THE PATHOLOGY OF PNEUMONIA IN THE PIG

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Summary of thesis submitted for the degree of Doctor of Philosophy in the Faculty of Medicine of the University of Glasgow, May 1965.

THE PATHOLOGY OF PNEUMONIA IN THE PIG

The literature dealing with pneumonia in the pig was reviewed, placing emphasis on the reports concerning the status of pig pneumonias in Britain but referring also to work in other countries where it was relevant.

The main objects of the thesis were (a) to study the histopathological changes occurring in the lungs of pigs with naturally occurring pneumonia with a view to classifying the lesions on this basis, (b) to study the pathogenesis of enzootic pneumonia in experimental animals, (c) to investigate the relationship between Pasteurella multocida, Haemophilus parainfluenza and pneumonia in the pig, (d) to study the pathogenesis of the pneumonia due to Metastrongylus apri in experimental animals and (e) to vaccinate young pigs against the pig lungworm M. apri using x-irradiated infective larvae of M. apri.

The naturally occurring pneumonias studied consisted of 376 sets of pneumonic lungs from pigs of all ages, which were obtained from three sources (i) pigs sent to the post-mortem room at the Veterinary Hospital, the University of Glasgow, for routine post-mortem examination, (ii) pigs

dying or destroyed on farms during the investigation of respiratory disease problems in the field, (iii) pigs killed in abattoirs. As a result of studying these lungs histopathologically the pulmonary lesions were classified into the following groups, enzootic pneumonia, interstitial pneumonia, pneumonia due to H. parainfleunza, simple acute bronchopneumonia necrotising bronchopneumonia, suppurative bronchopneumonia, embolic pneumonia, tuberculosis, pneumonia due to M. apri, pulmonary toxoplasmosis, giant cell pneumonia and pulmonary vascular lesions.

Enzootic pneumonia, interstitial pneumonia, the pneumonia due to H. parainfluenza and the pneumonia due to M. apri were studied in experimental animals.

Enzootic pneumonia was produced experimentally in pigs using suspensions of pneumonic lung injected intratracheally. These animals were killed at different time intervals after infection and the serial pathology of the disease was described. The significance of three of the important features of the pneumonia namely alveolar cell hyperplasia, peribronchiolar lymphoid nodular development and alveolar collapse was discussed, and a hypothesis was advanced to explain the pathogenesis of the pulmonary lesions.

Interstitial pneumonia was regularly associated with P. multocida but

attempts to produce the pneumonia experimentally in pigs using pure cultures of the organism were unsuccessful.

It was demonstrated, however, that H. parainfluenza could produce pneumonia in experimentally infected pigs identical to that occurring in the field.

A survey was carried out to determine the incidence of lungworms in the pigs being sent to an abattoir in central Scotland and 93 of 1,113 sets of lungs examined were found to contain lungworms giving an incidence of 8.4%. The lungworms collected during this survey were used to infect cultures of the earthworm Eisenia foetida which in common with several other species of earthworms, is the intermediate host for this parasite. Pigs were infected with known numbers of larvae, obtained from the earthworms, and were killed serially so that the pathogenesis of the disease could be studied.

Before x-irradiation could be used to attenuate larvae for vaccinating pigs against M. apri it was necessary to discover the most suitable level of irradiation. This was done by infecting four groups of pigs with normal larvae, larvae given 20kr of x-irradiation, larvae given 40kr of x-irradiation and larvae given 60kr of x-irradiation. The most suitable

level as judged by (a) the number of worms at autopsy and (b) the degree of clinical disturbance was found to be 60kr.

Using the information gained from the previous experiment a group of young pigs was vaccinated with infective larvae which had been previously irradiated with 60kr of x-rays. When the animals were killed after they were challenged with normal larvae, it was found that the results of the experiment were inconclusive.

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GENERAL INTRODUCTION

The respiratory disease complex is economically one of the most important of all the groups of diseases which affect the pig. Resulting from this and from the scientific curiosity of many investigators, considerable interest has been shown in the various entities which constitute this complex, but even today many of them are difficult to define and are improperly understood.

The scope of this thesis is not meant to cover all of the diseases which affect the pig's respiratory tract and attention will be directed to the disease processes resulting in pneumonia.

Research in this field was probably first conducted during the second half of the nineteenth century. At that time and during the first half of the twentieth century however research was, for the most part, directed towards isolating micro-organisms from pneumonic lungs and determining their role in the pathogenesis of the disease. In contrast histopathological descriptions of the actual lesions occurring in the lungs were infrequently made and no attempt was made to classify the pneumonic lesions of the pig on this basis. Since the techniques for isolating many of the possible pathogens are still at an experimental stage and are not standard laboratory practice, the histopathological appearance of the lesions is an important guide to diagnosis and to rational clinical procedure. A variety of micro-organisms have been isolated from pneumonic lesions, but the part played by them in the disease process is often obscure. It is obvious therefore that these organisms alone cannot be used as a criterion for classifying the pneumonias of the pig.

A study of the naturally occurring lesions of the pig lung on a histopathological basis was therefore undertaken and this was supported by studying the reaction of the pig's lung to experimental infections with a variety of agents such as bacteria of the genera Haemophilus and Pasteurella, the infective larvae of Metastrongylus apri and suspensions of pneumonic lung from cases of enzootic pneumonia.

Jarrett, Jennings, McIntyre, Mulligan and Urquhart (1957) have demonstrated that it is possible to produce active immunity in cattle against the lungworm Dictyocaulus viviparus by the administration of third stage larvae of D. viviparus partially inactivated by x-irradiation. In the pig a parasitic pneumonia is caused by M. apri which is in some respects comparable with bovine parasitic bronchitis and it was therefore decided to include in the study an investigation into the possibility of vaccinating pigs against this parasite with x-irradiated third stage larvae, of M. apri.

REVIEW OF THE LITERATURE ON PIG PNEUMONIAS IN BRITAIN WITH REFERENCES TO WORK IN OTHER COUNTRIES

It has been known for a long time that pneumonia occurs in pigs. Betts (1953) quoted Aristotle as mentioning "the decay of the lungs" in pigs and Virgil as describing a disease in which "a racking cough shakes the sickening swine". It was not until the second half of the 19th century, however, that investigators, spurred on by the advances being made in bacteriology at that time, had a closer look at the types of pneumonia occurring in pigs.

Several descriptions of lesions occurring in pigs' lungs can be found in the literature of this period. Kleine (1877) described lesions found in two experimental pigs infected with "the so-called enteric or typhoid fever of the pig", which showed "mapping out of the lobules and lobes of the lungs by oedema of interlobular tissue, the lung tissue of the corresponding parts being at the same time hyperaemic". Histologically he described this as a lobular pneumonia which in its later stages transforms the lung into a necrotic disintegrating mass, in which he could see masses of micrococci. In one of these cases he saw bundles of nematode worms in the larger bronchi and ova from these worms in the inflamed lung tissue. This must be one of the first histological descriptions of Metastrongylus infection in pigs. Walley quoted by Billings (1888) also described a pneumonia in which interstitial oedema was an important feature. The surface of the involved area was raised above the surrounding lung and there was usually pleurisy.

One of the most interesting papers of this period was concerned with what was called catarrhal pneumonia (McFadyean 1888), and which appears, from the description of the lesion, to be not incompatible with what is now called enzootic pneumonia. McFadyean wrote "almost the entire lobule exhibits changes of catarrhal pneumonia" and "the alveolar epithelium has lost its squamous character, its place being taken by large irregular germinating cells. Within the lumen of the alveolus there are more or less dense masses of catarrhal cells, obviously derived by proliferation from the altered epithelium". He added "smaller bronchi, even when the epithelium is comparatively healthy, show mass peribronchial infiltration with round cells".

Catarrhal pneumonia however, did not seem to be regarded as an important entity and most attention was focussed on the two recently recognised conditions of swine plague and swine fever. It was apparently not clear whether swine plague had occurred in Britain or not and in a paper entitled "Pneumonia in Swine Fever" published in 1896 this was pointed out. As a result of an investigation into this problem McFadyean (1897) reported that he had no evidence that there was in Britain "a second epizootic disease of the pig in the form of an infectious pneumonia." The other epizootic disease he was referring to was swine fever and at that time there was a considerable amount of confusion in Britain and abroad about the occurrence of pneumonia in swine fever and even if swine fever and swine plague were different diseases. These

conditions were confused by Billings (1888) but not by Salmon, quoted by Billings (1888) or McFadyean (1888). The latter recognised that the conditions were different and was in some doubt about the role of pneumonia in swine fever. In 1891 he appeared to contradict himself and reported that the lung lesion in swine fever was a catarrhal pneumonia and that pneumonia was a fairly constant finding except in very acute cases. Five years later however, in the paper "Pneumonia in Swine Fever" quoted earlier, the opinion was expressed that pneumonia whether catarrhal or croupous was a rare lesion in swine fever. This view was based on a series of experimental animals which were probably twenty pigs whose autopsies were reported earlier in the year by McFadyean (1896). At this time swine fever was thought to be a bacterial infection and in 1897 McFadyean claimed to have isolated a swine fever bacillus from the lungs of a pneumonic pig and to have produced lesions in the lungs of an experimental pig with this. He said that this was a rare lesion however, and devoid of practical interest.

The pigs used in these early experiments would not satisfy the more stringent criteria applied at the present time, and this makes it difficult to evaluate the results of early experiments. It seems, however, that at the end of the 19th century in Britain, pneumonia was recognised as a not uncommon entity in the pig, although it was thought that pneumonia only occurred infrequently in swine fever and some people were not convinced that swine plague had occurred in this country as a second epizootic disease

distinct from swine fever.

Except for a paper in 1902 by McFadyean describing three cases of tuberculosis in young pigs in which the lungs were severely involved and one in 1937 by Robertson who surveyed 1,009 pigs in the north-east of Scotland and found that 13.08% had Metastrongylus infection, most papers in the first half of the 20th century were concerned with pneumonias caused by filter passing agents. Much of the thinking on this subject was both stimulated and confused by Shope's work on swine influenza in the United States.

During the pandemic of human influenza 1918-1919 Dr. J.S. Koen, an inspector in the division of hog cholera control of the Bureau of Animal Industry in the U.S.A. recognised as a clinical entity a respiratory disease in pigs closely resembling human influenza which he called "Hog flu" (Laidlaw 1935, Shope 1931a). Several other observers also described the clinical features of the disease (Murray 1920, McBride, Niles and Markey 1928, Fulton 1930, Spray 1922) but it was not until Shope's series of papers that it was properly understood (Shope 1931a, Lewis and Shope 1931, Shope 1931b). He described the pathology of the condition and demonstrated that a haemophilic bacillus, Haemophilus influenzae suis and a filter passing agent were involved in the disease which he called swine influenza. He also showed that the complete clinical picture could only be reproduced by experimentally infecting pigs with the filter passing agent and H. influenzae suis. An important fact which Shope noticed from his field investigations and which he states in his first paper (1931a) was that "the term of 'flu as popularly

used embraces more than one clinical entity." He recognised another respiratory disease "often simulating the true epizootic swine influenza in some respects but differing markedly in others. The true nature of the condition could usually be recognised by the absence of prostration on the part of the affected animals, the greater chronicity of the disease and the failure of more than a small portion of the herd to become affected."

These were facts which later investigators failed to appreciate and a considerable amount of confusion arose subsequently because the term "swine influenza" was misused. In the years 1932, 1934, 1935, 1936, 1937 and 1938 Shope published papers describing the serology of swine influenza, the relationship of swine influenza to human influenza, the disease in mice, the immunisation of pigs against swine influenza, the presence of neutralizing antibodies to swine influenza in human sera in the United States, and on the experimental infection of pigs with human influenza strains PR8 and WS.

In Europe at this time there were several reports describing pneumonic conditions in pigs which were thought to be the same as or similar to Shope's swine influenza. Köbe and Waldman (1933, 1934 and 1938), described a pneumonia which principally affected young pigs. This was a chronic pneumonia and since the experimental disease using material passed through a Seitz EK filter was slight, it was thought that the disease in the field was a complex of a filter passing agent and a bacterium. This condition was called Ferkelgrippe (piglet influenza) but in retrospect it is more

likely to have been enzootic pneumonia. Momberg-Jorgensen (1938) was able to reproduce this disease using only material which had been filtered through collodium filters of approximate pore diameter 0.94 . Glasser (1939) pointed out that the disease was not confined to piglets but could affect older animals and unfortunately suggested that it should be called Schweingrippe (swine influenza).

Lamont (1938) described a pneumonia of young pigs in Northern Ireland and called it piglet influenza. According to this report 50-70% of pigs going through bacon factories in the North of Ireland were affected and the condition was also present in Scotland. In his opinion it was "the most common and serious disease of pigs in these islands and Europe". This disease, which was usually introduced into a herd by buying in pigs or by sending off breeding stock for service, most commonly involved housed pigs and had its most serious effects on young animals. An acute rise of temperature was not a common feature of the pneumonia and survivors had a chronic spasmodic cough, many of them becoming bad doers. After filtration through Seitz and Berkefield filters, the filtrate produced lung lesions in eight out of twenty-seven pigs infected. On several occasions in the field they failed to find Haemophilus suis or any organism at all. These features of the disease suggest that it resembled enzootic pneumonia more than swine influenza and later (Lamont 1952) it was pointed out that the two conditions had been confused. The next publication of interest in Britain was by Blakemore and Gledhill (1941)

in which they describe their investigations into five outbreaks of pneumonia in pigs. Haemophilus influenzae suis was isolated from some of these pigs and from pigs in three of the outbreaks a virus was recovered which could be transmitted to ferrets, and neutralizing antibodies were demonstrated in the sera of convalescent pigs, for the ferret adapted virus. They pointed out that the disease was not identical to swine influenza as described in America, since the chronic course was the most serious feature in four out of the five outbreaks. The lesions occurring in their cases resembled those seen in the North of Ireland which had been sent to them by Lamont. Glover and Andrewes (1943) reported on the relationship between two strains of viruses from pig lungs, one of which had been isolated in Cambridge by Blakemore and Gledhill and the other in Northern Ireland by Lamont. They concluded that neither of the two British strains was "more closely related to the other or to the Shope pig virus than it is to a human influenza A."

Earlier, in 1935, Andrewes, Laidlaw and Smith had demonstrated antibodies to swine influenza in the sera of human beings in Britain but they regarded these as probably being non-specific. Unsuccessful attempts were made during 1948 and 1949 to isolate swine influenza virus from the lungs of pigs involved in outbreaks of respiratory disease in the North of Ireland and East Anglia (Gulrajani and Beveridge 1951a) and the opinion was expressed (Gulrajani 1951) that swine influenza had been confused with another specific infection of the pig's lung which was a separate entity. The view that true

swine influenza due to Shope's virus had not been recorded in Britain was supported later by Andrewes and Worthington (1959).

After the confusion of the first fifty years it was conclusively established in the next decade that there was in Britain a widespread important disease of pigs which was a chronic pneumonia quite distinct from swine influenza. This was first described by Gulrajani and Beveridge (1951b) and was called infectious pneumonia of pigs. They were able to reproduce the pneumonia with bacteria-free suspensions, and found macroscopic lesions in experimental animals killed between twelve and sixteen days after infection. No lesions were seen in pigs killed up to seven days after infection. The agent, in one experiment, did not appear to pass a filter of approximate pore diameter 0.56 μ and antibodies to swine influenza could not be demonstrated in the sera of experimental animals. When it had been established that another transmissible pneumonia did occur the attention of most workers was focussed on the nature of the causal agent. Betts(1952) suggested the name virus pneumonia of pigs, or V.P.P. for the disease, assuming that the causal agent was a large virus.

In this paper some clinical and epidemiological aspects of the pneumonia, including some details of the causal agent, were given. The latter was described as a large virus not less than 200mμ in size, susceptible to aureomycin to some degree, and with the ability to produce lung lesions by itself although in most natural cases secondary bacteria were present. It was pointed

out that diseases which were probably similar had been reported in Finland, Sweden, Norway, Poland, Holland, France, U.S.A., Canada, South America and Australia. Betts, in the same paper, described a syndrome which he called "secondary breakdown", which was characterised by the development of an acute clinical picture in animals already suffering from V.P.P. From the yellow necrotic lesions in the lungs Pasteurellae sp. could be easily isolated. In the same year Betts and Beveridge (1952) gave more details of the disease, mentioning failure to immunise pigs using subcutaneous injections of infected lung as antigen. Although they called the disease virus pneumonia of pigs they pointed out that the characteristics of the causal organism suggested it was like the psittacosis agent, the grey-lung virus, the p.p.l.o. group and primary atypical pneumonia of man. Experiments were conducted later to investigate the economic loss which might be due to the disease (Betts and Beveridge 1953), and as a result of further experiments (Betts, Whittlestone, Beveridge, Taylor and Campbell, 1955) it was stated that although few pigs died from V.P.P., morbidity was high and pig farmers suffered financial losses because of the greater amount of food consumed and the increased time required to reach bacon weight. Following attempts to control virus pneumonia in the field which involved the eradication of the disease from forty four herds, it was found that there were too many pitfalls and the method was too difficult (Betts, Whittlestone and Beveridge, 1955). Although it was not easy to eradicate the disease in a

herd, it was possible, and two reports were made on the procedures used to accomplish this in two herds, in each of which the eradication procedures lasted nineteen months (Barber, Brande, Mitchell and Betts 1955, Whittlestone and Betts 1955). Shanks and McPherson (1955) noticed that the incidence of virus pneumonia was less in sows than in young pigs and suggested that old breeding stock should be used to establish herds which might be kept free of V.P.P. by striot supervision of litters and culling of coughing pigs. In spite of the large number of publications on this pneumonia very little detail had been published about the pathology. Jarrett (1953) described a pneumonia, occurring in the calf, associated with peribronchial lymphoid hyperplasia and when Pattison (1956) described the histopathology of the lesions occurring in a transmissible pneumonia of the pig, which was almost certainly the same condition as the Cambridge people were working with, he pointed out the similarity between this condition, the pneumonia occurring in the calf, and grey lung disease of mice (Niven 1950).

In the series of papers from the Cambridge workers references had been made to other pneumonic conditions of the pig. Betts (1952) considered that apart from tuberculosis the great majority of lesions in pig's lung were due to V.P.P. and Betts, Whittlestone and Beveridge (1955) found that lungworms were the most common cause of coughing apart from V.P.P. These latter workers described briefly in the same paper a pneumonia occurring in some herds in which large numbers of giant cells were seen histologically.

Whittlestone (1957b) discussed a wide range of porcine respiratory diseases and stated that he preferred the name "enzootic pneumonia" proposed by Wesslen and Lanek (1954) to "virus pneumonia", since it indicated epidemiological and pathological features of the disease, while being non-commital about the causal agent. Discussing this problem he reported that he had seen a pleomorphic organism in impression smears from naturally occurring and experimental cases of enzootic pneumonia. It was also pointed out that p.p.l.o. had been isolated from the respiratory tracts of pigs which had respiratory diseases but also from pigs which were normal, and that experimental inoculation of these cultures had not produced pneumonia. In his opinion the relationship between the various organisms had not yet been established and no definite conclusions could be drawn regarding the role played by them in the aetiology of enzootic pneumonia. The histological picture of the disease was thought by him to be typical but not diagnostic.

While this work was being carried out in Britain a considerable amount of research was being done abroad and diseases were reported from several countries which appeared to be similar to enzootic pneumonia. Prior to Gulrajani and Beveridge (1951b), Pullar (1948, 1949 a.b.c.) described an infectious pneumonia of pigs which was characterised by high morbidity and low mortality, with Pasteurella septica present in 90% of the cases. He was unable however, to reproduce the disease using filtrates of pneumonic lung alone, and he thought that there was a filter passing agent in the lung which

was unable to initiate infection by itself but could "assist the otherwise inert pasteurella to establish lesions". Rislakki (1953) described a disease in Finland which he called infectious pig cough, and which behaved in a similar manner to the British disease in his experimental work. Plowright (1953) recorded a pneumonia of pigs in Kenya resembling enzootic pneumonia and a similar disease was described in Canada (Fulton, Burton and Miller, 1953). Wesslen and Lannek (1954) proposed the name swine enzootic pneumonia for the disease and swine enzootic pneumonia agent (S.E.P. agent) for the causal organism which they claimed to have isolated from six out of eight field cases on tissue cultures of swine lungs, swine kidney, bovine skin, bovine lung, and human lung. In the same year, however, Hjarre, Dinter and Bakos (1954) were unable to confirm that the agent could be easily grown on tissue culture having failed in thirteen out of sixteen cases to do so with three doubtful cases. Lannek and Wesslen (1955) recognised changes in tissue culture produced by the agent and saw numerous small particles in the tissue culture cells after two days which were similar to the pleomorphic organism described by Whittlestone (1957a). These particles could be separated by centrifugation and agglutinated by antisera produced in rabbits by injecting pneumonic lung as antigen.

Schofield (1956) examined a series of lungs histologically and described a pneumonia resembling enzootic pneumonia in Canada, and also a giant cell pneumonia, which was seen in four pigs. In the same year Lannek and Bornfors (1956) found that although it was not possible to prevent the dissemination of

enzootic pneumonia by treating pigs with tetracycline and oxytetracycline, it was possible to inhibit the development of the disease by using the drugs prophylactically. The same authors (Lannek and Bornfors 1957) demonstrated that pigs which had recovered from enzootic pneumonia were immune to re-infection. It was claimed by Lannek and Wesslen (1957) that the agent of enzootic pneumonia had been grown on the yolk sac of embryonated eggs and that macroscopic lesions were produced in the lungs of pigs experimentally infected with the agent after it had been grown on tissue culture. The comparative histopathology of swine influenza and enzootic pneumonia was studied in experimentally infected pigs by Urman, Underdahl and Young (1958).

Although the problems associated with enzootic pneumonia attracted the interest of many workers, pneumonias of the pig caused by parasites were not completely neglected in Britain.

It was popularly thought at this time that the migrating larvae of Ascaris lumbricoides were responsible for much pneumonia in pigs and the clinical syndrome popularly known as Thumps. However, when pigs were experimentally infected with Ascaris lumbricoides (Betts 1954), it was shown that coughing only lasted five or six days, and the lung lesions, which were quite small, had disappeared in animals killed twenty one days after infection.

The incidence of lungworm infestation was studied in Cheshire, London and Hereford by Dunn, Gentles and White (1955) and was found to be 18.3%,

26% and 27% respectively of the lungs examined in these localities. The pathology of the lesions was described and Dunn (1956) went on to study the pathology of the experimental disease in eight pigs infected with single or repeated doses of earthworms containing infective third stage larvae. MacKenzie (1958 a.b. and 1959) also studied Metastrongylus infestation in pigs. In the first paper observations on the natural disease in a pig herd free from enzootic pneumonia were recorded. The lesions occurring in these animals were described and it was pointed out that consolidation occurred in some pigs which was indistinguishable from enzootic pneumonia, although as a rule the lesions were small and histologically dissimilar. The second paper contained a description of the clinical course of the disease in seven pigs experimentally infected with doses of larvae ranging from 200 to 8,000 and killed five weeks after infection. As a result of his experimental work 22 pigs had been infected and killed at intervals ranging from one day to eighty days. Using this material he described the progressive pathology of the condition in his third paper.

Bacteria were usually considered to play a secondary role in the development of many pneumonias in the pig, but a Haemophilus parainfluenza was found which could produce pneumonia by itself. This was first described by Pattison, Howell, and Elliot (1957) who reported the isolation of a Haemophilus-like organism along with swine fever virus from a pig with pneumonia. When a pure culture of this organism was given intra-tracheally

with swine fever virus, a pneumonia was produced with a definite histological picture, differing from that produced by giving swine fever virus by itself and was also differing from enzootic pneumonia. Later Mathews and Pattison (1961) classified the organism as Haemophilus parainfluenza and on this occasion were able to produce pneumonia using pure cultures of the organism by themselves.

About this time another attempt was made to control the spread of enzootic pneumonia in the field. In Britain the economic benefit of having a herd free from enzootic pneumonia was obvious to a group of farmers who formed an association termed "The Association for the Advancement of Virus Pneumonia-free Pigs". The term virus pneumonia was used because the disease was known to the lay public as virus pneumonia, although there were scientific grounds for calling it enzootic pneumonia, the name preferred by many research workers. This Association was advised scientifically by Dr. R.G. Goodwin and Dr. P. Whittlestone of the University of Cambridge Veterinary School. A paper was published (Goodwin and Whittlestone 1960) describing the experiences of the authors in supervising the pig herds belonging to the Association. In this they defined the standards of inspection required and discussed the difficulty of diagnosing the disease, referring to five different types of lesion encountered in six herds which they considered to be different from enzootic pneumonia on account of the clinical picture, the pathology and the experimental behaviour of the diseases. One of these conditions was described in

more detail later (Goodwin and Whittlestone 1962). Young and Underdahl (1960) also described a scheme for the certification of pig herds as free from "virus pneumonia" which was operating in the United States.

In recent years more information has accumulated about the nature of the causal agent of enzootic pneumonia confirming that it is not a virus, and it has been established that it can be grown on cell free media and still retain its virulence for pigs. Betts and Whittlestone (1963) found particles resembling the pleomorphic organisms described earlier by Whittlestone (1957) in tissue cultures from the turbinate mucosa and the lungs of pigs experimentally infected with enzootic pneumonia. Fluids from the third passage but not from the twentieth passage produced pneumonia in pigs but no attempt was made to transmit this serially in pigs.

Goodwin and Whittlestone (1964) suggested that certain criteria should be fulfilled before it could be claimed that the causal agent of enzootic pneumonia had been grown in vitro. These were (a) the material inoculated into experimental pigs must be at least a 10^{-12} dilution of the pneumonic material used to seed the culture (b) the pig inoculum should induce, after the expected incubation period, a macroscopic pneumonia that both grossly and histologically closely resembles enzootic pneumonia and (c) this induced pneumonia should be serially passaged at least once and preferably twice in pigs, each passage resulting in a macroscopic pneumonia that still retains the gross and histological characteristics of enzootic pneumonia. According

to them, Lennek and Woonlen (1957) may have grown the organism in suspended-cell cultures of pig lung or kidney. Their own work (Goodwin and Whittlestone 1963) satisfied the criteria laid down and in this paper they reported that the agent had been grown and passaged twice in plasma clot cultures and then five times in pig-lung-monolayers. Pleomorphic organisms (P.O.) were seen in the pneumonic lungs used to seed the cultures, in the monolayer cultures and in the pneumonias induced from the cultures. This work was done with what these workers called the J strain of enzootic pneumonia and later they were able to grow this agent in a media free from cells (Goodwin and Whittlestone 1964) and also to fulfill the criteria they laid down. Pleomorphic organisms were found at all stages of the procedure (as in their earlier paper) and they concluded that there was no justification for the use of the term virus pneumonia of pigs (V.P.P.). It was pointed out that although it was likely that the J agent was a Mycoplasma this point had not been conclusively proved.

The isolation of an organism causing enzootic pneumonia in pigs would appear to simplify the definition of the disease and enable a more positive attitude to be taken in its diagnosis. Goodwin and Whittlestone (1964) however, used the term "enzootic pneumonia complex" and indicated that they had evidence that the J agent may not be the only agent of the pleomorphic organism type which will cause the enzootic pneumonia complex.

The diagnosis was also complicated in their opinion (Goodwin and

Whittlestone 1963) because the pathology was not specific and they had encountered pneumonic conditions differing from enzootic pneumonia yet yielding morphologically similar organisms in tissue culture.

While it is obvious that the current views on pig pneumonia are more elaborate than those of the earlier workers, there are many unresolved problems to be tackled, problems which are of economic importance to the pig industry and also problems which are intrinsically important because of the relationship they have with other fundamental biological issues.

MATERIALS AND METHODS

In this section the general materials and methods used during the study are detailed. The parasitological, microbiological and other special techniques are dealt with at the beginning of the appropriate section of experimental work.

Pathological Examinations

The specimens examined were either pig carcases or sets of pig lungs obtained from abattoirs. The trachea, bronchi, lungs and bronchial lymph nodes were inspected in every case. The trachea, was opened with scissors and after external inspection and palpation, the lungs were either sectioned several times in each lobe with a knife or the bronchial tree was opened with scissors. Those lungs in which it was thought that pulmonary embolism might have occurred were examined by opening the pulmonary arteries with scissors before any other cuts were made into the lung parenchyma. The bronchial lymph nodes at the hilus of the lung and the apical lymph node at the bronchus supplying the right apical lobe were sectioned.

Pieces of tissue for histological examination were taken from each lobe of the lungs in every case, whether lesions were present or not, and from the bronchial or apical lymph nodes. The trachea was also examined histologically in many cases.

When the body of the animal was available for autopsy a complete macroscopic examination of all the systems was made and blocks of tissue were

taken from organs other than the lower respiratory tract when it was thought necessary. In these cases the upper respiratory tract was usually examined histologically, blocks of tissue being taken from the turbinates and the nasal septum.

All gross pathological changes found were recorded in writing and in addition pulmonary changes were recorded on diagrams of the dorsal and ventral surfaces of the lungs.

Histopathological Techniques

Fixation: Blocks of tissue were fixed in 10% aqueous formol or corrosive formol consisting of 9 parts of a saturated aqueous solution of mercuric chloride to 1 part of a 40% formaldehyde solution. Pieces of lung which floated were submerged in the fixative by placing cotton-wool saturated with the fixative on top of the tissue so that the cotton-wool occupied the space between the lid of the jar and the fixing fluid. After fixation the tissues were processed by one of the techniques described below. When it was desired to keep samples of lung without embedding them, 10% aqueous formol was used.

Dehydration and Embedding: Most of the material fixed was processed in a series involving dehydration in alcohol and clearing in chloroform. Afterwards it was vacuum embedded. Large pieces of lung, such as cross sections of whole lobes, were double embedded in necolloidin and a butanol series was used occasionally.

Section Cutting: Paraffin embedded blocks were cut on Cambridge Rocker Microtomes at 6-8μ thick and a sledge type microtome was used to section the larger blocks, such as cross sections of whole lobes of a lung.

Frozen Section Method: Frozen sections were sometimes used. These were prepared from pieces of lung tissue fixed in 10% aqueous formol and cut on a Leitz freezing microtome at 10μ thick.

Staining Procedures: Haematoxylin and eosin were used as a routine stain for histological sections. The following stains were used to visualise better and to give additional information about particular aspects of the structural changes which had occurred in the lungs being examined; picro-Mallory, Van Gieson, Weigerts elastica, Verhoeff-Van Gieson, Orcein, Gordon and Sweet's reticulin stain, Foot's reticulin stain, periodic acid Schiff, Southgate's mucicarmine, Alcian blue, Phosphotungstic acid haematoxylin, Sudan IV, Sudan Black, Oil Red O, Toluidene Blue, Gomori's aldehyde fuchsin, Gram-Twort, Gram Weigert, Ziehl-Neelsen, Carbol chromotrope, Unna Pappenheim, Feulgen.

Experimental Animals

Pigs four to eight weeks of age, of both sexes, were used as experimental animals. These were obtained from a herd of a Large White pigs managed by the Department of Animal Husbandry of the University of Glasgow. This closed herd is believed to be free from enzootic pneumonia since it was established from a nucleus of breeding animals obtained by hysterectomy. The only additions to the herd have been a few animals for breeding purposes.

which were obtained from other closely supervised herds.

In order to check the status of the herd concerning the presence or absence of pneumonias, in particular enzootic pneumonia, all animals dying have been autopsied and their lungs examined histologically. The lungs of all pigs sent for slaughter were collected and were similarly examined. This examination has now been carried out during a period of six years and no case of enzootic pneumonia has been encountered. When pigs were required for parasitological experiments with M. apri, the sows were farrowed in arks on concrete and the young pigs were kept on concrete until they were needed.

On the farm premises there is a fattening house surrounded by a concrete apron which has two completely separate sections. The pigs experimentally infected with M. apri were housed in one of these sections during the course of these experiments.

The animals used in all other experiments which involved using suspensions of pneumonic lung or suspensions of bacteria were housed at the Veterinary Hospital. This accommodation consisted of isolated pens with solid walls to the ceiling, which could only be entered through a door communicating with the exterior of the building. When occupied by experimental animals, they were attended by a stockman who had no contact with any other pigs in the Hospital. The animals were fed either a proprietory milk substitute or a proprietory sow and weaner meal depending on their age. When the weather was cold infra red lamps were put into the pens to maintain

a comfortable environment. The accommodation was ventilated by a small inlet ventilator in the door of each pen and an exit louvre type ventilator in the roof.

Methods of Experimental Infection

Suspensions of pneumonic lung, bacterial cultures or larvae of M. apri were administered to pigs orally or intravenously or intratracheally.

Oral Administration

The problems associated with giving pigs liquids orally are (a) they aspirate the fluid (b) they shake their heads and the fluid runs out of their mouths (c) they will not swallow it, and gargle with it until either of the previous accidents happens. In the parasitological experiment the whole volume of larval suspension given had to be swallowed by the pigs to ensure that the intended dose of larvae was received. The following procedure was adopted to achieve this. A 20 mls. polythene syringe was fitted with a needle to which had been attached a piece of firm polythene tubing about 20 cms. long and slightly curved. The required dose of larval suspension was drawn into the syringe with the tubing attached. The pig was restrained in a vertical position on a stockman's lap and a wooden gag with a hole in its centre was placed in the pig's mouth. The polythene tubing on the syringe was passed through the hole into the pig's pharynx and on into its oesophagus. The animal could be seen swallowing as the tube entered the oesophagus, and when this happened the suspension was injected.

The tube was removed and the syringe and tube rinsed with isotonic saline which was then injected into the pig as before to help wash out the syringe and flush the larvae down into the pig's stomach.

In an attempt to facilitate the procedure the pigs in one experiment were given 8 mgms. largactil per 10 lbs. body weight intramuscularly about 15 minutes before they were dosed.

Intravenous Administration

When blood samples were required or material had to be injected intravenously anterior vena cava punctures were done with the pig restrained on its back.

Intratracheal Administration

Before suspensions were injected intratracheally the animal was lightly anaesthetised with 5% thiopentone sodium given intravenously. It was then restrained on its back on a board inclined at 20° to horizontal with its head higher than its tail. A 10 mls. syringe was fitted with an 18 guage 1½" long needle and filled with isotonic saline. The needle was inserted vertically in midline either posterior to the larynx or immediately anterior to the manubrium of the sternum. When it entered the trachea large bubbles of air were easily drawn into the syringe which was then detached from the needle and replaced by a syringe containing the suspension for injection. After the suspension had been injected slowly, the needle was withdrawn and the pig was lifted onto its hind end. The animal usually coughed lightly

once or twice at this time. Recovery from the anaesthetic did not take longer than 15-20 minutes.

Temperatures

The temperatures of all experimental animals were recorded in degrees Fahrenheit.

INTRODUCTION TO PATHOLOGICAL STUDIES AND EXPERIMENTAL WORK

It has been pointed out already that one of the principal objectives of this study was to investigate the histopathological changes produced by naturally occurring diseases in pigs lungs. Since histological examination of lungs is the most frequent laboratory method available to clinical investigators of pig pneumonia, an attempt was made to classify the lesions according to the histopathology of the changes found, in the hope that this would provide a reasonable basis for diagnosis and prognosis.

A total of 376 sets of lungs were examined from the sources shown in Table 1. The pathological examinations and histopathological techniques were performed as previously described in Materials and Methods.

Routine postmortems usually consisted of individual animals arriving dead at the laboratory as a result of pneumonia or other diseases when lung lesions were also present. Material from field investigations consisted of dead pigs sent to the laboratory, and the specimens obtained when these cases were further investigated in the field. These specimens were pigs bought from the same farms for destruction and sets of lungs collected from the abattoir when pigs from the farm were slaughtered.

The material referred to in Table 1 as abattoir specimens comprised 40 lungs with pig lungworms collected specifically for this disease and 195 cases of pneumonia collected at random from local abattoirs.

Table 1

THE SOURCES OF PIG LUNGS USED FOR THE HISTOPATHOLOGICAL CLASSIFICATION OF NATURALLY OCCURRING LESIONS

Source of Lungs	Number of Lungs
Routine Post Mortems	74
Clinical Investigations on Farms	67
Abattoir Material	235
Total From All Sources	376

As a result of the histopathological investigation the pulmonary lesions were classified as shown in Table 2.

After studying the spectrum of pulmonary pathology which occurred naturally in the pig, it was decided to examine some of the conditions in more detail. The diseases selected for this purpose were, enzootic pneumonia, interstitial pneumonia, the pneumonia associated with Haemophilus parainfluenza infection, and Metastrongylus pneumonia.

In this thesis the pneumonias are dealt with consecutively in the order outlined in Table 2. The pathological features of the naturally occurring lesions are described first in each section and following this where it is applicable the experiments performed and the conclusions arrived at have been outlined.

Table 2

A HISTOPATHOLOGICAL CLASSIFICATION OF PULMONARY LESIONS IN THE PIG

	No. of Cases Examined
Enzootic Pneumonia	253
Interstitial Pneumonia	13
Pneumonia due to *Haemophilus parainfluenza*	1
Simple acute Bronchopneumonia	18
Haemorrhagic Bronchopneumonia	3
Necrotising Bronchopneumonia	6
Suppurative Bronchopneumonia	6
Embolic Pneumonia	5
Tuberculosis	14
Pneumonia due to *Metastrongylus apri*	77
Pulmonary Toxoplasmosis	1
Giant Cell Pneumonia	2
Pulmonary Vascular Lesions	2

ENZOOTIC PNEUMONIA

(1) The Pathology of the Disease Seen in the Field

Enzootic pneumonia was the commonest disease affecting the lungs examined. When 195 pneumonic lungs were collected at random in the abattoir, 137 (70%) were found to belong to this group. Overall however, the disease was seen in 253 pigs including adult breeding stock, bacon pigs, pork pigs, store pigs and unweaned pigs 6 weeks old. In some cases it was complicated by co-existing bronchopneumonias, interstitial pneumonia, or pulmonary abscess formation. Adhesive pleurisy was also found as a complicating lesion in a number of animals.

Macroscopic Findings

The lesions were nearly always bilateral, lobular and in the anterior parts of the lungs (Fig. 1). The apical lobes, cardiac lobes, antero-ventral region of the diaphragmatic lobes and the intermediate lobe were more frequently affected than other regions. The amount of consolidation varied considerably and in many cases only a few lobules in the apical or cardiac lobes were affected. This was particularly true of the abattoir specimens. Complete or almost complete consolidation of the apical and cardiac lobes was seen as a result of the lobular consolidation extending to affect the whole lobe. The consolidation in very severe cases involved a large amount of the diaphragmatic lobes, including the diaphragmatic surface and dorsal border of the lobes and extended along the basal border to the posterior tip of the lungs (Fig. 2). Cross

sections of the diaphragmatic lobes in these cases showed extensive consolidation of lobules within the lobes, with as much as four-fifths of the pulmonary parenchyma affected. The subpleural lobules in the diaphragmatic lobes were involved more frequently than the lobules within the lobes.

A small number of cases was found in which the distribution of the lesions was atypical. These were cases with only moderately extensive lesions of the apical, cardiac or antero-ventral parts of the diaphragmatic lobes and large patches of consolidation at the posterior tip of the diaphragmatic lobes on the dorsal border or along the basal border.

Affected areas of lung were well demarcated from normal tissue, although the appearance of the lesion varied, particularly with regard to colour. Sometimes they were fawn, sometimes greyish-pink and sometimes dark reddish brown or plum coloured. Although the consolidated tissue in any one animal was usually uniform in appearance it could vary. Closer inspection showed the presence of regularly distributed groups of three to four white or greyish small spots in some cases (Fig. 3). These varied in prominence, but were usually very small except in long-standing or complicated infections. Lesions were also seen which had a mottled appearance (Fig. 4) due to the juxtaposition of normal and abnormal groups of alveoli within lobules which were not wholly involved. This appearance was associated with the early stages of the disease.

The affected lobules were either on the same level as the surrounding

normal lobules or lower than them as a result of alveolar collapse. In the event of this happening, they were slightly smaller than normal and the adjacent lung showed compensatory emphysema. A more striking degree of compensatory emphysema was seen in normal lobules surrounded by consolidated lung tissue, particularly if they were at the edges of lobes. The consolidated and collapsed areas felt thin and hard and sometimes were slightly rough to touch when the white spots described earlier were large and protruded above the collapsed alveoli. These white spots were related to changes around and within bronchi and bronchioles.

Lesions often looked glistening as a result of pleural oedema. For the same reason interlobular septa were dilated and more prominent than usual in some lungs. When consolidated areas were incised they frequently oozed copious amounts of clear or greyish fluid and when pressed, thick, greyish or yellow catarrhal exudate came out of the bronchi.

The trachea was usually normal but sometimes the trachea and the major lobar and segmental bronchi, contained thick, grey or yellow, tenacious catarrhal exudate.

The main bronchial and apical lymph nodes were enlarged and oedematous in all cases (Fig. 5). The degree of enlargement was very striking in animals with extensive pulmonary consolidation.

Microscopic Findings

Trachea: The lumen of the trachea was usually empty but sometimes

contained a mixture of mucus, polymorphonuclear leucocytes in various stages of disintegration, small numbers of alveolar macrophages, plasma cells, lymphocytes and desquamated epithelial cells. The lamina propria was sometimes infiltrated with plasma cells and lymphocytes, and plasma cells could be found frequently around the tracheal glands in the submucosa even in the absence of other changes.

Bronchi: The bronchial lumen was either packed with polymorphonuclear leucocytes living or dead, mixed with variable amounts of mucus and basophilic amorphous debris or contained only small numbers of polymorphonuclear leucocytes by themselves or mixed with alveolar macrophages, plasma cells, lymphocytes and desquamated epithelial cells (Fig. 6). The bronchial epithelium was often thickened due to hypertrophy and hyperplasia of its constituent cells. Plasma cells were seen frequently in the epithelium, apparently migrating through it. The lamina propria often contained large numbers of plasma cells, some lymphocytes and other mononuclear cells, but polymorphonuclear leucocytes were not usually numerous. Plasma cells were present in large numbers around the glands in the submucosa. The cells of these glands appeared to be very active in some cases and the glandular ducts and acini were dilated and contained a few plasma cells or polymorphonuclear leucocytes. Aggregates of lymphocytes were also found in the submucosa and these sometimes formed lymphoid nodules particularly between adjacent plates of cartilage. Lymphoid nodules were more frequently seen between the plates of cartilage or a plate of

cartilage and the muscularis than in the small mount of fibrous connective tissue outside the cartilage. In some cases, however, a few lymphoid nodules were found in this situation.

Bronchioles: The characteristic changes produced by this disease were seen at the bronchiolar and alveolar level, and in the case of the former they occurred particularly in the peribronchiolar tissues. The bronchiolar lumina usually contained cellular exudate similar to that seen in the bronchi. The epithelial changes were also similar but the hypertrophy and hyperplasia was more marked in the bronchioles (Fig. 7). The lamina propria was diffusely infiltrated by cells, most of which were plasma cells, but lymphocytes, mononuclear cells, some polymorphonuclear leucocytes and often one or two eosinophil leucocytes could be seen. The changes in the peribronchiolar tissues were an important feature of the lesions and contributed more to the thickening of the bronchiolar walls than did the cellular accumulation in the lamina propria (Fig. 7). There was a diffuse collection of plasma cells and lymphocytes, which produced substantial thickening of the peribronchiolar tissue (Fig. 8 and Fig. 19).

A very striking development, however, was the formation of peribronchiolar lymphoid nodules (Fig. 9). These very frequently developed between the muscular layer of the bronchiole and the related branch of the pulmonary artery (Fig. 10 and Fig. 11). They also appeared very often to be associated with the lymphatic channels in the peribronchiolar tissues. The lymphatic

vessel was seen as a space lined by flattened cells, at the periphery of the nodules and even extending around groups of nodules which had developed close together (Fig. 12). Branches of these lymphatic channels were sometimes seen extending between these nodules.

Within the diffuse collections of cells in the peribronchiolar tissues, focal concentrations of lymphoid cells were seen which were presumably the precursors of the mature lymphoid nodules (Fig. 7 and Fig. 8). These smaller accumulations were either composed of many reticular cells and lymphoblasts with some large and small lymphocytes at the edge or scattered amongst the other cells, or were predominantly a mass of small lymphocytes with only a few of the other cell types. The fully developed lymphoid nodules had germinal centres with reticular cells and lymphoblasts, many of which were seen in mitosis, and small capillaries. The rest of the nodules were composed of the other cells of the lymphoid series. Lymphoid nodules were seen extending along the length of bronchioles in longtiudinal sections (Fig. 13). In many cross sections they often appeared to have developed on one side of the bronchiole where several coalesced to form a large mass of lymphoid tissue (Fig. 14). These nodules sometimes extended into the bronchiolar wall and when this happened the adjacent muscular layer atrophied (Fig. 11 and Fig. 14). The lymphoid cells often overflowed into the lamina propria and the integrity of the epithelium itself was often breached in a manner similar to that which is seen in the tonsil (Fig. 11 and Fig. 14). The lymphoid nodule frequently

bulged into the lumen of the bronchiole and distorted its wall so that the lumen appeared as a crescentric slit (Fig. 14).

Alveoli: In addition to the peribronchiolar reactions the alveolar changes were an important feature of the disease. The walls of the alveoli were thickened due to the proliferation of alveolar cells. The degree of thickening varied considerably. Plasma cells were also seen in the alveolar walls and occasionally a few eosinophils were present too. The alveolar cells had a large round nucleus, and clear vacuolated cytoplasm. A significant feature of the lesion was their presence on the alveolar walls protruding into the lumen either singly or in groups (Fig. 15). These cells were sometimes seen bulging the nucleus of an alveolar epithelial cell into the lumen of the alveolus. Alveoli adjacent to the pleura, septa or bronchioles showed this reaction to an advanced degree on the part of the wall next to these structures. At these sites the wall of the alveolus was covered by cuboid alveolar cells. Some alveolar cells protruding into alveoli were seen with a cap of material which was frequently basophilic (Fig. 16). This appeared to be in the apex of the cell, but by focussing, could often be shown to be on the outer surface of the apex, just outside the cell membrane.

The principle cell types within the alveoli were alveolar macrophages and plasma cells (Fig. 17). When polymorphonuclear leucocytes were present their numbers varied very much from case to case and also from section to section in any one case. They sometimes occurred in small numbers mixed

with the alveolar macrophages and plasma cells but were also seen completely filling alveoli and alveolar ducts. Alveolar macrophages assumed two main forms. The cytoplasm was either densely eosinophilic and slightly granular with perhaps one or two vacuoles or else it was extremely vacuolated and faintly eosinophilic. The cytoplasm in this latter type of cell sometimes had an eosinophilic rim at the periphery of the cell (Fig. 17). Alveolar macrophages were also seen which had phagocytosed polymorphonuclear leucocytes or plasma cells and some macrophages had more than one cell inside them. Binucleated alveolar macrophages occurred and sometimes even larger giant cell types were seen with four to twelve nuclei and granular eosinophilic cytoplasm which was sometimes very much vacuolated (Fig. 18). The relative numbers of alveolar macrophages to plasma cells in any one alveolus varied enormously throughout the lung, in most cases. Some alveoli were full of plasma cells, others full of alveolar macrophages and others contained representatives of both cell types.

Other cell types found in the alveoli were lymphocytes in small numbers and regularly one or two eosinophils. Alveolar oedema had developed in many cases and where there was no oedema fluid numerous eosinophilic granules were often found lying about between the cells.

The description of the alveolar changes so far applies to lobules in which the alveoli were expanded. In many cases however, there was alveolar collapse (Fig. 14). This happened even when the bronchioles of the lobule

and the bronchi were free from cellular exudate or mucus which might block them. Lobules so affected usually had only a small number of cells in the alveolar lumina except a few cases in which they contained many polymorphonuclear leucocytes. The cell types found were similar to those already described. The alveolar walls contained only a slight excess of alveolar cells within them or projecting into the lumen.

Blood vessels: The bronchus of the pulmonary artery were involved in the bronchial and peribronchiolar reactions already described. In the connective tissue around the pulmonary veins diffuse collections of plasma cells and lymphocytes were also frequently found. Lymphoid nodules developed in these sites but were not as prominent as those around the bronchioles, since they were not so numerous and did not usually form large masses. Vessels cut longitudinally were seen to have aggregates of lymphoid cells or occasionally nodules scattered focally along their walls.

Pleura and Septa: Oedema of the pleural connective tissue and the interlobular septa was seen in many cases, associated with dilatation of the pleural and septal lymphatics (Fig. 19). Moderate numbers of plasma cells, lymphocytes and mononuclear cells were present in the connective tissue, and lymphoid nodules sometimes developed. These were seen, in some sections, bulging into the lymphatic vessels. Plasma cells and lymphocytes were also seen within the dilated lymphatics.

Lymph nodes: Many nodules with large germinal centres containing

numerous mitotic figures, in the lymph nodes. In the sinusoidal tissue polymorphonuclear leucocytes and plasma cells could be found. Plasma cells were also seen amongst the small lymphocytes outwith lymphoid nodules in the dense lymphoid tissue and occasional eosinophils were also scattered about in this situation.

Associated Lesions

Other lesions were seen in some of the lungs which were considered different from enzootic pneumonia. These were due to simple acute bronchopneumonia, necrotizing bronchopneumonia, haemorrhagic bronchopneumonia, suppurative bronchopneumonia, interstitial pneumonia, tuberculosis and lungworms. A detailed description of these lesions is given later. Two other lesions were seen in seven cases which were otherwise indistinguishable from enzootic pneumonia.

(a) Fibrosis of alveolar walls with gross thickening of these structures and distortion of the alveoli architecture occurred in three cases (Fig. 20). In the fibrosed regions there was epithelialisation of the alveoli by small cuboidal cells.

(b) Fibrosis of the bronchial and bronchiolar walls was seen in four cases. The fibrosis predominately involved the lamina propria of the bronchioles, making it much thicker than normal and the muscularis was reduced to a thin rim (Fig. 21). Fibrosis of the alveolar walls and epithelialisation also occurred in these two cases. Peribronchiolar lymphoid nodules were present but were not large or numerous.

Discussion

The changes occurring within the lungs were best considered at a lobular level and on this basis it was possible to classify them into three groups according to the changes in the peribronchiolar tissues and the alveoli.

Group I

In this group there was a diffuse mononuclear cell infiltration in the bronchial and bronchiolar walls, with or without a large amount of catarrhal exudate in the lumina of these structures.

Associated with these changes the alveoli were expanded and there was hyperplasia of alveolar cells in or on the alveolar walls. The lumina of alveoli and alveolar ducts contained many alveolar macrophages, many plasma cells and variable numbers of polymorphonuclear leucocytes.

Group II

A large number of well developed peribronchiolar lymphoid nodules was present in this group and lymphoid nodules were also seen in the bronchial walls. In addition there was usually a variable amount of diffuse mononuclear cell accumulation in the bronchial and bronchiolar tissues. The lumina of bronchi and bronchioles sometimes contained a catarrhal exudate.

The associated alveolar reaction was similar to that seen in Group I.

Group III

In this group there were many well developed peribronchiolar lymphoid nodules and lymphoid nodules in the bronchial walls. The lumina of the

bronchi and bronchioles sometimes contained a catarrhal exudate, and the walls of some of these structures were diffusely infiltrated by mononuclear cells.

The alveoli were partially or completely collapsed with a variable number of alveolar cells in or on the alveolar walls. The lumina of the alveoli contained small numbers of alveolar macrophages and plasma cells and sometimes large numbers of polymorphonuclear leucocytes.

This classification is useful for descriptive purposes and also for evaluating the disease process in any one case. It could not be applied too rigidly however to the pneumonia as a whole in any individual animal, because although in some cases all of the sections examined showed lobular changes compatible with one of the three groups, in others many lobules could be found representative of two or even three of the groups.

The significance of the three groups of changes will be discussed later when the pathogenesis of the lesion is considered.

In the material described here the cases considered under "Associated Lesions" were obviously different histologically from the others and the cases with fibrosis of the alveolar walls and bronchiolar fibrosis had features resembling some of the cases described as Type XI pneumonia (Goodwin and Whittlestone 1962). However, from the histology alone one could not exclude the possibility that the lesions were due to a mixed infection including the enzootic pneumonia agent. A similar type of fibrosis was seen in pigs with obvious suppurative bronchopneumonia.

ENZOOTIC PNEUMONIA

(2) Introduction to Experimental Work

During the investigation of respiratory problems in the field a farm was visited on which there was an outbreak of pneumonia causing death in a litter of young pigs. This farm was known to have had a chronic respiratory problem of the enzootic pneumonia type for a long time but previously there had not been a high death rate in the young pigs. The deaths were confined to one litter although others were at risk and Haemophilus suis was isolated from four of six pigs post-mortemed. The histopathology of the lesions was compatible with the early stage of enzootic pneumonia complicated by an acute simple bronchopneumonia, and an interesting feature was the absence of many large peribronchiolar lymphoid nodules.

In view of the differences between the disease in this litter and the more typical type of enzootic pneumonia outbreak in older pigs i.e. unthriftiness, low mortality, and lesions characterised histologically by both an alveolar cell reaction and many peribronchiolar lymphoid nodules, it was decided to attempt to transmit the pneumonia experimentally to study (i) the part played by H. suis in the infection and (ii) the serial pathology of the disease in animals killed at different time intervals after infection.

ENZOOTIC PNEUMONIA

(3) Description of Original Outbreak

The litter involved consisted of eleven pigs six weeks old, and was from a Wessex Saddleback sow which had been mated with a Landrace X Wessex Saddleback boar. The farmer had thought the litter was quite healthy until one pig was found dead. Since this animal was seen to have severe pneumonia at autopsy the farm was visited and when all the piglets in the litter were examined, they had temperatures ranging from 104.5°F. to 106.8°F. Five of them were obviously pneumonic with respiratory rates varying from 60-80 per minute at rest going up to 110-120 per minute when excited. They were dyspnoeic and coughed frequently. The sow was normal. Each young pig was given 2 mgm/lb. of terramycin subcutaneously and the treatment was repeated at 1 mg/lb. when they were revisited on the second day. At this time the temperatures of four of them had returned within normal limits but the others were still as high as before. The respiratory embarrassment had not improved. On the third day the clinical picture was much the same and two of the pigs were bought so that they could be killed and post-mortemed. Within the next ten days three of the remaining pigs in the litter died. During this period the sow continued to be clinically normal and although sporadic coughing was heard in adjacent litters in the same house there were no deaths or other signs of severe respiratory disease.

At post-mortem on the pigs which died the pulmonary consolidation

involved from three fifths to four fifths of the lung volume, whereas in the two which were destroyed half of the lung volume was affected. The consolidation affected the apical, cardiac, intermediate and anteroventral parts of the diaphragmatic lobes in all pigs. In five of them however there was also extensive patchy consolidation throughout the diaphragmatic lobes. The consolidated lobules varied in appearance from grey to pinkish-fawn to plum coloured and sometimes were lower than the surrounding normal lung. Groups of small creamy spots could be seen in some of them and when they were sectioned and pressed oedema fluid and greyish mucopus exuded from the pulmonary parenchyma and bronchi. The bronchial lymph nodes were grossly enlarged and congested.

Histological examination of the lungs showed pulmonary oedema and large numbers of alveolar macrophages, plasma cells and polymorphonuclear leucocytes in the alveoli. The proportions of cells varied considerably from field to field in any one case. The alveolar walls were thickened due to alveolar cell proliferation and often these cells were seen projecting into the alveolar lumen. Small basophilic caps were seen on these cells and were very numerous in three cases. Plasma cells and polymorphonuclear leucocytes were seen in the alveolar walls also. The peribronchioler tissues were thickened due to the accumulation of plasma cells and cells of the lymphocyte series. Around some bronchioles there were single lymphoid nodules with widely separated cells and many mitotic figures at their centres. The lamina propria

contained many plasma cells and some lymphocytes. The bronchiolar epithelium was hyperplastic and lymphocytes and plasma cells could be seen migrating through to the lumen which contained in many instances alveolar macrophages, plasma cells, lymphocytes and sometimes large numbers of polymorphonuclear leucocytes. Often small foci of necrosis and desquamation of epithelial cells were seen. Similar changes were seen in the epithelium and lumina of the bronchi and there were many plasma cells around the bronchial glands and in the lamina propria. There were very few lymphoid nodules in the bronchial walls. Small accumulations of plasma cells and lymphocytes were seen around blood vessels and there were variable degrees of septal oedema.

In many lobules with a lot of polymorphonuclear leucocytes in the alveoli, bronchioles, and bronchi there was marked congestion of the alveolar and bronchial walls. The polymorphonuclear leucocytes infiltration and congestion was patchily distributed throughout the lobules and had the appearance of a simple acute bronchopneumonia.

Sections of trachea which were examined showed ballooning and desquamation of the epithelial cells. There was little reaction in the lamina propria but numerous plasma cells were seen around the tracheal glands. Occasional aggregates of lymphoid cells were seen in the larynx below the epithelium.

Some of the bronchial lymph nodes examined were full of active nodules with large pale germinal centres whereas others were masses of small lymphocytes, mixed with variable numbers of plasma cells. A small but consistent

number of eosinophils were seen scattered in ones and twos throughout the nodes. In the loose lymphatic tissue there were usually large numbers of polymorphonuclear leucocytes and some macrophages.

Bacteriological examination of pieces of lung removed aseptically from the pigs yielded the results shown in Table 3. When impression smears were examined after being stained by a Giemsa method (Whittlestone 1957a), small structures resembling the pleomorphic organisms described by Whittlestone (1957a) were seen in all pigs. Attempts to grow mycoplasma from two of the pigs on solid media were unsuccessful.

Table 3

RESULTS OF BACTERIOLOGICAL EXAMINATION ON THE ORIGINAL PIG LUNGS

Pig	History	Bacteriological Findings
9226/53	Died	Haemophilus suis
9226/54	Destroyed	Negative
9226/55	Destroyed	Haemophilus suis
9226/56	Died	Bordetella bronchiseptica
9226/57	Died	Haemophilus suis
9226/58	Died	Haemophilus suis
		Pasteurella multocida

ENZOOTIC PNEUMONIA

(4) Transmission Experiments

The experimental transmissions carried out with material from the outbreak of pneumonia described in the previous section can be classified into two groups. In one, 10% suspension of pneumonic lung in sterile normal saline were used and in the other pure cultures of *H. suis* in broth.

Materials and Methods

The pigs used were 2-4 weeks old when infected and were housed at the Veterinary Hospital in the accomodation described in Materials and Methods. All of the pigs were infected intratracheally under light anaesthesia as described earlier. Prior to infection they were examined clinically and their temperatures recorded. During the course of the experiment these procedures were repeated every day, and temperatures over 104.0°F. were considered significant. The cultures of *H. suis* were supplied by Mr. M. Grindlay, Department of Bacteriology, University of Glasgow Veterinary School. The whole lung suspensions were made by grinding pneumonic portions of lung, which had been removed aseptically from the pigs, in a sterile mortar with sterile carborundum and 2-3 mls. of sterile normal saline. When the tissue had been satisfactorily ground more saline was added to give a 10% suspension and this was allowed to settle out on the bench for five minutes. The

supernatant was poured into sterile universal bottles, and these were centrifuged for ten minutes at 1500 r.p.m. to remove any remaining carborundum and large tissue particles. After centrifugation the supernatant was transferred to another sterile universal bottle and this was used to infect the experimental animals. On one occasion a suspension of sterile normal lung was required and this was prepared by autoclaving pieces of normal lung in sterile universal bottles prior to grinding the lung with carborundum.

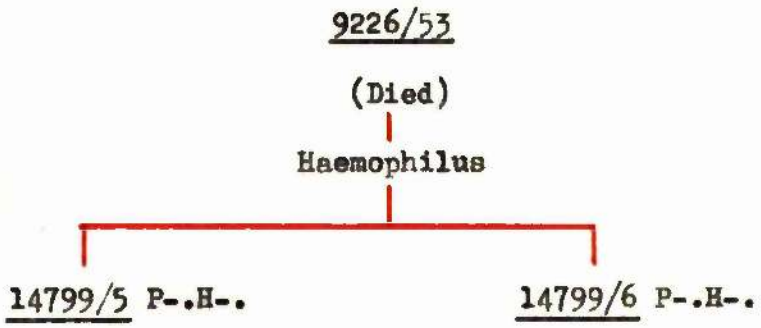
Experimental animals were killed by anaesthetising them with 6% pentobarbitone sodium and then bleeding them to death from the femoral or brachial blood vessles. The specimens for bacteriology and pieces of pneumonic lung for transmission were then removed aseptically. Impression smears were made of the cut surface of pneumonic lesions and stained by Giemsa. Seven blocks of tissue were taken from the lungs, one from the trachea and one from the bronchial lymph nodes of each pig for histological examination. The lung histology consisted of tissue from each lobe of the lungs.

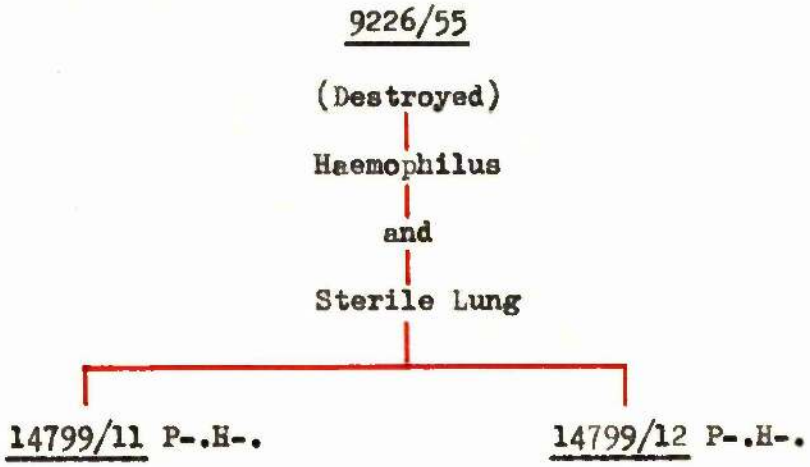
Haemophilus Transmissions

5 mls. of a broth culture of H. suis, isolated from the first pig which died, 9226/53, were given intratracheally to two pigs, Table 4. When they were killed seven days later there were no lesions in the lungs and the organism could not be recovered from pieces of lung examined bacteriologically. The animals, 14799/5 and 14799/6, had been apparently normal during the course of the experiment but both showed a slight temperature rise, to 104.2

Table 4

RESULT OF INFECTIONS WITH HAEMOPHILUS SUIS





P-. No pneumonia.

H-. Haemophilus not cultured.

and 104.6 respectively. This occurred in 14799/5 on the third and fourth days and in 14799/6 on the fourth and fifth days.

Haemophilus suis was also recovered from case 9226/55, one of the two pigs which were destroyed, and 5 mls. of a broth culture from this pig were given intratracheally to two pigs 14799/11 and 14799/12, Table 4. In an attempt to provide a stimulus for the organisms to proliferate 3 mls. of a 10% suspension of sterile normal lung were given at the same time. At post mortem seven days later these animals had no pulmonary lesions and no organisms were recovered from the lungs. They had been clinically normal during the period of the experiment and their temperatures had stayed within normal limits.

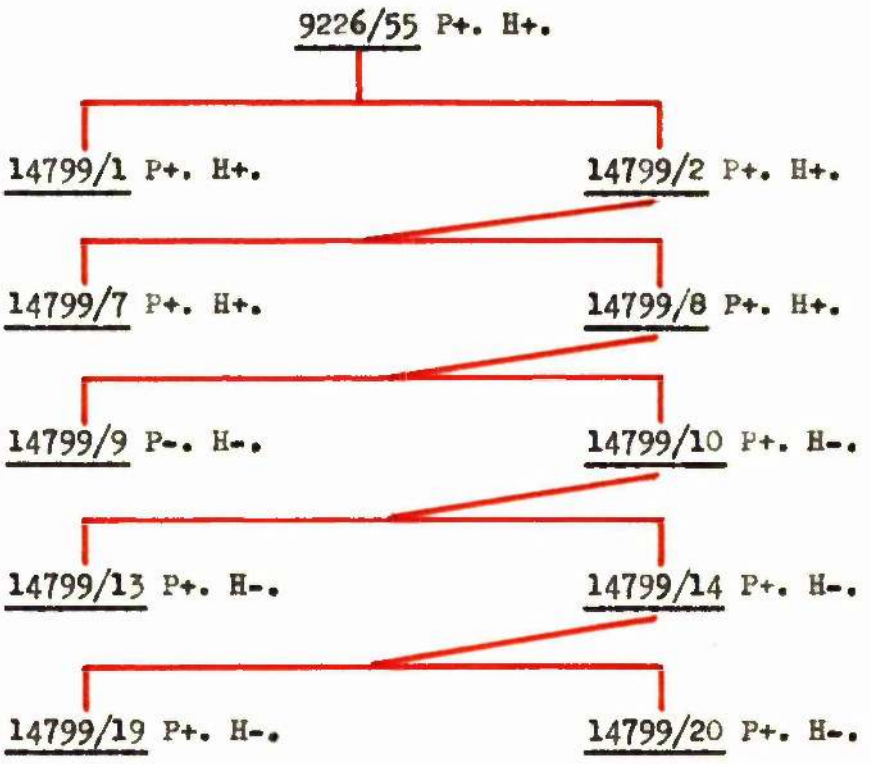
Transmissions with Suspensions of Pneumonic Lung

Case 9226/55 was also used as the source of material for this series of transmission, which is summarised in Table 5.

Two pigs were given 10 mls. of a 10% suspension of pneumonic lung from 9226/55 and killed seven days later. Both pigs had moderately extensive pale pink patches of consolidation and when the lungs were examined bacteriologically H. suis was recovered. 5 mls. of a suspension from one of them, 14799/2, were passaged into two more pigs with the same results when they were killed seven days later. When the passage was continued in two pigs, using material from 14799/8, which had contained H. suis, the organism was

Table 5

RESULTS OF SERIAL TRANSMISSIONS OF PNEUMONIC LUNG SUSPENSIONS AT SEVEN DAY INTERVALS



P+. Pneumonia present

P-. Pneumonia absent

H+. Haemophilus cultured

H-. Haemophilus not cultured

not recovered at autopsy seven days later and only one of the pigs had patches of pneumonia. Pneumonic lung from this animal 14799/10, was passaged into two more pigs and seven days later both pigs had pneumonia at autopsy which was quite extensive in one animal. Haemophilus suis was not recovered from either of these pigs. A suspension of lung was prepared from the pig with good lesions, 14799/14, and 10 mls. were given intratracheally to two more pigs. These were both found to have pneumonia seven days later although H. suis was again absent.

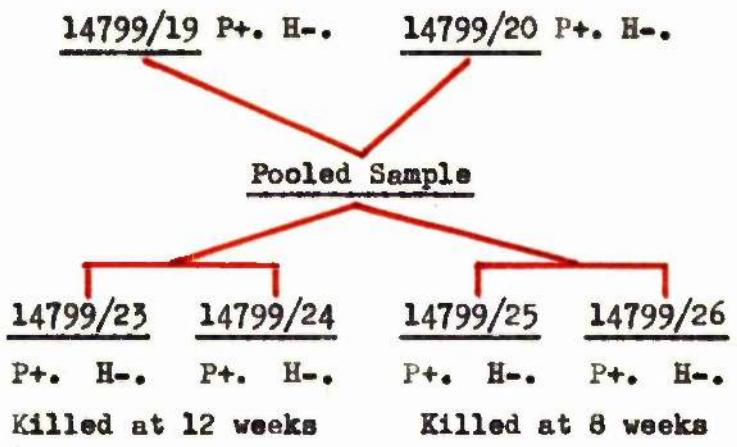
Pneumonic lung from the two pigs involved in this last passage was pooled and a 10% suspension was prepared from which four pigs were given 10 mls. intratracheally, Table 6. Two were killed eight weeks later and the other two were killed 12 weeks after infection. At post-mortem all of them had extensive pneumonia and Haemophilus suis was not present. A non-haemolytic Streptococcus was isolated from one pig 14799/24 killed at 12 weeks.

Pleomorphic organisms were seen in the impression smears made from the lungs of all of the pigs involved in the passages outlined in Table 5 and Table 6. This includes 14799/9 which had no macroscopic lesions but had histological changes associated with the early stages of the disease.

While the previous series of transmissions was being carried out it

Table 6

FINAL PASSAGE OF MATERIAL FROM 9226/55



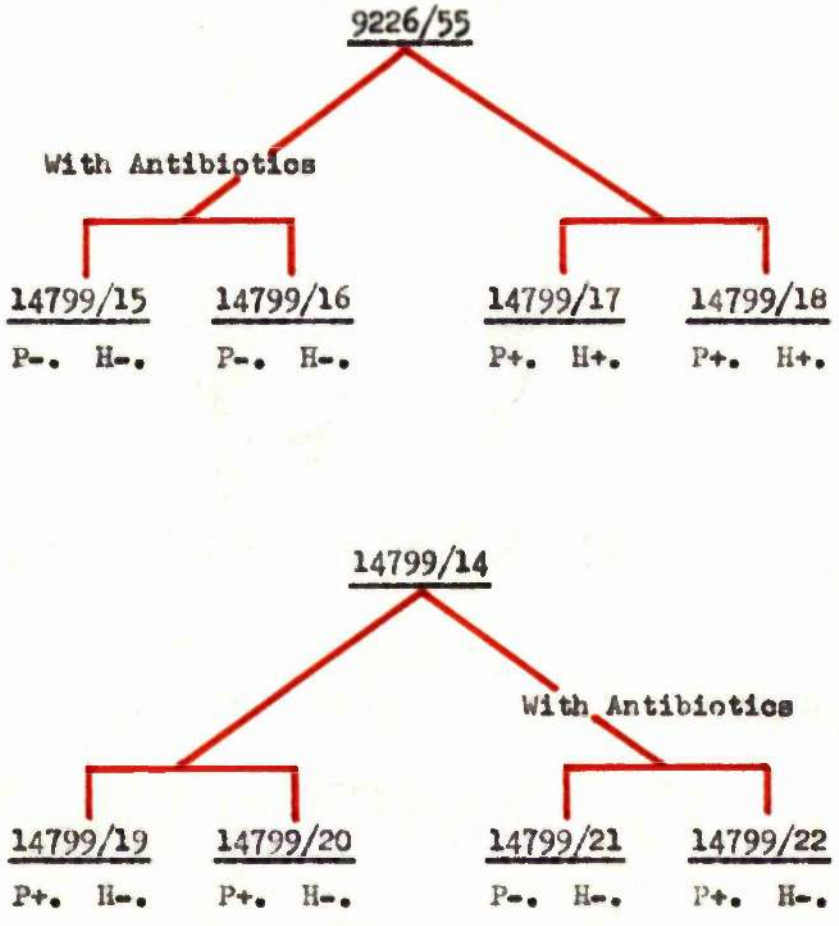
P+. Pneumonia present. H-. Haemophilus not cultured.

was decided to test the effect of adding antibiotics to the original lung material in order to suppress the Haemophilus organism. Accordingly a 10% suspension of lung from 9226/55 was prepared. This lung had been kept for three weeks at - 30°C. The suspension was divided into two aliquots and 100 I.U. of penicillin/ml. and 100 µgm. streptomycin/ml. were added to one aliquot half an hour before the pigs were infected. Each pig was given 10 mls. of the suspension as shown in Table 7. When they were killed after three weeks there were no lesions in the animals given the suspension to which antibiotics had been added and H. suis could not be recovered. Patches of pneumonia were found in the other two animals however and H. suis was recovered from these.

It was decided to test the effect of the same antibiotics on suspensions with Haemophilus; as material was being prepared from 14799/14 in the seven day passage series, some of it was treated as before with penicillin and streptomycin and 10 mls., were given intratracheally to two pigs, 14799/21 and 14799/22, at the same time as 14799/19 and 14799/20 were infected with the untreated suspension, Table 7. When the pigs were killed seven days later those given the untreated suspension had patches of pneumonia whereas one of the pigs given the material pretreated with antibiotics had no lesions and the other had only a small lesion. Haemophilus suis was not recovered from any of the pigs.

Table 7

TRANSMISSIONS IN WHICH PENICILLIN AND STREPTOMYCIN WERE USED



P+. Pneumonia present. H+. Haemophilus cultured.

P-. Pneumonia absent H-. Haemophilus not cultured.

Controls

During the period of these experiments four pigs were housed in the same accommodation as the experimental animals but not in direct contact with them. When they were killed at the end of the transmissions no lesions were seen in the lungs and they were bacteriologically sterile.

Two pigs were infected with 10% suspension of normal lung and when they were killed seven days later there were no macroscopic lesions in the lungs. Histological examination of the lungs showed that they were essentially normal. A few alveoli contained an occasional alveolar macrophage but there were no changes in the alveolar walls or peribronchiolar tissues.

Discussion

The Haemophilus organism used in these experiments was isolated in Glasgow and sent to the Agricultural Research Council's Institute at Compton, Berks., for typing and comparison with their strains of H. parainfluenzae (Mathews, and Pattison 1961). It was found that it would grow in the absence of X factor but not without V factor. There was a weak positive precipitation with H. suis type A sera but no reaction with sera prepared from H. parainfluenzae and the conclusion reached was that the Glasgow organism was a H. suis similar to strains from the National Collection Type Cultures. These findings were in agreement with the pathology since the lesions in the original outbreak and in the subsequent passages when H. suis was present, were not like

the pneumonia described later due to H. parainfluenzae, which is similar to the pneumonia produced by the Compton organism.

Haemophilus suis was not necessary for the development of pneumonia in experimental animals and even when it was present the same respiratory distress as seen in the original outbreak did not occur. The organism in pure culture was incapable of producing pulmonary lesions. These results could be interpreted as showing that H. suis unlike H. parainfluenzae is unable to cause disease by itself and can only act as a secondary invader. On the other hand they might have been due to the resistance of the experimental animals or to the fact that there was a loss of virulence after culture. A problem of a similar nature has been encountered with P. multocida, another agent associated with pulmonary lesions in the pig. Haemophilus suis is thought by some workers to be responsible for Glässer's disease, characterised by polyserositis, meningitis and arthritis (Bakos, Nilsson and Thal 1952). There was no arthritis or serositis in the pigs involved in the original outbreak and when the brains of the animals were examined histologically they were normal. The pigs infected experimentally with H. suis cultures or lung containing H. suis developed no lesions of Glasser's disease either.

The success in passaging the pneumonia with bacteria free material and the presence of pleomorphic organisms suggest that the enzootic pneumonia agent was involved in the original outbreak and this was confirmed by the

pathology, (to be described), in the experimental animals, particularly those killed 8 and 12 weeks after infection. One of the interesting features of this organism is its capability for readily producing disease in experimental animals compared with other agents such as H. suis and P. multocida and an even more closely related organism Mycoplasma mycoides.

The experiments using suspensions treated with antibiotics gave rather unusual results. The enzootic pneumonia agent is not susceptible to antibiotics in vitro (Betts and Campbell 1956) although aureomycin and terramycin have a prophylactic effect in vivo, and one would therefore not have expected the result shown in Table 7 when the transmission was made from 9226/55. This indicated that the agent was either dependent on H. suis for the establishment of infection or that it itself was susceptible to penicillin and streptomycin. The result of the second transmission with antibiotics i.e. from 14799/14, is equivocal in that small lesions were established in one pig and therefore the agent was not, at any rate, completely susceptible to penicillin and streptomycin although its infectivity was apparently reduced. The number of pigs involved is too small however to allow any definite conclusions to be made on this point.

An interesting and important feature concerning the antibiotic sensitivity of the agent is that the series of transmissions using pneumonic lung was started from a pig which had been treated for two days with terramycin immediately before

the first transmission was made. This confirms the finding of other workers, quoted earlier, that this antibiotic has no therapeutic value in the treatment of this disease.

ENZOOTIC PNEUMONIA

(5) The Serial Pathology of the Experimentally Produced Disease

The pathology of the pneumonia which occurred in the experimental pigs infected with suspensions of pneumonic lung described in this section. This work had two objects (i) to confirm that the original outbreak of pneumonia was due to the enzootic pneumonia agent and (ii) to study the pathogenesis of the lesions by serial killing of the experimental animals.

As a result of the transmission experiments described in the previous section, the lungs of fifteen pigs (Table 8) which had been killed at four different stages after infection, namely one week, three weeks, eight weeks and twelve weeks, were available for investigation. All of these pigs had been infected with pneumonic lung suspensions in the manner described in the previous section, and the clinical examinations outlined earlier were made during the course of the experiments.

Clinical Observations

Temperatures in excess of 104.0°F. were recorded in all but four of the pigs on sporadic days throughout the period of infection but not earlier than two days after infection. Eight of the pigs which were kept for one week were heard coughing during this period. This took the form of a light cough on the third or fourth day but was usually quite harsh by the sixth to seventh days. In the animals kept for longer periods of time coughing was

Table 8

THE PIGS ON WHICH THE SERIAL PATHOLOGY IS BASED

Case No.	No. of Weeks when Pigs were killed after Infection
14799/1	1 week
14799/2	"
14799/7	"
14799/8	"
14799/10	"
14799/13	"
14799/14	"
14799/19	"
14799/20	"
14799/17	3 weeks
14799/18	"
14799/25	8 weeks
14799/26	"
14799/23	12 weeks
14799/24	"

heard and continued until they were slaughtered. Towards the end of the first week the respiratory sounds on auscultation were usually broncho-vesicular inspirations with vesicular expirations but bronchial breathing was heard in three of the animals kept for longer periods of time. Sibilant ronchi were also heard, even during the first week. Respiratory rates were recorded initially but because of the fluctuations due to excitement this was not continued for all of the pigs.

Pathology

In the following description the histopathology of the pneumonic lungs will be given after the macroscopic features of the lesions are outlined.

1 Week

The affected lobules were usually pale pink and were found in all the lobes of the lungs although the apical, and cardiac lobes were involved in every case. The lesions in one animal were dark red and in another greyish pink. Occasionally the lobules were slightly lower than the surrounding lung. Taking the group as a whole the consolidation was not extensive, since the amount of lung involved was usually only two to three tenths of the total volume. The bronchial lymph nodes were enlarged.

3 Weeks

The consolidation in these pigs was distributed in the apical lobes, cardiac lobes, intermediate lobe, and anteroventral portions of the dia-

phragmatic lobes. The whole of the apical and cardiac lobes were involved. The lesions were quite extensive with about four to five tenths of the lung volume involved in each animal. The affected lobules were fawn coloured, felt quite firm and were on the same level as the surrounding normal lung. On section oedema fluid could be expressed from the cut surface and a small amount of grey mucopus from the bronchi. Completely consolidated tissue sank in corrosive formal.

The bronchial lymph nodes were markedly enlarged.

8 Weeks

There was moderately extensive consolidation with three to four tenths of the lung affected in both animals, and the anterior parts of the lungs were involved (Fig. 22). The lesions were darker in colour than those seen at three weeks, being dark red, and when they were inspected closely, small groups of white spots could be seen in some lobules. Parts of the affected lung were slightly collapsed. On section oedema fluid could be expressed from the cut surface but there was not much muco-pus in the bronchi.

The bronchial lymph nodes were moderately enlarged.

12 Weeks

The pneumonia in these pigs was similar in extent and distribution to the lesions seen at 8 weeks. However all the affected lobules were obviously collapsed and were dark red. In addition they felt thin. The bronchial

system contained virtually no muco-pus.

The bronchial lymph nodes were moderately enlarged.

Histological examination of the lungs, trachea and bronchial lymph nodes from the pigs showed the following changes.

1 Week

The most obvious changes at this early stage were seen in the alveoli and the lumina of bronchioles and bronchi.

The alveolar changes could be divided into two basic types both of which were seen in any individual animal but usually one was more extensive than the other. They were (i) alveolar collapse of varying degrees, from partial to almost complete, which affected whole lobules or parts of lobules (Fig. 23). The alveolar walls were thickened by the multiplication of alveolar cells within them. These usually did not appear to protrude into the lumen. The lumina of the alveoli contained a mixture of alveolar macrophages and polymorphonuclear leucocytes but sometimes only the latter cells. The alveolar cells and macrophages had large nuclei in relation to the amount of cytoplasm which was not vacuolated but was sometimes lightly basophilic. (ii) the alveoli were fully expanded and there were many cells in their lumina (Fig. 24). These were alveolar macrophages and polymorphonuclear leucocytes mainly (Fig. 25). The alveolar macrophages had variable amounts of cytoplasm and many were vacuolated. Increased numbers of alveolar cells

were seen in the alveolar walls and often they protruded into the lumen (Fig. 25). The alveolar walls were thinner than in the collapsed areas and the alveolar cells were scattered about in ones or twos and only occasionally in small groups.

In some pigs there were patches of alveolar oedema and the septal and peribronchial lymphatics were slightly dilated with oedema fluid and contained cells similar to those seen in the alveoli. Small numbers of other cell types usually only occurring singly were seen in the alveoli. They were eosinophils, lymphocytes, plasma cells, and large cells with very basophilic cytoplasm and a densly staining nucleus which were either plasmablasts or lymphoblasts. A multinucleated giant cell with vacuolated cytoplasm which had phagocytosed a polymorphonuclear leucocyte was seen in one pig.

The lumina of the bronchioles was sometimes filled with a mass of cells like those in the alveoli but usually there were more polymorphonuclear leucocytes. The bronchiolar epithelium was hyperplastic to varying degrees and polymorphonuclear leucocytes could be seen migrating through it. There was usually not much thickening of the peribronchiolar tissues and they only contained a few cells of the lymphocyte series plus plasma cells. Reticular cells with large elliptical, palely staining nuclei and no obvious cytoplasm were seen. Small numbers of lymphocytes and plasma cells occurred in the lamina propria of the bronchioles. Occasional discrete lymphoid nodules had developed in the peribronchiolar connective tissues. In most pigs only

one was found and they neither contained germinal centres nor infiltrated the adjacent bronchiolar wall.

Small numbers of lymphocytes and plasma cells were found in other connective tissue sites such as around pulmonary veins, and in the interlobular septa and pleura. Single eosinophils were seen in these sites mixed with the other cells and also lymphoblasts or plasmablasts.

The lumina of the bronchi contained an exudate similar to that seen in the bronchioles but there was more mucus mixed with the cells. Small numbers of plasma cells were seen around the bronchial glands and in the lamina propria lymphocytes and plasma cells were present in small numbers. At some points polymorphonuclear leucocytes were seen migrating through the epithelium.

When the trachea was examined changes similar to those occurring in the bronchi were seen.

The sinusoids of the bronchial lymph nodes were flooded with polymorphonuclear leucocytes. The dense lymphatic tissue contained many mitotic figures and lymphoblasts but no definite organisation into lymphoid nodules with germinal centres. Once more a small but significant number of eosinophils were seen scattered amongst the other cells.

3 Weeks

At this stage the two striking features seen when sections were examined with the low power were a heavy cellular accummulation within expanded alveoli and considerable thickening of the peribronchiolar connective tissue (Fig. 26).

The latter was due primarily to diffuse accumulation of cells but lymphoid nodules also occurred singly or in small groups.

There were many more cells in the alveolar walls and lumina at this stage than in the expanded alveoli at one week. The alveolar walls were very cellular and swollen by large numbers of alveolar cells with clear or vacuolated cytoplasm. Mitotic figures could be seen in some of these cells. Many of them protruded into the alveolar lumen either singly or even in small groups and some alveoli particularly those adjacent to bronchioles, blood vessels, interlobular septa or pleura, had an almost continuous cuboidal layer of these cells on their walls. Some of the cells had caps of material on their luminal apices which were usually basophilic, to varying degrees of intensity. Plasma cells, lymphocytes and polymorphonuclear leucocytes were also seen in the alveolar walls and in the walls of alveolar ducts.

Alveolar macrophages occurred in large numbers within the alveoli and in addition considerable numbers of plasmablasts, immature and mature plasma cells and lymphocytes could be seen (Fig. 27). Polymorphonuclear leucocytes were present and a small but consistent number of eosinophils were observed scattered singly among the other cells. Occasionally multinucleated giant cells were found. Some alveoli contained oedema fluid and in others there was a granular eosinophilic precipitate between the cells. Although the overall picture was dominated by alveolar expansion some foci with partial alveolar collapse of parts of lobules were seen.

The marked thickening of peribronchiolar tissues was due to the accumulation of large numbers of lymphocytes, plasma cells and cells which were either lymphoblasts or plasmablasts (Fig. 28). Large reticular cells were also prominent. At some points well formed lymphoid nodules had developed, often with germinal centres. These contained reticular cells and lymphoblasts and there was considerable mitotic activity. Small groups of lymphoid nodules occurred together and the lymphocytes and plasma cells at the periphery of some of them infiltrated the lamina propria of the bronchiole causing atrophy of the muscularis and distortion of the bronchiole wall. In the lamina propria of most bronchioles there were plasma cells and lymphocytes particularly the former, and these could be seen migrating through the bronchiolar epithelium which was hyperplastic. Within the bronchiolar lumina there was a mixture of polymorphonuclear leucocytes, alveolar macrophages, and lymphocytes and plasma cells. Polymorphonuclear leucocytes often predominated.

Considerable accumulations of cells similar to those in the peribronchiolar tissues occurred around blood vessels and could also be seen in smaller numbers scattered diffusely or in small groups in the connective tissue of the pleura and septa and around bronchi. Single eosinophils were seen taking part in the reaction in the peribronchiolar tissues, and in the septa, in the pleura, and in the perivascular tissues.

Within the bronchi there was a variable amount of mucus, polymorphonuclear leucocytes and smaller numbers of the other cells seen in the alveoli. The epithelium was hyperplastic and immediately below it were many plasma cells and some lymphocytes. These cells and occasional polymorphonuclear leucocytes could be seen migrating through the epithelium. Large numbers of plasma cells occurred around the bronchial glands and there was increased glandular activity.

The changes in the trachea were essentially the same as in the bronchi but the exudate in the lumen was scanty.

The bronchial lymph nodes showed the features seen at one week but in addition to polymorphonuclear leucocytes, plasma cells were found in the sinusoids. Lymphoid nodules with germinal centres had developed and small numbers of eosinophils were detected throughout the node.

8 Weeks

The general appearance of the lesion at eight weeks was due to an active alveolar reaction similar to that seen at three weeks but many larger patches of collapse had developed. There was a significant increase in the number of peribronchiolar lymphoid nodules which were very numerous and coalescing to form large groups. Lymphoid nodules accounted for most of the peribronchiolar reaction. The lumina of most of the bronchi and bronchioles contained very little exudate.

The cells taking part in the alveolar reaction were those seen at three weeks, but in many alveoli there were greater numbers of plasma cells. In others although they were still expanded the number of cells of all types was decreasing. A similar decrease had taken place in the number of alveolar cells in the alveolar walls and protruding from the alveolar walls. The lumina of the alveoli in areas showing partial or almost complete collapse usually contained only a few plasma cells, and alveolar macrophages. The alveolar walls had only a slight excess of alveolar cells. The alveolar ducts in many of these areas were still patent so that the lung had a patchy appearance.

Associated with the alveolar changes was the development of a large number of lymphoid nodules with germinal centres (Fig. 29). They were seen principally in the peribronchiolar tissues but also sometimes beside pulmonary veins or in the gaps between the bronchial cartilages, or in the connective tissue of the septa and pleura. The peribronchiolar nodules often occurred in groups of three to four causing distortion of the bronchial wall and collapse of adjacent alveoli. They could sometimes be seen bulging into peribronchiolar lymphatics. The cells of the follicles were composed of a high percentage of reticular cells, lymphoblasts and large lymphocytes. Mitotic figures were numerous. Many plasma cells were mixed with the lymphocytes at the periphery of some follicles and the adjacent peribronchiolar tissues contained large numbers of these cells. In addition, infiltration

of the lamina propria by these cells was frequently seen producing local thickening of this layer. Atrophy of the muscularis had taken place and sometimes there was extensive infiltration of the bronchiolar epithelium so that it was almost completely disrupted. Around the rest of the bronchiole the epithelium was hyperplastic and there were fewer plasma cells and lymphocytes in the lamina propria. Most bronchioles contained very little exudate, but when it occurred it was similar to that seen at three weeks.

There were large numbers of plasma cells, lymphocytes and plasmablasts or lymphoblasts around the pulmonary veins and groups of these cells also occurred in the septa and pleura, sometimes forming small aggregates. Eosinophils were seen in the situations described earlier.

The bronchial changes only differed from those at three weeks in the absence of a copious exudate in the lumina of most of them. The tracheal changes were also similar except that in one case a small group of lymphoid cells, mostly lymphoblasts was seen among the many plasma cells around the tracheal glands.

The bronchial lymph nodes resembled those examined at three weeks.

12 Weeks

Although most of the bronchioles and bronchi seen in section were patent there was widespread alveolar collapse at this stage. Most of the bronchioles in these lobules had lymphoid follicles either singly or in groups in the peribronchiolar tissues (Fig. 30). Adjacent to collapsed lobules expanded

lobules or lobules with foci of expansion and collapse were seen, with a similar peribronchiolar reaction but virtually no alveolar reaction.

The lumina of collapsed alveoli were frequently devoid of cells and the alveolar walls appeared to be almost normal. Some of them however did contain a slight excess of alveolar cells with plasma cells and alveolar macrophages. A few were full of polymorphonuclear leucocytes.

The peribronchiolar follicles on the whole were more discrete in that they often did not spill over into the lamina propria of the bronchiole and the adjacent muscularis was intact. In addition many of them contained greater numbers of small lymphocytes than at earlier stages and there was a decrease in the numbers of plasma cells and lymphocytes in the bronchiolar wall as a whole. The reticular cells had become prominent in the centres of some follicles as clear spaces with a central nucleus outlined by dark masses of small lymphocytes.

The other connective tissue zones, around blood vessels, in the septa and pleura showed similar changes. Occasional eosinophils could still be found. The alveoli which were expanded, in lobules with peribronchiolar lymphoid nodules, were substantially normal or contained scattered representatives of the cell types described earlier as taking part in the alveolar reaction.

The most obvious changes in the bronchi and trachea were the many plasma cells around the mucous glands and the presence of lymphoid aggregates

or even a few true nodules in these sites. There was very little exudate in the lumina of these structures and most bronchi were virtually empty. Plasma cells and lymphocytes could still be found in the lamina propria and also migrating through the epithelium which was not hyperplastic. Small numbers of polymorphonuclear leucocytes were also seen in the epithelium.

The bronchial lymph nodes differed from those seen earlier because of the large number of small lymphocytes in the lymphoid nodules. The phenomenon by which the reticular cells became outlined by small lymphocytes was also to be seen.

Discussion

The pathology of the disease in the experimental animals was similar to that in pigs which had become infected with enzootic pneumonia in the field as described in section (1). It would appear therefore that the enzootic pneumonia agent was involved in the outbreak of pneumonia which was the source of the experimental material. The results of the transmission experiments confirmed this because the pneumonia was serially passaged with bacteria free suspensions of pneumonic lung.

Most of the features of the original pneumonia were found in the pigs killed at three weeks. The patches with severe congestion of alveolar walls, very dense infiltration by polymorphonuclear leucocytes in alveoli, bronchioles and bronchi and the congestion of blood vessels in bronchiolar walls with

focal necrosis of bronchiolar epithelium were not seen. It is inferred therefore that these changes were produced by a secondary bronchopneumonia caused by H. suis in most of the pigs or B. bronchiseptious or P. multocida in those from which these organisms were isolated. It was not possible to prove this experimentally either by injecting cultures of H. suis or even by infecting animals with pneumonic lung containing H. suis. There was no histological difference between the lungs of the pigs killed at one week from which H. suis was isolated and those in which it was absent; it is therefore assumed that the organism played little part in the lesions seen at 3 weeks, since none of the features characteristic of an acute simple bronchopneumonia were seen.

Under favourable conditions the lesions of enzootic pneumonia will resolve. This is known from the lower incidence of lesions found in sows (Shanks and Macpherson 1955) and from the experimental work of Lannek and Bornfors (1957). The latter authors showed that 18 of 23 pigs, all of which had radiographic evidence of pneumonia at one month, were radiographically negative at four months. This was confirmed in three of them at post-mortem. One month later i.e. five months after experimental infection, no macroscopic lung lesions were seen in the remaining fifteen when they were killed.

Assuming that there is some temporal relationship between the different histopathological features of the lesions it is possible that the sequence of

events is as follows:-

(i) Small patches of alveolar collapse and alveolar cell hyperplasia with some alveolar macrophages and polymorphonuclear leucocytes accumulating in the alveolar lumina.

(ii) This is followed by marked hyperplasia of alveolar cells, re-expansion of alveoli, and the accumulation of many alveolar macrophages and polymorphonuclear leucocytes within them. The macrophages are derived from some of the proliferating alveolar cells. Alveolar oedema develops.

(iii) At the same time as (i) and (ii) plasma cells and cells of the lymphocyte series begin to accumulate in the connective tissues of the lobule in increasing numbers, particularly in the peribronchiolar tissues but also around pulmonary veins, and in the septa and the pleura.

(iv) The composition of the cellular exudate in the alveoli changes due to the appearance of plasma cells and their precursors. At this stage there is definite diffuse thickening of the peribronchiolar tissues by reticular cells, lymphoblasts, and plasma cells. The proliferation of alveolar cells continues.

(v) Many large lymphoid nodules with germinal centres including a high proportion of lymphoblasts, form in the peribronchiolar and other connective tissue sites. There are now many plasma cells in the alveoli among the other cell types.

(vi) The number of cells in the alveoli decreases and extensive alveolar

collapse occurs. There is also a reduction in the number of alveolar cells in the alveolar walls.

(vii) At this stage the alveoli are still collapsed but many are otherwise normal. The peribronchiolar lymphoid nodules which contain a high proportion of small lymphocytes, are relatively more discrete and are beginning to regress.

(viii) These changes are followed by alveolar re-expansion, but some lymphoid nodules persist in the peribronchiolar tissues.

The speed at which these changes occur may depend on (a) the magnitude of the initial infection (b) the ability of the animal to respond to the infection, (c) the opportunity for re-infection and the magnitude of re-infection (d) complicating secondary infections. Factors (b) and (c) may account for the persistence of lesions and explain the epidemiology of the disease in the field.

In this connection Whittlestone's (1957a) observation that the rate of resolution is faster in pigs kept in small groups, is extremely interesting.

In the pneumonia described here stages (i) (ii) (iii) were seen at one week, stage (iv) at three weeks, (v) and (vi) at eight weeks and (vii) and (viii) at twelve weeks.

There is of course some overlap of one phase with another in any individual and probably each secondary lobule of pulmonary tissue, which is well delineated by connective tissue from its neighbours, in the pig, goes

through the sequence of changes independently from the others so that as the disease spreads throughout the lung by the air passages the lesion may be seen at different stages of development in different lobules. This was in fact what was observed in the naturally occurring cases of enzootic pneumonia described in section (1) most of which would have been in an environment offering ample opportunity for reinfection and persistence of the lesions. From this pathogenesis experiment the three groups of changes found in the pigs infected in the field would seem to represent different phases in the development of the disease with Group I being an earlier phase than Group II and Group II an earlier phase than Group III.

The most striking and characteristic features of enzootic pneumonia are (i) the peribronchiolar proliferation of lymphoid tissue associated with the appearance of large numbers of plasma cells in the lungs and (ii) the alveolar cell hyperplasia.

Lymphoid tissue and plasma cells in particular, are known to be associated with antibody production. Since these cells occur in large numbers within the alveoli, in the connective tissue of the lobule, particularly the peribronchiolar tissue, and around the bronchial and the tracheal glands, it is almost certain that a considerable amount of antibody is being produced in the lungs of pigs with enzootic pneumonia and in fact (Lannek and Bernfors, 1957, Whittlestone, 1957a) have shown that recovered animals are resistant to reinfection. This may seem at odds with experience in the field, where

infection in pigs up to six to seven months of age is very common and the disease is endemic on many farms. The degree of immunity is not known however and it may be easily overcome especially if the opportunity for heavy reinfection is present. It is also possible that the enzootic pneumonia agent may exist in several different antigenic forms. No circulating antibodies have been reported and Betts (1952) was unable to demonstrate neutralising antibodies in convalescent serum. It seems reasonable to conclude from the histopathology however that the prolonged course of the disease, which occurs in many pigs, and a major part of the pulmonary consolidation are the results of an immune reaction by cells of the antibody producing system within the lung to a persisting antigenic stimulus.

The tremendous hyperplasia of alveolar cells which begins during the early stages of the infection and the alveolar collapse which develops later on in the disease process may be related. Alveolar collapse, is an important feature of enzootic pneumonia and has been attributed to bronchioles being blocked with exudate (Whittlestone, 1957a). It often occurs however, in the absence of any significant amounts of secretion in the bronchial or bronchiolar pathways and although it is difficult to exclude obstruction as the aetiological factor on the basis of histological examination, in the cases of bronchopneumonia described later, considerable amounts of exudate accumulated in the respiratory passages without collapse occurring in the

associated lobule. An alternative explanation for the collapse is damage to the lipoprotein alveolar lining layer. This has been the subject of several original papers and review articles (Clements, Brown and Johnson, 1958, Pattle and Thomas 1961, Avery 1962, Clements 1962, Bolande and Klaus 1964). Clements et al (1958) in describing the function of this layer said 'the alveolar lining layer operates as an anti-atelactasis factor' and obviously therefore its absence or damage would result in collapse even if the bronchioles, bronchi and trachea were patent.

Any interpretation of how this happens is highly speculative but it may be connected with the striking hyperplasia of alveolar cells which occurs in this disease. Generally two main functions are attributed to these cells (a) they migrate into the lumen and become macrophages (b) they secrete the alveolar lining layer. In enzootic pneumonia many of the alveolar cells which migrate into the lumen do exhibit phagocytic activity. However it is possible that many of the cells which are on the walls and protruding into the lumen have a secretory function and the basophilic caps of material seen on some of the alveolar cells projecting into alveoli could be reasonably interpreted as secretion from these cells.

If one assumes that the hyperplasia of alveolar cells which occurs in enzootic pneumonia is at least in part due to increased secretion of the alveolar lining layer either (i) to replace the lining because it is destroyed by the pleomorphic organism growing in it or (ii) as a direct effect of the

agent on these cells, it is possible that a stage could be reached when their secretory capacity is exhausted and the integrity of the lining membrane could no longer be maintained. When this happened alveolar collapse would occur. If the organism was then eliminated by the immune reaction, already described, and the alveolar cells recovered, the lining layer would be replaced and re-expansion would occur.

ENZOOTIC PNEUMONIA

(6) General Discussion

On the basis of the field observations and experimental work outlined in section (1) to (5) it can be said that enzootic pneumonia is a widespread disease of pigs with definite histological features. The disease in the field was not histologically similar in every pig but this probably only reflected different stages in the progression of the pneumonia and was not surprising when one considered the pathogenesis of the lesions in the experimental animals.

The histopathological entity was characterised by (i) extreme alveolar cell hyperplasia and the accumulation of these cells (a) within alveolar walls, but often protruding into the alveoli and (b) free within the alveoli (ii) the presence of numerous plasma cells and cells of the lymphocyte series in the fibrous connective tissue regions of the secondary lung lobule particularly, in the peribronchiolar position. These cells eventually became organised into lymphoid masses distorting the adjacent bronchiole (iii) alveolar collapse.

All of these features were not necessarily seen in every individual pig but usually there was a combination of at least two of them.

Since histopathology must play an important role in the differential diagnosis of pneumonia in the pig, it is pertinent to ask whether or not enzootic pneumonia defined on these criteria is a single disease aetiologically.

Schofield (1956) suggested that it was highly probable that several different viruses could cause pneumonia in the pig and Pattison (1956) indicated that in his opinion it had not been determined at that time that the infective agent of V.P.P., as it was then called, was the only cause of lesions in the pig lung associated with lymphoid hyperplasia. Whittlestone (1957b) also considered that the pathology although typical was not in itself diagnostic, and stressed the importance of finding pleomorphic organisms (P.O.) in impression smears of lungs with enzootic pneumonia. These organisms were found in the original outbreak of pneumonia described in section (3) and in the disease produced experimentally with bacteria free suspensions of pneumonic lung. However P.O. may sometimes be difficult to separate from non-pathogenic mycoplasma on smears, and culture on solid mycoplasma media is required to achieve this. In addition pathogenic and non-pathogenic mycoplasma may occur concurrently in the same pig (Goodwin and Whittlestone 1964) and in this case it would seem that experimental pig transmission is necessary to establish a diagnosis. The diagnosis in an individual case is also complicated by the fact that there may be no P.O. to be seen (Goodwin and Whittlestone 1960).

The success of these two workers in cultivating the agent of the J strain of enzootic pneumonia seemed to offer hope of simplifying the diagnosis of the disease but in the same paper (Goodwin and Whittlestone 1963) it was stated that they "had encountered pneumonic conditions in the field that

differ from enzootic pneumonia, yet yield morphologically similar agents in tissue cultures". These authors considered that the condition probably had several different aetiological agents and said they had "some evidence that the J agent may not be the only agent of the P.O. type that can cause the Enzootic Pneumonia complex" (Goodwin and Whittlestone 1964).

While it is possible that several different organisms might exist which could produce lesions resembling one phase in the development of the lesion of enzootic pneumonia, and so confuse the diagnosis in the individual pig, no such organisms have been found yet and no one has shown that any organisms other than those acquired from indisputable cases of enzootic pneumonia can produce pulmonary lesions in the pig morphologically similar to enzootic pneumonia in every respect.

The problem of whether the histopathological entity is one disease or a complex can only be answered by the cultivation, and detailed characterisation of organisms from different outbreaks of pneumonia and the experimental reproduction of pneumonia with these agents. At the moment this has only been achieved with one type of organism the J strain of enzootic pneumonia (Goodwin and Whittlestone 1963).

The biological relationship between the "J agent" and the other strains of enzootic pneumonia which have been worked with is not clear, and whether or not this organism exists in several forms differing in antigenicity, transmissibility, infectivity and virulence is not known.

Some of the cases classified as enzootic pneumonia in section (1) did have slight differences from the overall pattern which might have suggested that these were aetiologically different. There were differences in such features as, the extent of bronchiolar epithelial hyperplasia, the number of alveolar cells causing thickening of the alveolar walls but not actually protruding from them, fibrosis of the alveolar walls, fibrosis of bronchiolar walls and the degree of lymphoid nodule formation. Nevertheless it was not possible to be categorically sure that the enzootic pneumonia agent was absent from the lesion and that the differences were not due to mixed infections, variations in the virulence of the organisms or variations in the hosts immunity producing an altered reaction.

If one accepts that it should be possible to find P.O. in smears from the pneumonic lungs of a group of pigs with enzootic pneumonia, the absence of P.O. in a group of pigs with pneumonia histologically similar to enzootic pneumonia is significant, unless our concepts of enzootic pneumonia are too narrow, and would suggest that another agent exists. This situation occurred in the disease described as Type XI (Goodwin and Whittlestone 1962).

However several herds in which Type XI pneumonia was diagnosed and which were believed to be free of enzootic pneumonia subsequently developed a disease indistinguishable from enzootic pneumonia (Goodwin and Whittlestone 1965).

Although histopathology is of limited value in exploring the hypothesis that enzootic pneumonia is an aetiological complex, it is still a useful basis

for defining the disease, at the present state of knowledge, and until adequate laboratory tests are available for typing the organism or organisms responsible the information gained from the histopathological examination of diseased lungs must play an important part in determining the manner in which clinicians tackle outbreaks of respiratory disease in the field.

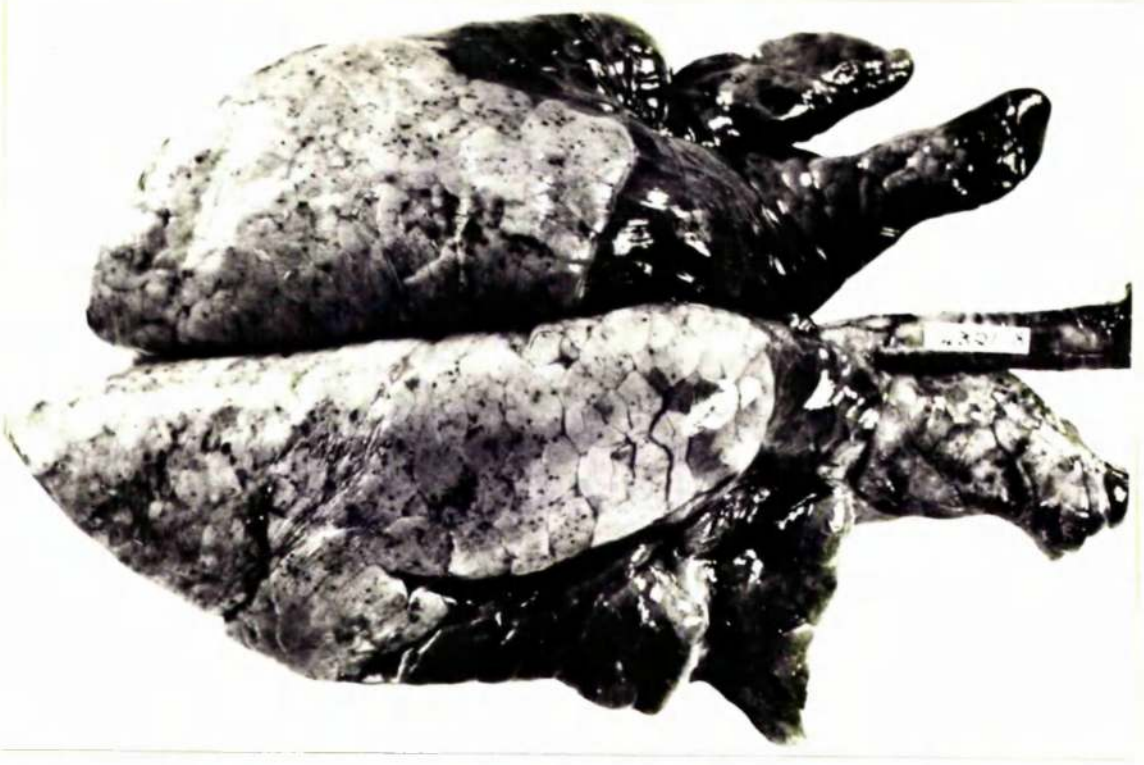


Fig. 1 Enzootic pneumonia: showing bilateral distribution of consolidation in the anterior parts of the lungs.

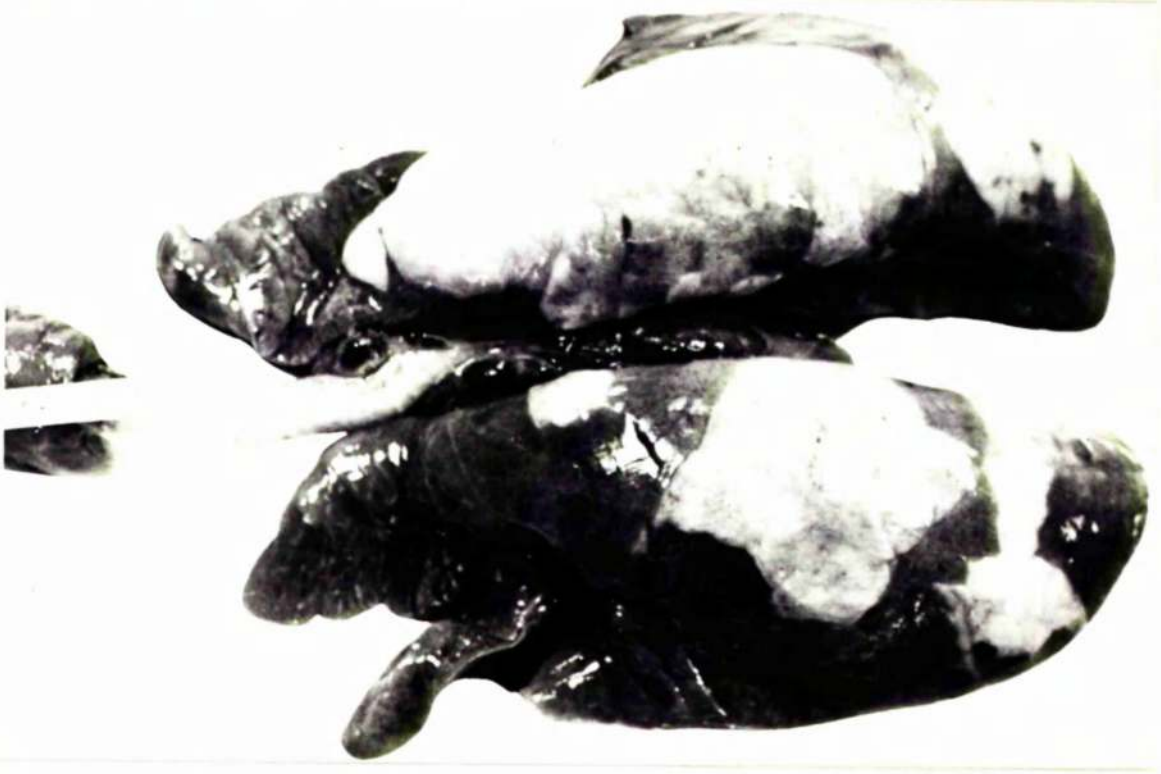


Fig. 2 Enzootic pneumonia: severe case with consolidation in posterior parts of the lungs. There is also septal oedema in the left lung.

Fig. 3 Enzootic pneumonia: groups of grey spots in consolidated lobules.

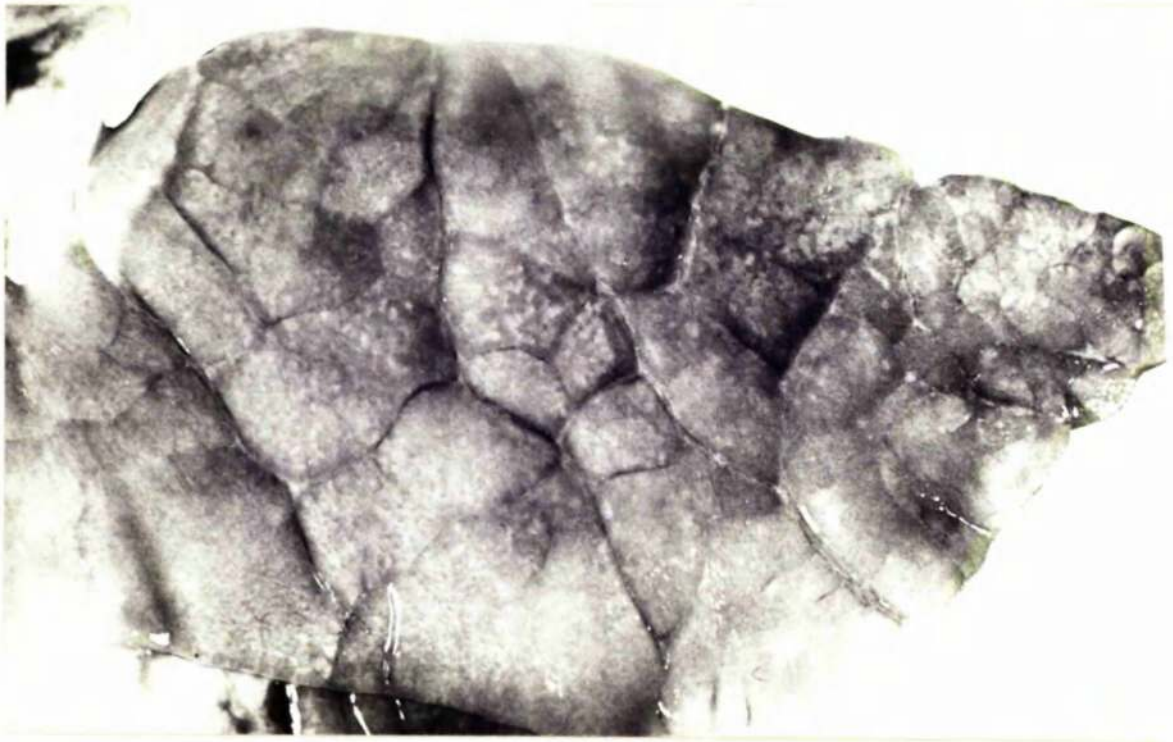


Fig. 4 Enzootic pneumonia: mottled appearance of lobules associated with the earlier stages of the disease.

Fig. 5 Enzootic pneumonia: enlarged bronchial lymph nodes.

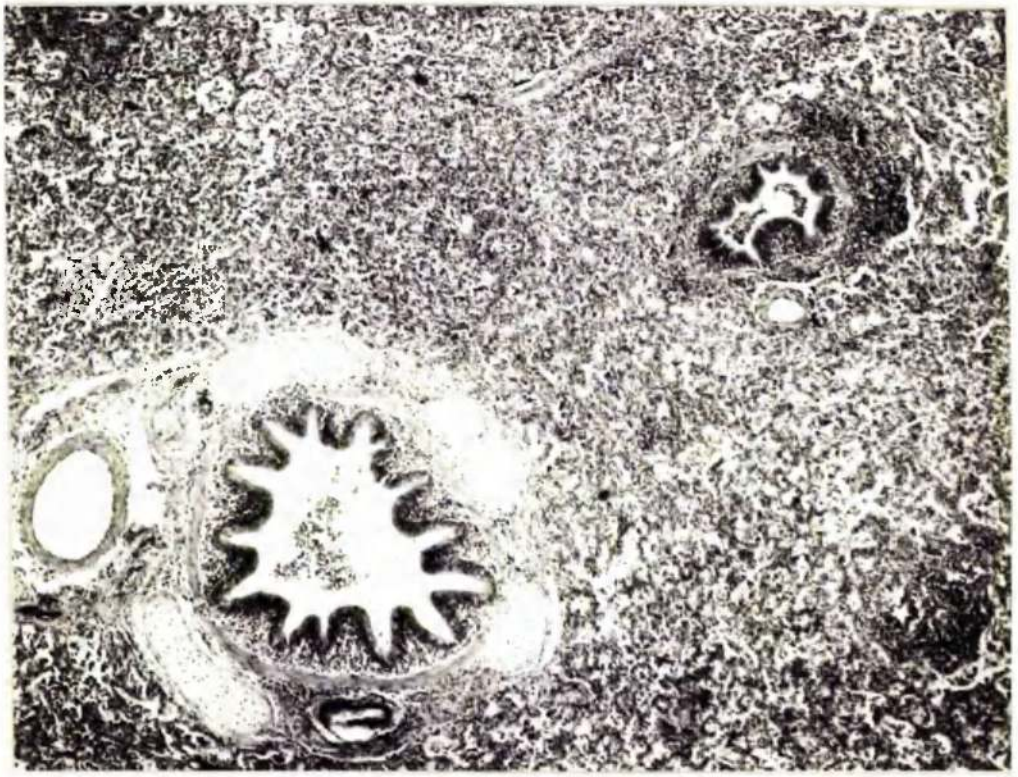


Fig. 6 Enzootic pneumonia: small amount of cellular exudate in the lumen of bronchus and cellular infiltration in the lamina propria. H. & E. x 50

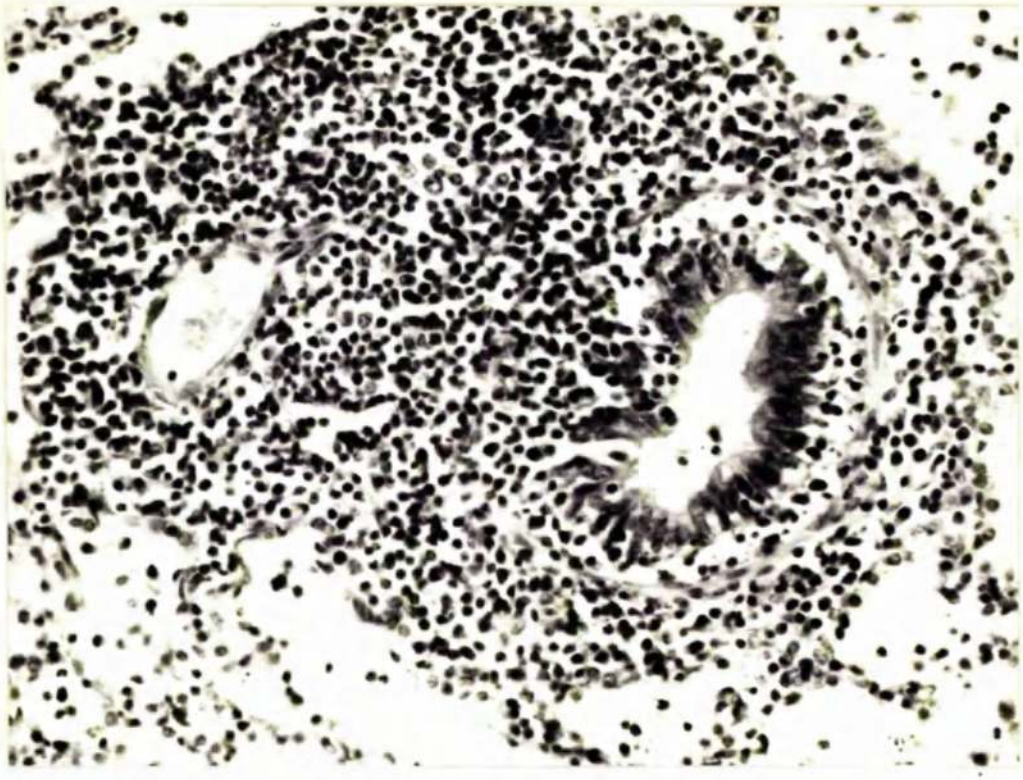


Fig. 7 Enzootic pneumonia: extensive diffuse accumulation of cells in the peribronchiolar tissue. H. & E. x 300

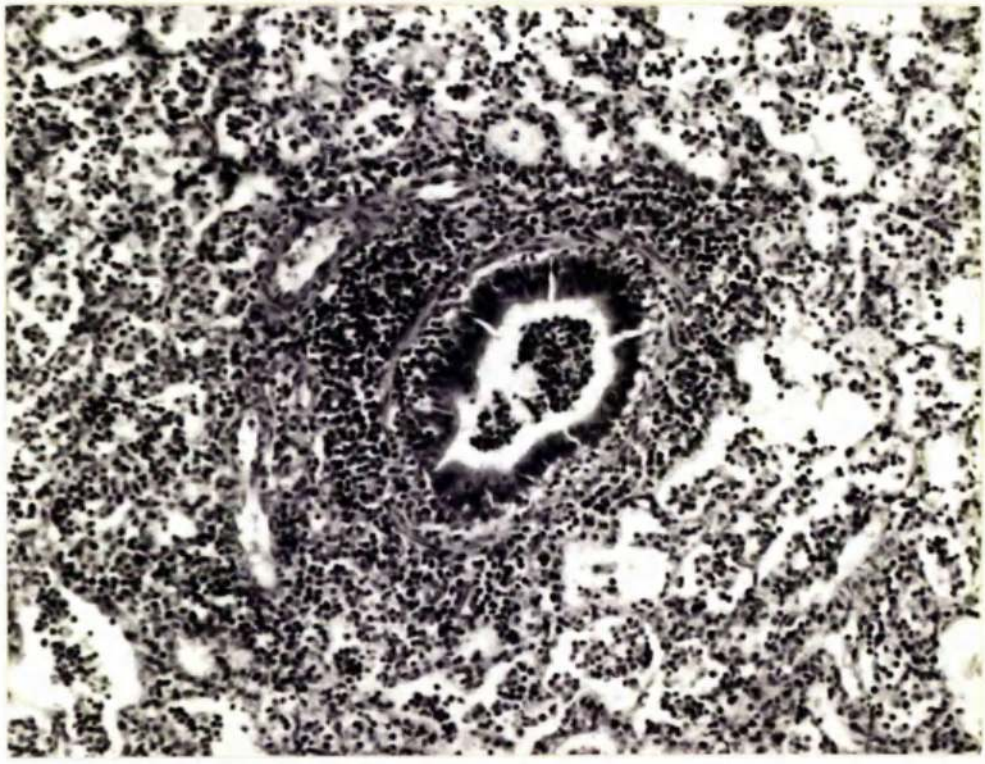


Fig. 8 Enzootic pneumonia: diffuse peribronchiolar accumulation of cells mainly between the pulmonary artery and the bronchiolar wall. H. & E. x 150

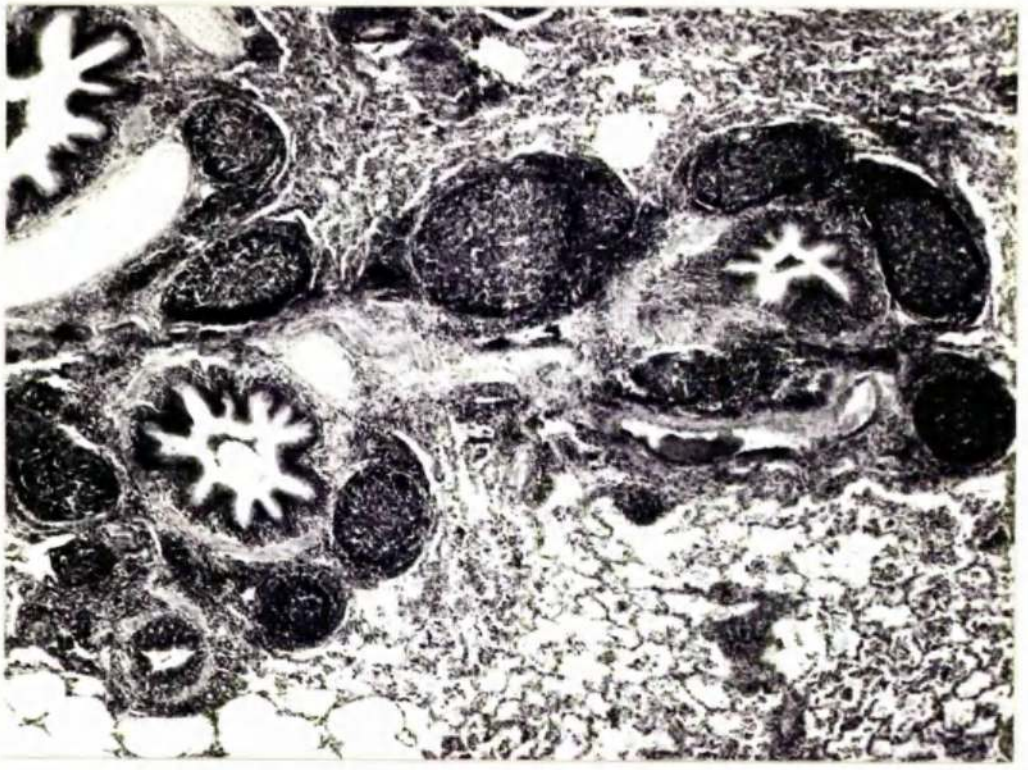


Fig. 9 Enzootic pneumonia: showing large number of peribronchiolar lymphoid nodules. H. & E. x 50

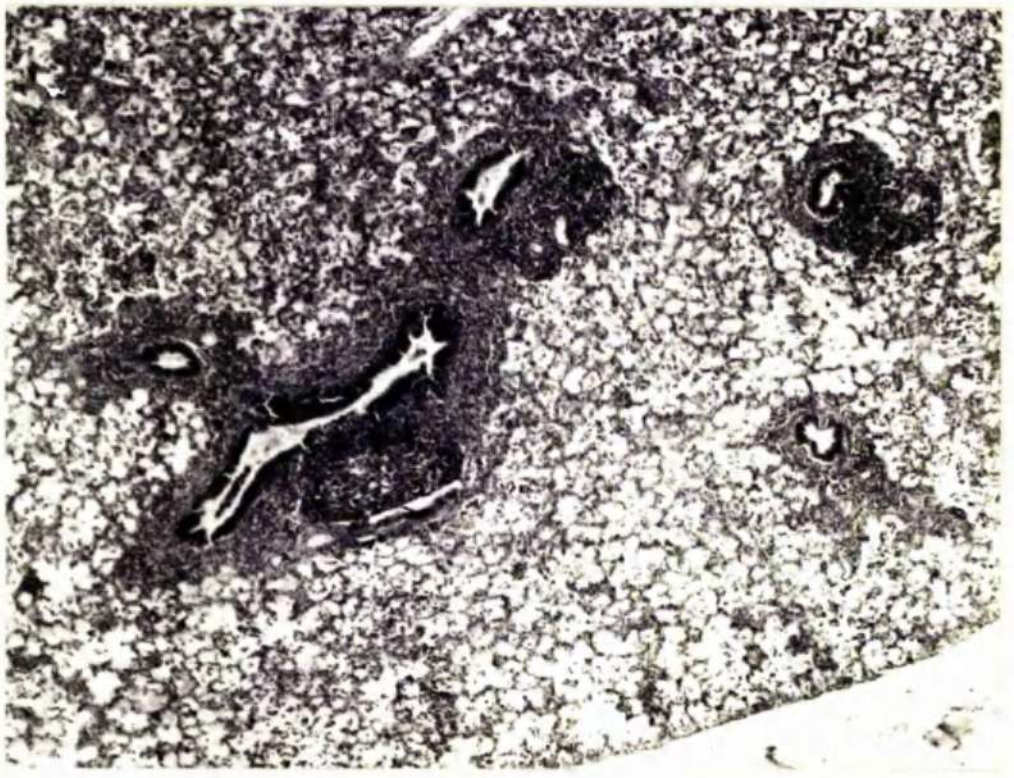


Fig. 10 Enzootic pneumonia: lymphoid nodule between bronchiolar wall and branch of pulmonary artery. H. & E. x 50

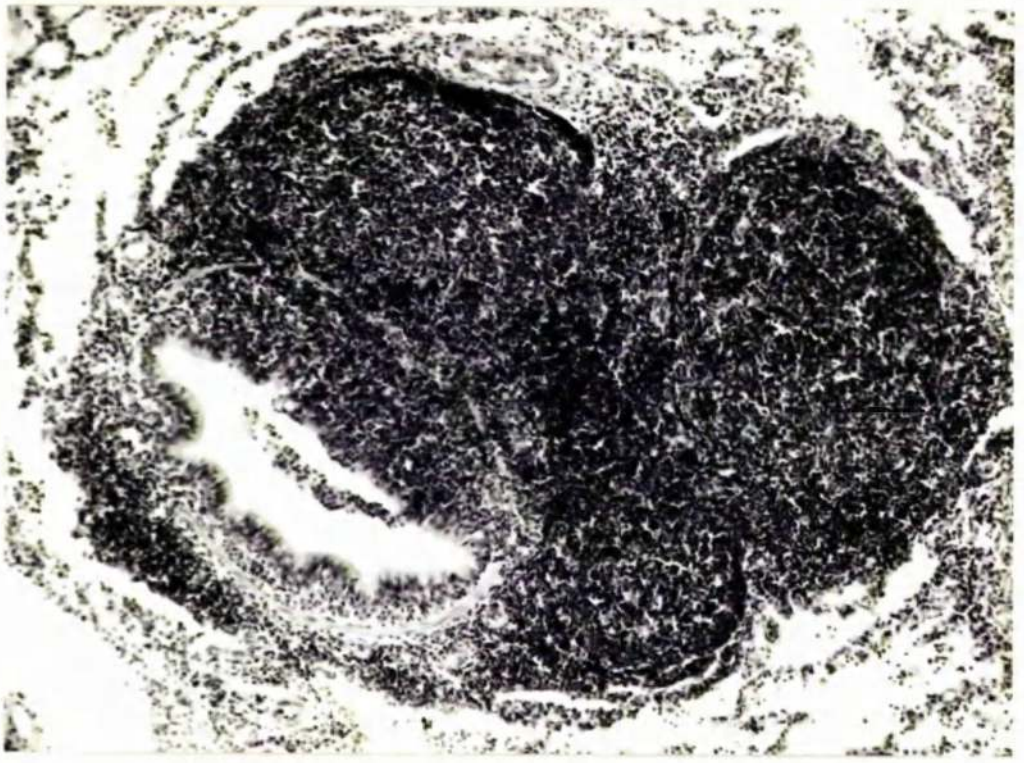


Fig. 11 Enzootic pneumonia: group of lymphoid nodules, showing invasion of bronchiolar wall by lymphoid cells and atrophy of muscularis. H. & E. x150

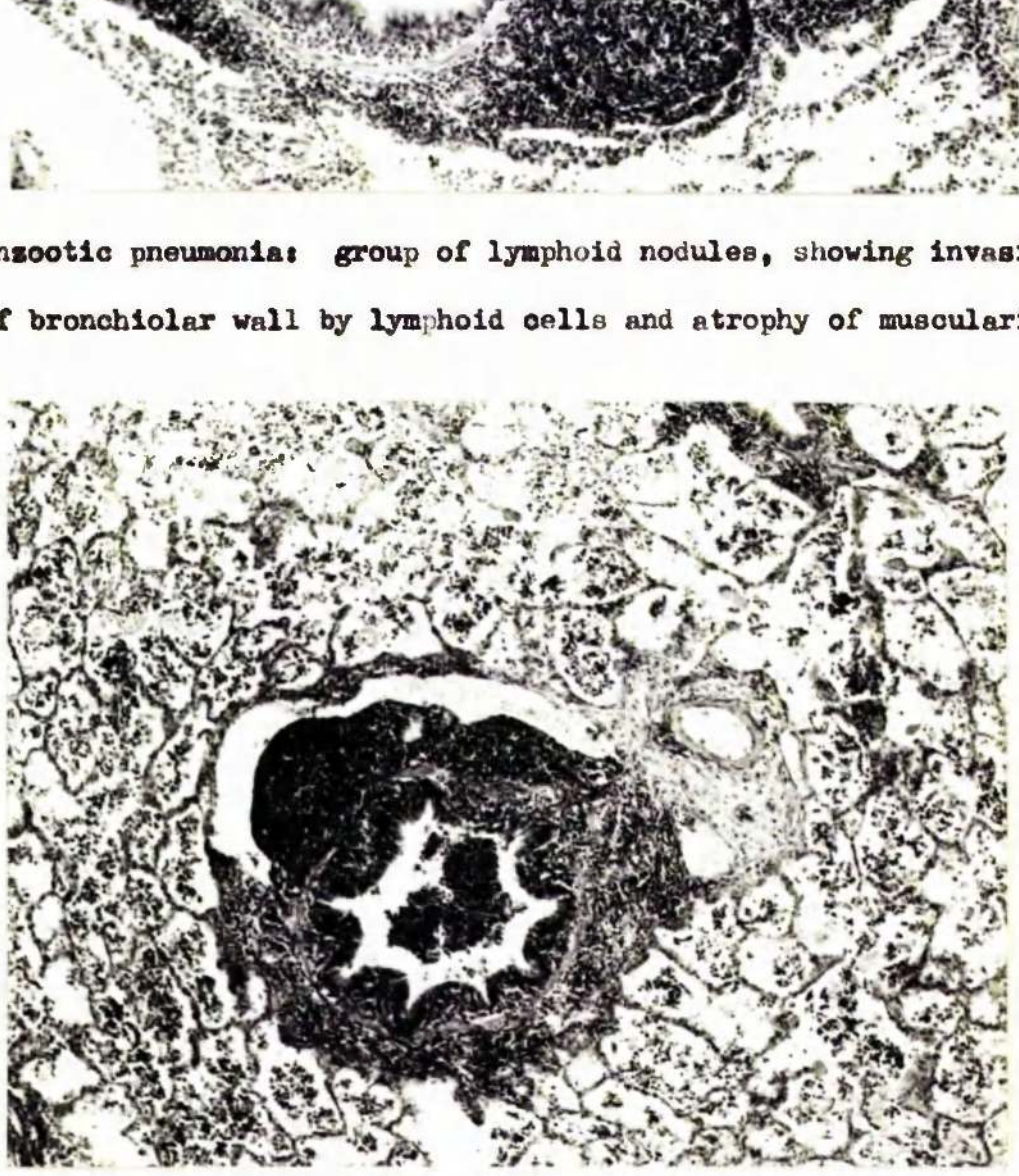


Fig. 12 Enzootic pneumonia: peribronchiolar lymphoid nodules bulging into peribronchiolar lymphatic vessel. H. & E. x100

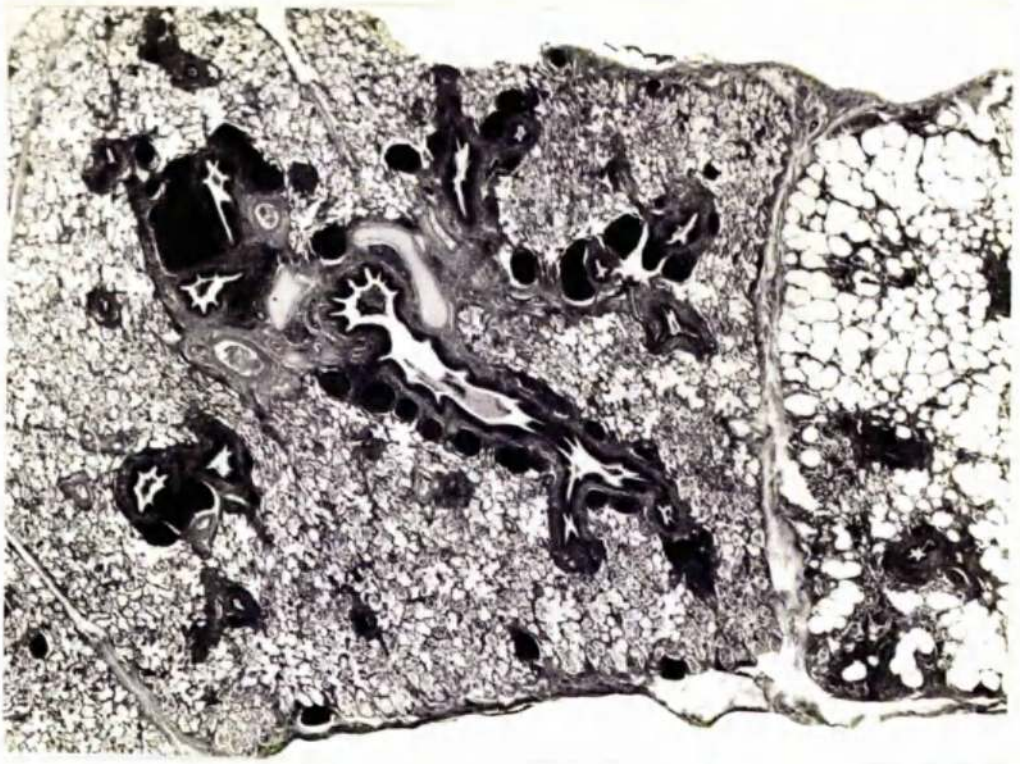


Fig. 13 Enzootic pneumonia: numerous peribronchiolar lymphoid nodules and lymphoid nodules in pleura. H. & E. x 8

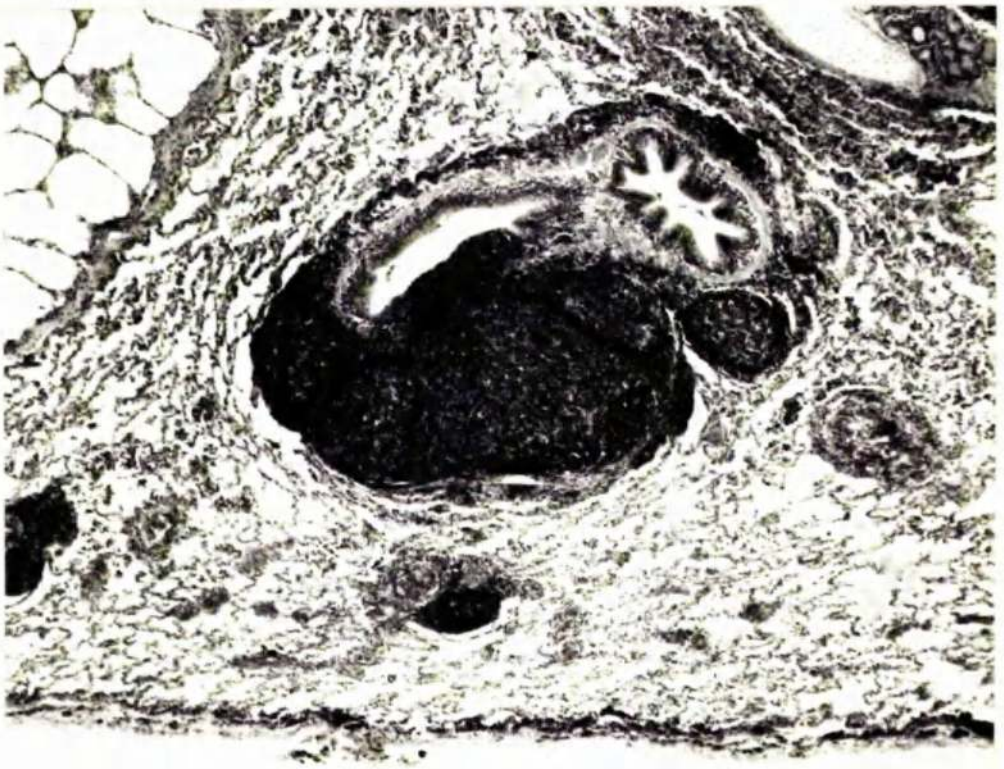


Fig. 14 Enzootic pneumonia: distortion of bronchiolar lumen by lymphoid nodules and alveolar collapse. H. & E. x 35

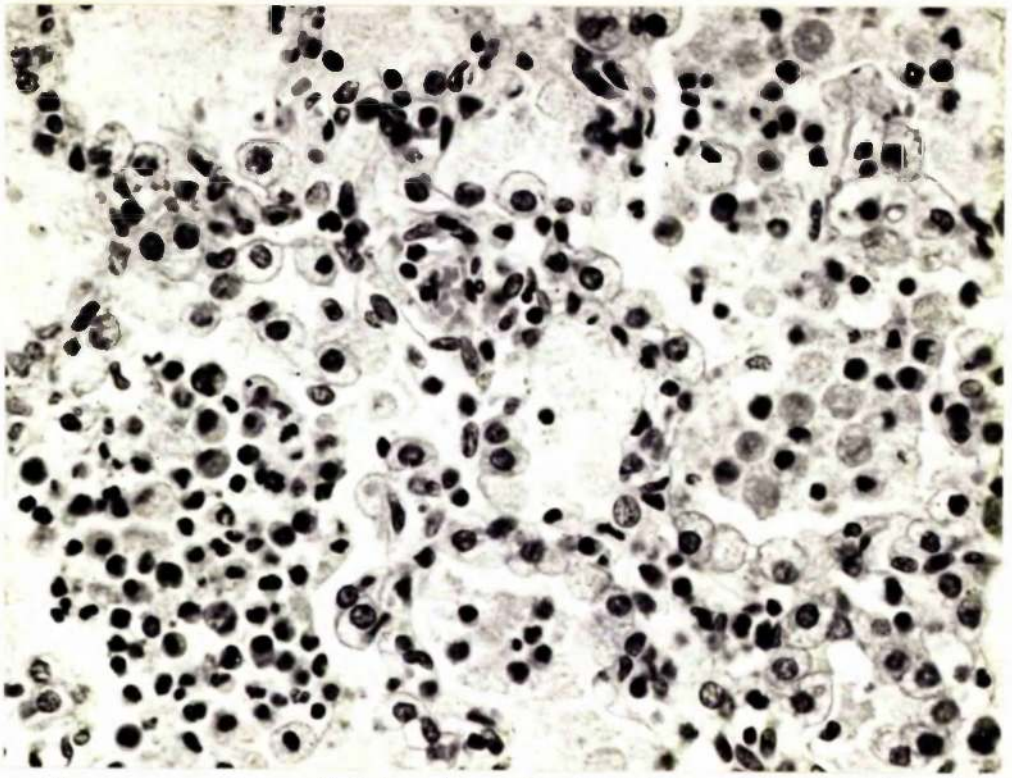


Fig. 15 Enzootic pneumonia: thickening of alveolar walls due to alveolar cell hyperplasia. H. & E. x 500

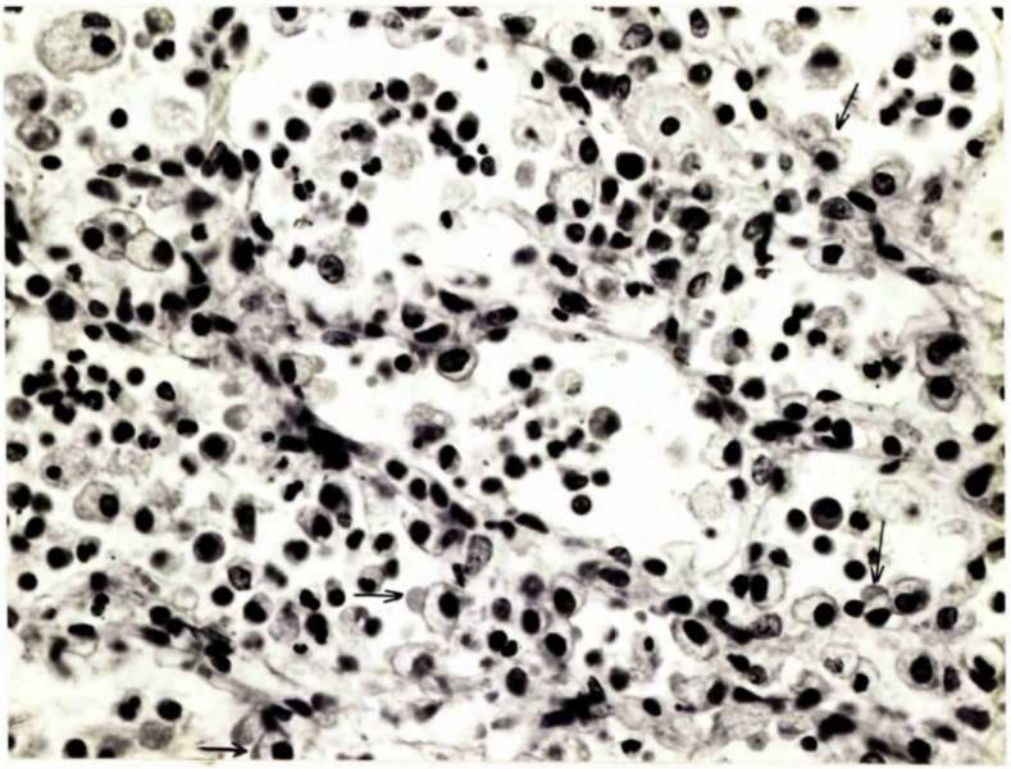


Fig. 16 Enzootic pneumonia: alveolar cells protruding into alveolar lumen with caps of material on their apices. H. & E. x 500

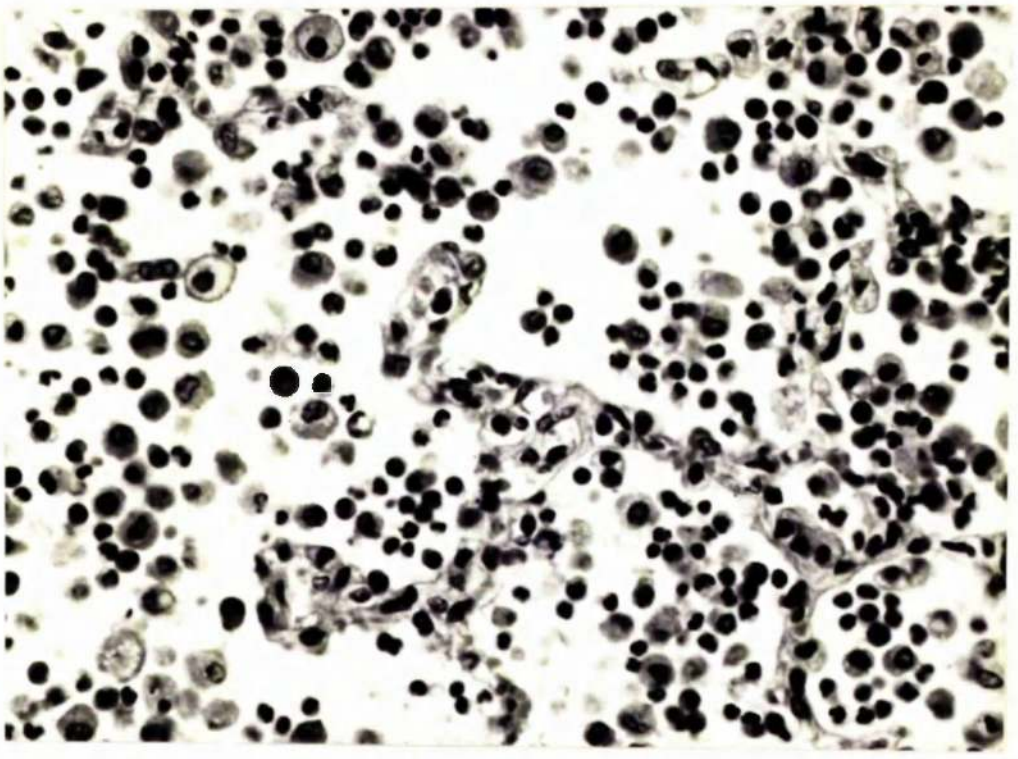


Fig. 17 Enzootic pneumonia: alveolar macrophages, plasma cells and polymorphonuclear leucocytes in alveolar exudate. H. & E. x 500

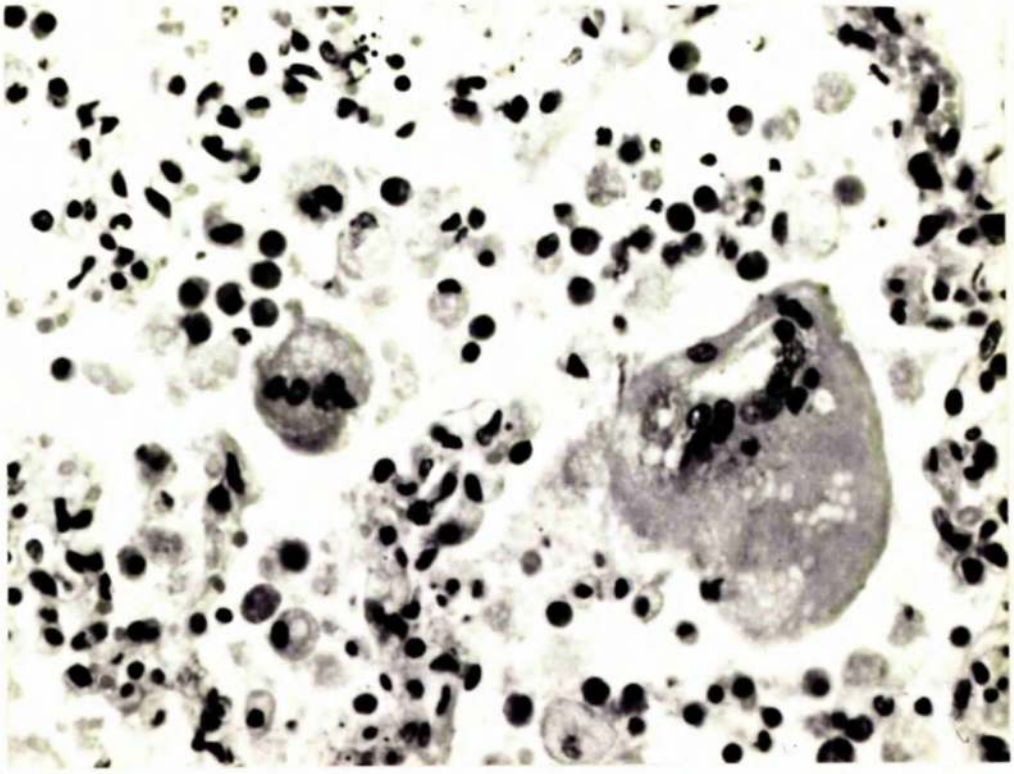
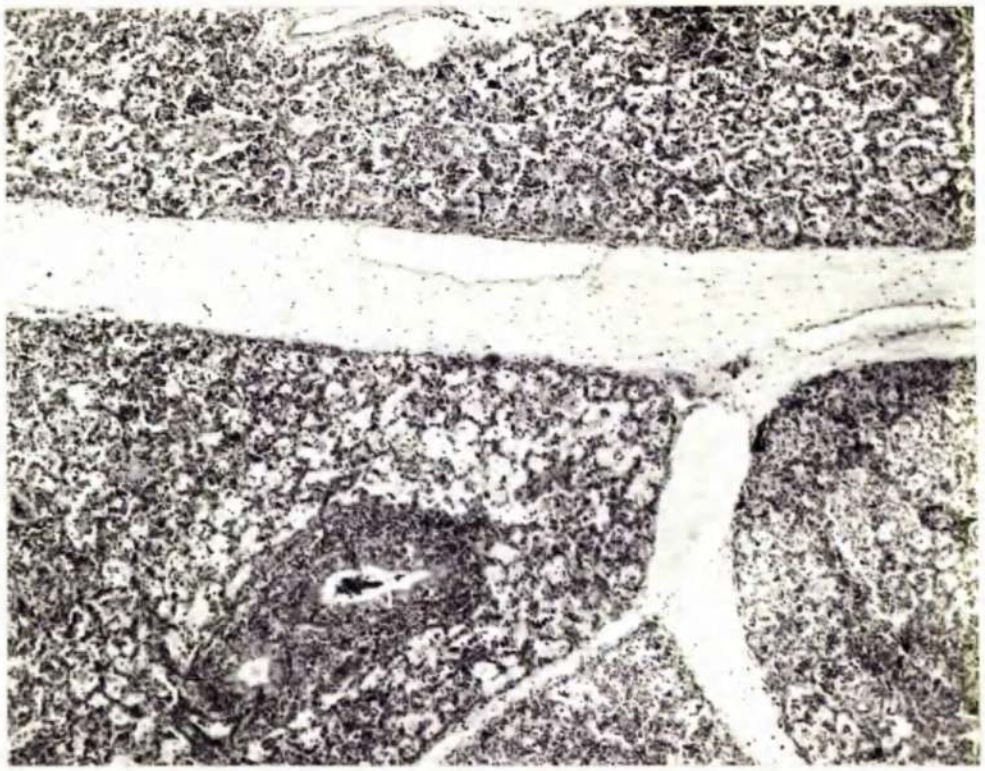
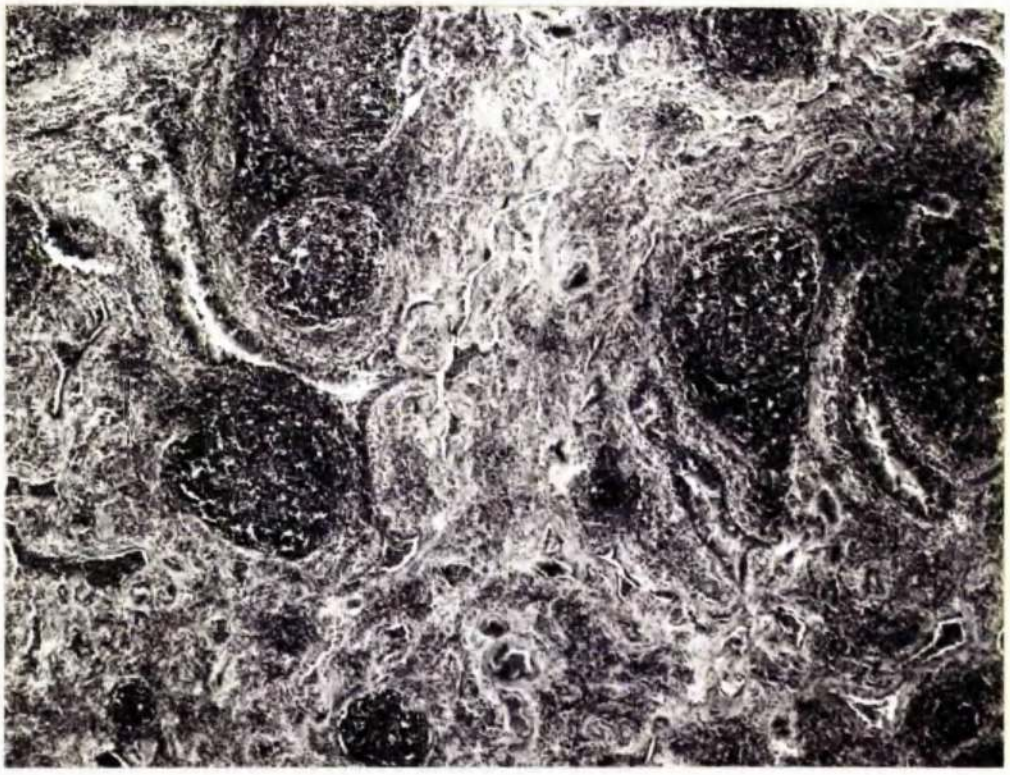


Fig. 18 Enzootic pneumonia: giant cells and plasma cells within alveoli. H. & E. x 500



19 Enzootic pneumonia: mild septal oedema and dilatation of septal lymphatics. H. & E. x 50



20 Enzootic pneumonia: alveolar collapse and fibrosis of alveolar walls. H. & E. x 50

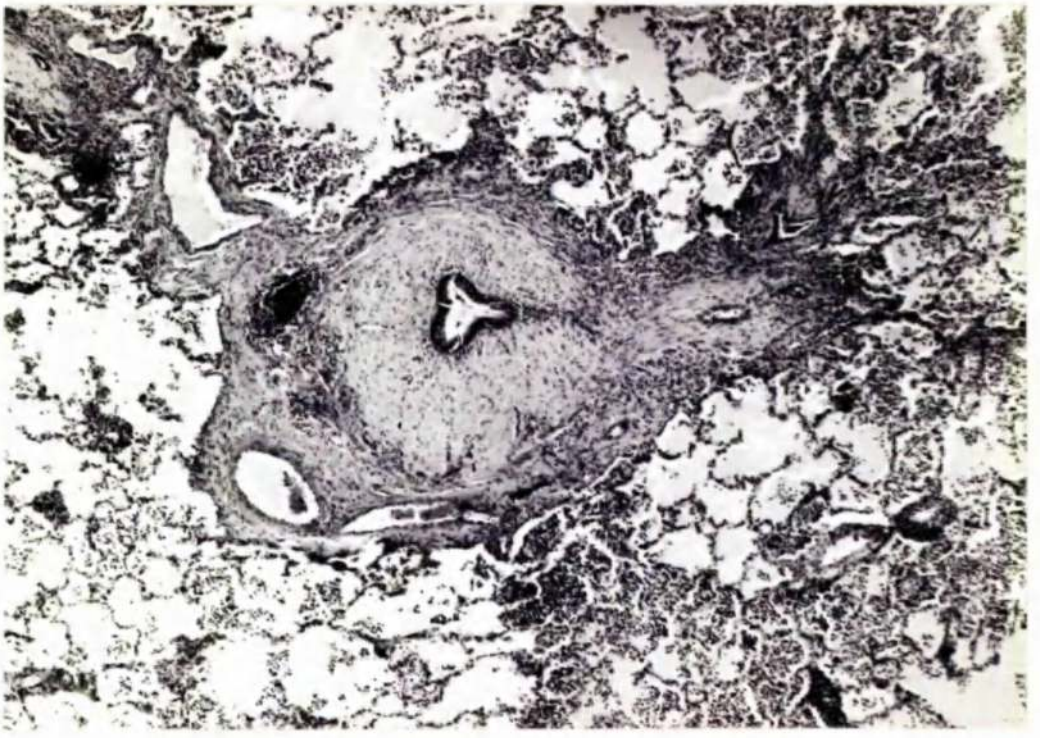


Fig. 21 Enzootic pneumonia: thickening of bronchiolar wall due to fibrosis of the lamina propria. H. & E. x100

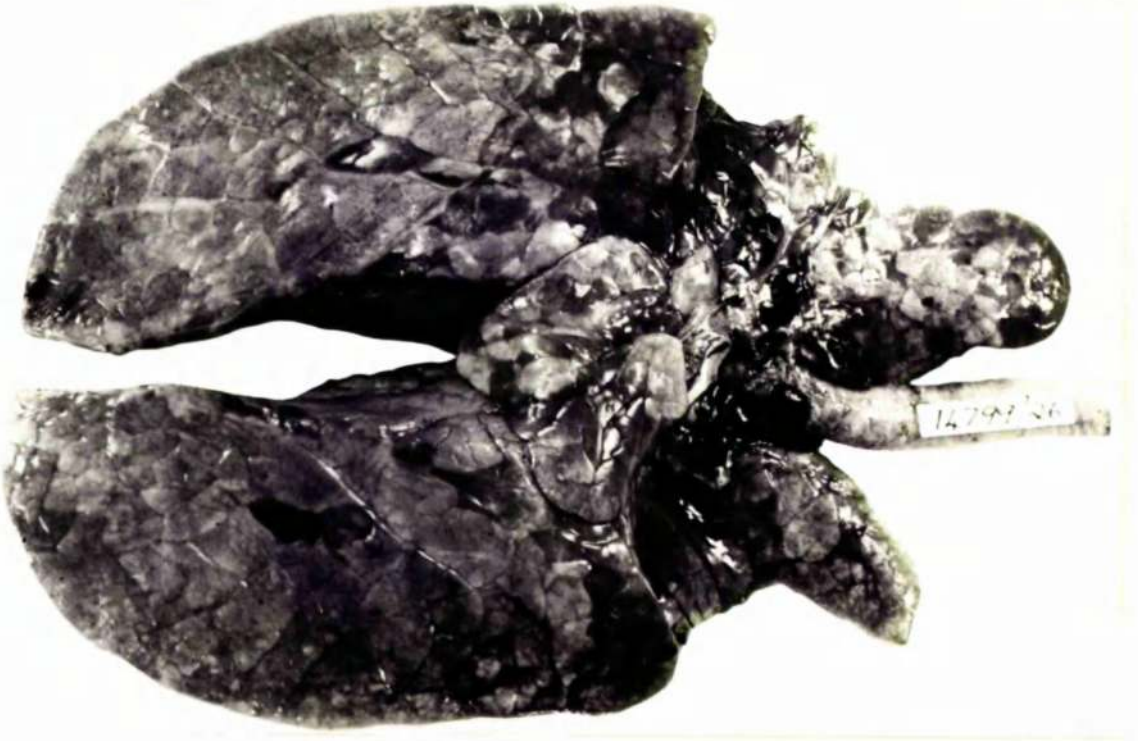


Fig. 22 Enzootic pneumonia: macroscopic appearance 8 weeks after experimental infection.

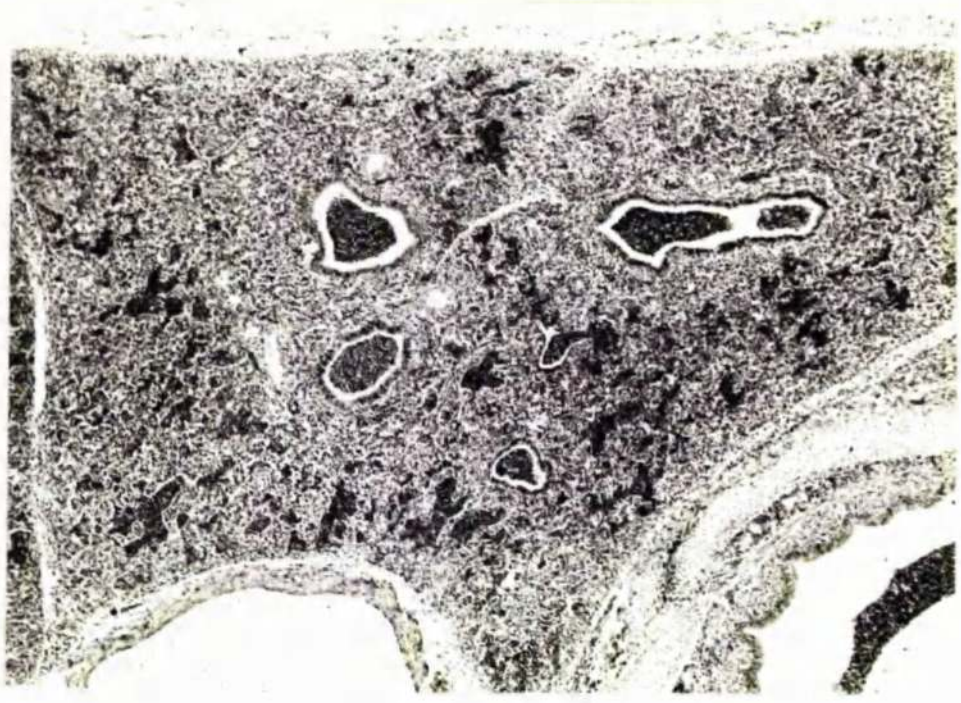


Fig. 23 Enzootic pneumonia (1 week): alveolar collapse and polymorphonuclear leucocytes in alveoli and bronchioles. H. & E. x 50

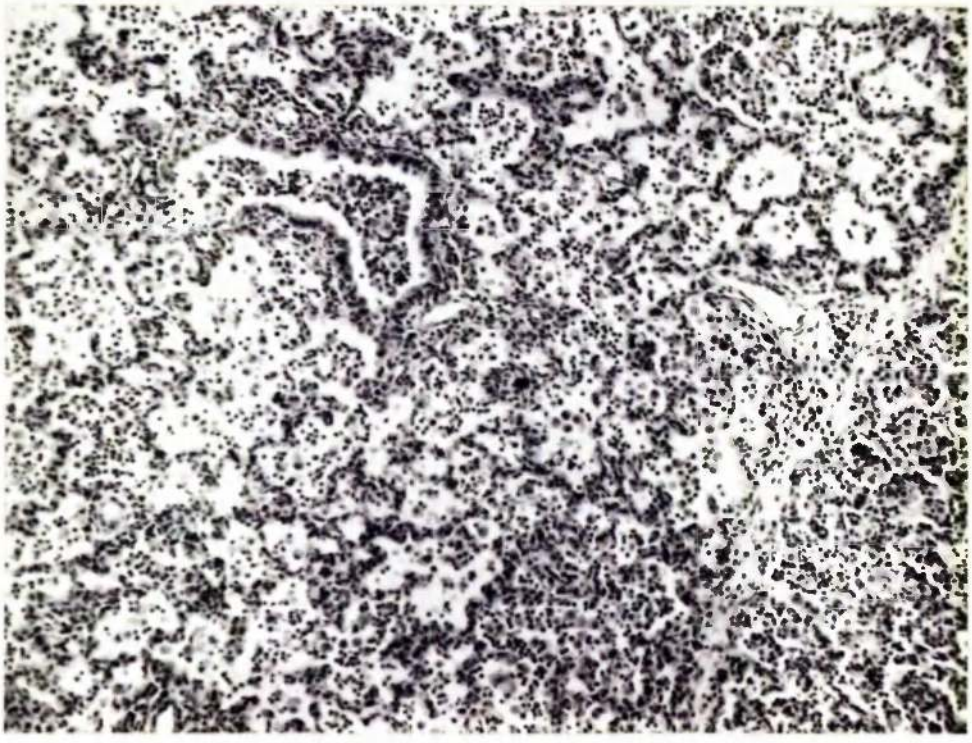


Fig. 24 Enzootic pneumonia (1 week): expanded alveoli full of cells and only a few cells in the peribronchiolar tissue. H. & E. x 150

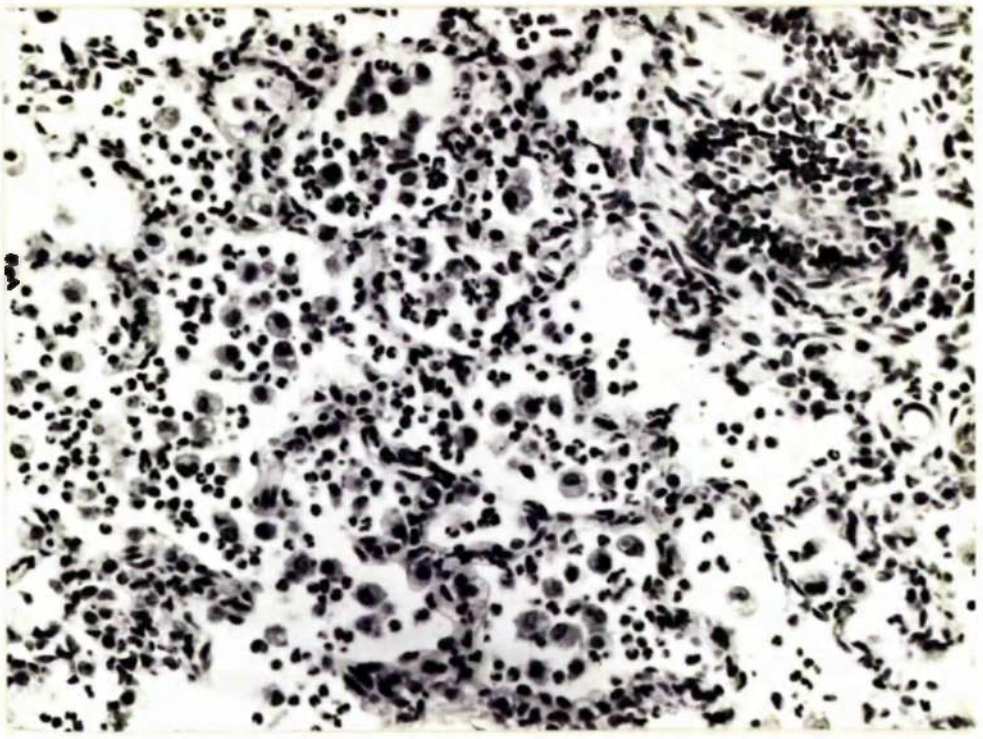


Fig. 25 Enzootic pneumonia (1 week): polymorphonuclear leucocytes and alveolar macrophages within alveoli. There is also alveolar cell hyperplasia. H.&E. x

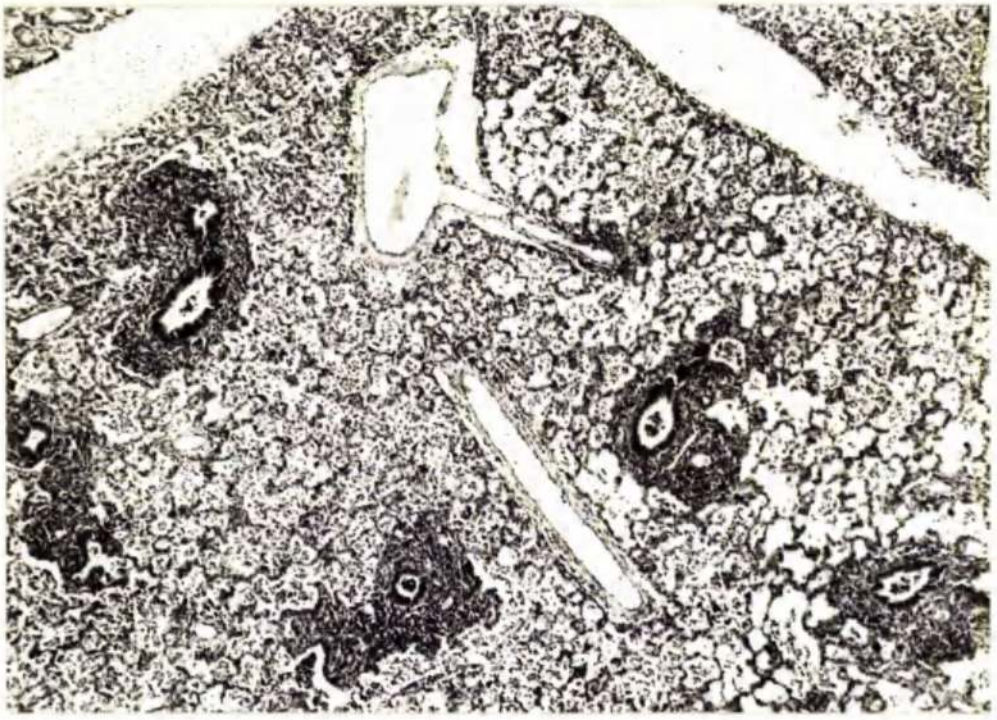


Fig. 26 Enzootic pneumonia (3 weeks): considerable thickening of peribronchiolar tissue by plasma cells and cells of the lymphoid series. H. & E. x 50

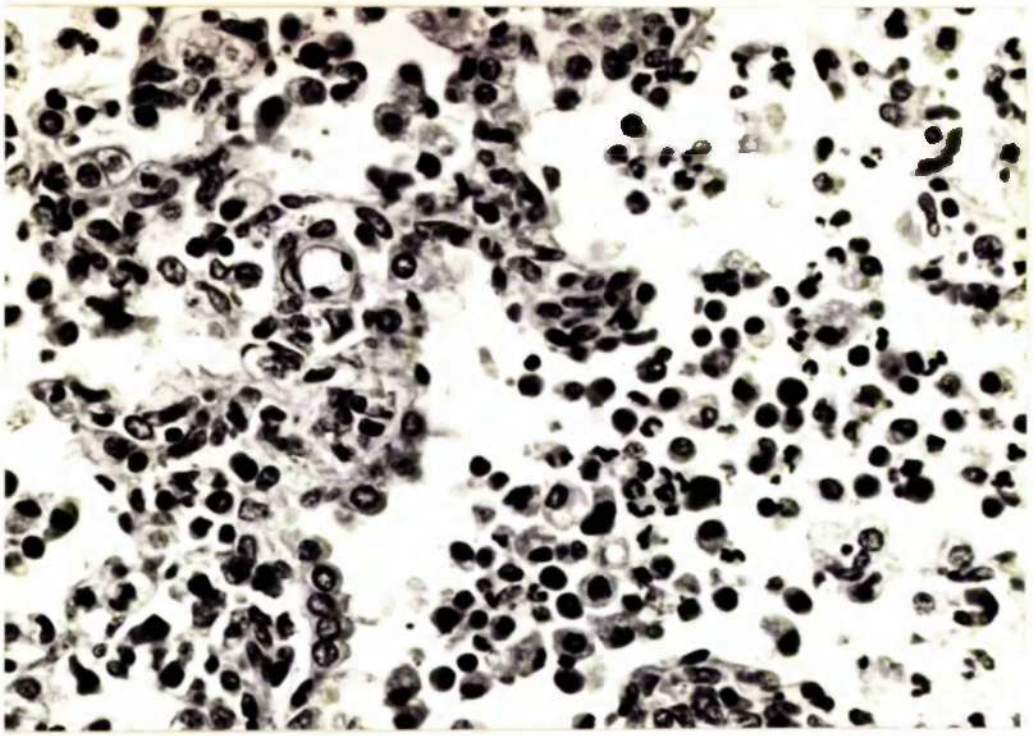


Fig. 27 Enzootic pneumonia (3 weeks): plasma cells, alveolar macrophages and polymorphonuclear leucocytes within alveoli. H. & E. x 500

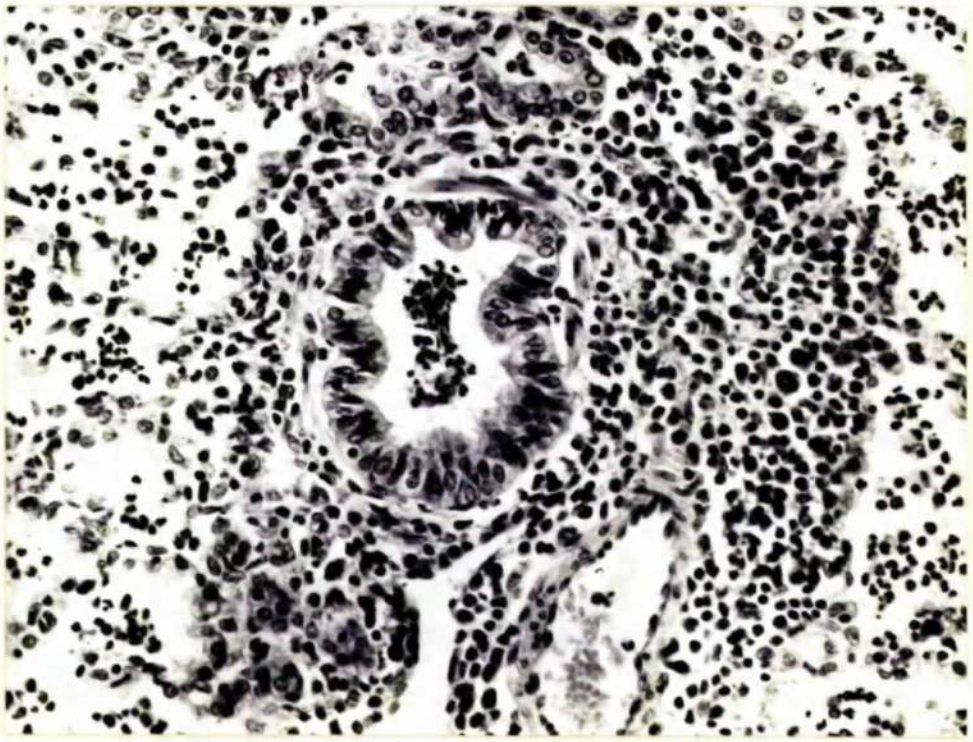


Fig. 28 Enzootic pneumonia (3 weeks): early peribronchiolar accumulation of cells and hyperplasia of alveolar cells in adjacent alveoli. H. & E. x 300

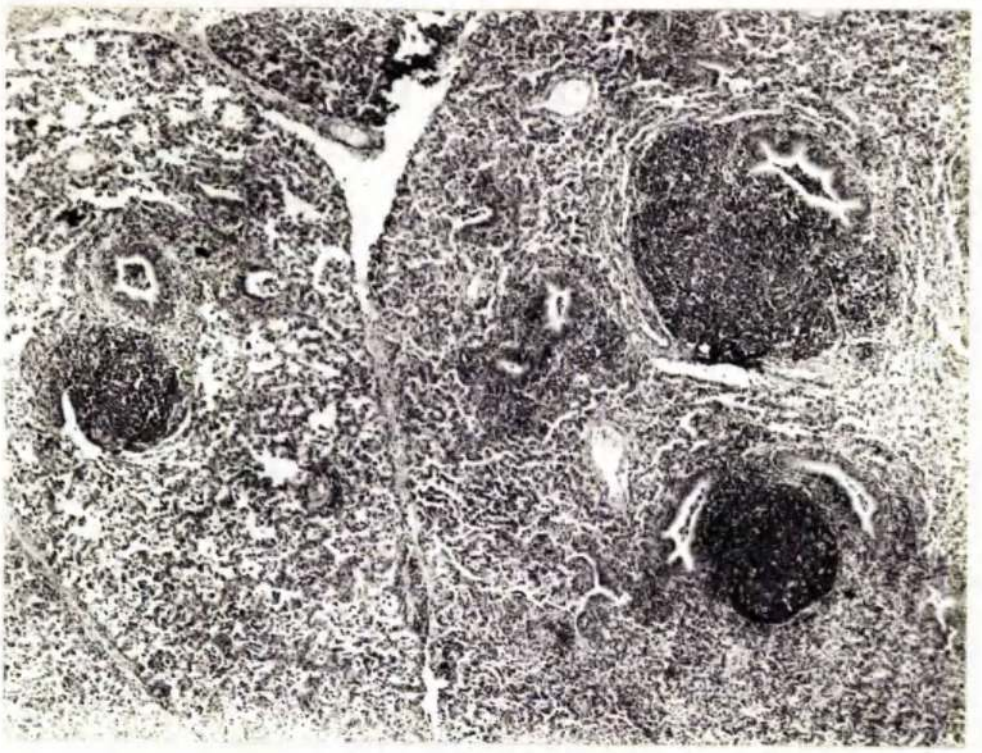


Fig. 29 Enzootic pneumonia (8 weeks): peribronchiolar lymphoid nodules and expanded alveoli. H. & E. x 50

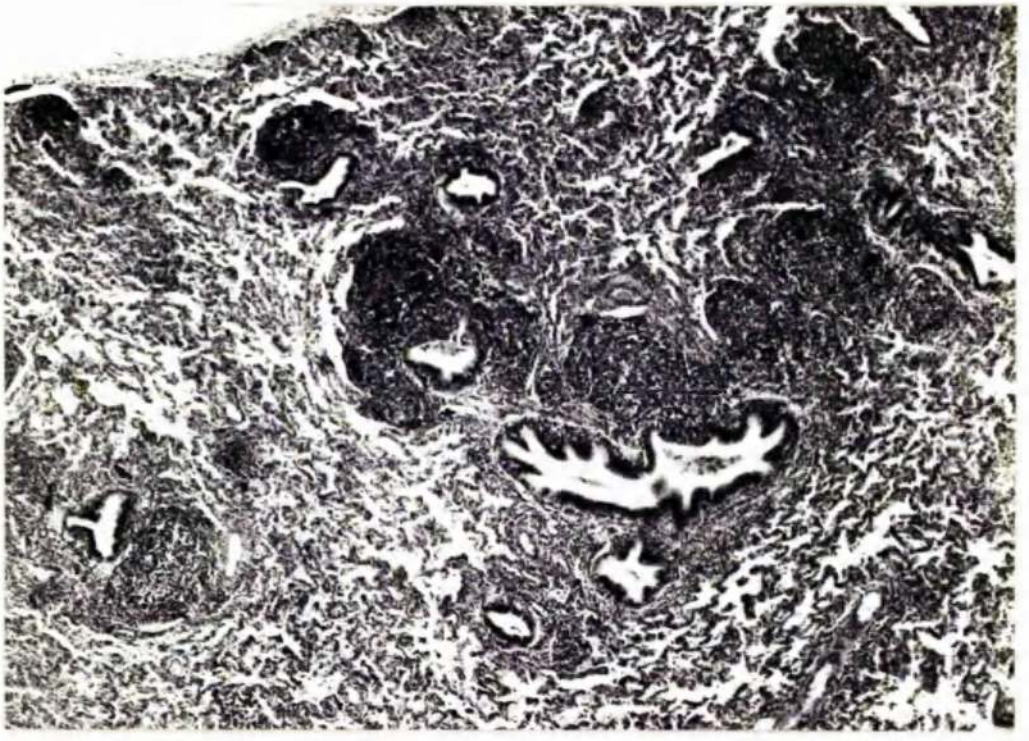


Fig. 30 Enzootic pneumonia (12 weeks): many peribronchiolar lymphoid nodules and alveolar collapse. H. & E. x 50

INTERSTITIAL PNEUMONIA

(1) The Pathology of the Disease Seen in the Field

Interstitial pneumonia was seen in thirteen pigs whose ages ranged from eight weeks to seven months. The disease was found in five of twenty two pigs selected at random on a farm with a pneumonia problem.

The range of clinical disturbances associated with the lesion were (a) sudden death (b) sudden deterioration of an existing pneumonic condition with the development of dyspnoea, coughing, fever and eventual collapse (c) a milder clinical course with no obvious respiratory disturbance apart from coughing, unless the animal was auscultated.

The history given on one farm was that there had been five deaths in a group of pigs three months old over a period of two months.

Macroscopic Findings

The consolidation produced by interstitial pneumonia generally assumed one of two types. One form was seen in severe cases with consolidation of lobar dimensions and the other tended to occur in cases where the lesions were more localised. In the latter instance the lesions sometimes only involved part of a lobule but more frequently a group of lobules was affected.

The most severe form produced bilateral consolidation of large areas of lung which could involve completely the apical lobes, the cardiac lobes, the intermediate lobe and as much as half of the total volume of the diaphragmatic

lobes at the same time. The consolidated portions were raised above the adjacent normal lung and were dark red or reddish brown. The interlobular septa were dilated and appeared as greyish or pale yellow lines. On the overlying pleura there was fibrinous pleurisy (Fig. 31). When the consolidated lung was palpated it felt full, and solid. It was heavier than normal and blocks of lung for histological examination sank in corrosive formol. The cut surface of a consolidated lobe had a velvety, granular appearance. It was mostly dark red in colour, but scattered about on the surface were reddish brown or yellowish patches which produced a mottled effect (Fig. 32). The interlobular septa were prominent as thick greyish or pale yellow lines and a similar linear appearance was sometimes seen extending into the lobules and around the bronchi.

When the lesion was seen in its second form it was present as a single patch of consolidation in one lobe or multifocally in several lobes of the lungs. Multifocal lesions were sometimes sufficiently large and numerous to produce almost lobar consolidation. In general the lesions were very obvious as a result of their yellow colour or because they were outlined by yellow lines. They felt solid and were raised above the surrounding lung. The pleural surface was yellow or appeared as an irregularly polygonal red area surrounded by prominent yellow bands which tended to follow the interlobular septa (Fig. 33). Within the lesion yellow bands could be seen outlining bronchi and blood vessels. A similar appearance was seen when

the lesions were sectioned and the interlobular septa were easily identified as greyish or yellow lines (Fig. 34). In some cases the surrounding yellow band was thickened by an outer layer of white fibrous connective tissue. The mucous membrane of the trachea and major bronchi was congested in severe cases and many dilated blood vessels could be seen in their walls. Blood-stained oedema fluid was sometimes found in the lumen.

The bronchial lymph nodes were enlarged, oedematous, congested and sometimes flecked with yellow patches of necrosis.

Microscopic Findings

The pneumonia had a striking appearance when examined with the lowest power (Fig. 35). There was a central group of alveoli whose walls were congested and whose lumina contained oedema fluid with variable amounts of fibrin and red blood corpuscles. Surrounding this and outlining the connective tissue structures of the lobules were basophilic bands. This appearance was produced by the alveoli adjacent to the interlobular septa, subpleurally, or around bronchi and bronchioles, and around blood vessels being filled with inflammatory cells and their debris (Fig. 36 and Fig. 37). Bands of alveoli packed with inflammatory debris however did sometimes extend across the centres of lobules. In addition the interlobular septa and their lymphatics were grossly dilated.

The reddish brown or yellowish areas in the severe type of lesion and the small wholly yellow lesion in the discrete form were foci of irregular

groups of alveoli packed with inflammatory cells and their debris. In the former type the alveolar walls were severely congested but in the latter there was necrosis of the alveolar walls. Bronchioles in the congested zone were usually either empty or full of oedema fluid, but those nearer the periphery were packed with cells.

Alveoli: The appearance of the congested alveoli in the lesions varied, in different fields (Fig. 37 and Fig. 38). Thrombosis of the capillaries in the alveolar walls and necrosis of the walls was a common occurrence. The necrotic walls however remained in position forming the outline of the alveoli. The lumina of the alveoli usually contained oedema fluid, variable amounts of fibrin, bacteria and red blood corpuscle, but in some alveoli there was frank haemorrhage. Groups of these alveoli had polymorphonuclear leucocytes in their lumina or else a mixture of polymorphonuclear leucocytes and small mononuclear cells with round, dark nuclei and a small amount of eosinophilic cytoplasm.

The alveoli at the edges of the lesions were packed with cells in various stages of disintegration. In some alveoli it was possible to identify the cells as resembling those seen nearer the centre of the lesion. In most, however, the cells were dead and had fused into a mass within the alveolar lumen. This mass had an eosinophilic background on which there pyknotic narrow nuclei all oriented in the same direction so that a "stre-a-ing" effect was produced. Some nuclei appeared cremated but others were

quite big and elliptical, with a diffuse basophilia over the cells, probably due to nuclear lysis. In the same sites fragments of nuclear debris, numerous bacteria and sometimes clear spaces were seen in these alveoli. The alveolar walls remained distinct in some fields but in others they were necrotic and often were unrecognisable. Another interesting feature of the necrotic cells in these alveoli was that the nuclei of some of them had a spermatazoan like shape due to a long basophilic stream of nuclear material extending from one end. Sometimes groups of these cells occurred together and the basophilic threads from them seemed to converge at roughly the same point in the adjacent alveolar wall.

The connective tissue of the structures adjacent to the alveoli was infiltrated with polymorphonuclear leucocytes and small mononucleors. In addition there were masses of nuclear debris and sometimes a lace like appearance was seen due to the presence of numerous clear circular spaces. The contents of the spaces varied, some of them contained shrunken necrotic cells or cell debris, but others were empty.

Bronchi and bronchioles: The bronchi and bronchioles in congested areas were either empty or contained oedema fluid, fibrin and red blood corpuscles. Bronchi and bronchioles particularly at the edges of the lesions were seen full of cells and cell debris similar to that described in the alveoli. In the bronchi desquamation of the epithelium was sometimes seen and there were excessive numbers of mitotic figures, in the surviving epithel-

ial cells. The lamina propria was congested and contained small numbers of plasma cells. Desquamation of the bronchiolar epithelium also occurred.

Septa and pleura: The changes in these structures were responsible for many of the characteristic features of the lesions. The interlobular septa and subpleural connective tissue were grossly oedematous and thickened. The lymphatics were dilated with oedema fluid and contained networks of fibrin often forming fibrin plugs. Masses of polymorphonuclear leucocytes and mononuclear cells were also found within the dilated lymphatics and in the connective tissue itself, in some fields (Fig. 36). The changes occurring at the edges of these structures and in the adjacent alveoli have been described. Sometimes this type of reaction was seen in a septum between two lobules which were otherwise normal. A fibrinous exudate infiltrated by varying numbers of inflammatory cells was seen on the pleura.

Blood vessels: Oedema of the perivascular tissues occurred and the perivascular lymphatics were dilated and sometimes contained fibrin plugs. Thrombi were found in the blood vessels particularly the veins and the venules but also, occasionally, the intralobular branchs of the pulmonary artery, in every case (Fig. 38). The thrombi often occluded the lumen but were sometimes mural. Necrosis of the walls of vessels and infiltration by polymorphonuclear leucocytes was also found.

The changes in the lobules adjacent to the discrete type of lesion

were either congestion of the alveolar walls with or without oedema fluid in the lumen, or thickening of alveolar walls due to increased numbers of alveolar cells and the accumulation of alveolar macrophages in their lumina.

Some lesions were surrounded by a zone of fibrous connective tissue, composed of young fibroblasts with basophilic cytoplasm, plasma cells, and capillaries.

Bronchial lymph nodes: The bronchial lymph nodes were congested and there were large numbers of polymorphonuclear leucocytes, small macrophages and red blood corpuscles in the sinusoids. There were not many lymphoid follicles with germinal centres but scattered throughout the dense lymphatic tissue were many lymphoblasts in mitosis, small lymphocytes and plasma cells. When areas of necrosis were present the reticulum of the lymph node was also destroyed.

Associated Lesions

Interstitial pneumonia was seen in lungs which were also affected with enzootic pneumonia and lungworms. Apart from pulmonary lesions with localised pleurisy, diffuse fibrinous pleurisy, fibrinous pericarditis and once fibrinous peritonitis were present. In four pigs diffuse or focal lesions of necrotic enteritis were found in the small intestine and in one of these a necrotising arteritis, similar to that seen in the lungs, involving blood vessels in the lamina propria.

Bacteriology

Pasteurella multocida was isolated from the lesions in the lungs of all but one case examined.

INTERSTITIAL PNEUMONIA

(2) Experimental Work

Since P. multocida was regularly associated with the lesions of interstitial pneumonia, it was decided to try to reproduce the disease using this organism.

Two groups of experiments were therefore performed, using the experimental animals described earlier.

Group 1

The lungs from a ten week old pig which had died of acute interstitial pneumonia were taken and a 1 in 3 suspension of the pneumonic lung was made in sterile isotonic saline. Ten mls. of this suspension were given intratracheally to two five weeks old pigs. One of the pigs showed a febrile response up to 105.6 on the second day and both were killed on the evening of the fourth day. The animal with the febrile response had a large patch of consolidation in the apical and subapical bronchopulmonary segments (Tallanti 1959) of the diaphragmatic lobe of the left lung. The lesion had a soft yellow centre and was surrounded by a red zone of congestion. Two similar but smaller lesions were found in the diaphragmatic lobe of the right lung and in the cardiac lobe of the right lung. There was also fibrinous pleurisy and fibrinous pericarditis. The other pig had only a small lesion of a similar type in the intermediate lobe of the right lung and it also had fibrinous pleurisy and pericarditis.

When the lesions were examined histologically they were seen to be like a necrotising bronchopneumonia rather than interstitial pneumonia. This was probably due to the fact that the material which was used had become rather badly contaminated before the lung suspension could be made. Pasteurella multocida, however, was re-isolated from the lungs of the above pigs and 10 mls. of a culture on blood agar from these animals were given intra-tracheally to two other five weeks old pigs. The daily temperatures of these pigs remained normal and when they were killed one week later no lesions were found in their lungs and the organism could not be re-isolated.

Group II

A similar type of pneumonia is known to occur in the calf and attempts were made to reproduce the lesion in pigs with a strain of P. multocida isolated from a calf with fibrinous pericarditis, fibrinous pleurisy, fibrinous peritonitis and fibrinous arthritis. During experiments with this organism in calves interstitial pneumonia, identical to that occurring in the pig, was produced in one animal. As a result of this finding an attempt was made to reproduce the disease in pigs with this strain of P. multocida.

The organism had been passaged twice in calves and after re-isolation 2 mls. of a broth culture were given intravenously to each of two ten weeks old pigs. The pigs showed a febrile response, up to 107.3 in one and 106.8 in the other, and were dull and anorexic. When they were destroyed four

days later both had fibrinous arthritis and one had endocarditis of the mitral valve. There were, however, no lung lesions. Pasteurella multocida was re-isolated from the joint lesion.

The isolate from these pigs was used to infect two more ten weeks old pigs. They were given 2.5 mls. of a six hours broth culture intravenously and when they were killed five days later, mild fibrinous arthritis of the hock joints, and in one animal the stifle joints, was found but there were no lung lesions. Both animals were febrile during the course of the experiment with temperatures up to 105.8 in one and 105.6 in the other. P. multocida was recovered from the hock joints of one animal.

Two other pigs of the same age had been infected intratracheally at the same time with 2.5 mls. of the culture. The pigs were afebrile during the five days which the experiment lasted. When they were killed a small abscess about 0.5 cm. in diameter was found in the lung of one animal and P. multocida was recovered from the abscess. No lesions were found in the lungs of the other animal and no organisms were recovered. The joints in both of these animals were also normal.

Conclusions

It was not found possible to reproduce interstitial pneumonia in the experimental pigs either by infecting animals intratracheally with suspensions of pneumonic lung containing P. multocida or by using cultures of P. multocida

injected intravenously or intratracheally.

A strain of this organism however originally isolated from a calf, was able to produce fibrinous arthritis in experimental pigs when given intravenously.

INTERSTITIAL PNEUMONIA

(3) Discussion

The term interstitial pneumonia has been used to describe two different types of reaction in the lung.

In one the lesion is an acute exudative inflammation in which the changes in the interstitium particularly the interlobular septa dominate the pathology. This is typified by the lesions seen in contagious bovine pleuro-pneumonia and interstitial pneumonia in the calf (Jarrett 1956), and is the sense in which the term is used here.

The second type of reaction to which the term interstitial pneumonia has been applied is a proliferative reaction in the interstitial tissues particularly that of the alveolar walls and the bronchial system and is characterised by primary atypical pneumonia in man and other pneumonias, of viral or rickettsial origin. In this sense enzootic pneumonia in the pig is an interstitial pneumonia.

It would seem preferable however to use the term as it is used here since the domestic animals, particularly the pig and the calf have well developed interlobular tissue which may play an important part in the development of a lesion and in its final anatomical appearance.

Interstitial pneumonia is a histopathological entity invariably associated with P. multocida and it is likely that this organism is

responsible for the condition. A similar pneumonia in calves (Jarrett 1956) is also associated with P. multocida but attempts to reproduce the lesions in pigs and calves with the organism have been mostly unsuccessful. The syndrome in pigs described as 'secondary breakdown' which is associated with yellow necrotic patches in the lungs teaming with bipolar organisms (Betts 1952) was probably this lesion. Schofield (1956) described a primary Pasteurella pneumonia in the pig with a histopathological picture which he considered typical for the disease, but was unable to reproduce it even with strains of P. multocida which were lethal for mice.

The macroscopic appearance of this pneumonia in its severe diffuse form is reminiscent of lobar pneumonia in man except that the lesion is bilateral. It is likely that spread to contigous tissue in the lung is by direct diffusion of organisms through the tissues via the pores of Kohn, in oedema fluid filling the bronchioles and the bronchi and spilling down into adjacent lobules, and also by extension along the lymphatic channels in the septa. This latter route would allow organisms to enter a septum and proceed from there to the adjacent lobule. Differences in the susceptibility of animals and differences in the virulence of the infecting strains of P. multocida probably account for the variations between the diffuse and the discrete form of the disease.

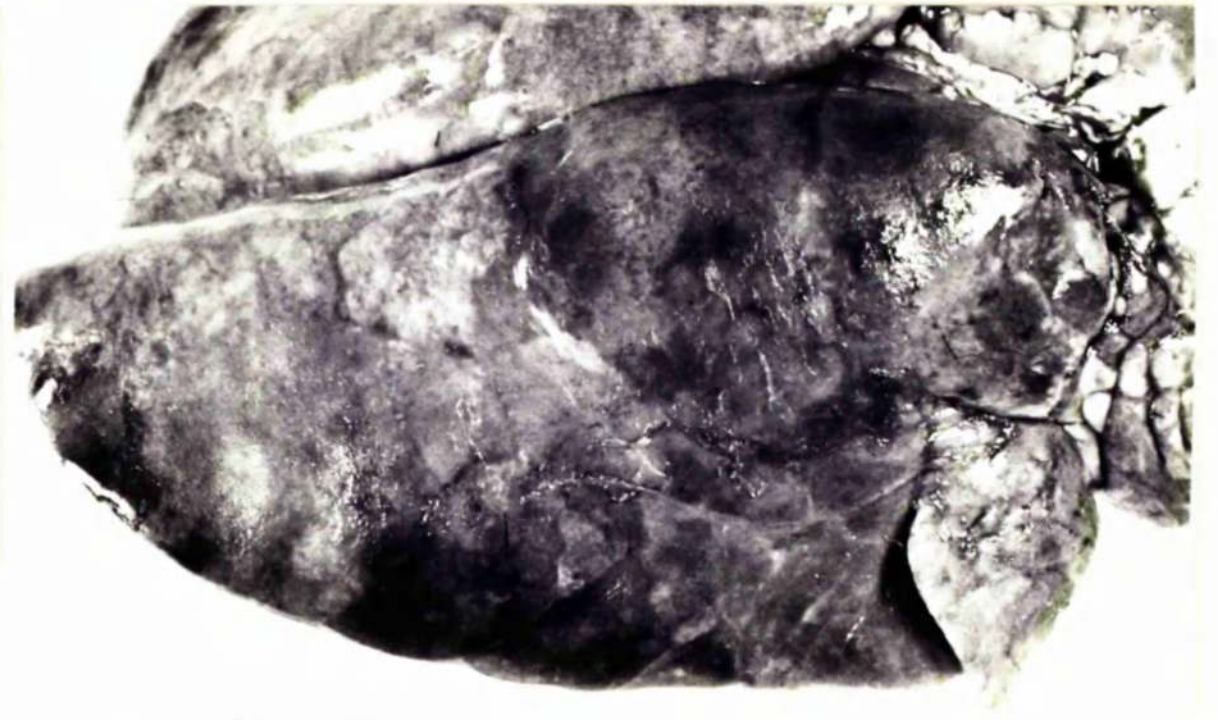


Fig. 31 Interstitial pneumonia: consolidation with overlying fibrinous pleurisy, involving most of the right lung.

Fig. 32 Interstitial pneumonia: cross section of a diaphragmatic lobe showing complete consolidation and mottled appearance of cut surface.

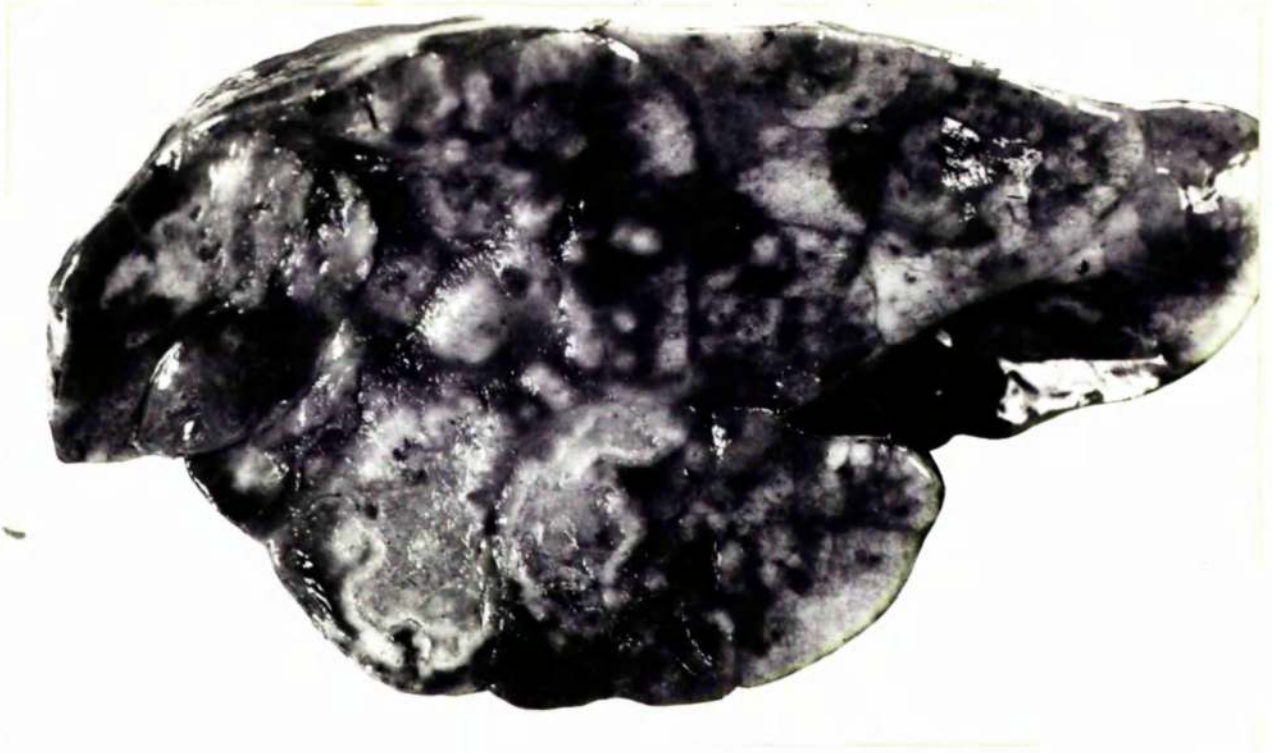


Fig. 33 Interstitial pneumonia: several discrete lesions in an intermediate lobe.

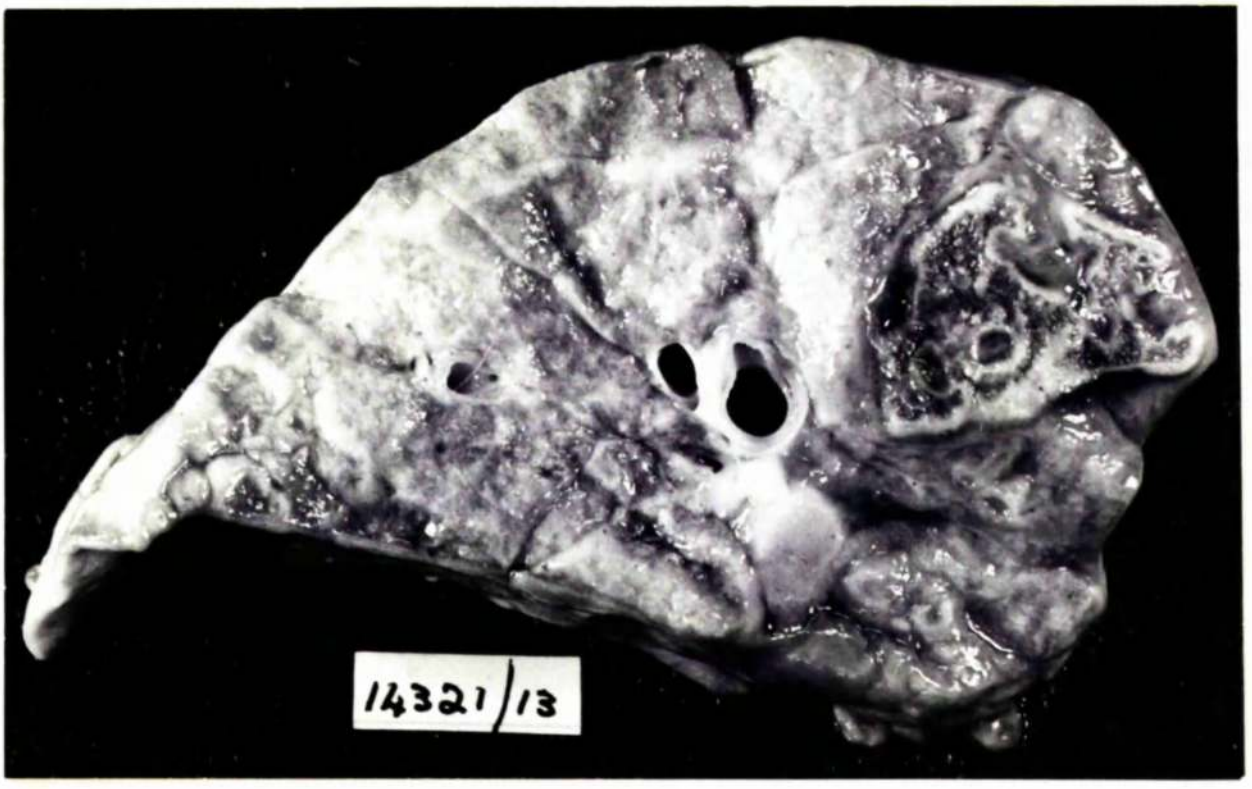


Fig. 34 Interstitial pneumonia: cross section of a well demarcated lesion with a red central area surrounded by yellow lines.

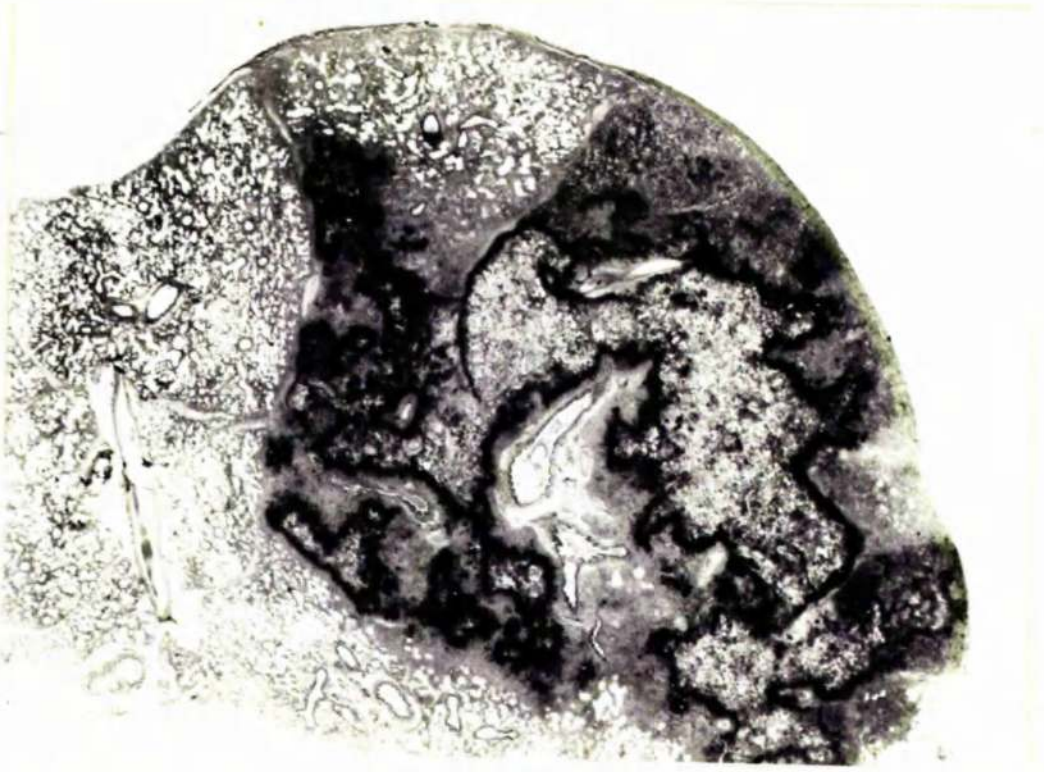


Fig. 35 Interstitial pneumonia: typical appearance when viewed under the lowest power objective. H. & E. x 10

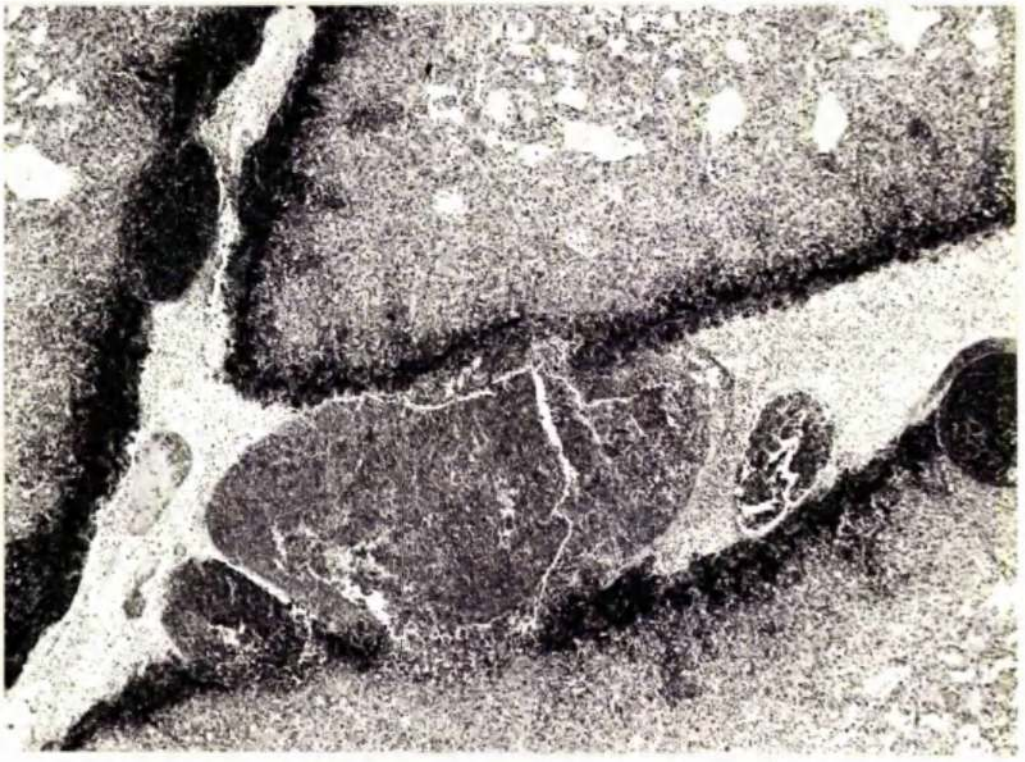


Fig. 36 Interstitial pneumonia: dilated septal lymphatics full of polymorphonuclear leucocytes and mononuclear cells. H. & E. x 50

Fig. 37 Interstitial pneumonia: severe congestion of alveolar walls and many cells within the alveoli, particularly adjacent to the blood vessels. H. & E. x 50

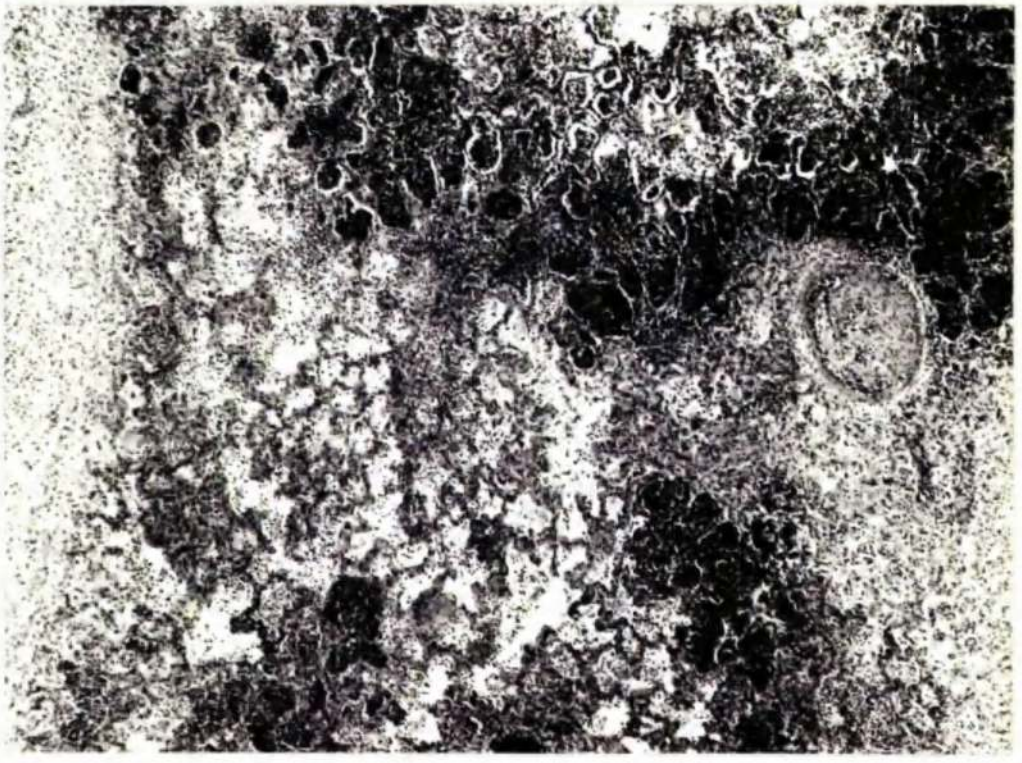


Fig. 38 Interstitial pneumonia: complete occlusion a pulmonary vein by a thrombus. H. & E. x 50

PNEUMONIA DUE TO HAEMOPHILUS PARAINFLUENZA

(1) The Pathology of the Disease seen in the Field

One pig was seen with this condition. According to the farmer the animal had been well, until one morning when it was seen "vomiting". He did not observe any respiratory disturbance and it was found dead next day.

Macroscopic Findings

The apical lobes, most of the cardiac lobes, part of the intermediate lobe and the antero-ventral parts of the diaphragmatic lobes were consolidated. The affected regions were very firm and felt full. The lung was reddish brown and mottled by small yellow dry-looking areas of necrosis which occasionally had a tendency to have straight edges (Fig. 39). There was overlying fibrinous pleurisy. The cut surface of the lung was very oedematous and was also mottled with yellow areas of necrosis. Portions of lung for histological examination sank in corrosive-formol. In the trachea and around the animal's lips there was a large amount of white foam.

The bronchial lymph nodes were enlarged and congested and contained flecks of yellow necrosis.

Microscopic Findings

The most striking feature noticed on low power examination was the dense basophilic masses of cells packing alveoli so that nodular looking

predominantly basophilic areas of various sizes were produced (Fig. 40 and Fig. 41). Only a few alveoli were involved in some fields while in others large sections of lobules were affected. This appearance was due to masses of inflammatory cells many of which were necrotic packing the alveoli, alveolar ducts and sometimes the bronchioles. The alveolar walls within the foci were congested and prominent in some areas, but in others they were necrotic and had fused with the surrounding mass of inflammatory cells. In some of these lesions there was a central group of alveoli which contained only a few cells, but had congested walls and were full of oedema fluid which gave the lesions the appearance of having a clear central area (Fig. 41). Bacteria could be seen in some of these central alveoli (Fig. 42). The cells in the alveoli responsible for the basophilic mass were a mixture of polymorphonuclear leucocytes and small mononuclear macrophages, which had round intensely staining nuclei and eosinophilic cytoplasm. The mononuclear cells predominated. Many of these cells were completely necrotic and had undergone karyolysis. In addition mixed with them were cells whose nuclei were crenated or simply elongated pynknotic ellipses (Fig. 43). Often groups of these were found lying together with the nuclei oriented in the same direction and these alveoli had a whorled or "streaming" appearance. Numerous bacteria were seen in the alveoli amongst the necrotic cells. The surrounding alveoli often had congested walls and were full of oedema fluid, containing fibrin strands and intralveolar haemorrhage was seen in several

fields.

There were many dilated and congested blood vessels in the walls of the bronchi and the bronchioles. In the lumina of many bronchioles cells and debris similar to that in the alveoli and alveolar ducts had accumulated. The interlobular septa were oedematous and often considerably thickened. The lymphatics within the septa and in the pleura were dilated, and contained red blood corpuscles, polymorphonuclear leucocytes, mononuclear cells and sometimes plugs of fibrin. Polymorphonuclear leucocytes and mononuclear cells were also frequently found in the septal connective tissue. On the pleura over the lesion there was a fibrinous exudate.

There was severe congestion and haemorrhage in the bronchial lymph nodes. The loose lymphatic tissue was full of polymorphonuclear leucocytes. Decreased numbers of small lymphocytes were seen around the follicles in the nodes and the cells of the follicles, most of which were lymphoblasts or reticulum cells were separated from each other to a greater degree than normal. Several areas of necrosis were also found.

Bacteriology

An organism identified as Haemophilus parainfluenza was isolated from the lungs of this animal.

PNEUMONIA DUE TO HAEMOPHILUS PARAINFLUENZA

(2) Experimental Work

It was decided to attempt to reproduce this pneumonia with pure cultures of H. parainfluenza isolated from the field case.

The pigs and the method of infection used were those described in Materials and Methods.

Two six weeks old pigs 16960/5 and 16960/6 were injected intratracheally with 15 mls. of a 24 hour broth culture of H. parainfluenza isolated from the lungs of the pig which died. Two hours after the animals were infected their temperatures were 107.2 and 107.0 respectively. Their temperatures returned to normal within 24 hours however, and remained normal until they were killed four days after infection. One animal, 16960/6, was heard coughing on the third day of the experiment and also on the fourth day. At post-mortem a small yellow lesion about 0.4 cm. in diameter was seen in the diaphragmatic lobe of 16960/5. The lesions in the other pig, 16960/6, were more extensive. Two slightly raised, firm patches of consolidation 0.5 cm. in diameter were found in the apical lobe of the right lung. They had soft yellow central areas surrounded by a grey edge. Similar lesions were seen on the dorsal border of the diaphragmatic lobe of the right lung and in the diaphragmatic lobe of the left lung. Some of them were covered by a localised patch of pleurisy.

Histological examination of these lesions showed that they consisted of a central area of necrosis with destruction of the pulmonary architecture and were surrounded by a zone of proliferating fibrous connective tissue. They did not resemble the pneumonia found in the original pig. Haemophilus parainfluenza could not be re-isolated from them. Two pigs from the same litter were kept in the same accomodation but not in contact with the infected pigs as control animals. When they were killed there were no lesions in their lungs.

Haemophilus parainfluenza was re-isolated from pieces of the original lung which had been kept at -40°C. Two six weeks old pigs, 16960/7 and 16960/8, were infected intratracheally with 15 mls. of a 24 hour broth culture and two other pigs from the same litter were kept as controls. Eighteen hours later 16960/7, was found dead although it had apparently made an uneventful recovery from the manipulations required for the intratracheal injection. The other pig, 16960/8, was clinically normal for the duration of the experiment and was killed seven days after infection along with the two control animals who were also normal. At post mortem no lesions were found in the controls. Two small patches of consolidation similar to those found in 16960/5 and 16960/6 were present on the dorsal border of the diaphragmatic lobe of both lungs of 16960/8. Haemorrhagic fluid was found in the trachea of 16960/7 and there was severe pulmonary oedema with dilatation of the interlobular septa. There were several large patches of consolidation

in the cardiac and diaphragmatic lobes of both lungs which were either greyish-pink or dark red. The overlying pleura was covered with a fibrinous exudate. Histological examination of the lesions in this pig showed that they were essentially similar to those occurring in the original case (Fig. 44). Haemophilus parainfluenza was recovered from the lungs of this animal but not from 16960/8.

Conclusions

Haemophilus parainfluenza was capable of producing lesions in the lungs of experimental pigs by itself, and in one animal from which the organism was re-isolated the pulmonary lesions were histologically similar to those seen in the field case. The pneumonic patches in the other animals were very localised and were not progressing, being in fact surrounded by fibrous tissue. The organism could not be re-isolated from these lesions.

PNEUMONIA DUE TO HAEMOPHILUS PARAINFLUENZA

(3) Discussion

A pneumonia histopathologically and bacteriologically similar to the case described here has already been recorded (Pattison, Howell and Elliot, 1957, Mathews and Pattison 1961). These workers considered that the histopathology was specific for the disease and although initially they were only able to reproduce the lesion by infecting pigs with swine-fever virus and the Haemophilus organism at the same time, they succeeded later in producing the typical lesion with the Haemophilus alone.

The organism isolated from the case described here was also capable of causing the disease by itself, and confirmed the findings of the workers referred to earlier that this pneumonia is a specific entity aetiologically and pathologically.

The histology of the dense cellular masses in the alveoli resembled the changes seen in some alveoli in cases of interstitial pneumonia but the general pattern of the lesion was different and vascular involvement was not an important feature of the disease.

Fig. 39 H. parainfluenza: a cardiac lobe in which small yellow areas of necrosis can just be seen against the reddish brown background.

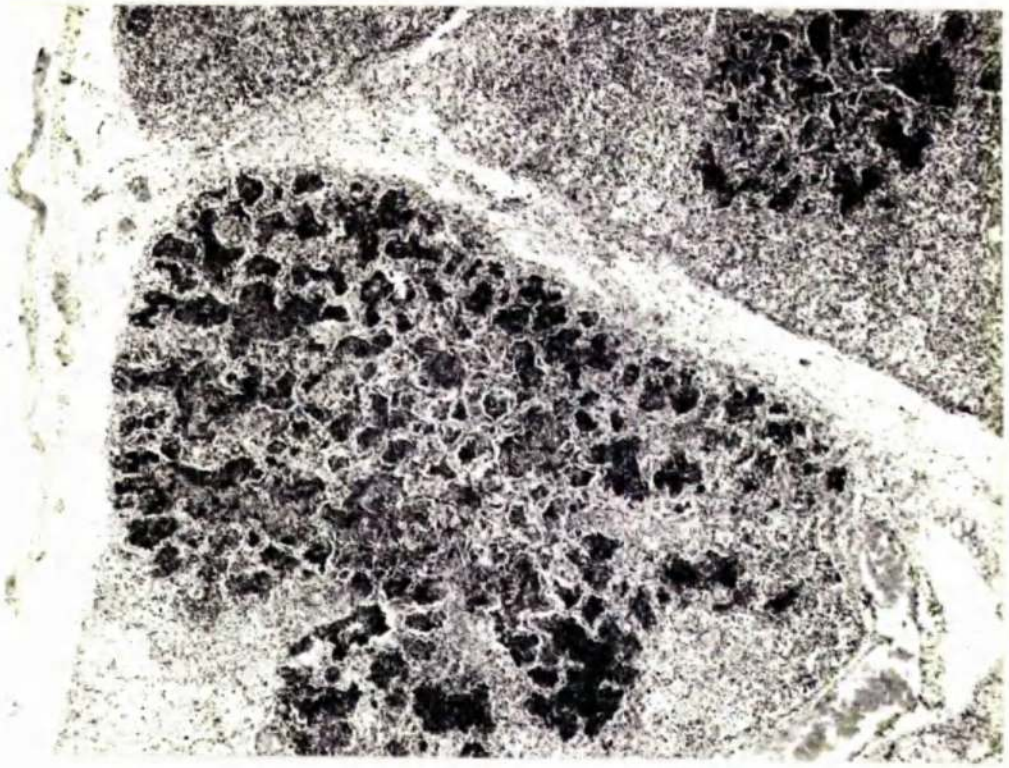


Fig. 40 H. parainfluenza: part of a lobule with alveoli full of basophilic mass of inflammatory cells and debris. H. & E. x 50

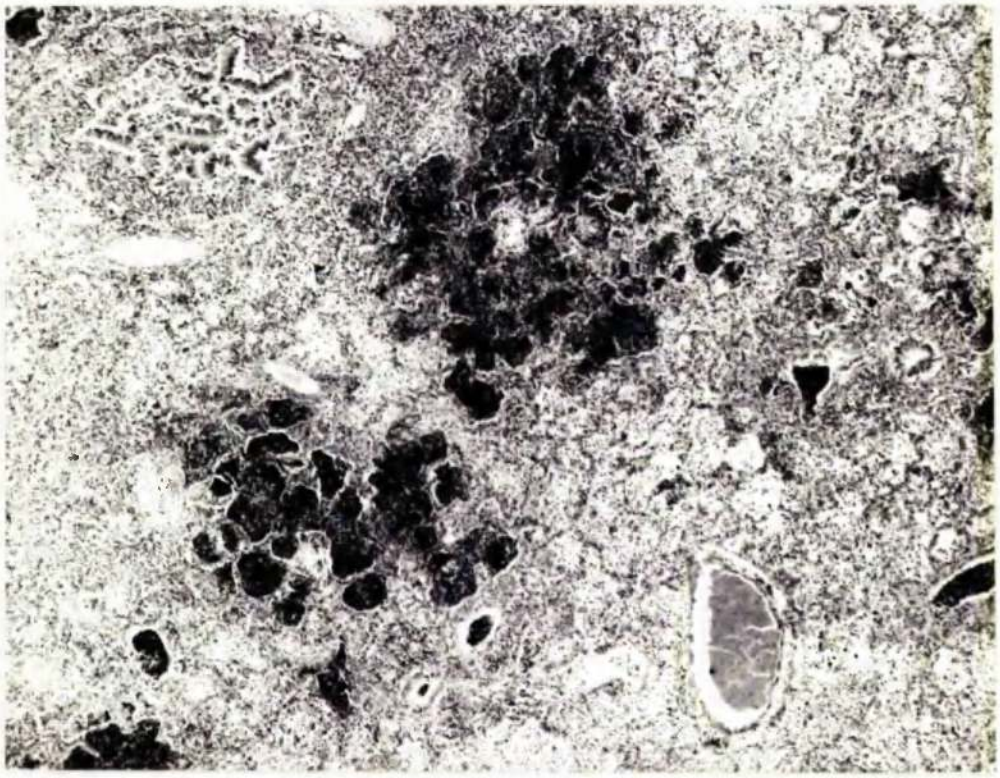


Fig. 41 H. parainfluenza: two basophilic zones, one of which has a small central clear area. H. & E. x 50

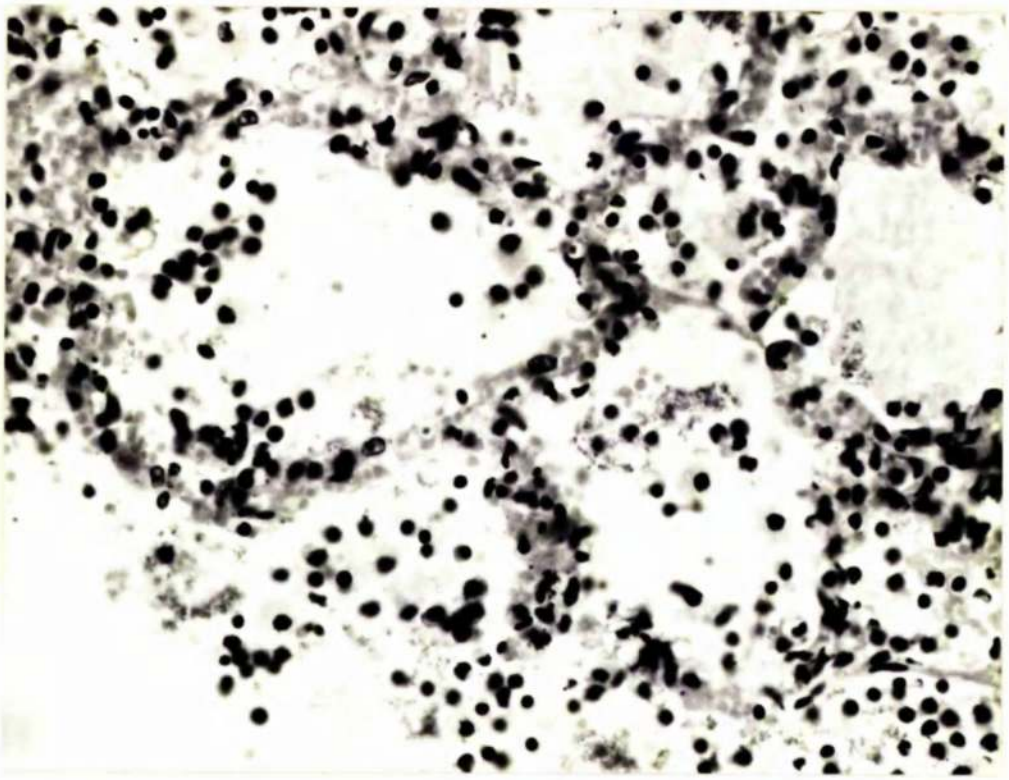


Fig. 42 H. parainfluenzae: clumps of bacteria in alveolar oedema fluid.

H. & E. x 500

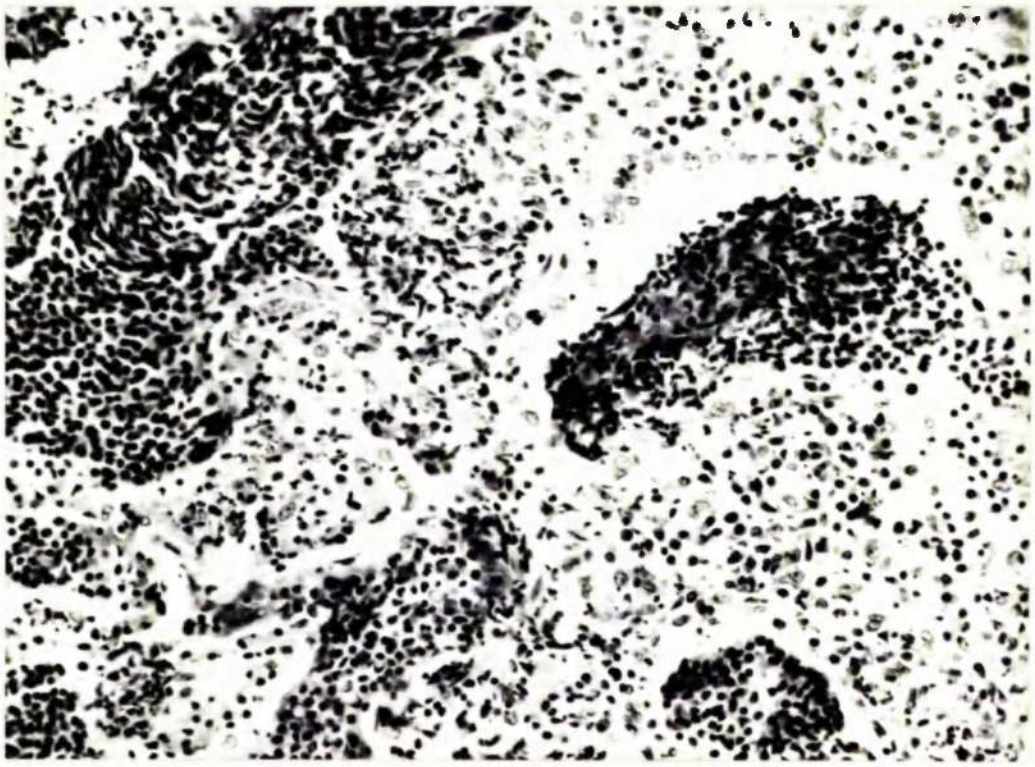


Fig. 43 H. parainfluenzae: detail of the b sophilic masses of cells within the alveoli. H. & E. x 300

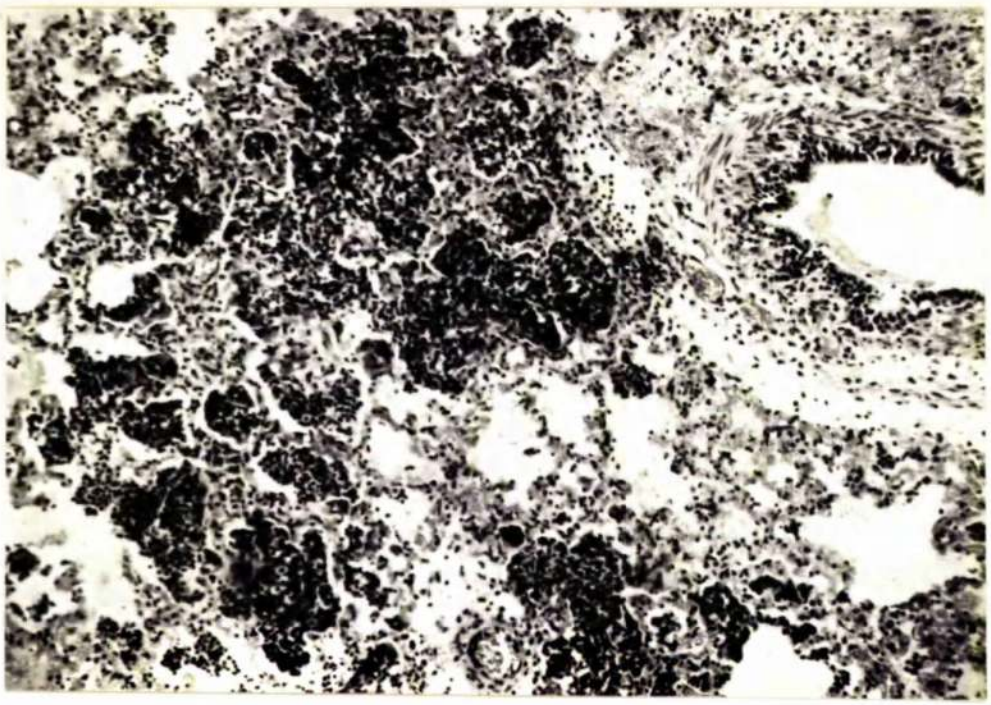


Fig. 44 Pneumonia produced by injecting H. parainfluenza intratracheally.

H. & E. x 150

SIMPLE ACUTE BRONCHOPNEUMONIA

Simple acute bronchopneumonia as a primary pulmonary lesion was seen in 18 pigs. The ages of eleven of these animals ranged from one month to three months and the remaining seven specimens were found in abattoirs.

The clinical signs were sometimes pyrexia, dyspnoea, coughing and dullness, but in four cases the pigs were found dead, and had apparently been normal prior to death.

Macroscopic Findings

Macroscopically in some lungs the lesions had a patchy distribution in all of the lobes with groups of three or four consolidated lobules occurring together. Oftener, however, the consolidated lobules had become confluent and large portions of lung were affected (Fig. 45). When this happened in the apical or cardiac lobes the whole lobe could be consolidated. The apical, cardiac, and antero-ventral parts of the diaphragmatic lobes of the lungs were the sites most frequently involved. The lesions were usually on the same level as the surrounding normal lung, but in a few cases were slightly lower than the surrounding normal tissue. They felt firm and the colour varied from intense dark red to brick red to greyish pink.

On the pleural surface and cut surface of the lesion groups of small white spots could usually be seen.

The cut surface appeared granular and sometimes exuded copious amounts of thin turbid fluid. Material which was viscus and grey or yellow could be

expressed from the cut ends of the bronchi and the interlobular septa were moderately thickened due to oedema in many cases.

The bronchial lymph nodes were enlarged, congested and oedematous.

Microscopic Findings

The histological appearance of the lesions varied according to the part of the lung examined since these often represented different stages in the development of the pneumonia.

The earliest stage seen was congestion of the capillaries in the alveolar walls, the bronchiolar and bronchial walls and also in the interlobular septa and pleura. In these lobules or parts of lobules there was oedema fluid in the alveoli and usually also in the bronchioles and bronchi. Small numbers of polymorphonuclear leucocytes were found in this fluid in the alveoli, alveolar ducts and terminal bronchioles.

The number of polymorphonuclear leucocytes increased and lobules seen at this stage had a heavy but patchy infiltration by polymorphonuclear leucocytes in alveolar ducts and alveoli, particularly those near bronchioles (Fig. 46). Large numbers of polymorphonuclear leucocytes were also seen within the lumina of the bronchioles themselves. The interlobular septa were dilated with oedema and the lymphatics in them, and the pleura were widely patent. Small amounts of fibrin were found in the alveolar oedema fluid and in the dilated lymphatics.

As the cellular infiltration increased all of the alveoli within a lobule became packed with polymorphonuclear leucocytes and alveolar macrophages were found in moderate numbers amongst the polymorphonuclear leucocytes. The lumina of the bronchioles were also packed with a similar mixture of cells and these could also be found in the bronchi, mixed with variable amounts of mucus (Fig. 47). The bronchial walls were congested and polymorphonuclear leucocytes were seen in the lamina propria along with some macrophages. In some cases desquamation of the bronchial epithelium had occurred and was quite severe. An occasional small peribronchiolar follicle was detected, in some pigs. These showed separation of the cells by oedema fluid and contained only a few mitotic figures. The peribronchiolar lymphatic vessels were dilated.

Intra-alveolar haemorrhage occurred in some cases and macrophages containing haemosiderin were found. Some of the alveolar cells were more prominent and numerous than usual but did not protrude into the alveolar lumina in large numbers as in enzootic pneumonia. In advanced lesions focal necrosis of the alveolar walls was seen.

The lymphatic vessels in the septa and pleura of well established cases were often full of polymorphonuclear leucocytes and fibrin networks.

There was little reaction in the walls of the larger bronchi and the trachea. Plasma cells were found around the glands in moderate numbers. The lumina of the larger bronchi and trachea however contained a mixture

of mucous, precipitated protein, many polymorphonuclear leucocytes, some alveolar macrophages, desquamated epithelial cells and necrotic cellular debris.

Histological examination of the bronchial lymph nodes showed that the sinusoids were full of polymorphonuclear leucocytes. Many mitotic figures could be seen in the germinal centres of follicles.

Bacteriology

Bacteriological examinations were made on six of the cases.

Bordetella bronchiseptica was found in the lungs of two pigs, Salmonella cholerae suis in two, Haemophilus suis in one and from another both Corynebacterium pyogenes and Pasteurella multocida were isolated.

Discussion

Patches of acute bronchopneumonia which were often quite small were frequently seen as complications of established enzootic pneumonia and young pigs under eight weeks of age dying with extensive pneumonic lesions had enzootic pneumonia and a concomitant severe acute simple bronchopneumonia. The cases described here however, were animals in which the acute bronchopneumonia was the only significant pulmonary lesion. The amount of septal oedema and septal lymphatic dilatation was not as great in these cases as in animals with acute interstitial pneumonia. In addition in the latter disease and also in the pneumonia due to Haemophilus parainfluenza there was a greater

degree of fibrin formation in the alveoli and the fibrin plugs in the lymphatics were much larger and occurred with greater frequency.

Although acute bronchopneumonia was a primary pulmonary lesion it sometimes occurred along with other diseases such as anaemia, acute suppurative meningitis, and purulent arthritis.

The pulmonary lesions in both the pigs from which Salmonella cholerae suis was isolated were intense dark red in colour and histologically there was a greater number of alveolar macrophages amongst the polymorphonuclear leucocytes than in the other cases.

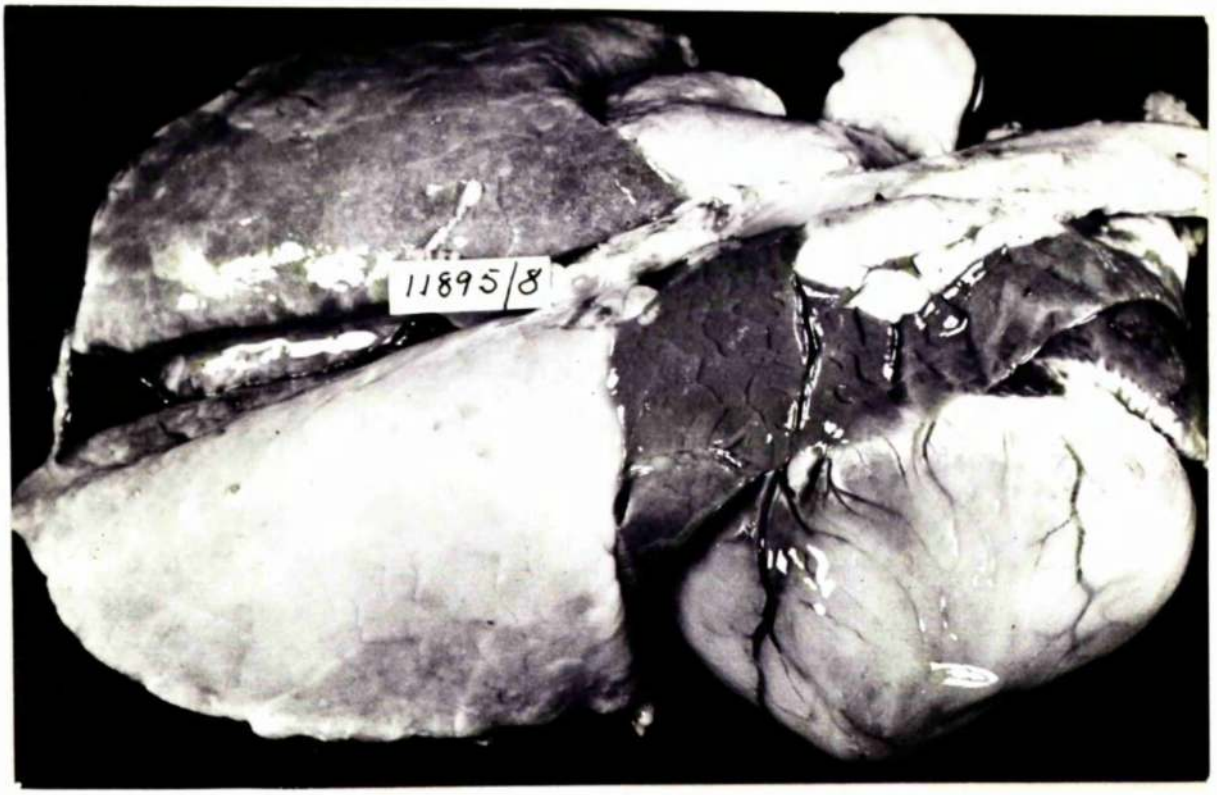


Fig. 45 Simple acute bronchopneumonia: involving the apical and cardiac lobes of the right lung and the diaphragmatic lobe of the left lung, of a pig with anaemia and cardiac hypertrophy.

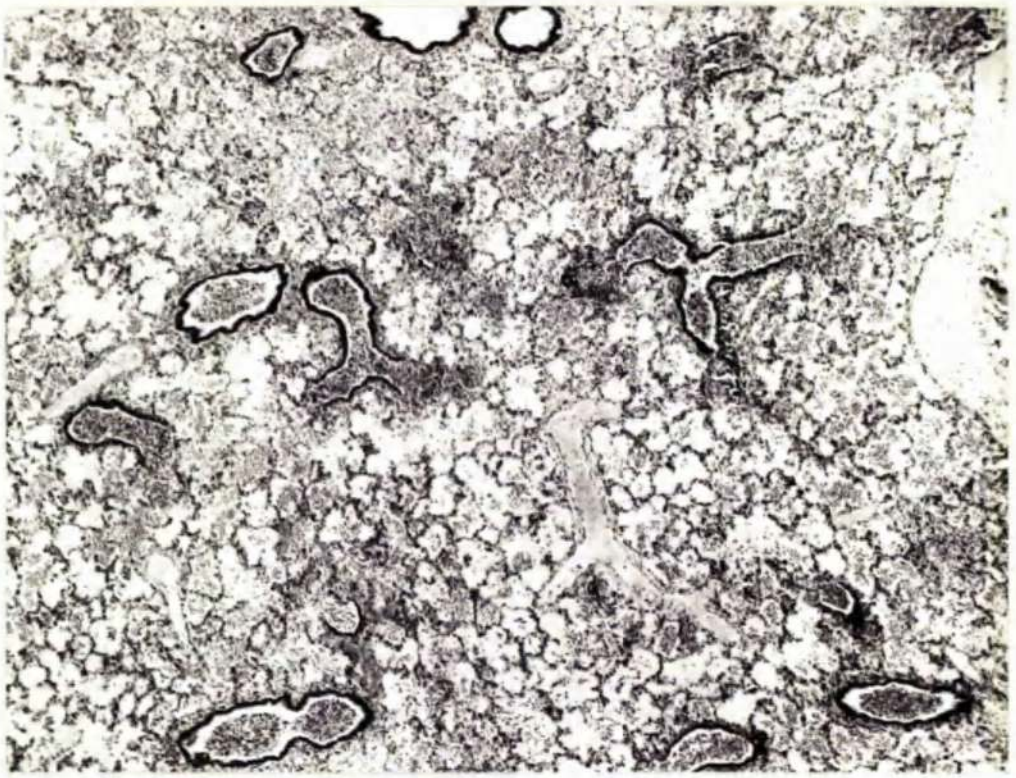


Fig. 46 Simple acute bronchopneumonia: the bronchioles are full of polymorphonuclear leucocytes and similar cells are present in the alveoli. H.& E. x 50

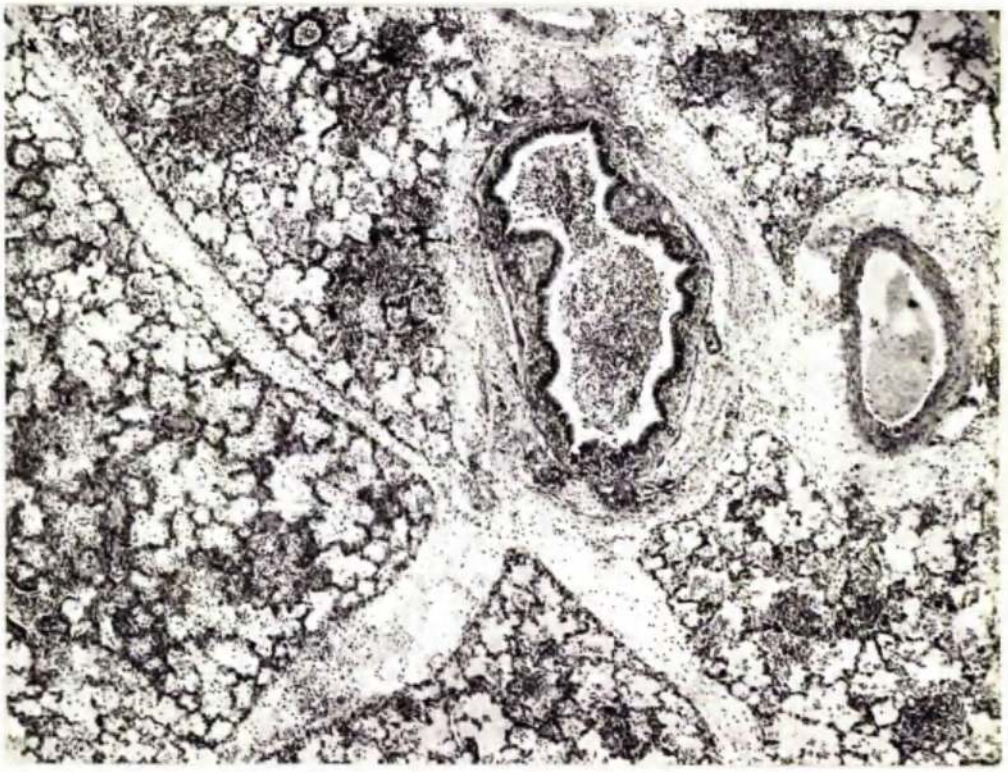


Fig. 47 Simple acute bronchopneumonia: showing congestion of the alveolar walls, mild septal oedema and a mixture of mucus and cells in a bronchus. H.& E. x 5

HAEMORRHAGIC BRONCHOPNEUMONIA

Haemorrhagic bronchopneumonia was seen in three pigs. Two of the specimens were obtained during the examination of lungs at a bacon factory and the third was a male pig four weeks old which was found dead. The latter case occurred in a herd established from pigs believed to be free of enzootic pneumonia.

Macroscopic Findings

The appearance of the lungs in the young pig which died was similar to the other two cases except that the lesions were more extensive. In this pig half of the total volume of the lungs was involved in the pneumonic process. There were lobules of consolidation in all the lobes of the lungs and in some places these had coalesced to form large pneumonic patches particularly in the antero-ventral part of both diaphragmatic lobes (Fig. 48).

The lesions felt firm, were at the same level as the surrounding lung and were dark red. A few patches were slightly grey. The interlobular septa were moderately dilated and the pleural surface of the lungs looked shiny. When the lungs were lifted they were heavier than normal. The cut surface was dark red, granular and exuded a small amount of thin turbid fluid from the bronchi and the alveolar surface.

The bronchial lymph nodes were enlarged and haemorrhagic.

Microscopic Findings

Histological examination of the lungs showed intense congestion of the capillaries and larger blood vessels in the alveolar walls, the bronchiolar walls and the bronchial walls. There was a considerable amount of oedema in the affected lobules and many polymorphonuclear leucocytes were seen in the alveoli, the bronchioles and the bronchi. Moderate numbers of alveolar macrophages were also present mixed amongst the polymorphonuclear leucocytes, and many of them contained haemosiderin. Occasionally focal areas with necrosis of alveolar walls were seen in some sections.

The most striking feature of the lesion however was the extreme intra-alveolar haemorrhage. In many lobules the alveoli were packed with red blood corpuscles and polymorphonuclear leucocytes. In some nearly all of the alveoli were full of red blood corpuscles only. The haemorrhagic exudate was also seen extending up into the bronchioles and bronchi.

The interlobular septa were moderately dilated and the lymphatic vessels distended. A similar appearance was seen in the pleural connective tissue. In addition to these changes however there were large numbers of free red blood corpuscles in the interlobular septal connective tissue and lymphatics (Fig. 49).

The connective tissue around the pulmonary arteries and the veins was also full of red blood corpuscles.

Those lobules which macroscopically were grey contained a higher proportion of alveoli full of polymorphonuclear leucocytes and macrophages than the others.

Histological examination of the bronchial lymph nodes showed that the sinusoidal tissue was full of red blood corpuscles and polymorphonuclear leucocytes. The capillaries in the dense lymphatic tissue were congested and there were moderate numbers of mitotic figures in the follicles.

Bacteriology

Bordetella bronchiseptica and Haemophilus suis were isolated from the lungs of the pig which died as a result of the pneumonia. A bacteriological examination was not carried out on the other two specimens.

Discussion

Haemorrhagic bronchopneumonia was found to be the cause of death in a four week old pig. This animal was not suffering from another pneumonic condition but was also affected with Inclusion Body Rhinitis.

In the two specimens obtained at a bacon factory there were lesions in the lungs of enzootic pneumonia as well as haemorrhagic bronchopneumonia.

Most of the changes seen in these pigs were also seen in animals with simple acute bronchopneumonia and intra-alveolar haemorrhages were seen in some cases of the latter condition. The haemorrhages were comparatively

small however and in view of the extensive haemorrhage occurring in the cases described here they were classified separately. In addition intra-septal haemorrhage was a feature of these cases and was not seen to any significant degree in simple bronchopneumonia. Macrophages filled with haemosiderin were more numerous within the alveoli in these cases than in those with simple bronchopneumonia.

The organisms involved in the case cultured were also recovered from cases of acute bronchopneumonia and it is possible that the different lesion was due to the combined infection or more likely due to the fact that these were different strains of the organisms.

Fig. 48 Haemorrhagic bronchopneumonia: showing the distribution mainly in the ventral part of the diaphragmatic lobe. H. & E. x 12

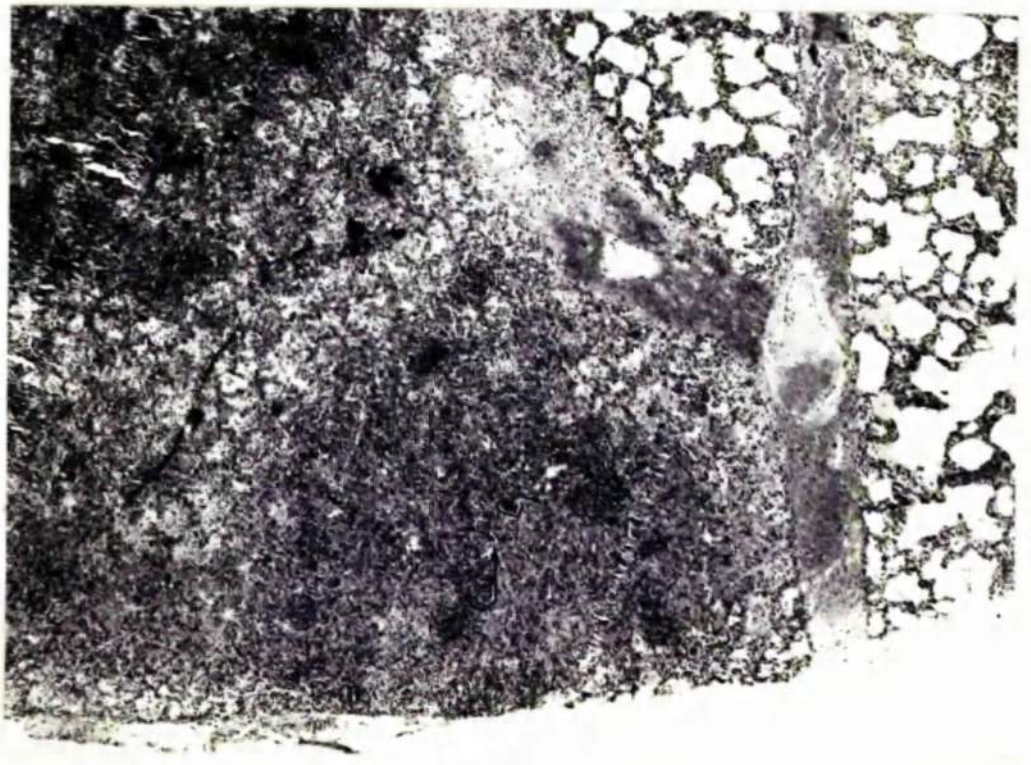


Fig. 49 Haemorrhagic bronchopneumonia: showing intraalveolar and intraseptal haemorrhage. H. & E. x 50

NECROTISING BRONCHOPNEUMONIA

Although small foci of necrosis of the alveolar walls was seen in cases of simple bronchopneumonia and haemorrhagic bronchopneumonia, they were usually not numerous and only detected by microscopy. Six cases of bronchopneumonia were seen in which necrosis of pulmonary tissue was an important feature of the lesions both microscopically and macroscopically, and consequently these were classified as necrotising bronchopneumonias.

Three cases were seen in pigs eight weeks old and the other three occurred in pigs about six months of age. The lungs of the latter three animals were obtained in an abattoir.

Macroscopic Findings

The lesion in one animal consisted of a patch of consolidation, whose pleural surface was 6.0 cms. in diameter, in the middle third of the diaphragmatic lobe of the left lung near its dorsal border. The visceral and parietal layers of the pleura were adherent at this point by fibrous bands. The cut surface of the lesion was very white due to the presence of large amounts of fibrous connective tissue and contained several yellowish-brown patches of coagulative necrosis.

In another case there were many firm nodular lesions about 1.0 cm. in diameter in all the lobes of the lungs which on section had a central

yellow patch of necrosis surrounded by a zone of congestion and haemorrhage.

There was extensive consolidation of the lungs in the remaining four cases with pneumonic areas in the apical, cardiac, intermediate and anterior parts of the diaphragmatic lobes. These areas had become confluent in the smaller apical and cardiac lobes to produce lobar consolidation. In one animal more than half of the total lung volume was consolidated.

The lesions were dark red in one case but in the other three were grey, very hard and raised above the surrounding normal tissue. On section large areas of dry, yellow coagulative necrosis could be seen and when the tissue was pressed thick yellow material exuded from the bronchi. The grey colour was due to extreme fibrous connective tissue proliferation around the areas of necrosis. The bronchial lymph nodes were enlarged in every case.

Microscopical Findings

Histological examination of the lungs showed that the lesions had been present for some time and were surrounded by variable amounts of fibrous connective tissue in all cases except the animal with extensive pneumonic lesions which were dark red macroscopically. This latter animal had an acute necrotising bronchopneumonia. In this cases there was dilatation and congestion of the capillaries in the alveolar walls, the bronchiolar walls and the bronchial walls and there were some patches of intra-alveolar haemorrhage. Most alveoli however were packed with masses of polymorpho-

nuclear leucocytes. At the periphery of the lesion the alveoli contained oedema fluid, fibrin strands and a few alveolar macrophages. Many extensive patches were seen where there was necrosis of the alveolar walls and the cells within the alveoli (Fig. 50). The centres of these necrotic lesions were invariably eosinophilic to different degrees but pale basophilic dots which were nuclear remains could be seen. Sometimes the outline of the alveoli w s present but in other lesions it was completely lost. Groups of bacteria could be seen in the lesions in some fields.

The lumina of bronchioles and bronchi contained large numbers of polymorphonuclear leucocytes and sometimes masses of necrotic debris. The epithelium had almost completely desquamated in some bronchioles and there was necrosis of the bronchiolar walls.

There was only moderate septal oedema with dilatation of lymphatic vessels which were full of polymorphonuclear leucocytes and fibrin. The pleural capillaries were congested and there were patches of fibrinous pleurisy.

The changes in the other cases were essentially similar except that there was not marked congestion of alveolar and bronchial walls and the fibroblast in the lung adjacent to the necrosis had proliferated to form new fibrous connective tissue. This resulted in septal and pleural fibrosis and the organisation of the fibrinous exudate in the alveoli at the periphery of the areas of necrosis. The alveolar walls at the edges of the

lesion were also thickened by fibroblast proliferation and migration of fibroblast from the walls into the fibrinous exudate in the lumen was seen. Some of these alveoli were epithelialised.

The central necrotic area was very palely eosinophilic in some fields but otherwise resembled the necrosis seen in the acute cases. At the edge there was a basophilic band of living and dead polymorphonuclear leucocytes.

In three cases lesions due to enzootic pneumonia were also present in the lungs.

The bronchial lymph nodes were congested and the sinusoids were full of polymorphonuclear leucocytes and some macrophages. Numerous mitotic figures could be seen in the follicles and often considerable numbers of eosinophils were present in the dense lymphatic tissue.

Bacteriology

Information about the bacteria associated with the lesions was available for two cases. Cultural examination had yielded Corynebacterium pyogenes and Pasteurella multocida from both sets of lungs and in addition a filamentous organism resembling Fusiformis necrophorus was seen in smears from one case, but could not be grown.

Discussion

Necrotising bronchopneumonia is most frequently seen in cattle

particularly calves, and in this species is frequently caused by F. necrophorus or C. pyogenes (Jarrett, 1956). The development of the disease in calves is often precipitated by the aspiration of milk into the lungs when these animals are artificially fed with buckets or bottles. Since artificial feeding is not practiced on the same scale with young pigs, this species is not exposed to this risk. In addition C. pyogenes and F. necrophorus are not as common pathogens in the pig as they are in cattle, and there is no disease in the young pig analogous with calf diphtheria, which produces necrotising lesions in the mouth and may provide necrotic debris loaded with F. necrophorus for aspiration into the lungs. Aspiration pneumonia has been described in pigs fed swill but the lesion is a granulomatous one and develops around inspired fragments of vegetable material (Whittlestone, 1957b).

Clinical histories were not available for all of the animals. The case of acute necrotising bronchopneumonia was reported as a sudden death and one of the other animals with extensive necrotising bronchopneumonia was thin, coughing and dyspnoeic. Haematological examination of this animal revealed a leucocytosis of 65,200 white blood corpuscles/c.m.m. and a differential count gave a result of 86% neutrophil polymorphonuclear leucocytes and 14% lymphocytes.

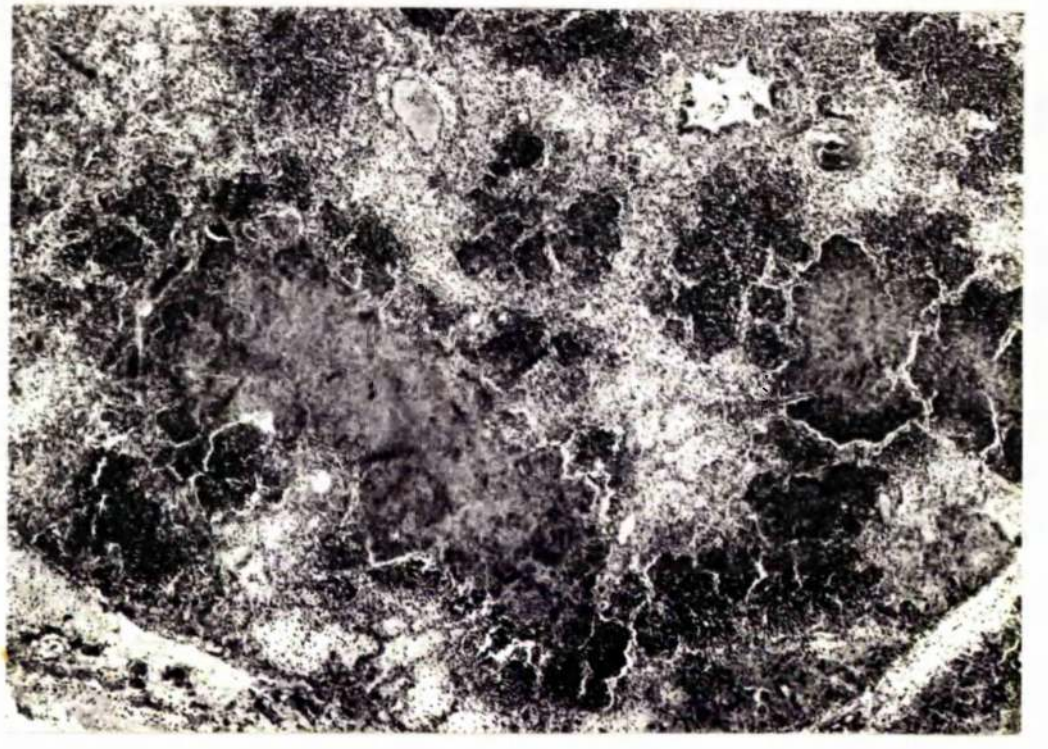


Fig. 50 Necrotising bronchopneumonia showing heavy cellular infiltration and necrosis of the lung. H. & E. x 50

SUPPURATIVE BRONCHOPNEUMONIA

Suppurative bronchopneumonia is a bronchopneumonia associated with a septic process in the lung resulting in abscess formation. The pulmonary lesions in six animals were classified in this category. Five of the pigs were between five and six months old and the other one was two months old.

Three of the older animals were detected on a farm with a severe respiratory disease problem. Enzootic pneumonia was present on the farm but many of the pigs examined at autopsy had extensive complicating pulmonary lesions such as interstitial pneumonia, acute bronchopneumonia and suppurative bronchopneumonia. When these animals with suppurative bronchopneumonia were seen alive they were in poor condition, coughing, dyspnoeic, febrile and haematological examination revealed a leucocytosis and a high B.S.R.

Macroscopic Findings

The gross appearance of the lungs varied from patches of consolidation, one to two lobules in size scattered in all the lobes of the lungs to confluent lobar consolidation in the apical, cardiac, and intermediate lobes of the lungs with a variable amount of the antero-ventral part of the diaphragmatic lobes also involved (Fig. 51). When the whole of the apical and cardiac lobes was not affected the lesions were in the ventral part of the lobes. The colour of the affected lobules varied from dark red to pale

greyish-pink to fawn. They felt firm and were either on the same level as surrounding round lung or sometimes slightly raised above it. On the surface of the lesions yellow abscesses which varied in size and number from case to case could be seen (Fig. 51). Sometimes however they were not apparent until the lungs had been sectioned. These abscesses varied from 0.2 cms. to 1.5 cms. in diameter, were spherical or elliptical and contained thick yellow pus. In four cases fibrous capsules could be seen around them. When the lungs were pressed thick yellow pus exuded from the bronchi and bronchioles.

There was a fibrinous pleurisy over the lesion in two animals, and in another case abscesses were found in the lobes of the liver and subserosally in the alimentary tract particularly in the spiral colon. The bronchial lymph nodes were congested and enlarged in all cases.

Microscopic Findings

Histological examination of the lungs from these pigs showed abscesses at different stages of development. In two animals the lesion appeared to be very recent and there was little fibrous connective tissue but in the others the abscesses were surrounded by fibrous connective tissue capsules.

At the centre of the suppurative focus there was complete loss of normal pulmonary architecture and the lung tissue was replaced by an amorphus palely staining eosinophilic material in which diffuse basophilic strands

could sometimes be seen (Fig. 52). At the edge of this there was a dense basophilic zone of polymorphonuclear leucocytes in various stages of disintegration. In recent lesions the alveolar architecture could be discerned here and the bands of polymorphonuclear leucocytes could be seen to be within alveoli. The adjacent alveolar walls were congested and their lumina contained oedema fluid with strands of fibrin. Variable numbers of alveolar macrophages also occurred and there was sometimes intra-alveolar haemorrhage.

At the periphery of the basophilic zone in older lesions the alveolar pattern was lost due to organisation and the development of a fibrous connective tissue capsule containing capillaries, large numbers of lymphocytes, plasma cells and macrophages particularly in its inner part (Fig. 53). This fibrous connective tissue proliferation could be seen developing from the adjacent fibrous connective tissue structures such as the interlobular septa, the pleura, the bronchial walls and the walls of blood vessels. The fibroblast in these sites had large well stained nuclei and abundant, obvious basophilic cytoplasm. Mitotic figures were also seen in these cells. Fibroblast proliferation also occurred in the walls of alveoli at the periphery of the abscesses so that the walls were thicker than normal and organisation of the exudate in the lumina of the alveoli occurred by fibroblasts migrating out into the fibrinous exudate from the alveolar walls. Some alveoli in this situation were epithelialised. Many abscesses both acute and chronic were seen communicating with bronchioles (Fig. 52).

Away from the abscesses the histological picture varied from that seen in acute bronchopneumonia to enzootic pneumonia if the latter disease was present.

The suppurative process sometimes started in bronchioles and there was necrosis of part or all of the bronchiolar walls. Most bronchioles and bronchi were full of polymorphonuclear leucocytes, macrophages and purulent debris.

A feature of some bronchioles was fibroblastic proliferation in the lamina propria with consequent increase in the thickness of this layer. This usually occurred in bronchioles involved in a septic process and the fibrous thickening extended into the bronchi.

The interlobular septa were markedly oedematous and the dilated lymphatic channels contained polymorphonuclear leucocytes and some fibrin.

The sinusoids of the bronchial lymph nodes were full of polymorphonuclear leucocytes and macrophages. Often the follicles in the dense lymphatic tissue seemed to be reduced in number and the cells here were separated rather more than usual by oedema fluid.

Bacteriology

Pasteurella multocida was isolated from one case and from another a small gram positive organism morphologically resembling Corynebacterium pyogenes and Erysipelothrix rhusiopathiae but having the biochemical

characteristics of neither.

Discussion

The finding of fibrosis in the alveolar walls and thickening of the lamina propria in the bronchiolar and the bronchial walls by fibrous tissue is interesting when compared with the lesions described in the section on conditions associated with enzootic pneumonia. It is possible that a suppurative bronchopneumonia in which the tissue necrosis was mostly confined to the interior of the bronchioles and bronchi was responsible for some of these lesions. If this is the case attempting to separate proliferative lesions broadly resembling enzootic pneumonia by using the amount of fibrous connective tissue in the bronchiolar or alveolar walls as a criterion is not feasible and may lead to errors in diagnosis. Even when there is no gross fibrous thickening in the bronchioles it is likely that simple bronchopneumonias if they persist long enough and do not resolve will stimulate fibrosis of the alveolar walls, and most cases of enzootic pneumonia showing this feature are complicated by simple bronchopneumonias.

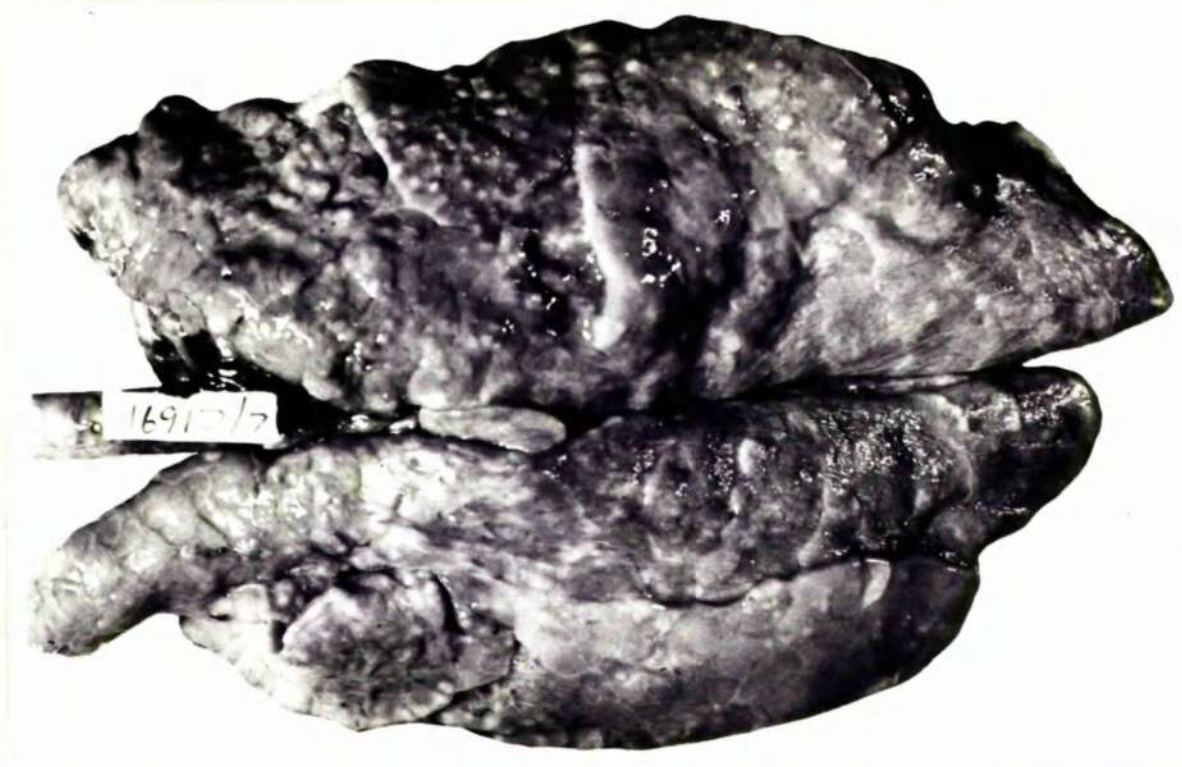


Fig. 51 Suppurative bronchopneumonia: producing extensive pulmonary consolidation.

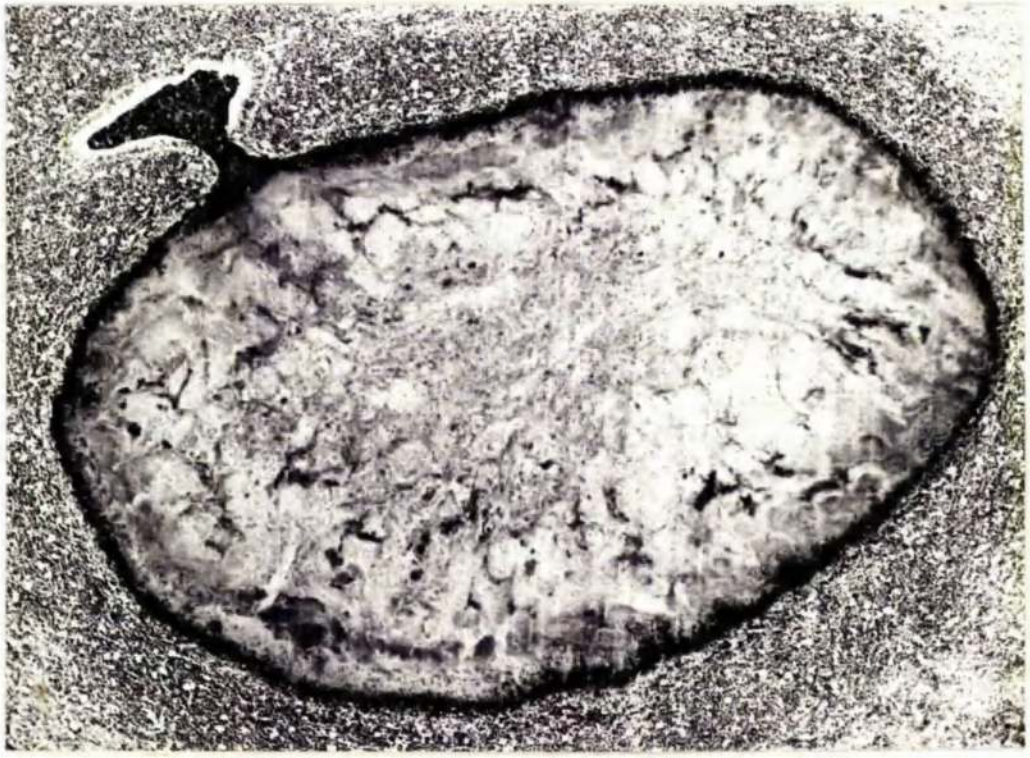


Fig. 52 Suppurative bronchopneumonia: abscess communicating with a bronchiole. H. & E. x 50

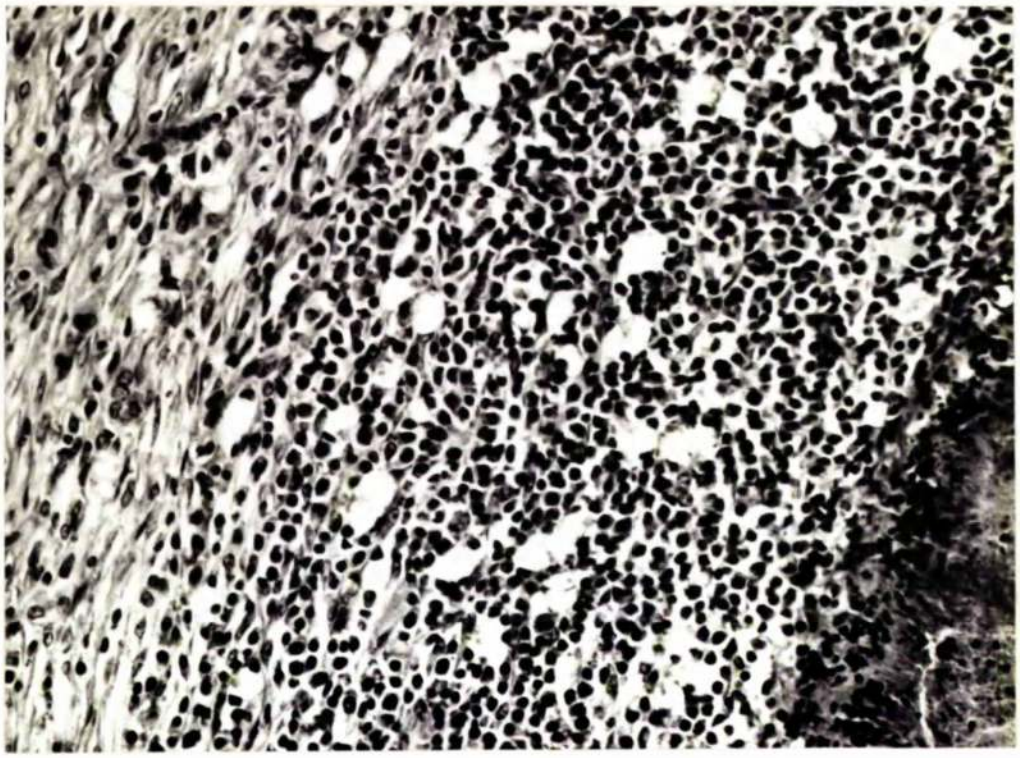


Fig. 53 Suppurative bronchopneumonia: details of the reaction at the edge of a suppurative focus. H. & E. x 250

EMBOLIC PNEUMONIA

The pig may develop pulmonary lesions as a result of infectious agents being carried into the lungs by the pulmonary or less frequently by the bronchial circulation. These lesions can be classified as embolic pneumonia and may vary in appearance from abscesses to patches of consolidation of considerable size.

Five cases of embolic pneumonia were seen and were divided into three groups to facilitate their description. Group 1 consisted of three cases in which the lesions were pulmonary abscesses. Group 2 consisted of one case in which there was extensive vascular involvement and Group 3 consisted of one case with haemorrhagic lesions secondary to haemorrhagic enteritis.

Group 1

Three pigs, two from the same group three months of age, and one four months of age were examined.

Macroscopic Findings

The two younger pigs were in very poor condition. Small patches of enzootic pneumonia were present in the apical and cardiac lobes of the lungs. Scattered in the consolidated lobules and also in normal lobules in all the lobes of the lungs were a very large number of yellow globular abscesses 0.5-0.8 cm. in diameter. Many of these protruded considerably from the

lung surface and the pleura over them was thickened. There were organised adhesion between the lobes of the lungs at the sites of the abscesses and also between the lungs and the thoracic wall. Sectioning the abscesses showed that they contained thick yellow pus and were surrounded by areas of consolidation of varying size. The bronchial lymph nodes were slightly enlarged.

Both of the pigs had purulent skin wounds around their hock joints and there were purulent wounds in their tails, the points of which had been bitten off.

The older pig had several lobules of consolidation in the dorsal border and medial edge of both diaphragmatic lobes. These lobules were firm, slightly raised above the surrounding lung and were surrounded by fibrous connective tissue. On section abscesses were seen in the lobules. The bronchial lymph nodes were slightly enlarged. The hock joint of the right hind leg was grossly swollen due to chronic purulent arthritis.

Microscopic Findings

Histological examination showed that the pulmonary lesions in the three pigs were similar. They consisted essentially of abscesses in different stages of development and healing. Most of them were scattered randomly in the lungs but some were seen around intralobular branches of the pulmonary

artery which were thrombosed. Many of the abscesses communicated with neighbouring bronchioles. The centre of the abscess was either a collection of polymorphonuclear leucocytes or a palely eosinophilic structureless area surrounded by polymorphonuclear leucocytes in various stages of disintegration. Next to this there was a mass of macrophages, lymphocytes and plasma cells in varying proportions, and a small number of eosinophils was also seen. Mixed with these cells and forming a prominent band around some abscesses were fibroblasts and capillary endothelial cells. The degree of fibrous encapsulation varied considerably.

Alveoli adjacent to the abscesses showed several different features. Their lumina was either full of polymorphonuclear leucocytes with some alveolar macrophages or there was a fibrinous exudate which was undergoing organisation in places. The alveolar walls were thickened by proliferating fibroblasts. Adjacent bronchioles and bronchi were full of polymorphonuclear leucocytes and often necrotic debris from the abscesses. The lamina propria and peribronchiolar tissues were infiltrated by plasma cells and some lymphocytes. When the lesions approached interlobular septa or pleura these structures were thickened by fibrous tissue proliferation.

Some lobules showed changes attributed to co-existing enzootic pneumonia.

Bacteriology

Bacteriological examination of swabs from the abscesses was negative.

Group 2

One case of embolic pneumonia which occured in a three months old pig is described here.

Macroscopic Findings

There was bilateral adhesive pleurisy. In all the lobes of the lungs there were large areas of greyish-red consolidation which felt very firm and looked granular. On sectioning the lung pus exuded from the bronchi and the bronchioles. The bronchial lymph nodes were enlarged. Subcutaneously in the left leg there was a large abscess 3.0 cms. in diameter.

Microscopic Findings

Histological examination of the lungs revealed a suppurative thrombo-arteritis involving the intralobular branches of the pulmonary artery. There was thrombosis of the branches of this vessel with necrosis of their walls and infiltration by large numbers of polymorphonuclear leucocytes (Fig. 54). The inflammatory process had spread to the adjacent pulmonary tissue and in these lobules the alveolar walls were congested and sometimes necrotic. Intra-alveolar haemorrhage was seen and in other alveoli there was oedema fluid rich in fibrin. The alveoli were also infiltrated with polymorpho-nuclear leucocytes and sometimes were completely packed with these cells.

Alveolar macrophages were also seen in moderate numbers and the septal cells were prominent in the walls of many alveoli although not markedly increased in number. The bronchioles and bronchi were full of polymorphonuclear leucocytes and there were moderate numbers of plasma cells in the lamina propria and peribronchiolar tissues. The septal and the pleural lymphatics were dilated and contained plugs of fibrin. Some sections from the apical and cardiac lobes had lesions of a co-existing enzootic pneumonia. The bronchial lymph nodes contained many follicles with large germinal centres.

Bacteriology

Pasteurella multocida was isolated from the lungs.

Group 3

The animal described here was three weeks of age and came from a litter affected with haemorrhagic enteritis. Three pigs had died as a result of this disease.

Macroscopic Findings

In addition to the lesions in the alimentary tract there were several small dark red patches scattered randomly in the lobes of the lungs. The bronchial lymph nodes were not noticeably enlarged.

Microscopic Findings

Histological examination of the lung lesions showed haemorrhages into the alveoli of the affected lobules and infiltration by large numbers of polymorphonuclear leucocytes. At the periphery of the lesions the alveoli contained oedema fluid and many alveolar macrophages. The lumina of the bronchioles and the bronchi were full of polymorphonuclear leucocytes. Within the lesion small branches of the pulmonary artery were thrombosed and the thrombi contained bacterial colonies. The vessel walls were infiltrated by polymorphonuclear leucocytes.

Bacteriology

A haemolytic strain of Escherichia coli was isolated from the intestines, liver, and spleen. The organism was typed by Dr. W.J. Soyka at the Central Veterinary Laboratory for the Ministry of Agriculture as serotype E681 (0141: K85(B) XIL).

Discussion

The pulmonary lesions in every case were secondary to a focus of infection in another part of the body. In Groups 1 and 2 there were purulent foci in the extremities and in Group 3 it was an enteritis due to a haemolytic E. coli. The animals in Group 1 were destroyed because they were not thriving but those in Groups 2 and 3 died. The pneumonia in Group 2 was probably significant in this respect.

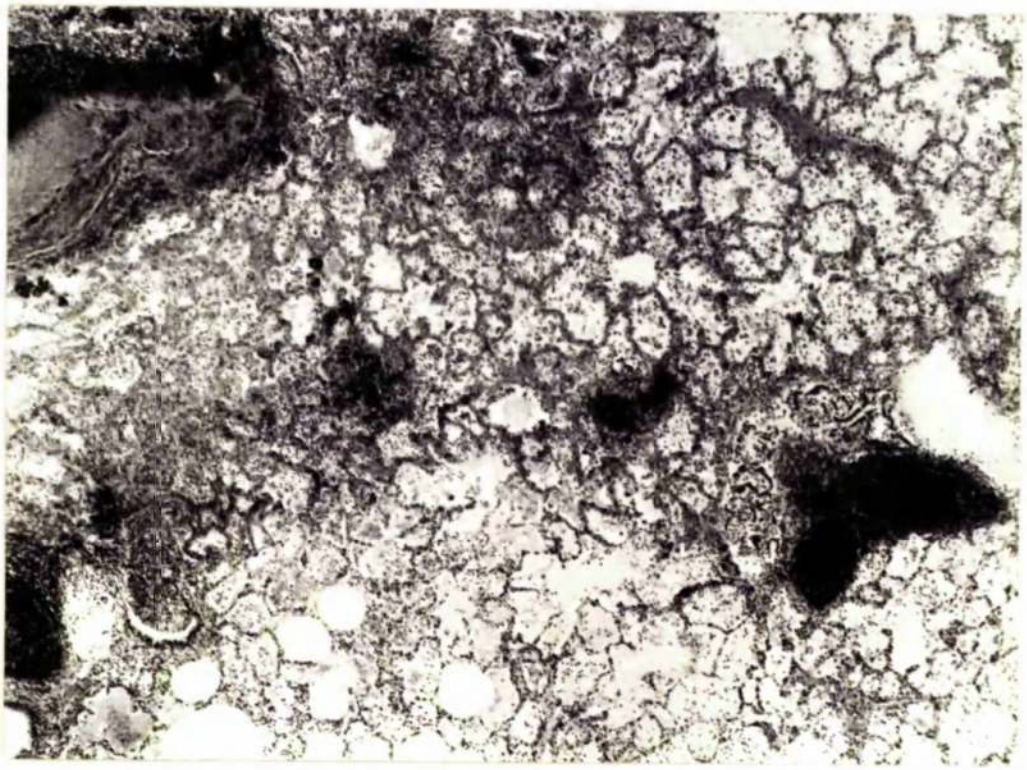


Fig. 54 Embolic pneumonia: thrombosis of branches of the pulmonary artery and capillaries in alveolar walls with congestion, and oedema in the surrounding alveoli. H. & E. x 50

TUBERCULOSIS

The pig is susceptible to infection by the mammalian and avian strains of Mycobacterium tuberculosis. Fourteen cases were seen which were classified as pulmonary tuberculosis and of these, one described under Group 1 was shown to be caused by the human type of M. tuberculosis and the remaining thirteen cases described in Group 2 had lesions histologically similar to those produced by the avian type of M. tuberculosis although cultural examinations were not done to confirm this.

Group 1

The case described here was a sow and the lungs were obtained from an abattoir.

Macroscopic Findings

Both lungs were extensively consolidated and felt full. There were patches of consolidation in all the lobes and the pleural surface was uneven due to the affected lobules being slightly higher than the normal lung tissue. The affected lobules were a pale yellow colour and contained many large white areas of calcification. Adhesive pleurisy was present between the cardiac and diaphragmatic lobes. On section two types of surface were seen. One looked smooth, solid, fleshy and grey and the other was granular white, and gritty, due to calcification. The septa were grey and thickened due to

fibrosis and there was also fibrosis around the larger bronchi from which a thick clear viscid fluid could be pressed.

The bronchial lymph nodes were enlarged and on section were grey and follicular. A few foci of calcification about 1.0 mm. in diameter were seen.

Microscopic Findings

Histological examination showed that the predominant features of the pulmonary lesion were fibrosis and calcification. In most lobules the lung architecture was completely obliterated by dense fibrous connective tissue surrounding large areas of calcification near which were the pyknotic remains of nuclei (Fig. 55). Scattered throughout the fibrous tissue were groups of plasma cells, lymphocytes, endothelioid cells and occasionally eosinophils. Giant cells were infrequently seen. The bronchial system had survived in these lobules and lymphocytes, plasma cells and mononuclears were scattered diffusely in the peribronchiolar tissues sometimes forming large cellular cuffs but usually not forming nodules (Fig. 56). The lamina propria of the bronchioles and bronchi was also heavily infiltrated by similar cells and within the lumina of these structures there was a mixture of mucus and polymorphonuclear leucocytes. The bronchial epithelium was hyperplastic in most fields.

Where there was no destruction of alveoli and replacement by fibrous tissue the alveolar lumina were full of alveolar macrophages with bright

eosinophilic cytoplasm and scattered amongst them were lymphocytes and plasma cells. In these regions there was also a diffuse peribronchiolar accumulation of plasma cells and lymphocytes. Fibrosis of the alveolar walls is commencing in some of these lobules.

Tuberculous follicles with a central amorphous, bright pink area of caseation surrounded by endothelioid cell mainly but also some lymphocytes and plasma cells were found in the dense fibrous connective tissue and in the areas where the alveoli were full of cells. These were not as numerous however as the foci of calcification.

The pleural and septal tissues are thickened by fibrous tissue proliferation and an adhesive pleurisy was present on some parts of the lung.

Tuberculous follicles were found in the bronchial lymph nodes. Giant cells were present in many of these and were seen more frequently in the nodes than in the lung. Small foci of calcification were found and there was extensive fibrosis, with obliteration of sinusoids and dense lymphatic tissue and thickening of the trabeculae.

Acid fast organisms were seen in sections of the bronchial lymph nodes stained with Ziehl-Neelsen.

Bacteriology

M. tuberculosis was isolated from the lung and the bronchial lymph nodes of this animal and was shown to be the human type by animal inoculation.

Group 2

The thirteen sets of lungs described here were from pigs approximately six months old and were collected from abattoirs.

Macroscopic Findings

Twelve sets of lungs had gross lesions of enzootic pneumonia and the tuberculous lung lesions were only discovered after microscopic examination. The thirteenth however had numerous hard grey nodules up to 1.5 cms. diameter in all lobes of the lungs. On section these nodules were firm and greyish white. The liver of this animal contained many white spots and the head had been condemned for tuberculous lesions in the submandibular lymph nodes. The bronchial lymph nodes were enlarged and contained several white circular foci.

Microscopic Findings

The lesions seen in all of the animals were essentially proliferative with little or no necrosis and qualitatively similar in each case. The following description is based on the case with lesions in the lungs and liver.

The nodular areas of consolidation were produced by fusion of masses of follicles (Fig. 57). At the centre of each follicle was a group of endotheliod cells in a small amount of amorphous eosinophilic material and

scattered between the endothelioid cells were lymphocytes. The periphery of the follicle was formed by circumferentially arranged fibroblasts and collagen fibres, amongst which were scattered lymphocytes. A few polymorphonuclear leucocytes were often seen in the follicles amongst the other cells and giant cells were readily found associated with them also (Fig. 58). Where the lung parenchyma was not obliterated by the large numbers of follicles it was possible to discern that some of them had developed within the interstitium of the alveolar wall and others were seen in septal and peribronchial lymphatic vessels. The alveoli adjacent to the coalescing follicles were full of alveolar macrophages and endothelioid cells. The alveolar walls and the interstitial tissue around blood vessels were thickened by cellular accumulations of lymphocytes, fibroblasts but also some plasma cells and endothelioid cells.

Follicles were found in the bronchial lymph nodes and in one case a small focus of calcification occurred.

The liver lesion consisted of tuberculous nodules similar to those in the lung. Acid fast organisms were found in only two cases in sections stained by Ziehl-Neelsen.

Discussion

The eradication of tuberculosis in the bovine and the decline of the disease in man should result in a corresponding lowering of the incidence

of tuberculosis due to the mammalian type of M. tuberculosis in the pig. Tuberculosis in the domestic fowl is still frequently seen however and this probably accounts for the preponderance of infection with this type in the pig as is illustrated in these cases. Modern intensive methods of pig and poultry husbandry make it less easy for the two species to mix and consequently one might expect the incidence of tuberculosis in the pig due to the avian type of organism to decrease also. From the cases seen in this series it would appear that pigs are only exposed to a very low level of infection since only one of the thirteen cases had extensive lesions.

Fig. 55 Tuberculosis: complete obliteration of pulmonary structure by calcification, fibrosis and cellular infiltration. H. & E. x 50

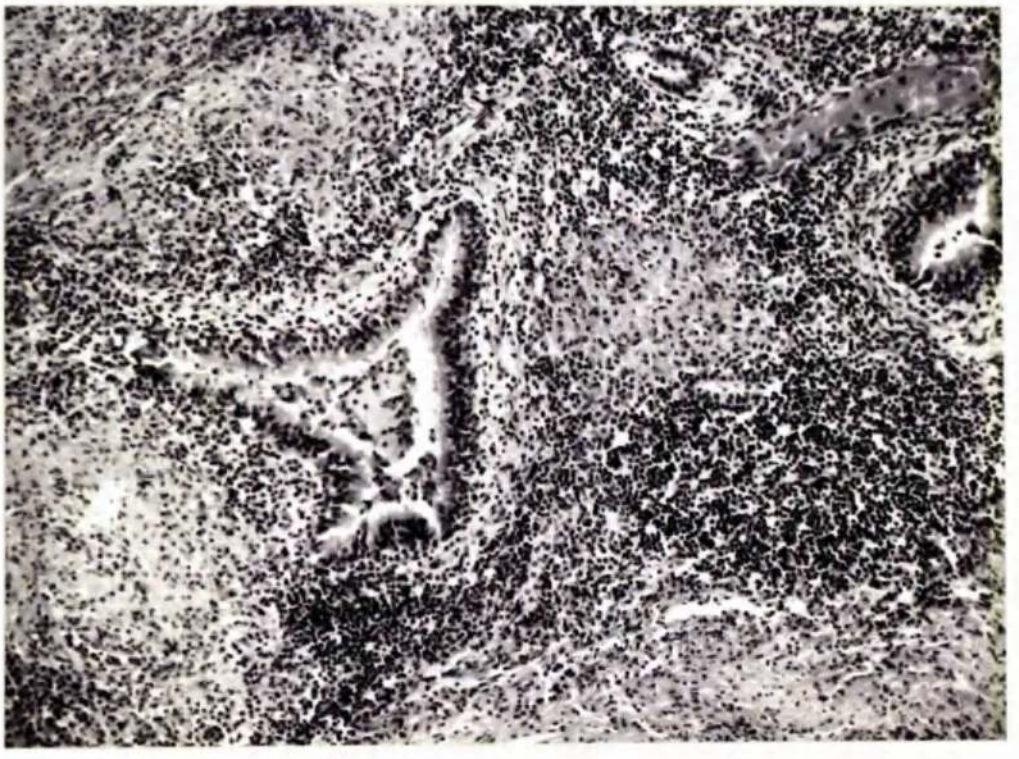


Fig. 56 Tuberculosis: bronchiole with marked infiltration of its wall by lymphocytes plasma cells and large mononuclears. H. & E. x 150

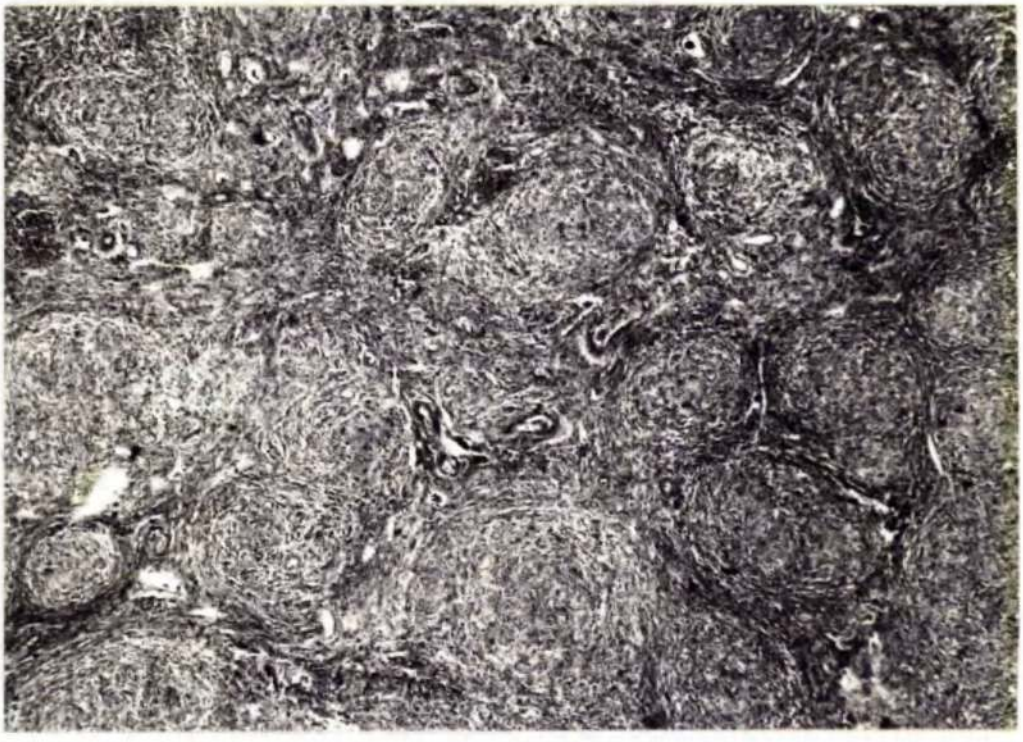


Fig. 57 Tuberculosis: confluent tuberculous follicles producing pulmonary consolidation. H. & E. x 50

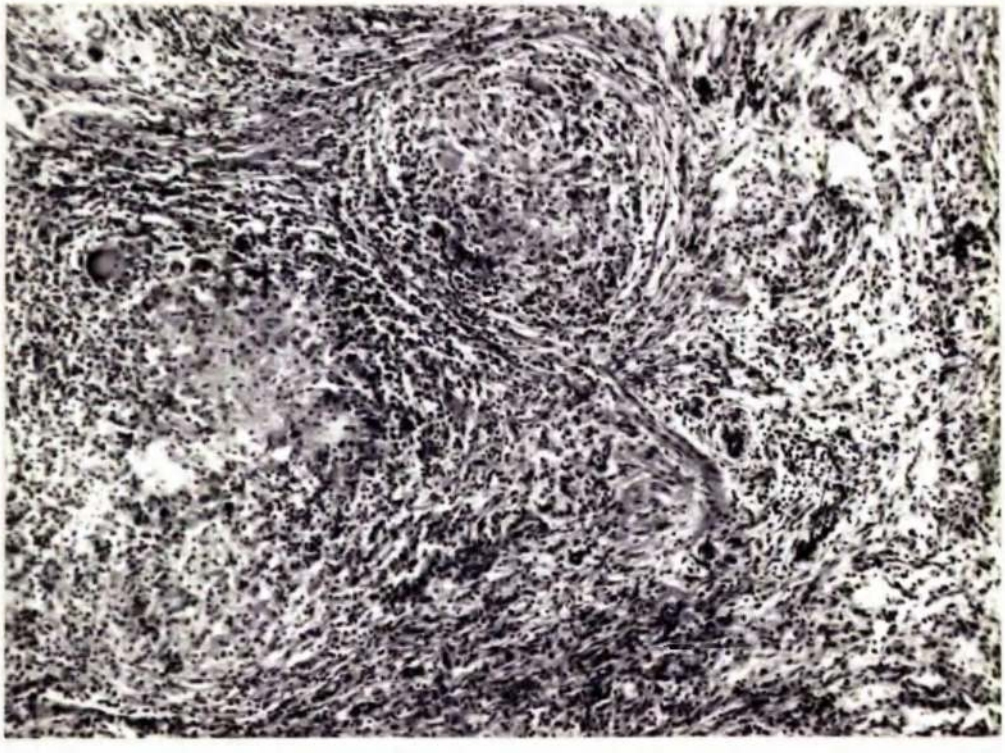


Fig. 58 Tuberculosis: tuberculous follicles one of which has a giant cell at its periphery. H. & E. x 150

PNEUMONIA DUE TO METASTRONGYLUS APRI

(1) The Pathology of the Disease Seen in the Field

The pig, in common with several other animals, is parasitised by lungworms. In the case of the pig these belong to the genus Metastrongylus; by far the commonest species found in Britain being Metastrongylus apri. This parasite is a frequent cause of coughing and pulmonary lesions in pigs which are kept out of doors and it is also responsible for some lung lesions seen in pigs kept indoors which had previously been reared outdoors.

The pathology of seventy seven cases of naturally occurring Metastrongylosis was studied. Sixty-five of the cases were collected at abattoirs and the remaining twelve were animals coming direct to the laboratory from pig farms.

Macroscopic Findings

The lesions resulting from lungworm infestation were of three types (1) lobular areas of vesicular emphysema (2) small patches of consolidation and (3) nodules which protruded above the surface of the lung.

Occasionally all three types of lesion were seen in the same case but in the specimens described here nodules alone or nodules and patches of consolidation occurred more frequently. All of the lesions were most frequently found in the diaphragmatic lobes, particularly the posterior half of the diaphragmatic lobes at the posterior tip of the lobes and along

the basal border. The apical lobes, the cardiac lobes and the intermediate lobes were affected in some cases.

Vesicular emphysema usually appeared as truncated triangular shaped areas conforming to the outline of one or more lobules invariably at the edge of a lobe with the apex of the triangle pointing medially. The effected lobules were white raised above their neighbours and the free edge of the lung at this point was rounded and protruded out of line with the general contour. When the pleural surface or a cut surface was inspected closely the alveolar dilatation could be appreciated macroscopically. The bronchi supplying these lobules were packed with lungworms. Nodules and sometimes small patches of consolidation were seen in the emphysematous lobules and then the well defined shape of the area was lost but the pallor and puffiness compared with surrounding normal tissue was still preserved.

Patches of consolidation were usually small sublobular in size and scattered in the predilection site for the lesions. They were pink, greyish-pink, or greyish-purple, and sometimes small white spots could be seen in them. They were frequently slightly raised and if large enough to palpate, felt very firm. They were most commonly seen in the posterior half of the diaphragmatic lobes on the costal or diaphragmatic surfaces and at or near the basal border, but also occurred in the anterior lobes of the lungs. When they occurred at the extreme edge of the lobe they sometimes bulged outwards, distorting the shape of the edge. A small number of lungs were

seen with larger patches of consolidation in the diaphragmatic lobes. These were three to four lobules in size and similar to the smaller lesions except that a larger number of white spots was often discernable.

Nodules occurred most frequently in the diaphragmatic lobes either singly or in small groups near areas of consolidation or emphysema, if these were present (Fig. 59). They varied from 0.1 cm. - 1.0 cm. in diameter but were mostly 0.4 - 0.6 cm. A considerably portion of the nodule protruded from the lung, particularly in the case of the larger ones. Nodules could also be seen in the lung if it was sectioned and when the small bronchi containing the lungworms were opened, they were sometimes seen protruding slightly into the lumen. Their colour was generally grey or grey-purple. Although they were usually found in the posterior half of the diaphragmatic lobes they were also seen in the apical or cardiac or intermediate lobes in some cases.

Lungworms were found in the small bronchi near the lesions and were always present if there was well developed vesicular emphysema. If nodules only were found lungworms were sometimes absent, and in some cases with only one or two worms there were no macroscopic lesions in the associated pulmonary tissue. The lungworms were found in the smallest bronchi and consequently the bronchial tree had to be opened to its finest ramification before they were discovered. The walls of these small bronchi containing worms were often obviously thickened and sometimes the lumen appeared dilated. The

commonest site in which the worms lived was again the posterior half of both diaphragmatic lobes and particularly the posterior tips of this region (Fig. 60).

The bronchial lymph nodes were either only slightly enlarged or not increased in size at all.

Microscopic Findings

The lesions seen microscopically were quite complex and were best described under four headings (i) bronchial and bronchiolar changes (ii) emphysematous areas (iii) consolidated areas (iv) nodules.

Bronchial and bronchiolar changes: Sections of adult worms cut in various planes were seen in the bronchi and bronchioles (Fig. 61). The females were easily identified by the larvated eggs in their uteris. In longitudinal sections the worms could be seen extending from the bronchi into the bronchioles and the anterior end was sometimes seen in the alveolar ducts and alveoli. The bronchioles were often considerably dilated by the mass of worms. Surrounding the worms was an exudate rich in mucus and containing a variety of cells; eosinophils, polymorphonuclear leucocytes, lymphocytes, plasma cells and macrophages. In some cases the cells were predominantly eosinophils. Larvated eggs were also seen in the exudate and some bronchioles were seen which did not contain worms but were dilated and packed with the exudate and large numbers of eggs.

The following changes were seen in the bronchi and bronchioles in the affected lobules irrespective of the presence or absence of worms.

Mucoid metaplasia occurred resulting in a striking increase in the number of mucus secreting cells in the bronchiolar and bronchial epithelium at the expense of ciliated cells (Fig. 62). When worms were present focal patches of atrophy and hyperplasia occurred in the epithelium. The former being due to pressure by the worms on the luminal surface.

The lamina propria was infiltrated by eosinophils. In some cases these were present in large numbers by themselves but usually there were moderate numbers mixed with plasma cells and some lymphocytes. The cells were sometimes seen migrating through the epithelium. Hypertrophy and hyperplasia of the muscularis was an arresting and frequently found alteration resulting in the smooth muscle layer being up to five or six times thicker than normal (Fig. 63). This hypertrophy was best developed in the bronchioles but also occurred in the bronchi and was seen in the alveolar ducts too. Cellular accumulations developed in the peribronchiolar tissues and again eosinophils were sometimes the dominant cell although if the accumulation was diffuse a mixture of eosinophils, lymphocytes and plasma cells was usually seen. In most of the cases peribronchiolar lymphoid nodules had developed either in a group unilaterally or forming a ring of nodules around the bronchiole. If longitudinal sections were studied this lymphoid sheath was seen extending from the bronchioles into the bronchi where it

was present between the muscularis and the cartilage and frequently resulted in destruction of most of the bronchial glands.

Emphysematous Areas: The bronchial system showed the changes already described. The alveoli, alveolar ducts and sometimes terminal bronchioles were markedly dilated and there was thinning of the alveolar walls (Fig. 64). The overdistension had resulted in rupture of some of the walls producing small emphysematous bullae.

Consolidated Areas: The patches of consolidation had several different appearances. In some lobules the alveoli and alveolar ducts were full of oedema fluid or mucus mixed with large numbers of eosinophils, polymorphonuclear leucocytes, plasma cells, lymphocytes, alveolar macrophages and scattered amongst these variable numbers of multinucleated giant cells with slightly basophilic hyaline cytoplasm. In addition to these cells larvated eggs were present, occasionally in large numbers. The eggs sometimes resulted in a local concentration of eosinophils and the egg shells appeared to have a thick layer of eosinophilic material on them similar to eosinophilic granules. Eggs were also seen inside giant cells and alveolar cells were increased in the alveolar walls.

In addition to this diffuse type of reaction consolidation resulted from large numbers of eosinophilic granulomas or eosinophilic abscesses or a combination of these developing in a lobule. These were reactions to aspirated eggs which were often seen at the centres of the lesions. The

eosinophilic granulomas consisted of a central mass of eosinophils surrounded by macrophages like endothelioid cells (Fig. 65). They had large elliptical nuclei with well stained nuclear membrane and had hyaline lightly basophilic cytoplasm. Sometimes these cells appeared to have fused together to form a syncytium. At the periphery were lymphocytes, plasma cells and some fibroblasts arranged concentrically. Eosinophils could be seen migrating between the various cells to the centre. Multinucleated giant cells with nuclei and cytoplasm similar to the endothelioid cells occurred either at the centre of the lesion or at the periphery.

Eosinophil abscesses were focal lesions with a large central brightly eosinophilic mass of eosinophils which were necrotic (Fig. 66). It was usually more difficult to identify egg remains in these. Around the central mass of necrotic eosinophils were lymphocytes, plasma cells and macrophages.

An additional lesion found accompanying those described above and which contributed to consolidation was obliterative bronchiolitis affecting terminal bronchioles and the alveoli and alveolar ducts near these. The proliferation of fibroblasts in the walls of the alveolar ducts and alveoli had thickened these structures and produced polypoid masses protruding into the air passage and extending up the terminal bronchioles. The epithelium over these masses and in the affected alveoli and alveolar ducts was very noticeable. The polyps and walls of these structures were infiltrated by eosinophils, plasma cells, lymphocytes and macrophages. Small patches of epithelialisation also

occurred.

Nodules. The obliteration of pulmonary parenchyma by developing lymphoid nodules sometimes produced consolidation but more frequently this appeared macroscopically as a definite raised nodule.

Histologically these were seen at different stages of development. The earliest specimens had a central eosinophilic mass which was obviously a dead lungworm and occasionally the outline of the inner part of the bronchiolar wall could be discerned. Surrounding this was a mass of coalescing but discrete lymphoid nodules separated by a capillary network so that the nodule often looked like a bunch of grapes. Older nodules or tangential sections through early nodules simply appeared as a diffuse mass of lymphoid cells or as a mass of coalescing microscopic nodules (Fig. 67).

At the periphery of the nodules the alveoli were collapsed and contained alveolar macrophages.

As well as the changes described above cellular infiltrations were seen in the pleura, and the septa. Usually this was a mixture of eosinophils, plasma cells, lymphocytes and macrophages but occasionally eosinophils predominated and sometimes small lymphoid aggregates were formed. These were also seen occasionally in the alveolar walls scattered throughout a lobule. Around blood vessels, pulmonary veins and the smaller branches of the pulmonary artery, collections of cells similar to those in the septa and pleura were observed.

Many lymphoid follicles with large germinal centres were seen in the bronchial lymph nodes and there were large numbers of eosinophils in the loose lymphatic tissue.

Discussion

The pathology of the lesions in naturally occurring lungworm infestation in the pig has been briefly described by other workers (Dunn, Gentles and White, 1955, Whittlestone, 1957b, MacKenzie, 1958a).

The pneumonia produced by M. apri resembles enzootic pneumonia in some features, particularly the peribronchiolar lymphoid hyperplasia and also to some extent in the alveolar reaction with plasma cells, lymphocytes and alveolar macrophages. In the latter instance however eosinophils are also usually present in large numbers compared with the occasional eosinophil which is regularly seen in the alveoli in enzootic pneumonia.

Although peribronchiolar lymphoid hyperplasia is a feature of both diseases the reaction is more extreme when caused by lungworms. Initially the changes in the peribronchiolar and bronchial tissues resemble each other but as the diseases progress they diverge. There is more involvement of the bronchial walls in the pneumonia due to M. apri and the final stage of the lymphoid nodular development is associated with the lungworms dying in the bronchioles with complete destruction of the bronchiolar wall which is then replaced by a mass of microscopic lymphoid nodules to produce a macro-

scopically visible nodule.

Despite the similarities Metastrongylosis does not present a major problem in diagnosis because even if worms are absent the smooth muscle hypertrophy, the mucoid metaplasia, the aspirated eggs associated with granulomas or eosinophilic abscesses, and the massive nodular development are characteristic. The obliterative bronchiolitis and the patches of epithelialisation are also useful pointers but might be simulated by organising exudates and alveolar fibrosis and epithelialisation that may develop in long standing simple or suppurative bronchopneumonias. While it is relatively easy to diagnose the presence of lungworm infestation, in some cases with lesions in the anterior lobes of the lungs, one might have reservations about stating unequivocally from the histopathology alone whether or not there was co-existing enzootic pneumonia.

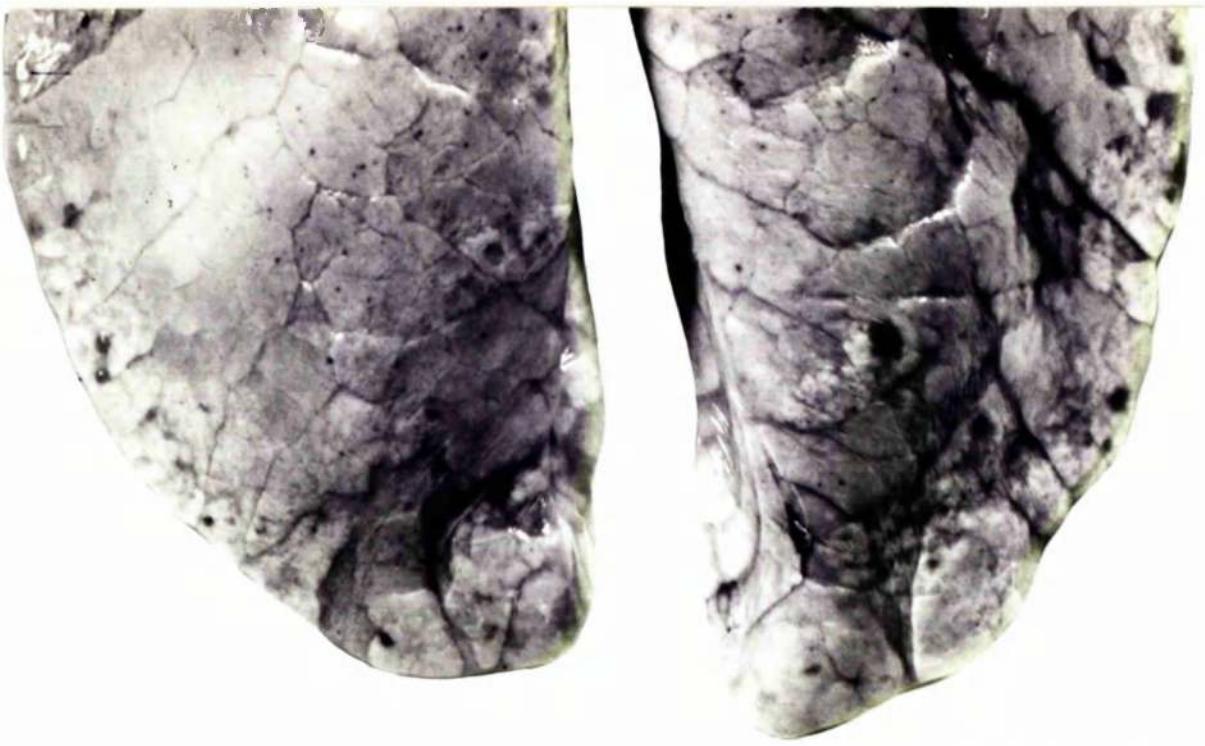


Fig. 59 M. apri: several nodules and mild vesicular emphysema in the posterior parts of the diaphragmatic lobes.

Fig. 60 M. apri: lungworms in a bronchus at the posterior tip of a diaphragmatic lobe.

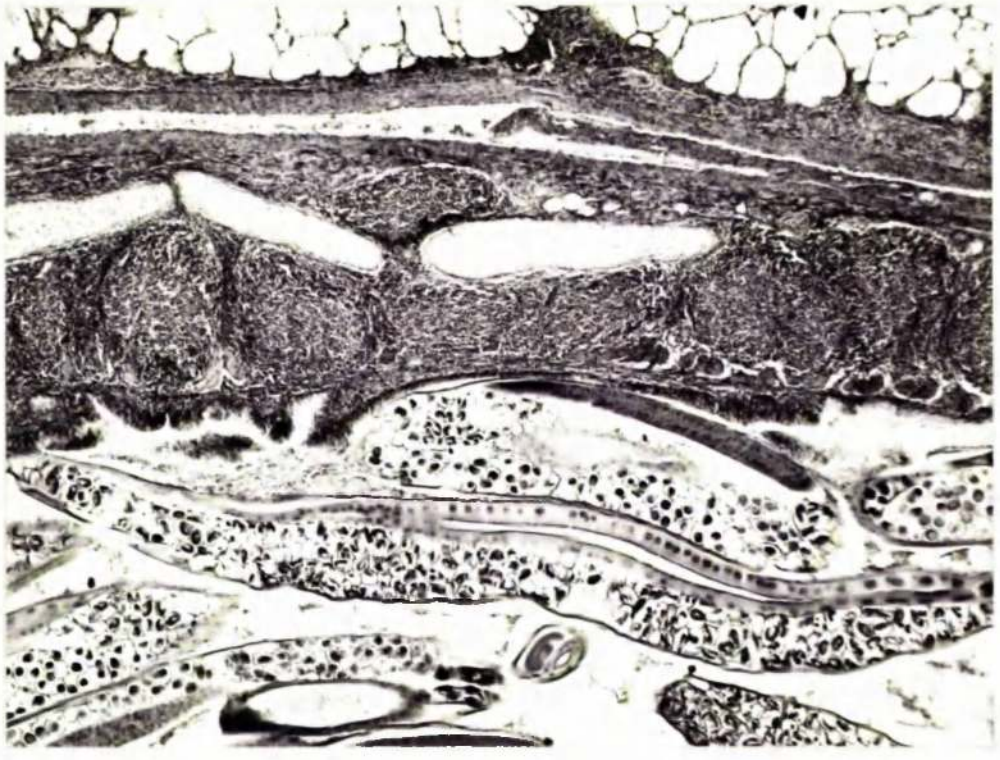


Fig. 61 M. apri: longitudinal section of bronchus containing adult female lungworms with atrophy and hyperplasia of the epithelium. H. & E. x 50

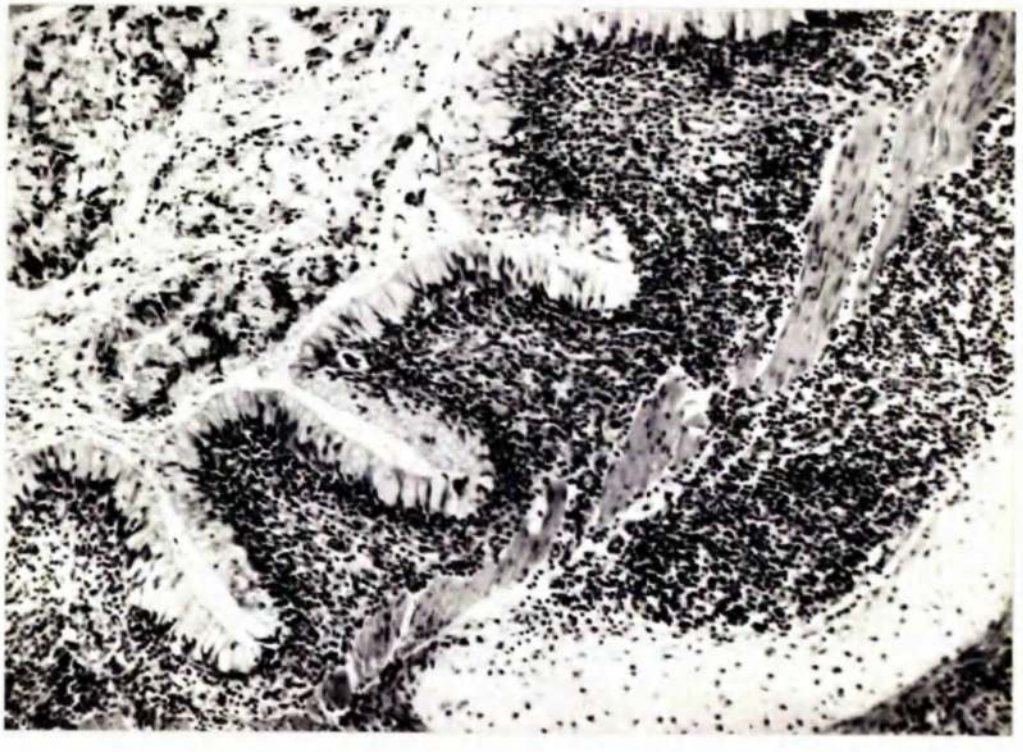


Fig. 62 M. apri: mucoid metaplasia in the epithelium of a bronchus. H. & E. x 150

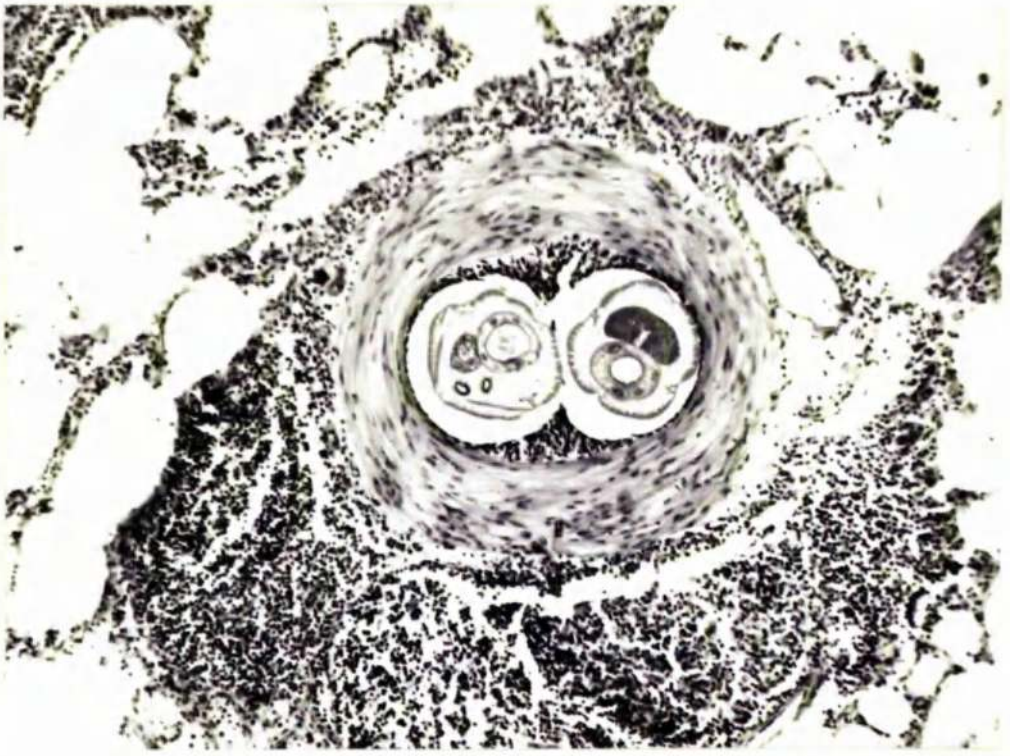


Fig. 63 M. apri: hyperplasia and hypertrophy of the muscularis of a bronchiole containing two lungworms. H. & E. x 150

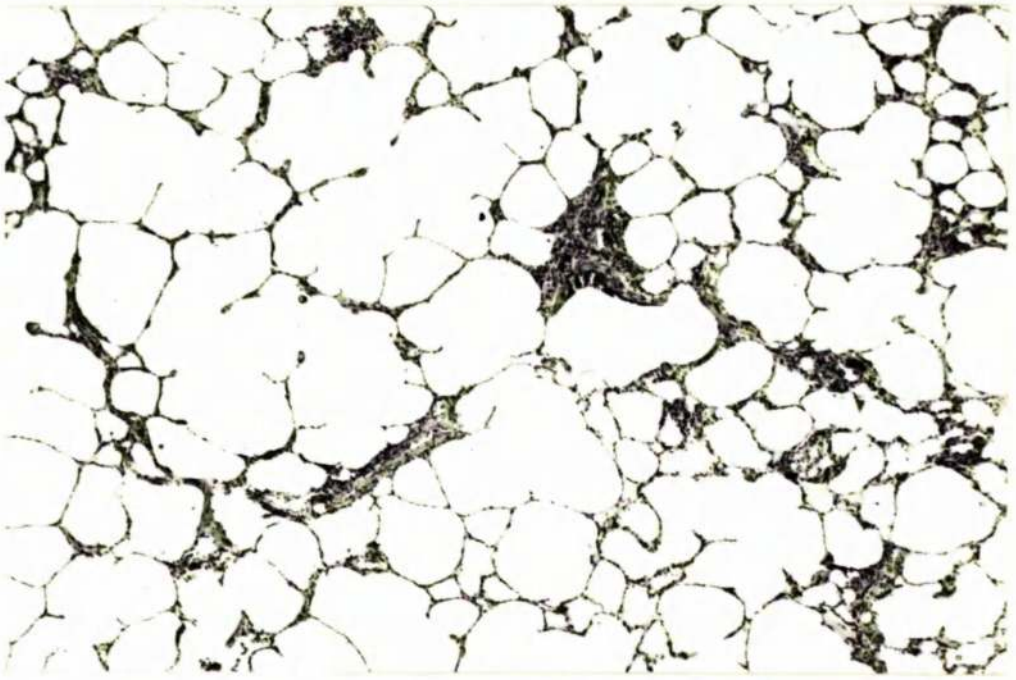


Fig. 64 M. apri: vesicular emphysema with distension of the alveoli and alveolar ducts. H. & E. x 50

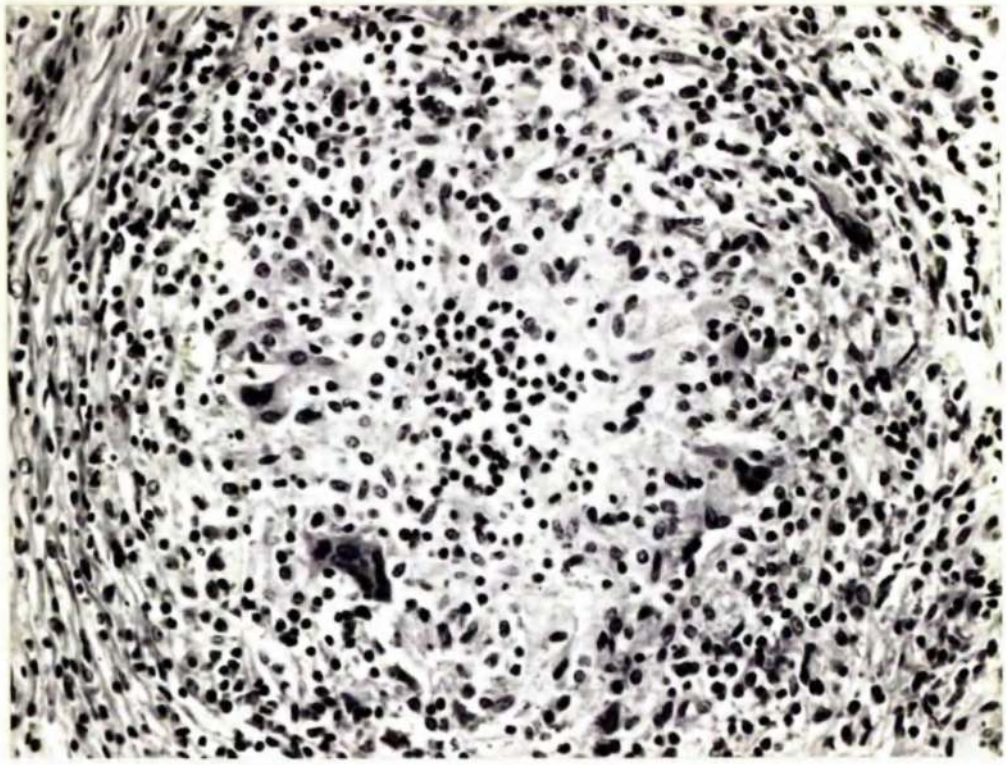


Fig. 65 M. apri: eosinophilic granuloma with a central group of small dark cells which are in fact eosinophils . H. & E. x 300

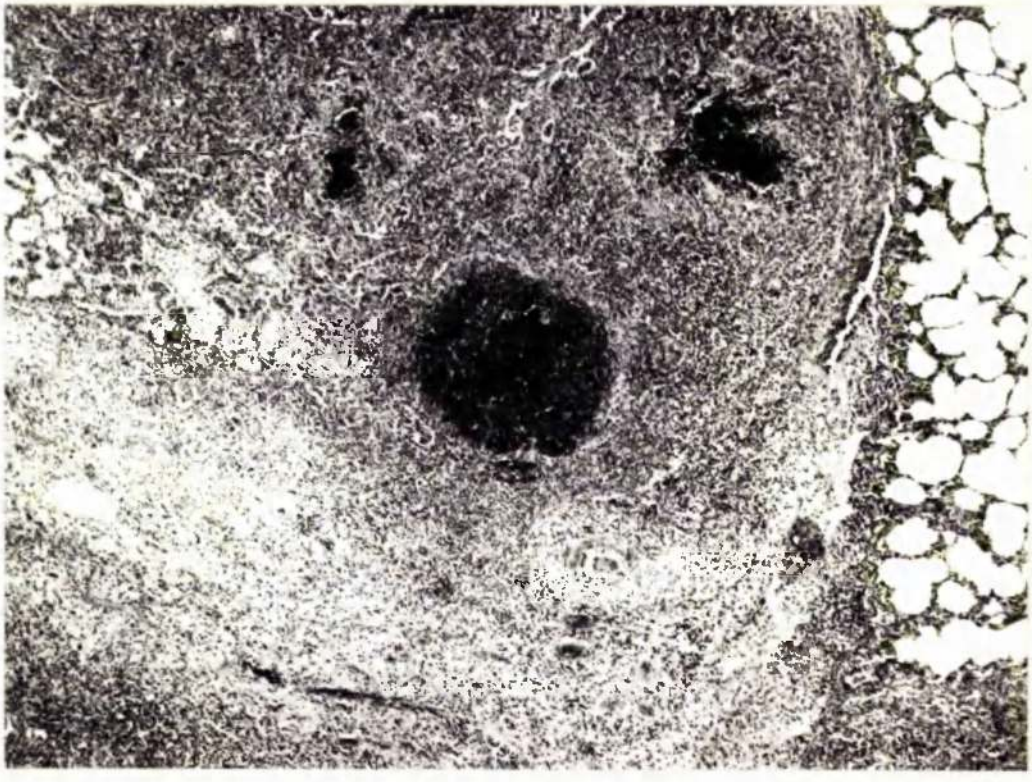


Fig. 66 M. apri: three eosinophilic abscesses, the largest of which has an egg near the periphery of the dark mass of degenerating eosinophils. H.& E. x 5

Fig. 67 M. apri: histological appearance of large lymphoid nodule which was discernable macroscopically. H. & E. x 50

PNEUMONIA DUE TO METASTRONGYLUS APRI

(2) Introduction to Experimental Work

It was pointed out in the review of the literature that some studies had already been made on Metastrongylus infection (Dunn, Gentles and White 1955, Dunn 1956, MacKenzie 1958 a,b, and 1959). In these studies however although the incidence of lungworm infestation found at abattoirs was described no indication was given about the severity of the parasite burden in individual animals, and at the time the investigations described in this thesis were started the progressive pathology of the condition had not been studied experimentally in detail.

A survey of the incidence and severity of Metastrongylus infestation in pigs in central Scotland was therefore carried out and the adult female M. apri collected were used to provide eggs for earthworm infection so that third stage infective larvae could be obtained for the experimental infection of pigs.

The serial pathology of the pneumonia associated with the adult stage of the parasite was studied in experimentally infected pigs.

It has been shown that it is possible to vaccinate cattle against Dictyocaulus viviparus using x-irradiated third stage larvae of D. viviparus to stimulate active immunity (Jarrett, Jennings, McIntyre, Mulligan and Urquhart, 1957). The possibility of this process being used to protect

the pig against its own lungworms seemed feasible and consequently two more experiments were planned. In one the effect of graded doses of x-irradiation on the third stage larvae of M. apri was studied and in the second an attempt was made to demonstrate that pigs vaccinated with x-irradiated third stage larvae of M. apri were immune to challenge with normal infective third stage larvae of M. apri.

PNEUMONIA DUE TO METASTRONGYLUS APRI

(3) The Incidence and Severity of Metastrongylus Infestation in Pigs Slaughtered in Central Scotland

The object of this survey was (i) to determine the number of worms occurring in the lungs of pigs going to an abattoir in Central Scotland, (ii) to discover the overall incidence of the disease in this population and (iii) to find out the sex ratio of the worms in these naturally occurring cases.

The lungs of pigs slaughtered at an abattoir in Stirling were examined in the course of ten visits which were made between July 1959 and June 1960. At each visit the lungs of the pigs were examined consecutively as they were removed after slaughter and there was no special selection of lungs. There was one exception to this method of examination and that was a group of ten sets of lungs which were deliberately collected because they contained lungworms. These lungs were not included in the incidence figures but the worms in them were included in the figures for the levels of parasitism found and the sex ratios of the worms.

Methods

The method of examination was to incise the diaphragmatic lobes of each set of lungs about 2.0 cms. from their basal borders throughout their entire length, i.e. from the posterior tip to that part, adjacent to the

cardiac lobe. The apical and cardiac lobes of each lung were also incised about 2.0 cms. from their ventral tips. While the incisions were being made the lung was pressed and any lungworms present were easily seen in the bronchi.

Lungs which were found to contain worms were retained to be taken back to the laboratory where they were opened by cutting down all the branches of the bronchial tree with scissors. When lungworms were found they were removed and put into isotonic saline. A record was kept of the lobe of the lung they were found in and whether it was the left lung or the right lung. The worms were later counted, sexed and representative samples were examined below a stereo microscope for identification.

Results

93 sets of lungs out of a total of 1,113 examined contained lungworms, which gave an overall incidence of 8.4%. The percentage of lungs with lungworms at each visit is shown in Table 9.

The actual numbers of worms found in each set of lungs is summarised in Table 10 and it can be seen that most lungs had less than 50 worms. This group of animals is further broken down in the histogram (Fig, 68), which shows that in the group, nearly 50% of the animals had less than 10 worms.

When the sex ratio male:female was calculated for cases with both sexes present, there was a wide scatter of results (Fig. 69). The ratios

Table 9

THE INCIDENCE OF METASTRONGYLOSIS IN PIG LUNGS EXAMINED AT AN ABATTOIR IN SCOTLAND

Date of Examination	No. of Cases Examined	No. with Lungworms	Percentage with Lungworms
July 1959	89	6	6.7
July 1959	131	17	13.0
August 1959	119	5	4.2
August 1959	122	13	10.7
August 1959	173	9	5.2
August 1959	167	7	4.2
December 1959	79	21	26.6
April 1960	86	7	8.1
May 1960	120	5	4.2
June 1960	27	3	11.1
Totals and Overall Incidence	1,113	93	8.4

Table 10

THE SEVERITY OF LUNGWORMS INFESTATION IN 103 PIGS

No. of Worms in Each Set of Lungs	No. of Pigs Affected
1 - 50	89
51 - 100	7
101 - 150	1
151 - 200	2
201 - 250	1
251 - 300	-
301 - 350	-
351 - 400	-
401 - 450	-
451 - 500	1
501 - 550	1
551 - 600	1

Figure 68

THE SEVERITY OF LUNGWORM INFESTATION IN 89 PIGS WITH LESS THAN 50 WORMS

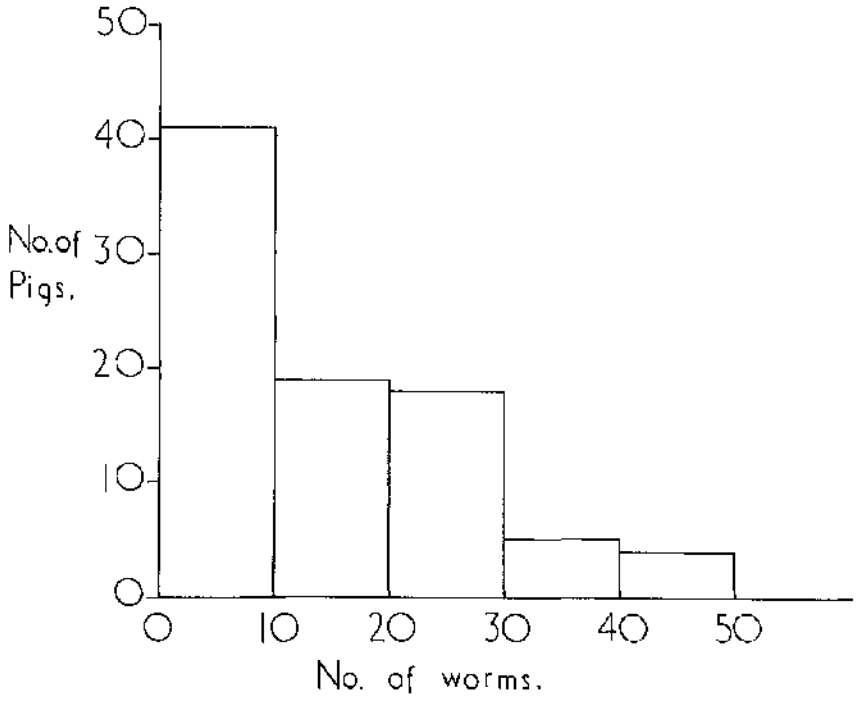
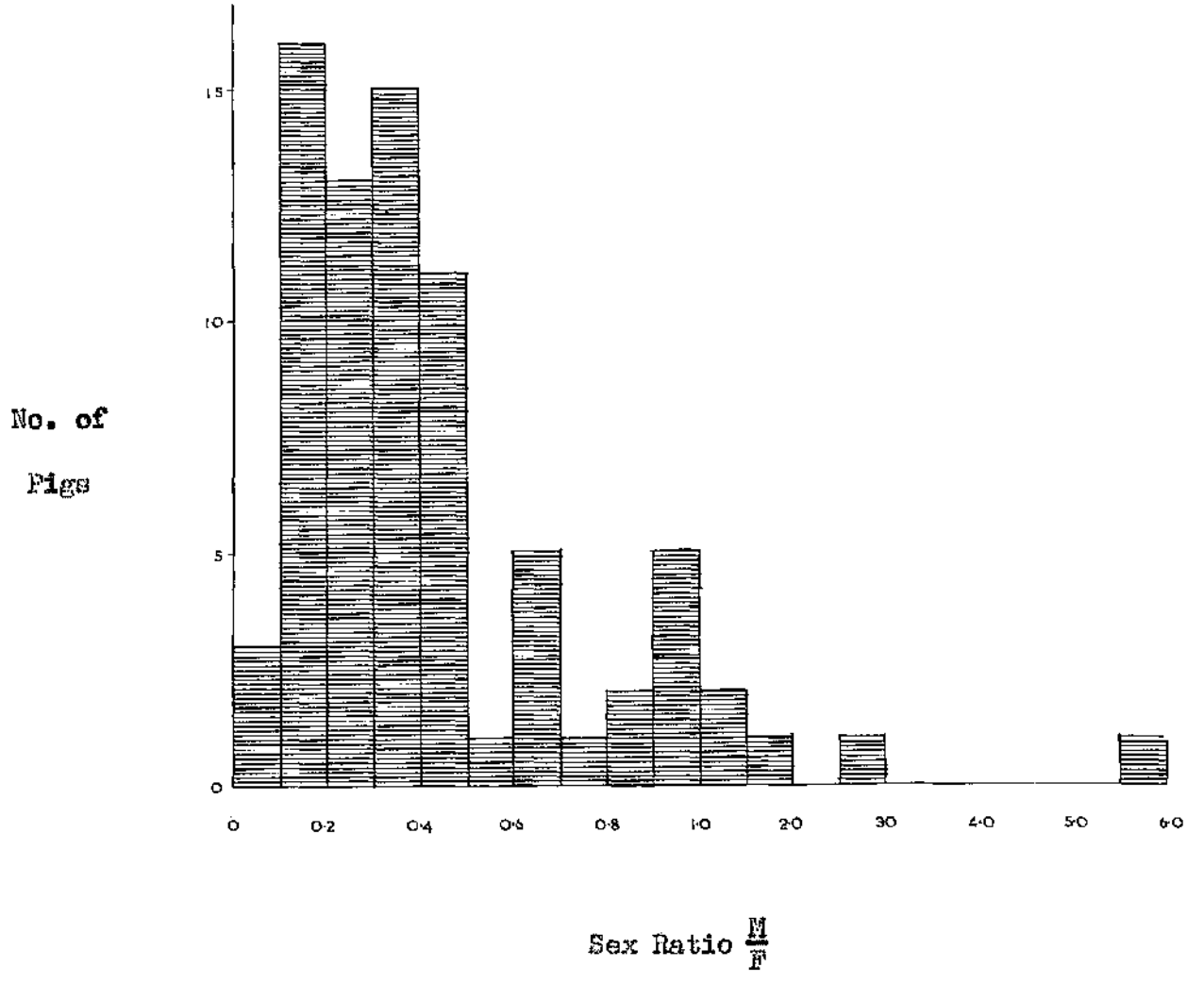


Figure 69

THE SEX RATIO $\frac{M}{F}$ IN NATURALLY OCCURRING METASTRONGYLOSIS



ranged from 0.04 to 6.0 although in most animals it was less than 1.0. In the group with one sex absent there were 25 cases which had no males and only one case with no females. All of these pigs except one with no males had less than 10 lungworms.

Lungworms were found in the diaphragmatic lobes in all of the cases, particularly at the posterior tips of the lobes. In addition, in one case, worms were found in a cardiac lobe, in another they were present in an apical lobe and in three cases they occurred in both the apical and cardiac lobes of the lungs. Three animals were seen with worms in all of the lobes of the lungs including the intermediate. When lungworms were present in all the lobes including or excluding the intermediate the parasitic burden was heavier than average, and more than one hundred lungworms were found in four of the six cases involved.

The species was identified as M. apri in every case but one. This animal had nineteen lungworms two of which were identified as female M. pudendotectus. These worms were much smaller than female M. apri and were approximately the same size as male M. apri, but appeared thicker than these worms to the naked eye (Fig. 70). When examined under a microscope the large spherical provagina (Fig. 71), characteristic of the species was easily seen.

Discussion

The incidence of lungworm disease in various regions of the United

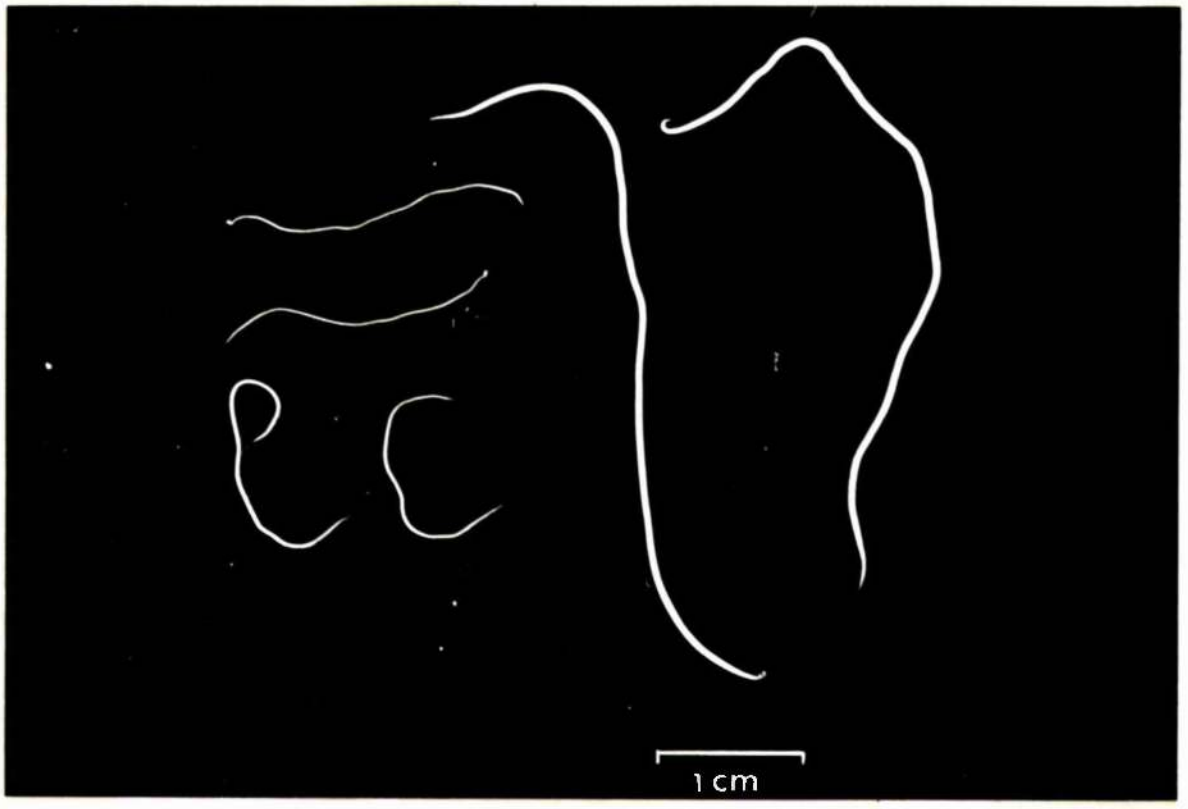


Fig. 70 Two female M. pudendotectus (lower left) below two male M. apri (upper left), compared with two female M. apri (right) for size.

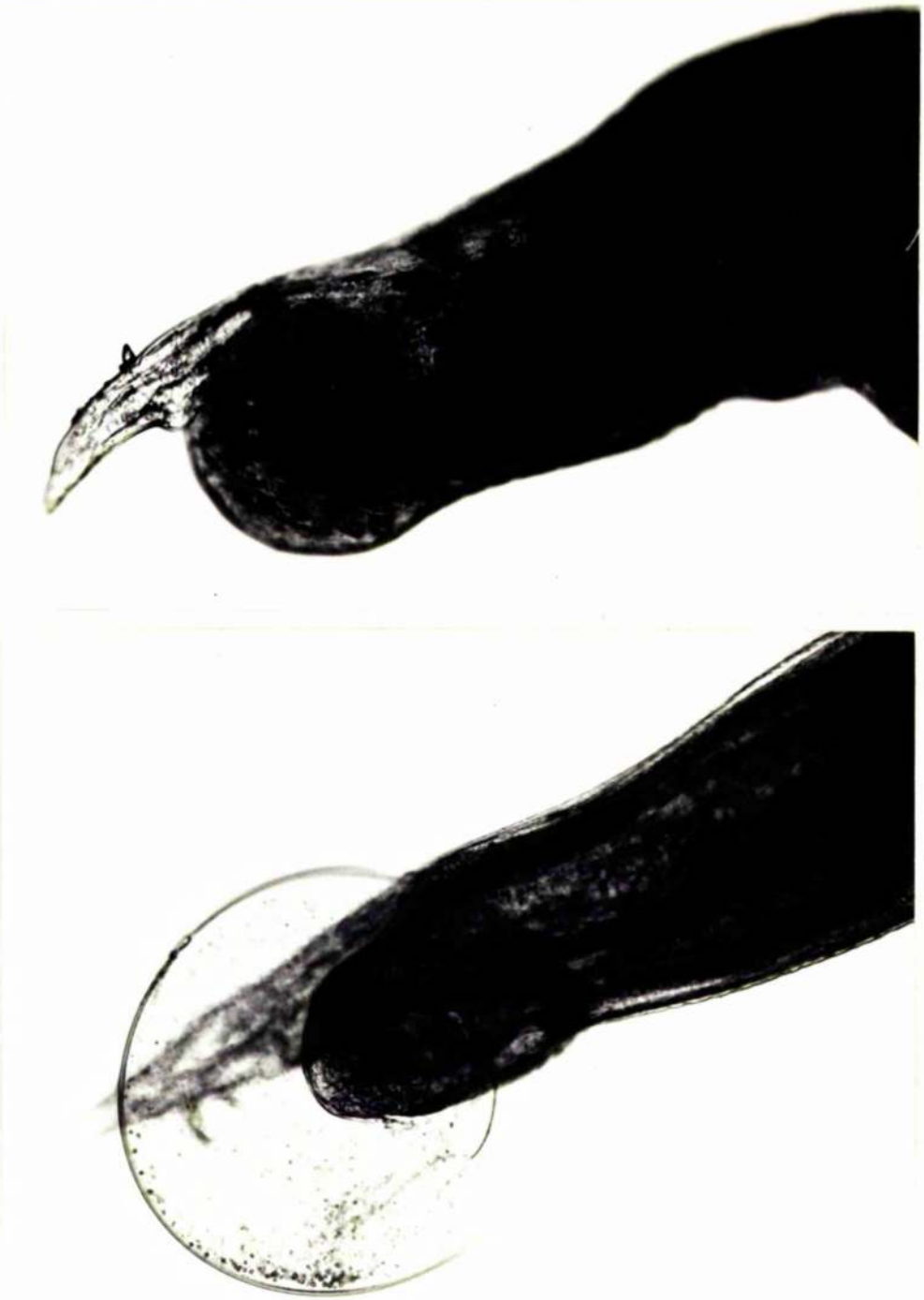


Fig. 71 Posterior end of a female M. apri (above) compared with the posterior end of a female M. pudendotectus (below) showing the spherical provagina of the latter. x 60

Kingdom has been investigated by several workers. The only other survey in Scotland was carried out by Robertson (1937) who found an incidence of 13.1% when he examined 1,009 sets of pig lungs at an abattoir receiving pigs from farms in north-east Scotland. This figure is not markedly different from the one found in this survey although it is likely that more pigs are kept indoors on concrete now than in the thirties.

Lewis (1926) found an incidence of 50% when he examined 137 pigs in mid-Wales and a more recent survey in England (Dunn, Gentles and White, 1955) reported the incidence as 18.3% of 1,238 pigs examined in a bacon factory in Cheshire, 26.0% of 366 pigs examined in London and 27.0% of 118 pigs examined in Hereford. When Whittlestone (1957b) examined 4,457 lungs representative of pigs from 15 counties in England he found that 29.0% had lungworms. He was able to trace his material back to the farms and discovered that 29 out of 32 farms were infected. Dunn et al. (1955) also traced their pigs to the farm of origin and found that 99 out of 224 farms were sources of infection. MacKenzie (1958a) examined pigs of all ages from one herd with lungworm and reported that 69.7% of store pigs, 59.5% of pork pigs and 53.2% of bacon pigs were affected and in addition 13.0% of the bacon pigs had no lungworms but had lesions attributable to previous infection. These figures demonstrate that on any one farm the number of pigs affected can be high.

There was considerable fluctuation in the daily incidence in this survey as can be seen in Table 9. To some extent this probably reflected the

management policy of the abattoir since there were days when large numbers of pigs were slaughtered from intensive herds where the pigs may not have had access to earthworms and inevitably this would mean a lowering of the incidence.

Most other surveys have not given figures for the level of parasitism in individual animals. Robertson (1937) said that the average was 11 lungworms per pig in his survey with a range of 1 to 65, and Dunn et al (1955) described light infestations in most pigs with 10-50 lungworms.

MacKenzie (1958a) gives average figures for the lungworms found in the store pigs, the pork pigs, and the bacon pigs on the farms he investigated and these were 15.4, 13.7, and 5.4 respectively. The highest individual counts of 100 or 200 lungworms were obtained in the two younger groups. In this survey also, although nearly all of the pigs examined were baconers the five highest lungworm counts recorded were in pork pigs. Counts higher than 25 were not seen by MacKenzie (1958a) in bacon pigs, but did occur in those reported here. Most of the pigs in this survey had a relatively small number of lungworms and this was probably due to (i) a low level of challenge and (ii) the fact that many of the cases were seen when the worm burden was dying off.

Ewing and Todd (1961) examined the sex ratio of Metastrongylus in 100 pigs at Wisconsin, U.S.A. and found that the overall ratio was 3:2 in favour of the female. They reported considerable variation in the ratio within a

given animal, but did not give the figures and also described some animals which were parasitised by a single sex. These findings are similar to the situation described here. In experimentally infected animals killed eight weeks after infection the sex ratio male:female is 0.4±0.2. If this is true in the field 99% of the population should be between 0 - 0.8. The pigs with sex ratios higher than this might represent the late stage of an infection with preferential loss of females, a phenomenon that is known to occur with other species of nematodes (Urquhart 1965, personal communication). The large number of pigs with no males however cannot be explained on this basis, but since these were pigs with small numbers of worms it is possible that a male was missed because they are much smaller than the female.

There was no correlation between the sex ratios and the number of lungworms present when this was plotted as a graph.

The distribution of the lungworms within the lungs in this series is the same as that reported by the other workers cited. None of them, however, recorded finding lungworms other than M. apri and although Lapage (1956) M. pudendotectus occurs in Britain, no details are given.

PNEUMONIA DUE TO METASTRONGYLUS APRI

(4) The Life Cycle of Metastrongylus sp. and the Method of Culturing the Infective Larvae of M. apri, with some Observations on the Intermediate Host

The three main species of the genus Metastrongylus which parasitise the pig are M. apri, M. salmi and M. pudendotectus, although others have been described such as M. madagascariensis (Chabaud and Gretillat 1956). Detailed descriptions for the identification of the three main species were made by: Gedoelst (1932) and Dougherty (1944). Using their criteria it was found in the survey described in the previous section that M. apri was by far the commonest of the trio occurring in Britain. The workers quoted earlier had similar results and it was decided therefore to use this species for all experimental work.

The definitive hosts of M. apri are either domestic or wild pigs and an intermediate host is required before the life cycle can be completed. Although pigs are the normal hosts for the adults, one case has been recorded describing their occurrence in the lungs of a child in Spain (Gonzalez, 1951) and there are reports of them reaching maturity in the lungs of a dog and in guinea pigs infected experimentally, (Schwartz and Alicata 1934, Dunn and White 1954).

The intermediate host is various species of earthworms which become in-

fected by ingesting the larvated eggs occurring in the faeces of parasitised pigs. The life cycle was studied in detail by Schwartz and Alicata (1929, 1932, 1934) who maintained that the eggs did not usually hatch in the faeces of the pig before ingestion by the earthworm, although it was possible to infect the earthworms with the first stage larvae experimentally. Pig lungworm eggs are susceptible to desiccation and high temperatures (Kates 1941). After desiccation they do not survive longer than 25 days at 17°C to 22°C and only a small number of eggs will survive 50°C for 10 minutes. They can, however, survive freezing at temperatures of -8°C to -20°C for as long as 108 days.

When earthworms ingest the eggs the larvae hatch out and burrow into the tissues of the intermediate host, accumulating in the wall of the oesophagus and in the lumina of the large vessels encircling the oesophagus called the hearts (Schwartz and Alicata, 1934). Occasionally larvae may be found in the wall of the crop or the intestine but rarely are they seen in the gizzard. In these sites the larvae molt twice to become the third stage, which is infective. The first molt occurs between 8 and 15 days after ingestion, depending on the ambient temperature and the second molt takes place on the succeeding day.

A wide variety of earthworms have been shown experimentally to be capable of acting as intermediate hosts (Schwartz and Alicata 1934, Dunn 1955 and Dayton 1957). This includes the species Allolobophora terrestris,

A. caliginosa, A. chlorotica, Eisenia rosea, E. foetida, E. lonnbergi, Lumbricus terrestris, L. rubellus, Helodrilus foetidus and H. calinginosus var. trapezoides. All species are not equally susceptible however, and Dunn (1955) found that E. foetida was the most easily infected experimentally. This earthworm was therefore used as the intermediate host in the work described here.

Darwin (1882) estimated that there might be as many as 26,000 earthworms per acre in a field, in which case there is obviously no shortage of intermediate hosts for the parasite if pigs are reared out of doors. Although the lifespan of an earthworm is not known, some are reported to have lived for ten years under laboratory conditions (Nicholas 1949) and McKenzie (1958a) reported that pigs had become infected from pastures free from pigs and pig manure for three years. Preceding the three year interval the land had been intensively grazed by pigs. If the infective larvae survive as long as the earthworm it is possible therefore for land to harbour infective earthworms for at least ten years.

Method of Culture

In order to produce infective third stage larvae a means of maintaining a large number of earthworms in a healthy and viable condition had to be devised. The species selected was E. foetida which is commonly found in manure heaps and compost and is easily recognised by the yellow and reddish brown bands on its body. The earthworms were collected in large pails from

the manure heap of a local farm on which there had been no pigs in living memory. They were distributed from the pails into groups of 200 in large flower pots. The flower pots had a diameter of 7 inches at their open end and a layer of paraffin wax was poured into the bottom of the pot to seal the drainage hole in the base. Each pot was filled to within 2 inches of the top with manure from the site where the worms were collected and the 200 worms lived in this until they were required for experimental purposes. The top of the pot was covered by a sheet of polythene which was perforated in the centre to allow free aeration of the culture and was secured to the pot by an elastic band.

Earthworm cultures were infected by adding large numbers of the female M. apri collected during the abattoir survey, to the manure in the pots. Under the conditions of culture these disintegrated rapidly and liberated the larvated eggs in their uteri (Fig. 72). The fluid in which the lungworms had been collected and counted was also added to the cultures since this usually contained large numbers of eggs from ruptured females. The cultures were kept for at least two months from the time of infection to allow the earthworm to pick up a large number of eggs, before they were used to infect pigs.

The flower pots were kept in a centrally heated building where the temperature fluctuated between 60°F and 70°F and it was important to inspect them regularly each week to make sure that the culture medium did not dry up,

otherwise the worms died very quickly. In addition to spraying the culture with water an additional supply of nourishment was made available since it was thought that the organic material in the manure might not keep such a large population of worms alive for several months. The extra food was a mixture of equal parts of two proprietory diets for laboratory animals, Diet 86 and Diet 18. Since these were in cube form, they were crushed into a rough powder before being sprinkled over the cultures once a week.

Four thousand infected earthworms were successfully kept under these conditions, and some of them were cultured for seven months. The infective third stage larvae were still viable at the end of this period.

Method of Examining Earthworms and Collecting Larvae

Two methods were used when examining earthworms for the presence of infective larvae. In each case since the number of larvae in the posterior half of the earthworm is negligible only the anterior half containing the oesophagus was examined.

Method 1

The earthworm was removed from the culture and put into a beaker of water to clean grit and debris from its skin. It was then picked up with a pair of forceps and cut in two with scissors. When doing this a small portion at the head was snipped of first to remove the cerebral ganglia.

The anterior half was put on a slide and cut open longitudinally with scissors and the internal organs were removed. The body wall and the viscera were pressed between two microscope slides which were then held together by rubber bands placed over their ends. This pressed preparation could be examined under a microscope and although larvae were easily recognised in the tissues or the hearts (Fig. 73), if large numbers were present they were difficult to count.

Method 2

The anterior half of the earthworm was obtained as in the previous method and was then chopped into small pieces using scissors. The fragments were left in a petri dish either in water or in isotonic saline for several hours, at room temperature. If larvae were present in the worm they migrated out and could be seen curled up or moving on the bottom of the petri dish, using a sterioscopic microscope. When the bottom of the petri dish was lined using a glass-diamond it could be used as a simple counting chamber.

The numbers of larvae leaving the worm fragments were increased by putting them in a 2% pepsin solution buffered at pH 2.0 and incubating them for half an hour at 37°C. A 2% trypsin solution was also used sometimes for enzymatic digestion of the fragments, and had a similar effect.

Most workers in the past (Sullivan and Shaw 1952, Dunn 1956) gave pigs infected earthworms or fragments of the earthworms to eat when they wanted

to produce the disease experimentally and estimated the dose given by calculating the mean number of larvae per worm in their culture population of earthworms, from a small sample of the earthworms which were killed and examined microscopically. It was felt that this method was not precise enough for quantitative studies. The following method was used to provide a suspension of larvae which could be counted accurately by a dilution method before it was divided into the required doses.

Since a considerable amount of time was involved in preparing the larvae some idea of the number of earthworms required was necessary. This was done by calculating the mean number of larvae and the standard deviation on a sample of five earthworms from each culture which was available for use. The number of larvae in each worm was counted using method 2 without employing enzymes. Twice the standard deviation was subtracted from the mean and this figure was taken as the minimum number of larvae in each worm in the culture. Using this, the number of earthworms likely to be required was calculated.

The anterior halves of the desired number of earthworms were chopped into the smallest pieces possible with scissors as has already been described. These fragments were put into 3.0 - 4.0 cm. of water in large crystallizing dishes 19.0 ins. diameter x 10.0 in. deep and left on the laboratory bench for 36 hours to let the larvae escape from the fragments.

After 36 hours the contents of the dishes were poured into Baermann

funnels through a large sieve, 24.0 ins. in diameter with 60 mesh/sq. inch and a mesh aperture of 0.0098". The worm fragments were allowed to settle on the sieve and then the funnel was topped up with water so that the sieve and the fragments were just immersed. Finally, filter paper (Whatmans No. 1 10" diam.) was placed on top of the fragments to prevent them floating off the sieve. The bottom of the funnel was closed by a piece of rubber tubing and a screw clip. The larvae wriggled through the sieve and could be collected by drawing of fluid at the bottom of the Baermann funnel. This was done hourly until sufficient numbers were collected.

The earthworm fragments were washed off the sieve and digested for one hour at 37°C in 2% pepsin solution buffered at pH 2.0. The mixture was allowed to cool and then Baermannised as before. This procedure gave an additional supply of larvae for infecting pigs which would provide adult M. apri for new earthworm cultures.

Observation on the number of M. apri larvae in earthworms

From the epidemiological point of view it would be interesting to know how many larvae an earthworm is capable of carrying, since a pig at pasture might not have the opportunity to eat large numbers. Dunn (1955) examined 229 earthworms from a farm with Metastrongylosis and found Metastrongylus larvae in 21% of them. Most of the worms had less than ten larvae but two contained 250 and 380 respectively. In order to investigate the ability of

earthworms to carry larvae the following two sets of observations were made:

Ten earthworms were chopped in ten lined petri dishes, as described before. Twenty six hours later the number of larvae which had emerged from the worms were counted and the worm fragments were removed to ten other lined petri dishes. To these a 2% pepsin solution buffered at pH 20 was added and the dishes were incubated for half an hour at 37°C. After they were removed from the incubator the new lot of larvae which had emerged from the worm fragments were counted. The results are shown in Table 12 in which it can be seen that all of the worms except one had contained over three hundred larvae. Simply chopping the fragments and leaving them at room temperature was reasonably efficient in all cases except one when the pepsin digestion liberated more larvae than had got out prior to this.

After this experiment some of the earthworm fragments which had been pepsinised were examined using pressed preparations and when considerable numbers of larvae were seen in them it became obvious that the figures obtained in this experiment were not the maximum numbers of larvae in the earthworms.

The whole procedure was repeated on six earthworms. This time trypsin was used as the enzyme and all the fragments were examined as press preparations after enzymatic digestion. The fragments were left for 56 hours before the larvae were counted and the digestion with trypsin was for one hour. The results are shown in Table 13. All of these worms had been

Table 12

THE NUMBER OF M. APRI LARVAE EMERGING FROM EARTHWORMS AFTER FRAGMENTATION AND ENZYMATIC DIGESTION

Worm No.	No. of Larvae After 26 hours	No. of Larvae After Pepsin	Total No. of Larvae/Worm
1	399	110	509
2	528	62	570
3	382	51	433
4	360	87	447
5	192	216	408
6	608	115	723
7	79	63	142
8	728	161	889
9	324	40	364
10	317	50	367

Table 13

THE NUMBER OF M. APRI LARVAE IN SIX EXPERIMENTALLY INFECTED EARTH WORMS

Worm No.	No. of Larvae After 56 Hours	No. of Larvae After Trypsin	No. of Larvae in Press Preparations	Total No. of Larvae/Worm
1	664	161	141	966
2	677	44	59	780
3	806	80	128	1,014
4	336	191	316	843
5	197	18	276	491
6	273	65	200	538

carrying over four hundred larvae and one had over one thousand larvae. Even with the longer period on the laboratory bench and trypsinisation earthworms 4, 5 and 6 still retained a large number of larvae in their tissues. In natural infections all of these would be liberated because of complete digestion of the earthworm. Although infected earthworms have been found in the field, in most cases the number of larvae they contained was small. In the conditions under which earthworms live in the laboratory, however, one would expect higher levels of infection to occur than in the field since they are exposed to a very large number of larvated eggs which are readily available in their food.

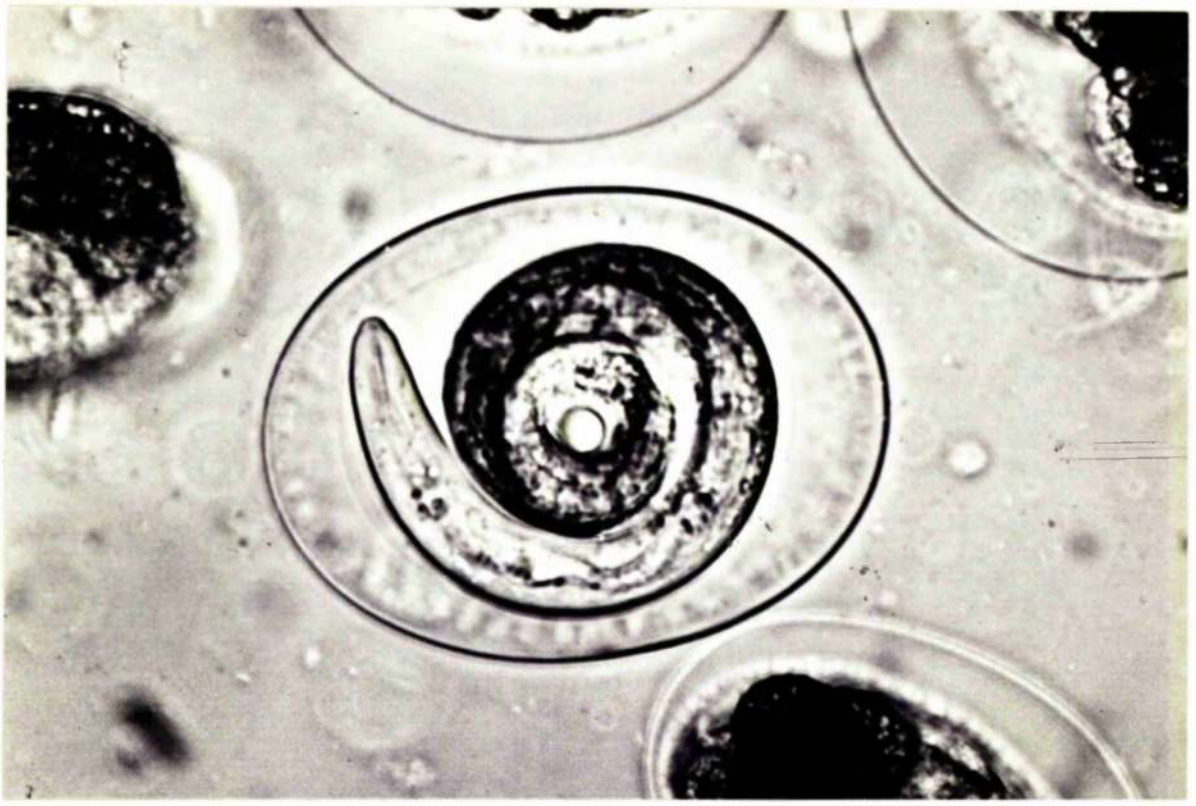


Fig. 72 Egg containing a first stage larvae of M. apri obtained from the uterus of an adult female lungworm. x 900

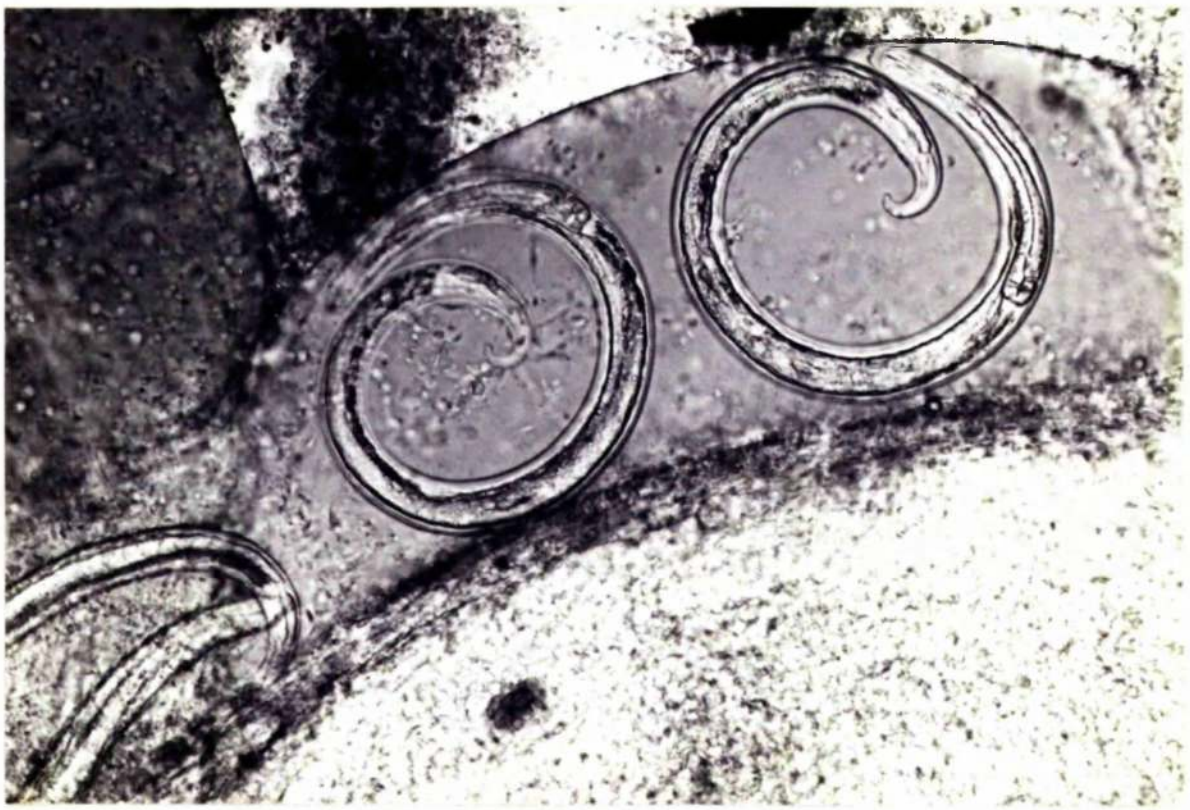


Fig. 73 Third stage infective larvae of M. apri coiled in an earthworm's heart. x 150

PNEUMONIA DUE TO METASTRONGYLUS APRI

(5) The Serial Pathology of Experimental Infections with Normal Larvae

The study of the pneumonia produced by Metastrongylus apri in the pig included observations on the changes occurring in the lungs of animals experimentally infected with normal larvae and killed at different times after infection. Dunn (1956) briefly described the histopathology of the disease in eight pigs infected in a variety of ways which involved feeding single, or repeated doses of earthworms containing lungworm larvae and Mackenzie (1959) in a more detailed study reported the pathological changes occurring in 22 young pigs infected with a single dose of larvae and killed at intervals of 1 to 80 days after infection. Although a large number of pigs were used in this experiment the number killed at each stage of the disease was small because of the time interval covered.

During the course of the experiments recorded here a series of 27 pigs, which had been infected with normal larvae were available to study the serial pathology of the pneumonia caused by M. apri, and the opportunity was taken to investigate the lesions produced by the adult stage of the parasite in more detail since it was thought that vaccination and x-irradiation would have their most significant effects at this stage of the disease.

Methods

The experimental pigs used were those described in Materials and Methods

and at the time of infection their ages ranged from four to eight weeks. They were housed on concrete during the course of the experiments in the accomodation which was described earlier and were killed at the time intervals after infection shown in Table 14.

The infective third stage larvae were obtained from earthworms as described in section 4. The suspensions of larvae collected from the Baerman funnels were counted by a dilution method and the required doses for the pigs were transferred to universal containers. The animals were infected orally using the technique described in Materials and Methods.

Before infection two faeces samples were examined from each pig using the flotation method described by Dunn, Gentles and White (1955). After the pigs were infected faeces samples from most animals were examined at four weeks, five weeks, six weeks, eight weeks, ten weeks, twelve weeks, fourteen weeks and sixteen weeks using the McMaster method employing saturated magnesium sulphate to float the eggs in the counting chambers.

After the pigs were killed small blocks of tissue for histological examination were taken from all the lobes of the lungs and the bronchial lymph nodes. The entire bronchial system was then opened with scissors and the lungworms which were present were removed, placed in isotonic saline, and counted later.

Results

The number of lungworms found in each pig at autopsy is shown in Table 14.

Table 14

THE RESULTS AFTER INFECTING PIGS WITH NORMAL THIRD STAGE LARVAE OF M. APRI

Pig	No. of Larvae Given	No. of Lungworms at Autopsy	Duration of Infection (weeks)	Faecal Egg Count at 6 weeks e.p.g.
M53	9,200	2,519	4	-
M54	9,200	1,023	4	-
L1	8,000	2,151	5	-
L2	8,000	142	5	-
L3	3,500	809	5	-
L4	8,000	2,771	5	-
L5	9,000	386	5	-
L6	9,000	816	5	-
M55	8,700	1,533	6	5,100
M56	8,700	1,346	6	3,250
M41	2,900	85	6	100
M42	2,900	109	6	50
M43	2,900	38	6	50
M49	2,900	530	6	550
M50	2,900	2	6	-
M4	4,200	205	8	400
M7	4,200	1,208	8	1,600
M12	4,200	85	8	250
M17	4,200	692	8	350
M23	4,200	378	8	450
M24	4,200	99	8	250
M5	4,500	7	12	350
M30	4,500	129	12	300
M3	4,500	14	14	700
M10	4,500	144	14	50
M16	4,500	534	16	1,750
M25	4,500	5	16	600

Larvated lungworm eggs were first detected in the faeces of some pigs four weeks after infection using the McMaster technique but in others no eggs were found until six weeks. The peak period of egg production was six weeks and the faecal egg counts at this time for all of the animals are shown in Table 14. Eggs were found in the faeces of all the pigs except M5, M3 and M25 until they were slaughtered. When the faecal egg counts at six weeks, and the worm counts at autopsy, of the three animals mentioned were compared with the equivalent observations on the animals killed at six weeks it seems likely that the former pigs had shed a considerable part of their lungworm burden by the time they were killed.

The pathology of the lesions found at autopsy will now be described.

Macroscopic Findings

4 weeks

In both animals there was a large number of lungworms in the smaller branches of the bronchial system in all the lobes of the lungs. Vesicular emphysema causing severe over-distension of the affected lobules was the most important lesion seen. This occurred in all the lobes of both lungs and was mostly distributed along the edges of the lobes (Fig. 74). The affected areas were very pale, puffy and in most situations were obviously lobular in configuration involving one lobule or a group of lobules. The bronchi supplying these lobules were packed with lungworms. One animal,

M54, had practically no consolidation in the lung, but the other had small pale pink oedematous patches of consolidation 1.0-2.0 cms. across in some of the lobules at the tips of the apical lobes, the cardiac lobes, and the intermediate lobe. They also occurred scattered along the basal edge of the diaphragmatic lobes and on the diaphragmatic surface of the diaphragmatic lobe of the left lung. The bronchial lymph nodes were enlarged.

5 weeks

Worms were found in every lobe of the lungs in all but one case, L5, in which there were no worms in the apical lobe of the left lung. Pale, lobular areas of severe vesicular emphysema were seen distributed similarly to that in the animals killed at four weeks. The pulmonary consolidation was more extensive in this group of pigs and was distributed similarly to that seen in the previous group. The apical lobes and the cardiac lobes were quite extensively involved in a strip like manner along their edges in L1 and L4. The consolidated lesions were pink or greyish pink, felt firm and exuded thin turbid fluid. Sometimes small yellow, white or greyish spots could be seen in them. The bronchial lymph nodes were larger than normal.

6 weeks

Although lobules with vesicular emphysema were seen, the outstanding feature of the lesions in this group particularly in M55, M56 and M49 was

pulmonary consolidation. The consolidation was mostly confined to the edges of the lobes but also occurred in the diaphragmatic surfaces of the diaphragmatic lobes (Fig. 75). Lesions could be seen in the lungs from the apical lobes to the posterior tip of the diaphragmatic lobes but the ventral tips of the apical and cardiac lobes and the antero-ventral edges of the diaphragmatic lobes were more severely involved in some pigs, particularly those with a large parasite burden. The consolidation was essentially similar in appearance to that seen at five weeks but often had a more granular texture, was very firm and was raised above the surrounding normal lung. Coalescing patches of consolidation formed strips along the edges of the lobes. Lobules of vesicular emphysema were also seen particularly in the diaphragmatic lobes at the posterior tip and along the posterior half of the basal border. The emphysematous lobules were pale but the degree of distension could only be described as moderate since it was less marked than that seen earlier. A few greyish purple nodules about 0.2 cms. in diameter were seen in two pigs M49 and M41, on the costal surfaces of the posterior halves of a diaphragmatic lobes near their edges. In one animal they also occurred in the apical lobe of the right lung.

The bronchial lymph nodes were enlarged.

8 weeks

The dominant feature of the lesions in this group was pulmonary con-

solidation which was similar in distribution to that seen at six weeks. The amount in each animal varied however depending on the number of lungworms present. Several lobules with moderate vesicular emphysema were seen in the diaphragmatic lobes of the lungs. One pig, M24, had a few greyish purple nodules 0.3-0.5 cm. in diameter in the posterior half of the diaphragmatic lobes.

The bronchial lymph nodes were enlarged.

12 weeks

The principal lesion at this stage was the formation of greyish-purple nodules 0.1-0.5 cm. in diameter which protruded above the surface of the lungs. These were seen in the apical lobes, the cardiac lobes and the diaphragmatic lobes of both lungs. They could be found at any position in the lungs but tended to occur near the edges of lobes (Fig. 76). Nodules were seen on the costal and diaphragmatic surfaces of the diaphragmatic lobes and also sometimes within these lobes. Two interesting observations were that there were at least twice as many nodules in M5 as in M30 and that the latter contained many more lungworms at autopsy.

Small patches of consolidation were found in M5 but none were seen in M30. Several lobules at the edges of the lobes of the lungs in the predilection site for emphysema were pale and slightly raised above the

others. These were patches of residual vesicular emphysema and were seen in both pigs.

The bronchial lymph nodes were only slightly hyperplastic.

14 weeks

The changes seen in the two pigs killed at 14 weeks were the same as those seen at 12 weeks.

16 weeks

In M16 a small number of pulmonary nodules and several greyish purple patches of consolidation at the edges of the apical lobes, the cardiac lobes and the antero-ventral edges of the diaphragmatic lobes were the only lesions seen. The pulmonary nodules in M25 were quite numerous and sometimes occurred in small groups of four or five close together. A few pale lobules with traces of emphysema were observed. The bronchial lymph nodes were not enlarged in M16 and only slightly enlarged in M25.

The histopathological changes producing the macroscopic features which have been described are reported next.

Microscopic Findings

4 weeks

Adult male and female lungworms sectioned in various planes were seen in the bronchi and bronchioles. Some bronchioles and smaller bronchi were

considerably distended by the mass of worms in the lumen (Fig. 77). The worms were usually surrounded by mucus in which larvated eggs could sometimes be seen. The mucus was usually devoid of cells but in some bronchioles there were also eosinophils, polymorphonuclear leucocytes, mononuclears and desquamated epithelial cells. The epithelium was hyperplastic and in the bronchi packed with worms the surface was irregular due to projecting patches of hyperplasia alternating with foci of atrophy produced by pressure from the worms. Mucoid metaplasia was not often seen but had occurred in a few bronchioles. Eosinophils, lymphocytes and plasma cells were found in the lamina propria of many bronchi and bronchioles and were also seen migrating through the epithelium. Moderate hypertrophy of the bronchial and bronchiolar smooth muscle had developed. Between the muscularis of the bronchi and the plates of cartilage cellular infiltrates similar to those in the lamina propria were found and there was also some lymphoid nodule formation. These early nodules were composed of widely separated cells and contained a high proportion of lymphoblasts. Lymphoid nodules had formed to a moderate degree in the peribronchiolar tissues of some bronchioles. The cells in this site however were more frequently diffuse accumulations of eosinophils, lymphocytes, and plasma cells. The changes described above which were seen in the walls of the bronchi and bronchioles were also seen, particularly in the case of the latter, when there were no sections of worms in the lumen.

Severe vesicular emphysema with distensions of the alveoli, the alveolar ducts and the terminal bronchioles (Fig. 77) and rupture of alveolar walls so that small bullae were formed was an outstanding feature of the histopathology of this stage of the disease. The bronchioles in these lobules were frequently packed with lungworms.

Lungworms were sometimes seen extending down into the alveolar ducts and alveoli, distorting the walls of the latter. In some lobules without emphysema there was patchy thickening of the alveolar walls particularly adjacent to alveolar ducts or blood vessels. This was due to a heterogeneous accumulation of cells including large mononuclear cells, described as endothelioid types, with indistinct cytoplasm, and a pale elliptical nucleus with a well stained nuclear membrane, alveolar cells, lymphocytes, plasma cells, eosinophils and polymorphonuclear leucocytes. Other lobules showed partial alveolar collapse, alveolar oedema and moderate infiltration within the alveoli by polymorphonuclear leucocytes, alveolar macrophages, large mononuclear cells, eosinophils, lymphocytes and plasma cells in various proportions. The alveolar cells were slightly more numerous in the alveolar walls. Some alveolar ducts were packed with polymorphonuclear leucocytes.

A few aspirated eggs were seen in the patches of consolidation. These eggs had either an obvious larvae inside them or contained granular eosinophilic material. The latter appearance was similar to that seen in immature eggs within the uteri of female worms and their presence in the lung was

probably associated with the onset of egg production. The reaction in the lobules where eggs were seen was usually a combination of cellular accumulations in the lumina of alveoli and thickening of the walls in the manner described above. In addition however multinucleated giant cells had appeared. One patch with obliterative bronchiolitis was seen. Eosinophils, lymphocytes and plasma cells were found in variable numbers in the septa and pleura.

The bronchial lymph nodes contained many nodules with pale germinal centres. Eosinophils were seen in the loose lymphatic tissue and there was hyperplasia of the reticulo-endothelial cells.

5 weeks

The changes in the bronchi and bronchioles were basically similar to those seen at four weeks. Larvated eggs, however, were more abundant in these structures mixed with the mucus and cells in the lumina. Mucoid metaplasia was a striking feature in both bronchi and bronchioles and was present to a greater degree in the animals of this group than in those seen at four weeks. Hypertrophy of the smooth muscle of the bronchi bronchioles and alveolar ducts was also very well developed in these animals. The hypertrophy of the bands of smooth muscle in the bronchioles had made obvious small gaps between them which could be seen in longitudinal sections. Diverticula of the bronchiolar epithelium had occurred through some of these into the peribronchiolar fibrous connective tissue. These diverticula

were little sac like protrusions sometimes containing mucus or mucus and cells and often surrounded by an accumulation of lymphoid cells in the peribronchiolar tissue. Peribronchiolar lymphoid nodular formation was still at a low level and the cellular accumulations in these sites were similar to those seen earlier.

Severe vesicular emphysema was seen in many lobules in which the bronchioles were often packed and distended with lungworms. Pulmonary consolidation was more extensive in this group and histologically had several appearances. The alveoli were either partially collapsed or more frequently they were expanded and contained a variety of cell type, in oedema fluid (Fig. 78). In others the focal accumulations of cells within the alveolar walls had produced considerable thickening of these structures. Both of these changes were seen earlier. In this group however there was more extensive consolidation with complete obliteration of the pulmonary parenchyma in places, by a reaction to a large number of aspirated eggs (Fig. 79). Where this had happened there was gross thickening of the alveolar walls by large mononuclear endotheliod type cells, associated with which were lymphocytes, eosinophils and plasma cells. The general pattern of the lung could sometimes be discerned but frequently the endotheliod type cells had formed a syncytium and the overall pattern was lost. Large multinucleated giant cells were often found in this reaction. Numerous eosinophilic granulomas had developed and were seen around eggs or occasionally around a larvae which had hatched from the

eggs (Fig. 80). These granuloma were within the lumina of alveoli or had become incorporated into the general consolidation. Where the alveolar pattern remained the alveoli and alveolar ducts sometimes contained oedema fluid rich in fibrin and cells. The exudate in many places was being invaded by the endothelioid cells from the adjacent alveolar wall and this process was probably responsible for the eventual obliteration of the alveoli and also for forming patches of obliterative bronchiolitis. Epithelialisation was sometimes found at these sites.

Many aspirated larvated eggs were observed in these pigs in the consolidated areas. They were seen in alveoli surrounded by a moderate number of eosinophils, lymphocytes, plasma and macrophages. Giant cells were not always present but sometimes they occurred in considerable numbers in the vicinity of the eggs and occasionally eggs were seen inside giant cells. In other lobules the eggs were surrounded by a mass of eosinophils and necrosis of the eggs and the lung tissue had occurred forming an eosinophilic abscess. Eggs were also seen in which the larvae had been destroyed and the egg was full of eosinophils and some lymphocytes or plasma cells. Very frequently however the eggs were associated with eosinophilic granulomas of various sizes and stages of development.

Mild septal oedema had developed but otherwise the changes in the septa and pleura were similar to those described earlier.

The changes in the bronchial lymph nodes did not differ from these seen at four weeks.

6 weeks

At this stage in some pigs there were more peribronchiolar lymphoid nodules than were seen previously and more lymphoid nodules were also seen in the bronchi. Longitudinal sections of bronchioles often revealed a diffuse mass of lymphocytes and plasma cells with some eosinophils all along the length of the bronchiole producing considerable thickening of the peribronchiolar tissues. The other changes in the walls of the bronchi and bronchioles seen at five weeks were still present and metaplasia and hypertrophy of the smooth muscle of the bronchi, bronchioles and alveolar ducts were prominent features of the lesions. Diverticula of the bronchiolar mucous membrane were more numerous in these cases and were often larger than those seen in the previous group (Fig. 61).

Patches of moderate vesicular emphysema were found but pulmonary consolidation was a more important feature of this stage of the disease. In general two types of consolidation were seen. In one the alveoli were expanded and were full of oedema fluid containing eosinophils, lymphocytes, plasma cells, macrophages, giant cells, endothelioid cells and polymorphonuclear leucocytes in moderate amounts, although in a few fields the latter cells were sometimes particularly numerous. Moderate alveolar cell hyper-

plasia had also occurred. Aspirated eggs were frequently found in these lobules. In the second type of consolidation the lesion was more granulomatous and all of the change described as occurring at five weeks with the formation of eosinophilic granulomas, syncytial masses of endothelioid cells, giant cells and the obliteration of the lung parenchyma were seen. Eosinophilic granulomas were particularly numerous in these pigs and many aspirated eggs were seen. A few free larvae involved in granulomatous reactions were found. This reaction was extreme in M55 and M56 and there was thickening of the septa around these lobules due to fibrosis. Eosinophilic abscesses had also developed. One animal, M50, had an exceptionally large number of giant cells in the patches of consolidation.

Foci of obliterative bronchiolitis were present in all of the pigs. Occasional lymphoid nodules were seen in the interlobular septa, in addition to the eosinophils, plasma cells and lymphocytes scattered diffusely in small numbers.

The appearance of the bronchial lymph nodes had not changed from that seen at five weeks but in one animal the amorphous eosinophilic remains of a larva were seen in the bronchial node.

8 weeks

Adult parasites were seen in the bronchi and bronchioles associated with all of the changes described earlier. More peribronchiolar lymphoid

nodules were seen forming groups on one side of a bronchiole, or surrounding the bronchiole completely in transverse sections, or extending along their length in longitudinal or tengential sections. In some bronchioles lymphoid cells from these nodules were infiltrating the wall causing replacement of the hypertrophied muscularis. Numerous lymphoid nodules were seen in the bronchi between the muscularis and the plates of cartilage and sometimes the bronchial glands had been replaced by the nodules.

Sections were examined in which it could be seen that the anterior ends of the lungworms in bronchioles had turned backwards and were pointing towards the trachea. Where this had happened there was extreme dilatation of the bronchiole and even the hypertrophied muscularis was very thin and only a few cells thick.

Patches of vesicular emphysema were found showing the features described earlier.

Obliterative bronchiolitis was seen and in the consolidated lobules the consolidation was either primarily due to eosinophilic granulomas with thickening of the alveolar walls and eventual obliteration of the alveoli by endothelioid cells, eosinophils, lymphocytes, plasma cells, polymorphonuclear leucocytes and giant cells or it was the type associated with alveolar oedema moderate alveolar cell hyperplasia and infiltration by the cells described before. Many larvated eggs or eggs in which the larvae had been destroyed or the crumpled eosinophilic remains of eggs were seen

in the consolidated lobules. Eosinophilic abscesses also occurred.

Moderately large multifollicular lymphoid nodules had developed in the lungs of two pigs.

Mild septal oedema and infiltration by eosinophils, plasma cells and lymphocytes was seen and in a few small lymphoid nodules were found in the septa and pleura.

A large number of active nodules had developed in the bronchial nodes. Many eosinophils were also present but appeared to be reduced in number compared with the earlier stages of the disease.

12 weeks

The diffuse cellular infiltrations in the bronchial and bronchiolar walls were still quite extensive. Bronchiolar diverticula were frequently found and there was still considerable hypertrophy of the bronchial, bronchiolar and alveolar smooth muscle. Mucoid metaplasia however was more sporadically observed, being completely absent in many fields. The development of lymphoid nodules in the bronchial walls and in the peribronchiolar tissue was much greater at this stage than at the earlier phases of the disease. This lymphoid tissue often formed sleeves of considerable thickness all along the bronchioles.

An important difference in the histopathology of this stage was the finding of dead adult parasites in bronchioles and the development of large lymphoid nodules which were macroscopically visible around these dead parasites.

The dead parasite took the form of a brightly eosinophilic amorphous structure surrounded by a mass of lymphoid cells of all types; reticular cells, lymphoblasts and lymphocytes which were either distributed randomly or arranged into nodules with germinal centres (Fig. 82). A considerable capillary network had often developed in between the lymphoid nodules and sometimes focal areas with macrophages full of haemosiderin were found in the lymphoid mass. Traces of the bronchiolar epithelium, which frequently appeared to be very hyperplastic, were found among the lymphoid cells. The development of these nodules is considered in more detail in the next section.

Portions of bronchioles being replaced by lymphoid proliferation were also seen. Sometimes there was no worm in the section examined and the bronchiole appeared to have lost its epithelium in places and be infiltrated in its inner part by masses of eosinophils, plasma cells and lymphocytes. At the periphery and replacing the muscularis was a dense collection of reticular cells, lymphoblasts and lymphocytes.

The patches of consolidation had the appearance of those examined at earlier stages and eosinophilic granulomas around eggs were still present. There was fibrosis of the septa in the lobules which were severely involved.

The bronchial lymph nodes resembled those seen at eight weeks.

14 weeks

In these pigs there was massive peribronchiolar lymphoid nodular

development. Many bronchiolar diverticula were seen and in some longitudinal section several of these could be seen distributed along the length of the bronchiole. Although smooth muscle hypertrophy was still pronounced mucoid metaplasia was not seen. Many large lymphoid masses, which sometimes contained the necrotic remains of a lungworm were found. Some of the lymphoid masses had replaced whole sections of lobules and it was difficult to know whether they had developed around a bronchiole or in patches of consolidation.

Aspirated eggs, eosinophilic granulomas, and giant cells were present in the patches of consolidation. Portions of lobules with alveolar oedema, alveolar cell hyperplasia and infiltration by moderate numbers of eosinophils, polymorphonuclear leucocytes, lymphocytes, plasma cells and macrophages were also seen. Small patches, usually sublobular in size, of moderate emphysema were present.

Small lymphoid nodules had developed in the septa and in the pleura.

The bronchial lymph nodes did not differ from those examined at 12 weeks.

16 weeks

The changes described as occurring at 14 weeks were seen in the pigs killed at 16 weeks and the principal features of the lesions were the same.

Discussion

The sequence of events occurring in the lungs of pigs during the adult

phase of lungworm infestation were clearly reflected in the macroscopic findings. Initially at four and five weeks as the parasite matured and increased in size it had an obstructive effect on the bronchioles which resulted in vesicular emphysema developing in the related lobules. At six and eight weeks when the lungworms were sexually mature and as their egg laying capacity increased, pulmonary consolidation due to some eggs being aspirated into the lungs became the dominant feature of the lesions and later still as the adult worms began to die in the lungs from twelve to sixteen weeks large lymphoid nodules were the principal lesion observed. The progression of the disease as outlined above is similar to that proposed by Mackenzie (1959) but he did not describe finding the necrotic remains of adult lungworms in the nodules in his cases and did not associate the major part of the massive lymphoid nodular development with this phenomenon. In this connection it is interesting that although the pigs M5 and M30, killed at 12 weeks, had similar egg counts at six weeks, M5 had only a few worms at autopsy compared with M30 but had at least twice as many macroscopic lymphoid nodules, suggesting that M5 had got rid of its parasite burden quicker than M30.

Although some of the pigs in this experiment had very heavy worm burdens the only clinical signs noticed were severe paroxysms of coughing. Between the bouts of coughing the animals were apparently normal. Coughing was sometimes quite violent and the animal's body would shake with the force required for expiration. The overall effect was reminiscent of the asthmatic

type of spasm in man. The only report of pigs dying as a result of lungworm infestation was made by Sullivan and Shaw (1952) who were studying the effect of lungworm burdens on weight gains. In these experiments the pigs were infected by feeding them earthworms and in the group given heavy infestations two pigs died after the onset of patency. One animal had 7,000 lungworms at autopsy and the other was reported as having too many to count. The death of these pigs after patency was probably associated with the development of pulmonary consolidation around aspirated eggs. Sullivan and Shaw (1952) did not find any significant difference in the weekly weight gains between their three groups of eight pigs one group of which was a control group.

Larvated eggs were frequently seen in the consolidated portion of lung but sometimes larvae were also found. These were presumably first stage larvae that had hatched from the eggs. Larvae were also described by Dunn (1956) and MacKenzie (1959) during this phase of the disease. In one animal in this series a larva had migrated to the bronchial lymph nodes. Eosinophilic granulomas were more numerous in the lungs than the lesions described as an eosinophilic abscess.

In the consolidation where the alveoli were expanded and full of oedema fluid and cells there were some similarities with enzootic pneumonia. The alveolar cell hyperplasia was not as extensive as in enzootic however and there was more variation in the cell types within the alveoli. Macro-

scopically the granulomatous type of consolidation could be differentiated from enzootic pneumonia and the other pneumonias which have been described, because it was very firm and granular, but the pale pink oedematous type of lesion as seen at four weeks and patchily in the other cases was not readily separable by simple visual inspection.

The production of vesicular emphysema in pig lungworm infestation is generally attributed to the parasites having a ball valve like effect in the bronchioles which allows air to enter during inspiration and then prevents its exit during expiration. Whether this is simply due to the presence of the worms and mucus in the lumen or whether there is also spasm of the bronchiolar musculature is not known. Likewise the muscle hypertrophy which was seen at four weeks but had become more pronounced by six weeks and was a prominent feature of the lesions from them until sixteen weeks could have been due either to stretching of the muscularis by the worms, or to repeated spasms of the muscularis during the period of infestation.

Although the muscularis sometimes appears to completely encircle bronchioles (Fig. 63) it is really a spirally arranged mass of muscle bands and after they become hypertrophied it is possible to see, particularly in tangential sections, that there are small potential gaps between them. Diverticula of the bronchiolar epithelium were seen protruding through these gaps into the peribronchiolar fibrous connective tissue especially during the later stages of the disease. Because the lungworms and the mucus block

the lumina of bronchioles and since there may be bronchiolar spasm it is likely that the pressure within the bronchioles is considerably increased. This possibly had the effect of forcing the epithelium through the gaps in the muscularis to produce the diverticula. Bronchiolar diverticula were not described by Dunn (1956) or MacKenzie (1959).

Mucoid metaplasia was an outstanding feature of the lesions in the pigs killed at five weeks, six weeks and eight weeks, but later although it was still seen, the epithelium in most bronchioles had reverted to its normal character. The proliferation of mucus secreting cells at the expense of the other cells in the epithelium may be analogous with the similar phenomenon which occurs in the abomasal epithelium of cattle infected with Ostertagia ostertagii.

In conclusion the pulmonary lesions produced by M. apri are complex and vary depending on the stage of the disease being examined. They are however quite typical and even in the absence of the adult parasite itself it should not be difficult to diagnose the pneumonia due to M. apri histologically.

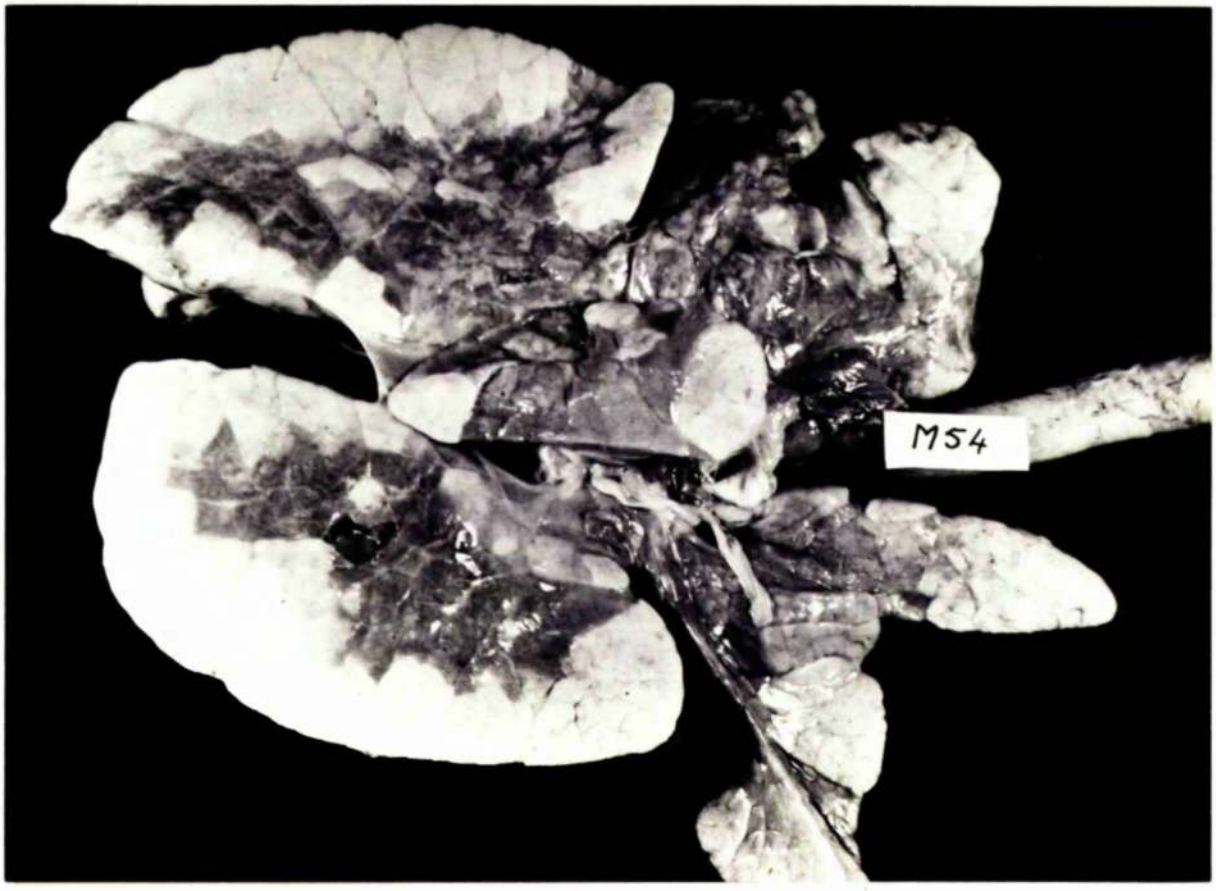


Fig. 74 M. apri (4 weeks): severe vesicular emphysema in all the lobes of the lungs.

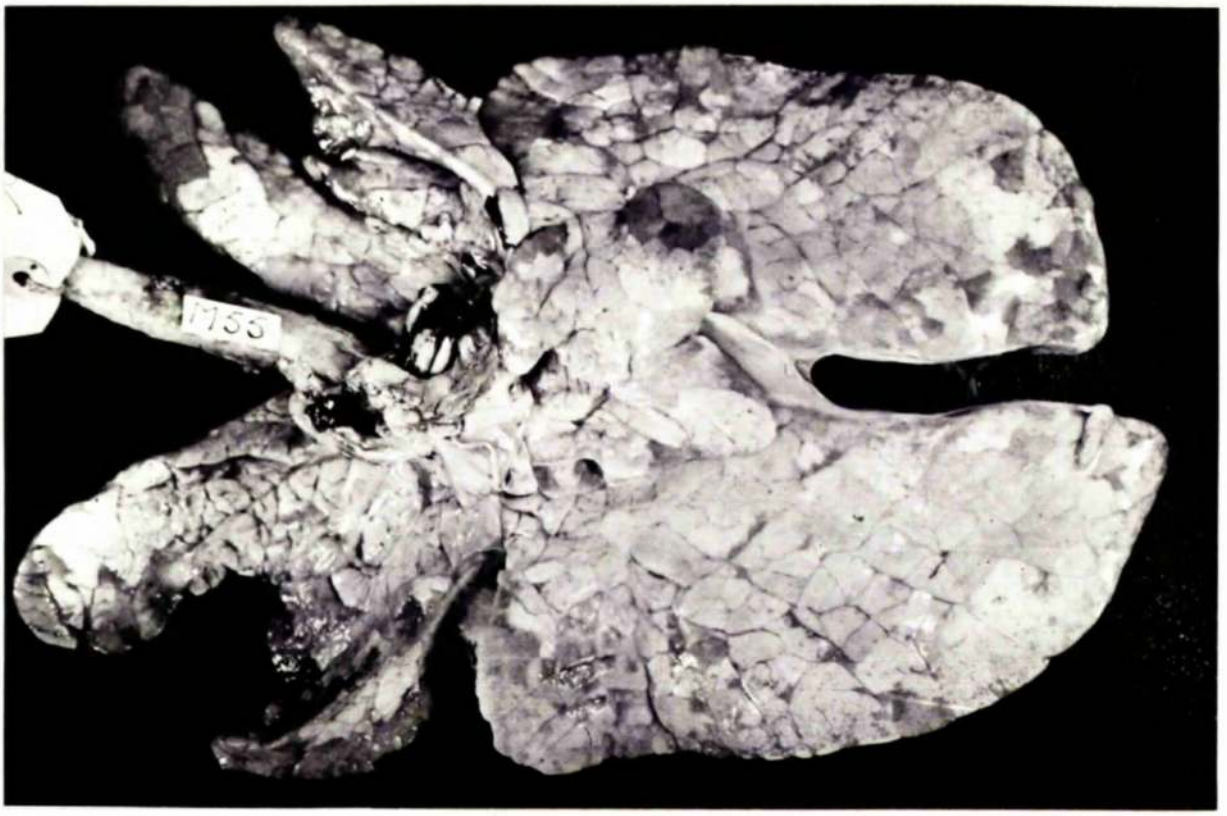


Fig. 75 M. apri (6 weeks): pulmonary consolidation in all the lobes of the lungs.

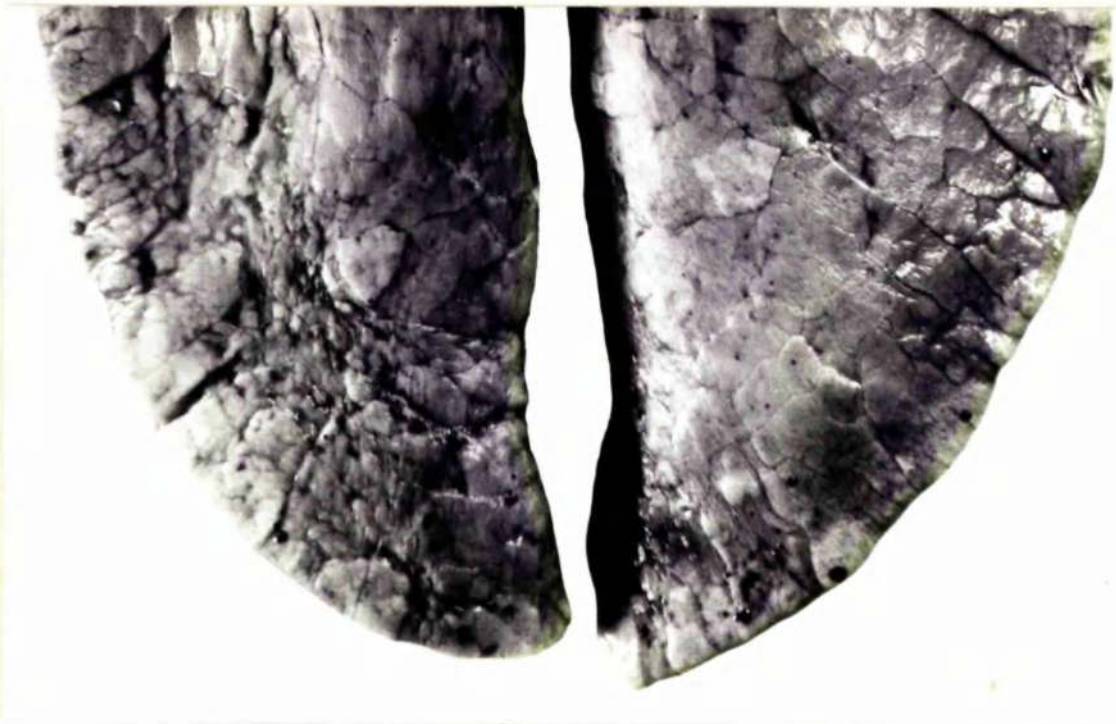


Fig. 76 M. apri (12 weeks): lymphoid nodules in the posterior halves of the diaphragmatic lobes.

Fig. 77 M. apri (4 weeks): a bronchiole in a lobule with emphysema distended and blocked with lungworms. H. & E. x 50

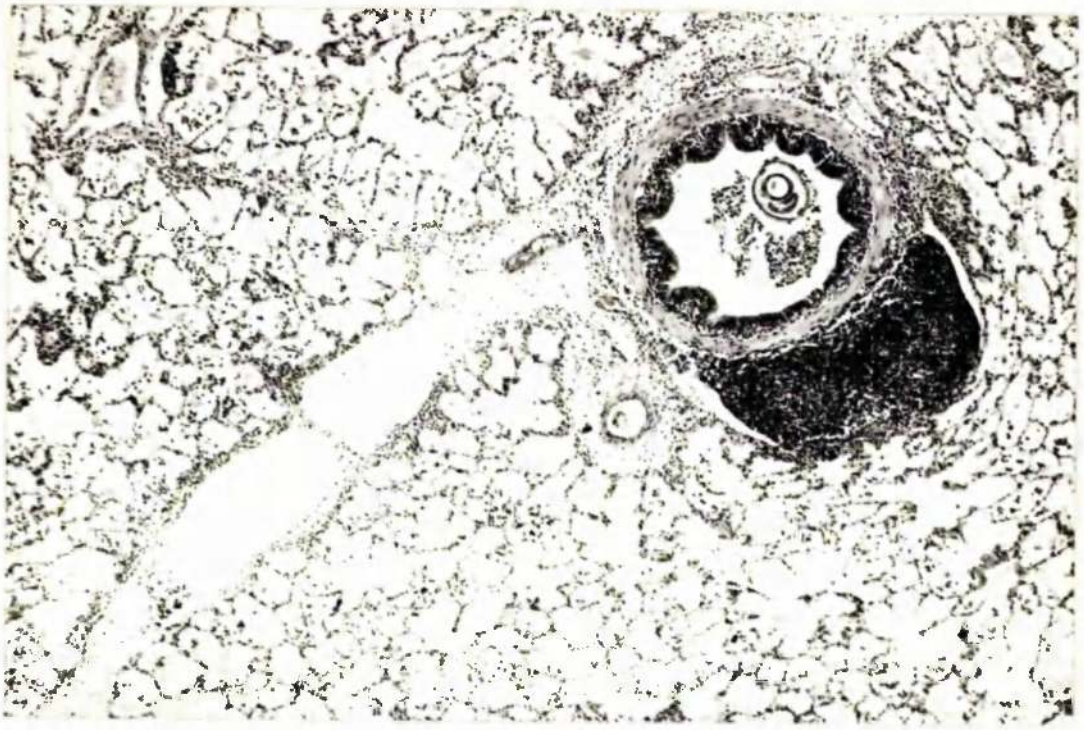


Fig. 78 M. apri (5 weeks): moderate alveolar and septal oedema with cellular infiltrates in the alveoli. H. & E. x 50

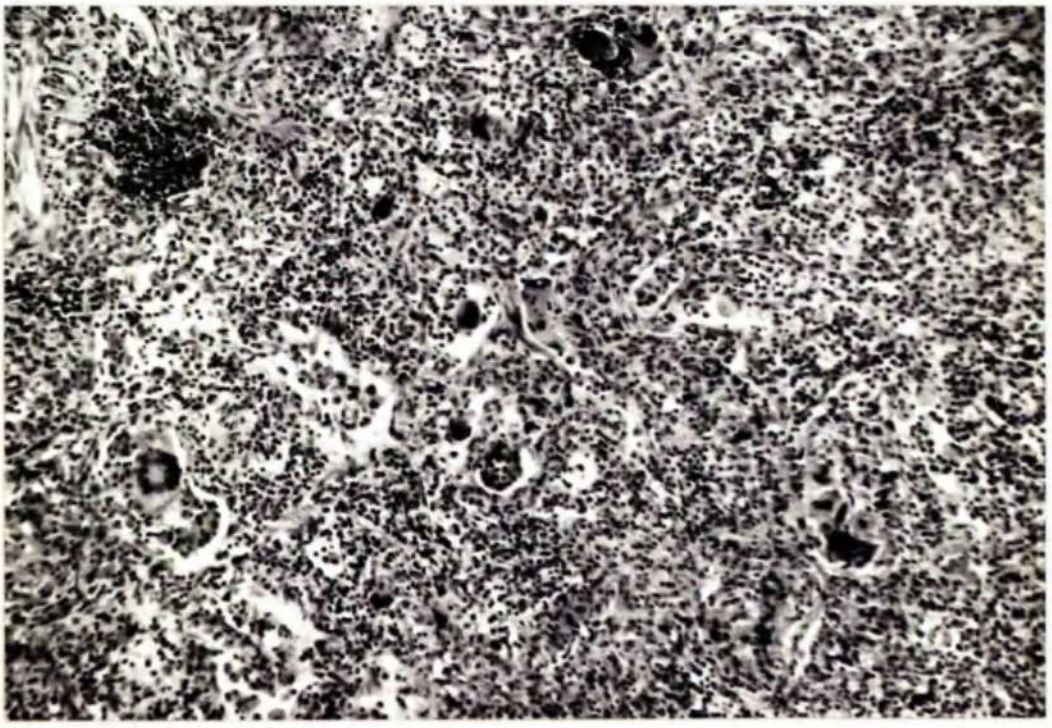
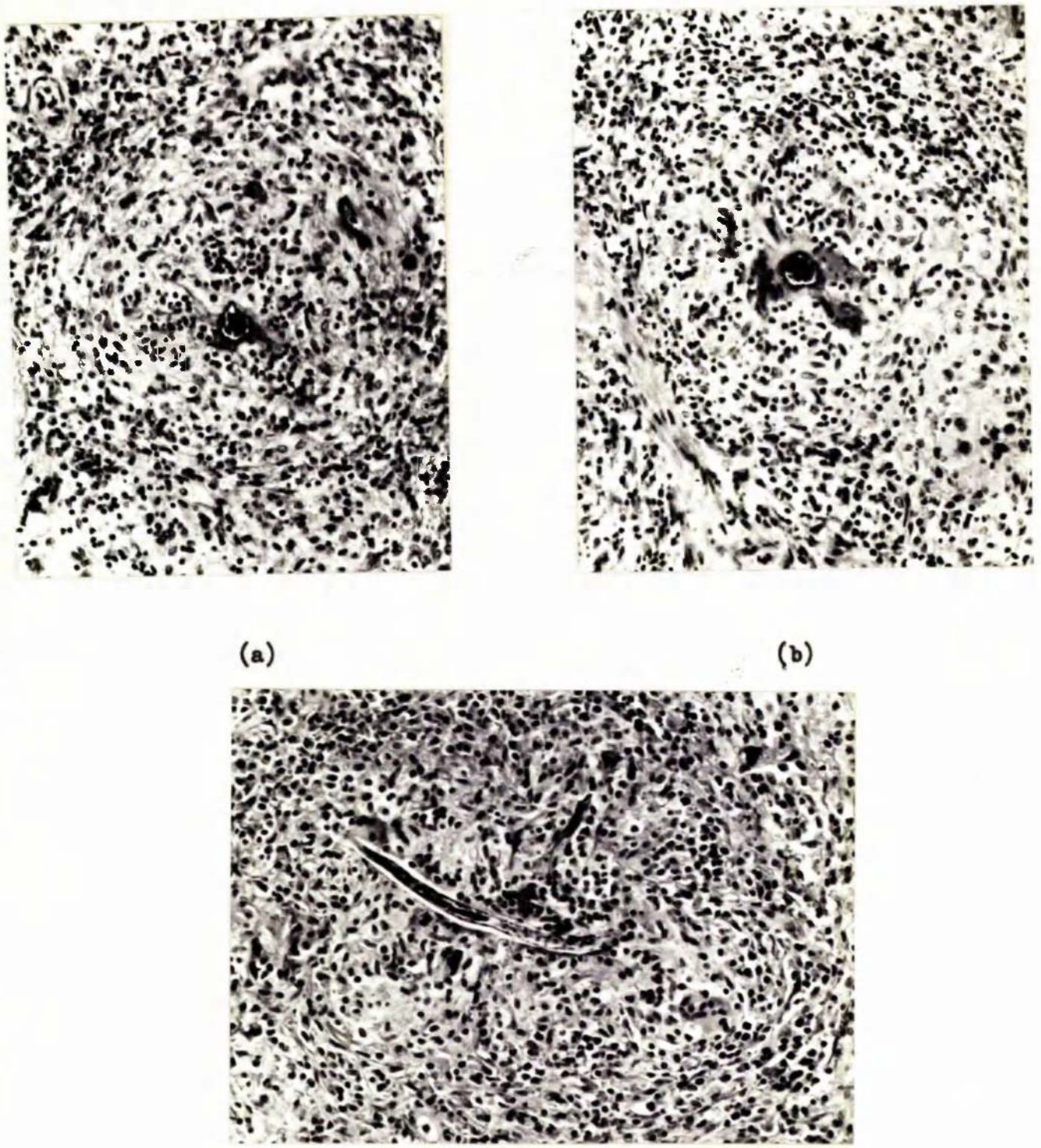


Fig. 79 M. apri (5 weeks): consolidation associated with aspirated eggs including eosinophilic granulomas, giant cells and a small eosinophilic abscess. H. & E. x 150



(a) (b)

(c)

Fig. 80 M. apri (5 weeks): detail of two eosinophilic granulomas around eggs (a and b) and around a larva (c). The egg in (b) is engulfed in a giant cell. H. & E. x 300

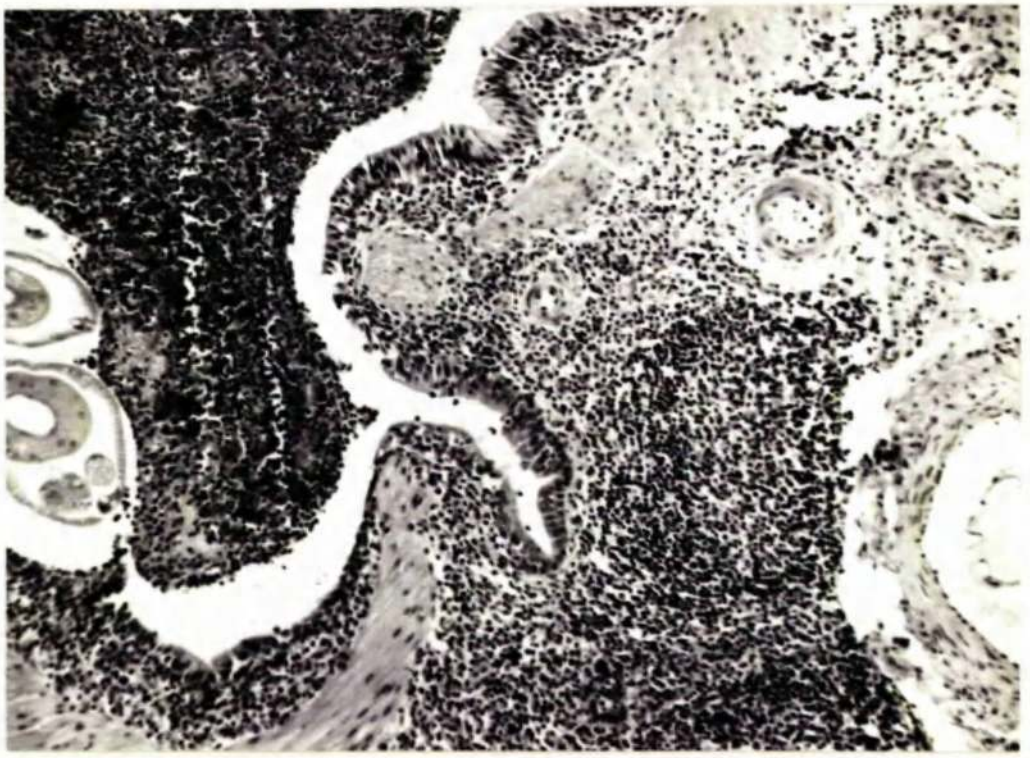


Fig. 81 M. apri (6 weeks): a small diverticulum of the epithelium of a bronchiole. H. & E. x 150

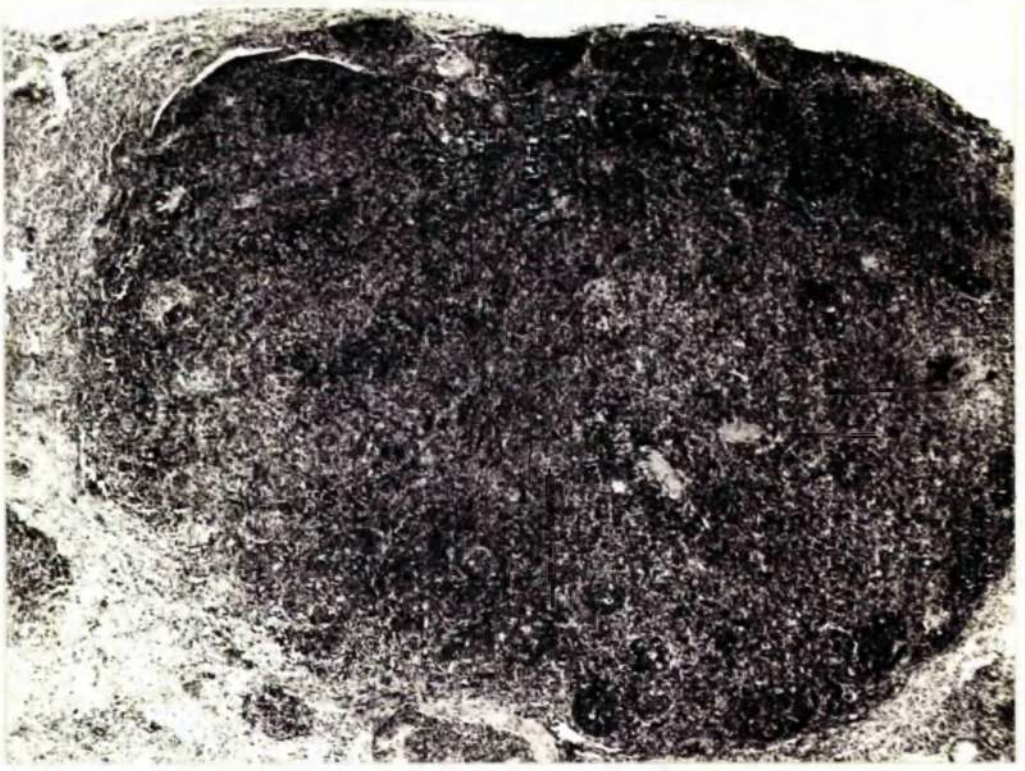


Fig. 82 M. apri (12 weeks): a large lymphoid nodule containing two sections of dead parasites. H. & E. x 50

PNEUMONIA DUE TO METASTRONGYLUS APRI

(6) The Effect of x-irradiation on the Infective Larvae of M. apri

At the beginning of this work it was stated that one of the objectives was to vaccinate pigs against Metastrongylus apri using x-irradiated larvae. Although pig lungworms are not a serious cause of economic loss in the pig industry, this new approach to the control of parasitism appears to have great potential and it was decided that experience with the technique as applied to parasites other than Dictyocaulus viviparus with which it was first shown to be successful (Jarrett, Jennings, McIntyre, Mulligan and Urquhart, 1957) would be useful.

After the method for infecting pigs experimentally had been developed, the next step was to discover what level of x-irradiation would be suitable for inactivating the infective larvae. Using the experiment quoted above as a guide it was decided to test that level of x-irradiation from 20,000r to 60,000r.

During the course of the experiment the following observations were made in each group:

(i) the number of lungworms at autopsy

(ii) the length of the lungworms

(iii) the sex ratio, males:females, of the lungworms

(iv) the faecal egg counts

(v) the amount of coughing

(vi) the weight gains

(vii) the pulmonary lesions

Methods

The infective third stage larvae of M. apri were collected from earthworms in the manner described earlier. They were concentrated into an aqueous suspension of 12 mls. by centrifugation and they were irradiated according to the technique described by Jarrett, Jennings, McIntyre, Mulligan and Urquhart (1960).

The larval suspension was put into a small "Perspex" dish 5.0 cms. in diameter which resulted in the suspension being 1.0 cm. deep. The dish was then placed in a depression in a wooden block filled with bolus (material which prevents "back-scatter"), and this was placed below the window of an x-ray machine at a point where the dose rate on the surface of the larval suspension was 200r. per minute. The x-ray machine was operated at 140 kV and 5 mA with external filtration of 0.25 mm. Cu and 1.0 mm Al. Calibration of the machine was carried out with a Baldwin-Farmer sub-standard dose meter. The doses described in this experiment are those delivered to the surface of the larval suspension. The x-ray machine was switched off for 5-10 minutes in each hour of running and the larval suspension was stirred by hand at

some time during this period. At time intervals corresponding to the three irradiation doses to be given, a volume of the larval suspension containing one-third of the initial number of larvae present, was removed and replaced with water. The samples of irradiated larvae were diluted immediately with tap water to a concentration suitable for counting and aliquots containing the requisite number of larvae for each experimental animal were then transferred to universal containers and made up to 25 mls. with tap water. Because of the time required to collect the larvae and irradiate them the pigs were not dosed with the normal and irradiated larvae until the day after irradiation, i.e. 64 hours after the earthworms were chopped up with scissors.

Twenty four pigs from two litters, seven weeks and eight weeks of age, were distributed into four comparable groups of six animals on the basis of their weights. Each pig in one group received 4,200 normal infective larvae, in the other groups the pigs were given a similar number of larvae which had been irradiated with 20,000r, 40,000r and 60,000r respectively. The pigs were housed together in one pen in a fattening house with a concrete floor, until they killed eight weeks after infection.

Before the animals were infected two faeces samples were examined from each individual by the flotation technique described by Dunn, Gentles and White (1955). During the course of the experiment weekly faeces examinations were made on all of the pigs using this technique and from the sixth to the eighth week the controls were examined by the McMaster technique using

saturated magnesium sulphate to float the eggs in the chambers.

The pigs were weighed before the start of the experiment and fortnightly thereafter. Since the animals could not be visited each day, in order to estimate the amount of obvious clinical disturbance the pig attendant who did not know how the animals had been treated was asked to record on a chart the ear number of each pig he heard coughing.

At autopsy the total number of worms of each sex was counted in each pig and after the lungworms from each group were pooled a representative sample of fifty of each sex were selected for measuring their lengths.

In some groups it was not possible to get fifty and then the largest number possible was collected.

Several blocks of tissue were taken for histological examination from the lungs and bronchial lymph nodes of each pig.

Results

The number of lungworms found at post mortem, the lengths of the worms and the sex ratio are shown in Table 15. Although the difference between the mean worm burden in each group is marked, the "take" in two groups was very scattered, resulting in large standard deviations and when the controls were compared with the other groups by the 't' test only the 60 kiloroentgen (kr) group were probably significantly different ($p = 0.05$). The difference between the 20kr group and the 60kr group was also probably significantly

Table 15

THE EFFECT OF X-IRRADIATION ON THE INFECTIVE LARVAE OF M. APRI

Group	Worm Count (Mean and S.D.)	Length of F. (m.m.) (Mean and S.D.)	Length of M. (m.m.) (Mean and S.D.)	Sex Ratio $\frac{M}{F}$ (Mean and S.D.)
Control	444.5±436.9	36.5±4.1	16.0±2.5	0.42±0.19
20,000r	212.0±161.1	26.8±3.7	10.4±2.9	0.07±0.06
40,000r	114.5±16.5	22.3±3.3	-	0.02±0.03
60,000r	14.7±18.1	17.5±4.7	-	-

different ($p = 0.05$) but the difference between the 20kr group and the 40kr group was not significant. The 60kr group was highly significantly different however, ($p = 0.001$) from the 40kr group.

The detrimental effect of the different levels of x-irradiation on the growth of the surviving lungworms was very clearly demonstrated. There was a highly significant difference ($p = 0.001$) between the length of the females found in the controls and those in the 20kr group. A similar difference was found between the 20kr and the 40kr group and also between the 40kr and the 60kr group. There were not enough male lungworms in the groups receiving the higher levels of x-irradiation to enable a statistical analysis to be made but the difference between the length of males in the controls and those in the 20kr group was highly significant ($p = 0.001$).

The male larvae were more susceptible to x-irradiation than the females with the result that, relatively fewer male worms were found in the irradiated groups at autopsy. In fact there were no males in the 60kr group. The difference between the sex ratio in the controls and that in the 20kr group is singificant ($p = 0.01$) and the difference between the controls and the 40kr group is highly significant ($p = 0.001$). The 20kr group does not, however, differ significantly from the 40kr group.

When the worms which had developed from irradiated larvae were examined microscopically no larvated eggs were seen in the females. Eggs were present within the uteri but they were often misshapen and only contained granular

material (Fig. 83). This failure to produce viable larvated eggs was reflected in the faeces examinations (Table 16). No eggs were found in the faeces of any of the pigs which had received irradiated larvae. On the other hand two pigs in the control group gave a positive result using the flotation technique in the fourth week and all were positive in the fifth week. At six weeks sufficient numbers of eggs were found to enable the McMaster technique to be used and as the table shows eggs were found until the end of the experiment.

Using the amount of coughing as the criterion there was an observable difference in the clinical effects of the infections in the controls compared with the pigs given irradiated larvae. Within the latter animals the 60kr group coughed much less than the other groups which were affected to approximately the same extent. Every pig coughed at least once starting on the ninth or tenth day, but the severity tended to be greater in those given normal larvae, many of which had paroxysms lasting for as long as a minute. Coughing continued in each group until the end of the experiment, although it was very infrequent in the 60kr pigs. Eighty two observations were made of pigs coughing in the controls, fifty three in the 20kr group, forty five in the 40 kr group, and only twelve in the 60kr group.

There was no significant difference between the weight gains for the different groups (Table 17).

The macroscopic appearance of the lungs of the pigs given irradiated

Table 16

RESULTS OF FAECES EXAMINATIONS. MEAN E.P.G. FOR THE GROUP

	Week of Experiment								
Group	0	1	2	3	4	5	6	7	8
Control	-	-	-	-	+ ve	+ ve	542	250	133
20,000r	-	-	-	-	-	-	-	-	-
40,000r	-	-	-	-	-	-	-	-	-
60,000r	-	-	-	-	-	-	-	-	-

Table 17

MEAN FORTNIGHTLY WEIGHTS (LBS.)

Group	Before Infection	2w.	4w.	6w.	8w.
Controls	69.3	82.5	98.8	123.3	135.8
20,000r	68.0	81.0	98.2	117.8	134.3
40,000r	68.7	82.2	97.7	112.5	134.2
60,000r	70.0	80.8	96.7	114.8	130.7

larvae differed from the controls in two ways. Firstly there were many small nodules in the lungs and secondly there was no consolidation of the type associated with the reaction to aspirated eggs.

The most obvious lesion in the controls was consolidation which took the form of small greyish-pink or fawn coloured patches 2.0-3.0 cms. across mostly distributed along the edges of the lobes and sometimes coalescing to form strips of consolidated tissue at this site (Fig. 84). The tips and edges of the apical, cardiac and intermediate lobes were involved as well as the diaphragmatic lobes. These patches were very firm and sometimes looked granular particularly on section and contained very small yellow spots. When pressed they exuded copious amounts of oedema fluid. All of the controls had wedge shaped patches of moderate emphysema. Only one pig however had nodules. They occurred in the diaphragmatic lobes were about 0.2-0.3 cm. in size and few in number. The bronchial lymph nodes were enlarged.

The pulmonary changes in the 20kr group and the 40kr group were very similar. Small patches of consolidation were seen distributed similarly to that in the controls but not so extensively. The consolidation was pinkish and oedematous but was not granular and firm with little yellow spots as in the previous group. Pale wedge shaped emphysematous lobules were seen in both groups. The main differentiating feature from the controls were the small grey or greyish purple nodules 0.1-0.4 cm. in diameter in all

the lobes of the lung in every pig (Fig. 85). Their numbers varied from 1-20 per pig and they tended to occur towards the edges of the lobes. They were slightly raised above the lung surface in most cases but when the lungs were opened some nodules were also seen within the substance of the lobes. The bronchial lymph nodes were moderately enlarged.

Small patches of consolidation were seen in only two pigs in the 60kr group but small mildly emphysematous patches were seen in all of the animals. The consolidation was similar to that in the 20kr group and 40kr groups. The most outstanding feature in this group was the very large number of nodules scattered in all the lobes of the lungs at or near the sites where the adult worms are usually found. The number varied from 16-60 per pig. The bronchial lymph nodes were only slightly enlarged.

Histological examinations of the lesions in the controls revealed the presence of the changes which have been described earlier as occurring at eight weeks after infection. Adult lungworms were seen in the bronchi and bronchioles and the females contained large numbers of larvated eggs. A variable amount of cellular exudate and mucus was seen around the lungworms and the epithelium had undergone mucoid metaplasia or hyperplasia or focal atrophy. The lamina propria of the bronchi and bronchioles was densely infiltrated by eosinophils, plasma cells or lymphocytes, particularly eosinophils. Smooth muscle hypertrophy was seen and there were dense accumulations of lymphocytes between the smooth muscle layer and the cartilage either in a

diffuse form or as nodules. In the peribronchiolar tissues lymphoid nodules occurred either singly or in small groups and sometimes they formed a ring around the bronchiole.

Hypertrophy of the alveolar duct muscle also occurred and alveolar emphysema was seen. The consolidated lesions were composed of eosinophilic granulomas around eggs, some eosinophilic abscesses, obliterative bronchiolitis and in addition in some lobules a diffuse cellular infiltrate into alveoli, full of oedema fluid, by eosinophils, polymorphonuclear leucocytes, plasma cells, alveolar macrophages and sometimes giant cells. Alveolar cell proliferation was also seen in these sites. Moderately large multifollicular lymphoid nodules were seen in two cases. The bronchial lymph nodes contained a large number of follicles with broad germinal centres and there were many eosinophils in the sinuses and dense lymphatic tissue.

The bronchial and bronchiolar lesions seen in the 20kr group were similar to those seen in the controls except that the lungworms did not contain larvated eggs (Fig. 86). Their ovaries showed degenerative changes such as pyknosis, karyorrhexis and clumping of chromatin and the eggs in their uteri were basophilic granular masses. Mucoid metaplasia, hyperplasia or atrophy were all seen in the epithelium and the cellular infiltration were similar to the controls. Smooth muscle hypertrophy and emphysema also occurred. The peribronchiolar lymphoid nodular development was well marked and sometimes very extensive. Reactions to dead worms were seen developing in some bronchioles.

No eosinophilic granulomas or abscesses associated with eggs were seen but the papillary lesions of obliterative bronchiolitis did occur. The consolidation seen macroscopically was mostly due to diffuse cellular infiltration into the alveoli of eosinophils, polymorphonuclear leucocytes, plasma cells, alveolar macrophages and sometimes giant cells. Multifollicular nodules of lymphoid cells containing dead worms were seen frequently. The changes in the bronchial lymph nodes were similar to those occurring in the control group.

The bronchial lesions in the 40kr group were much less severe than in the previous groups. The lungworms, which were only seen occasionally in sections, were smaller in diameter than normal and many of them contained abnormal refractile globules. The most prominent lesion was peribronchiolar lymphoid hyperplasia which formed sleeves of lymphoid tissue extending down to the terminal bronchioles. A moderate degree of mucoid metaplasia and smooth muscle hypertrophy was seen and also mild vesicular emphysema. Reactions to dead worms in bronchioles were again frequently found as were multifollicular lymphoid nodules. The alveolar reactions were not extensive but lobules or parts of lobules were seen containing oedema fluid and diffuse cellular infiltrates as described earlier. Papillary lesions of obliterative bronchiolitis were seen in one pig and there were no eosinophilic granulomas or abscesses. The changes in the bronchial nodes did not differ from those seen earlier.

In the 60kr group the bronchial lesions were not numerous or severe and when they did occur resembled those described earlier for the other irradiated

groups. The important lesions seen histologically in this group were associated with bronchioles and were (i) dead worm reactions and (ii) extensive peribronchiolar lymphoid nodular development. The latter was frequently seen around bronchioles with no parasites. Dead worm reactions were seen in the other irradiated groups but were most numerous in this group of pigs. They appeared to start in the smaller bronchioles or alveolar ducts around the anterior end of the worm and were seen in the following stages of development.

(i) Some internal structure could be identified in the dead lungworm or it was an amorphous eosinophilic mass retaining the general outline of the parasite. Whatever the appearance of the parasite it was surrounded by a mass of eosinophils (Fig. 87). Eosinophils also occurred in the lamina propria in large numbers. The hypertrophied muscularies could be identified and there were large numbers of eosinophils, and plasma cells in the peribronchiolar tissues. In addition large reticular cells have become obvious at this site.

(ii) The dead worm was surrounded by eosinophils and there was extreme hyperplasia of the bronchiolar epithelium (Fig. 88). Eosinophils and plasma cells were present in the lamina propria in large numbers and there was disruption of the muscularis by plasma cells and lymphocytes infiltrating through from the peribronchiolar tissues. Many plasma cells and lymphocytes were present in the peribronchiolar tissues. The reticular cells appeared to be forming

a syncytium and were also seen in the lamina propria.

(iii) The central mass consisting of the remains of the lungworm surrounded by eosinophils, was itself surrounded by numerous plasma cells and at the periphery, was a mass of lymphoid tissue starting to form nodules.

(iv) The dead worm was still surrounded by eosinophils but there was complete destruction of the bronchiolar wall and the mass of lymphoid cells in the peribronchiolar region, was developing a nodular organisation.

(v) Eventually only the eosinophilic debris of the worm was seen at the centre of a mass of lymphoid tissue which had a nodular arrangement particularly at its periphery (Fig. 89).

(vi) Finally nothing remained but a large nodule composed of microscopic lymphoid nodules between which was a well developed capillary network.

There was very little response in the alveoli other than that around the bronchioles with the dead worm reaction. During the early stages, in particular, the adjacent alveoli were full of oedema fluid, eosinophils, plasma cells, lymphocytes and alveolar macrophages. The alveolar walls were sometimes thickened due to infiltration by these cells and there was alveolar cell hyperplasia. The reaction in the bronchial lymph nodes was similar to the controls.

Discussion

When using x-irradiation to produce a parasitic vaccine the object is to

prevent the maturation of the adult parasites without completely destroying the early larval forms in the host since it is thought that the 'excretions and secretions' of these forms may be responsible for the immunity produced by the vaccines (Urquhart, Jarrett and Mulligan, 1962). Ideally when assessing the correct level of x-irradiation to use the animals should be challenged with normal larvae after vaccination. However, because of the difficulty in producing large numbers of M. apri larvae it was decided to judge the effectiveness of x-irradiation by the number of lungworms developing from the x-irradiated larvae. The results showed that 60kr was the level of choice since this inhibited maturation more than the other doses but did not stop development completely. In addition the larvae irradiated in this way produced fewer and milder clinical signs than the others and there was minimal pulmonary consolidation.

The 'takes' in the control and 20kr groups were disappointing because of the great variation in the number of worms at autopsy. In the controls the range was 85 to 1,208 worms and in the 20kr group 7 to 462. Although the larvae were out of the intermediate host for quite a long time extra doses which had been made up were found to contain viable larvae, as judged by motility, several hours after the pigs had been infected. The method of preparing and counting the doses was the same for each group and any inaccuracy might have been expected to show in all of the groups instead of two only. Mild lungworm infestation had been found in three pigs from the

farm which was the source of experimental animals and although the young pigs used in this experiment were on concrete all of their lives they may have had some protecting antibodies passed on to them from their mothers. It was not possible to use pigs from any other source because of the prevalence of respiratory disease in pigs and since the object of the investigation was also to study the pathology it was essential to ensure that the pigs were free from enzootic pneumonia.

All of the lungworms developing from irradiated larvae were sterile. This is a well known effect of x-irradiation on nematodes which was demonstrated some time ago by Levin and Evans (1942) working with Trichinella spiralis. Urquhart et al (1962) reported that cattle lungworms developing from D. viviparus larvae irradiated with 40kr were stunted to about one third normal size and that the sex ratio (males to females) altered from 1:1 to approximately 1:11. The phenomenon of stunting was demonstrated here and it is interesting that it was directly related to the level of x-irradiation given. In this experiment however, even with 60kr the degree of stunting was less than in D. viviparus, the 60kr females being about half the size of the normal females.

The greater susceptibility of male larvae to x-irradiation also occurs with M. apri. The reason for this sex difference is not clear and does not seem to occur in higher animals such as rats or mice.

The absence of eosinophilic granulomas and eosinophilic abscesses in

the pigs given irradiated larvae reflects the importance of aspirated larvated eggs in causing these lesions. It is interesting that the papillary .. lesions of obliterative bronchiolitis were seen in the alveolar ducts and the alveoli of pigs given irradiated larvae. This lesion must be associated with the passage of the larval forms through these tissues or a reaction to adult antigens rather than to eggs. Likewise mucoid metaplasia and muscle hypertrophy are reactions to the adult parasites themselves irrespective of their egg producing potential.

The reaction to dead parasites which resulted in nodule formation was similar to that occurring in natural infections. In the latter instance however and in experimental infections with normal larvae large numbers of them form at a later stage of the disease. The cellular accumulations which develop are almost certainly antibody producing sites but are probably not concerned with protective antibody formation since the antigens they are reacting to are those from the disintegrating adult or late larval stages and not from the living early larval phases. Similar nodules are seen in the lungs of cattle as a response to dead *D. viviparus* and the reaction takes place irrespective of the nature of the lethal agent whether it is, preceding x-irradiation, drug therapy or the hosts own immune reaction (Jarrett, McIntyre and Sharp, 1962).

On the basis of the results of this experiment it was decided that when an attempt was made to vaccinate pigs against *M. apri* 60kr of x-irradiation

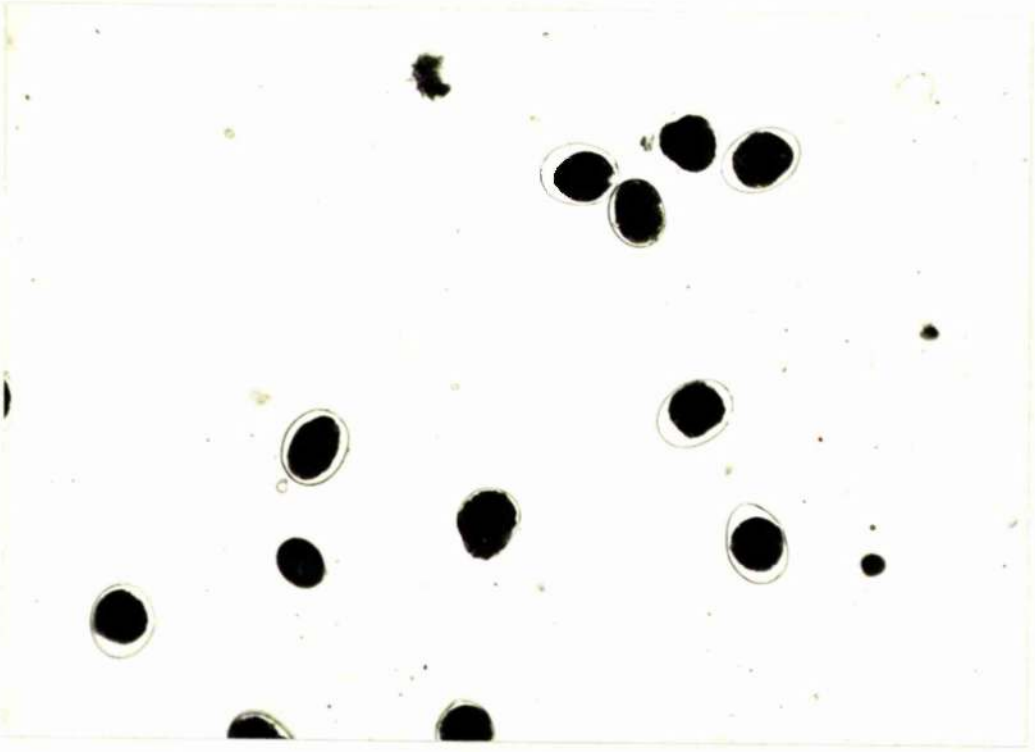


Fig. 83 M. apri: eggs from the uterus of a lungworm which had developed from a larva exposed to 40kr of x-irradiation. (cf. Fig. 72). x 150

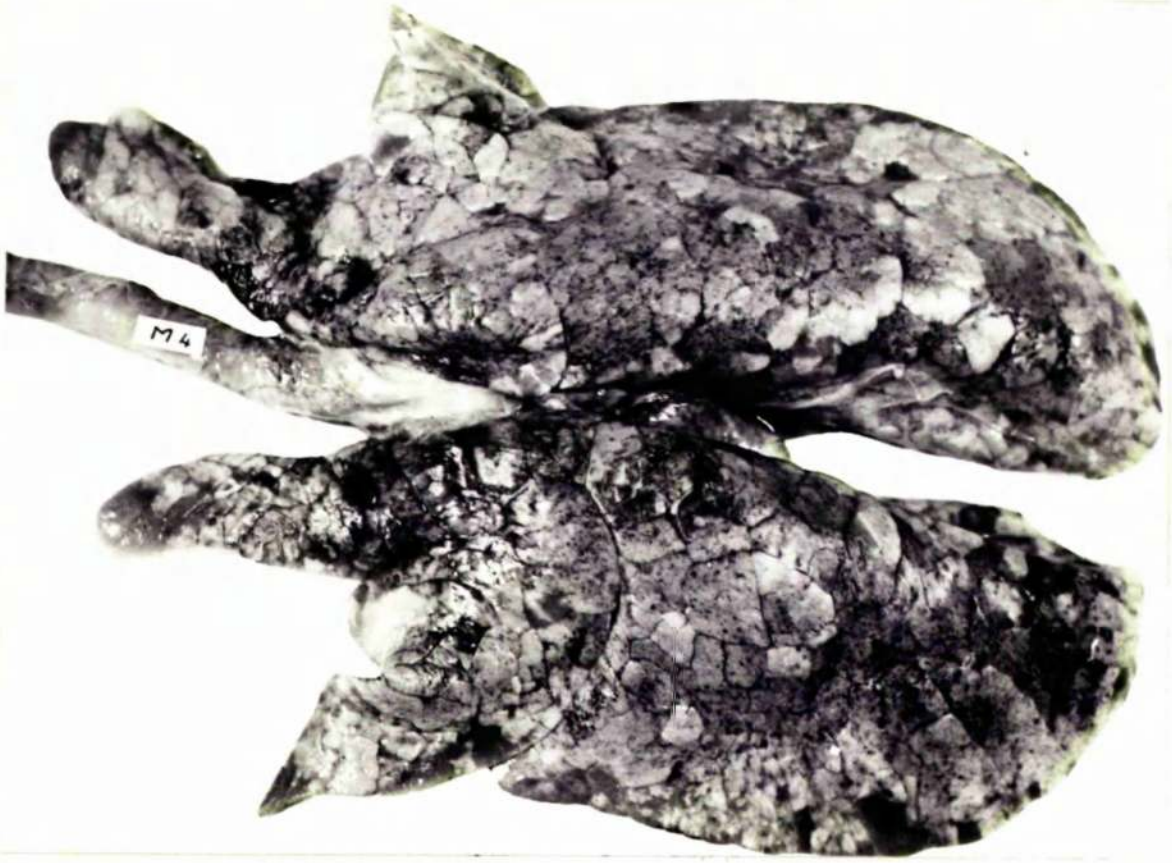


Fig. 84 M. apri: lungs from a pig in the control group showing patches of consolidation at the edge of the lobes.

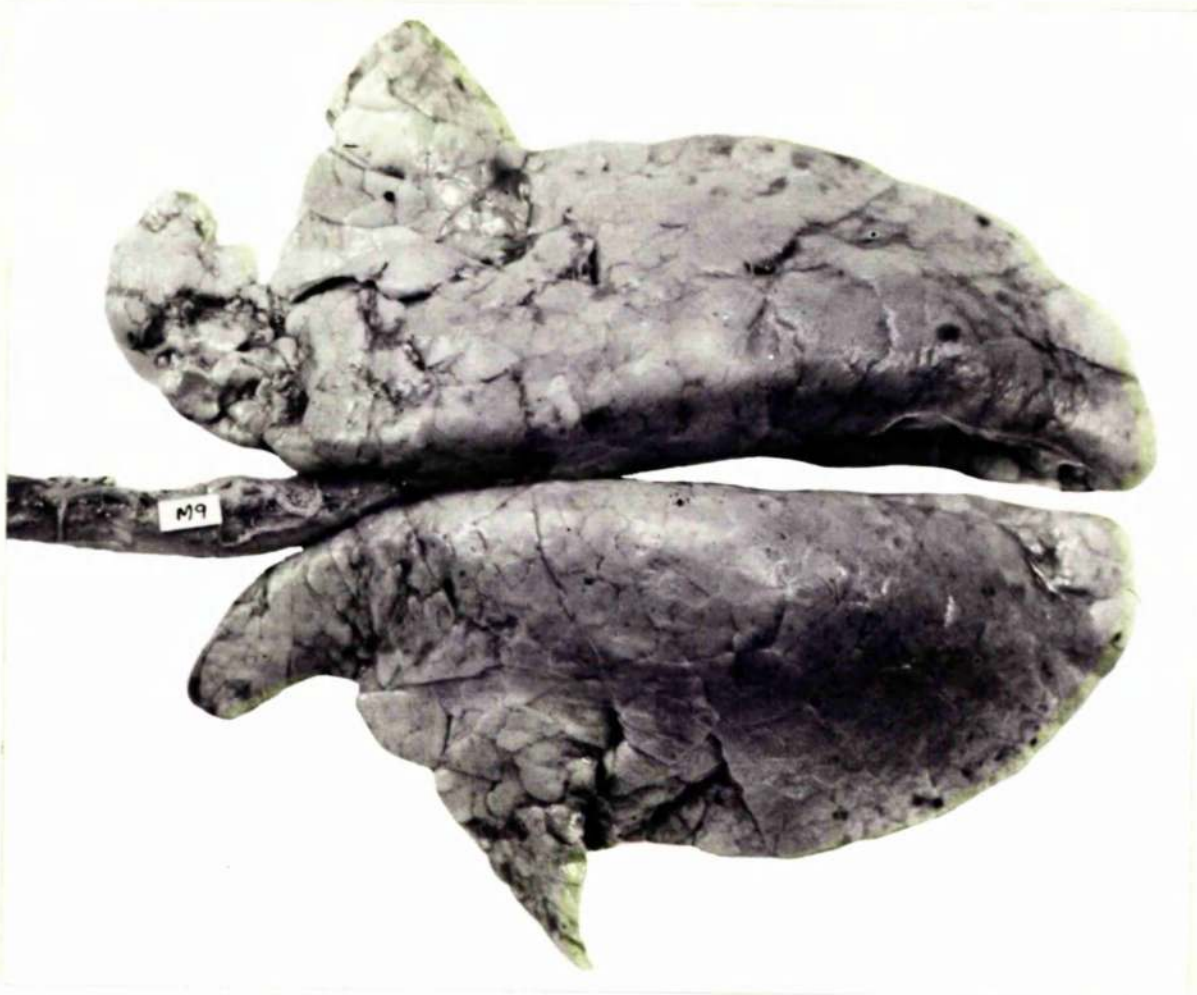


Fig. 85 M. apri: lungs from a pig infected with larvae exposed to 20kr showing lymphoid nodules.

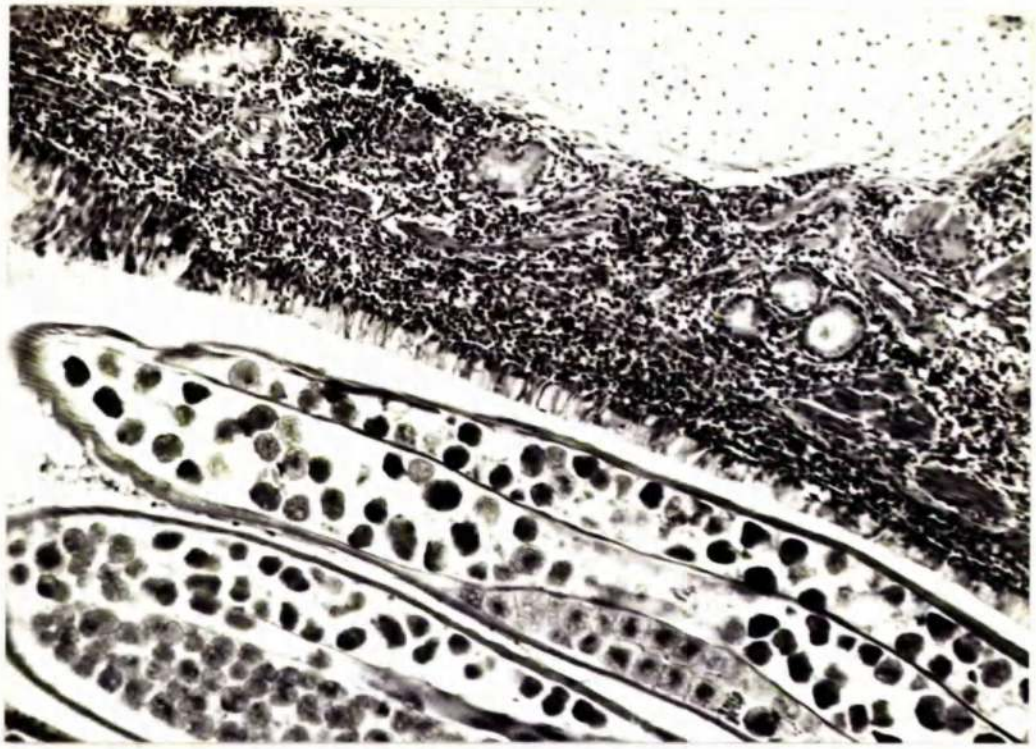


Fig. 86 M. apri: sections of lungworms with non-larvated, granular eggs in their uteri. H. & E. x 150

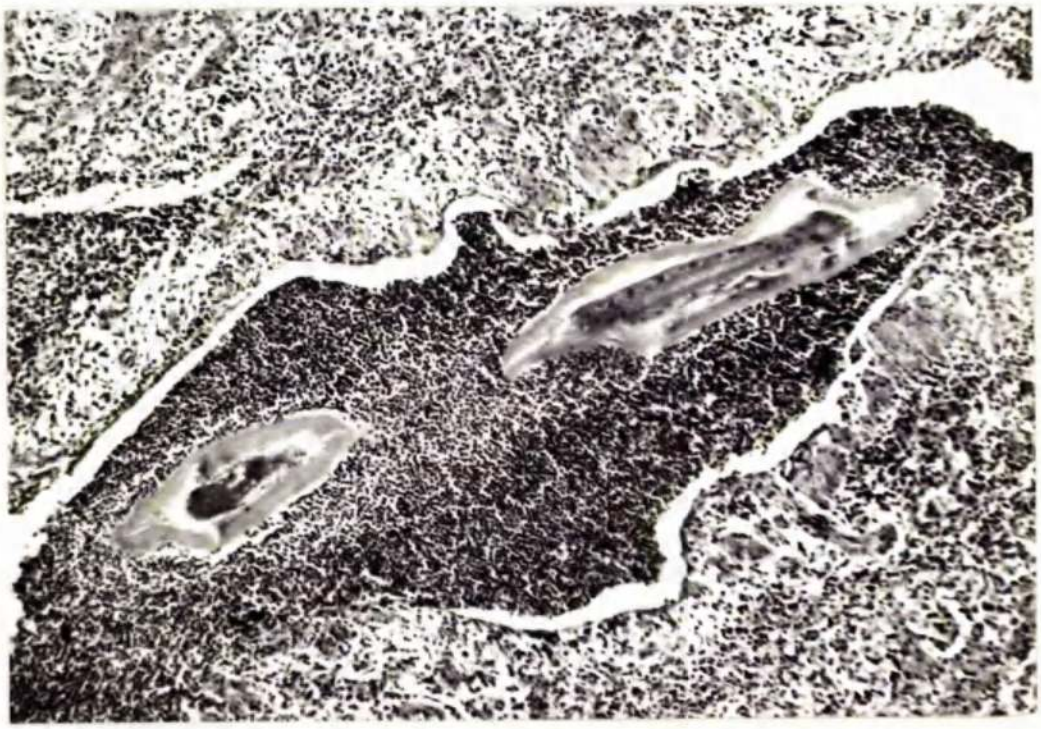


Fig. 87 M. apri: a degenerating parasite surrounded by a host of eosinophils. H. & E. x 150

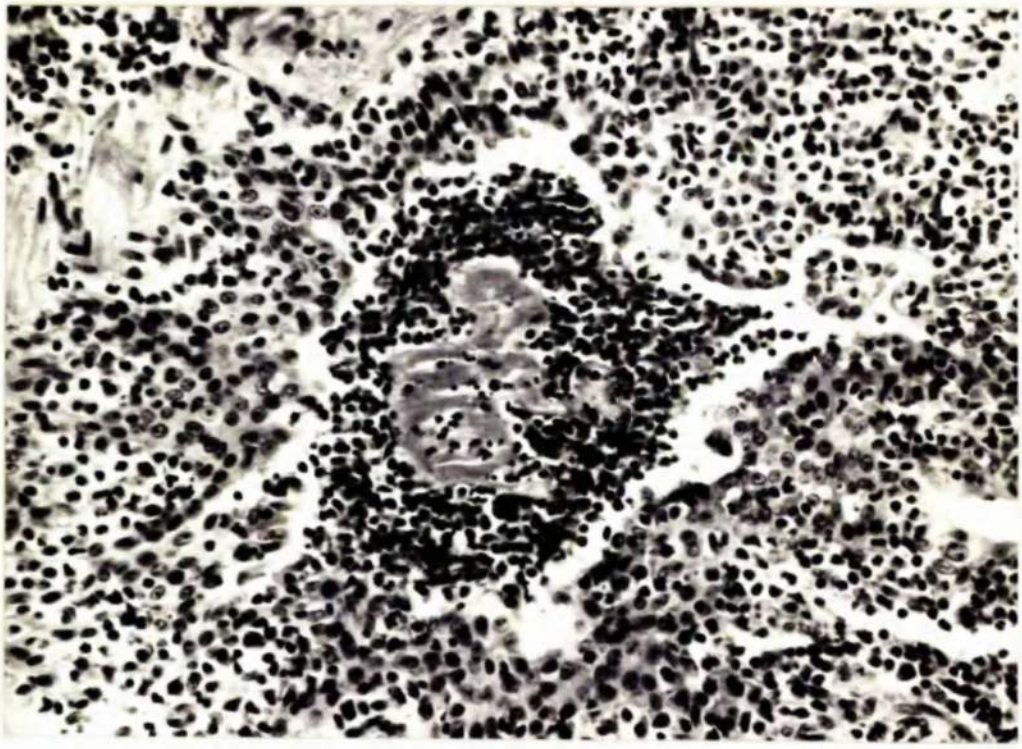


Fig. 88 M. apri: part of a dead parasite surrounded by eosinophils and hyperplasia of the bronchiolar epithelium. H. & E. x 300

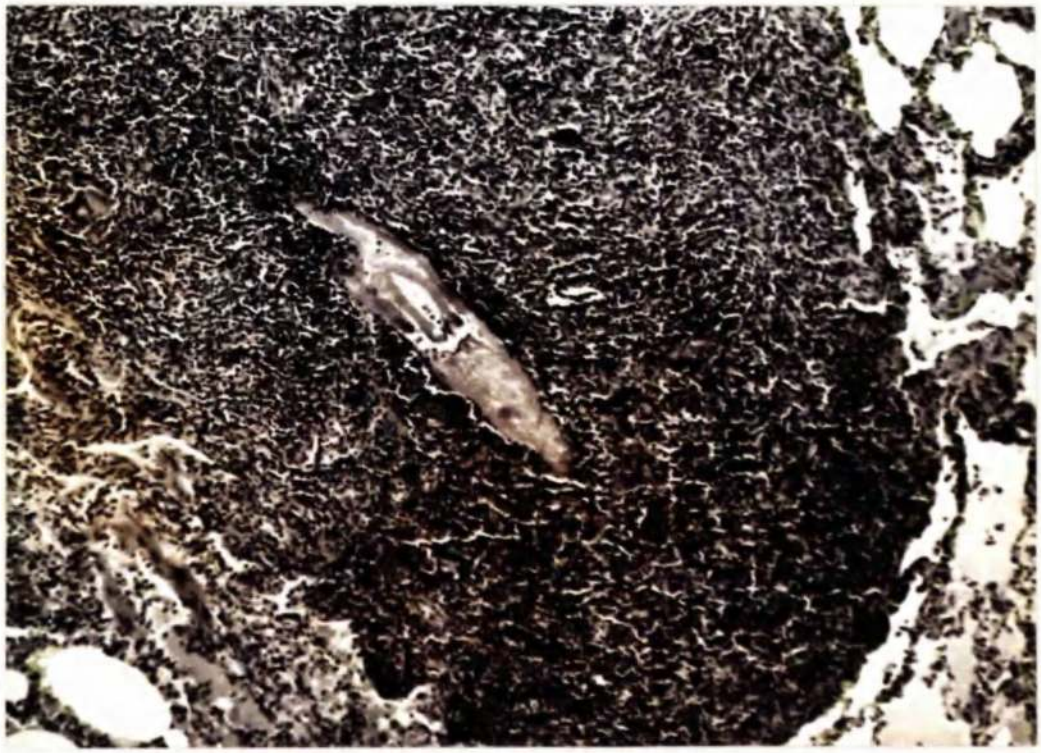


Fig. 89 M. apri: a dead lungworm surrounded by a mass of cells of the lymphoid series. H. & E. x 150

PNEUMONIA DUE TO METASTRONGYLUS APRI

(7) The Vaccination of Young Pigs with X-irradiated Larvae

Since the first demonstration that x-irradiated larvae could be used to protect cattle against Dictyocaulus viviparus (Jarrett, Jennings, McIntyre, Mulligan and Urquhart 1957) the technique has been successfully applied to some parasites of other species e.g. Haemonchus contortus in lambs (Jarrett, Jennings, McIntyre, Mulligan and Sharp 1961) and Uncinaria stenocephala in the dog (Dow, Jarrett, Jennings, McIntyre and Mulligan 1959). It was thought therefore that the parasites of the pig might be controlled in a similar manner and consequently the following experiment was designed with the object of vaccinating young pigs with x-irradiated third stage larvae of Metastrongylus apri and testing the immunity produced by challenging them, after a suitable period, with normal third stage larvae. In experiments with the parasites referred to earlier it was found that two doses of x-irradiated larvae gave better protection than one and consequently this procedure was followed in the experiment described here. The amount of x-irradiation employed with other parasites has varied from 20 kr. to 60 kr. It was concluded from the experiment described in section (5) however, that 60 kr. was the most suitable amount of x-irradiation for M. apri larvae.

Methods

The methods for obtaining infective larvae, irradiating them with 60 kr.

and making up the required doses were similar to those described earlier. The larval preparation, irradiation and the dosing of the pigs with the larvae occupied a period of time similar to that in the previous experiment.

All of the pigs used came from one litter which was four weeks old when the experiment started. They were divided into two comparable groups of six pigs on a weight basis. Unfortunately one of the pigs receiving the x-irradiated larvae died five days after its first dose. Death was not due to the Metastrongylus larvae and the experiment was continued with two groups of five pigs. Initially, the group to be vaccinated were given 3,000 x-irradiated larvae when they were four weeks old. Four weeks later they were given their second dose, which consisted of 6,000 x-irradiated larvae and four weeks after that both groups of pigs were challenged with 2,900 normal infective larvae. All of the animals were killed six weeks after being challenged.

Faeces examinations were made using the techniques described earlier. The sow, whose litter was used for the experiment, was examined and shown to be free of Metastrongylus eggs using the flotation technique. All of the experimental pigs were examined before vaccination started and during the second period of vaccination, by the same method. When they were expected to become patent i.e. five weeks after challenge with normal larvae, the McMaster technique was adopted for all faeces examinations.

The pigs were weighed before the experiment was started and then at

fortnightly intervals during the course of the experiment.

After the animals were killed several blocks of tissue were taken from the lungs and the bronchial lymph nodes of each one, for histological examination. The lungs were then opened and the number of lungworms counted. As the worms were counted they were sexed and later the sex ratio, male:female was calculated for each pig.

Results

The number of worms found at autopsy in each group is shown in Table 18. It can be seen that the mean figure for the control group is slightly higher than the mean for the vaccinates but there is a wide scatter of 'takes' in the controls. When the figures for the two groups were compared using the 't' test there was no significant difference between them.

While the worms in the vaccinates were being counted it was noticed that some of the females were abnormally small and were sterile, and it was concluded that these were worms persisting from the doses of x-irradiated larvae. The number found in each pig was 12, 8, 10, 8, and 1 respectively according to the order of the worm counts in Table 18. When these worms were subtracted from the figures in Table 18 the 'take' in the vaccinates after challenge became 112.6 ± 20.98. This was still not significantly different from the controls.

The sex ratio in the controls was 0.35 ± 0.20 when it was calculated

Table 18

RESULTS OF METASTRONGYLUS APRI VACCINATION EXPERIMENT

Group	No. of Lungworms	Mean And S.D.	Sex Ratio $\frac{M}{F}$	Mean And S.D.
	530		0.41	
	109		0.42	
Controls	85	152.8 ± 214.6	0.52	0.35 ± 0.20
	38		0.41	
	2		0	
	148		0.15	
	143		0.25	
Vaccinates	110	120.4 ± 23.4	0.11	0.15 ± 0.06
	103		0.09	
	98		0.15	

for all five pigs (Table 18). If the animal with only 2 worms was ignored however, the sex ratio became 0.44 * 0.05 and when this was tested against the sex ratio for the vaccinates there was a highly significant difference ($p = 0.001$).

Larvated Metastrongyle eggs were found in the faeces of all animals six weeks after challenge (Table 19). More controls than vaccinates however were found to be passing eggs in the fifth week.

The mean weights for the two groups during the course of the experiment are shown in Table 20 and although it appears that the controls were lighter than the vaccinates at the end of the experiment when the standard deviations were taken into account there was no significant difference. Similarly the weight gains for the two groups during the last six weeks of the experiment were not significantly different.

Some coughing was heard before challenge in the pigs given x-irradiated larvae but it was not severe and after challenge pigs in both groups were heard coughing.

The predominant lesion in the control group was patches of consolidation at the edges of the lobes of the lungs usually not extending into the lungs further than the peripheral one or two lobules. They occurred in the diaphragmatic lobes, often at the posterior tip of the lobe, but were also quite extensive in some pigs in the apical, cardiac and intermediate lobes. The consolidated patches were either pale greyish pink and oedematous, or a similar colour but firm, slightly raised above the surrounding lung, with a

Table 19

RESULTS OF FAECES EXAMINATION (E.P.G.) AFTER CHALLENGE:

VACCINATION EXPERIMENT

Group	Week 1	2	3	4	5	6
Controls	-	-	-	-	500	550
	-	-	-	-	50	50
	-	-	-	-	50	100
	-	-	-	-	50	50
	-	-	-	-	-	-
Vaccinates	-	-	-	-	-	50
	-	-	-	-	-	50
	-	-	-	-	50	100
	-	-	-	-	-	50
	-	-	-	-	-	50

Table 20

MEAN FORTNIGHTLY WEIGHTS (LBS) OF PIGS IN VACCINATION EXPERIMENT

	Week							
Group	0	2	4	6	8	10	12	14
Controls	10.1	12.2	20.2	30.8	38.8	49.6	60.1	74.4
Vaccinates	10.6	14.2	23.9	34.4	44.0	56.8	69.8	84.6

granular texture and occasionally contained small yellow spots. A few small greyish purple nodules were seen in the diaphragmatic lobes of two pigs. Pale lobules of moderate vesicular emphysema were seen in all of the pigs at the edges of the diaphragmatic lobes. The bronchial lymph nodes were all moderately enlarged.

A similar type of consolidation was seen in the vaccinated pigs but it was slightly less extensive. Every pig in this group however had in addition a considerable number of greyish nodules 0.1 - 0.3 cm. in diameter mostly located in the diaphragmatic lobes. Several lobules of vesicular emphysema were seen in all of the pigs in the diaphragmatic lobes. The bronchial lymph nodes were moderately enlarged.

The histological appearance of the lesions in the controls had all of the features associated with patent lungworm infection. Sexually mature worms of both sexes were seen in the bronchi and bronchioles often surrounded by many eosinophils or a mixture of mucus, eosinophils, plasma cells and macrophages. Mucoid metaplasia had occurred. The lamina propria of the bronchi and bronchioles was infiltrated by eosinophils and plasma cells. There were diffuse accumulations of plasma cells and lymphocytes in the peri-bronchiolar tissues and also between the cartilage of the bronchi and the smooth muscle layer. Lymphoid follicles had also developed in these sites. Bronchial and bronchiolar smooth muscle hypertrophy was seen and there was also some hypertrophy of alveolar duct muscle. A few multifollicular lymphoid

nodules were found. The oedematous areas of consolidation seen macroscopically, were due to alveolar oedema and a cellular exudate in the alveoli and alveolar ducts consisting of eosinophils, plasma cells, lymphocytes, polymorphonuclear leucocytes and alveolar macrophages. Giant cells were also present. The walls of many of these alveoli were thickened by alveolar cell proliferation and accumulations of plasma cells, and eosinophils. In the more granular lesions with occasional yellow spots there were aspirated eggs, large numbers of giant cells, eosinophilic granulomas and eosinophilic abscesses. Patches with obliterative bronchiolitis were also seen. There was moderate distension of alveoli and alveolar ducts in some lobules due to emphysema.

When the bronchial lymph nodes were examined histologically large numbers of follicles were seen with active looking germinal centres. Eosinophils were present in moderate numbers in the loose lymphatic tissue and in one case the eosinophilic remains of a parasite were seen surrounded by masses of lymphocytes.

The lesions in the vaccinates had all the features seen in the controls. However, there were many more multifollicular lymphoid nodules some of which had dead parasites at their centres. In addition the peribronchiolar and bronchial lymphoid follicular development was more extensive. The lungworms were mostly sexually mature males and females but lying beside them were worms which had developed from irradiated larvae and did not contain

larvated eggs. The gonads had a hyaline eosinophilic appearance and contained many lipid globules. The uteri were distended with eosinophilic granular material sometimes containing small pynknotic nuclei. Eosinophilic abscesses were more numerous. This was particularly true in one pig which had in addition a large area of necrosis associated with many larvaeted eggs and the remains of a dead parasite. A lymphoid nodule was developing around the lesion.

The changes in the bronchial lymph nodes did not differ from the controls.

Discussion

The results of this experiment did not show that it was possible to vaccinate young pigs against M. apri using x-irradiated larvae. Part of the reason for this was the large variation in the number of worms found in the control animals. This was similar to the result in the control group in the previous experiment. Considerable care was taken in the computation and administration of the doses and there was no doubt that each pig received all of the larvae it was supposed to get. The two pigs with the lowest 'takes' i.e. 2 lungworms and 38 lungworms at autopsy had more pulmonary consolidation than one would have expected from the small worm burdens and it seems likely that part of the worm burden was shed before the pigs were killed perhaps as a result of immunity derived from the parent sow. Even allowing for the disappointing result in the controls it is obvious that the pigs given x-irradiated larvae were not completely protected since some of the challenging larvae

developed into sexually mature male and female lungworms.

When calculating the sex ratio for the controls it was justifiable to omit the pig with two lungworms because of the size of the sample. On this basis there was a highly significant difference between the sex ratio in the controls and the one in the vaccinates. The lower figure for the controls was due to excessive number of females which cannot be explained by the small number of obviously stunted worms which were found. This suggests that there was persistance of females which were not obviously stunted, from the x-irradiated doses, and that these were counted as normal females. If this is true the 60 kr. of x-irradiation was not so efficient in stunting the worms as in the previous experiment where the worms developing from larvae receiving 60 kr. were of half normal size. Unfortunately by the time the sex ratios had been calculated the worms were no longer available for examination and it was not possible to check them microscopically for larvated eggs.

It has not been found possible to vaccinate young lambs two to three months of age against H. contortus using x-irradiated larvae, although a degree of immunity was produced in sheep over six months of age (Urquhart, Jarrett and Mulligan 1962). These workers suggested that this might have been due to either (i) immunologic immaturity, (ii) interference with antigenic stimulation by colostrally acquired antibodies or (iii) a degree of immunologic unresponsiveness due to the presence of excessive amounts of antigen. The pigs used in this experiment were only four weeks old when

they were first given irradiated larvae and the development of immunity may conceivably have been affected by the first two factors mentioned. If this is true an x-irradiated larval vaccine would be of limited value in pig husbandry since most pigs are exposed during the first eight weeks of life if they are exposed at all. Only breeding stock which might be put on to pasture for the first time later in life would benefit.

In the previous experiment it was shown that one of the main pathological features seen in pigs given irradiated larvae was pulmonary lymphoid nodule formation. It is not surprising therefore that these nodules were found more frequently in the vaccinated group than in the controls. The slightly greater number of eosinophils in eosinophilic abscesses and the relatively greater numbers of these abscesses compared with eosinophilic granulomas, in the vaccinated animals are compatible with some degree of immunity having been produced. When mice were infected with Ascaris lumbricoides eggs and allowed to recover, on reinfection many of the larvae were halted in the liver and there was a cellular reaction around them which was absent in primary infections, (Sprent and Chen 1949). Eosinophils played a prominent role in the reaction.

The results of this experiment were inconclusive since one could not conclude that it was impossible to vaccinate young pigs with x-irradiated larvae of M. apri. The experiment would be worth repeating using a smaller second vaccinating dose to reduce the amount of antigen being given and to

decrease the number of worms which might develop from the vaccine. When the final kill is made the worms should be checked for sexual maturity. Ideally control groups to check each stage of x-irradiation should also be included.

A major obstacle is the variation in the 'takes' with normal larvae and before further experiments are carried out this problem would need to be investigated more fully. If possible piglets from another herd free of enzootic pneumonia and whose mothers have been reared on concrete all their lives should be used in order to exclude the possibility of the passive transfer of immunity from the sow to the piglets to be used as experimental animals.

PULMONARY TOXOPLASMOSIS

A five weeks old male pig was autopsied and found to have a pneumonia in which structures identical to the pseudocysts of the protozoan Toxoplasma gondii were seen. An attempt to isolate the organism in mice, however, using lung material which had been stored for four weeks at -50°C was unsuccessful.

The animal which had been dull and unwilling to walk for some time became tachypnoeic several days prior to death.

Macroscopic Findings

The lungs were heavy and the pleural surface was shiny. In all the lobes of the lungs there was a diffuse mottling produced by lobules with greyish-pink patches of infiltration. The trachea and bronchi contained thin grey fluid and the cut surface of the lung exuded similar fluid when pressed. This grey turbid fluid was also found in the nasal cavity.

In addition there was purulent arthritis in the right shoulder joint and the right elbow and subcutaneous abscesses were present at the manubrium of the sternum and in the left foreleg.

Microscopic Findings

Even at a microscopic level the lesion had a patchy appearance due to incomplete involvement of all the alveoli within the lobules affected. The alveoli contained oedema fluid, polymorphonuclear leucocytes and alveolar

macrophages (Fig. 90). The alveolar macrophages were not large and round with abundant cytoplasm but mostly were medium sized with bean shaped nuclei and moderate amounts of cytoplasm. The nuclei often stained heavily and the cytoplasm was frequently strongly basophilic. Occasionally bi-nucleated forms were seen and one or two multinucleated giant cells were also found. There was patchy congestion of alveolar walls and the walls were thickened by polymorphonuclear leucocyte infiltration and the proliferation of mononuclear cells within the walls. These did not have the appearance of the alveolar cells seen in enzootic pneumonia but resembled the alveolar macrophages described above. Lymphocytes and plasma cells were also seen but in smaller numbers than the other two cell types mentioned. A few regions in which there was swelling of the alveolar epithelium were found (Fig. 91). Some of the alveolar epithelial cells had a single abnormally large hyperchromatic nucleus (Fig. 92) and one multinucleated epithelial giant cell was seen.

The lumina of the bronchioles and bronchi contained cells similar to those seen in the alveoli. The cells of the bronchial and bronchiolar epithelium had larger and more strongly basophilic nuclei than normal and the cytoplasm of many of them was also strongly basophilic. Several mitotic figures were seen in these epithelial cells. The lamina propria of the bronchioles was moderately infiltrated by polymorphonuclear leucocytes, which could be seen migrating through the epithelium and there was a mild to

moderate diffuse infiltration of the peribronchiolar tissues by polymorphonuclear leucocytes, macrophages and lymphocytes. The perivascular tissues were infiltrated in a similar manner. Mild oedema of the interlobular septa was seen and although the septa were infiltrated by considerable numbers of polymorphonuclear leucocytes and macrophages (Fig. 90) they were not grossly thickened.

Most of the protozoan structure were seen lying free in the alveolar lumina (Fig. 93). They were either round, 10-15μ in diameter, or pear shaped and 15-20μ long x 9.0-14.0μ broad. They were slightly eosinophilic and contained 11-50 basophilic bodies. A few of these pseudocysts appeared to be extending from the alveolar wall into the lumen (Fig. 94) and some were seen in the alveolar wall. The pseudocysts were obviously forming in cells of the lung tissue and two cells were seen, one an alveolar epithelial cell and the other free in the alveolar lumen, with groups of the basophilic bodies developing in their cytoplasm and the nucleus squashed at one end of cell.

Discussion

Toxoplasma gondii is known to be capable of infecting a large number of different species and infection in the pig has been described (Momberg-Jørgensen, 1956, Harding, Beverley, Shaw, Edwards and Bennett 1961). The latter authors reported the first outbreak recognised in pigs in Great Britain.

In the cases they described, dyspnoea was a prominent feature and from their review of the literature they stated that "Most authors have found lesions in the lungs when overt disease was present and it seems likely that pulmonary signs may be a common feature of acute toxoplasmosis in young pigs".

The infiltration of the interlobular septa described in their paper was seen in this case but fibrosis of the septal, pleural and perivascular tissues had not occurred.

Toxoplasmosis is sometimes associated with necrosis in the lung but necrosis was not seen in this case, and apart from the abscess and purulent arthritis seen macroscopically no lesions were found in the other organs and no pseudocysts were seen in the brain, myocardium, liver or kidneys.

Toxoplasmosis had not been seen previously in the herd to which the pig belonged and no cases have been diagnosed since although the herd is closely supervised and every animal dying is post-mortemed since it is owned by the University of Glasgow Veterinary School and is the source of experimental pigs.

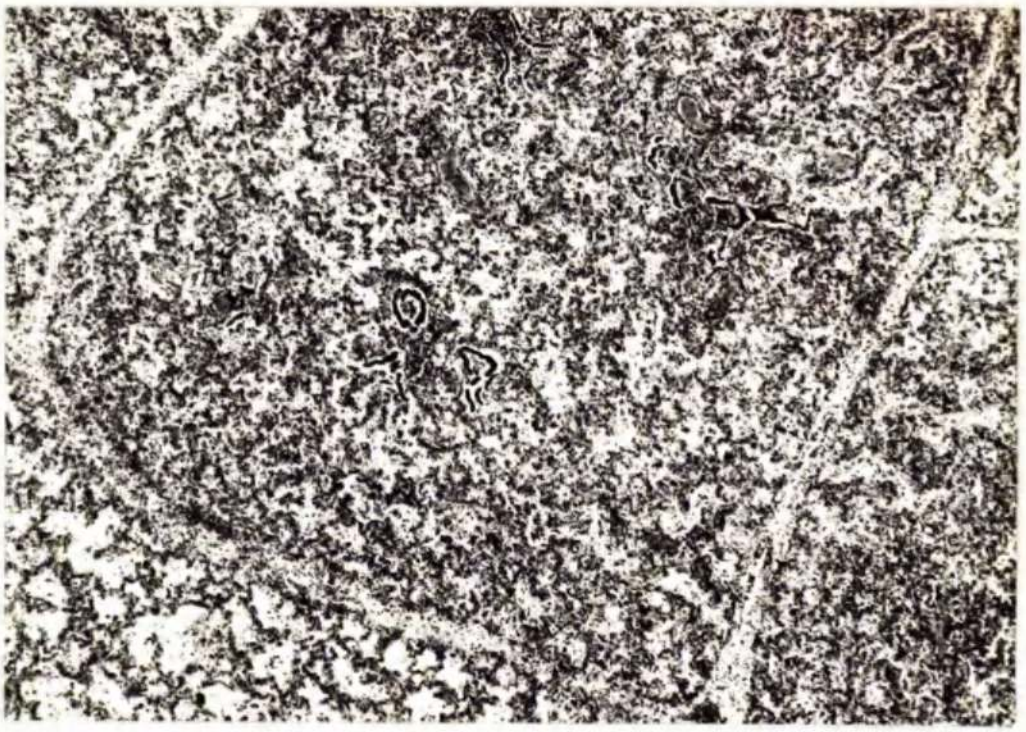


Fig. 90 Toxoplasmosis: cellular infiltration into the bronchioles, alveoli and septa. H. & E. x 50

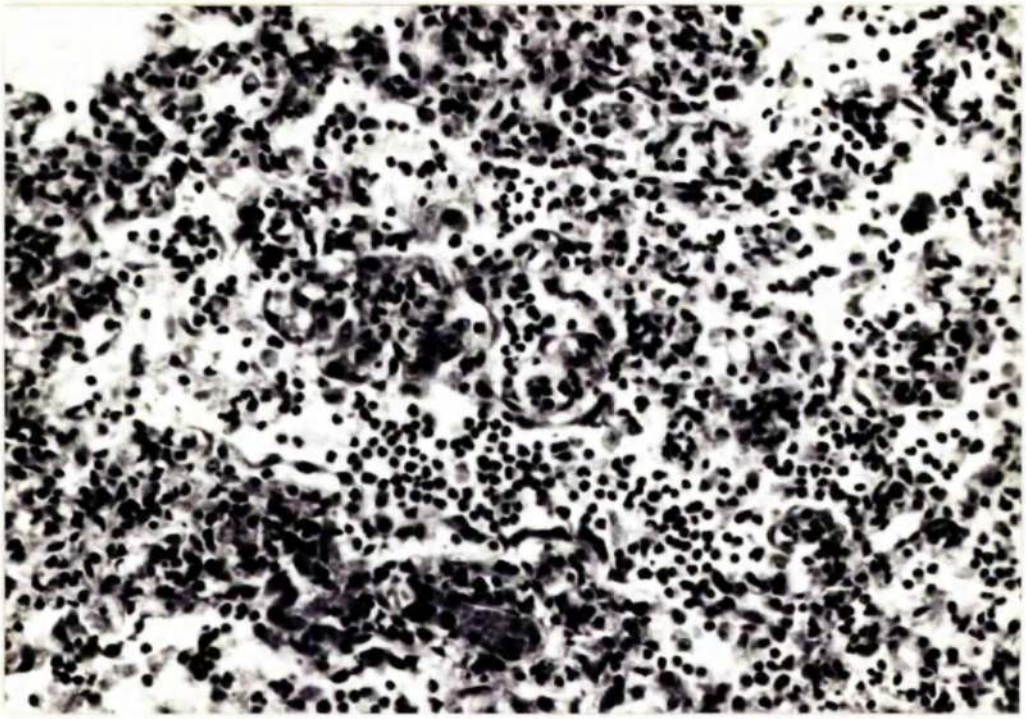


Fig. 91 Toxoplasmosis: swelling of the epithelial lining of an alveolar duct, filled with polymorphonuclear leucocytes and macrophages. H. & E. x 300

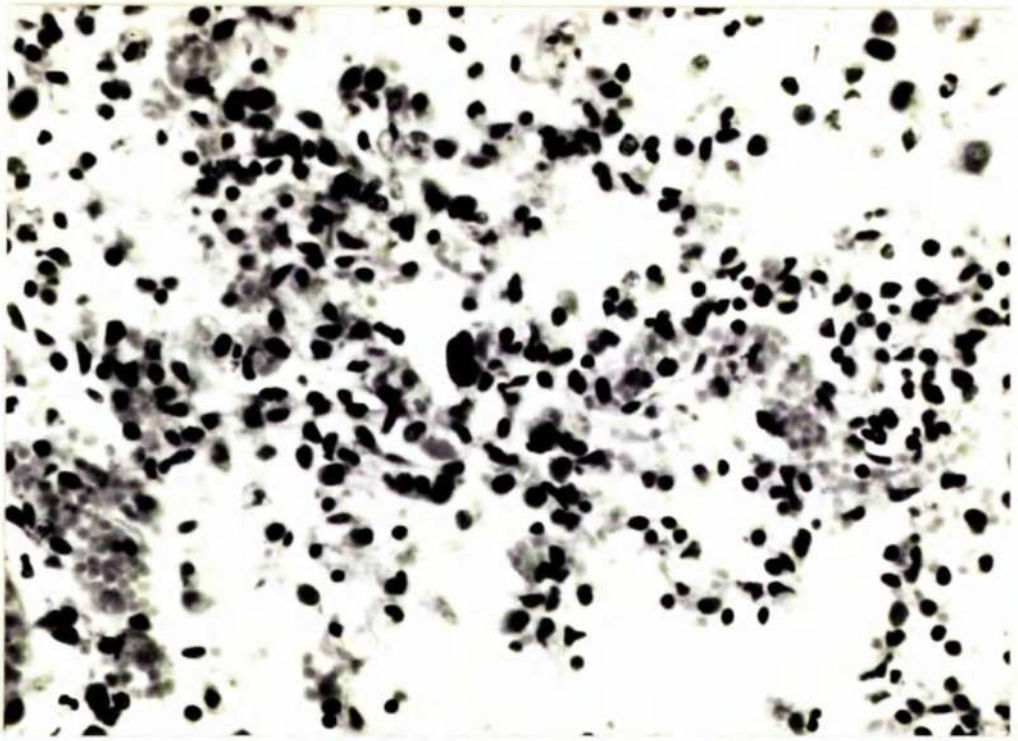


Fig. 92 Toxoplasmosis: large, dark, nucleus of an alveolar epithelial cell. H. & E. x 500

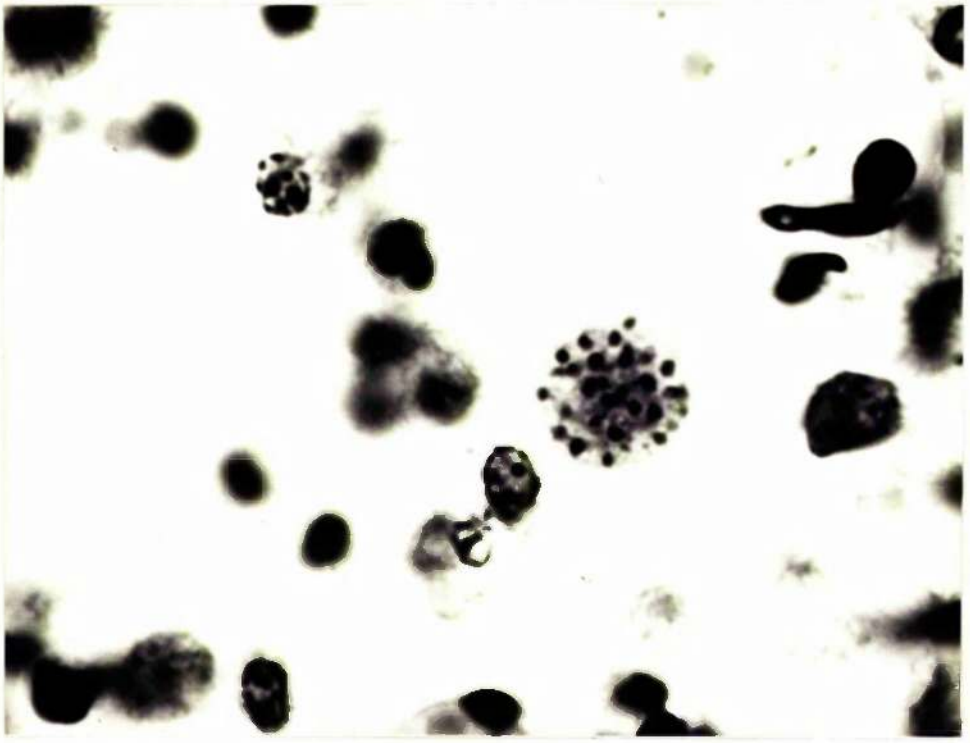


Fig. 93 Toxoplasmosis: pseudocyst of Toxoplasma gondii seen within an alveolus. H. & E. x 2,000

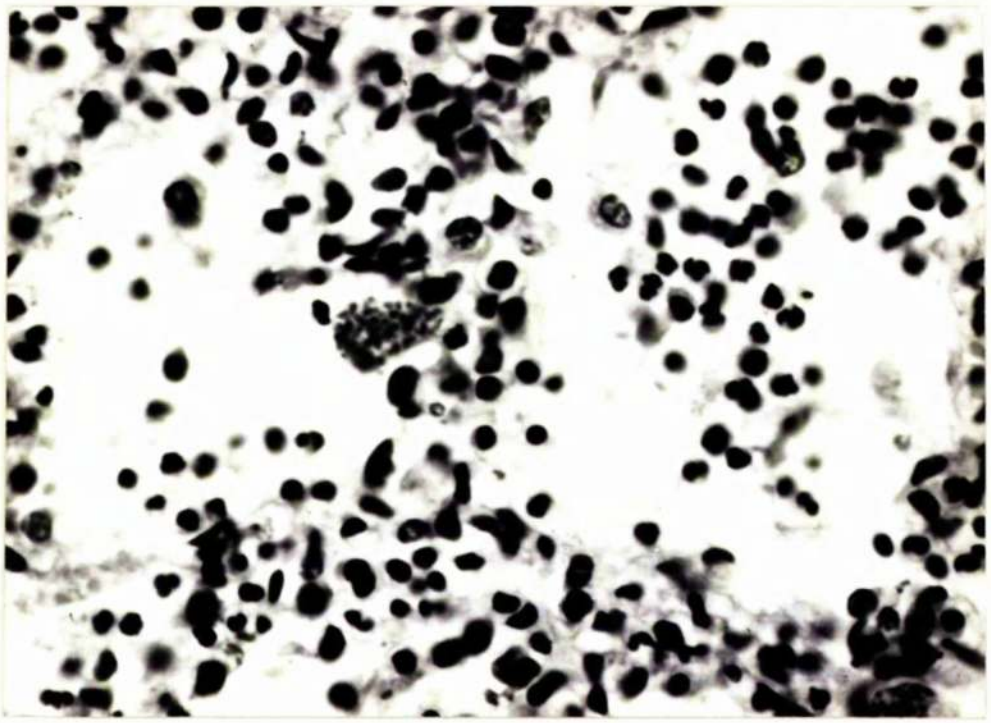


Fig. 94 Toxoplasmosis: a pseudocyst projecting into an alveolus from its wall. H. & E. x 800.

GIANT CELL PNEUMONIA

A giant cell pneumonia was described in pigs by Schofield (1956), who quoted Whittlestone (personal communication) as saying that he had seen a similar condition in two herds free from enzootic pneumonia. Schofield however did not give any details of the lesions which he had classified in this way.

In this series two sets of lungs were found in an abattoir survey which had a giant cell pneumonia.

Macroscopic Findings

Small, scattered reddish, lobular areas of consolidation were present in the apical, cardiac, and diaphragmatic lobes of the lungs. The bronchial lymph nodes were moderately enlarged.

Microscopic Findings

The most striking feature histologically was the presence of many large multinucleated giant cells within the alveoli (Fig. 95). Along with these were numbers of alveolar macrophages, eosinophils, plasma cells and lymphocytes (Fig. 96). Some alveoli were full of polymorphonuclear leucocytes. The alveolar walls were thickened in places due to infiltration by plasma cells and eosinophils. This infiltration was particularly noticable around the small blood vessels in the alveolar walls. The alveolar epithelium was prominent in some

alveoli and papillary projections into the alveolar lumina consisting of a central eosinophilic mass infiltrated by plasma cell and macrophages and covered by alveolar epithelium were found. The septal and pleural connective tissue were fairly heavily infiltrated in places by plasma cells, lymphocytes and a small but significant number of eosinophils. The peribronchiolar tissues were diffusely infiltrated by plasma cells, lymphocytes and eosinophils and similar cell types could be found in the lamina propria of the bronchioles and bronchi. The bronchial epithelium was hyperplastic and the lumina of the bronchi and the bronchioles was sometimes full of polymorphonuclear leucocytes, mixed with which were some eosinophils and an occasional giant cell.

The bronchial lymph nodes contained a large number of follicles with large clear germinal centres containing numerous mitotic figures. Considerable numbers of eosinophils were present in the dense lymphatic tissue around these follicles.

Discussion

The aetiology of this lesion is not clear. The fact that a small but significant proportion of the inflammatory cells were eosinophils suggests that a parasite might be responsible. No parasitic larvae however could be found in any of the sections examined and the lesion did not look as if it were due to Metastrongylus sp. It has been known for a long time that the

larvae of Ascaris lumbricoides migrate through the lungs and can produce pneumonia in the pig. It was shown however, (Betts 1954, Underdahl and Kelly 1957), that clinically this pneumonia did not last very long and that the pulmonary lesions had resolved by 21 days after experimental infection. The histology of the lesions was unfortunately not described. If these cases were a result of Ascaris larvae the infection must have occurred less than three weeks prior to slaughter.

No cases of pneumonia unequivocally due to the larvae of Ascaris lumbricoides were found in this investigation.

Fig. 95 Giant cell pneumonia: many giant cells in the alveoli and diffuse cellular peribronchiolar infiltration. H. & E. x 50

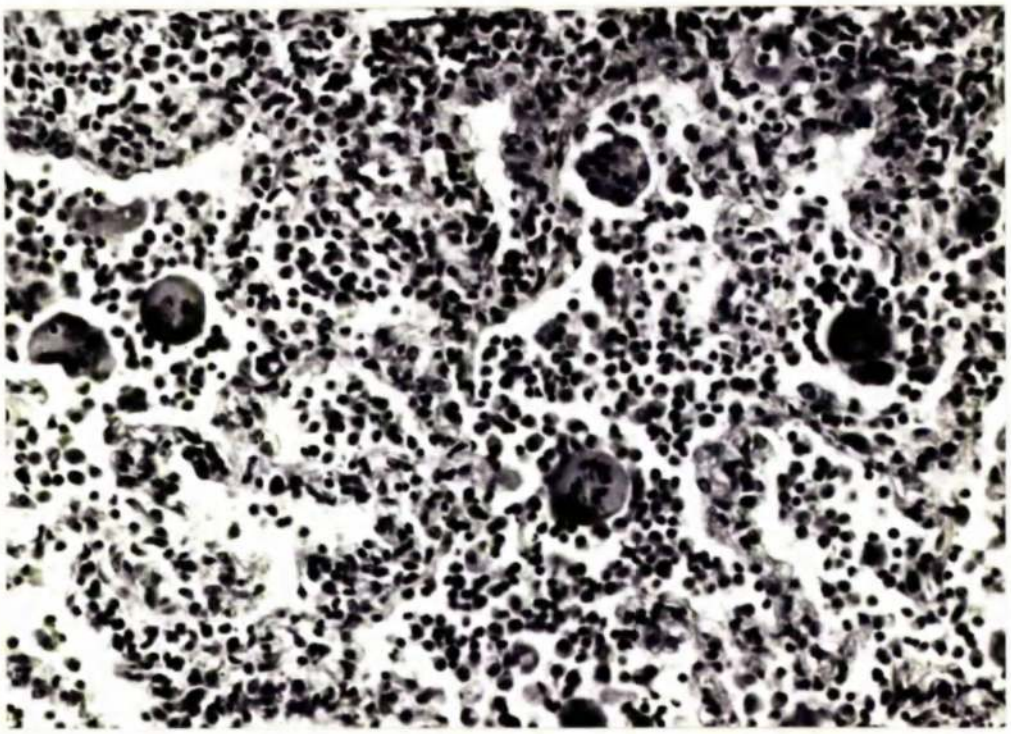


Fig. 96 Giant cell pneumonia: multinucleated giant cells within the alveoli. H. & E. x 500

PULMONARY VASCULAR LESIONS

Arterial disease is not a common cause of clinical disease in the pig although there have been several reports describing lesions in the systemic arterial system resembling the early stages of atherosclerosis (Gottlieb and Lalich 1954; Jennings, Florey, Stehbens, and French 1961). Pulmonary arterial lesions were found in two pigs in this series. In the one described as Case 1 the lesion was an arteritis and in the other described as Case 2 it was due to pulmonary embolism.

Case 1

This animal was a three months old pig which was found dead.

Macroscopic Findings

There was a small quantity of serous fluid in the abdomen and in the liver there were several focal patches of fibrosis. In all the lobes of the lungs there were small reddish-purple patches which did not feel particularly firm. The bronchial lymph nodes were moderately enlarged and oedematous.

Microscopic Findings

Histological examination of the lungs showed the presence of a pulmonary arteritis (Fig. 97). Many of the intralobular branches of the pulmonary artery were affected. There was fibrinoid necrosis of the vessel wall which often involved the whole circumference (Fig. 98) and in longitudinal sections could

be seen to be distributed focally along its length. The necrotic wall was intensely eosinophilic, was P.A.S. positive and stained red with Mallory's trichrome stain. It was blue with phosphotungstic acid haemotoxylin. Surrounding the lesion was a cellular infiltrate of polymorphonuclear leucocytes, lymphocytes, plasma cells and some macrophages. The connective tissue around the vessel was oedematous and the fibroblasts had become prominent. Most of the lesions were in that part of the vessel adjacent to a bronchiole. The neighbouring alveoli were either normal or contained a few alveolar macrophages and lymphocytes. Several small patches of simple bronchopneumonia were seen in some of the affected lobules.

The bronchial lymph nodes were oedematous and there were many polymorphonuclear leucocytes in the loose lymphatic tissue. There were only moderate numbers of lymphoid follicles present.

No lesions were seen histologically in the other organs.

Case 2

This case was a sow. The farmer noticed that the animal was losing weight and it dropped dead quite unexpectedly before he called for veterinary advice.

Macroscopic Findings

The carcase was emaciated. The pericardium was distended with yellow serous fluid and there was a mild fibrinous pericarditis. The coronary veins were distended. The right ventricle was hypertrophied and on the

pulmonic valve there was a large creamy coloured, friable vegetation 4.0 cms. high and almost completely blocking the pulmonary trunk. Several vegetations which were much smaller were found on the mitral valve and on the aortic valve. The lungs were congested and there was pulmonary oedema. Scattered over the surface of the lungs particularly in the diaphragmatic lobes were several dark red patches about 1.0 cm. square. On section they only extended into the lung about 0.5 cm. and in one of them a creamy coloured thrombus 0.2 cm. in diameter was seen in a branch of the pulmonary artery. When transverse sections were made through the diaphragmatic lobes of the lungs large emboli were seen in many branches of the pulmonary artery. The largest emboli were seen in vessels 0.8 cm. in diameter. An embolus was also found in the dorsal segment of the cardiac lobe of the left lung. There was no pulmonary infarction. The bronchial lymph nodes were enlarged.

Several small, pale infarcts were seen in the left ventricle and these were also renal infarcts at different stages of development.

Microscopic Findings

Histological examination of the lungs demonstrated the presence of oedema fluid in the alveoli, alveolar ducts and bronchioles. There was pulmonary congestion and the dark red patches seen macroscopically were areas of haemorrhage. Where haemorrhage had occurred haemosiderin containing macrophages had collected. Scattered, small accumulations of polymorphonuclear leucocytes and alveolar macrophage were seen in the alveoli. The most striking

feature histologically was the emboli in the branches of the pulmonary aftery (Fig. 99). These consisted of a pale pink mass of conglutinated platelets blocking the lumen of the vessel and usually the vessel wall merged with the embolus because of infiltration from the vessel wall by polymorphonuclear leucocytes, macrophages, fibroblasts and capillaries. Many emboli contained large olumps of bacteria. In smaller vessels the inflammatory reaction had spread to the adventitial connective tissue. Some bronchi had oedema fluid and small numbers of polymorphonuclear leucocytes in their lumina. The bronchial lymph nodes had many follicles with large, pale germinal centres and there were considerable numbers of polymorphonuclear leucocytes in the loose lymphatic tissue.

Histological examination of the heart confirmed the presence of infarction and pericarditis but also revealed an embolic myocarditis. Similarly, infarction and embolic nephritis were seen in the kidneys.

Bacteriology

Cultural examination yielded a profuse growth of an alpha haemolytic streptococcus belonging to Lancefield's group C. The organism was grown from the vegetations on the valves of the heart, the pulmonary emboli and the kidneys.

Discussion

The lesions in Case 1 were similar to those occurring in polyarteritis

nodosa and it is probable that this case is an example of this condition which has already been described in the pig, (Stunzi 1949). The aetiology is unknown although an allergic reaction in the vessel wall is postulated.

Bacterial endocarditis in the pig most frequently affects the mitral valve and consequently pulmonary embolism due to this condition is not common. Although in this case, large branches of the pulmonary artery were occluded there was no infarction in the related portion of the lung illustrating the excellent collateral blood supply from the bronchial arteries which the pig has in common with other species.

Atherosclerotic lesions were not seen in the pulmonary circulation in any of the animals. This was probably due to (a) the young age of the vast majority of the pigs examined in this investigation and (b) the known markedly lower incidence of atherosclerosis in the pulmonary circulation compared with the systemic circulation attributable to the much lower blood pressure in the former.

The most important cause of pulmonary haemorrhages in the pig is the swine fever virus. This virus may produce lobular patches of haemorrhage scattered focally in all the lobes of the lungs. Many cases of swine fever seen in the field however, have pneumonia and it has been claimed by some workers that the swine fever virus will produce pneumonia.

During this investigation a limited number of lungs from one outbreak of swine fever became available for study and it was found that the pneumonia

occurring in some of the pigs was similar to enzootic pneumonia histologically.

McFadyean (1896) experimentally infected twenty pigs with swine fever and only found small haemorrhages and collapse in the lungs at post mortem. He was of the opinion that when pneumonia occurred it was a secondary infection.

Kernkamp (1939) in a description of the frequency of different lesions in 334 pigs with swine fever consisting of 286 naturally occurring cases and 48 artificially infected pigs, found hyperaemia of the lungs in 44.9%, pulmonary haemorrhage in 27.2% and pulmonary inflammation in 40.4%. In the experimental series, pulmonary inflammation was found in 14.5% while in the field cases it occurred in 44.7%. The lesions were typical bronchopneumonias and occasionally a lobar type of pneumonia. He also expressed the opinion that pneumonia is a secondary or complicating factor.

Pattison, Howell and Elliot (1957) however, described pneumonic areas in seven pigs given swine fever virus intratracheally which were indistinguishable from enzootic pneumonia macroscopically, but had a different histological appearance. They summarised them as being "characterised microscopically by collapse, with varying degrees of cellular exudate". The cells being described as polymorphonuclear leucocytes, histiocytes and septal cells. Small haemorrhages were present in some sections and occasionally cuffing of blood vessels with small round cells was noted.

Bacteriological examination of the lungs was negative in all but one

case from which a Pasteurella was isolated. The authors concluded that the swine fever virus was responsible for the lesion and said that they confirmed this in three pigs infected intraperitoneally with the virus.

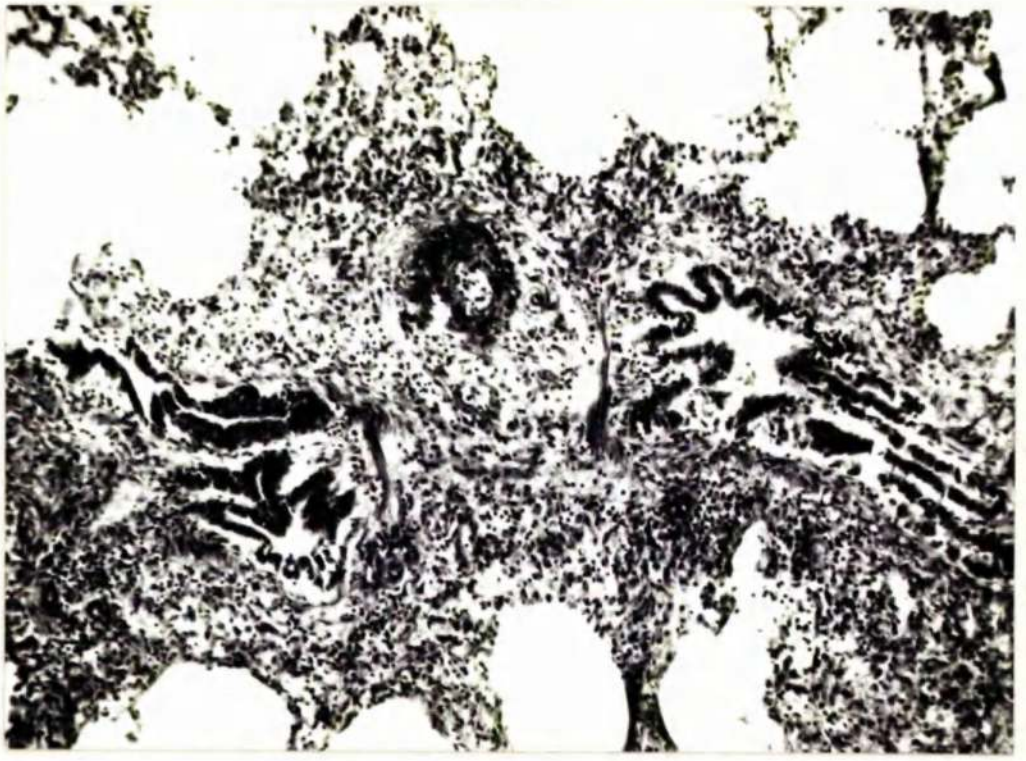


Fig. 97 Pulmonary arteritis affecting a small branch of the pulmonary artery. H. & E. x 150

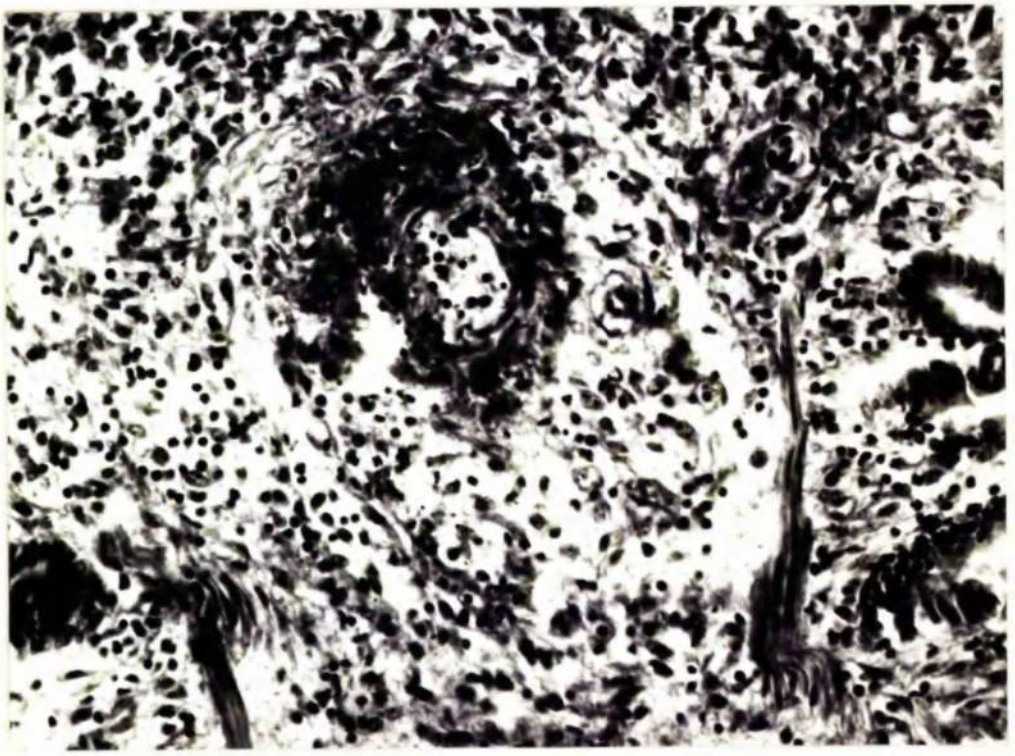


Fig. 98 Pulmonary arteritis: fibrinoid necrosis of the arterial wall and perivascular cellular infiltration. H. & E. x 300

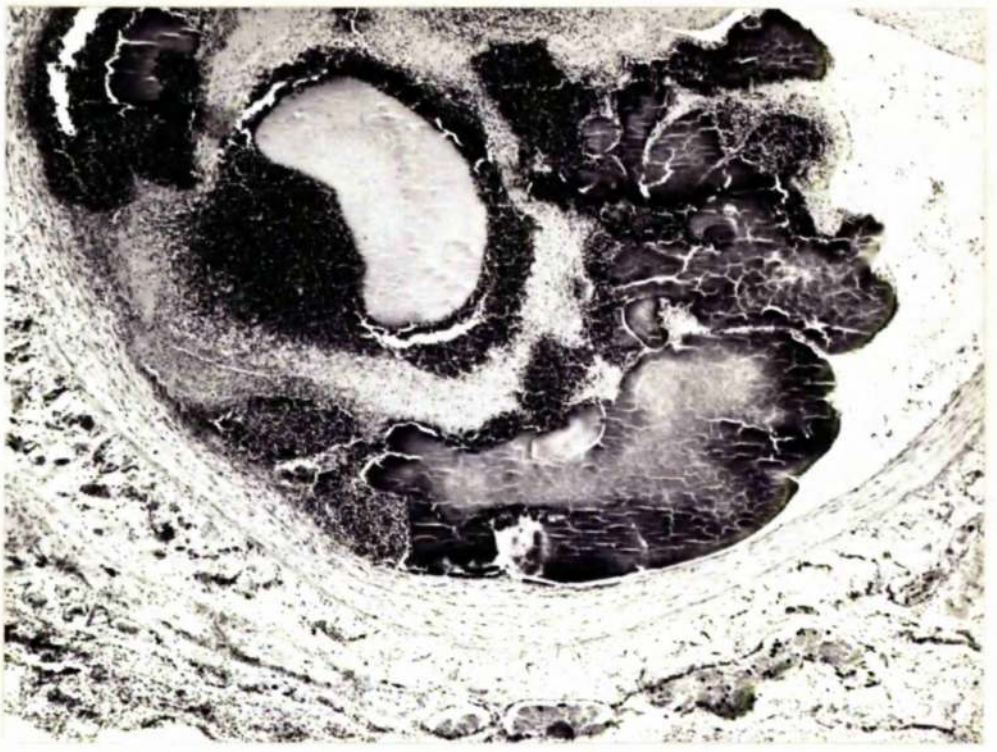


Fig. 99 A large embolus containing bacterial colonies and infiltrated by polymorphonuclear leucocytes, in a branch of the pulmonary artery.
H. & E. x 50

CONCLUSIONS

The pulmonary lesions of the pig had not previously been classified on a morphological basis and it was felt that this was a necessary prerequisite to understanding pneumonia in the pig, so that these conditions which are important economically can be controlled in the field. In order to do this a survey of the naturally occurring pulmonary disease in the pig was carried out and a histopathological classification was prepared offering adequate and satisfactory criteria for defining the different pulmonary diseases of the pig. Using these criteria the pneumonias were divided into thirteen groups, which were apparently specific entities. The other studies which were carried out supported the concept that these were in fact separate entities.

Enzootic pneumonia and the pneumonia produced by Metastrongylus apri were more frequently found than other pneumonias and were therefore studied in more detail.

The experimental study of enzootic pneumonia showed that this disease is associated with a series of changes in the lungs; the histological characteristics of these changes vary with the stage of development of the disease and the features of the lesion depend, at least in part, on the duration of the infection. By correlating these observations with the changes found in pigs which had developed pneumonia under field conditions it was possible to delineate and characterise enzootic pneumonia as a histo-

pathological entity differing in several important ways from the other naturally occurring pneumonias of the pig. Until the etiology of enzootic pneumonia is firmly established histopathology must be the main diagnostic criterion.

Some of the features of enzootic pneumonia are similar to those of the pneumonia produced by infection with M. apri. The experimental studies on this disease however showed that the pneumonia due to M. apri was associated with other pulmonary changes which are characteristic of the condition and enable it to be separated readily from enzootic pneumonia. The pneumonia due to M. apri was also found to have a sequential series of changes whose appearance depended on the time after infection at which the lesions were examined.

While attempting to protect young pigs against M. apri by using x-irradiated infective larvae of M. apri, to stimulate active immunity, it was found that x-irradiation (i) reduced the number of worms which developed from a given number of larvae (ii) prevented the worms from becoming sexually mature (iii) and resulted in the worms which developed from x-irradiated larvae being smaller than those which developed from normal larvae; the degree of stunting depended on the level of x-irradiation administered to the larvae. In addition it was shown that the male larvae were more susceptible to x-irradiation than the female larvae.

ACKNOWLEDGEMENTS

I should like to acknowledge my debt to Dr. W.F.H. Jarrett on two accounts, firstly for the training in pathology which I have received in his department and secondly for his advice and criticism during the period when I was working on the problem which is the subject of this thesis.

I should also like to record my thanks to Professor W.L. Weipers from whom I have received much encouragement in my post-graduate studies.

A major problem encountered by workers in pig respiratory diseases is the task of finding a source of experimental pigs free from enzootic pneumonia. I was fortunate in having access to the pig herd maintained by the Department of Animal Husbandry and Preventative Medicine, and I must thank Professor J.S.S. Inglis and Mrs. Mary Weipers for their co-operation in supplying me with experimental animals from this herd.

The bacteriological examinations of the pig lungs studied in this work were made by M. Grindlay Esq., M.R.C.V.S., and L. Nagy Esq., B.Sc., and I am indebted to them for the useful and interesting information made available by their assistance.

A thesis dealing with macroscopic and microscopic pathology must of necessity contain a considerable number of photographs. In this respect I would like to thank A. Finnie Esq., for his collaboration in providing me with the excellent photographs published here. I am also indebted to

J. Murphy Esq., both for his help in the post-mortem room and for his aid during many of the experimental manipulations performed in connection with this study.

While carrying out the work necessary for this thesis I received expert technical assistance from Miss Fay Overend and C. Ferguson Esq., during the parasitological studies, and from all the technicians in the histopathology laboratory of the Department of Hospital Pathology, particularly Mrs. Sheila Cranston and Miss Freyda Davie, in the preparation and sectioning of material for microscopic examination. I would like to thank them all for their help. I must also thank Mrs. Norma Gilmour, Mrs. Helen Macleod, Miss Helena Stewart and Mrs. Robina Williamson for their invaluable assistance in the preparation of the material for publication.

A major part of the preparation of a thesis involves typing the work in its final form. This was done for me by Miss Irene Douglas to whom I express my sincerest thanks.

I should also like to record my gratitude to the Agricultural Research Council for financing me during my early years of post-graduate study and in addition to thank Messrs. Allen and Hanbury's Ltd., who made available money for experimental animals.

REFERENCES

Andrewes, C.H., Laidlaw, P.P., and Smith, W. 1935. Brit. J. exp. Path., 16 566.

Andrewes, C.H., and Worthington, G. 1959. Bulletin of W.H.O. on Influenza, 20 436.

Avery, M.E. 1962. Pediatrics., 30 324.

Bakos, K., Nilsson, A., and Thal, E. 1952. Nord. Vet. Med. 4 241.

Barber, R.S., Braude, K., Mitchell, K.G., and Betts, A.O. 1955. Vet. Rec., 67 690.

Betts, A.O. 1952. Vet. Rec., 64 283.

Betts, A.O. 1953. Brit. vet. J., 109 99.

Betts.,A.O. 1954. Vet. Rec., 66 749.

Betts, A.O., and Beveridge, W.I.B. 1952. J. Path. Bact., 64 247.

Betts, A.O., and Beverdige, W.I.B. 1953. Vet. Rec., 65 515.

Betts, A.O., and Campbell, R.C. 1956. J. comp. Path., 66 89.

Betts, A.O., Whittlestone, O., and Beveridge, W.I.B. 1955. Vet. Rec., 67 685.

Betts, A.O., Whittlestone, P., Beveridge, W.I.B., Taylor, J.H., and Campbell, R.C. 1955. Vet. Rec., 67 661.

Billings, F.S. 1888. 'Swine Plague' State Journal Publishing Co., Lincoln, Nebraska.

Blakemore, F., Gledhill, A.W., Glover, R.E., and Taylor, E.L. 1941. Proc. roy. Soc. Med., 34 611.

Bolande, R.P., and Klaus, M.H. 1964. Amer. J. Path., 45 449.

Chabaud, A.G., and Gretillat, S. 1956. Ann. Parasit. Hum. Comp., 31 572.

Clements, J.A. 1962. Scientific American. December, 3.

Clements, J.A., Brown, E.S., and Johnson, R.P. 1958. J. Appl. Physiol., 12 262.

Darwin, C. 1882. The formation of Vegetable mould through the action of worms, with observations on their habits, London.

Dayton, D.A. 1957. J. Parasit., 43 282.

Dougherty, E.C. 1944. Proc. helminth. Soc. Wash., 11 66.

Dow, C., Jarrett, W.F.H., Jennings, F.W., McIntyre, W.I.M., and Mulligan, W. 1959. J. Amer. vet. med. Ass., 135 407.

Dunn, D.R. 1955. Brit. vet. J., 111 97.

Dunn, D.R. 1956. Brit. vet. J., 112 327.

Dunn, D.R., Gentles, M.A., and White, E.G. 1955. Brit. vet. J., 111 271.

Dunn, D.R., and White, E.G. 1954. Nature (Lond.), 174 1193.

Ewing, S.A., and Todd, A.C. 1961. Amer. J. vet. Res., 22 606.

Fulton, J.S. 1930. J. Amer. vet. med. Ass., 77 368.

Fulton, J.S., Burton, A.N., and Millar, J.L. 1953. J. Amer. vet. med. Ass., 123 221.

Gedoelst, L. 1923. Bull. Soc. Path. Exot., 16 622.

Glasser, K. 1939. Dtsch. tierärztl. Wschr., 47 209.

Glover, R.E., and Andrewes, C.H. 1943. J. comp. Path., 53 329.

Gonzalez, J.C. 1951. Rev. Iberica. Parasit., 11 265.

Goodwin, R.F.W., and Whittlestone, P. 1960. Vet. Rec., 72 1029.

Goodwin, R.F.W., and Whittlestone, P. 1962. J. comp. Path., 72 389.

Goodwin, R.F.W., and Whittlestone, P. 1963. Brit. J. exp. Path., 44 291.

Goodwin, R.F.W., and Whittlestone, P. 1964. Vet. Rec., 76 611.

Goodwin, R.F.W., and Whittlestone, P. 1965. Res. vet. Sci., 6 100.

Gottlieb, J., and Lalich, J.J. 1954. Amer. J. Path., 30 851.

Gulrajani, T.S. 1951. J. comp. Path., 66 48, 60 and 101.

Gulrajani, T.S., and Beveridge, W.I.B. 1951a. Nature (Lond.), 167, 856.

Gulrajani, T.S., and Beveridge, W.I.B. 1951b. J. comp. Path., 61 118.

Hjarre, A., Dinter, A., and Bokos, K. 1954. Arch. Exp. Veterinarmed., 6 82.

Jarrett, W.F.H. 1953. Vet. Rec., 65 153.

Jarrett, W.F.H. 1956. Brit. vet. J., 112 431.

Jarrett, W.F.H., Jennings, F.W., McIntyre, W.I.M., Mulligan, W., and Sharp, N.C.C. 1961. Am. J. vet. Res., 22 186.

Jarrett, W.F.H., Jennings, F. W., McIntyre, W.I.M., Mulligan, W., and Urquhart, G.M. 1957. Vet. Rec., 69 1329.

Jarrett, W.F.H., Jennings, F. W., McIntyre, W.I.M., Mulligan, W., and Urquhart, G.M. 1960. Immunology,, 3 147.

Jarrett, W.F.H., McIntyre, W.I.M., and Sharp, N.C.C. 1962. Amer. J. vet. Res., 23 1183.

Jennings, M.A., Florey, H.W., Stehbens, W.E., and French, J.E. 1961. J. Path. Bact., 81 49.

Kates, K.C. 1941. J. of Parasit., 27 265.

Kernkamp, H.C.H. 1939. J. Amer. vet. med. Ass., 95 159.

Kleine. 1877. Vet. J., 5 45.

Kobe, K. 1933. Zbl. Bakt., 1 129 and 161.

Kobe, K. 1934. Dtsch. tierärztl. Wschr., 42 603.

Laidlaw, P.P. 1935. Lancet, 1 1118.

Lamont, H.G. 1938. Vet. Rec., 50 1377.

Lamont, H.G. 1952. Vet. Rec., 64 442.

Lannek, N., and Bornfors, S. 1956. Vet. Rec., 68 53.

Lannek, N., and Bornfors, S. 1957. Nord. Vet. Med., 9 91.

Lannek, N., and Wesslen, T. 1955. Acta path. microbiol. scand., 34 343.

Lannek, N., and Wesslen, T. 1957. Nord. Vet. Med., 9 177.

Lapage, G. 1956. Veterinary Parasitology, P. 149. Oliver & Boyd, Edinburgh.

Levin, A.J., and Evans, T.C. 1942. J. Parasitol., 28 477.

Lewis, E.A. 1926. J. Helminth., 4 123.

Lewis, P.A., and Shope, R.E. 1931. J. exp. Med., 54 361.

MacKenzie, A. 1958a. Vet. Rec., 70 843.

MacKenzie, A. 1958b. Vet. Rec., 70 903.

MacKenzie, A. 1959. Vet. Rec., 71 209.

Mathews, P.R.J., and Pattison, I.H. 1961. J. comp. Path., 71 44.

McBride, C.N., Niles, W.B., and Markey, H.E. 1928. J. Amer. vet. med. Ass., 73 331.

McFadyean, J. 1888. J. comp. Path., 1 101.

McFadyean, J. 1891. J. comp. Path., 4 316.

McFadyean, J. 1896. J. comp. Path., 9 119 and 220.

McFadyean, J. 1897. J. comp. Path., 10 156.

McFadyean, J. 1902. J. comp. Path., 15 159.

Momberg-Jørgensen, H.S. 1938. Berlin u. Munch. tierärztl. Wschr., 573-576.

Murray, C. 1920. J. Amer. vet. med. Ass., 57 539.

Nicholas, W.H. 1949. Nat. Geographic Mag., 95 269.

Niven, J.S.F. 1950. Brit. J. exp. Path., 31 759.

Pattison, I.H. 1956. Vet. Rec., 68 490.

Pattison, I.H., Howell, D.G., and Elliot, J. 1957. J. comp. Path., 67 320.

Pattle, R.E., and Thomas, L.C. 1961. Nature (Lond.), 189 844.

Plowright, W. 1953. Vet. Red., 65 313.

Pullar, E.M. 1948. Aust. vet. J., 24 320.

Pullar, E.M. 1949a. Aust. vet. J., 25 53.

Pullar, E.M. 1949b. Aust. vet. J., 25 123.

Pullar, E.M. 1949c. Aust. vet. J., 25 262.

Rislakki, V. 1953. Nord. Vet. Med., 5 113.

Robertson, D. 1937. Scottish J. Agric., 20 373.

Schofield, F.W. 1956. Canad. J. comp. Med., 20 252.

Schwartz, B., and Alicata, J-E. 1929. J. of Parasit., 16 105.

Schwartz, B., and Alicata, J-E. 1932. J. of Parasit., 18 21.

Schwartz, B., and Alicata, J-E. 1934. Technical Bulletin of the United States Dept. of Agric. Washington No. 456. Life history of lungworms parasitic in swine.

Shanks, P.L., and McPherson, R., 1955. Vet. Rec., 67 533.

Shope, R.E. 1931a. J. exp. Med., 54 349.

Shope, R.E. 1931b. J. exp. Med., 54 373.

Shope, R.E. 1932. J. exp. Med., 56 575.

Shope, R.E. 1934. J. exp. Med., 59 201.

Shope, R.E. 1935. J. exp. Med., 62 561.

Shope, R.E. 1936. J. exp. Med., 63 669.

Shope, R.E. 1937. J. exp. Med., 66 151.

Shope, R.E. 1938. J. exp. Med., 67 739.

Spray, R.S. 1922. J. Infect. Dis., 31 10.

Sprent, J.F.A., and Chen, H.H. 1949. J. Infect. Dis., 84 111.

Stünzi, H. 1949. Schweiz. Arch. Tierheilk., 91 367 and 436.

Sullivan, J.F., and Shaw, J.M. 1952. N. Amer. Vet., 33 704.

Tallantd, S. 1959. Anat. Anz., 106 68.

Underdahl, N.R., and Kelly. 1957. J. Amer. vet. med. Ass., 130 173.

Usman, H.K., Underdahl, N.R., and Young, G.A. 1950. Amer. J. vet. Res., 19 913.

Urquhart, G.M., Jarrett, W.F.H., and Mulligan, W. 1962. Advanc. vet. Sci., 1 87.

Waldmun, O. 1934. Dtsch. tierärztl. Wschr., 42 606.

Waldmen, O. 1936. Dtsch. tierärztl. Wschr., 44 847.

Wesslen, T., and Lannek, N. 1954. Nord. Vet. Med., 6 481.

Whittlestone, P. 1957a. Thesis submitted to the University of Cambridge.

Whittlestone, P. 1957b. Vet. Rec., 69 1,354.

Whittlestone, P., and Betts, A.O. 1955. Vet. Rec., 67 692.

ERRATUM

Betts, A.O. and Whittlestone, P. 1963. Res. Vet. Sci. 4 471-479.

Harding, J.D.J., Beverley, J.K.A., Shaw, I.G., Edwards, B.L. and Bennett, G.H. 1961. Vet. Rec. 73 3.

Waldman, O. 1938. Rept. XIIIth Intern. Vet. Congr. Zürich-Interlaken 1 491-497.

Young, G.A. and Underdahl, N.R. 1960. J. Amer. vet. med. Ass. 137 186.