virus can act as a lung pathogen in naturally infected sheep.

SUMMARY

Isolations of parainfluenza 3 (PI3) virus were made from pneumonic lung tissue of 2 lambs and from the nasal passages of a further 4 lambs in a group of 10 involved in an outbreak of respiratory disease. *Pasteurella haemolytica* was isolated from the lungs of each of the 4 lambs which were killed for post-mortem examination.

On histological examination changes of both an acute exudative and proliferative nature were observed in each lung. In one lung, from which virus was isolated, acidophilic cytoplasmic inclusions were present in bronchiolar epithelial cells.

No PI3 virus antibodies were detected in serum samples collected prior to the onset of respiratory disease in the group. One of the lambs which was killed and 5 of the 6 which were not slaughtered subsequently developed antibodies against PI3 virus. Each lamb first showed antibody response in serum samples collected at the time of, or immediately following, respiratory disease or a severe febrile illness.

The possible relationship of PI3 virus infection to the clinical and pathological findings is discussed.

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PATHOLOGY OF PNEUMONIA IN INTENSIVELY-REARED CALVES

By

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INTRODUCTION

One of the most serious causes of economic loss in intensive beef production is death or unthriftiness resulting from pneumonia. The aetiology of calf pneumonia is complex and is likely to remain so until the causal agents can be related to specific lesions. The purpose of this paper is to describe two types of pneumonia which occurred in intensively-reared calves on two farms in East Lothian and to attempt to relate the lesions to possible aetiological factors. The lesions are classified on a morphological basis.

MATERIALS AND METHODS

During the winter of 1964-65 this Institute in conjunction with the Veterinary Investigation Service, East of Scotland College of Agriculture, was asked to investigate outbreaks of pneumonia in intensively-reared calves.

Male Friesian calves ranging in age from 10 to 16 weeks had been purchased in North Cheshire and South Lancashire and transported by road to 2 farms in East Lothian. Clinical examination of the calves was carried out weekly at first and then at fortnightly intervals. Post-mortem examinations were carried out on 8 animals which died when about 4½ months old. The lungs of a further 28 calves, approximately 1 year of age, were examined when slaughtered at the end of the feeding period.

Pieces of lung, pulmonary lymph nodes, trachea and other tissues when available, were fixed in 10 per cent. formol-saline with secondary fixation in saturated mercuric chloride, dehydrated, cleared and embedded in paraffin. Sections were cut at 6 μ and routinely stained with celestin blue—haematoxylin and eosin. Other stains used were van Gieson, phloxine-tartrazine, Gordon and Sweet, McNeal, Heidenhain's iron haematoxylin, P.A.S. and pyronin-methyl green. To demonstrate fibrin Mallory's phospho-tungstic acid haematoxylin, picro-Mallory (McFarlane, 1944), fuchsin Miller (Slidders, 1961) and picro-Mallory V and Martius-Scarlet-Blue (MSB) (Lendrum, Fraser, Slidders and Henderson, 1962) were used.

Bacteriological examination of the lungs of 9 animals comprising Group A were carried out.

RESULTS

Clinical Signs

On arrival some of the calves were coughing and several had a serous nasal discharge. Within 7 to 10 days the majority were coughing, and had a serous or mucoid nasal and sometimes ocular discharge, but within 2 weeks most of them were clinically normal except for a persistent cough. In the few calves which developed a secondary broncho-pneumonia the serous or mucoid nasal discharge became purulent, the temperature rose to 105°F. or higher and there was dyspnoea with rales. Partial to complete anorexia and depression preceded death.

Diarrhoea was observed in 2 cases, one of which had a concurrent pneumonia while the other was clinically normal except for an occasional cough.

On one farm, 8 out of 44 calves died whereas on the other there were no deaths in 21 animals.

Lesions

The lesions in the lungs can be divided into 2 distinct types on gross and histopathological findings. Group A consists of 9 animals, 8 of which died on one farm. Pneumonia was the cause of death in 7, while the other death was due to primary ruminal tympany. The remaining calf was from the other farm and was clinically normal when slaughtered.

Group B is made up of 27 animals; 14 from one farm, 13 from the other. Except for an occasional cough these animals, when slaughtered, were clinically normal.

Group A

Gross pathology. In 7 calves the only normal areas were scattered lobules in the dorsal part of the diaphragmatic lobes. The affected areas failed to collapse when the thorax was opened, were lobular in distribution, very firm and dark red to almost purple. Numerous small, greyish foci were seen beneath the pleura and on the cut surface of consolidated lobules in 5 of the 7 cases. A mucopurulent exudate could be expressed from cut bronchi and bronchioles. In the remaining 2 cases the gross lesions were similar, but were confined to either the right apical or cardiac lobes and necrotic foci were not present. Interstitial emphysema, sometimes with the formation of large bullae and thickening of the pleura, was present in the diaphragmatic lobes of 3 animals. The pulmonary lymph nodes were congested and oedematous.

Escherichia coli was isolated from 4 cases, *Pasteurella multocida* from one and *Pasteurella haemolytica*, type A serotype I and *Streptococcus viridans* from another case. The remaining 3 lungs were bacteriologically sterile. Two animals from one farm had recently been treated by a veterinary surgeon while the third animal was clinically normal and there was no history of previous antibiotic therapy.

Histopathology. The histopathological findings were bronchitis, bronchiolitis, a cellular exudate in alveoli, focal areas of necrosis and epithelialization of alveoli. Small intracytoplasmic, eosinophilic inclusions were occasionally seen in bronchial and bronchiolar epithelial cells.

The trachea was examined in 3 cases. The changes were similar and consisted of neutrophilic and lymphocytic infiltration of the mucosa. The epithelium was often tattered and in 1 case there were small areas devoid of epithelium while in another there were small acidophilic, intracytoplasmic inclusions. Lymphocytes, plasma cells and small numbers of macrophages were present in the submucosa.

The bronchi and particularly the bronchioles contained a necrotic exudate composed of dead or dying neutrophils, macrophages, epithelial cells and lymphocytes. Occasionally erythrocytes, fibrin and small colonies of bacteria made up part of the exudate. The epithelium was infiltrated with neutrophils and lymphocytes, and had undergone degenerative changes ranging from vacuolation of

epithelial cells to complete necrosis of the epithelium and, in 2 cases, of the mural structures. There was epithelial metaplasia in 3 cases; very tall columnar cells were seen in one of the cases while in the other 2 the epithelium consisted of a single-layer of low cuboidal cells. In the peribronchial and peribronchiolar tissues there was congestion of the blood vessels and infiltrations of round cells, lymphocytes, plasma cells and occasionally macrophages, and fibrous tissue proliferation in older lesions.

Within the alveoli the reaction was exudative, but the type of exudate varied considerably. In all the calves there was a serous exudate, while in 8 out of 9 there was haemorrhage and necrosis of alveoli: in 7 there was a fibrinous exudate which in places was beginning to undergo organization. Macrophages were the predominant cell type with variable numbers of neutrophils. Two types of macrophages were observed (Fig. 1). The majority had a large pale-staining oval or indented nucleus with abundant, often foamy, cytoplasm which stained faintly with eosin. These cells often contained ingested material. The others were smaller and had a more densely staining oval or round nucleus and deep-pink, homogeneous cytoplasm (H & E). These cells did not usually contain phagocytosed material. In 7 out of 9 cases multinucleated macrophages and large syncytial giant cells were present, usually lying free in the alveoli (Fig. 3). In 6 of the cases the large syncytial giant cells appeared to be of epithelial origin while in the remaining case the majority were, according to the classification of Omar (1964), alveolar giant cells. In one of the cases a small acidophilic, intracytoplasmic inclusion was seen in a syncytium. Phagocytosed material was rarely seen in the syncytial giant cells. In 4 of the 7 cases containing syncytia there were focal areas of alveolar epithelialization and the cells lining the alveoli were low cuboidal in type (Fig. 2). In 3 cases there were groups of alveoli packed with alveolar macrophages, many of which were elongated, situated in an amorphous exudate which stained densely basophilic with haematoxylin (Fig. 4). The cells and the exudate were seen in the pores of Kohn, involving adjacent alveoli and were occasionally seen in bronchioles. While it was possible to demonstrate fibrin in adjacent alveoli no strands of fibrin could be seen within the basophilic amorphous exudate. Small, strongly acidophilic inclusions were seen in the cytoplasm of the alveolar macrophages. There were fibrin thrombi in the perivascular, peribronchial, interlobular and pleural lymphatics. Within or near these areas there were usually foci of necrosis. The capillaries were congested and there were increased numbers of macrophages and lymphocytes in the interalveolar septa. In 3 of the cases there were small focal areas of alveolar collapse. The interlobular septa were thickened due to distended lymphatics, congestion of the blood vessels and leucocytic infiltration.

In 4 of the 9 cases there were a few small, phloxinophilic, intracytoplasmic (2 to 5 μ) inclusions in bronchial and bronchiolar epithelial cells and occasionally, in alveolar epithelial cells (Fig. 5). The inclusions were usually situated in the cytoplasm near the apex of the cell. Although small and few in number, they were easily recognized in sections stained with phloxine-tartrazine, but less so with haematoxylin and eosin. In one case intracytoplasmic inclusions were also present in the nasal mucosa, tracheal mucosa and in the liver. The inclusions in the liver were always situated in or near areas of centrilobular degeneration.

The pulmonary lymph nodes were oedematous and the blood vessels were

congested. The peripheral sinuses contained fibrin, neutrophils, macrophages and lymphocytes. Within the cortex there were increased numbers of plasma cells. The medullary sinuses contained large numbers of neutrophils, lymphocytes, macrophages and plasma cells.

Group B

Gross pathology. In this group the lesions were very uniform and varied only in their distribution and size. The affected areas were greyish-blue, depressed below the level of the surrounding alveoli and did not crepitate when squeezed. The cut surface was dull red and many bronchi and larger bronchioles appeared to be dilated and often contained a mucoid or mucopurulent exudate. In 21 animals these atelectatic areas were confined to the anterior lobes, mainly the right apical lobe. Of the remaining 6 animals, 3 had lesions in the anterior lobes and in either the diaphragmatic or the intermediate lobes: 3 appeared normal. The pulmonary lymph nodes were enlarged in the majority of animals.

Histopathology. The pathology of the lungs of this group was characterized by peribronchial and peribronchiolar lymphocytic hyperplasia, atelectasis, focal vesicular emphysema and mild interstitial pneumonia.

The peribronchial and peribronchiolar lymphocytic hyperplasia varied in degree from very slight to very marked, with the formation of lymphocytic "cuffs" which appeared to start as peribronchial and peribronchiolar infiltrations of mature lymphocytes (Fig. 6). As the infiltrations enlarged, blast cells could be recognized among the mature lymphocytes and increased in number until a pale germinal or reactive centre was formed. At this stage there was usually a solid ring or "cuff" of mature lymphocytes surrounding the affected airway. As shown by serial sections these "cuffs" extended for some distance down a particular bronchus or bronchiole. In the final stage of "cuff" formation plasma cells appeared around the periphery and between the "cuff" and the affected bronchus or bronchiole. In those cases where lymphoid hyperplasia was considerable, there was complete peribronchial, peribronchiolar and, in 4 out of 27 lungs, perivascular cuffing, in addition to partial or complete collapse of affected bronchioles and the surrounding alveoli.

The alveolar changes were not striking except for the extent of atelectasis. Where there was slight or moderate "cuffing" there were focal areas of atelectasis, whereas in areas where the "cuffs" were considerable the atelectasis was lobular in distribution (Fig. 7). In 4 lungs atelectasis was not observed. In 14 lungs there was no cellular reaction: 9 contained small numbers of macrophages in collapsed alveoli and in the remaining 4 the alveoli contained small numbers of macrophages and either neutrophils or giant cells. In 19 lungs there were focal areas of vesicular emphysema. The interalveolar septa in all cases were slightly or moderately thickened due to increased numbers of macrophages and lymphocytes, and in 14 lungs small numbers of neutrophils. Eosinophils were present in 4 lungs.

The inflammatory response of the bronchi and bronchioles varied with the degree of "cuffing". Where there was little or no inflammatory response, "cuffing" was minimal. As the degree of "cuffing" increased so did the inflammatory response. In one lung, in which there was a marked inflammatory response in the

bronchi, eosinophilic, intracytoplasmic inclusions were seen in bronchial epithelial cells (Fig. 8).

In 6 lungs there were lesions affecting medium-sized arteries consisting of either intimal fibrosis (4 cases) or medial hypertrophy (2 cases). In 12 lungs there was an apparent increase in the number and size of arterioles especially in the areas near the anterior border of the right apical lobe. In addition, there was usually perivascular, peribronchial and peribronchiolar fibrosis and lobular collapse of alveoli. Bronchiectasis was also a feature in these areas.

Inflammatory changes in the tracheal mucosa were very slight or absent. In 14 lungs there was slight lymphocytic infiltration of the submucosa, but this was considered to be normal (Trautmann and Fiebiger, 1952).

There were no significant differences between the mediastinal and left and right bronchial lymph nodes. Lympho-follicular hyperplasia was present and 15 contained small numbers of neutrophils within the medullary sinuses. Increased numbers of macrophages and plasma cells within the medullary sinuses were seen in over 50 per cent. of the lymph nodes while in 2 there was eosinophil cell infiltration of the cortex, particularly in and around the trabeculae.

DISCUSSION

The two types of pneumonia described in this paper are very similar to atypical pneumonia of calves described by Jarrett (1954), a name given to two morphologically distinct types of pneumonia. Atypical pneumonia, group A or inclusion body pneumonia (Jarrett, 1956) is characterised mainly by cuboidal epithelialization of alveoli. Acidophilic, intracytoplasmic inclusions and syncytium formation may also be present. Group B, atypical pneumonia or "cuffing" pneumonia, first described by Jarrett, McIntyre and Urquhart (1953) is characterised by peribronchial and peribronchiolar lymphocytic hyperplasia and, in the later stages, by atelectasis and mononuclear cell infiltration of alveoli.

The gross and histological findings in group A of this investigation are those of either acute or subacute bronchopneumonia. The variations in the alveolar exudate may be due to any one or a combination of 3 factors: the stage of inflammation, the virulence of the organism(s) involved or the effects of treatment. Lesions suggestive of a virus pneumonia, although partly obscured by the bacterial infection, were present as well. Syncytium formation was present in 7 out of 9 cases. In one, only synctia were seen while in 4 there was also cuboidal epithelialization of alveoli: in the remaining 2 there was syncytium formation, cuboidal epithelialization of alveoli and acidophilic intracytoplasmic inclusions within bronchial and bronchiolar epithelial cells and within a syncytium.

Jarrett (1954, 1956) postulated a virus aetiology for his cases of inclusion body pneumonia because of the histological similarities between them and canine distemper (including canine infectious demyelinating encephalitis), giant cell pneumonia of young children and human measles, all proven viral infections. Eosinophilic intracytoplasmic inclusions similar to those described by Jarrett (1954) were found in the bronchiolar and alveolar epithelial cells in colostrum-deprived calves and in hysterotomy-derived, colostrum-deprived calves inoculated with the T1 strain (Dawson, Darbyshire, Lamont and Paterson, 1964; Dawson, Darbyshire and Lamont, 1965) and the J121 strain of PI-3 respectively

(Betts, Jennings, Omar, Page, Spence and Walker, 1964; Omar, Jennings and Betts, 1966). According to Omar *et al.* (1966), as cited by Darbyshire, Jennings, Dawson, Lamont and Omar (1966), the principal lesions in calves infected with PI-3 virus are epithelialization, syncytium formation and specific intracytoplasmic and intranuclear, acidophilic inclusions which are of transient duration. Thus it would appear that in 6 out of 9 cases of pneumonia in group A of this survey, PI-3 virus infection may have been an underlying factor, death being due to a secondary bacterial infection. Jolly and Ditchfield (1965) by histopathology, and by positive fluorescent-antibody and complement fixation tests were able to diagnose bronchopneumonia due to PI-3 in 2 clinical cases of calf pneumonia.

The gross and histopathological findings in group B resemble the "cuffing" pneumonia of calves first reported by Jarrett *et al.* (1953) and described in more detail by Jarrett (1954). Since that time a few reports of this condition have been published (Carter and Rowsell, 1958; Martin, 1963; McIntyre, 1963). Whether the lesions in group B are due to a specific aetiological agent or are the end result of a previous bronchopneumonia is not known.

In many respects the lesions in group B are similar to those of bronchiectasis in man in which there is dilatation with fibrous thickening of bronchiolar walls along with nodular and diffuse peribronchiolar, lymphocytic infiltration (Gunn, 1961). As cited by Gunn (1961), Ogilvie (1941) states that about 80 per cent. of cases of bronchiectasis have their beginning in childhood, while Boyd (1931) found bronchopneumonia to be the commonest form of disease which provided the conditions for bronchial dilatation in childhood. Bronchopneumonia is a common condition in young calves; moreover, according to Jubb and Kennedy (1963) bronchiectasis is principally a disease of young bovines. Chronic peribronchial, peribronchiolar and perivascular fibrosis, arterial lesions and bronchiectasis were observed in 12 lungs in this survey indicating that the initial pneumonia does not resolve but becomes chronic in a large number of animals. Two cases of bronchiectasis associated with "cuffing" pneumonia have been reported by Jarrett (1956). The possibility that "cuffing" pneumonia of calves may be the end result of a mycoplasmosis has been discussed by Omar (1966).

SUMMARY

During an investigation of pneumonia in intensively-reared calves two morphologically distinct types of pneumonia were encountered.

Of 9 cases in the first group 6 had lesions resembling inclusion body pneumonia of calves which, by their histopathological appearance, were considered to be due to parainfluenza 3 virus.

The second group consisted of 27 animals and resembled "cuffing" pneumonia of calves. The possibility that this condition was the sequel to a previous bronchopneumonia is discussed.

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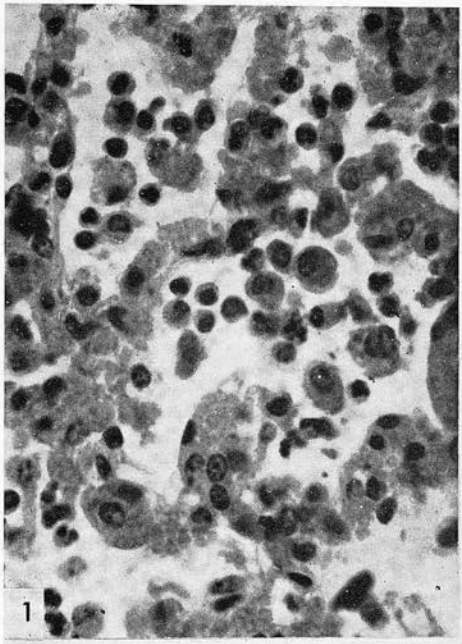

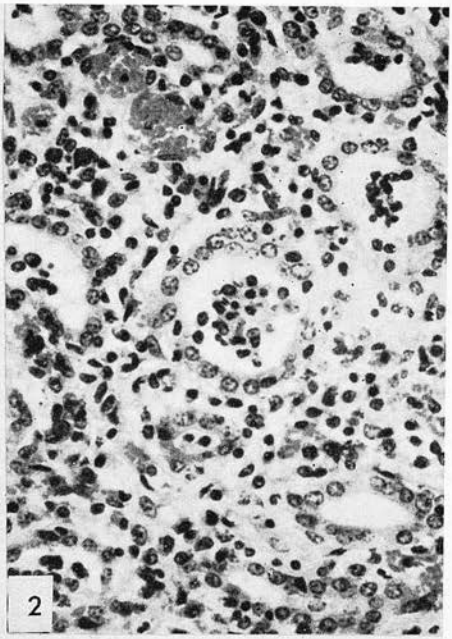

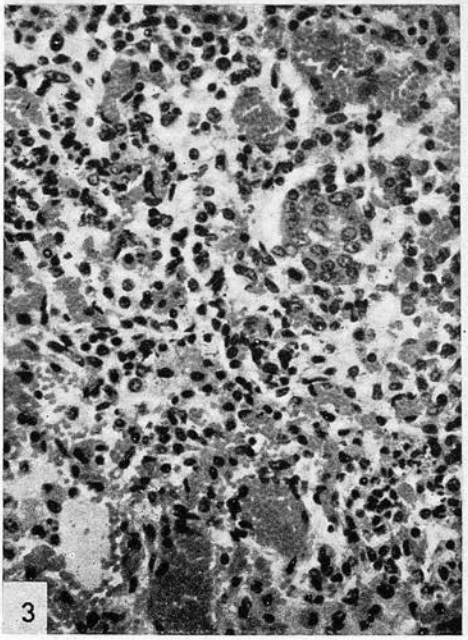

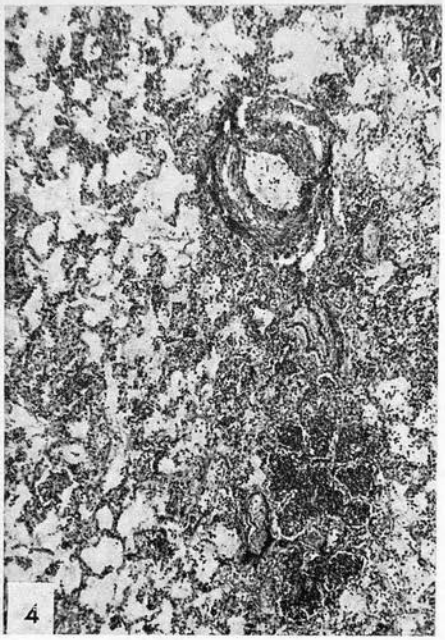

Fig. 1. Lung, group A. There are two types of macrophages within alveoli and hyperaemia of the alveolar capillaries. H. & E. × 550.
Fig. 2. Lung, group A, showing cuboidal epithelialization of alveoli. H. & E. × 320.
Fig. 3. Lung, group A, showing a syncytium and mononuclear cell infiltration of alveoli. H. & E. × 3C0.
Fig. 4. Lung, group A, showing oedema and fibrin strands in alveoli. Note the mononuclear cell infiltration and elongated alveolar macrophages within an amorphous exudate in other alveoli. Picro-Mallory × 60.

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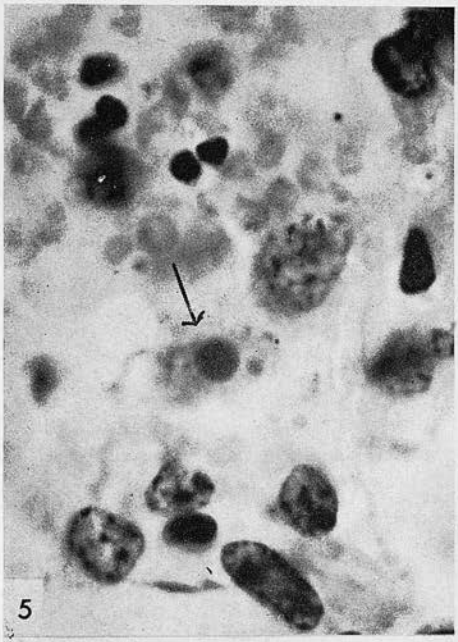

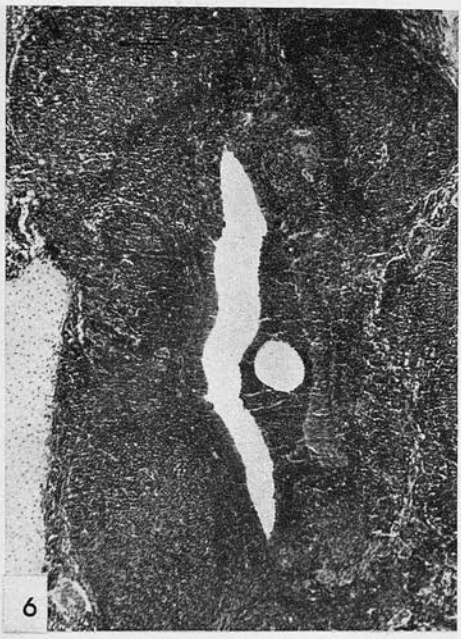

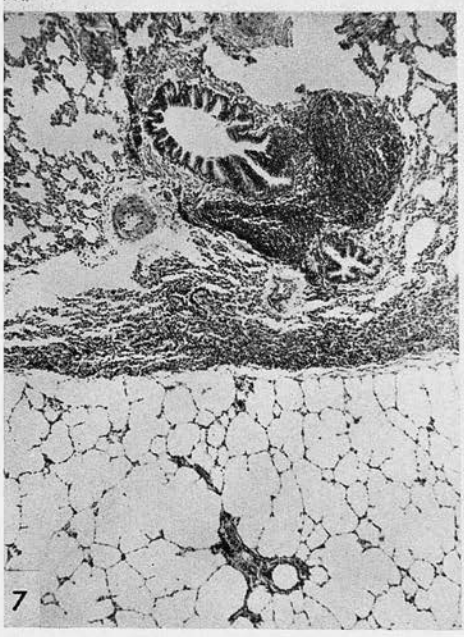

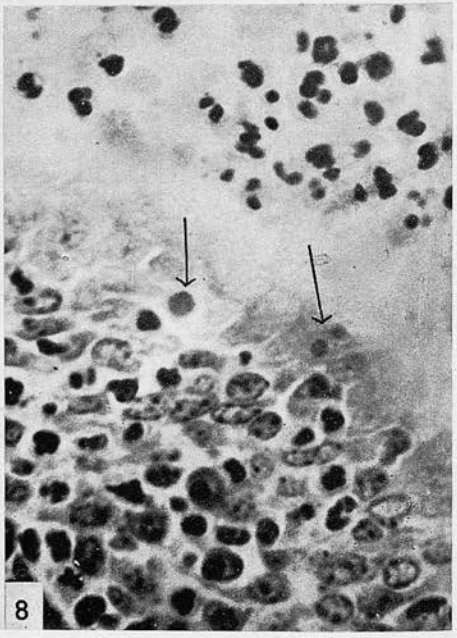

Fig. 5. Lung, group A, showing an intracytoplasmic inclusion lying above and slightly to the right of the nucleus. Phloxine-tartrazine × 1750.

Fig. 6. Lung, group B. Extensive peribronchial lymphoid hyperplasia. Note the pale germinal centres within the lymphoid "cuff" and the exudate, composed mainly of neutrophils in the lumen of the bronchus. H. & E. × 55.

Fig. 7. Lung, group B, showing peribronchiolar lymphoid hyperplasia, focal atelectasis and mild interstitial pneumonia. Note the lobular distribution of the lesions. H. & E. × 38.

Fig. 8. Lung, group B. Intracytoplasmic inclusions within bronchial epithelial cells. Note the neutrophil and plasma cell infiltration of the mucosa. Phloxine-tartrazine × 1000.

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