SOME ASPECTS OF THE PATHOLOGY AND PATHOGENESIS OF BOVINE TUBERCULOSIS

(and other published papers)

Submitted as a Thesis for the Degree of D.Sc.
of Edinburgh University
by

JOHN T. STAMP, B.Sc., M.R.C.V.S.

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INTRODUCTION

Many of the problems concerned with the pathogenesis of bovine tuberculosis remain unsolved. The following short review of the pathology and pathogenesis of the bovine disease gives a summary of present knowledge.

CONGENITAL TUBERCULOSIS

In congenital tuberculosis of the calf, it is presumed that infection occurs in the majority of cases by way of the umbilical cord and so by the foetal circulation to the liver and portal lymph nodes and there gives rise to lesions. The process may stop here but in many instances nodular lesions appear also in the spleen substance, in the lung substance, and occasionally in heart muscle along with widespread lesions in carcase lymph glands.

The characteristic lymph gland lesion consists of enlargement of the gland with diffuse cellular infiltration and early caseation. It is not a nodular type of lesion. A characteristic post-mortem finding is early caseation of portal, caudal mediastinal, and bronchial lymph glands with isolated nodules in lung, liver spleen, etc.

POST-NATAL CALFHOOD TUBERCULOSIS

Examination of the literature shows that there is great confusion as regards the method of infection in these cases. Nieberle (1929) has done extensive morbid anatomical examination of calves at slaughter,

and published an account of his conclusions. The site of entry of primary post-natal calfhood infection is in the majority of cases by the intestinal canal where ulceration may or may not occur. The regional lymph glands show extensive tuberculosis and the importance of the portal lymph gland as being the regional lymph gland draining the duodenum is stressed. Primary pulmonary infection is relatively uncommon but when it does occur a pulmonary focus is usually found in addition to tuberculous change in the bronchial glands. Innes (1937), giving an account of the ideas of the Nieberle School, states that while in man and dog only a small percentage of primary infections occur in extra-pulmonary tissues, in the case of the bovine species it seems to be the unanimous opinion that alimentary infection is the more prevalent, this conclusion being based on the situation of the primary complex. White and Minett (1941), discussing the pathogenesis of tuberculosis in the calf, state that the Germans emphasise the importance in young calves of primary tuberculosis of the intestine and mesenteric lymph glands. These workers also stress the fact that the portal lymph nodes are not necessarily indicative of liver infection since the nodes also drain the duodenum and they suggest that isolated tuberculosis of these nodes in a young calf may indicate either alimentary infection from the duodenum or congenital infection

by the umbilical vein. It is, however, impossible to reconcile these statements with morbid anatomical findings such as those described by Gofton (1937) who from abattoir experience states that at least 40 per cent. of all tuberculous calves not showing hepatic lymph gland lesions show lesions confined to lungs and lung glands, or with the findings of Schulz (1937) who out of 107 cases of tuberculosis in the calf found only seven intestinal complexes. Nieberle (1937), in a later article than the one above, appears no longer to agree with his own previous findings since he now states that respiratory infection of the calf is much more frequent than alimentary and that all calves which show lesions in the liver or portal glands are examples of true congenital infection. Innes (1940) in a more recent article quotes the new findings of Nieberle. The findings of Nieberle (1937) indicate that lesions found in the portal lymph gland of calves over 14 days old are not in the majority of cases due to post-natal intestinal infection. Since these cases constitute a large proportion of all calfhood lesions it becomes obvious that if they are no longer classed as alimentary then the percentage incidence of this route of infection drops accordingly. This question of the routes of infection of tuberculosis in the calf has led to extensive experimental evidence being put forward to support the various theories, but Glover (1941) in a comprehensive review considers

that the problem is still a very open one.

Primary infection in the calf is frequently followed by early generalisation, and Nieberle (1929) divides this generalisation into three distinct types.

(1) Protracted Generalisation. This is by far the most frequent form. The primary complex is present with caseous lesions in portal lymph gland and/or mesenteric glands and in addition the caudal mediastinal lymph glands frequently show similar lesions. The bronchial glands are much less extensively altered but frequently show some change. In addition to these lesions, discrete nodules, usually only in small numbers, are seen in the lungs, spleen, liver kidneys, and peripheral lymph glands.

(2) Acute Miliary Tuberculosis. This type is relatively uncommon in the early post-primary generalisation period. When it does occur large numbers of small miliary tubercles are present in the organs. In the lungs the scatter of lesions is not uniform nor are the nodules equal in size.

(3) Large Nodular Generalised Tuberculosis. There are a number of foci of comparatively large size scattered throughout the lung giving the appearance of a focal tuberculous pneumonia.

ADULT PULMONARY TUBERCULOSIS

It appears to be generally accepted that tuberculosis of the adult is merely a slow progression of lesions acquired in calfhood and in consequence the

disease in the calf has assumed undue prominence. That this is not the case is very easily demonstrated in that the percentage number of calves reacting to the tuberculin test is very low in comparison to that of adult reactors. It would appear that the most important age at which infection of cattle takes place is from six months to two years and it is the route of infection in these cases which is of far greater importance than the route of infection in calves. There is however no published work on the pathogenesis of the disease in these older animals except for the present thesis.

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On what foundation does this rest?

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THE PATHOGENESIS OF BOVINE TUBERCULOSIS AS BASED ON ABATTOIR RETURNS

J. T. STAMP and A. WILSON[§§]

(Vet. Rec. 1946, Vol. 58, No. 2)

ROUTES OF INFECTION

REVIEW OF PRESENT KNOWLEDGE

It has never been seriously debated that the primary site of infection is intestinal when the only lesions present in the body are in the mesenteric lymph glands, whether alone or in combination with intestinal lesions. The significance of tuberculous bronchial and mediastinal lymph glands, in the absence of intestinal or mesenteric lymph gland lesions, has, however, been much debated. Villemin (1868) first suggested that these lesions originated by inhalation of tubercle bacilli, and he was later supported in this view by Koch (1901). Von Behring (1903) formulated the theory that the portal of entry in such cases was by the alimentary tract and that the bacilli having entered the body by this route passed through the mucous membrane of the intestine and also through the related lymph glands, without producing in them any lesions. Such bacilli were supposed either to pass to the lungs by the thoracic duct and the right heart, or to pass to the mediastinal and bronchial glands by direct

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[§§]A. Wilson, B.Sc., M.R.C.V.S., supplied abattoir returns from Birmingham on which some of the observations are based.

lymphogenous channels and here produce their first lesions. Calmette and Guerin (1905 and 1906) strongly supported this view and reported many animal experiments to support their contentions. These inconclusive experimental findings have been strongly criticised; M'Fadyean (1910) believed that their evidence tended, if anything, to show that lung and lung gland lesions do not occur after ingestion experiments without previous lesions in the mesenteric glands. Many subsequent experiments have been performed but none has afforded absolute evidence for either theory. It has been conclusively shown that larger numbers of tubercle bacilli are needed to infect an animal by the alimentary than by the respiratory tract. The problem has been approached in other ways, such as by morbid anatomical study of naturally occurring tuberculosis, mostly in the human subject, but this also has failed to provide incontrovertible evidence. Nevertheless, much valuable work has been done, most of which suggests that the thoracic lymph glands are infected directly from inhalation lesions of the lung. One of the main methods of morbid anatomical approach has been to demonstrate in the lung primary aerogenous lesions to account for the involvement of the bronchial glands. Parrot (1876) was the first to describe these primary lung lesions and, as a result of extensive and thorough work, he stated that the tracheo-bronchial gland lesions could be

trusted to indicate the presence of lung lesions. Since Parrot numerous workers have made similar careful morbid anatomical studies, and it is now generally accepted that, in man, if caseation is present in the bronchial glands, then the lungs are the portal of entry. More recently Blacklock (1932), attacking the problem in a different way, has given further very strong evidence in support of this view: he found that many primary alimentary lesions in children are caused by the bovine type of bacillus, while primary lung and lung gland lesions are chiefly caused by the human type of bacillus. This is to be expected, if bronchial and tracheal lymph gland caseation is due to inhalation infection, since this route of infection is most likely to arise during contact with cases of open pulmonary tuberculosis which in the human being are predominantly caused by the human type of bacillus. On the other hand, mesenteric lymph gland lesions arise from ingestion of tubercle bacilli, milk infected with the bovine type of bacillus often being the source of infection.

Veterinary literature is almost entirely lacking in carefully recorded post-mortem findings of large numbers of tuberculous cattle and little use has been made of the existing recorded abattoir findings, so that the common portals of entry of the tubercle bacillus into the bovine body have not been well assessed. A survey of veterinary literature shows that it was fairly well appreciated by observers at

the end of the last century that in cows pulmonary and bronchial/mediastinal lymph gland tuberculosis was the most frequent form of tuberculosis. Since that time, and especially since Behring's hypothesis, supported strongly by Calmette, was put forward, it has become a widely held opinion that the common site of infection in cattle is the intestine (Edwards, 1937; Calmette, 1923; Mettam, 1906; Cadeac, 1909; Muller, 1906; Valee, 1905; Innes, 1937; Udall, 1939; Edit., 1908; Edit., 1910). The majority of the more recent writers on this question, however, give no evidence to support their conclusions, so that the reader is at a loss to know on what grounds the writers base their opinions. In consequence, it is not surprising that so much confusion exists as to the frequency of the various sites of tuberculosis in cattle.

ORIGINAL OBSERVATIONS

It was considered that an analysis of the meat inspection returns of two large abattoirs (Edinburgh and Birmingham) might give useful evidence as to the common sites of infection in cattle. At both of these abattoirs meat inspection is carried out under strict veterinary control and it is certain that lesions of macroscopic size in the lymph glands of the head and lung are seen and recorded. Personal observation has shown that characteristic tuberculous lesions of the retropharyngeal, bronchial and mediastinal lymph glands are rarely missed.

Mesenteric gland lesions are more frequently overlooked but it is considered that the percentage missed is not great.

Tables of incidence, etc., for heifers, bullocks, cows and bulls, have been compiled from the detention records of the two abattoirs; but since, on the whole, they agree closely, only one set of figures will be given, and where differences do occur these will be given in the text. In the case of heifers, bullocks and cows, the returns of 3,000 consecutive tuberculous animals were examined but in the case of bulls it was only possible to obtain figures for 1,000 animals. The returns for tuberculous calves, aged from a few days to several months, were compiled from the records of the late Chief Veterinary Officer of Edinburgh (Mr. Gofton).

Many important deductions, including the incidence of tuberculosis in each group, can be stated from examination of the results (see Tables I to V) and it is interesting to note that the incidence in the same group varied little from year to year over a long period. The data obtained from the detention records were analysed and grouped so that lesions clearly indicating a definite portal of entry of the disease were classified together under each age group (calves, heifers, bullocks, cows and bulls) and compared one with the other. In this classification, retropharyngeal gland lesions are taken as arising from pharyngeal infection, which

may indicate ingestion or inhalation; bronchial and mediastinal lymph gland lesions are taken as indicating lung infection by inhalation; mesenteric gland lesions are taken as indicating infection by ingestion. In addition to these uncomplicated portals of entry, various groups of lesions are also associated, so that such combinations of lesions as head and lung, head and intestine, lung and intestine, etc., are grouped together. In this way, one can determine which lesions are frequently associated together and from this some idea of the pathogenesis of the disease can be gained.

CALVES

The incidence of tuberculosis in calves up to three to four months old is low, only 189 out of 10,000 calves being tuberculous, an incidence of 1.89 per cent. Mesenteric lymph gland lesions occur in only 32 cases out of 189, that is in 15.0 per cent. of tuberculous calves or 0.3 per cent. of all calves. Of these, 22 also show portal lymph gland lesions, which gives support to the contention that these glands may be infected from the duodenum during primary infection (White and Minett, 1941). Lesions confined to the thorax occur in 51 per cent. of tuberculous calves, or 0.98 per cent. of all calves. Only 2 per cent. of tuberculous calves or 0.04 per cent. of all calves show lesions confined to the retropharyngeal lymph glands. The remaining 28 per cent. of tuberculous calves show lesions confined to the liver or liver and lung glands; this probably indicated that congenital infection occurs in 0.5 per cent. of all calves.

TABLE I

Edinburgh — CALVES — Incidence 1.89% (189 Tuberculous Animals)

Organ Complex	No.	% T.B.	% of Total
Head	4	2	0.04
Lungs and lung glands	98	51	0.98
Head and lungs	1	-	-
Intestine	1	-	-
Intestine and head	1	-	-
Intestine, liver and head	-	-	-
Intestine and lungs	6	2.6	0.06
Head, intestine and lungs	2	1	0.02
Lungs and liver	39	20	0.39
Head, lungs and liver	-	-	-
Lungs, liver and intestine	17	8.9	0.17
Head, lung, intestine and liver	5	2.5	0.05
Liver	15	8	0.15

HEIFERS AND BULLOCKS

Analysis of the meat inspection returns shows that in the two groups, the incidence of the disease and the organ complexes affected are similar, suggesting that the pathogenesis of the disease in heifers and bullocks is the same. The incidence of tuberculosis in the two groups varies slightly over a period of years but averages at 6 per cent. In heifers and bullocks primary intestinal infection is relatively low, since lesions confined to the mesenteric lymph glands occur in only 5.3 per cent. of tuberculous animals or 0.3 per cent. of all animals of the group, while not more than 32 per cent. of such tuberculous animals or 1.9 per cent. of all heifers and bullocks have mesenteric lymph gland lesions, even in conjunction with lesions in other situations.

The incidence of primary respiratory infection in the heifer and bullock, as indicated by lesions confined to the thoracic glands, differs in the two abattoirs. At Birmingham the incidence is 35 per cent. of tuberculous animals or 2.1 per cent. of all animals, while at Edinburgh (as indicated in the Table), the incidence is 22 and 26.3 per cent., and 1.4 and 1.5 per cent. respectively. Primary pharyngeal infection, as indicated by lesions confined to retropharyngeal lymph glands, also varies as regards incidence at the two abattoirs. At Birmingham 18 per cent. of tuberculous animals or

1.0 per cent. of all animals have such lesions, while at Edinburgh, as indicated in the Table, pharyngeal infection occurs in 33.6 per cent. of tuberculous bullocks and 20.4 per cent. of tuberculous heifers, that is in 1.9 per cent. and 1.3 per cent. respectively of all animals. In addition, 7.2 to 8.2 per cent. of tuberculous animals or 0.4 to 0.5 per cent. of all animals in these groups have lesions confined to the head and lungs. Lesions confined to hepatic lymph glands occur in 2.3 to 4.1 per cent. of tuberculous heifers and bullocks, or 0.1 to 0.2 per cent. of all animals; while liver and lung lesions occur in 6 to 13.4 per cent. of tuberculous cases or 0.3 to 0.8 per cent. of all animals.

Simultaneous primary infections involving head, lungs, intestine and hepatic lymph gland, as well as secondary reinfection of these organs, make up the remaining 1.8 per cent. of all heifers and bullocks slaughtered. Abattoir returns are useless for assessing the significance of these multiple lesions.

TABLE II

Edinburgh — HEIFERS — Incidence 6.2% (3,000 Tuberculous Animals)

Organ Complex	No.	% T.B.	% of Total
Head	615	20.4	1.3
Lungs and lung glands	657	22.0	1.4
Head and lungs	246	8.2	0.5
Intestine	159	5.3	0.3
Intestine and head	66	2.2	0.1
Intestine, liver and head	32	1.0	0.1
Intestine and lungs	120	4.0	0.2
Head, intestine and lungs	150	5.0	0.3
Lungs and liver	401	13.4	0.8
Head, lungs and liver	57	1.9	0.1
Lungs, liver and intestine	201	6.7	0.4
Head, lungs, intestine and liver	234	7.8	0.5
Liver	69	2.3	0.1

TABLE III

Edinburgh — BULLOCKS — Incidence 5.7% (3,000 Tuberculous Animals)

Organ Complex	No.	% T.B.	% of Total
Head	1,008	33.6	1.9
Lungs and lung glands	789	26.3	1.5
Head and lungs	218	7.2	0.4
Intestine	160	5.3	0.3
Intestine and head	32	1.0	0.1
Intestine, liver and head	54	1.7	0.1
Intestine and lungs	80	2.7	0.2
Head, intestine and lungs	48	1.6	0.1
Lungs and liver	180	6.0	0.3
Head, lungs and liver	60	2.0	0.1
Lungs, liver and intestine	172	5.7	0.3
Head, lungs, intestine and liver	100	3.3	0.2
Liver	125	4.1	0.2

COWS

The incidence of tuberculosis in adult dairy cows killed at the abattoir is very high; in the period under investigation it was 43 per cent. and the incidence over a ten-year period shows little variation from this figure. Uncomplicated primary intestinal complexes account for only 3.7 per cent. of tuberculous cows or 1.6 per cent. of all cows. Intestinal lesions associated with pharyngeal lesions are also few, 0.9 per cent. of tuberculous cows or 0.4 per cent. of all cows killed having these lesions. Mesenteric gland lesions in association with pulmonary and pharyngeal tuberculosis are very common, 22.7 per cent. of tuberculous cows or 9.8 per cent. of all cows reaching the abattoir show such lesions. Uncomplicated pulmonary complexes occur in 36.8 per cent. of all tuberculous cows or in 15.8 per cent. of all abattoir cows. Associated pharyngeal and pulmonary tuberculosis occurs in 9.4 per cent. of tuberculous cattle, 4 per cent. of all cows. Uncomplicated pharyngeal infection occurs in 9.2 per cent. of tuberculous cows or 4 per cent. of all cows. Primary hepatic lesions occur in 0.5 per cent. of tuberculous cows or 0.2 per cent. of all cows killed. The remaining 17 per cent. of tuberculous cows show complex lesions which cannot be assessed from abattoir returns, that is 7 per cent. of all cows killed show such lesions.

TABLE IV

Edinburgh — COWS — Incidence 43% (3,000 Tuberculous Animals)

Organ Complex	No.	% T.B.	% of Total
Head	277	9.2	4.0
Lungs and lung glands	1,103	36.8	15.8
Head and lungs	283	9.4	4.0
Intestine	110	3.7	1.6
Intestine and head	27	0.9	0.4
Intestine, liver and head	20	0.6	0.3
Intestine and lungs	384	12.8	5.5
Head, intestine and lungs	297	9.9	4.3
Lungs and liver	76	2.6	1.1
Head, lungs and liver	29	1.0	0.4
Lungs, liver and intestine	113	3.8	1.6
Head, lungs, intestine and liver	265	8.8	3.8
Liver	16	0.5	0.2

BULLS

The incidence of tuberculosis in bulls, by the time these animals reach the abattoir, is 27 per cent.; this varies little from year to year. Uncomplicated intestinal tuberculosis in the bull occurs in 3.2 per cent. of tuberculous bulls or 0.9 per cent. of all bulls reaching the abattoir, while associated pharyngeal and intestinal tuberculosis occurs in 1.1 per cent. of tuberculous bulls or 0.3 per cent. of all bulls. Mesenteric gland lesions, in association with pulmonary or pulmonary and pharyngeal lesions, occur in 5.1 per cent. of tuberculous bulls or 1.4 per cent. of all bulls. Uncomplicated pulmonary tuberculosis occurs in 25.6 per cent. of tuberculous bulls or 6.9 per cent. of all bulls reaching the abattoir. Uncomplicated primary pharyngeal lesions occur in 37 per cent. of tuberculous bulls or 10 per cent. of all bulls. Primary hepatic lesions occur in 1.1 per cent. of tuberculous bulls or 0.3 per cent. of all bulls. Complex lesions occurring in lung, head, intestine and liver, occur in 16.2 per cent. of tuberculous bulls or 4 per cent. of all bulls.

TABLE V

Edinburgh — BULLS — Incidence 27% (1,000 Tuberculous Animals x 3)

Organ Complex	No.	% T.B.	% of Total
Head	1,113	37	10
Lungs and lung glands	768	25.6	6.9
Head and lungs	312	10.4	2.8
Intestine	96	3.2	0.9
Intestine and head	33	1.1	0.3
Intestine, liver and head	30	1.0	0.3
Intestine and lungs	75	2.5	0.7
Head, intestine and lungs	78	2.6	0.7
Lungs and liver	120	4.0	1.1
Head, lungs and liver	60	2.0	0.5
Lungs, liver and intestine	141	4.7	1.3
Head, lungs, intestine and liver	135	4.5	1.2
Liver	33	1.1	0.3

FINDINGS

In both sexes the incidence of tuberculosis is very low in calfhood; it rises to some extent in young cattle but it is not until the animal becomes mature that the incidence is so very high. The widely held belief that the common route of infection in the calf is by the intestine is not borne out by the morbid anatomical findings. The infrequency of the intestinal complex has also been shown by Schulz (1937). Thoracic gland tuberculosis is common in young calves, which suggests that infection is commonly by the respiratory tract. Hepatic lesions, either alone or in conjunction with thoracic lymph gland lesions, are also commonly found in young calves and these lesions are probably an indication of congenital infection. It can be seen from Table I that thoracic lymph gland lesions occur frequently in combination with hepatic lesions and also, but less frequently, in combination with mesenteric gland lesions; this, however, must not be taken to confirm Behring's hypothesis, since in the above instances lesions first occur in the abdominal cavity. It would appear from all the evidence that intestinal infection plays only a minor part in young calves and that congenital and pulmonary infections are the most important.

It could be argued that calves killed at the abattoir are too immature to be a representative sample since older calves are not usually killed, but

if there is any marked difference in the route of infection in older calves, then this should be easily identifiable in the post-mortem records of tuberculous heifers and bullocks. Since the incidence of tuberculosis in heifers and bullocks is about 6 per cent. it is in fact evident that a proportion of bovines must become infected between early calfhood and young adult life, although the majority of these tuberculous animals have probably not been closely housed along with milking herds. The rise in incidence of the disease is due, in part, to an increase in retropharyngeal lymph gland lesions indicating that, in heifers and bullocks, the pharynx is a common site of entry of tubercle bacilli. This increase in incidence of retropharyngeal lymph gland tuberculosis is not, however, associated with any increase in the number of mesenteric lymph gland lesions. If then retropharyngeal lymph gland involvement is indicative of ingestion, as is by no means certain since these glands drain both respiratory and alimentary regions, then simultaneous involvement of the intestine or mesenteric lymph glands with concomitant lesions does not occur. Respiratory infection of the heifer and bullock, as indicated by the primary pulmonary complex, is also a common route of infection. In the bullock and heifer, with an increase in incidence of tuberculosis of approximately 4 percent. over that in the calf, it is clear that about two-thirds of this increase is

due to uncomplicated primary infections of pharynx and/or lung. Primary intestinal infection is infrequent.

The incidence of tuberculosis in the abattoir cow is very much higher than in the heifer and this is due in part to a very distinct rise in the incidence of uncomplicated pulmonary tuberculosis (i.e., heifers 1.4 per cent. and cows 15.8 per cent. of all animals killed), while the incidence of pharyngeal tuberculosis is also higher. Primary intestinal infection plays no important part in the increased incidence of tuberculosis in the cow, although the cow, differing from the younger animal, frequently has mesenteric lymph gland lesions secondary to pulmonary tuberculosis; thus 40.4 per cent. of tuberculous cows, or 17.4 per cent. of all cows reaching the abattoir, have tuberculous mesenteric lymph nodes. Personal observation at a large number of post-mortem examinations of tuberculous cows has convinced me that the majority of mesenteric gland lesions are not due to primary intestinal infection but are due to swallowing of tuberculous debris from progressive and open lung lesions. Hepatic lymph gland tuberculosis is also frequently observed along with these mesenteric gland lesions, which would appear to indicate that the hepatic lymph gland becomes infected from the intestinal tract.

The incidence of tuberculosis in the bull is

lower than in the cow but is very appreciably higher than in bullocks or heifers. The common sites of entry are the pharynx and the lung, while intestinal complexes are infrequent. The bull differs from the cow in that extension of the disease from the lungs to the intestinal tract is not so frequent; so that mesenteric gland tuberculosis, a common feature of the older cow, is not seen so frequently in the bull.

In all groups, the incidence of lesions confined to the hepatic lymph gland is relatively constant (0.1 to 0.3 per cent. of animals killed) and, since congenital tuberculosis is not necessarily lethal to the calf, it is possible that these isolated hepatic lymph gland cases of older animals are in fact cases of surviving congenitally infected animals. Such cases recognised on post-mortem examination have been described by Gofton (1937) and McKay (1943).

DISSEMINATION OF TUBERCLE BACILLI AFTER PRIMARY INFECTION

REVIEW OF PRESENT KNOWLEDGE

It has for long been stated in veterinary literature that tubercle bacilli only disseminate along preformed lymphatic channels and the explanation for the situation of naturally occurring lesions has depended entirely upon the presence of known and unknown lymphatic pathways, spread being in no way embarrassed by the direction of the lymph flow, even in perfectly healthy regions. An accurate knowledge

of the dissemination of bovine tuberculosis is essential for those who in the capacity of meat inspectors have to judge the carcases of slaughtered tuberculous animals. The idea that lymphatic extension is the only important method of dissemination in the bovine is incorrect.

ORIGINAL OBSERVATIONS

A focus of tuberculosis, once established at a primary site of entry, may progress by direct infiltration of surrounding tissues and this is seen frequently around the primary lung lesions of the bovine. Dissemination may also occur by lymphatic channels and examples of this are numerous, e.g., the initial lung focus reaching the related hilar glands, intestinal infection reaching mesenteric and hepatic glands and progressing from these to the caudal mediastinal and other thoracic glands, etc. Tuberculous lung lesions in the bovine may disseminate extensively by the bronchial passages to give the characteristic and very frequently occurring bronchopneumonic lung lesions from which infection passes to the trachea, pharynx and intestine, and thence to the peritoneum. Dissemination by natural passages, other than bronchi, also occurs; for example, infection may pass from peritoneum to uterus, by the Fallopian tube; from kidney to bladder by the ureters, and throughout the udder by the milk ducts. In all these examples, however, the dissemination is "local". In the bovine the lesions which occur in

haematogenous dissemination may be few in number and benign in type, but on the other hand haematogenous dissemination may cause widespread miliary tuberculosis with severe illness. All stages between discrete and isolated nodules and acute miliary tuberculosis may be seen.

A survey of abattoir figures, with special reference to haematogenous dissemination of lesions in the bovine, has been carried out. It is considered that meat inspection methods, at some centres, have overlooked the possibility of this type of dissemination. At many abattoirs lesions confined to lungs, head or gut, are not taken as necessitating general carcase examination. The Public Health (Meat) Regulations (Scotland) 1932, the only statutory rules for meat inspection in Great Britain, also appear to minimise the importance of this method of spread, in that if lesions are confined, on first examination, to the organ of entrance, i.e., lung, head or intestine, then only the regional glands must be examined, so inferring that lymphogenous spread alone is of importance.

Abattoir records were available for examination from two large abattoirs in this country, one (Birmingham) carrying out comprehensive carcase lymph gland examination in nearly all cases of tuberculosis, the other (Edinburgh) doing so only when lesions are present on the pleura and/or peritoneum or in the liver and/or hepatic lymph gland. It was

considered that these records might give an indication of the frequency of haematogenous dissemination and, at the same time, by comparing the two one might indicate the frequency with which carcase lymph gland lesions are overlooked in the less complete systems of meat inspection. In addition, if the results were so tabulated that the portals of entry were also indicated, it would be possible to determine from which organs dissemination most frequently occurs. For this purpose, only those carcase lymph gland lesions which obviously did not arise by direct lymphatic drainage from a primary site of entry, were taken as a true indication of haematogenous dissemination, i.e., lesions in popliteal, prescapular, precrural and inguinal lymph glands; in addition, lesions of bone and central nervous system were also taken as an indication. It is known that kidney, udder, uterus, etc., undoubtedly can be infected from the blood stream, but since the route of infection of such organs is not constant, lesions in these organs could not be used as evidence of blood spread. No true assessment of the frequency of haematogenous dissemination to lymph glands can be made from the records of advanced pleural and peritoneal cases of tuberculosis since at neither abattoir is carcase lymph gland examination carried out in these cases, as the carcase is totally condemned without further examination. The numbers of cases in which haematogenous dissemination can be

definitely said to have occurred are therefore minimal.

Table VI shows the frequency of haematogenous dissemination as indicated by involvement of peripheral carcase lymph glands along with the relative frequency of this type of dissemination from the various portals of entry of the disease. This can obviously only be assessed from the returns of the Birmingham abattoir where complete carcase examination is carried out in the majority of cases of tuberculosis. (The returns of this abattoir are given second under each heading in Table VI and should be compared with the first figures which are obtained from the returns of the Edinburgh abattoir where, as described above, carcase lymph gland examination is less complete.) In each case the percentage of tuberculous animals showing haematogenous dissemination is followed by the percentage of all animals killed showing such lesions. In the case of the calves only one set of figures is given since carcase lymph gland examination at both abattoirs is complete.

TABLE VI

Calves

Organ Complex	No. of T.B. cases	No. of haem.	% haem. of T.B. Animals	% haem. of total
Head	4	Nil	Nil	Nil
Lungs and lung glands	90	1	1.1	Neg.
Head and lungs	1	Nil	Nil	Nil
Intestine	1	Nil	Nil	Nil
Intestine and head	1	Nil	Nil	Nil
Intestine, liver and head	0	Nil	Nil	Nil
Intestine and lungs	5	2	40	0.02
Head, intestine and lungs	2	Nil	Nil	Nil
Lungs and liver	39	21	53	0.2
Head, lungs and liver	8	7	87	0.07
Lungs, liver and intestine	17	10	59	0.1
Head, lungs, intestine and liver	5	5	100	0.05
Liver	15	Nil	Nil	Nil
Liver and head	Nil	Nil	Nil	Nil

TABLE VI (contd.)

Heifers

Organ Complex	No. of T.B. cases	No. of haem.	% haem. of T.B. Animals	% haem. of total
Head	615	Nil	Nil	Nil
	551	7	1.3	0.2
Lungs and lung glands	657	3	0.5	0.1
	1,072	44	4.1	1.5
Head and lungs	246	3	1.2	0.1
	273	18	6.6	0.6
Intestine	159	Nil	Nil	Nil
	80	2	0.3	Neg.
Intestine and head	66	Nil	Nil	Nil
	35	1	2.8	Neg.
Intestine, liver and head	32	6	18.8	0.2
	69	2	2.8	Neg.
Intestine and lungs	120	3	2.5	0.1
	104	3	2.9	0.1
Head, intestine and lungs	150	9	6.0	0.3
	42	1	2.4	Neg.
Lungs and liver	401	21	5.2	0.7
	194	11	5.6	0.4
Head, lungs and liver	57	15	26.3	0.5
	91	7	7.8	0.2
Lungs, liver and intestine	201	24	12.0	0.8
	215	30	14.0	1.0
Head, lungs, intestine and liver	234	42	18.0	1.4
	243	31	13.0	1.0
Liver	60	Nil	Nil	Nil
Liver and head	9	Nil	Nil	Nil

TABLE VI (contd.)

Bullocks

Organ Complex	No. of T.B. cases	No. of haem.	% haem. of T.B. Animals	% haem. of total
Head	1,008	Nil	Nil	Nil
	541	11	2	0.4
Lungs and lung glands	789	1	Neg.	Nil
	1,025	65	6.3	2.2
Head and lungs	218	1	Neg.	Neg.
	329	30	9	1.0
Intestine	160	Nil	Nil	Nil
	78	1	1.3	Neg.
Intestine and head	32	Nil	Nil	Nil
	57	1	1.8	Neg.
Intestine, liver and head	54	Nil	Nil	Nil
	63	Nil	Nil	Nil
Intestine and lungs	80	1	1.2	Neg.
	99	4	4	0.1
Head, intestine and lungs	48	2	4	Neg.
	69	Nil	Nil	Nil
Lungs and liver	180	14	8	0.5
	207	19	9.1	0.6
Head, lungs and liver	60	14	23.3	0.5
	85	11	13.7	0.3
Lungs, liver and intestine	172	21	12	0.7
	213	25	11.7	0.8
Head, lungs, intestine and liver	100	17	17	0.6
	206	49	24	1.6
Liver	99	Nil	Nil	Nil
Liver and head	26	Nil	Nil	Nil

TABLE VI (contd.)

Cows

Organ Complex	No. of T.B. cases	No. of haem.	% haem. of T.B. Animals	% haem. of total
Head	277	Nil	Nil	Nil
	151	3	2	0.1
Lungs and lung glands	1,103	3	0.3	0.1
	1,139	49	4.3	1.6
Head and lungs	283	1	Neg.	Neg.
	228	13	5.7	0.4
Intestine	110	Nil	Nil	Nil
Intestine and head	27	1	3.7	Neg.
	27	1	3.7	Neg.
Intestine, liver and head	20	1	5	Neg.
	20	Nil	Nil	Nil
Intestine and lungs	16	1	6	Neg.
	384	2	Neg.	Neg.
Head, intestine and lungs	160	6	4	0.2
	297	5	1.7	0.2
Lungs and liver	83	5	6.0	0.2
	76	1	1.3	Neg.
Head, lungs and liver	222	7	3.1	0.2
	29	1	3.5	Neg.
Lungs, liver and intestine	171	13	8.0	0.4
	113	2	1.8	Neg.
Head, lungs, intestine and liver	252	16	6.4	0.5
	265	21	8	0.7
Liver	463	40	8.7	1.3
	16	Nil	Nil	Nil
Liver and head	5	Nil	Nil	Nil

TABLE VI (contd.)

Bulls

Organ Complex	No. of T.B. cases	No. of haem.	% haem. of T.B. Animals	% haem. of total
Head	1,113	3	Neg.	0.1
	288	9	3	0.3
Lungs and lung glands	768	3	Neg.	0.1
	1,275	87	6.9	2.9
Head and lungs	312	3	1	0.1
	408	51	13	1.7
Intestine	96	Nil	Nil	Nil
Intestine and head	27	Nil	Nil	Nil
	33	Nil	Nil	Nil
Intestine, liver and head	33	3	9	0.1
	30	Nil	Nil	Nil
Intestine and lungs	45	3	6	0.1
	75	3	4	0.1
Head, intestine and lungs	198	9	4.5	0.3
	78	3	4	0.1
Lungs and liver	111	3	2.8	0.1
	120	3	2.5	0.1
Head, lungs and liver	132	18	13.6	0.6
	60	9	15.0	0.3
Lungs, liver and intestine	72	12	16.6	0.4
	141	18	12	0.6
Head, lungs, intestine and liver	141	9	6	0.3
	135	12	9	0.4
Liver	261	39	15	1.3
	36	Nil	Nil	Nil
Liver and head	Nil	Nil	Nil	Nil

Neg.-- Negligible percentage

FINDINGS

Uncomplicated retropharyngeal and mesenteric lymph gland lesions, in all groups, are only occasionally associated with lesions in carcase lymph glands but lung and lung gland lesions with, or without, concomitant retropharyngeal lesions quite frequently show this association. Thus when examination is complete 4.1 per cent. of heifers, 6.3 per cent. of bullocks, 4.2 per cent. of cows and 6.9 per cent. of bulls with lung gland involvement show lesions also in carcase lymph glands, which lesions are missed by the incomplete examination. Similarly, 6.6 per cent. of heifers, 9 per cent. of bullocks, 5.7 per cent. of cows and 13 per cent. of bulls, with head and lung lymph gland lesions have lesions also in carcase lymph glands which are not detected on incomplete examination. In the numerous cases where both lung and liver are involved carcase lymph gland lesions are frequent, but since the liver is involved carcase lymph gland examination is complete under both systems of meat inspection. From the above it appears quite evident that tuberculous lesions of the lung and its regional lymph glands are of great significance in meat inspection, since these lesions are frequently associated with haematogenous dissemination.

GENERAL CONCLUSIONS

An analysis of extensive abattoir returns of

tuberculous cattle has been made and for this purpose the route of infection is taken as being indicated by the presence of a primary complex. From the figures obtained and from personal observation, it is suggested that the pathogenesis of tuberculosis in cattle may be as follows.

In calves the incidence of the disease is very low, infection arising chiefly in two ways, congenitally or by inhalation after birth. Haematogenous dissemination is frequently encountered in the calf following primary infection. The incidence figures for heifers, bullocks, bulls and cows show with certainty that tuberculosis of the adult is not merely a slow progression of lesions acquired in calfhood. It is obvious that the incidence increases quickly after the animal has attained maturity, so that while in heifers and bullocks the incidence is 6 per cent., in bulls it is 27 per cent. and in cows 43 per cent. In the older animals the figures are obviously not representative of the cows and bulls on our farms, where undoubtedly the incidence is lower, but it is clear that 43 per cent. of our cows do become infected during their lifetime. In these cases the primary complex is predominatingly pulmonary in position. Post-primary haematogenous dissemination giving rise to carcase lymph gland lesions also occurs in these older animals, although not so frequently as in the calf. In addition to these primary lung infections there are also in the

adult bovine a considerable number of cases where lymph gland changes are only seen in the retro-pharyngeal lymph glands. These glands drain both respiratory and alimentary areas so that it is uncertain by which of these routes infection enters, although the fact that retropharyngeal gland lesions are much more frequently associated with lung and lung gland lesions than with mesenteric gland lesions might indicate that the majority of these lesions are due to inhalation infection.

Tuberculosis of the liver and/or hepatic lymph glands are also frequently occurring lesions but the route of infection is complex: (1) Directly from the umbilical vein in congenital tuberculosis; (2) From primary intestinal tuberculosis; (3) From early or late post-primary haematogenous dissemination; (4) From secondary infection of the bowel from swallowed tuberculous sputum. This variety of routes of involvement of the liver and hepatic glands makes these lesions unreliable for the evaluation of the pathogenesis of bovine tuberculosis.

SUMMARY

(1) The portals of entry of the tubercle bacillus in tuberculosis are reviewed.

(2) The abattoir returns for 189 tuberculous calves, 3,000 tuberculous heifers, 3,000 tuberculous bullocks, 1,000 tuberculous bulls and 3,000 tuberculous cows have been abstracted, grouped and analysed for the

first time in order to assess the frequency of the various routes of infection.

(3) The dissemination of the disease in the bovine body is described, the importance of haematogenous dissemination being shown.

THE DISTRIBUTION OF THE BRONCHIAL TREE IN THE BOVINE LUNG

J. T. STAMP

(Jn. Comp. Path. & Ther. 1948,
Vol. 58, No. 1)

INTRODUCTION

In this section it is proposed to give an account of certain aspects of the anatomy of the bronchial system of the normal bovine lung. The distribution of the bronchial tree is neglected in anatomical text books and it is possible that it is of importance in the explanation of the localisation and subsequent distribution of tuberculous lesions.

EMBYROLOGY AND COMPARATIVE ANATOMY OF THE LUNG

In mammals the lungs arise as a diverticulum from the ventral aspect of the pharynx. This lung bud bifurcates and the respiratory organs are then represented by a tubular trachea and two lung buds or primary bronchi. In the primitive animals this may be the end of development, when the respiratory system consists of a trachea and two bronchi, each with a simple pulmonary sac. In higher forms, the thorax becomes larger and lung buds grow out in regular series from the two main bronchi. The reptilian lung is still more complex but the lung is nevertheless entirely hyparterial, that is to say, all the bronchi arise from the trachea below the point at which it is crossed by the pulmonary artery. In about 95 per cent. of mammals, however, the right

lung differs from the left, in that an eparterial bronchus is present which supplies the apical portion of the right lung. This bronchus arises from the main bronchus (90 per cent. of living mammals) or from the trachea, cranial to the point at which the pulmonary artery crosses the trachea. The reasons for this asymmetry are not very clear, but it is recognised that in the left lung, the ascending branch of the first hyparterial bronchus develops more fully to supply a portion of the lung similar to, but smaller than, that served by the eparterial bronchus on the right. It is suggested that the apical portion of the left lung is smaller than the right because it has a lessened opportunity for pulmonary expansion on the left side, where the thoracic volume is smaller than on the right side, in consequence of the rotation of heart and oesophagus in opposite directions. Thus in the mammal an increased respiratory area is obtained chiefly by an increase in size of the right lung. This new respiratory area is at first supplied by the cranial branch of the first right hyparterial bronchus, which enlarges until it becomes as large as the parent bronchus. It is now only a small step in evolution for the area to be supplied by its own eparterial bronchus.

METHODS OF EXAMINATION OF THE MAIN BRONCHI AND THEIR PULMONARY SEGMENTS

The anatomy of the bronchial tree may be investigated by dissection, by making corrosion casts, by the study of bronchograms, and by the injection of air into bronchi to ascertain their distribution. Most of the results in this article have been obtained by dissection methods while doing detailed pathological examinations of tuberculous lungs, but full use has been made of the injection of air method in normal lungs. In the early stages the lungs were fixed in formalin and blunt dissection was carried out; in the bovine lung it is comparatively simple to dissect out even small lung lobules by this method since the interlobular connective tissue is so plentiful. The results so obtained have been confimed and amplified further by reconstruction of the bronchial tree from multiple slicing of fixed lungs, using a large ham slicing machine. In all, 60 lungs have been examined.

BRONCHIAL TREE OF THE BOVINE LUNG

The larger subdivisions of the bronchial tree are relatively constant and it is the main lobar bronchi along with their primary branches which are described below.

The trachea gives rise to two primary hyparterial bronchi which extend down to the most caudal part of each diaphragmatic lobe of lung. Along their length, the primary bronchi give rise to

numerous branches which, with the exception of the first branch on either side, supply the diaphragmatic lobes. On the right side the trachea has the extra eparterial bronchus. The terms primary, secondary, tertiary, etc., as applied to bronchi by some authors, tend to be misleading, since the main bronchus to each diaphragmatic lobe and to the right apical lobe is part of the primary stem and should, therefore, be called a primary bronchus. The main bronchi to the other lobes (left apical, left cardiac, right cardiac and intermediate) however, although analogous to the main diaphragmatic and right apical bronchi, are in fact branches of the primary bronchi and must of necessity be called secondary and their branches tertiary. For this reason these terms will not be used in this communication; the bronchi will be named according to the portion of lung supplied.

Glass (1934) gives the conception of the "broncho-pulmonary segment" defined as the area of lung tissue supplied by a given bronchus. This, however, is an arbitrary conception and the size of any segment depends upon the size of the bronchus which supplies it; thus a whole lobe may be regarded as a broncho-pulmonary segment supplied by a lobar bronchus, while at the other extreme, a terminal lobule may be looked upon as a broncho-pulmonary segment supplied by a terminal bronchiole. When the term broncho-pulmonary segment is used in

this article it denotes a portion of lung supplied by one of the lobar bronchi to be described. In the description and diagrams, the broncho-pulmonary segments of each lobe will be lettered in alphabetical order according to the order of origin from the main lobe bronchus of their associated bronchi. These bronchi will be nominated with the same letter as that given to their pulmonary segments. The diagrams show the surface markings of these broncho-pulmonary segments from two viewpoints, so that every surface can be seen.

ANATOMICAL DESCRIPTION

Right apical lobe. (Figs. 1 and 2.) The eparterial bronchus arises from the right lateral surface of the trachea about 10 cm. from its bifurcation. It is about 1.5 cm. in length and then bifurcates into a cranial branch supplying the cranial division of the apical lobe, and a caudal branch supplying the caudal division of the apical lobe.

Cranial Division of the eparterial bronchus to the right apical lobe. This branch (A) is directed ventrally and slightly cranially; it runs down the caudal border of the lobe to its rather bulbous tip which lies ventrally upon the sternum. It has two main and well marked cranial branches. The first (B) is given off near the origin of the stem bronchus and passes cranially and slightly ventrally to supply the dorsal part of the lobe. The second and more

distal branch (C) is only slightly smaller than the main bronchus and it also passes ventrally and slightly cranially. Finally, a third and much smaller medial branch (D) supplies the medial portion of the bulbous tip of the lobe. In this way, the anterior lobe is divided into four main broncho-pulmonary segments, three (B, C and A) being demarcated on both the lateral and medial surfaces of the lobe, while the segment (D) is present only on the medial surface.

Caudal Division of the eparterial bronchus to the right apical lobe. This bronchus (A) runs in a ventral and slightly caudal direction to the most ventral tip of the lobe. At the hilum of the lobe, this bronchus gives off a large branch (B) which runs in a caudal direction to supply the most dorsal part of the caudal portion of the right apical lobe. More distally, second and third branches arise, the more proximal (C) of the two running in a cranial and ventral direction, while the other (D) passes in a caudal and ventral direction. The lobe is composed of four broncho-pulmonary segments (A, B, C and D), all being demarcated on both the lateral and medial surfaces of the lobe.

Right cardiac lobe. (Figs. 1 and 2.) This lobe is triangular in cross section, with one surface facing laterally and two surfaces meeting at a peak medially. The lobe is supplied by the first branch of the right main stem bronchus and arises from the

lateral surface of this bronchus soon after its bifurcation from the trachea. The bronchus (A) runs in a ventral direction to the tip of the lobe, at first medial and superficial but gradually becoming deep in the lung substance. The branches of this right cardiac bronchus are numerous. The first (B) is very short and quickly bifurcates into two, one running cranially and ventrally and the other running caudally and ventrally. The second (C) runs in a ventral and medial direction to supply the proximal portion of the medial peak of the lobe. The third (D) almost immediately divides into two, in a similar manner to the first, although the two branches may arise separately from the main lobe bronchus. The fourth (E) is similar to the second and runs in a ventral and medial direction to supply the distal portion of the medial peak of the lobe. The last four branches (F, G, H and I) all arise separately and alternately run caudally and ventrally and cranially and ventrally. The lobe in this way is divided into numerous broncho-pulmonary segments, all of which, except the two medial peak segments (C and E) can be demarcated on both lateral and medial surfaces of the lobe.

Right diaphragmatic lobe. (Figs. 1 and 2.) This lobe is by far the largest lobe in the bovine lung. It can be represented as being a triangular pyramid with three borders, three surfaces and a base. The mediastinal surface, bounded by the costo-

vertebral and mediastinal borders, has an elongated triangular shape, its base being cranial and its apex caudal. This surface closely follows the curve of the diaphragm in a caudal and dorsal direction, so that the apex of the surface becomes confluent with the apex of the other two surfaces to form a peak which fits into the angle where diaphragm, vertebral column and ribs meet, at about the eleventh thoracic vertebra. The diaphragmatic surface, bounded by the mediastinal and costo-phrenic borders, is closely moulded to the curve of the diaphragm so that it runs in a caudal and dorsal direction to help form the lobe peak in the vertebral costal diaphragmatic angle, while laterally the surface is curved concavely and runs in a ventro-lateral direction. The costal surface, bounded by the costo-vertebral and costo-phrenic borders, due to the influence of the diaphragm on the shape of the thoracic cavity, is roughly triangular. The apex of the triangle is dorsal and caudal and forms part of the lobe peak. The base of the lobe pyramid is small and is intimately attached to the apical lobes. It can be seen, therefore, that the mass of lung substance is towards the mediastinal surface and since this surface is also triangular with its apex caudal, it follows that the greater portion of lung substance in this lobe is medial and cranial.

The bronchus to this lobe is the direct continuation of the right bifurcation of the trachea.

It enters the lung substance at the hilum and passes directly to the lung peak by running in a caudal and dorsal direction parallel, but lateral, to the mediastinal surface; it runs through the thickest portion of the lobe substance. This bronchus can be termed the right caudal bronchus (A). From this bronchus, numerous branch bronchi arise which gradually decrease in size as the apex of the lobe is reached. In addition to this, the origins of these branches become more closely associated towards the apex so that a given length of caudal bronchus gives origin to more numerous but smaller bronchi caudally, than it does cranially. The general plan of the distribution of these branch bronchi is simple, in that the thick medial portion of the lobe is supplied by a series of relatively short bronchi, five of which pass dorsally, and six to eight ventrally. The numbers vary slightly due to the fact that the caudal ventral branches occasionally arise from common trunks, instead of independently. The remaining lateral portion of lobe substance is supplied by longer and larger lateral branches.

The demarcation of the broncho-pulmonary segments supplied by these various branch bronchi is also relatively simple. The corresponding segments of lung associated with the dorsal bronchi (B, E, G, J and N) are demarcated by bands passing from the ventral border of the mediastinal surface over this surface and then over the dorsal border on to the

costal surface of the lobe. The segments of lung supplied by the ventral bronchi (D, I, L, M, O, R and P) can be seen only on the diaphragmatic surface of the lobe and are directly associated with the dorsal broncho-pulmonary segments at the ventral border of the mediastinal surface. The broncho-pulmonary segments of the lateral branch bronchi (C, F, H, K and Q) occupy the remaining portion of lung surface and are demarcated on both the costal and diaphragmatic surfaces. A brief account of the course of these various branches follows but at the apex end of the lobe, as already mentioned, the origin of the various branches are closely related and are not constant, so that slight variations from the positions indicated in the diagrams will occur.

The first dorsal branch (B) is large and quickly divides into two parts, one of which passes caudally and the other cranially. A smaller branch is given off immediately after the origin of the bronchus and passes in a ventral direction. The 2nd, 3rd, 4th and 5th (E, G, J and N) dorsal branches are all similar, and pass in a dorsal caudal direction, the direction becoming increasingly more caudal and less dorsal as the lung apex is reached. The first three ventral branches (D, I and L) of the caudal bronchus almost immediately after their origin divide into two, one branch passing to supply the cranial part of its segment, while the other runs caudally to supply the rest of the segment. The other ventral branches

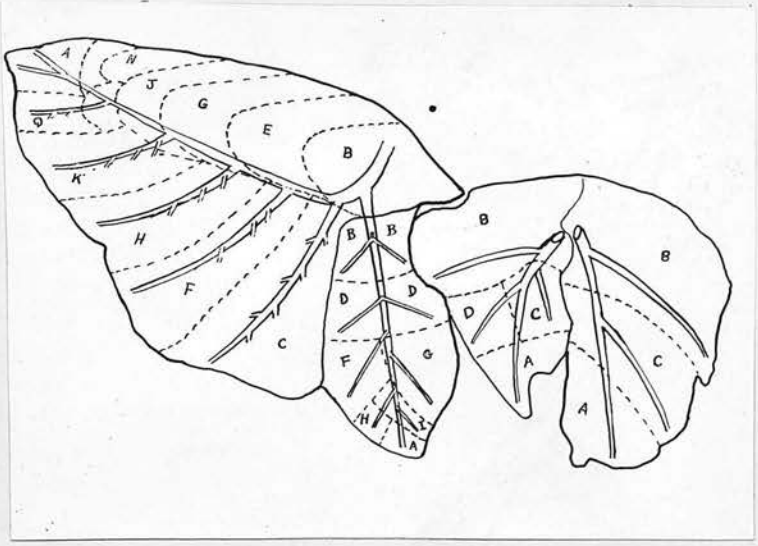
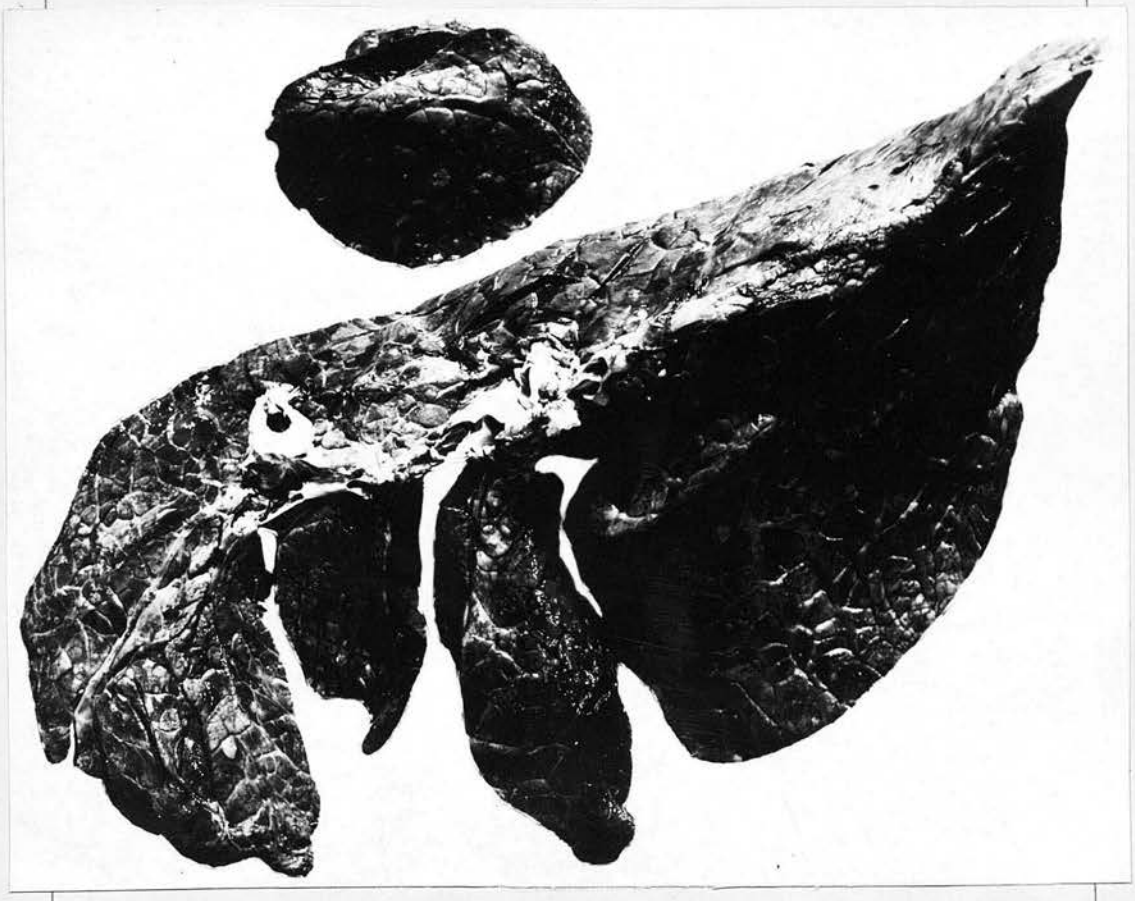


Fig.1. - Distribution of the bronchial tree in right lung. (Dorsal)



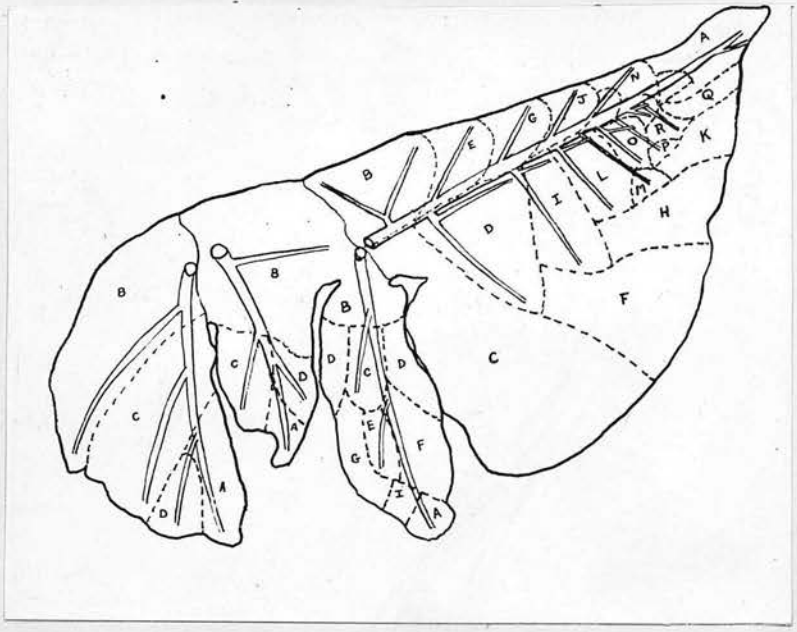


Fig.2. - Distribution of the bronchial tree in right lung (Ventral)

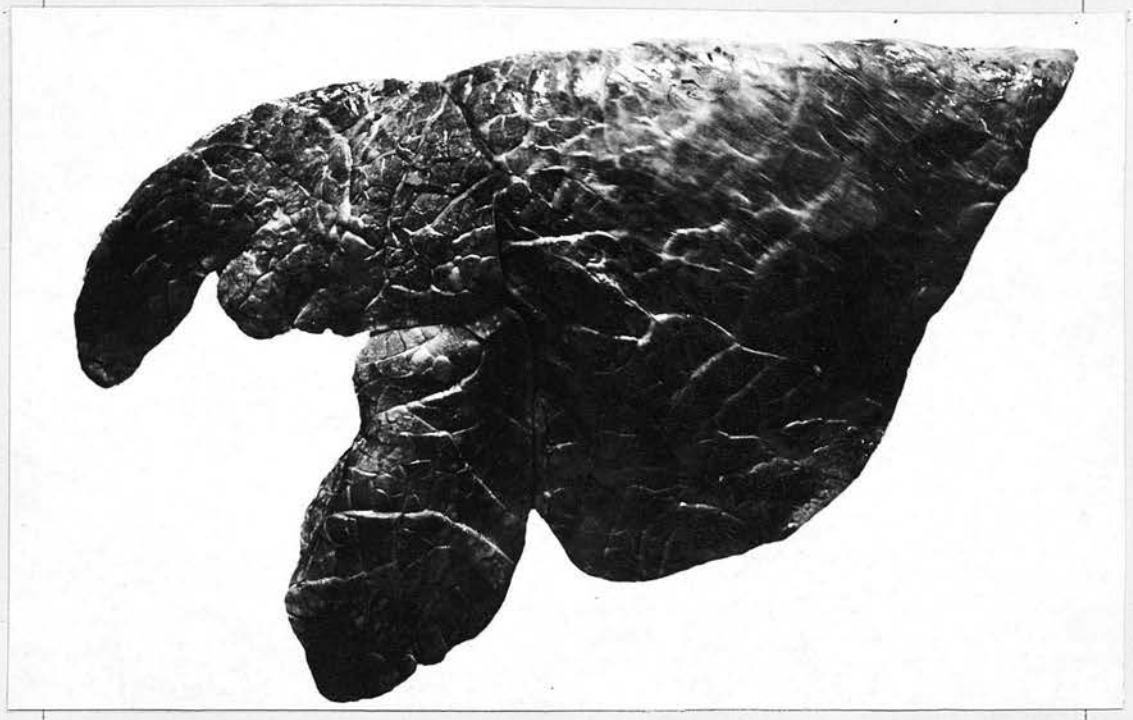
(M, O, P and R) are inconstant; they may be similar to the above, or the lateral and caudal subdivisions may arise separately. The first lateral bronchus (C) is long and terminates at the costo-phrenic angle supplying the most ventral part of the whole lung. It runs along the caudal border of its lung segment and gives off several branches which run cranially and ventrally to supply the cranial portion of the lung segment. The 2nd, 3rd, 4th and 5th (F, H, K and Q) branches are very similar, except that they become decreasingly shorter and at the same time begin to run more caudally and less ventrally; all, however, terminate in the costo-phrenic border. The 5th lateral (Q) branch is very short and along with the terminal bifurcation of the caudal bronchus supplies the lobe peak in the costo-diaphragmatic vertebral angle.

Intermediate lobe. The bronchus to this lobe is the second branch of the right caudal bronchus and runs to the tip of the lobe. A short distance from its origin cranial and caudal branches arise which supply the cranial and caudal parts of the lobe respectively.

Left apical lobe. (Figs. 3 and 4.) This lobe, unlike the right apical lobe, is undivided and is supplied by the cranial division of the first branch of the left bronchus. This first branch originates just caudal to the bifurcation of the trachea and immediately before the left caudal bronchus enters

the diaphragmatic lobe. The left apical lobe bronchus (A) passes in a cranial and slightly ventral direction to the apex of the lobe. The first two branches of this lobe bronchus are small; one (B) runs caudally and the other (C) dorsally to supply the base of the lobe. Immediately distal to this first branch, the lobe bronchus gives origin to its two main branches. These two branches arise together, one (D) passing cranially and dorsally and the other (E) cranially and ventrally. In this way, the lobe is divided into five broncho-pulmonary segments, dorsal (D), middle (A), ventral (E), and two caudal (B and C), in position. The three main pulmonary segments (A, D and E) are demarcated on both the lateral and medial surfaces of the lobe but the caudal segments are demarcated, one (B) on the lateral and one (C) on the medial surface.

Left cardiac lobe. (Figs. 3 and 4.) The bronchus to this lobe arises from the common trunk which also gives rise to the left apical bronchus already described. It (A) passes ventrally to the tip of the cardiac lobe as the main stem bronchus. The left cardiac lobe is divided into horizontal segments, five in number (C, E and F, H and I, K and L, M and N), which are supplied by pairs of small cranial and caudal branches, each two originating together from the main stem bronchus. These segments are demarcated on the lateral and medial surfaces of the lobe. In addition on the medial



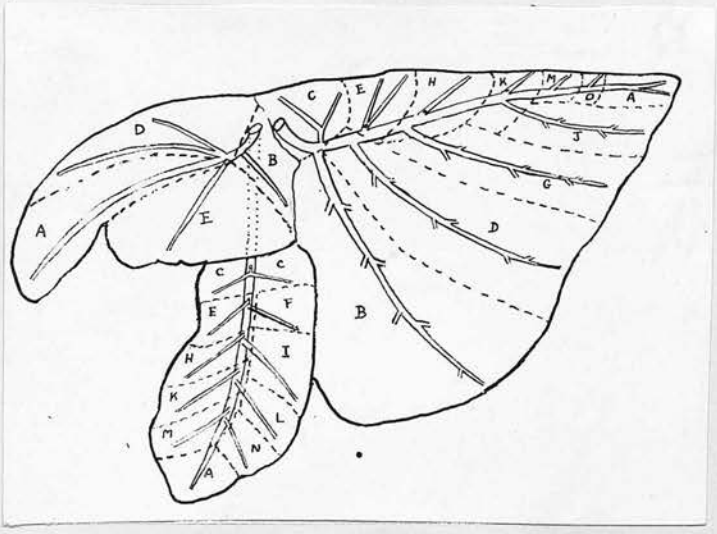


Fig.3. - Distribution of the bronchial tree in left lung. (Dorsal)

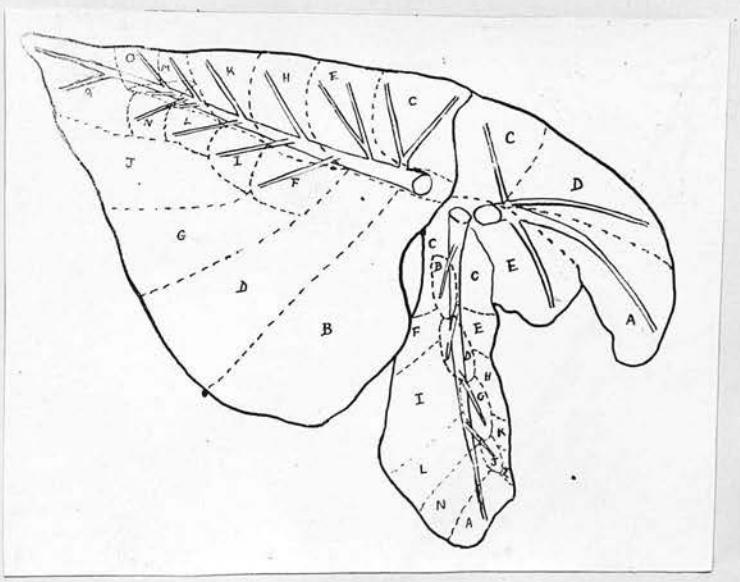


Fig.4. - Distribution of the bronchial tree in left lung. (Ventral)

surface of the lobe are four segments (B, D, G and J) which make up the peak of the lobe, since the latter is triangular in cross section with a medial apex. These segments are supplied by small medial branches of the lobe bronchus.

Left diaphragmatic lobe. (Figs. 3 and 4.) This lobe has a similar basic broncho-pulmonary structure to that of the right diaphragmatic lobe but varies in detail. There are six dorsal branches (C, E, H, K, M and O) of which both the first and second quickly bifurcate. The ventral branches (F, I, L and N) are smaller and less complex and fewer in number and supply smaller broncho-pulmonary segments than those of the right side. The first ventral branch of the right diaphragmatic lobe is not represented and the corresponding lung segment is incorporated into the 2nd lateral broncho-pulmonary segment. There are only four lateral bronchi (B, D, G and J) but their distribution is similar to those of the other lung.

SUMMARY

The anatomy of the bronchial system of 60 normal bovine lungs is described for the first time.

BOVINE PULMONARY TUBERCULOSIS

PRIMARY INFECTION

J. T. STAMP

(Jn. Comp. Path. & Ther. 1948,
Vol. 58, No. 1)

INTRODUCTION

The present investigation appeared to be warranted in view of the great difference of opinion by many workers as regards the common portal of entry of the tubercle bacillus into the bovine body.

A survey of veterinary literature shows that it was well appreciated by observers at the end of the last century that in cattle bronchial-mediastinal lymph gland tuberculosis was the most frequently occurring form and that such lesions were commonly not associated with lung lesions (M'Fadyean, 1891; Friedberger and Frohner, 1895; Nocard, 1895; Laithwood, 1896; M'Fadyean, 1898). This frequency of bronchial-mediastinal lymph gland tuberculosis in bovines has been fully supported in more recent literature (Stamp and Wilson, 1946; Spartz, 1934). At that time it was not doubted that these lesions arose by inhalation infection. The view that such lesions are produced, almost invariably, by tubercle bacilli entering the body through the mucous membrane of the alimentary tract was later put forward by Von Behring (1903) and by Calmette and Guerin (1905, 1906) and it is now widely stated that the common site of infection leading to bronchial-mediastinal

lymph gland tuberculosis is the intestine (Mettam, 1906; Muller, 1906; Schroeder and Cotton, 1906; Cadeac, 1909; Edit., 1908; Edit., 1910; Edwards, 1937; Innes, 1937; Udall, 1943). That the question of the intestinal origin of pulmonary tuberculosis is still unsettled is shown by the experimental work of White and Minett (1941) and Edwards (1937), the viewpoints of these workers being ably reviewed by Glover (1941) who considers that their evidence, like the evidence of much previous experimental work, is inconclusive and that the problem is still a very open one.

It has never been seriously debated that the primary site of infection is intestinal when the only or oldest lesions present in the body are in the mesenteric lymph glands, nor has it been suggested that similar lesions present in the retropharyngeal lymph glands are not indicative of pharyngeal infection. Theoretically, the bronchial-mediastinal lymph glands can become the seat of tuberculous disease by lymphatic drainage of lung infections either aerogenous or haematogenous in origin, or by lymphatic flow from a distant site of infection such as the alimentary tract.

In consequence of this vexed question of alimentary versus pulmonary origin of bronchial-mediastinal gland tuberculosis, it was thought that a detailed morbid anatomical examination, along with careful histological examination of cases of bovine

tuberculosis, might give some evidence to support one or other theory. It was considered that despite statements to the contrary (Law, 1912; Wallis Hoare, 1913; Calmette, 1923; Yamagiwa and Jasikawa, 1930), it might be possible to demonstrate some lesions in the lung which could be regarded as primary to the bronchial-mediastinal lymph gland lesions and due to aerogenous infection. Such lesions have been extensively described, both in experimental tuberculosis and in spontaneous tuberculosis in the human. In addition, it was thought that an extensive morbid anatomical investigation of bovine tuberculosis might indicate the routes of dissemination of the disease in the body.

ORIGINAL OBSERVATIONS

METHODS

Material from 100 cases was obtained from Edinburgh abattoir, and no history was available. The animals, which were selected from a larger investigation, were those in which, on initial examination for meat inspection purposes, lesions of tuberculosis or suspected tuberculosis were confined to the thorax. Such a selection is justified since the returns from such abattoir inspections have been the foundation of the many statements as to the frequency of thoracic tuberculosis in the bovine.

The following post-mortem examination was carried out on all cases. At the abattoir there was a careful examination of the carcase and carcase

lymph glands. The lungs and heart, alimentary canal and head, along with the udder and uterus if present, were taken for detailed macroscopical examination at the laboratory, and for histological examination.

Alimentary canal. This was stripped from the mesentery, opened and washed from end to end. The mucosa was then examined for any abnormality, and associated mesenteric and gastric lymph glands cut into multiple thin slices.

Liver. The hepatic lymph glands were examined by thin slicing while the liver substance was examined by slicing on a large ham machine.

Head. The retropharyngeal, parotid and submaxillary lymph glands, mucosa of the mouth, nose and pharynx, and the tonsils were examined.

Lungs and Heart. These were first infused with 2 per cent. formol saline through the pulmonary artery. The whole organs were then immersed in a large bin of formalin for several days until fixed. The regional lymph glands were first examined along with the thoracic duct or ducts. The left bronchial, right cranial bronchial, the right caudal bronchial (rarely present), the caudal mediastinal, middle mediastinal and cranial mediastinal lymph glands were all carefully examined by multiple slicing with a razor. The lung was next dissected into its various lobes, right diaphragmatic, accessory, right cardiac, right cranial and caudal apicals, left apical, left cardiac and left diaphragmatic lobes. Each lobe was

sliced into thin slices on the ham machine. All the lobes except the diaphragmatic ones were cut at right angles to the main lobe bronchus, starting at the lobe hilum. The diaphragmatic lobes were sliced parallel to the main lobe bronchus beginning at the mediastinal surface. In this way it was relatively simple to distinguish the major bronchial pulmonary segments and to record the distribution of lesions within these segments. Later in the investigation, the infusion of the lungs and heart was dispensed with and the tissues were hardened for slicing by freezing in a mixture of salt and ice.

All lesions found were carefully recorded on charts as regards situation, appearance and age.

ANATOMICAL CONSIDERATION

Before describing the observations on thoracic tuberculosis, it is convenient here to review briefly the anatomy of the bovine lung and the lymphatic system of the lung and thorax in so far as it is important to the understanding of the path of infection in tuberculosis. The bovine lungs are divided into lobes by deep interlobar fissures. The left lung is divided into three lobes named apical, cardiac and diaphragmatic. The right lung may be regarded as having five lobes, cranial and caudal apical, cardiac, intermediate and diaphragmatic. The individual lobes themselves can be readily sub-divided into broncho-pulmonary segments, each segment being supplied by a single bronchus. The

main broncho-pulmonary segments have been described (Stamp, 1948), and in this communication the lesions of tuberculosis will be related to these divisions of the lung. In addition, since the regional lung glands are the common seat of tuberculous lesions, it is opportune here to discuss briefly the drainage areas of these glands and their afferent lymphatic vessels. The lymphatics of the lungs have been divided into two groups, deep lymphatics draining lung substance, and superficial lymphatics draining pleura and subpleural lung tissue. The two sets communicate freely at the surface, but since valves are present lymph flow between the two is only from lung to pleura (Baum, 1912). The deep pulmonary lymphatics comprise plexuses which accompany the air passages, arteries and veins in the lung to the hilum. The superficial lymphatics pass over the pleural surface to the mediastinum. In general, the drainage paths of the various lobes can be stated, but it must be recognised that variations may occur. The lymph glands of importance in thoracic tuberculosis are cranial mediastinal, middle mediastinal, caudal mediastinal, left bronchial, right cranial bronchial, right caudal bronchial. The cranial mediastinal and right cranial bronchial are frequently fused while the right caudal bronchial is often absent.

Left apical and cardiac lobes. Both superficial and deep lymphatics drain to the left bronchial lymph gland.

Left diaphragmatic. Superficial lymphatics drain to the caudal mediastinal lymph gland except those draining the more cranial portion of the lobe which enter the left bronchial lymph gland. The deep lymphatics run to the hilum of the lung and for the most part go to the left bronchial lymph gland; a small number, however, go to the caudal mediastinal gland. As an exception, some of the deep vessels may drain to the middle mediastinal lymph gland.

Right apical (cranial and caudal parts). Both superficial and deep lymphatics drain chiefly to the right cranial bronchial lymph gland; a few enter the cranial mediastinal lymph gland.

Right cardiac lobe. Superficial vessels drain to the right caudal bronchial, right cranial bronchial and left bronchial. Deep vessels run to the right caudal bronchial and to the middle mediastinal lymph glands. If the right caudal bronchial gland is absent, all run into the middle mediastinal.

Intermediate lobe. Both superficial and deep lymphatics run to the caudal mediastinal and left bronchial lymph glands.

Right diaphragmatic. Subpleural vessels drain chiefly to the middle mediastinal gland but some cross to the left bronchial lymph gland.

THORACIC TUBERCULOSIS

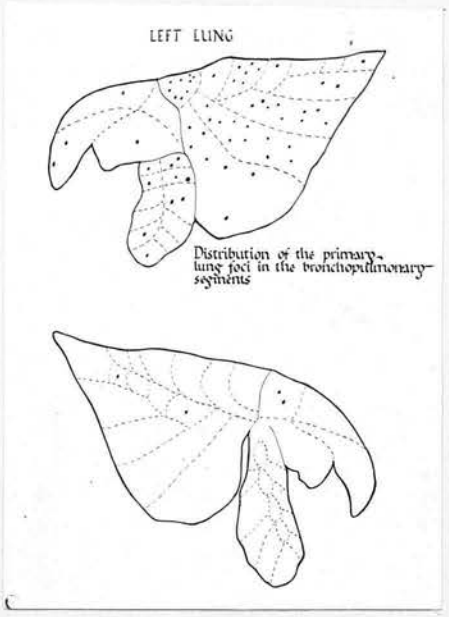
Cases with primary lung foci.

From the 100 cases of bovine tuberculosis with lesions confined to the thorax on abattoir examination, it is considered that primary lung foci were found in 89 cases (89 per cent.) and that these foci were the origin of the thoracic lymph gland tuberculosis. Of the 89 cases in which primary lesions were found, 72 had one focus, six had two foci, five had three foci, five had four foci, and one had five. In all cases both macroscopic and microscopic examination of lung lesions was carried out to assess whether a lesion could be primary to the regional lymph gland changes or not.

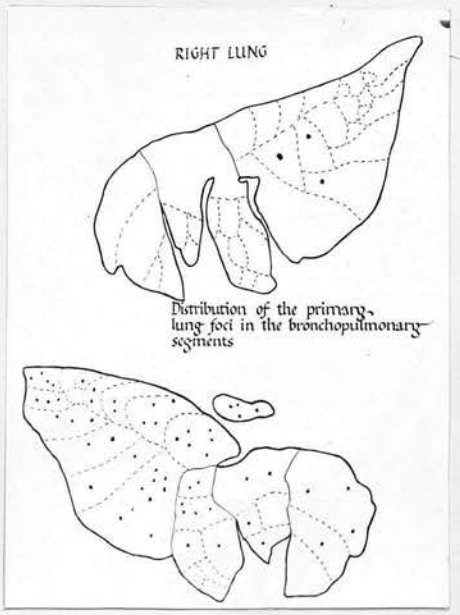
Of the remaining eleven cases, lung lesions were not found in three cases, while in eight cases they were so advanced that it was impossible to ascertain a primary focus. It is noteworthy that in one case where no primary lung lesion was present, a large productive tuberculoma of the larynx was the only lesion present in the body except for the caseation of the cranial and middle mediastinal lymph glands.

The distribution of the primary lung foci in the various lobes and their broncho-pulmonary segments.

The situation of the various primary foci in the lungs is shown in Diagrams I and II. As can be seen, both right and left lungs are almost equally involved. The diaphragmatic lobes are more frequently the site of primary infection than the



Diag.1. - Distribution of the primary lung foci in left lung.



Diag.2. - Distribution of the primary lung foci in right lung.

other lobes. No lobe of the lung is immune to primary infection.

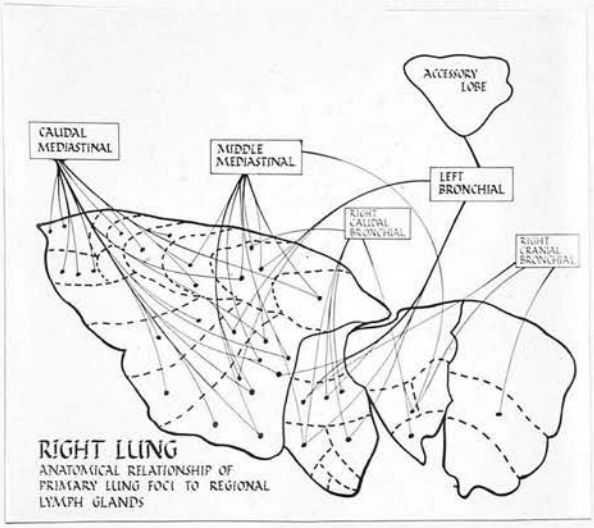
The anatomical distribution of the tuberculous disease in the bronchial-mediastinal lymph glands with relationship to the primary foci.

Lesions are found in association with the primary lung foci in the bronchial-mediastinal lymph glands, and the relationship is that the lymph glands directly draining the area of lung involved are affected first. In every case the anatomical appearance of the foci forming the lung-lymph gland complex leaves little doubt that they antedate any other lesions present in the body. The anatomical relationship of the primary foci to the lymph gland lesions is most clearly demonstrated in the 72 cases which show only one primary lung focus. A similar relationship is also seen when primary foci are multiple, but in these cases the lesions in the related glands are obviously more widespread and the association of primary focus to lymph gland change is not so obvious as in the cases with a single primary focus. The following is a brief summary of the findings in the 72 cases of single primary foci. (See Diagrams III and IV.)

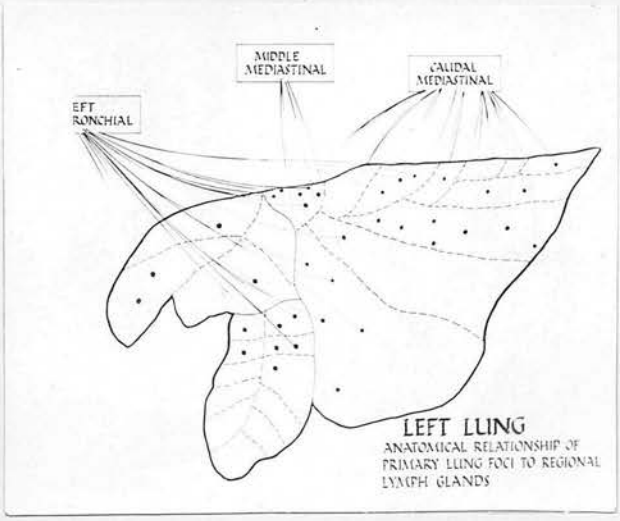
Right lung.

Right cranial apical foci give rise to lesions in the right cranial bronchial lymph gland.

Right caudal apical foci give origin to lymph gland lesions in right cranial bronchial, right



Diag.3. - Anatomical relationship of single primary lung foci in the right lung to the regional lymph glands.



Diag.4. - Anatomical relationship of single primary lung foci in the left lung to the regional lymph glands.

bronchial (if present) and middle mediastinal lymph gland.

Right cardiac foci drain to left bronchial, right bronchial and middle mediastinal lymph gland.

Right diaphragmatic. Primary foci occurring in the cranial broncho-pulmonary segments of this lobe give rise to associated lesions in the left bronchial, middle mediastinal and caudal mediastinal lymph glands, while those occurring in the distal broncho-pulmonary segments drain chiefly into the caudal mediastinal lymph gland. There is little doubt that lesions in the cranial broncho-pulmonary segments drain along both superficial and deep lymphatics to the middle mediastinal and left bronchial glands. Primary foci situated in the caudal broncho-pulmonary segments drain almost entirely along the superficial lymphatics to the caudal mediastinal lymph gland, and this route appears to be the most direct path from primary lesions so situated to a regional lymph gland.

Accessory foci drain to both left bronchial and caudal mediastinal lymph glands.

Left lung.

Left apical and cardiac lesions drain directly to the left bronchial lymph gland.

Left diaphragmatic foci in the cranial broncho-pulmonary segments drain chiefly to both the left bronchial and caudal mediastinal lymph glands. Primary foci in the caudal broncho-pulmonary

segments, as on the right side, drain directly into the caudal mediastinal lymph gland.

It will be seen that if these recorded observations of primary complexes are compared with the normal lymphatic drainage areas, they agree closely.

APPEARANCE AND SIZE OF THE PRIMARY LUNG FOCUS

In accordance with their macroscopic appearance and size, the primary lung foci can be classified as shown in Table I, which also shows the type of lesions found in the associated regional lymph gland or glands. In every case histological examination of lung and lymph gland lesions indicates that the lymph gland lesions are never older than those in the lung.

Encapsulated caseous calcified nodules 1 cm. or less in diameter.

Fifteen lesions of this type are found occurring in 13 cases of primary lung infection. They vary in size from $\frac{1}{2}$ to 1 cm., and all are characterised by a definite fibrous tissue capsule surrounding caseous or calcified caseous material. Obvious macroscopic cavitation is occurring in three of these lesions leading to local dissemination of the lesion. The regional lymph gland shows encapsulated caseous nodes of similar age and size to the lung lesions (see Figs. 1 and 2).

Young lesions 2 cm. or less in diameter obviously still progressing, and with no fibrous tissue encapsulation.

TABLE I

THE RELATION OF THE PATHOLOGICAL CHANGES IN THE PRIMARY LUNG FOCI TO THOSE IN THE ASSOCIATED TRACHEO-BRONCHIAL GLANDS

Number of cases	Pathological lesion in lung (primary focus)	Number of cases	Pathological lesion in regional lymph glands
13	Encapsulated caseous calcified nodules, 1 cm. or less in diameter	13	Encapsulated calcified caseous nodes, $\frac{1}{4}$ - 2 cm. in diameter
26	Early lesions, obviously still active and with little or no encapsulation	9	Early caseous tubercles
		2	Early confluent caseation
		6	Diffuse multifocal caseation of granulation tissue
		1	Caseation with slight encapsulation
		8	Nodular caseation
14	Encapsulated caseous calcified nodes, 1 - 2 cm. in size	14	1 - 3 cm. encapsulated calcified caseous nodes
18	Large encapsulated caseous calcified lesions	17	Large hyaline encapsulated abscess
		1	Massive caseation

TABLE I (continued)

Number of cases	Pathological lesion in lung (primary focus)	Number of cases	Pathological lesion in regional lymph glands
1	Acute cavitating caseous pneumonia	1	Enlarged soft and caseous
1	Fibrotic cavity	1	Large encapsulated caseous calcified abscess
13	Coalescing caseous acinar broncho-pneumonia	13	Diffuse caseating productive granulation tissue
3	Nodular broncho-pneumonia	3	Encapsulated caseous calcified abscess

Figs.1&2. - Encapsulated caseous calcified primary lung lesions, 1 cm. in diameter.

Forty-six such lesions are described as occurring in 26 cases of primary lung infection, three of these cases having four lung foci each; they vary considerably in appearance. Very young lesions consist of one or several areas of consolidation within a primary lung lobule, spreading peripherally and at the same time undergoing slight central caseation. In this group, however, the majority are at a later stage of development and consist of condensed lung tissue surrounding a focus of recent caseation which vary in size from 2 mm. up to 2 cm. in diameter. Obvious macroscopic cavitation is occurring in 12 of these lesions leading to slight local bronchial dissemination of infection. None show any degree of encapsulation with fibrous tissue, although the older lesions show a pale halo of tuberculous granulation tissue and condensation of surrounding lung tissue. This, however, does not represent healing but merely the progressing edge of an active focus.

The corresponding lymph gland lesions vary in appearance, depending upon the development of the lung focus. They may consist of early small caseous tubercles with or without granulation tissue capsules, or they may have developed into areas of early confluent caseation. In cases where the lung lesion appears to be progressing rapidly, those in the associated lymph gland are comparatively large and consist of areas of tuberculous granulation

tissue undergoing diffuse multifocal caseation (see Figs. 3, 4, 5 and 6).

Nodes of coalescing caseous acinar broncho-pneumonia.

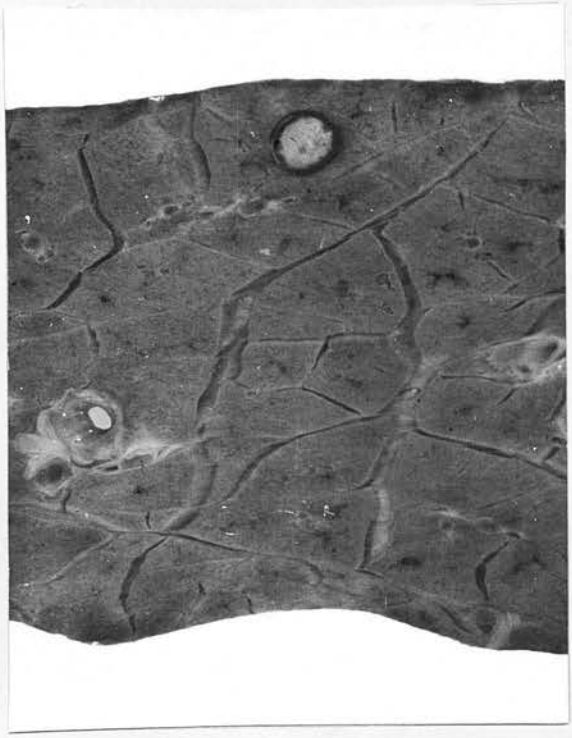
Fourteen such lesions, varying in size from 2 to 5 cm. in diameter, occur in 13 cases of primary lung infection: they consist of several or many foci of caseous acinar broncho-pneumonia which are coalescing, giving finger-like processes of caseation. They may involve one or many primary lung lobules so that the primary focus may be small or large. The whole lesion is usually encapsulated with a slight fibrous tissue capsule but there is no connective tissue capsule between the individual acinar caseous foci. All stages in the confluence of the individual acinar foci to a caseous abscess with definite surrounding encapsulation of fibrous tissue can be traced. Cavitation of these lesions has occurred in nine cases.

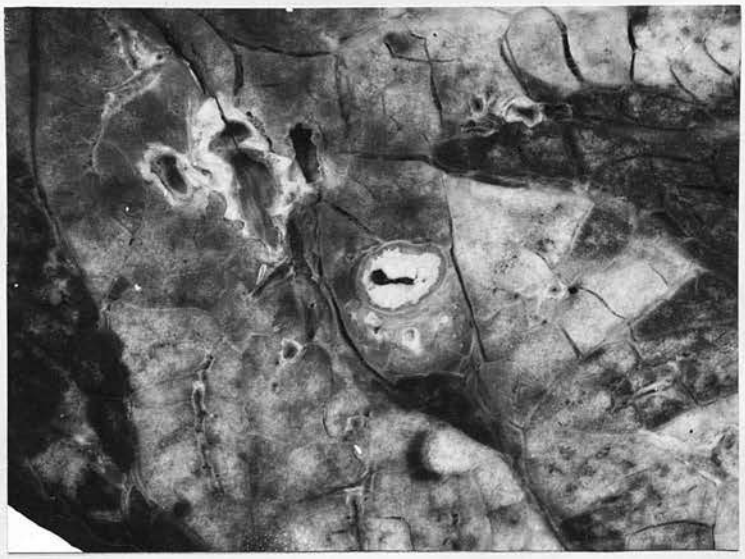
The regional lymph glands are almost completely replaced by cellular granulation tissue showing varying degrees of multifocal caseation (see Figs. 7, 8, 9 and 10).

Encapsulated caseous calcified nodes 1 to 2 cm. in diameter.

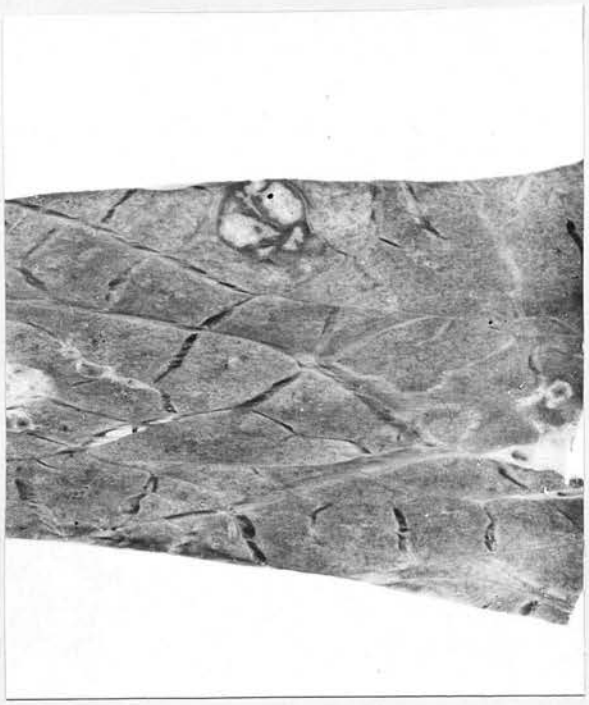
Eighteen such primary lung lesions are found in 14 cases of pulmonary tuberculosis, consisting essentially of caseous and frequently calcified nodes of tuberculosis all having a definite fibrous tissue capsule. In the younger lesions a multifocal

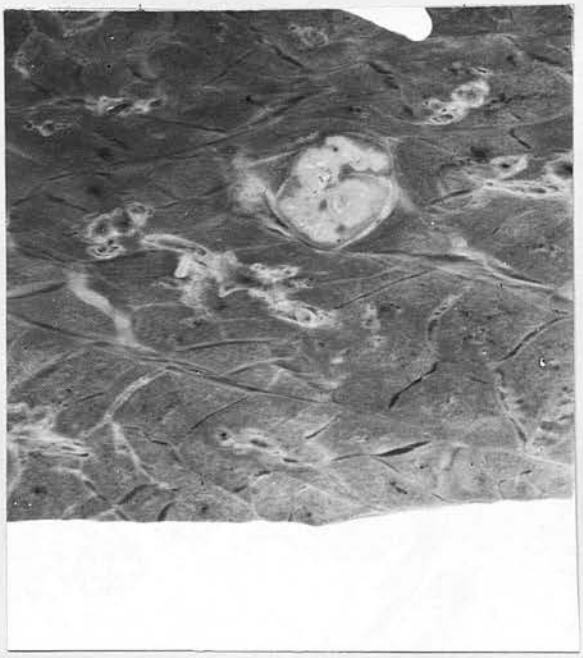
Figs. 3&4. - Young lesions still progressing and with no encapsulation.



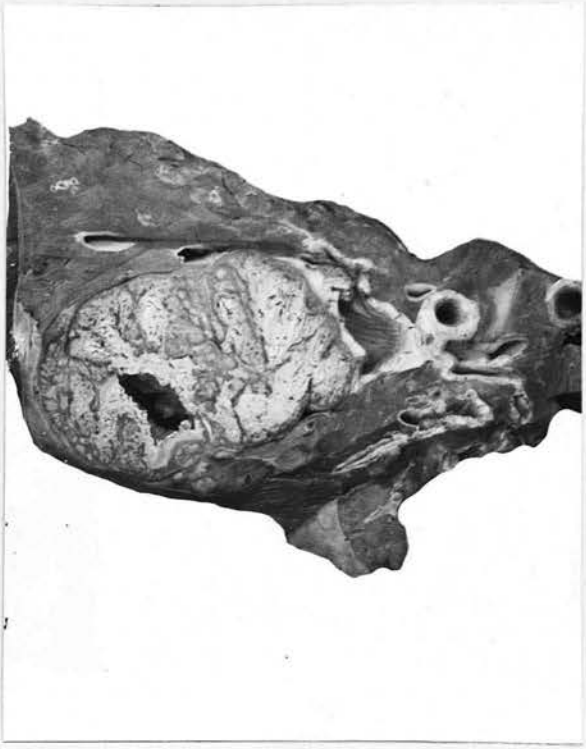


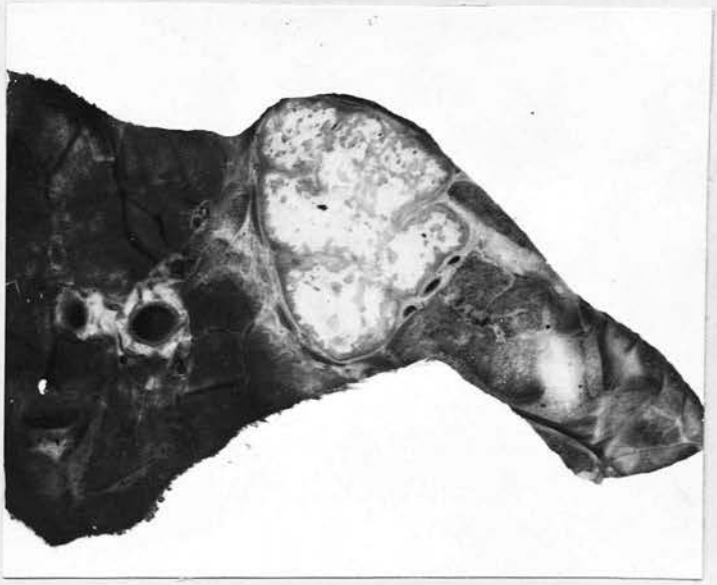
Figs. 5&6. - Young lesions still progressing and with no encapsulation.





Figs. 7&8. - Coalescing caseous acinar broncho pneumonia with encapsulation.





Figs. 9&10. - Coalescing caseous acinar broncho pneumonia with encapsulation.

origin is discernible. Cavitation along the associated bronchus occurs frequently and 17 of the 18 described in this category show very evident macroscopic signs of such progression (see Figs. 11, 12, 13 and 14).

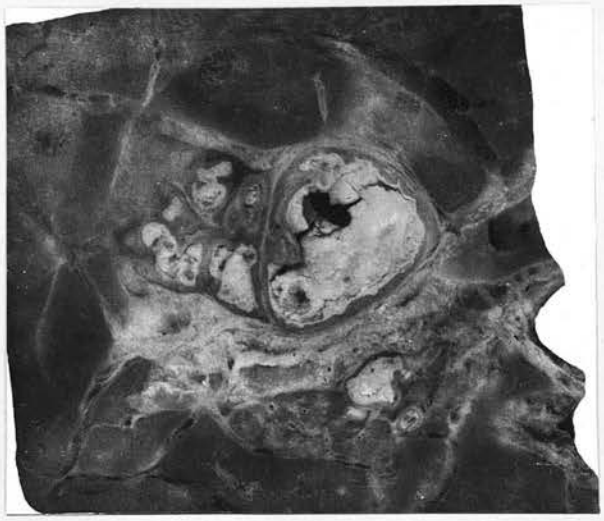
Large encapsulated caseous calcified lesions.

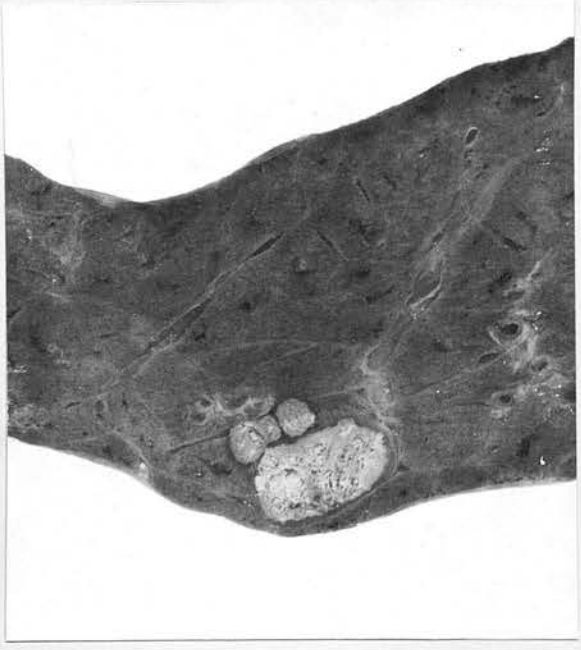
These lesions are essentially similar to those described in the last section except that they differ considerably in size, varying from 2 to 5 cm. in diameter; they are caseous and calcified and heavily encapsulated with dense fibrous tissue. The origin of these large aggregations from the confluence of several caseous foci is clearly seen in some of the younger lesions; 26 are found in 18 cases of primary lung infection. All the lesions show definite macroscopic evidence of bronchial cavitation.

The lymph gland lesions in both the above categories consist of hyaline encapsulated caseous calcified abscesses of varying size, while occasionally the whole gland is replaced by massive caseation surrounded by a very thickened fibrous tissue capsule (see Figs. 15, 16, 17 and 18).

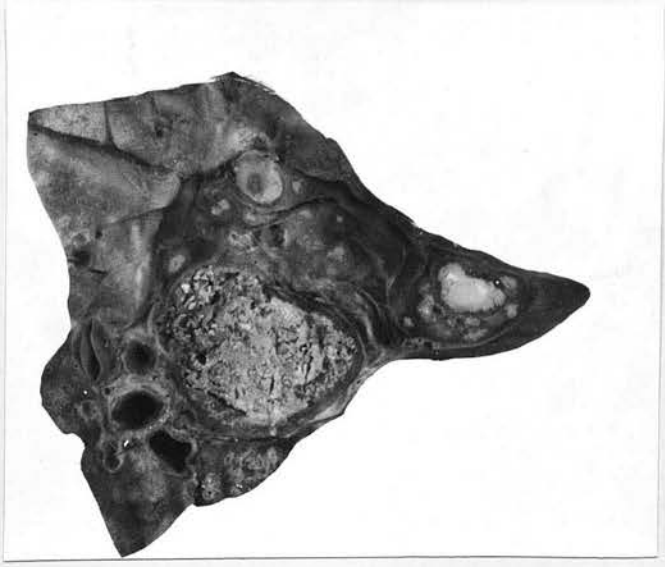
Fibrotic cavity.

One such primary lesion is seen. The cavity is small and heavily encapsulated with fibrous tissue and is drained by a small bronchus. Despite the heavy encapsulation healing is not complete for small active tubercles, in which acid-fast bacilli are present, line the cavity. Local bronchial

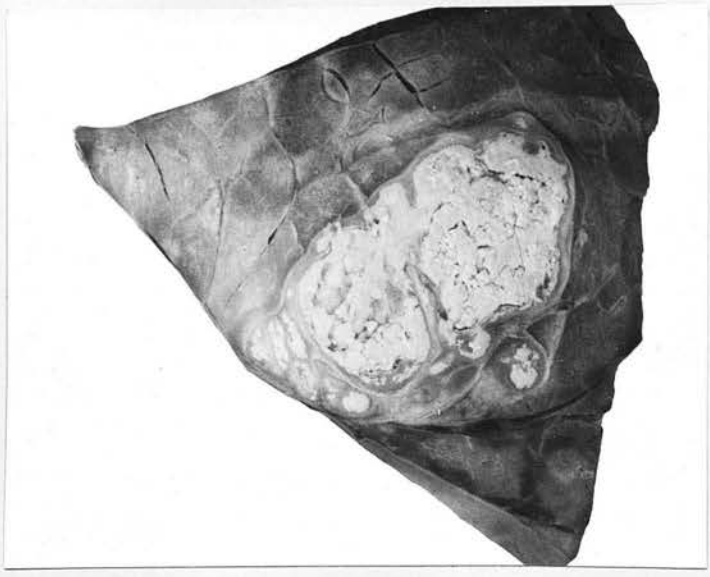


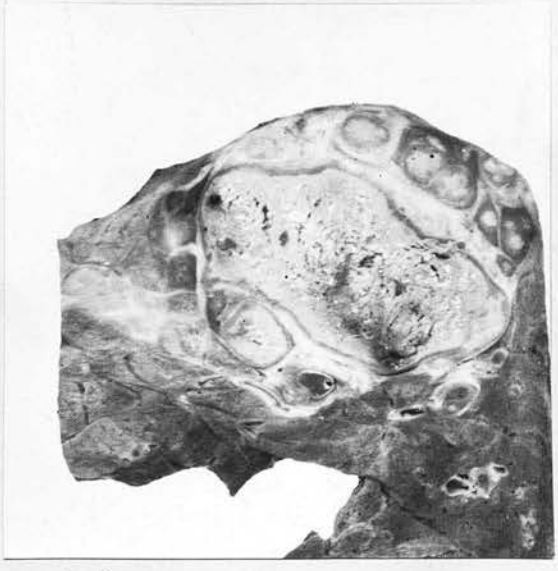


Figs. 11&12. - Encapsulated caseous calcified nodes, 1-2cms. in diameter.



Figs. 13&14. - Encapsulated caseous calcified nodes, 1-2cms. in diameter.





Figs. 15&16. - Large encapsulated caseous calcified lesions.

Figs. 17&18. - Large encapsulated caseous calcified lesions.

dissemination has occurred. The associated lymph gland lesion consists of a thick walled caseous abscess (see Fig. 19).

Acute cavitating caseous pneumonia.

Only one such primary lesion is seen and in this case a major broncho-pulmonary segment is undergoing massive caseation and acute cavitation. The regional lymph glands are grossly enlarged, soft and caseous with no fibrous tissue reaction (see Fig. 20).

Nodular broncho-pneumonia.

Three such lesions are found in three cases of primary lung infection. They vary in size from 2 to 5 cm. in diameter and consist of several neighbouring lung lobules which are undergoing caseation and encapsulation. The juxtaposed lesions appear of similar age. Confluence does not occur owing to the formation of the fibrous tissue capsules between individual nodes. Macroscopic bronchial cavitation is seen in all three cases. The associated lymph gland lesions consist of encapsulated caseous abscesses (see Figs. 21 and 22).

RESULTS

Primary lung lesions.

Of a total of 100 cases of thoracic tuberculosis, 89 (89 per cent.) were shown to have recognisable primary lesions in the lung, while in eight cases the lung disease was too advanced to enable one to recognise the primary focus. In only three cases was a lung lesion not found. In every

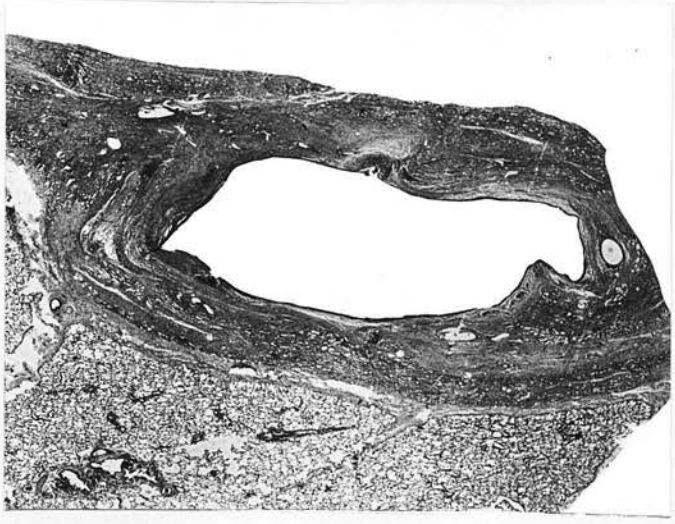


Fig. 19. - Fibrotic cavity.

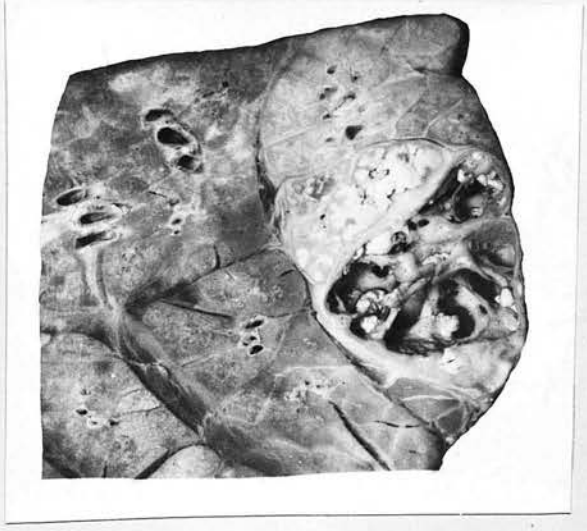
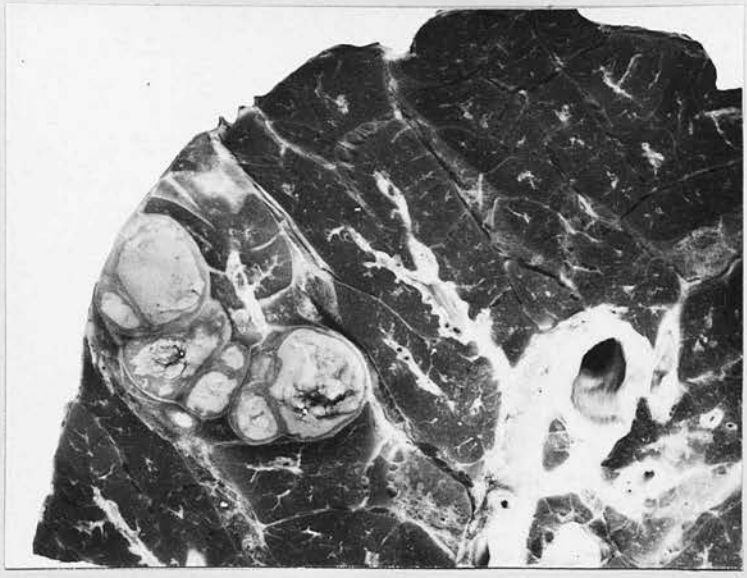
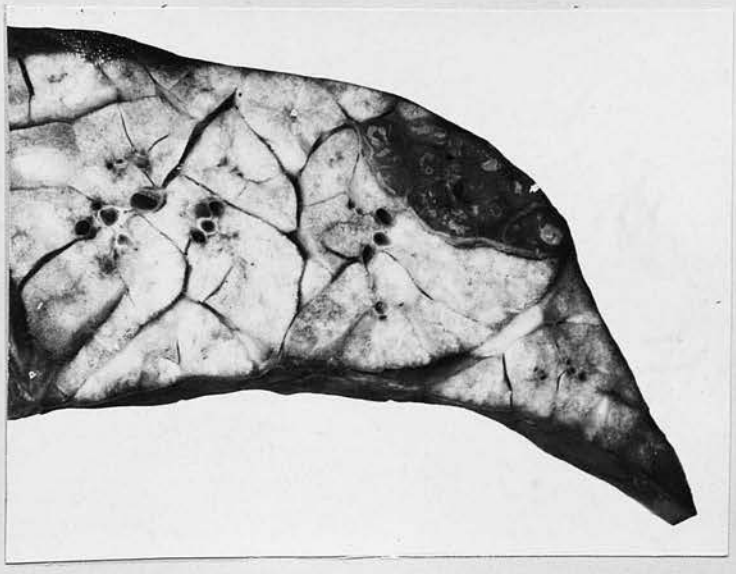


Fig. 20. - Acute cavitating caseous pneumonia.





Figs. 21&22. - Nodular broncho-pneumonia.

case careful macroscopic and microscopic examination of both lung and lymph gland lesions was carried out in order to assess the comparative age of the two lesions. For this purpose the general concepts of tuberculosis pathology have been followed. It is clear from these findings that when tuberculous lesions are found confined to the bronchial mediastinal lymph glands, then in nearly every case an associated lesion will be found in the lung substance. In addition, both naked eye and microscopical examination showed that the foci in the lung and in the related lymph glands were of the same age or those in the lymph glands more recent. (See Table I.)

In all 124 primary lung foci were seen. In the majority of cases they were single but in 17 were multiple. In size and appearance and age they varied very considerably. Thus in more than 25 per cent. of cases the primary focus was less than 1 cm. and in several were only 2 mm. in diameter and were difficult to locate. The remaining primary lesions varied in size from 1 cm. to 5 cm. In appearance and age the primary foci varied from an early cellular infiltration of lung lobule to a very large heavily encapsulated caseous calcified mass. Four cases had atypical primary foci; in one the primary focus consisted of a large, acute, cavitating tuberculous pneumonia, while in the three others it was an area of chronic nodular broncho-pneumonia

similar to the lesions seen in post-primary infection. Evidence of marked healing in the form of surrounding fibrosis was seen in 49 cases (55 per cent.). In one case the primary lesion consisted of a small cavity surrounded by dense fibrous tissue. In only one case was there any evidence of ossification. There was no, or only slight, evidence of healing in 40 cases (45 per cent.). Caseation and calcification in the primary focus was usual except in the very youngest lesions.

Relation of primary lung foci to bronchial mediastinal lymph glands.

The relationship between primary lung focus and regional lymph gland was such that the lymph glands in direct anatomical relation to the segment of lung involved were the most grossly diseased, but in many cases the infection had clearly progressed to other lymph glands. In no case was there any obvious chain of foci between lung and gland, so that it would appear that the tubercle bacilli must be carried directly in the lymph flow without causing intermediate lesions.

This is the crucial evidence

Dissemination from the primary infection.

In the bovine the most common form of dissemination from the primary lesion is bronchogenic arising by softening of the caseous material. Thus, out of a total of 89 cases where the primary lesion has been determined, 61 show evidence of very definite bronchial spread. It was more noticeable,

however, that if one excepts the 26 cases where the infection was obviously very recent, then out of the remaining 63 more advanced cases, 49 (80 per cent.) have developed bronchial pneumonia arising from ulceration of the primary lesion. Haematogenous dissemination determined by involvement of peripheral carcase lymph glands, kidney, etc., occurred in only five cases.

FINDINGS

Portal of infection.

The finding of primary lung foci in bovine tuberculosis is not in agreement with many previous statements in the literature of bovine tuberculosis (Friedberger and Frohner, 1895; Law, 1912; Wallis Hoare, 1913; Nocard, 1895; Calmette, 1923; M'Fadyean, 1891; Theives, 1939; M'Fadyean, 1898; Laithwood, 1896; Whipham, 1909; Yamagiwa and Jasikawa, 1930). From the evidence presented it appears unlikely that the lung lesions arise by lymphogenous spread from the lymph glands, nor indeed was there any suggestion that retrograde lymphatic spread back into the lung had occurred at all. Regarding the portal of entry of the tubercle bacilli which cause the primary lung focus, one is therefore left with two theoretical possibilities. Either the bacilli enter the body by the mucous membrane of the digestive tract and do not produce foci until they have been transmitted to the lungs by the blood stream as suggested by Von Behring, or the bacilli

N.B. Many

enter the lung directly by inhalation. Although the histological features of primary lung lesions in the bovine have not been described in this communication, it is necessary to say that in those cases where the primary focus was at its earliest stage it consisted histologically of a small area of tuberculous broncho-pneumonia and, so far as could be seen, there was no evidence to suggest a vascular origin to these lesions. In fact, the lesions appeared to be characteristic of inhalation infection.

Age distribution of primary foci.

Nieberle (1931) has divided bovine tuberculosis into calfhood and adult types with differences similar to those frequently described between childhood and adult tuberculosis. Thus in the calf there is gross caseation of the regional lymph nodes associated with a primary lung focus, the whole being commonly called a primary complex (Ranke, 1916) from which frequently metastatic haematogenous lesions disseminate. On the other hand, he states that adult bovine pulmonary tuberculosis is characterised by (a) spread being predominatingly intra-bronchial; (b) absence of macroscopic lesions in the pulmonary lymph nodes; (c) predominance of fibrosis; and (d) cavitation. In the human there would appear to be two possibilities for this division into types: (a) differences due to acquired resistance from a previous infection leaving a healed primary complex;

or (b) an age determined native resistance (Rich, 1944). It has, however, previously been shown that bovines are not commonly infected when young but acquire lesions for the first time in adult life (Stamp and Wilson, 1946) so that if such a division into calfhood and adult types as described by Nieberle does in fact occur, then these differences are due not to any acquired resistance but are due to an age determined resistance. The present investigation, however, does not support the contention of Nieberle, for out of a total of 100 animals examined, one was under one year of age, seven were under two years, 27 under three years, 29 under four years and 25 over four years of age, so that practically all the animals were adult; yet 89 per cent. of them showed the presence of caseation of regional lymph nodes associated with a lung focus. It could, of course, be argued that these lesions were formed during calfhood, but surveys of abattoir material do not support this contention, nor does the fact that 40 of the present cases had active and young lesions present. Further evidence against the view that age in itself alters the form of the disease is clearly given in Table II, when it can be seen that the type of the primary pulmonary lesion is in no way influenced by the age of the animal. Thus many young, actively progressing lesions are seen in the older age groups while comparatively static encapsulated lesions are found in the younger groups.

TABLE II

AGE DISTRIBUTION OF PRIMARY LUNG FOCI ACCORDING TO THEIR MACROSCOPIC CHARACTERS

Character of primary focus			Under 1 year	1-2 years	2-3 years	3-4 years	Over 4 years
Encapsulated caseous calcified nodules, 1 cm. or less in diameter	H	1		1			
	B	6	1		3	2	
	C	6				1	5
Encapsulated caseous calcified nodes, 1-2 cm. size	H	1		1			
	B	7		1	4	2	
	C	6				2	4
Large encapsulated caseous calcified lesions	H	1		1			
	B	10			4	6	
	C	7				2	5
Fibrotic cavity	H						
	B	1				1	
	C						
Nodular broncho-pneumonia	H						
	B	3				3	
	C						
Total		49	1	4	11	19	14

TABLE II (continued)

AGE DISTRIBUTION OF PRIMARY LUNG FOCI ACCORDING TO THEIR MACROSCOPIC CHARACTERS

Character of primary focus			Under 1 year	1-2 years	2-3 years	3-4 years	Over 4 years
Early lesions with little or no encapsulation	H	1			1		
	B	15			8	7	
	C	10					10
Acute cavitating caseous pneumonia	H						
	B						
	C	1				1	
Coalescing caseous acinar broncho-pneumonia	H	2			2		
	B	9		3	5	1	
	C	2				1	1
Total		40		3	16	10	11

H = Heifers, 6
B = Bullock, 51
C = Cow, 32

Thus, in the bovine, it would appear incorrect to divide tuberculosis into calfhood and adult tuberculosis since such a classification implies that there are anatomical differences in the disease due to differences of immunity caused by age. It must be stressed, however, that it is not doubted that a form of chronic isolated pulmonary tuberculosis does occur in the bovine, but this usually follows primary infection and is independent of age.

Anatomical distribution of primary foci.

No portion of the lung parenchyma failed to show primary lesions so that there is no predilection site for its location. Nevertheless, lesions were more frequent in the diaphragmatic lobes even after consideration is given to the greater volume of lung parenchyma in the diaphragmatic lobes (Medlar, 1940). The findings are also in agreement with those of Smith (1894), Medlar (1940) and Nieberle (1931) in that in the diaphragmatic lobes the most common area for lesion location is on the dorsal aspect of the lung. There was no distinct difference in the incidence of primary infection between left and right lungs.

Factors responsible for the characteristics of the primary lung foci in the bovine.

It has been stated by Rich (1944) that when a pathologist is faced at autopsy with an established lesion in an organ it would be necessary to know the following factors before any accurate understanding

of its development could be made: (a) the virulence of the infecting bacilli; (b) number of bacilli that initiate infection; (c) the degree of native resistance; (d) the degree of acquired resistance present or capable of being developed; (e) degree of hypersensitivity existing or capable of being developed; (f) the tissue in which infection occurs.

In the individual case these factors are usually undetermined, but it is possible to make some estimate of the degree of development of such factors as native resistance, acquired resistance and hypersensitivity in the bovine by observing the lesions in the spontaneous disease in large numbers of cattle. Since the same differences in the reaction of the first infection recur regularly, there can be little doubt that some conclusions can be made. It is thought that the present series might give some information on these aspects of the disease. In the first place, it would appear that the native resistance of the bovine, at least in adult life, is comparatively low for there is little pathological evidence in this series to suggest that the normal bovine body exerts any great restraint upon the progress of the first infection, for in the majority of cases caseation is prominent and healing is inconspicuous while regional lymph nodes frequently become massively involved.

The question of acquired resistance in tuberculosis has been a subject of much controversy,

and it is to Ranke (1916) that we owe the widely accepted view that tuberculous infection can be divided into three stages: primary infection, second or hypersensitive stage, and third stage of increased resistance depending upon the development of acquired resistance. It is thought that in the majority of childhood primary infections, acquired resistance develops promptly and effectively, for the lesions are arrested and encapsulated while still very small, giving rise to the familiar non-progressive, globular little lesions of the primary complex (Rich, 1944). In cattle, however, this is not usually the case, for in our series in only 13 cases could one say that the lesion had been promptly arrested as described above. Much more commonly (46 cases), the primary lesion was comparatively large and caseous, reaching in many cases to 5 or 6 cm. in diameter. It is, therefore, suggested that in cattle the primary focus progresses further than is the rule in the human. Nevertheless, these large caseous lesions do eventually become arrested and encapsulated so that if Ranke's hypothesis is accepted then it would appear that in cattle the second stage of hypersensitivity is prolonged, but that eventually acquired resistance strongly develops and a localisation of the caseous mass begins. It has, however, already been shown that this localisation in the majority of cases is only partially successful, for bronchial dissemination giving rise to a chronic

broncho-pneumonia is common. For these reasons it is considered that the use of the term primary complex should not be used in cattle since it is misleading to those who recognise its pathology in human tuberculosis.

By many, however, the views of Ranke are not accepted as the explanation for the difference between childhood and adult tuberculosis. Krause (1925) believes that the differences are due to (a) difference in the patency of the lymphatics at various ages; (b) difference in lymphoid locations; (c) difference in the ability to form fibrous tissue at different ages, while Rich (1944) suggests that the differences are due not to acquired resistance conferred by a previous immunising infection, but are due to an increase in native resistance resulting in some way from the ageing processes, i.e., there is pathological evidence that the adult reacts to primary infection in a manner different from the child. Rich states, however, that there has been no experimental proof that young and adult animals differ in their reaction to the tubercle bacillus. Most certainly, in the present investigation, the cattle examined varied in age from under one year to nine years old and, as can be seen by Table II, there is no evidence to suggest that age varies the type of reaction to primary infection in the bovine. Most certainly the pathological evidence in bovine tuberculosis is that acquired resistance, following

primary infection, plays the major role in altering the type of reaction that occurs in subsequent infection, and that the concepts of Krause and Rich cannot be applied in bovine tuberculosis.

SUMMARY

The anatomical considerations of bovine thoracic tuberculosis are reviewed.

For the first time the occurrence and distribution of primary lung lesions in bovine thoracic tuberculosis, and the macroscopic appearances of the primary lung lesions have been described.

The pathway of infection, the dissemination from primary infection and pathogenesis of bovine tuberculosis are given.

The anatomical relationships of primary lung and lymph gland lesions are described.

Summaries indicative but not informative

Original

BOVINE PULMONARY TUBERCULOSIS

MICROSCOPIC CHARACTERS OF THE PRIMARY LUNG FOCI

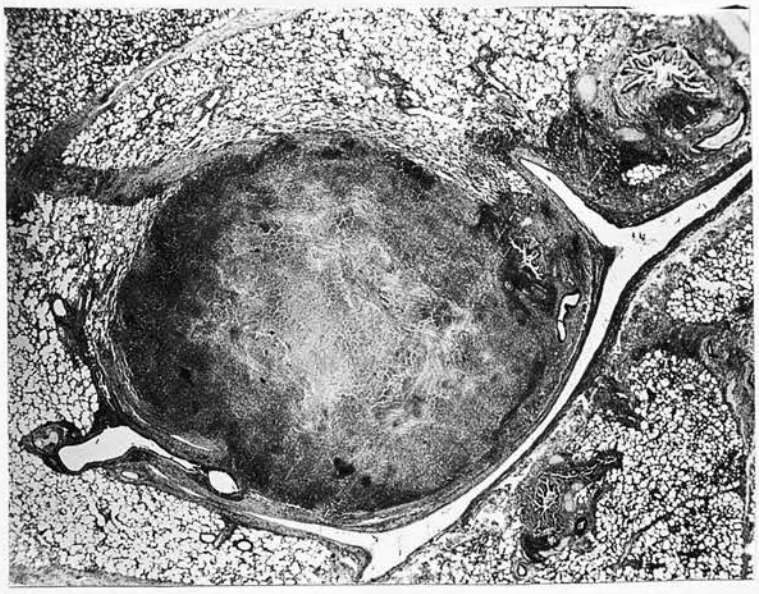
J. T. STAMP

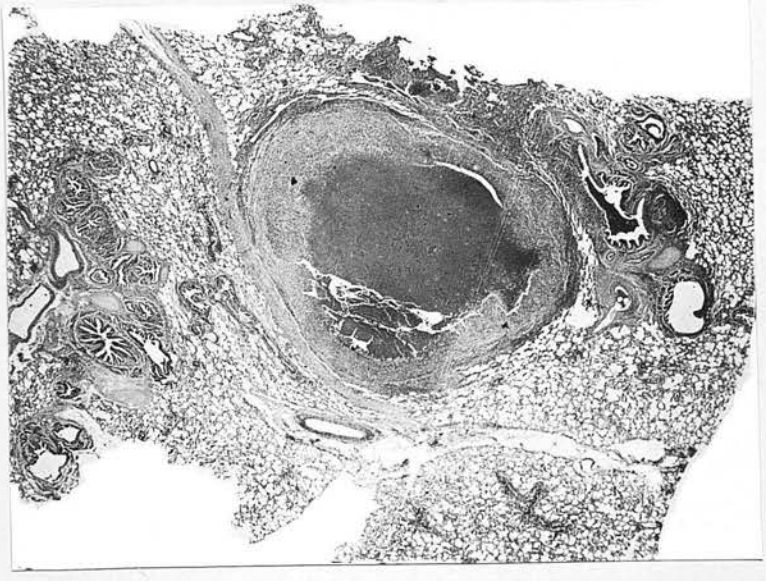
(Not published)

In the human the primary lung lesion consists of a small exudative focus of pneumonia which rapidly undergoes caseation, the exudative nature of the lesion however can always be shown by the preservation of the elastic tissue of the lung. The caseous area is surrounded in the early stages by tubercles, later the whole becomes encapsulated with ordinary fibrous tissue. Calcification and even ossification is frequently present. In the human there have been several descriptions of the very early phase of exudative pneumonia but no such lesions have been described in the bovine. Unfortunately in the present series (Stamp, 1948) such a lesion was not encountered. While in the human the majority of primary lung lesions heal or regress, in the bovine this is not the case. The histology of primary lesions in older bovines has not previously been described. In the following description the origin and early progress of such lesions are given.

ORIGINAL OBSERVATIONS

The earliest lesion seen consisted of a central area of necrosis and caseation surrounded by a zone of tuberculous granulation tissue (Figs. 1 and 2). The exudative nature of the original lesion is





Figs.1&2. - Early primary lung foci
H.& E. x 10.

however seen in sections stained for elastic tissue which method of staining also shows the intimate involvement of bronchioles. Other early primary foci are essentially similar except that the lesions obviously originate from several centres of inflammation rather than from a single site (Figs. 3 and 4). The individual areas of caseation however quickly coalesce to form a single focus surrounded by tuberculous granulation tissue. In the majority of early cases seen the focus is situated within a primary lung lobule but in some cases the primary focus obviously originates from several infection centres situated in neighbouring primary lung lobules. There is little attempt at fibrous tissue encapsulation. In the early lesions there is very little involvement of the neighbouring lymphatic tissues.

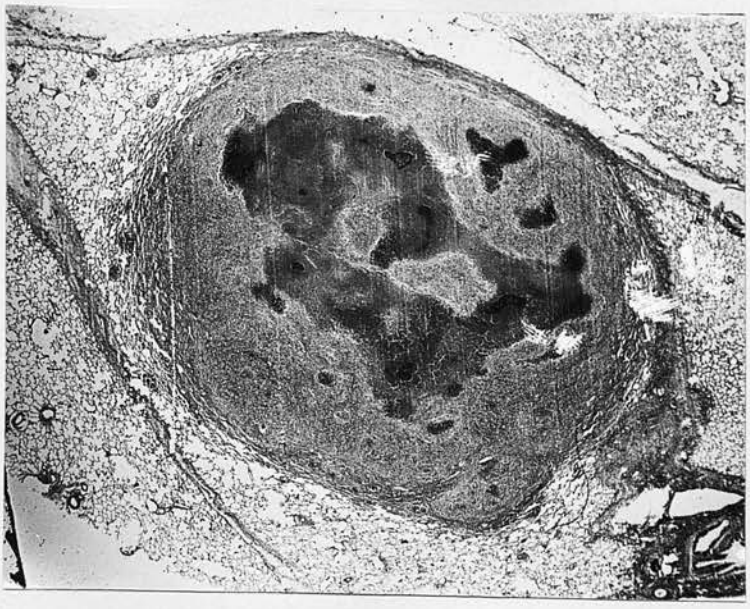
Extension of the primary lung focus is usual in the bovine. This occurs in several ways.

(a) Direct extension of the primary focus.

Histologically the caseous area becomes larger, not only by direct extension, but also by the coalescing of numerous peripheral foci which have originated by lymphatic spread from the first focus. Surrounding tuberculous granulation tissue is constantly being broken down and built up (Figs. 4, 5 and 6).

(b) Bronchial dissemination.

The lesions arising by direct extension of the



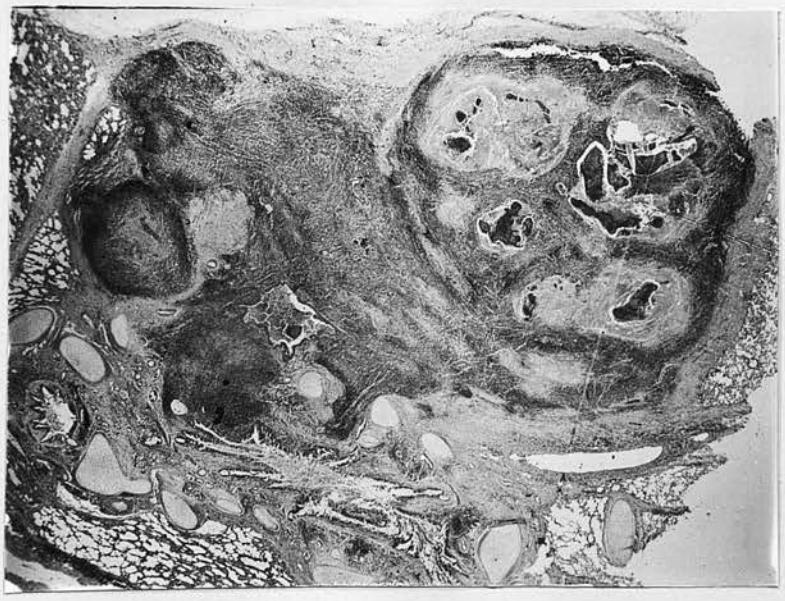
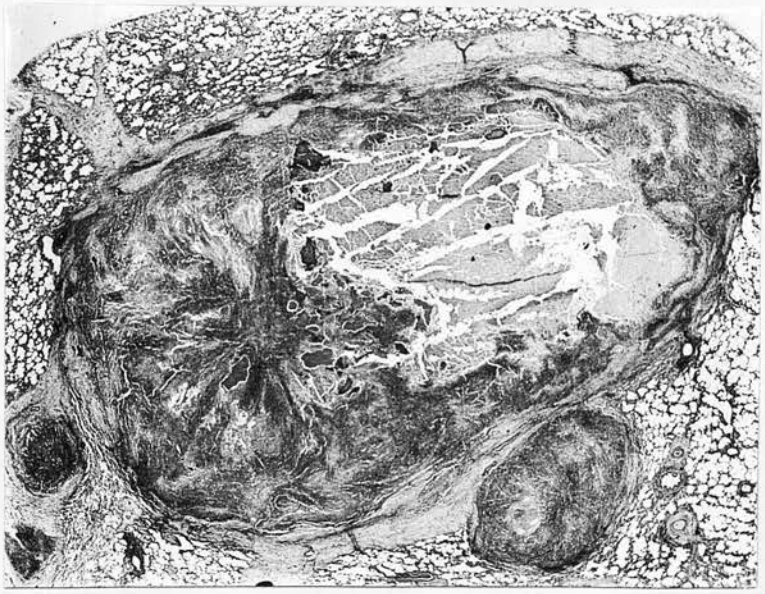
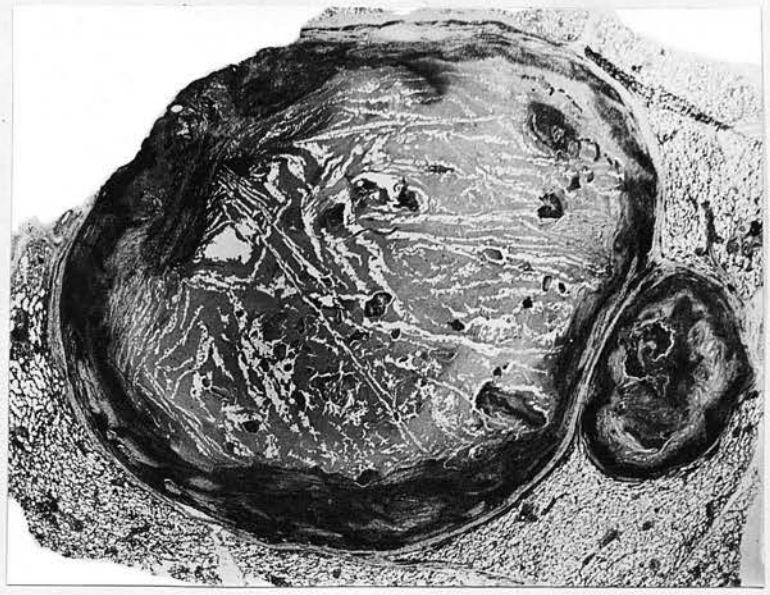


Fig.3. - Early primary lung foci.
H.&.E. x 10.

Fig.4. - Local progression of
early primary lung foci.
H.& E. x 10.





Figs.5&6. - Local progression of primary lung foci. H.& E. x 10
H.& E. x 4

primary focus quickly involve small bronchial vessels while in addition the primary focus, even in its comparatively early stages, discharges caseous material into its own branch bronchiole. In this way localised areas of broncho-pneumonia arise which can be clearly seen microscopically as caseating tubercles in the form of an acinar broncho-pneumonia.

RESULTS

The histology of the early primary focus is described as it occurs in the bovine lung.

(6)

BOVINE PULMONARY TUBERCULOSIS

PHTHISIS

J. T. STAMP

(Not published)

INTRODUCTION

In the majority of people who develop phthisis primary infection has occurred years previously. Phthisis usually develops as a small focus of caseous pneumonia which once formed has all the potentialities of developing into many forms of phthisis. The majority of these initial lesions are in subapical and apical location. The origin of these lesions is much debated whether they represent an exogenous super infection or whether they represent an endogenous exacerbation with bronchial dissemination of apical foci, the remnants of early haematogenous dissemination. Up to recent years it was however very clear that phthisis had no direct association with the primary pulmonary focus. Recently however it would appear that when primary infection is delayed until adult life the primary focus may lead directly to phthisis.

In the bovine where phthisis is also the commonest form of the disease there have been no reports regarding the origin of the pulmonary lesions.

ORIGINAL OBSERVATIONS

Material from 350 cases of bovine pulmonary

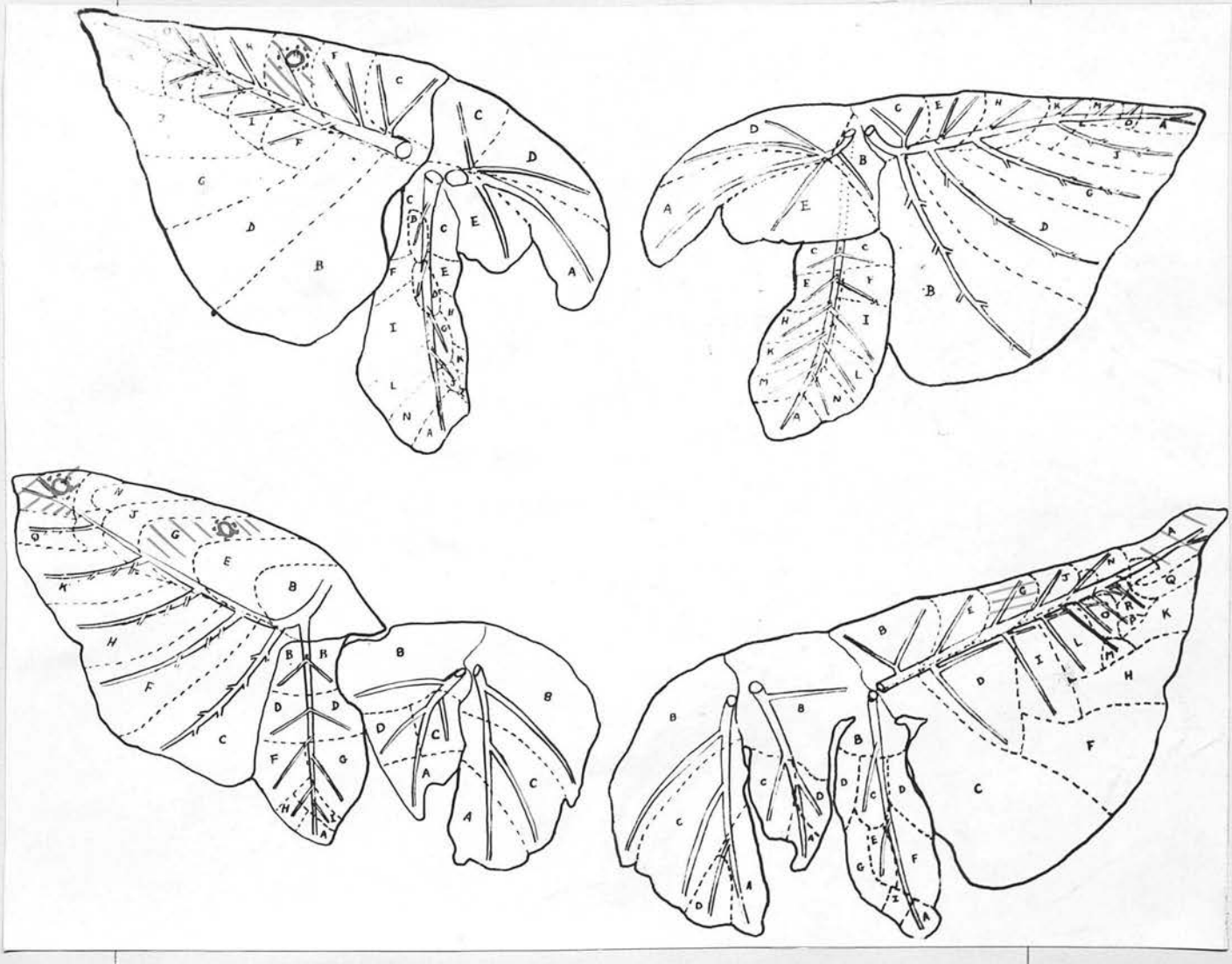
tuberculosis was obtained from Edinburgh abattoir, no history being available. As shown by Stamp (1948) 100 of these cases had lesions confined to the thorax, the remainder having more disseminated lesions throughout the body. Of the 100 cases with lesions confined to the thorax 89 were shown to have recognisable primary lesions in the lung and of these 61 showed definite evidence of bronchial spread with development of varying degrees of bronchial pneumonia. Of the 250 cases where lesions were more disseminated the primary focus could only be demonstrated with comparative certainty in 54 cases since in the remaining cases the pulmonary tuberculosis was greatly advanced. In those cases where the primary focus was determined it was quite clear that it frequently caseated, liquified and excavated so that intrabronchial aspiration followed giving rise to bronchopneumonia, and repetition of the same process gave rise to further areas of bronchopneumonia in newly involved parts of the lung. In this way progressive and destructive tuberculosis of the lung continued. The determination of the broncho-pulmonary segments of the bovine lung by Stamp (1948) made it comparatively easy to determine the route of spread which gave rise to the pneumonic areas. Examination of the cases of pulmonary tuberculosis, using the broncho-pulmonary segment as the basis of recording, made it very obvious that the pneumonia was even in advanced cases a segmental broncho-

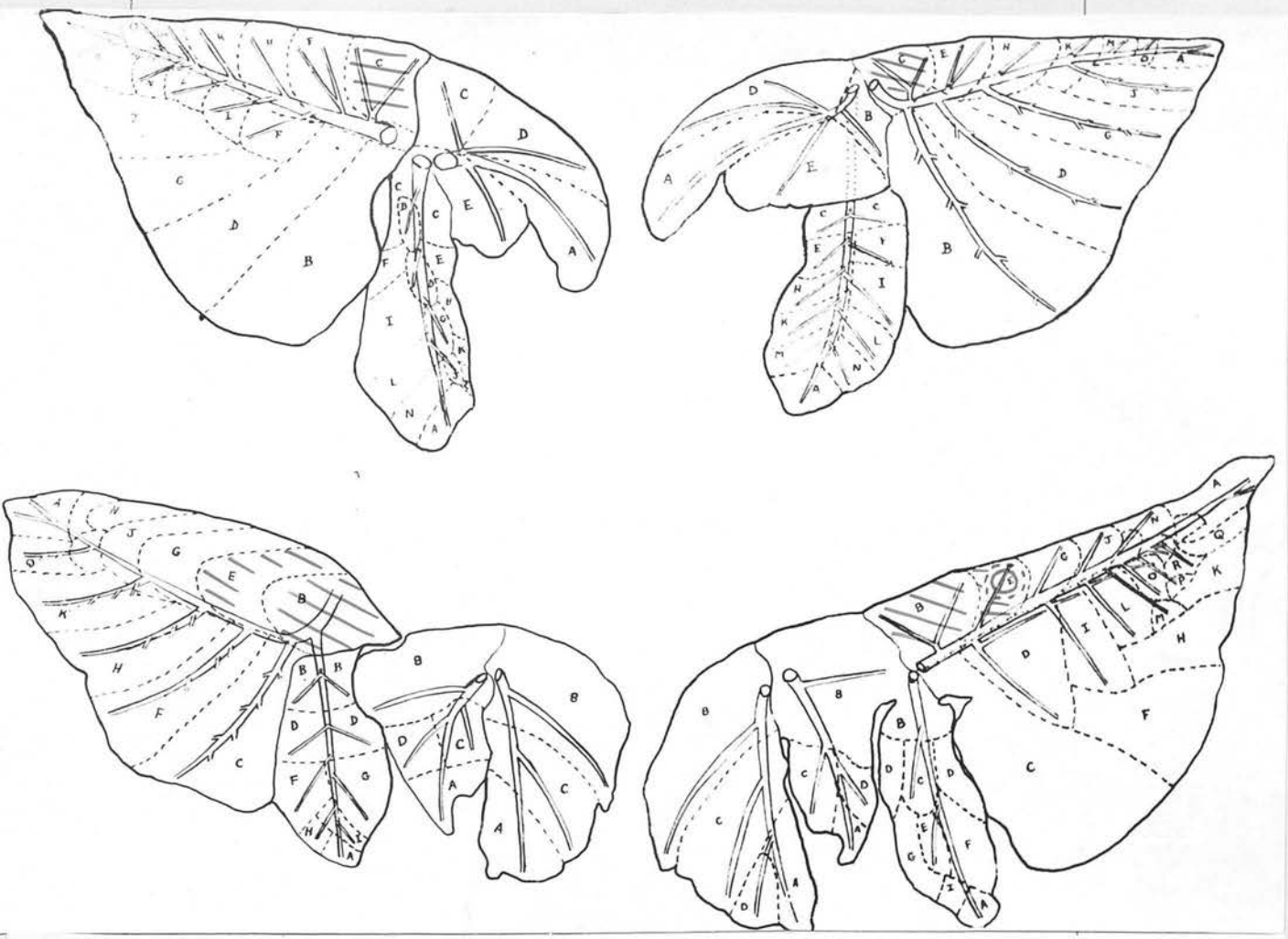
Diagrams 1 - 19

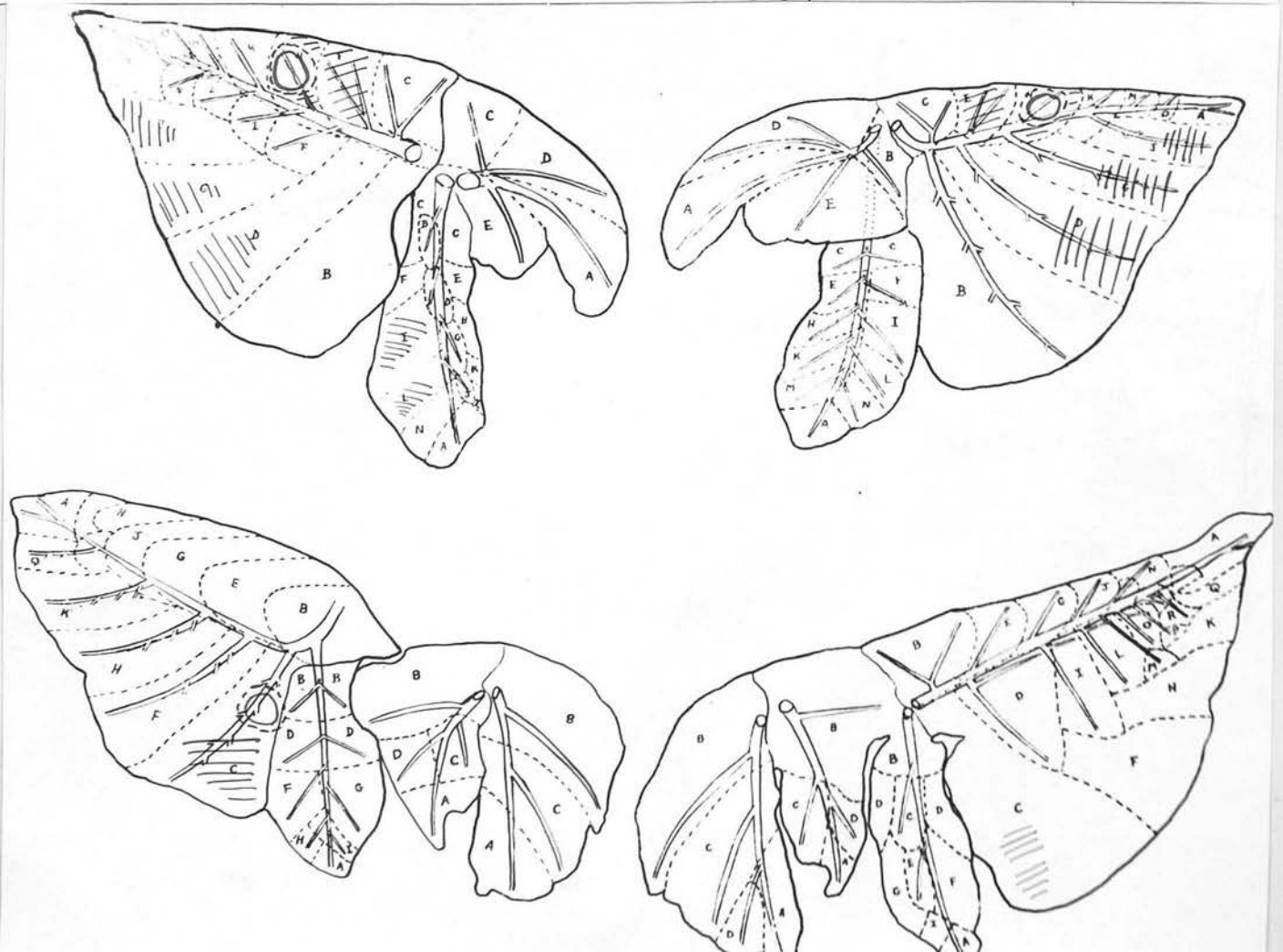
The following diagrams demonstrate the usual method of develpoment of phthisis in the bovine by bronchogenic progression of primary lung foci.

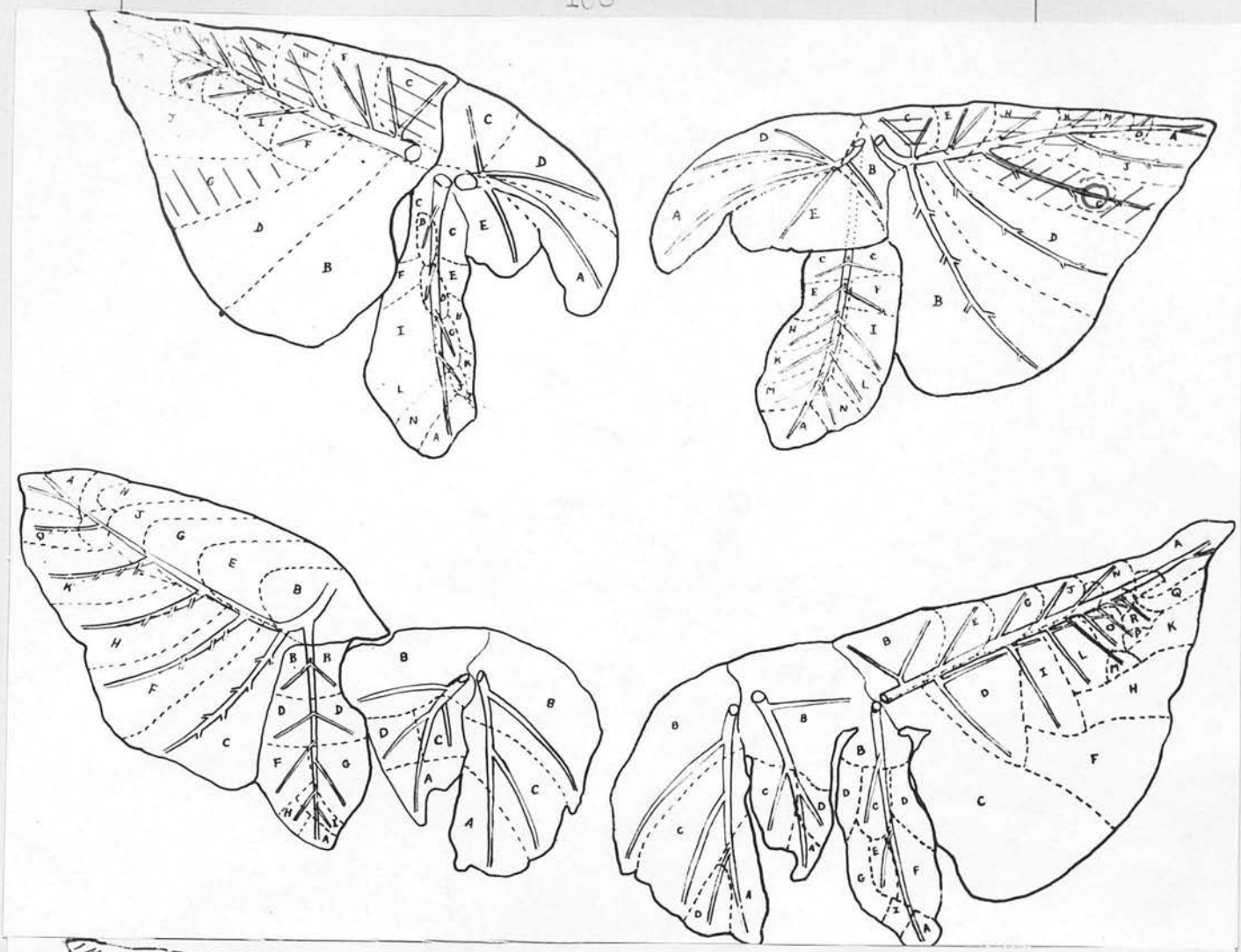
☼ --- primary focus

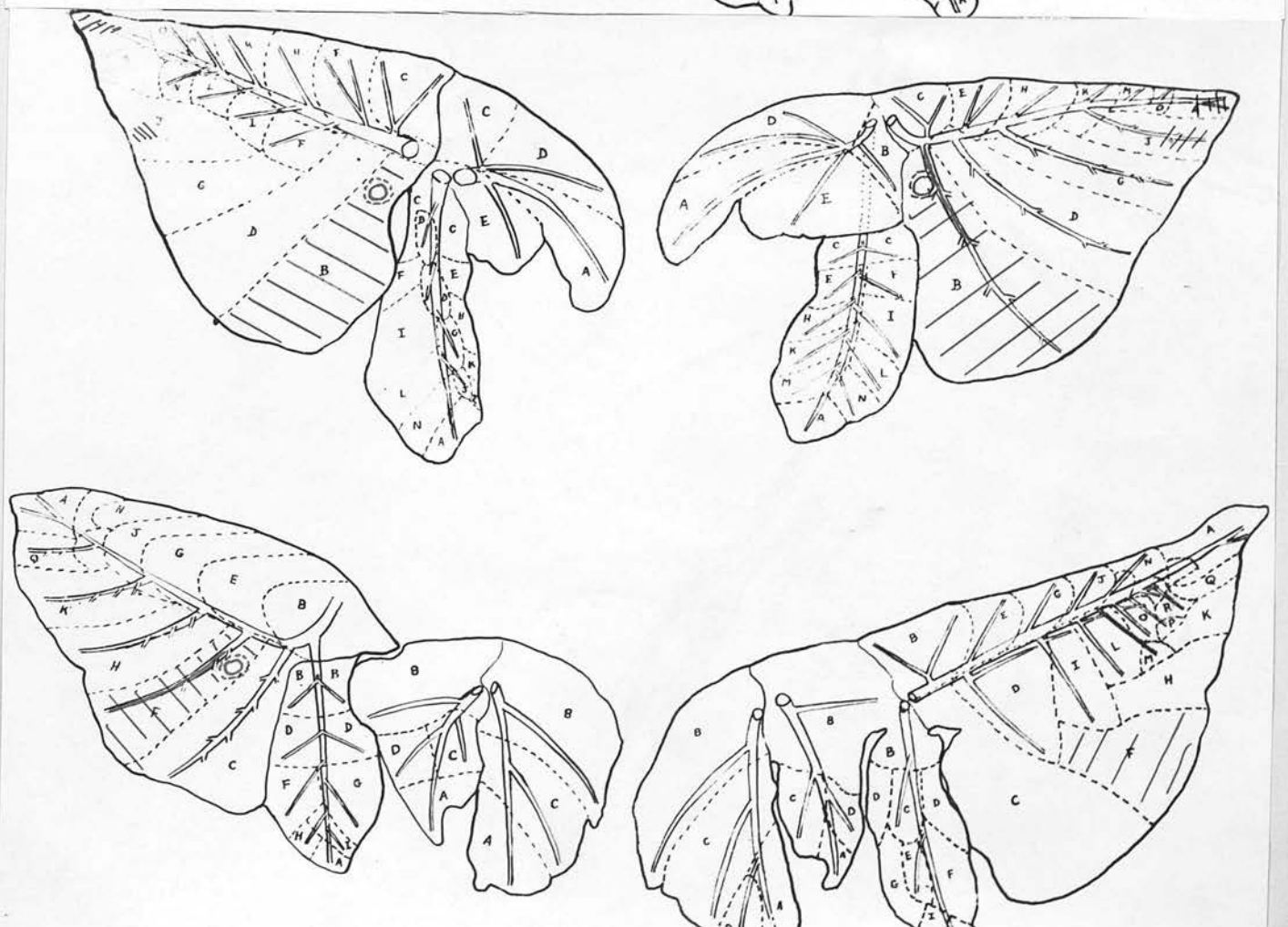
//// --- progressing lesions of tuberculous bronchopneumonia (phthisis)

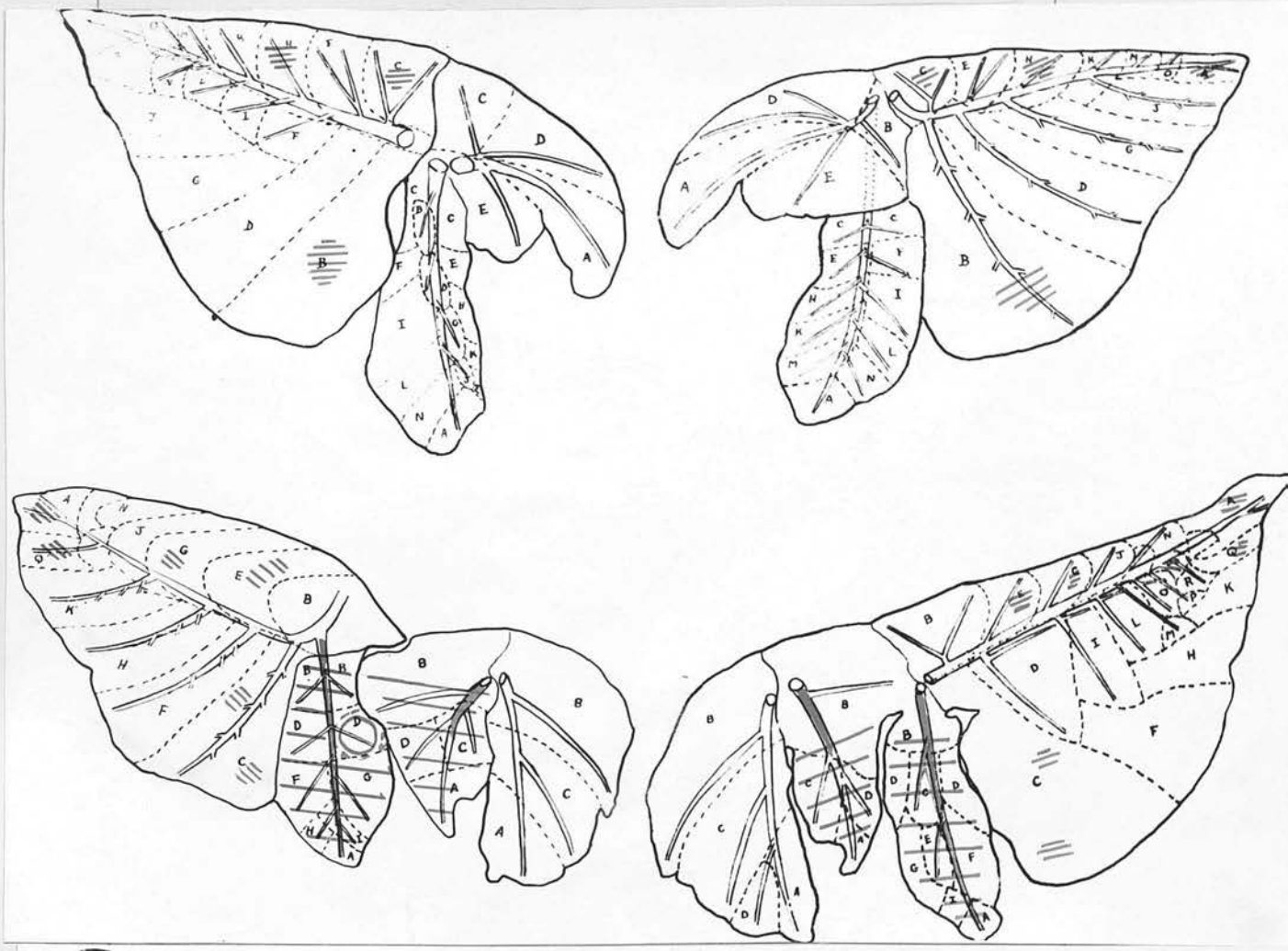


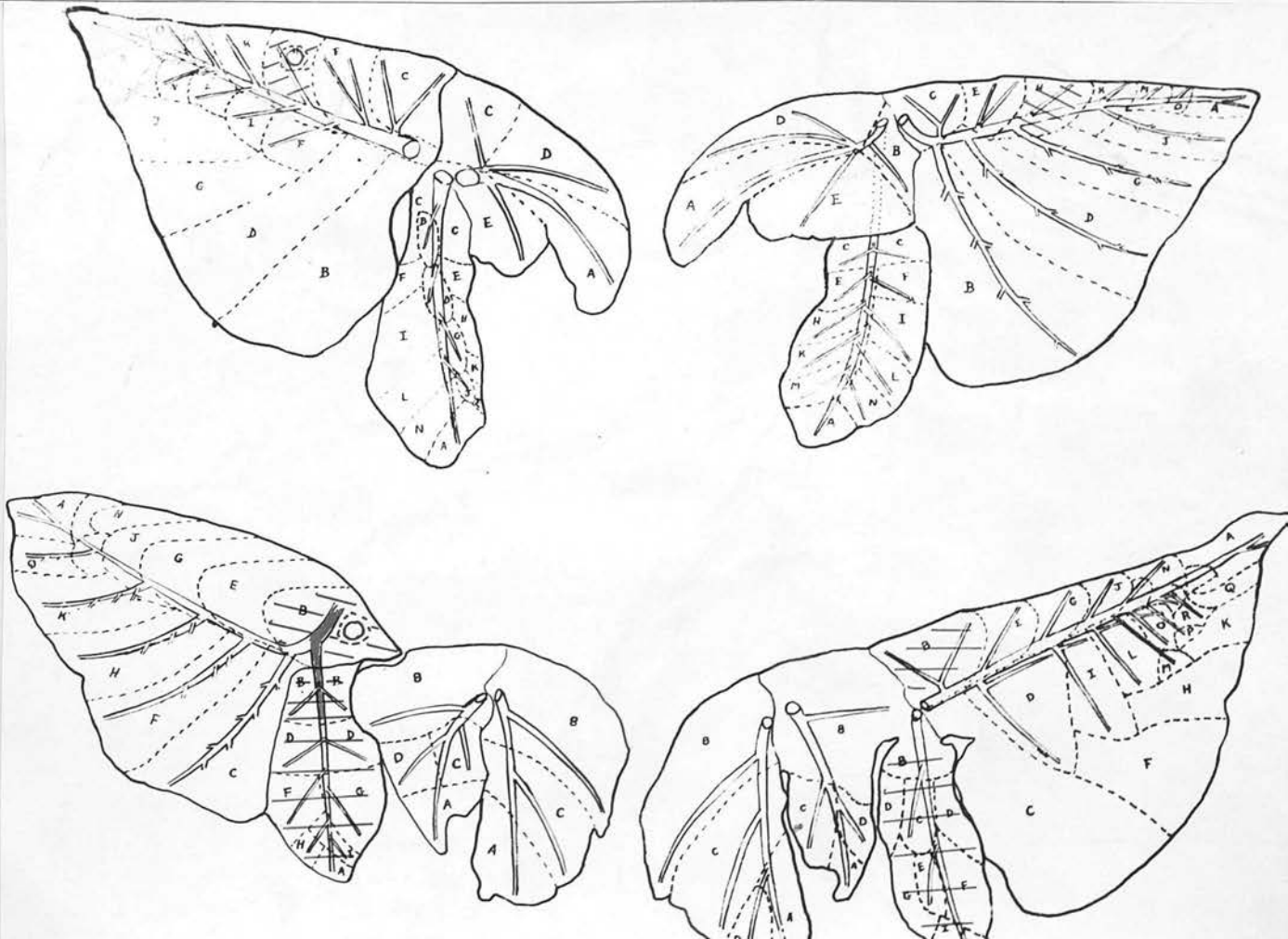


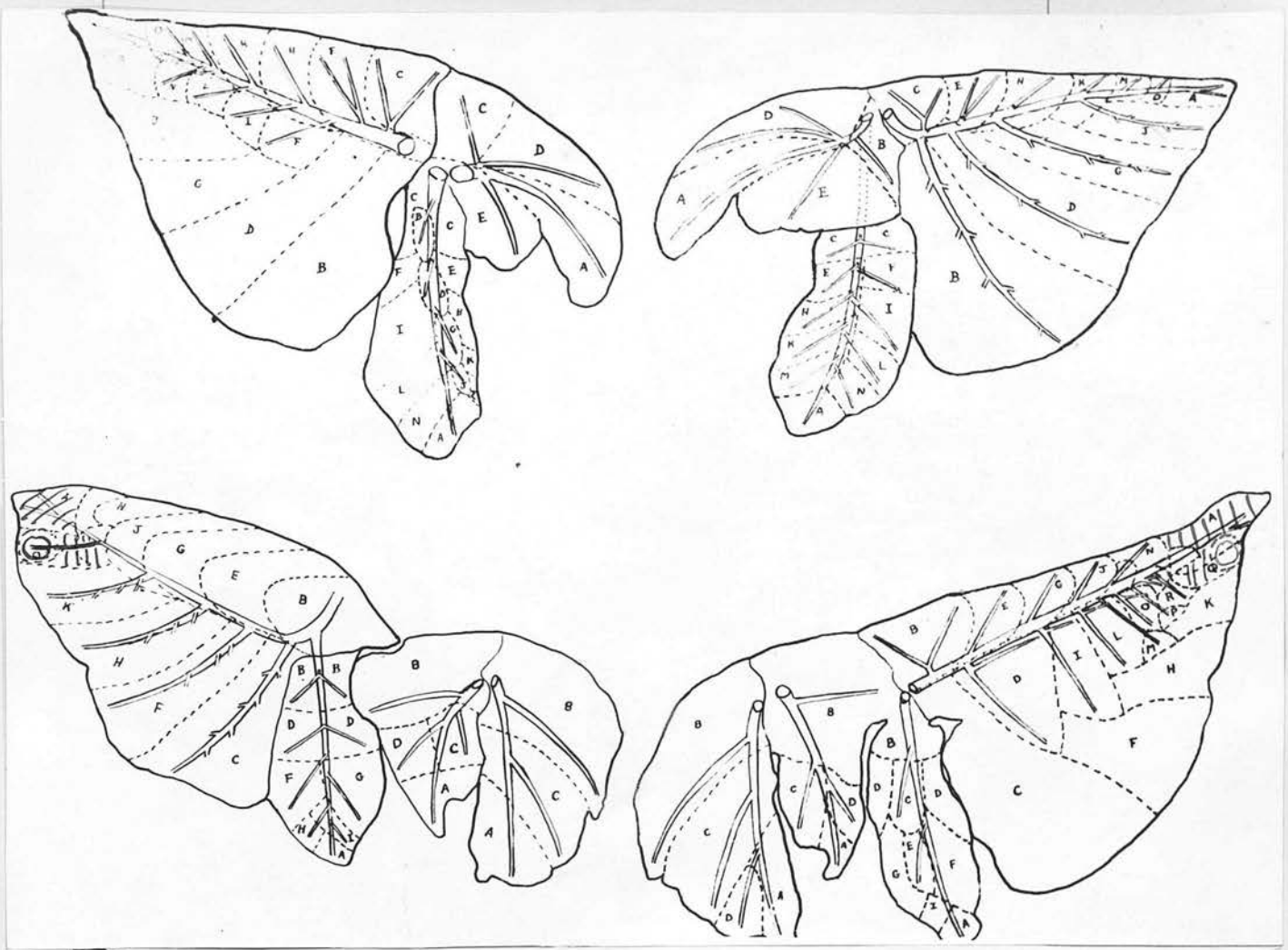


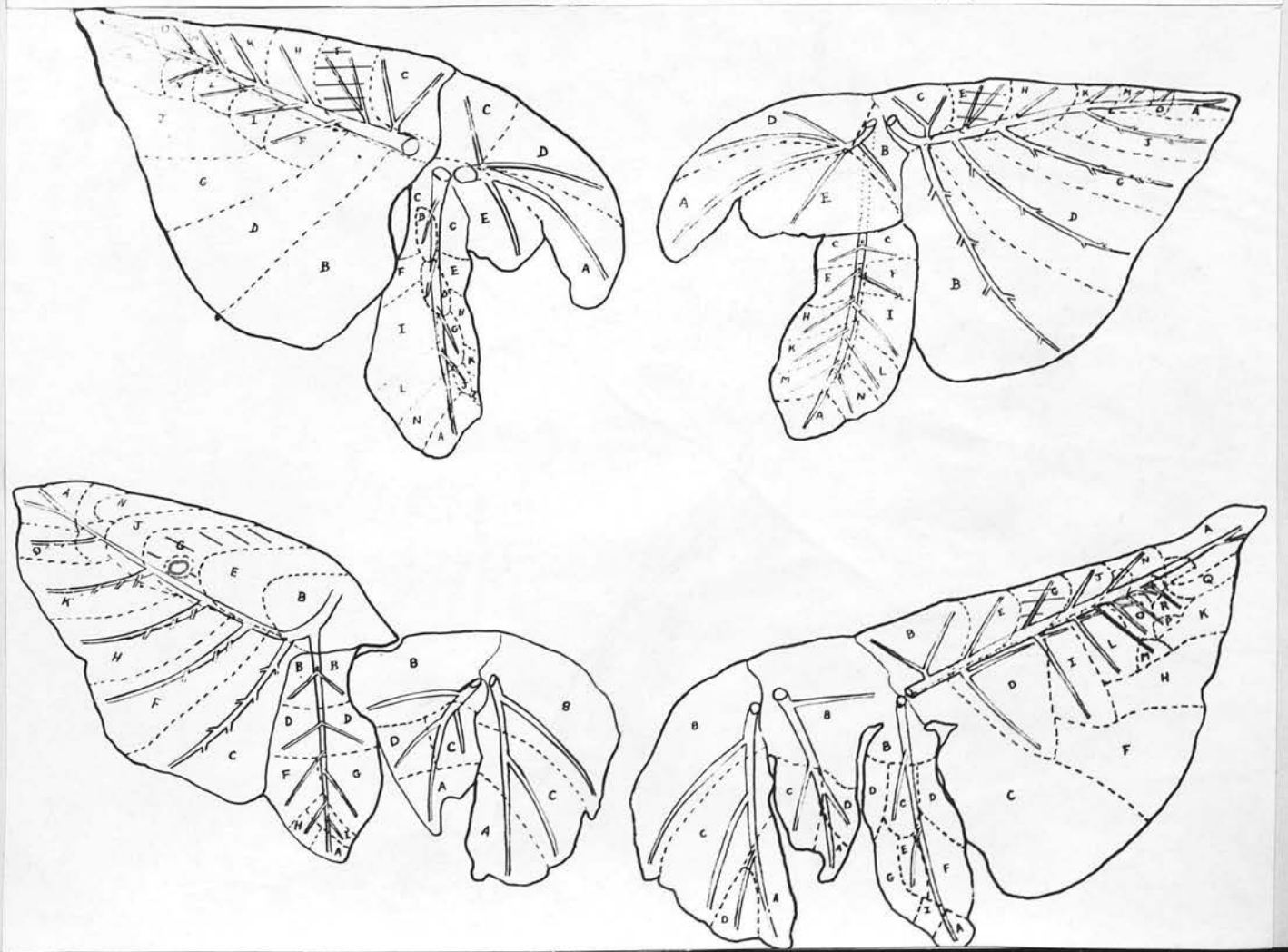


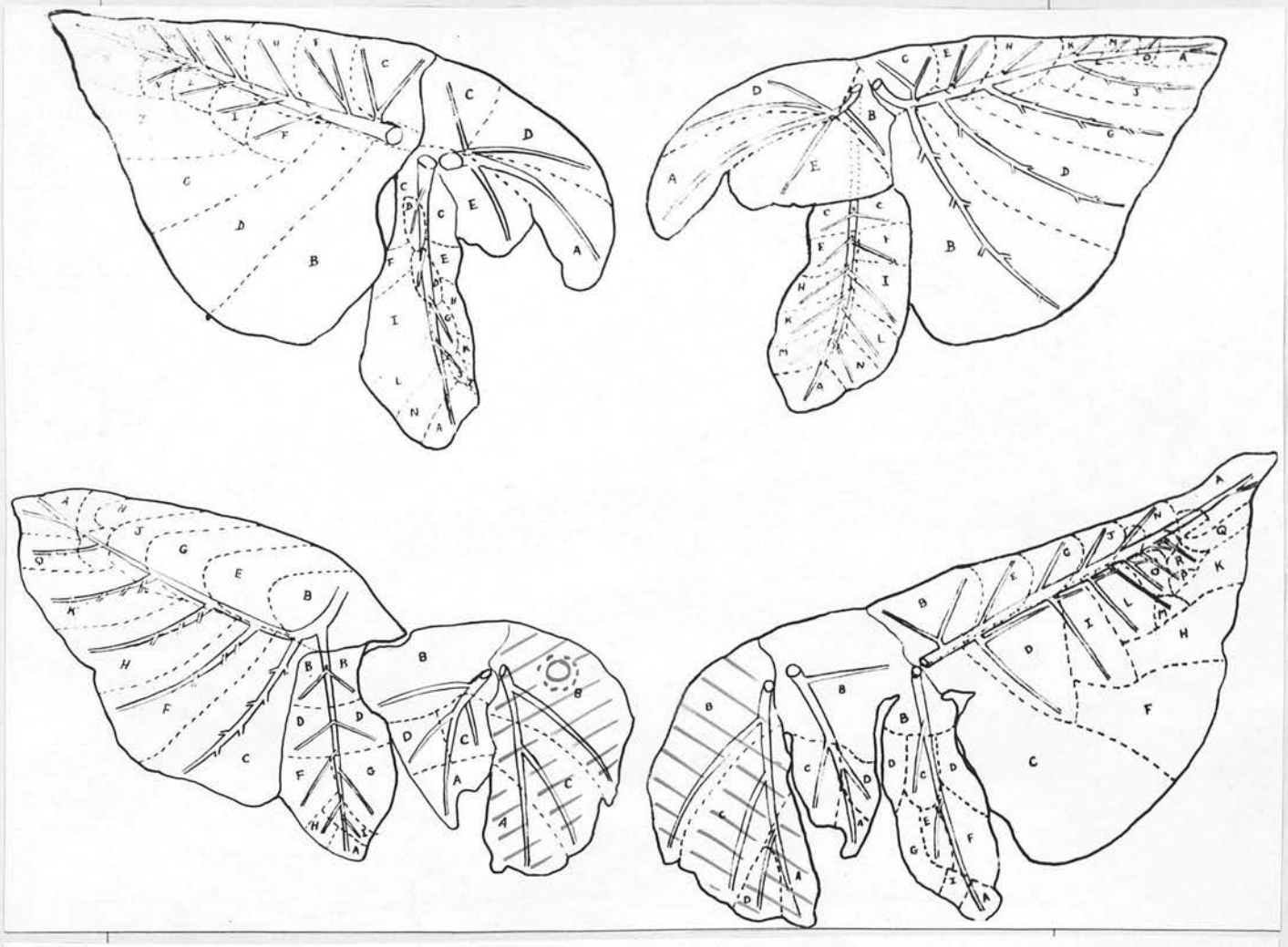


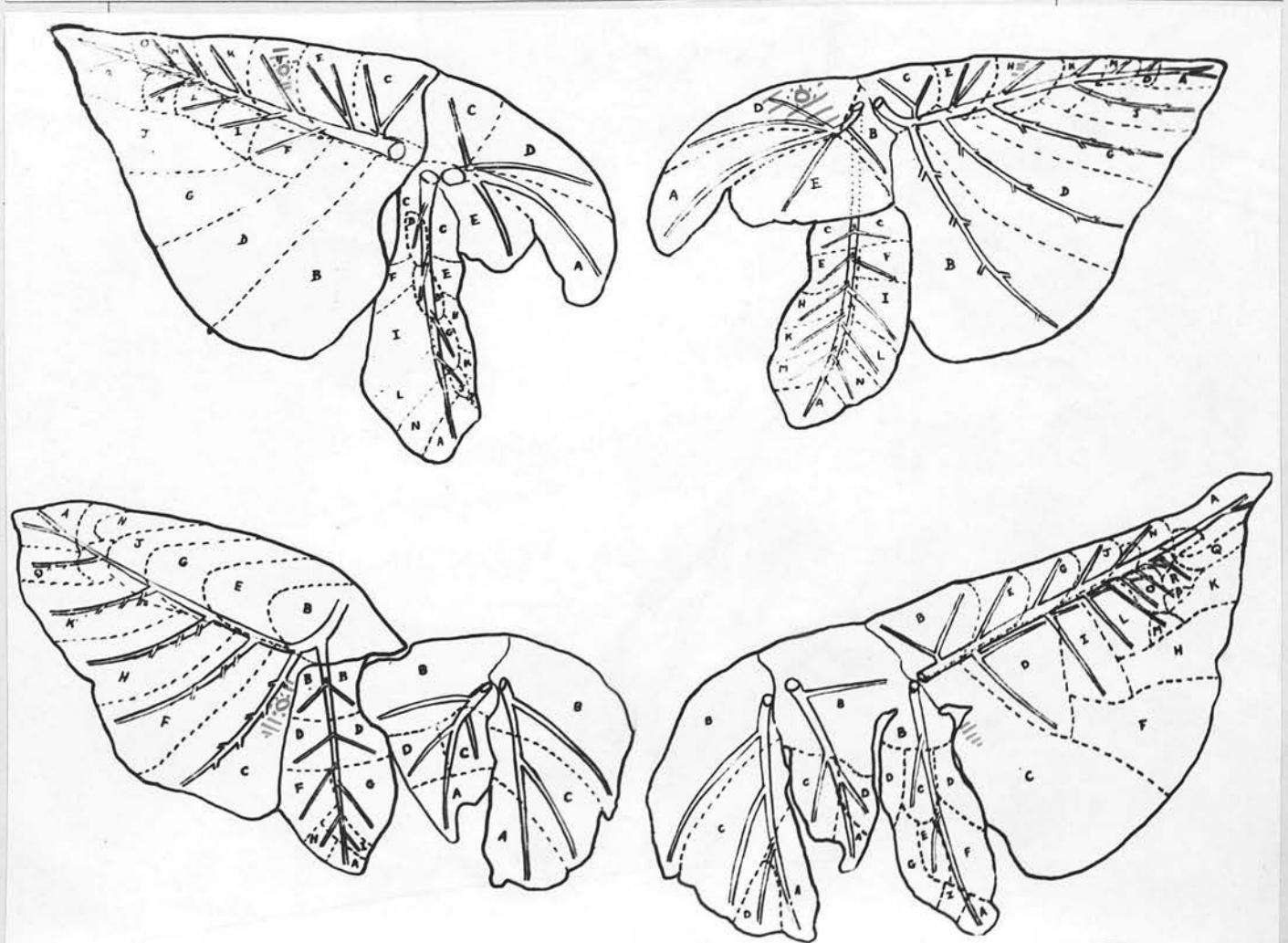


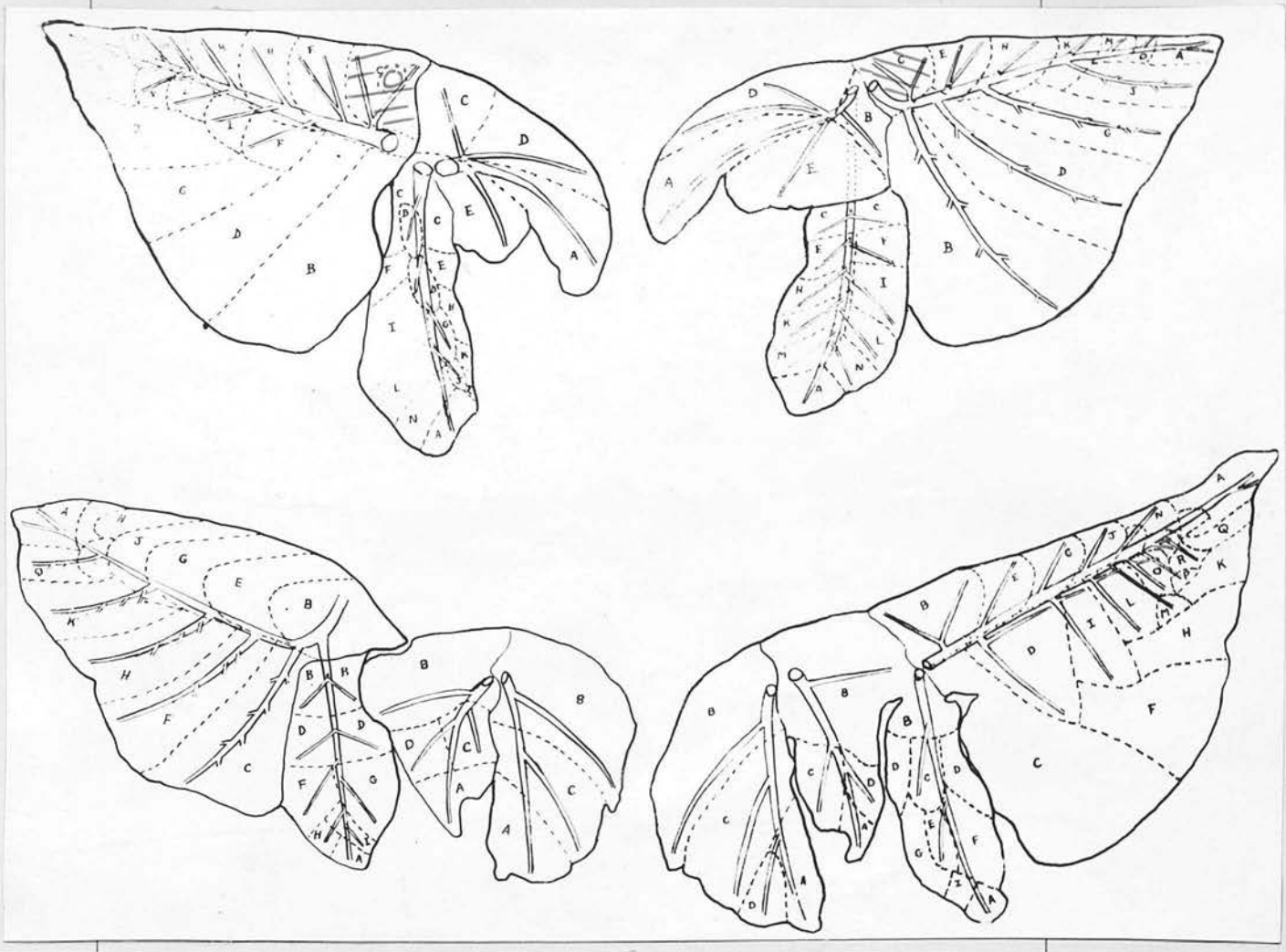


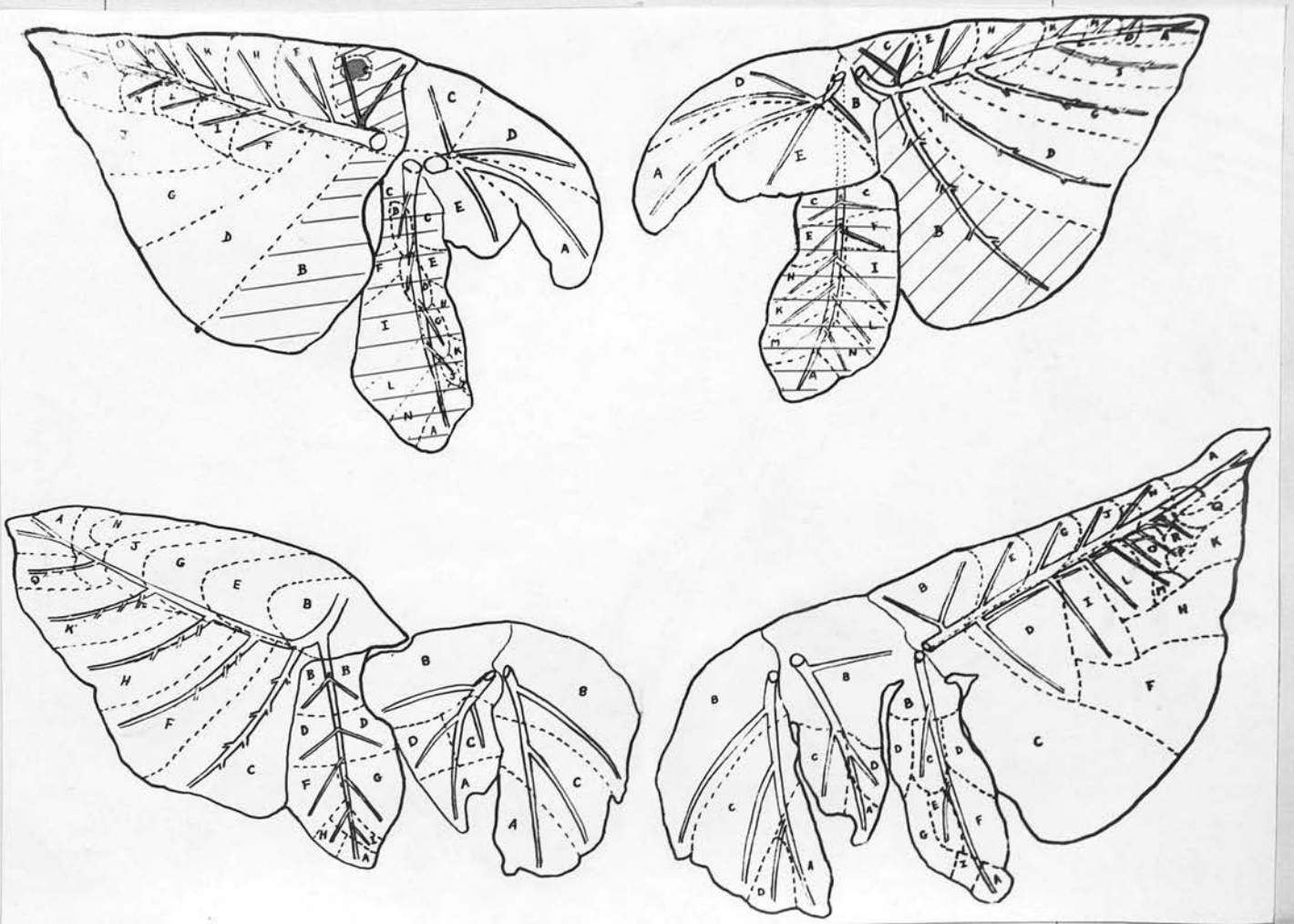


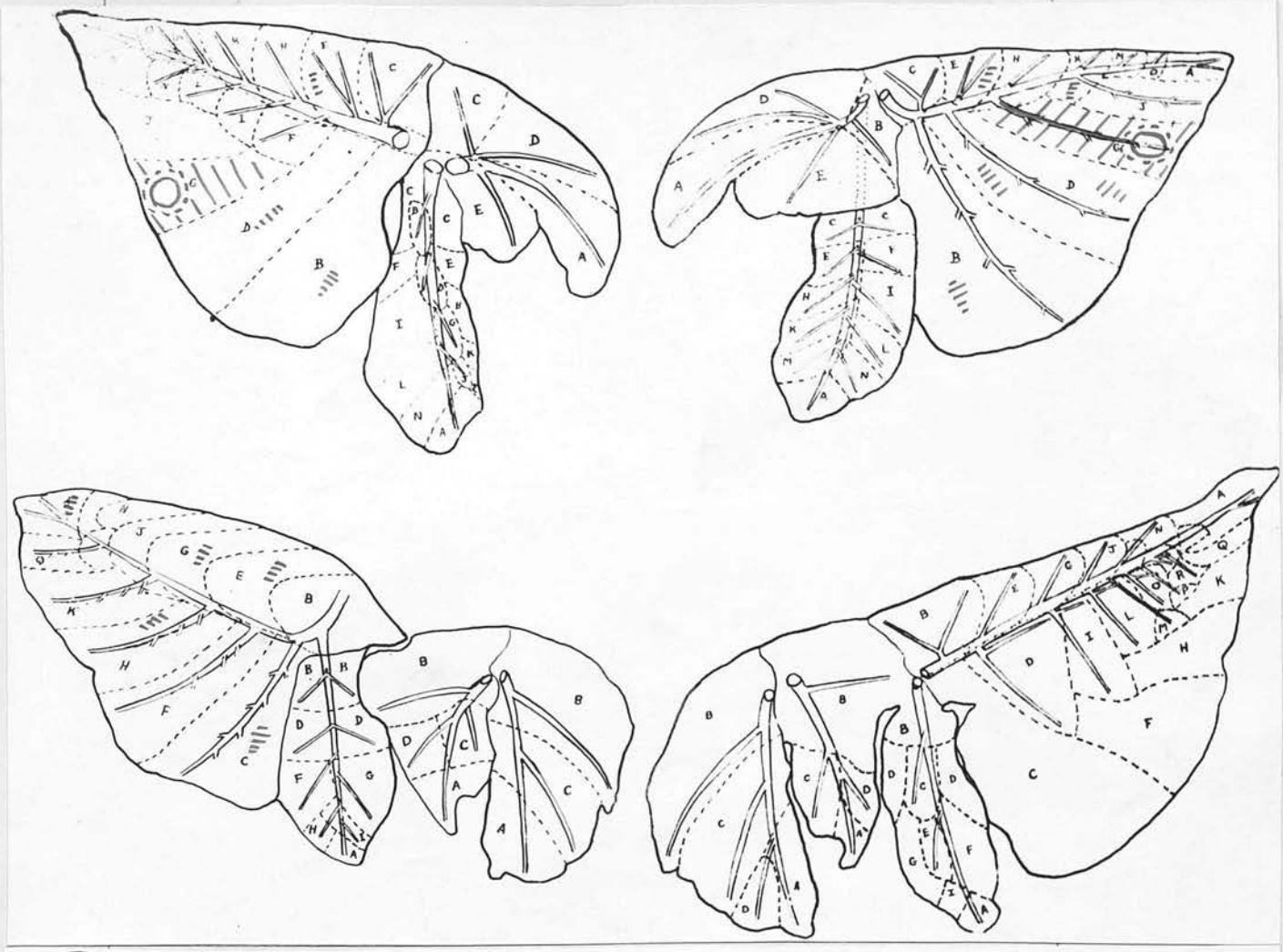


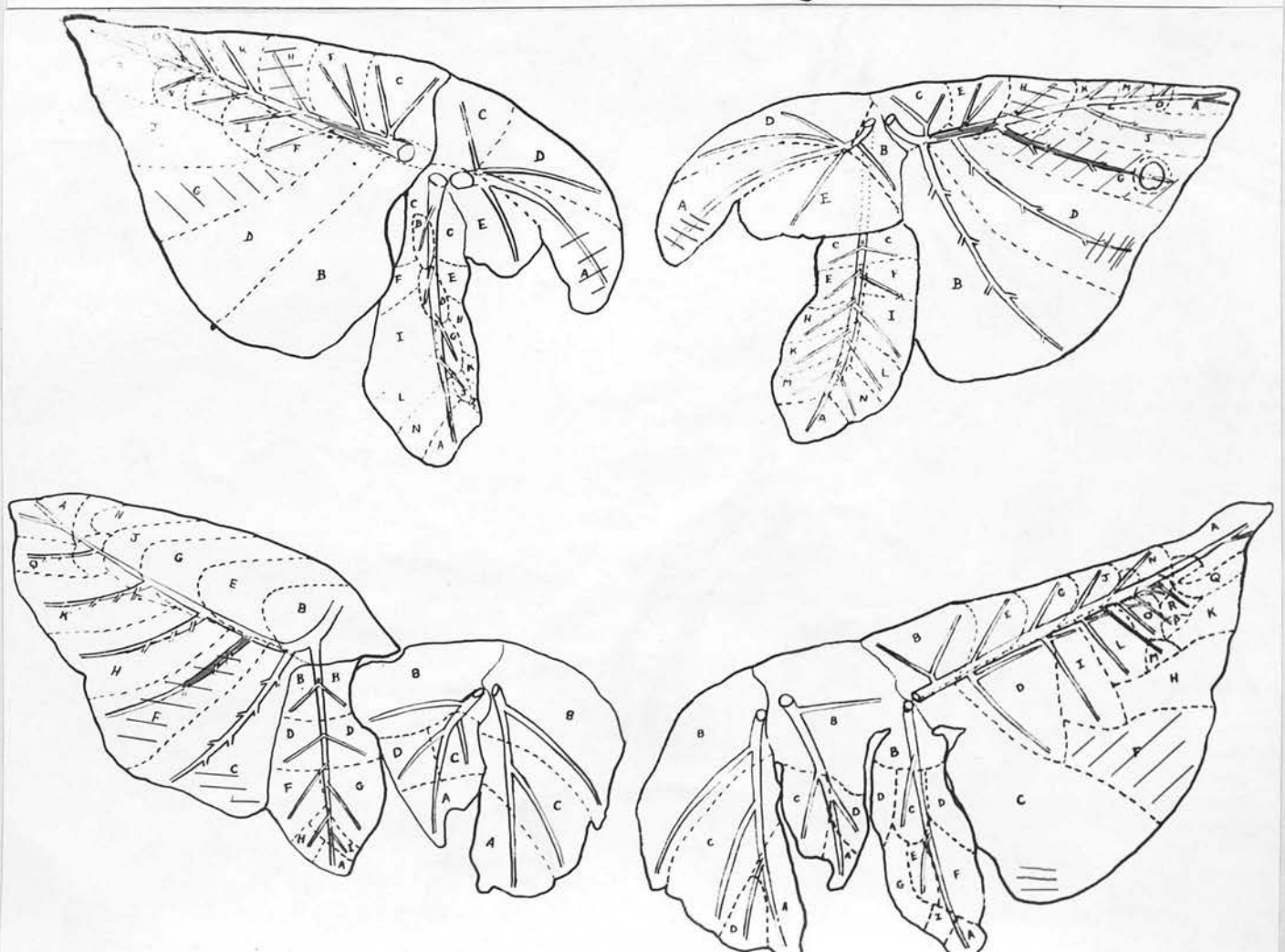


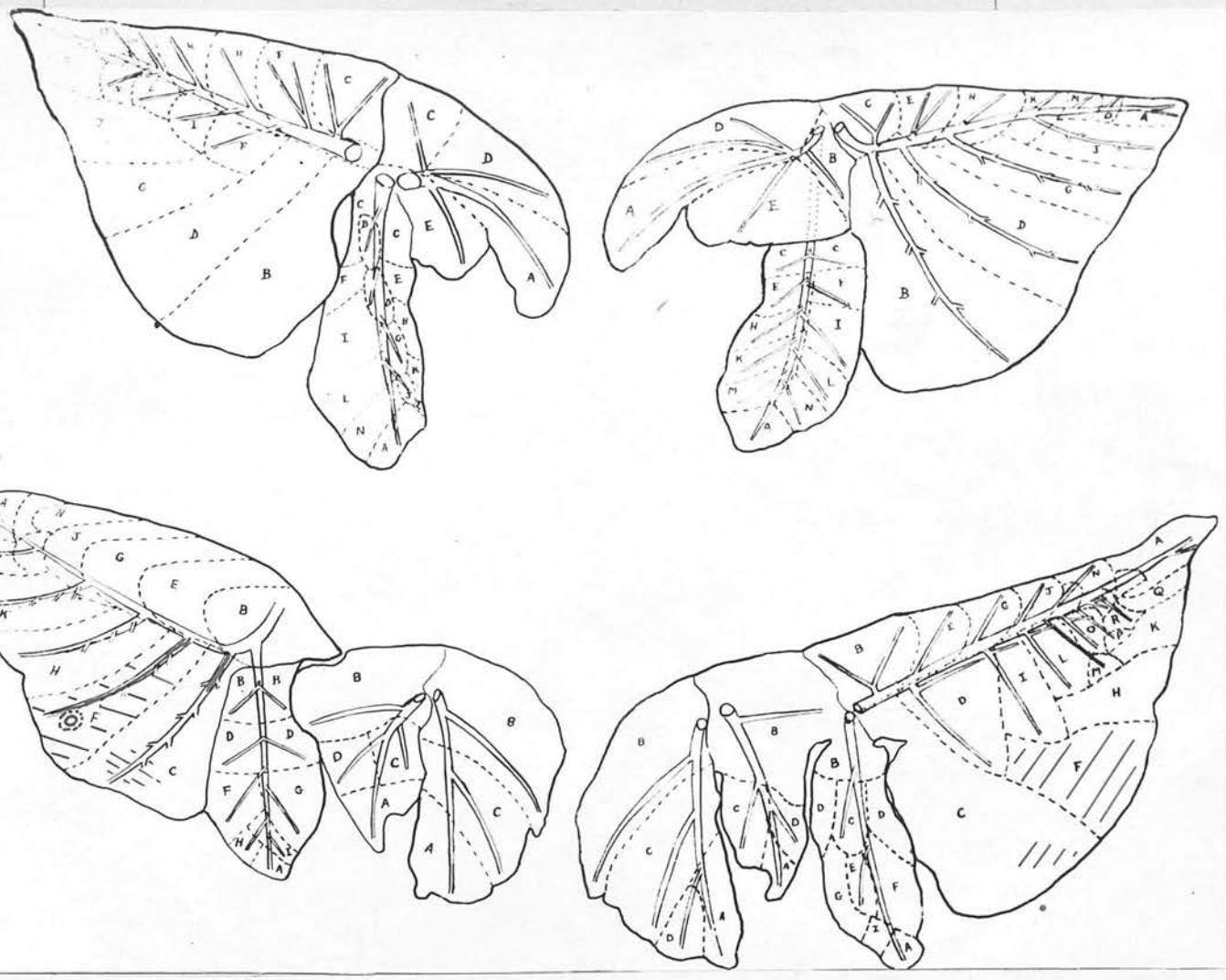


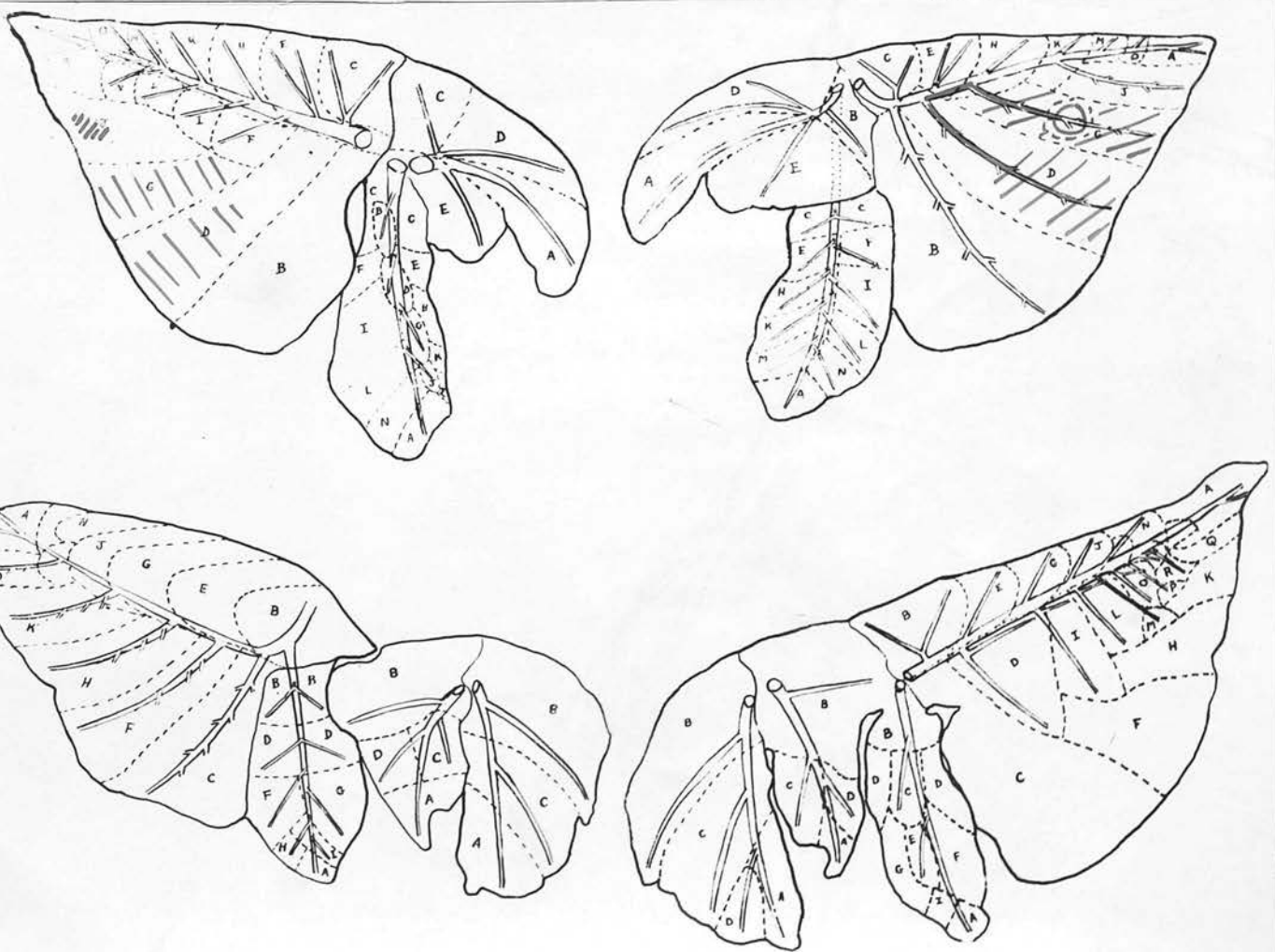


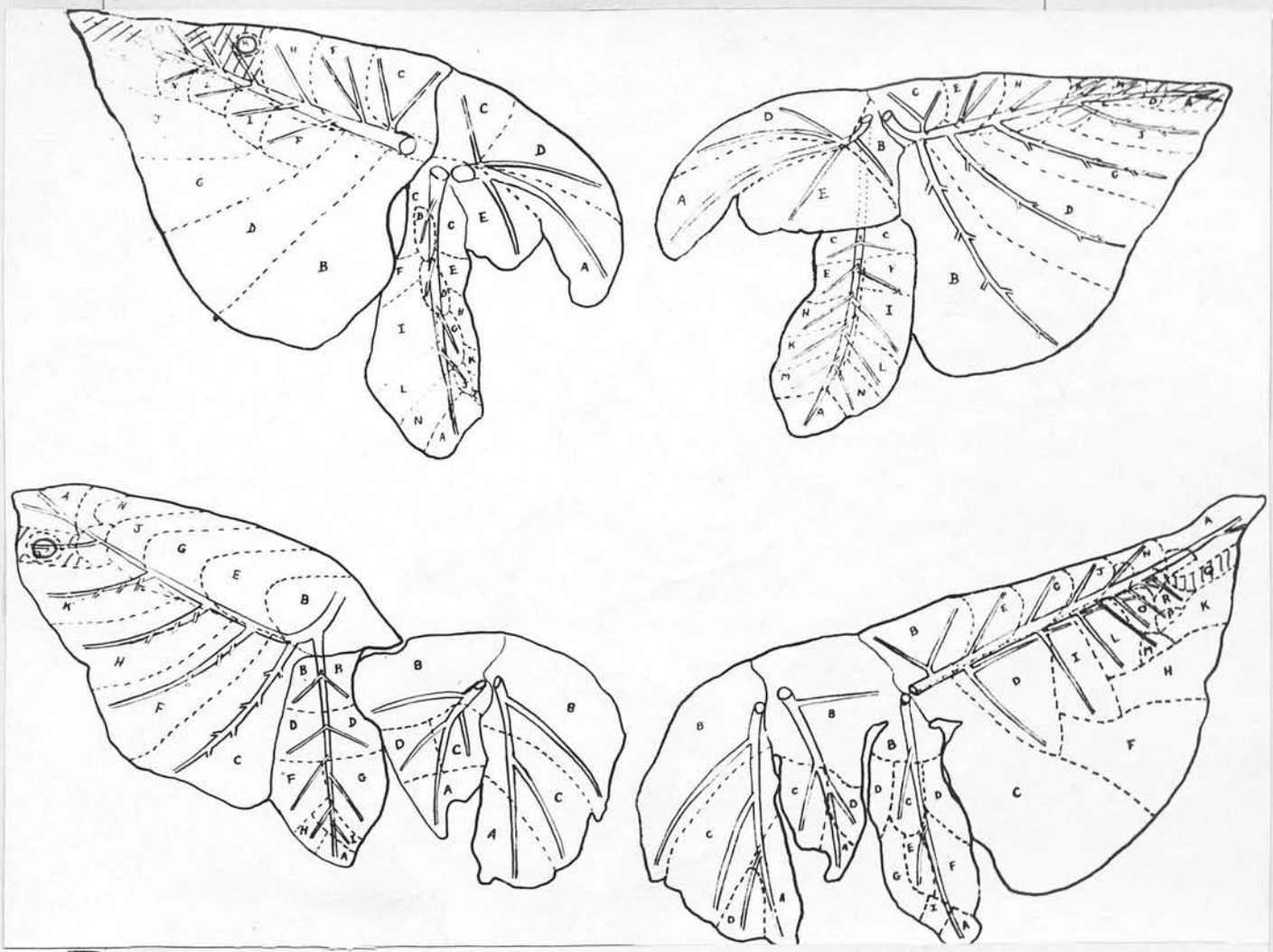


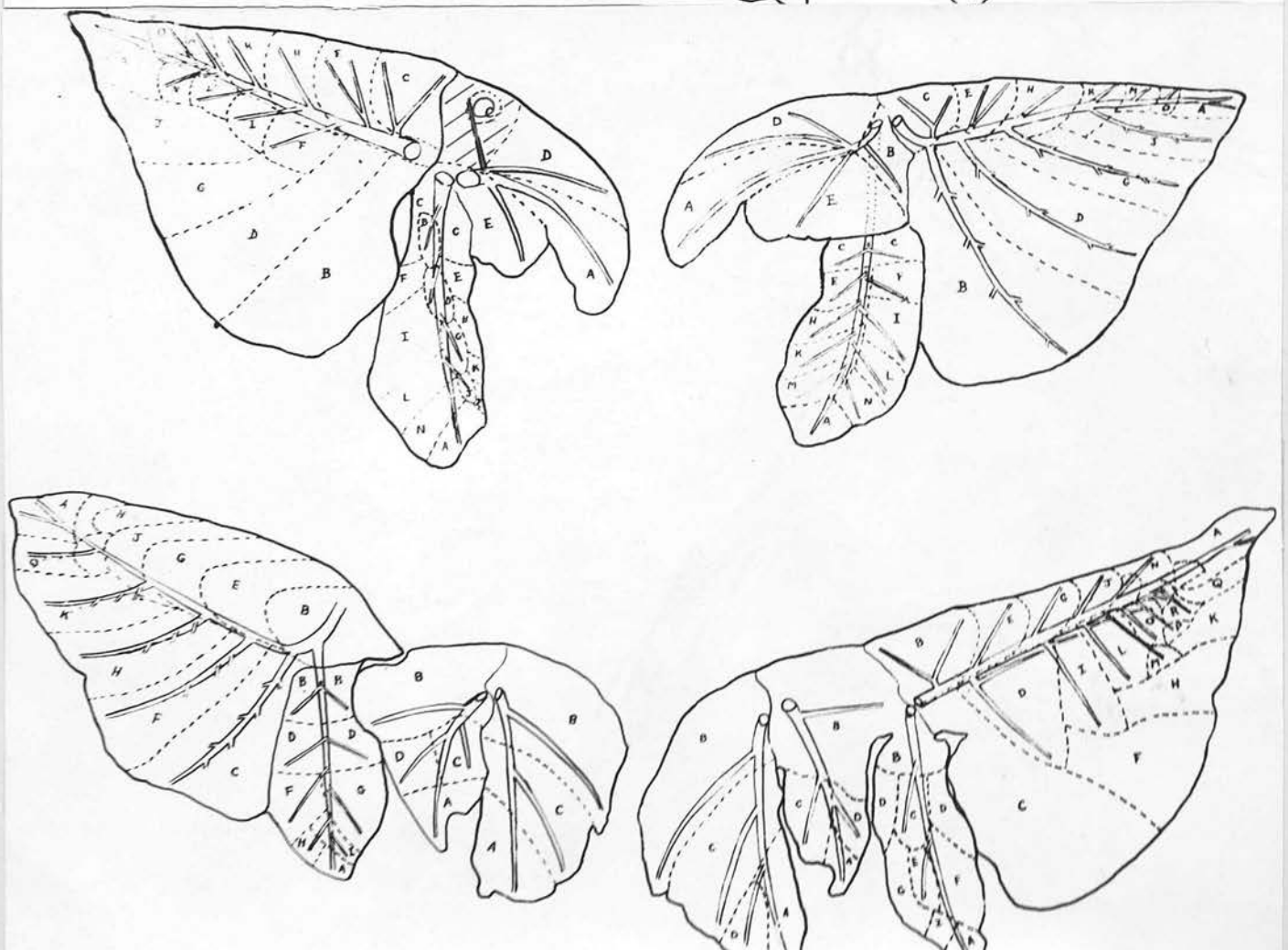












pneumonia neighbouring broncho-pulmonary segments becoming successively involved and showing more recent lesions. Diagrams 1 - 19 are representative of the method of dissemination of pulmonary tuberculosis from the primary infecting focus. It was found that such pulmonary lesions could be divided into the following types.

Caseous acinar bronchopneumonia.

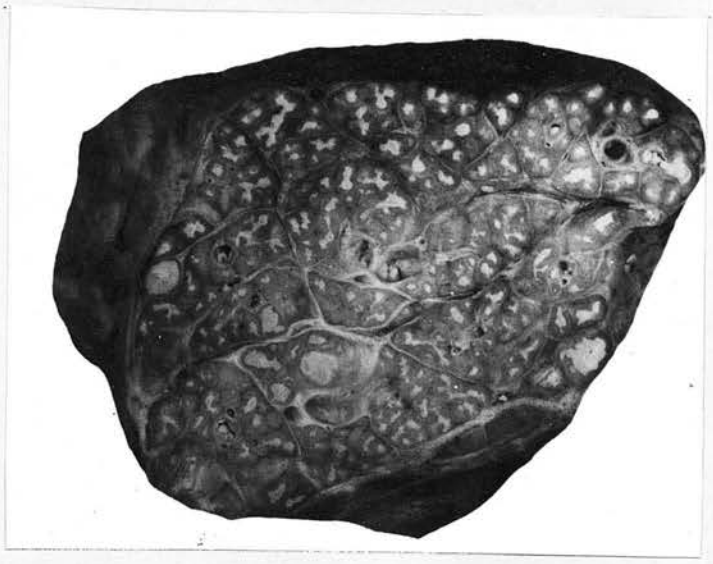
These lesions consist of a number of small regular caseous foci each surrounded by a zone of granulation tissue lying close together within a primary lung lobule. Depending upon the degree of bronchial infection either one, several or all the lobules of a broncho-pulmonary segment may be involved. (Figs. 1 and 2.)

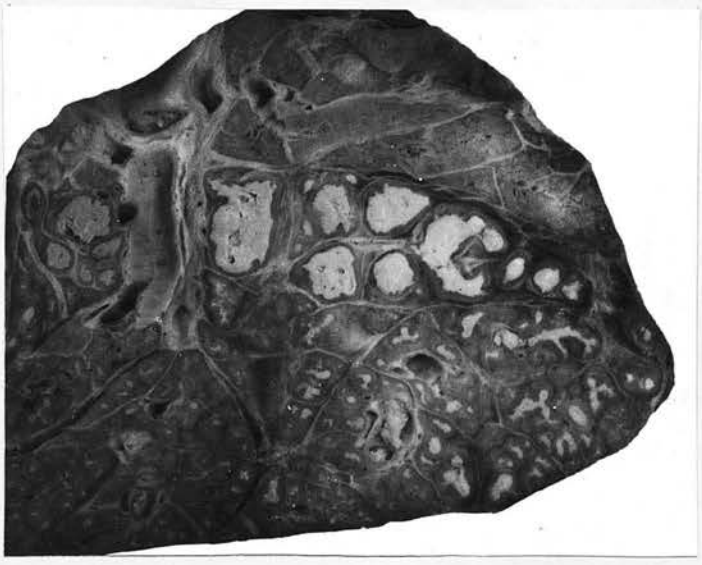
Caseous lobular bronchopneumonia.

This lesion is merely a progression of the previous type of lesion in which the several small caseous foci within a lobule coalesce to form a caseous lobule surrounded by tuberculous granulation tissue and condensed lung tissue. (Figs. 3 and 4.) Frequently these caseous lobules become surrounded by dense fibrous tissue. (Figs. 5 and 6.)

Chronic caseous pneumonia.

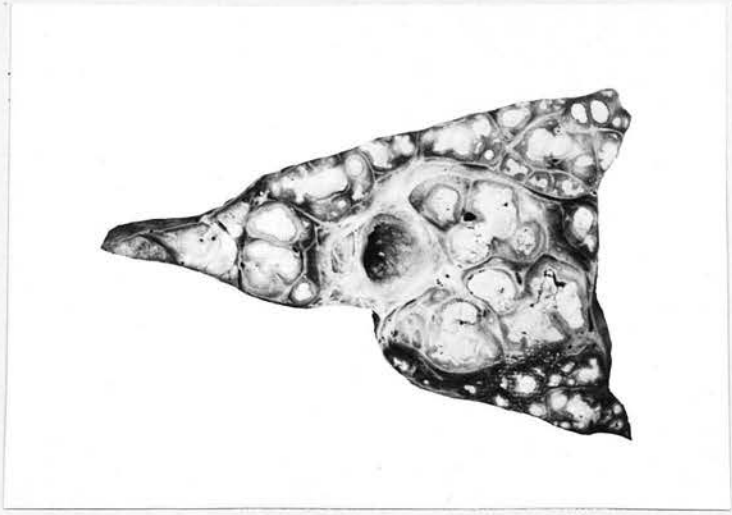
These lesions although still conforming to the major broncho-pulmonary segments consist of considerable irregular areas of caseous material, the existing lobular structure of the segment having been obliterated. Surrounding fibrous tissue formation

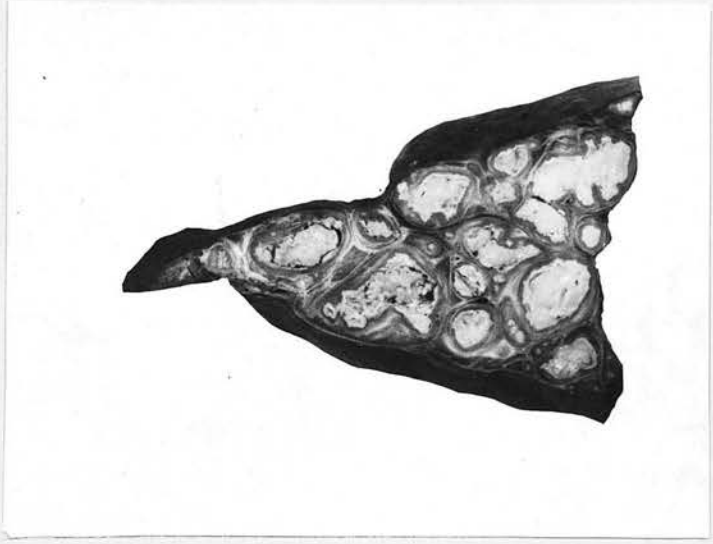




Figs.1&2. - Caseous acinar broncho pneumonia arising from massive bronchial dissemination.

Figs.3&4. - Caseous lobular broncho pneumonia.





Figs.5&6. - Chronic caseous lobular broncho pneumonia.

is often considerable. (Figs. 7 and 8.)

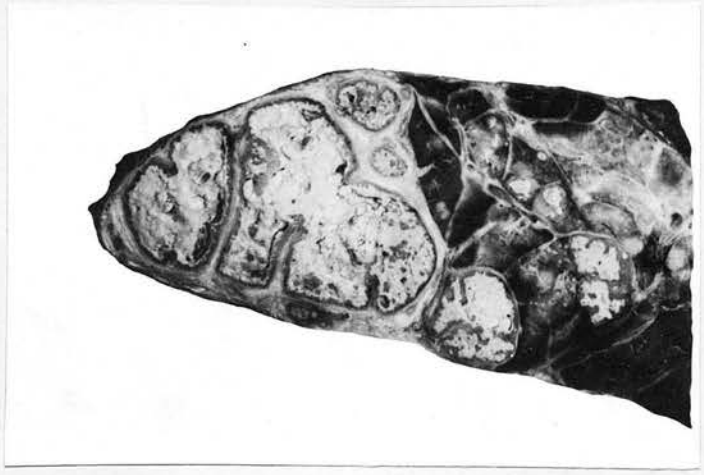
Cavities.

These develop as a result of the liquefaction of the caseous lobular lesions in the lung, the softened caseous material being discharged along the draining bronchus although the drainage is usually incomplete. The cavities vary in shape and size following the outlines of the caseous lesions which are softening. (Figs. 9, 10 and 11.) Haemorrhage has not been found in any cavity. Frequently the epithelium of the associated bronchus appears to extend down into the forming cavity so that on microscopical examination the cavity is seen to be lined with a type of stratified epithelium giving the appearance of a bronchiectatic cavity. Tuberculous ulceration of the draining bronchi extending to the major bronchi and even to the trachea is frequent.

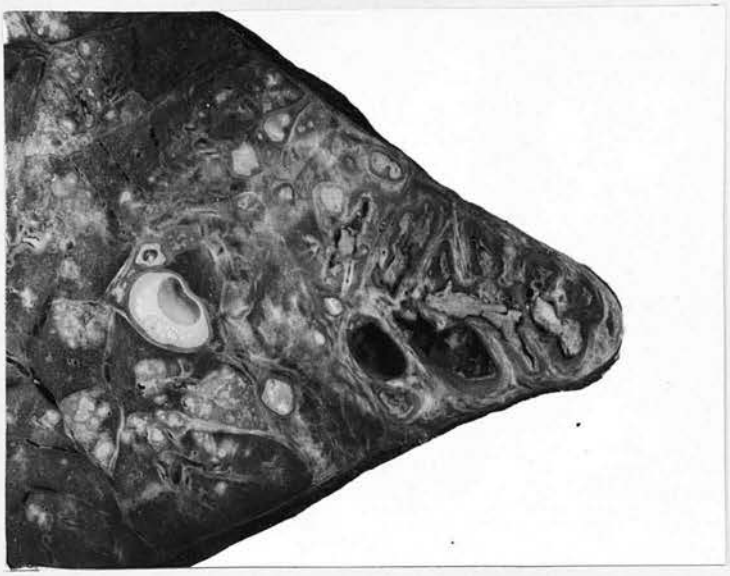
These varying pictures seen in the tuberculous bovine lung although varying considerably in appearance are all similar in that they merely represent variations of segmental-bronchopneumonia.

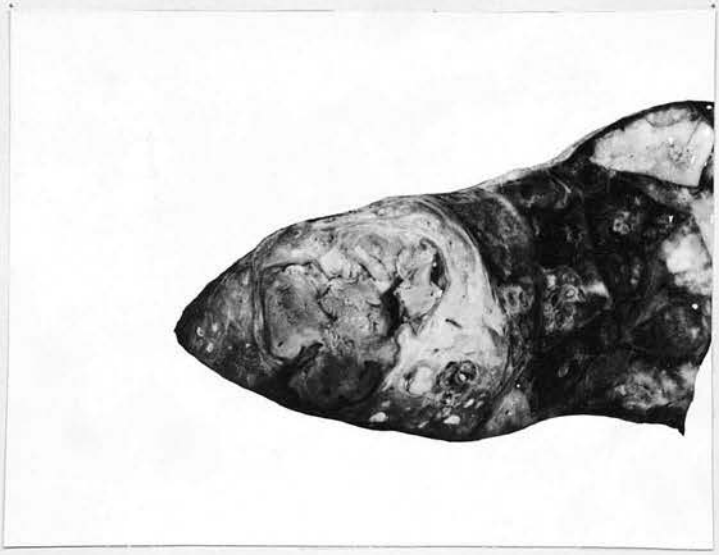
Pleurisy.

Tuberculous pleurisy is a common lesion in the bovine and in many cases this lesion arises early after primary lung infection by direct lymphatic drainage from the lesion to the pleura with subsequent direct spread over the pleura. Tuberculous pleurisy has not been seen to arise by direct rupture of a lung or lymph gland lesion.



Figs.7&8. - Chronic caseous pneumonia.





Figs.9&10. - Chronic cavity - essentially bronchogenic in origin.

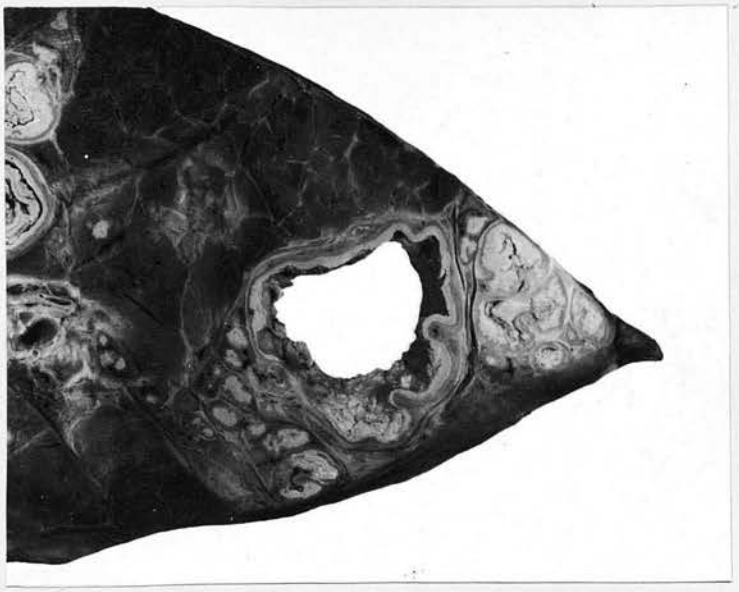


Fig.11. - Chronic cavity - essentially bronchogenic in origin.

SUMMARY

The pathogenesis of bovine pulmonary tuberculosis (phthisis) is discussed on the evidence given by 350 post mortem examinations of tuberculous cattle. This is the first attempt to determine the origin of the lesions of bovine phthisis.

Original for bovine, not for man.

TUBERCULOSIS OF THE BOVINE UDDER

J. T. STAMP

(Jn. Comp. Path. & Ther. 1943,
Vol. 53, No. 3)

INTRODUCTION

Tuberculosis of the bovine udder is of great importance on account of its connection with milk hygiene. The present work has been done in the hope that it will help to some extent in the diagnosis, both ante-mortem and post-mortem, of this condition.

The available literature dealing with tuberculosis of the udder is not large and reference to it shows some discrepancy of view as to its incidence, pathogenesis and morbid anatomy. M'Fadyean (1917), although discussing udder tuberculosis at some length, gives no indication as to its frequency in this country. According to Gofton (1926), abattoir statistics give the incidence as 1 to 2 per cent. of tuberculous cattle and later (1931) he states that 45 per cent. of the cases of udder tuberculosis occur in cattle in which the disease has not generalised. Nieberle (1930) states that the udder is the organ most commonly affected after the lungs, while Joest and Kracht-Palejeff (quoted by Hutyra and Marek) state that 25 per cent. of cases of generalised tuberculosis show udder lesions. According to Savage (1929), "veterinary data indicated that about 40 per cent. of cows in Great Britain were affected with tuberculosis, the great majority of these being

non-infective to the milk supply." Savage is of the opinion that probably less than 1 per cent. of infected cows in Great Britain developed 'open' tuberculosis.

It is generally accepted that infection of the udder is by the blood stream (Nieberle, 1930, and Ostertag, 1905). M'Fadyean (1917), after reviewing the literature, thinks that this view is erroneous and that the udder is usually infected from the lymph stream, giving as his reasons that the disease is often confined to one quarter, usually a hind quarter, that the lesions are rarely discrete tubercles, that the upper part of the quarter is affected first and that the supramammary lymph glands are usually involved before the udder.

As regards the morbid anatomy of the tuberculous lesions, M'Fadyean (1917) stresses the fact that tuberculous mastitis is not a nodular lesion.

The most complete account of the pathology of udder tuberculosis has been given by Nieberle (1930). He divides udder tuberculosis into the following three distinct types:-

(a) Disseminated miliary tuberculosis.

(b) Chronic udder tuberculosis.

(c) Acute caseating tuberculosis.

ORIGINAL WORK

The work undertaken was the examination, both gross and microscopic, of tuberculosis in the udder of the cow. Udders were taken from animals which, on slaughter, were found to be affected with fairly widespread tuberculosis. A pathological examination was carried out on 27 such udders and of these 12 were found to contain tuberculous lesions.

In each udder examined, whether obviously tuberculous or otherwise, the minimum examination comprised the following: (a) Detailed naked eye examination of both halves of the udder, by means of multiple slicings of the organ. (b) Smears from the cut surface of all quarters; stained by the Ziehl-Neelsen technique and examined for tubercle bacilli. (c) Similar examination, naked eye, of the supramammary lymph glands was carried out. (d) Tissue was taken from two large areas in each quarter of the udder. In most of the cases, however, many additional blocks were taken from all regions, particularly at various levels above the ampulla, so as to trace the changes in the larger milk ducts.

At least six blocks were taken from each of the two areas and examined by the following staining methods. (a) Haematoxylin and Eosin. (b) Mallory's connective tissue stain. (c) Ziehl-Neelsen's stain for acid-fast organisms. French's elastica stain was used in many sections, but not in

all.

It was found that tuberculous udder lesions could be divided by means of macroscopic and microscopic examination into the following distinct types.

TYPE 1. Acute Caseating (Cases 1 and 2).

Naked eye: There was present an extensive and widespread destruction of lobular tissue, with large areas of haemorrhagic necrosis, in which there might be no trace of calcification. Softenings occurred throughout the affected quarters with the formation of abscesses. Occasionally massive calcification was found. The supramammary lymph glands in one out of the two cases showed naked eye lesions of tuberculosis.

Histology: Microscopical examination showed a complete break-down of the normal gland substance in large areas. The lobules were no longer distinguishable individually since the tuberculous process had transgressed and destroyed the interlobular connective tissue septa. Groups of affected lobules were, however, still separated so that the gland was apparently made up of very large lobules separated by connective tissue. Each of these areas was made up, not of acini, but of necrotic and caseous material surrounded by a zone of tuberculous granulation tissue. Blood vessels were very numerous and haemorrhagic areas were commonly seen. Remnants of invaded lobules occurred here and there

between the lesions (Fig. 1).

In less affected areas the appearances, while of the same nature as described above, were less advanced and were explanatory of the evolution of the process. Rapidly proliferative and caseating lesions were seen spreading from focal centres of tuberculosis in the individual lobule. The lesions reached sometimes halfway, sometimes to the edges of the lobule, while still remaining restricted to the single lobule. Here and there affected lobules occurred in groups and the tuberculous process was becoming confluent, so as to produce the large caseating areas first described.

The interlobular ducts showed important changes. Where there were extensive lesions involving groups of lobules, the interlobular ducts were completely destroyed with the surrounding lobules. On the other hand, interlobular ducts which were free of the large masses, exhibited a chronic tuberculous lesion; their walls were thickened by granulation tissue formation and, while focal tubercles were not identifiable, the process was demonstrated to be tuberculous by the presence of acid-fast bacilli.

In Case 1, where massive calcification of the udder had occurred, the supramammary lymph glands contained large calcified and caseous nodules almost entirely replacing the lymphoid tissue. In Case 2, the supramammary lymph glands showed no evidence of tuberculosis. They did, however, show a well marked

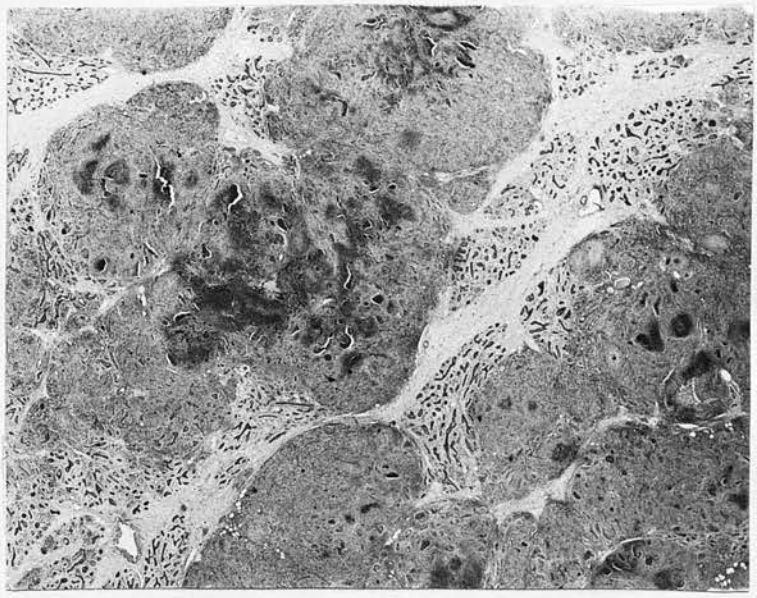


Fig.1. - Udder tissue showing large areas of haemorrhagic necrosis and caseation.

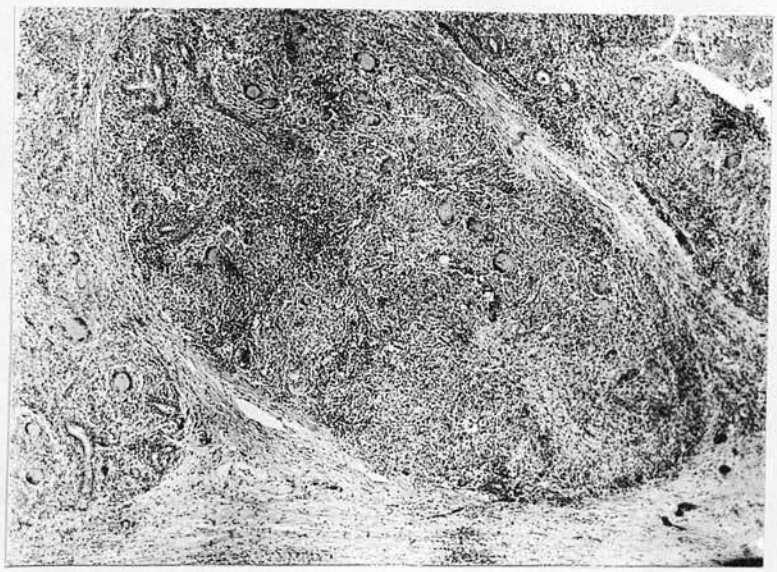
H.& E. x 7½

purulent lymphadenitis of a non-specific character.

TYPE 2. Chronic Diffuse Granuloma (Cases 3 and 4).

Naked eye: The udder tissue, instead of having the usual flaccid glandular character, was solid and stiff, while the lobulated structure was greatly exaggerated so that the lobules projected from the surrounding interlobular connective tissue. The colour of the whole surface was chalky white instead of the normal pinkish-white. The surface was dry and there was no oozing of milk. The supramammary lymph glands showed no lesions of tuberculosis.

Microscopic examination: The lobular structure of the udder was maintained and the interlobular fibrous tissue, although thinned out, was not encroached upon. Almost every lobule in the involved quarter was invaded by tuberculosis. In rare instances an isolated lobule of fairly normal lactating type was seen or only one or two small tuberculous follicles were present in it. In the majority, however, the acinar structures were completely destroyed or only a few partially atrophied ones survived. The lobule area was then replaced by aggregations of tuberculous follicles with many giant cells; frequently these follicles had diffuse edges and were confluent, so that the focal appearance was obscured (Figs. 2 and 3). In occasional lobules the central part, which would involve the lobular duct, had broken down to caseation.



Figs.2&3. - Udder tissue showing replacement of normal lobules with tuberculous granulation.

H.& E. 15
20

The interlobular ducts had a very striking appearance. The duct walls were greatly thickened to 150 mu or more (as compared to the normal 20 mu), being invaded by rather fibrous tuberculous granulation tissue. The lumen was thereby considerably diminished. The epithelial lining was usually completely destroyed, but in some instances persisted as a much altered layer of flattened squamous cells.

Supramammary lymph gland: Large secondary lymph nodules were present. The lymph sinuses showed some endothelial desquamation. Scattered through the peripheral part of the supramammary lymph gland were small tuberculous lesions (30μ- 60μ) consisting of small follicles of epithelioid cells, lymphocytes and plasma cells, along with one or two giant cells. These nodules were very numerous, as many as 20 being present in one section. Acid-fast organisms were found, but were few in number.

TYPE 3. Chronic Nodular (Cases 5, 6, 7, 8, 9 and 9a).

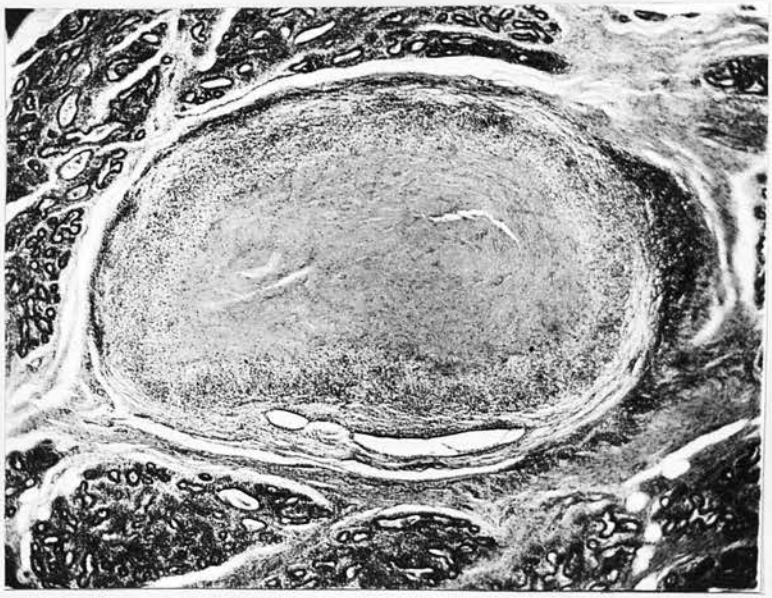
Naked eye: The cut surface showed nodules 2-10 mm. in diameter projecting from the groundwork of the udder; these were whitish-yellow in colour and were obviously gritty when cut with the knife. They were unevenly scattered over the cut surface, being more numerous in some areas than others. In other cases the lesion, although similar, differed in that the nodules were not gritty, being either yellow and caseous or pale, tough and fibrous.

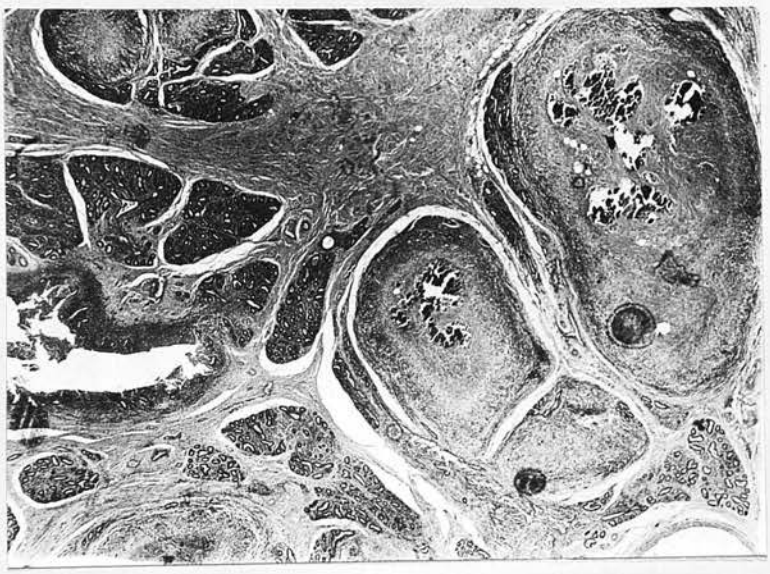
Except in the case where the nodules were fibrous, the groundwork had the features of a non-lactating udder.

The supramammary lymph glands showed extensive caseous and calcified nodules when the udder showed calcification. In the udder with fibrous nodules, no lesions could be found in the supramammary lymph glands. In the two udders which had caseating nodules the supramammary lymph glands in one case showed naked eye lesions of tuberculosis.

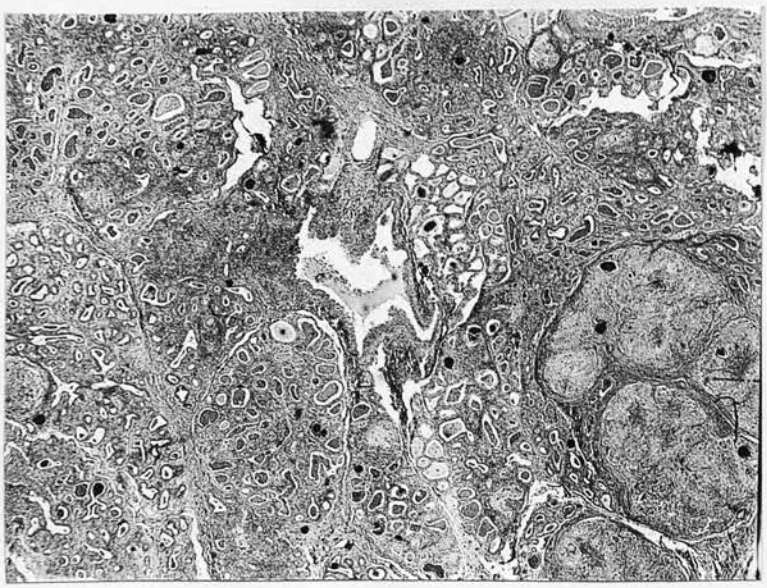
Microscopically, the normal lobular character of the udder was maintained but numerous tuberculous lesions were present. The lesions in each case were confined to single lobules and there was no encroachment on the interlobular connective tissue. The normal structure of the morbid lobule was completely obliterated by the tuberculous reaction; there was a peripheral zone of tuberculous granulation tissue surrounding a caseous area with central calcification (Fig. 5). At the periphery of a few lobules surviving acini could be seen. Some of the largest lobules showed two, three or four advanced lesions (Figs. 6 and 7). In Case 9 the lobule lesion was caseous without calcification (Fig. 4). In Case 5 the lesion was dense and fibrous with a thick wall of connective tissue (Figs. 8 and 9).

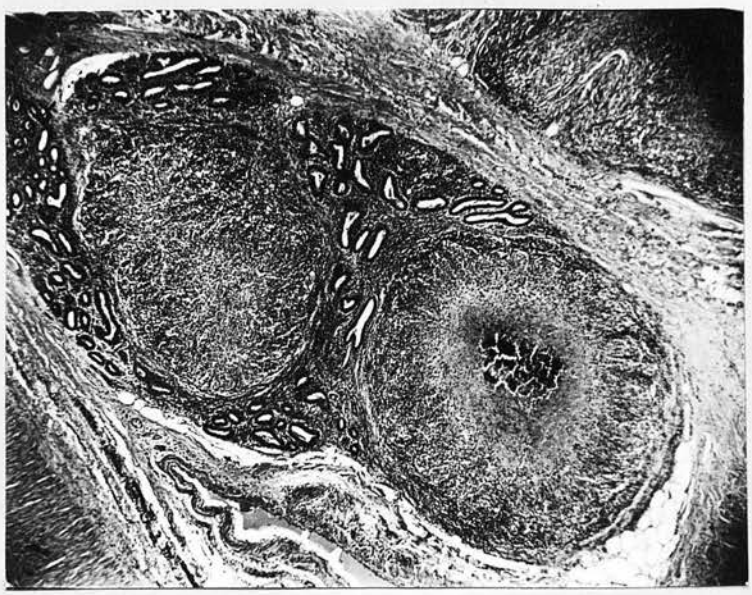
The interlobular ducts were almost universally tuberculous. Their lumina were dilated and filled



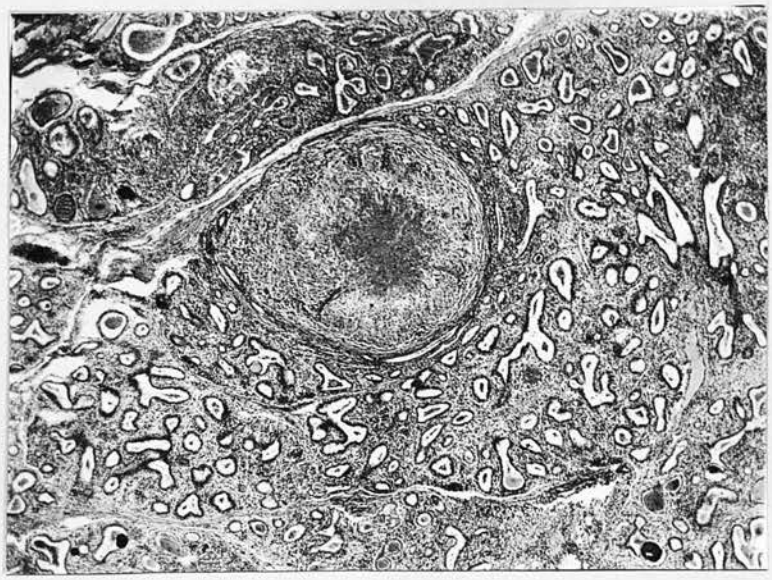


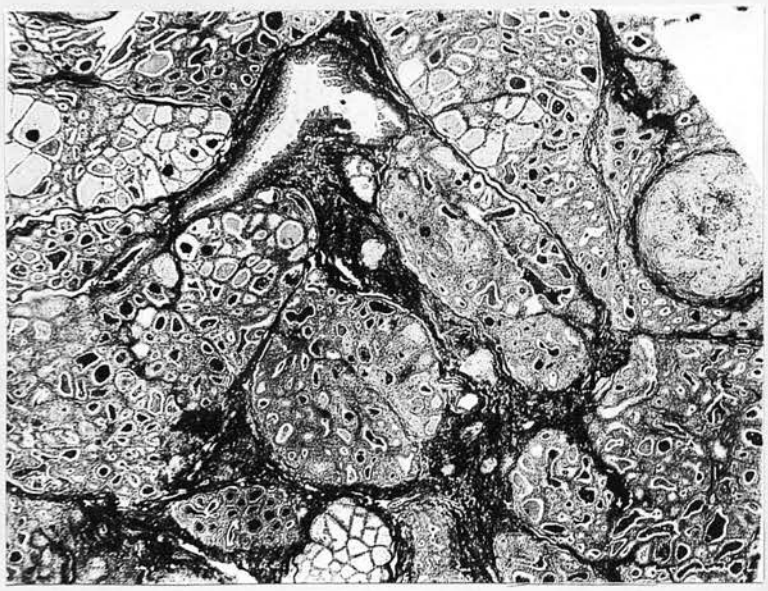
Figs.4&5. - Udder tissue showing chronic nodular tuberculous lesions.
H.& E. x 30
15





Figs.6&7. - Udder tissue showing chronic nodular tuberculous lesions.
H.& E. x 15
30





Figs.8&9. - Udder tissue showing chronic nodular tuberculous lesion.
H.& E. x 30
15

with a cellular exudate, neutrophils being present in large numbers as well as lymphocytes and large macrophages. The lining epithelium was proliferating and was many cells deep and often thrown up into papilliform projections (Figs. 6 - 9). Squamous metaplasia was also seen in places. The duct wall was greatly increased in thickness by tuberculous granulation tissue and fibrous overgrowth.

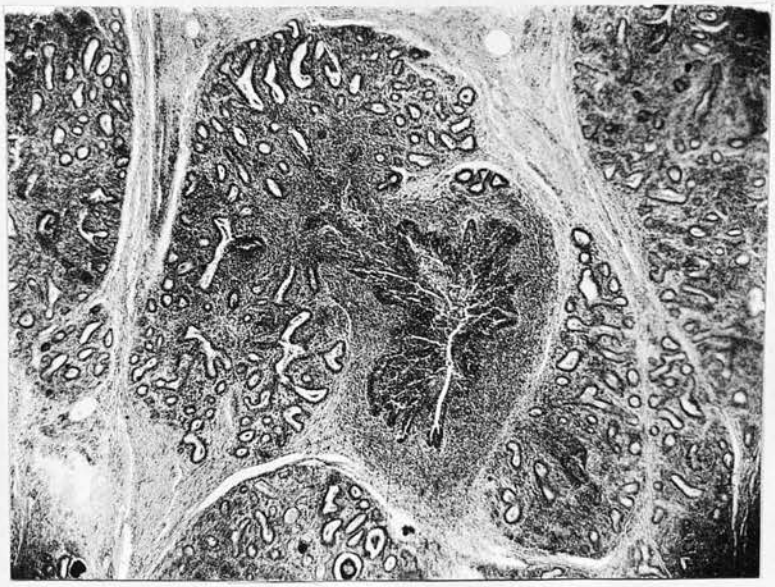
Supramammary lymph gland: Microscopic examination of the supramammary lymph glands showed very little remaining lymphatic tissue. The normal gland substance was almost completely replaced by calcified tuberculous nodules (1-10 mm.). In Case 5, however, where the nodules in the udder were fleshy and fibrous, no tubercles could be found in the lymph glands even microscopically. In Cases 7 and 9, where the nodules in the udder were caseous, the supramammary lymph glands in the former showed no lesions microscopically, while in the latter there was a chronic non-specific lymphadenitis.

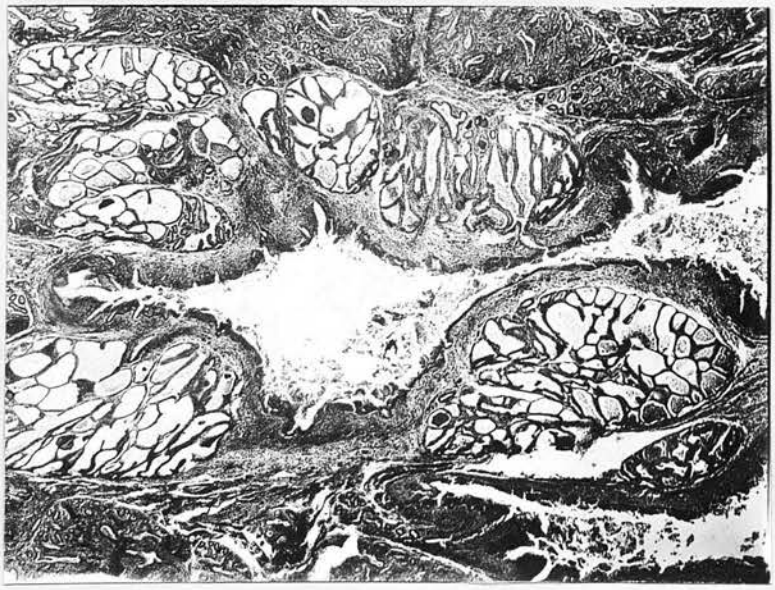
TYPE 4. Diffuse Tuberculous Galactophoritis without Nodules (Cases 10 and 11).

Naked eye: The affected quarters were rather fibrous and compact, lobulation being demarcated by the well developed connective tissue stroma. The general colour was a pale pink. Scattered over the surface were groups of lobules which were yellowish in colour, suggesting early stages of caseation. Unlike tuberculous nodules they did not project from

the cut surface.

Microscopically, the udder tissue retained its normal lobular character. Many of the lobules were in the regressive phase after lactation, while others, fewer in number, were still lactating. No old tuberculous lesions could be found in the lobules. The outstanding feature in these cases was the pathological change in the interlobular milk ducts (Figs. 10 and 11). These ducts were greatly dilated and thick-walled with the enlarged lumina filled with an inflammatory exudate, neutrophils, lymphocytes and monocytes and occasionally large masses of keratin. The epithelial lining of the ducts frequently showed metaplastic change from the normal low columnar to the stratified squamous type. Various stages in this metaplasia were seen; there was either a heaping up of columnar cells or a very definite keratinised squamous metaplasia. The walls were greatly thickened (500 μ) and showed chronic inflammatory changes consisting of fibrous granulation tissue massively infiltrated with round cells but only an occasional tubercle. On tracing out many of the tributary ducts of these large interlobular ducts to the corresponding lobules, by serial section, the inflammatory change could still be seen to be present. In the smaller ducts, however, the lumina were occluded by cellular debris. Examination of the lobules feeding the occluded ducts showed them to be in the non-secreting phase. Tuberculous





Figs.10&11. - Diffuse tuberculous galactophoritis without older tissues lesions.
H.& E. x 30
30

lesions were found in these lobules, but were restricted to the main lobular ducts, and the tuberculous process could be traced down in continuity to one of the larger interlobular ducts (Fig. 10). These intralobular lesions were all of much more recent character than those in the interlobular ducts. The majority of the lobules were affected as above, but occasional lobules were met with whose ducts, although emptying into large infected interlobular ducts, were themselves non-infected and consequently non-occluded. These lobules were in the stage of lactation. This was especially seen around the very large collecting ducts.

Supramammary lymph gland: Examination showed occasional microscopical tubercles just beneath or within the subcapsular sinus. The gland as a whole showed a chronic lymphadenitis of non-specific character.

UNCLASSIFIED

In one case a careful and thorough examination of the udder revealed no tuberculous lesion of any type, although the supramammary lymph glands showed naked eye lesions of caseating tuberculosis. It is, however, difficult to assess the importance of this observation since tuberculous lesions can be easily missed.

FINDINGS

Incidence: Of the 27 udders examined from tuberculous cattle retained, but not necessarily totally condemned, in the detention room of an abattoir, 12 were found to be tuberculous, while in one case tuberculous lesions were found only in the supramammary lymph glands. It is appreciated that the number examined is not large, but the udders were accepted without selection, so that the results are considered to have some significance although cows detained in the abattoir could not represent a cross section of tuberculous cows in the country. As can be seen the percentage figure of these cattle with advanced tuberculosis and showing udder lesions in this investigation is 44 per cent. This is considerably higher than the figure generally quoted in Great Britain, but Nieberle, although not stating definite figures, gives the udder as the organ most frequently affected after the lungs. In assessing the significance of this figure, it should be kept in mind that it was arrived at by careful and thorough histological examination as well as by naked eye examination. In this connection it is important to stress the fact that three cases would have escaped recognition if only naked eye examination had been relied upon, and three more cases were only suspected of being tuberculous and could easily have escaped detection in the ordinary methods of examination.

Distribution of the lesions. The distribution of the lesions in the udder varies widely; in some cases all four quarters were affected with tuberculosis, whereas in other cases the lesions were confined to two quarters. The following table (Table 1) shows the distribution in the cases examined, along with the presence or absence of tuberculous lesions in the supramammary lymph glands.

Nature of lesions. It has been found that tuberculous lesions in the udder show considerable differences in type, and this finding is in general agreement with that of Nieberle. An important practical aspect of this variation in different cases is its bearing on the possibility of physical diagnosis during life. The acute spreading types of lesions (Type 1) and the more chronic but widespread granulomatous lesion (Type 2) probably present little trouble, but other types (Types 3 and 4) must present a very difficult or impossible task in recognition during life.

The different types of involvement which have been described represent, in the main, different degrees of acuteness and chronicity in the nature of the lesion. There has, of course, been no possibility of obtaining in any one case a correlation between the end result observed and the clinical evolution of the disease, but the histological changes appear to give reliable evidence as to relative rate of progress of lesions. The acute

TABLE I

DISTRIBUTION OF LESIONS

	Case No.	L.F.Q.	R.F.Q.	L.H.Q.	R.H.Q.	Supramammary lymph gland - naked eye	Supramammary lymph gland - microscopic
Group I	1	+ early	+ early	+	+	+	+
	2	+ (L)	+ (L)	+ (1)	+ (1)	-	-
Group II	3	+	+	+	+	-	+
	4	+	+	+	+	-	+
Group III	5	+	+	+ (L)	+ (L)	-	-
	6	+ (1)	+ (1)	+ (1)	+ (1)	+	+
	8	+ (1)	+ (1)	+ (1)	+ (1)	+	+
	9A	+ (1)	+ (1)	+ (1)	+ (1)	+	+
	7	+ (1)	+ (1)	- (L)	- (L)	-	-
	9	- (L)	- (L)	+ (1)	+ (1)	-	+
Group IV	10	+ (1)	+ (1)	-	-	-	+
	11	- (L)	- (L)	+ (1)	+ (1)	-	+
	12	-	-	-	-	+	+

L = Large numbers of lactating lobules.
1 = Occasional lactating lobules.

caseating types show evidence of rapid progress and may be regarded as indicative of low resistance in the animal. The more chronic lesions (Type 3) point with certainty to a duration of months, thus suggesting some degree of immunity which results in a slow progress of the lesion, but which is insufficient to cause destruction of the tubercle bacilli. This is a common finding both in bovine and human tuberculosis in general.

Method of infection. The lesions have in all cases been fairly advanced, so that direct evidence as to the method of initial infection of the udder has not been obtained, but some deductions are possible from the facts observed. In the first place, the udder lesions have been found in cattle with fairly extensive internal tuberculosis and it appears fairly certain that the udder, as a peripheral organ, has been involved secondarily, the disease having spread from elsewhere. The possible routes would be by lymphatics or by the blood stream. The findings do not support the theory that the lymphatic vessels carry infection to the udder: the supramammary lymph glands frequently showed no naked eye or microscopic lesions of tuberculosis, even when the udder was extensively invaded, while in other cases only microscopic tubercles were present in the supramammary lymph gland. In addition, if infection were by the lymph stream one would expect the udder to show always a characteristic pathological picture

with older lesions nearest to the lymph gland, but this, in contradistinction to M'Fadyean, has not been found. It has also been shown that there may be very extensive involvement of ducts and lobules throughout a quarter without there being any encroachment on perilobular connective tissue in which lymphatic vessels lie. For this reason I believe that the initial settling of bacilli is from the blood stream with subsequent extension in the udder by the ducts. In this event it would appear that the organisms in a tuberculous bacillaemia may lodge only in two quarters (possibly one) and spread extensively there, while other quarters remain healthy and secreting. In Type 3 (chronic nodular) numerous old but not obsolete lesions were widely scattered and these could represent multiple sites of initial blood stream infection, but in Types 2 (chronic diffuse granuloma) and 4 (diffuse tuberculous galactophoritis without nodules) no older focal lesions in lobules were observed which could be accounted for in this way.

One of the most striking features in all cases has been the tuberculous invasion of the duct system. The existence of this was overlooked in the first few cases examined, owing to the rather nonspecific appearance of the changes in the ducts. Later, when occasional tubercle follicles in the duct walls and the invariable presence of acid-fast bacilli had been noted, the true nature of the duct

inflammation became apparent and re-examination of all material led to the finding of this lesion in every case. The extensive involvement of the duct system seems to point with certainty to the part played by it in the spread of bacilli in the udder. In cases where old nodular lesions occur in scattered lobules (Type 3) the duct system is also infected. On the other hand, two cases (Type 4) show the same universal duct involvement with no old lobule lesions, while there is evidence of quite recent and early spread back into the lobular ducts and acini. The exact site of the first lodgement of the bacilli thus remains uncertain, but a general view of the whole of the material suggests that it is high up in the duct system and that the first tuberculous lesion is in the duct wall, the lobules being affected secondarily to the ducts. One most suggestive feature is that the duct system involvement may affect only two quarters while two escape. Such cases leave no doubt that the process extends by some local mechanism in the tissue and that the line of transport of tubercle bacilli is the lactiferous ducts. The fact of existence of a lumen in which infection can spread is no doubt important, but the possibility has also to be considered that the tissue of the ducts is less resistant to bacterial invasion than that of the lobules.

It is considered that the most important

histological finding in all cases is this extensive involvement of the duct system. It is of practical importance to recognise that in every case examined in this work, no matter whether the lesions were old and partially healed or young and active, the ducts show a tuberculous inflammation in the affected quarter. In other words, every tuberculous udder is an open case of tuberculosis and as such is a possible danger to public health. A further interesting feature is that the tuberculous lesion in the terminal interlobular ducts leads frequently to a blocking of this duct, so that the milk is prevented from passing from the secreting lobule to the larger ducts. This appears to cause an atrophy of the secreting acini which enter the resting stage. It is deduced that this duct blockage with the resultant atrophy of the lobules leads to the progressive "drying up" of the udder rather than tuberculous destruction of secreting tissue. It is, however, important to note that even where much of the quarter is non-lactating, a few lobules immediately surrounding the larger ducts are still lactating so that there must be a continuous risk of milk contamination. This is the more important when one realises that other quarters of the same udder are sometimes still in full lactation, so that there is a danger that whatever little milk can be withdrawn from the infected quarter may reach the milk pail.

SUMMARY

1. Twenty-seven udders from advanced tuberculous cows were examined; of these 12 were affected with tuberculosis, while one showed lesions only in the supramammary lymph glands.

2. The presence of tuberculosis in the udder has been ascertained by naked eye examination more frequently than by manual examination and still more frequently by histological examination than by naked eye examination.

3. The presence or absence of naked eye lesions of tuberculosis in the supramammary lymph glands gives no indication as to the involvement of the udder.

4. The distribution of the lesions in the udder has been described. Tuberculosis does not necessarily invade all four quarters of the udder, nor is there any apparent relationship between the type of lesion and the quarters infected.

5. Four types of tuberculosis of the udder have been described both naked eye and microscopically. The type apparently depends on the resistance of the animal and not on the mode of infection. Tubercle bacilli have been found in all the cases described.

6. In all cases, whether chronic or more active, there has been an extensive tuberculosis of the duct system. In other words, all cases of tuberculosis in the udder are open lesions. The inflammatory change in the ducts is not very typically tuberculous histologically, but is characterised by the

presence of the tubercle bacilli.

7. Even very advanced lesions of tuberculosis in the udder may leave some lobules lactating. It would therefore appear that the affected quarter can still secrete a little infected milk. This is even more important when it is realised that other quarters are still, in some cases, fully lactating and non-infected, so that the cow may still be in use as a milker.

8. The original infection of the udder by tuberculosis is presumed to be by the blood stream. Once infected, however, the infection is evidently spread by the duct system.

AN OUTBREAK OF BOVINE TUBERCULOSIS DUE TO UDDER IRRIGATION

D. McFARLANE and J. T. STAMP §§

(Vet. Rec. 1944, 56, 369)

Preliminary investigation of an outbreak of tuberculosis in an attested herd suggested that the high incidence had resulted primarily from irrigation of the udders of certain of the animals with apparatus contaminated with M. tuberculosis. The circumstances in this outbreak were very favourable for study and in view of the seriousness of the outcome in this instance and the widespread application of udder irrigation in cases of mastitis, it was decided to carry out a full investigation.

HERD HISTORY

At the commencement of the investigation, the herd consisted of 87 animals, 33 in the milking herd, 22 heifers, 17 stirks, 14 calves and one bull. The herd had been attested for five years and there had been no reactors or breakdowns. At the routine tuberculin test in May, 1943, 49 of the 87 animals reacted positively. Of the 33 milking cows, 32 reacted; the only bull was positive; the 22 heifers were negative; three of the 17 stirks and 13 of the 14 calves showed a positive reaction.

Consequent upon this tuberculin test suspicion

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§§ This is a portion of a larger paper published by McFarlane, Garside, Watts and Stamp. This portion of the paper represents the combined work of McFarlane and Stamp.

was aroused of enlarged and indurated quarters in six animals. Bacteriological examination of the milk of these animals showed tubercle bacilli in each, including the only non-reactor in the milking herd.

It is necessary here to consider certain aspects of the herd management and history. The milking herd, calves, stirks and bull were on one farm, the heifers on another. The milch cows were kept in two byres, the bull being in one of them, but all grazed together. The standard of husbandry was good. There was a bad record of mastitis and udder irrigation had been practised on the majority of cases, e.g., three times in the spring and summer of 1942, and on each occasion a clinical cure had been affected. In the autumn of 1942 it was decided to irrigate 21 quarters of 16 cows showing clinical mastitis, which would be dry in October and November and were due to calve in the spring. Each animal was irrigated twice with a fortnight's interval between the two. On each occasion the 16 animals were irrigated on the same day and in the same order. Shortly after, the farmer noted a progressive enlargement and induration of all irrigated quarters which did not yield to treatment, including further irrigation by the farmer. On calving, three of the animals were slaughtered owing to the udder condition, while the remaining 13 produced milk which was bulked and used for feeding the spring calves and for human consumption. At varying intervals after

calving, seven of the irrigated cows were slaughtered because of the udder disease, i.e., progressive enlargement with induration. No evidence of tuberculosis in these animals was seen by the meat inspector at the routine abattoir examination. The six irrigated cows remaining in the herd at the time of our investigation were those found to be excreting tubercle bacilli in the milk. All showed marked induration and enlargement of the irrigated quarters. It is interesting to note that of these six animals the one (TBO 1) showing the most severe tuberculous mastitis and general emaciation was the only milch cow not reacting positively to the tuberculin test. The 17 stirks born in the spring of 1942 were kept in a separate paddock and had not been fed on milk from the herd since the late summer of 1942; i.e., before the suspected irrigation. The three positive reactors in this group were the three oldest and they alone of the stirks had run with irrigated cows in the autumn of 1942. The 14 calves born in the spring of 1943, 13 of which were positive reactors, were kept in a separate pen. The infection in these animals was obviously due to the feeding of bulked milk from the milking herd. The bull was kept in a byre with the cows and had contracted tuberculosis as shown by the test. The 22 heifers were kept on a separate farm and were negative to the tuberculin test. They were served by the infected bull.

MATERIAL AND METHODS

Pathological examination of the six remaining irrigated and two non-irrigated animals was carried out. Seven cows were killed at the local slaughter-house and one at the local knackery, under our supervision. The carcase lymph glands were examined by multiple incision and any suspicious or tuberculous glands were kept for microscopical examination. The tongue, trachea, heart and lungs, i.e., the pluck, were brought back to the laboratory, as were the intestines, mesentery and udder. The lungs and udder were perfused with 4 per cent. formaldehyde and when fixed were cut in a ham slicing machine into slices, about 1 cm. thick. The material so obtained was examined macroscopically and microscopically. The intestines were slit from end to end and examined macroscopically by two workers, any suspicious lesions being kept for histological examination.

PATHOLOGICAL FINDINGS (See Table I)

The six irrigated cows (TBO 1-2-3-5-6-7) all show a very similar picture. The primary site of entry of the tubercle bacilli is the udder in all cases; the lesions in this organ are massive and obviously much older than any others in the body. Histologically the lesions consist of a diffuse caseous galactophoritis variably associated with either caseation or productive lobular changes in the irrigated quarters; very early tuberculosis is noted in non-irrigated quarters. The regional lymph

TBO 1 Irrigated cow

Lungs	Substance	Early broncho-pneumonia of all lobes
	Regional Lymph Glands	Caudal mediastinal-marked but early caseation
Intestines	Substance	Negative
	Regional Lymph Glands	Negative
Udder	Substance	Massive caseous tuberculous galactophoritis in irrigated quarters
	Regional Lymph Glands	Very large with small tubercles
	Carcase Lymph Glands	Negative
	Remarks	The udder lesion is primary

TBO 2 Irrigated cow

Lungs	Substance	Very early exudative tuberculous broncho-pneumonia in diaphragmatic lobes
	Regional Lymph Glands	Caudal and middle mediastinal glands, 3 - 4 early caseous tubercles
Intestines	Substance	Negative
	Regional Lymph Glands	The mesenteric glands show three small caseous non-encapsulated tubercles
Udder	Substance	Massive caseous tuberculous lobular mastitis and galactophoritis in irrigated quarter
	Regional Lymph Glands	Very large and soft with a few miliary tubercles
	Carcase Lymph Glands	Retropharyngeal glands and tonsils enlarged by early caseous tubercles. Iliac glands large and soft with microscopic tuberculosis. Right prescapular gland shows tuberculosis
	Remarks	The udder tuberculosis is primary. Haematogenous spread has been present, as indicated by involvement of the right prescapular lymph gland

TBO 3 Irrigated cow

Lungs	Substance	Very early exudative tuberculous broncho-pneumonia
	Regional Lymph Glands	Negative
Intestines	Substance	Negative
	Regional Lymph Glands	Negative
Udder	Substance	Massive caseous tuberculous lobular mastitis and galactophoritis in irrigated quarter
	Regional Lymph Glands	Very large and soft with a few miliary tubercles
	Carcase Lymph Glands	Negative
	Remarks	The udder is primary

TBO 5 Irrigated cow

Lungs	Substance	Sub-acute miliary tuberculosis and very early exudative tuberculous broncho-pneumonia in one diaphragmatic lobe, pleurisy
	Regional Lymph Glands	Bronchial and mediastinal glands are enlarged and caseous
Intestines	Substance	Negative
	Regional Lymph Glands	The mesenteric glands show a few early caseous tubercles
Udder	Substance	Massive caseous tuberculous lobular mastitis and galactophoritis in irrigated quarter
	Regional Lymph Glands	Very large and soft with a few miliary tubercles
	Carcase Lymph Glands	Retropharyngeal glands and tonsils enlarged and caseous. Iliac glands large and soft with caseous tubercles
	Remarks	The udder is primary with subsequent haematogenous spread and inhalation tuberculous pneumonia

TBO 6 Irrigated cow

Lungs	Substance	Negative
	Regional Lymph Glands	Negative
Intestines	Substance	Negative
	Regional Lymph Glands	Negative
Udder	Substance	Massive caseous tuberculous lobular mastitis and galactophoritis in irrigated quarter
	Regional Lymph Glands	Very large and soft with a few miliary tubercles
	Carcase Lymph Glands	Iliac glands large and soft with caseous tubercles
	Remarks	The udder and its associated glands are the only tissues showing lesions in the body

TBO 7 Irrigated cow

Lungs	Substance	Very early exudative tuberculous broncho-pneumonia of the diaphragmatic lobes
	Regional Lymph Glands	Negative
Intestines	Substance	Negative
	Regional Lymph Glands	A few small caseous nodules
Udder	Substance	Massive caseous tuberculous lobular mastitis and galactophoritis in irrigated quarter
	Regional Lymph Glands	Very large and soft with a few miliary tubercles
	Carcase Lymph Glands	Iliac glands large and soft with caseous tubercles
	Remarks	Primary udder tuberculosis with reinfection by inhalation

TBO 4 Non-irrigated cow

Lungs	Substance	Early exudative and caseous tuberculous broncho-pneumonia almost confined to the diaphragmatic lobes
	Regional Lymph Glands	Typical productive tubercles with early calcification and encapsulation
Intestines	Substance	Negative
	Regional Lymph Glands	Negative
Udder	Substance	Negative
	Regional Lymph Glands	Negative
	Carcase Lymph Glands	Negative
	Remarks	A non-irrigated cow showing primary inhalation tuberculosis

TBO 8 Non-irrigated cow

Lungs	Substance	(1) There is an old ossified nodule 1.5 cm. in diameter. Sub-pleural in a diaphragmatic lobe. (2) Widespread sub-acute caseating broncho-pneumonia
	Regional Lymph Glands	Bronchial and mediastinal glands enlarged and caseous
Intestines	Substance	Negative
	Regional Lymph Glands	Mesenteric glands show a few small lesions without capsules
Udder	Substance	Negative
	Regional Lymph Glands	Negative
	Carcase Lymph Glands	Both retropharyngeal glands are enlarged and show tubercles and marked caseation and softening with distinct capsules. The tonsils show tubercles
	Remarks	A non-irrigated cow with a very old ossified lesion (probably tuberculous) and a more recent broncho-pneumonia

glands in all cases show tuberculosis. In five of the cows there is an inhalation infection; the lungs show very early tuberculous broncho-pneumonia with involvement of the regional lymph glands. Variations in this basic picture are seen; thus in two cases the retropharyngeal glands and tonsils are involved, in three the mesenteric glands show a few very early tubercles and in two cases post-primary haematogenous dissemination has taken place as shown by miliary tuberculosis of the lungs (TBO 5) and tuberculosis of the right prescapular lymph gland (TBO 2). In one case (TBO 6) the udder and regional glands alone show lesions.

The two non-irrigated cows (TBO 4 and TBO 8) show no udder lesions, but the other features are the same, i.e., early inhalation tuberculous bronchopneumonia and tuberculous lymphadenitis of regional lung glands. In one case (TBO 4) there are no other lesions, but the other (TBO 8) shows retropharyngeal, tonsillar and mesenteric gland lesions as well as the bronchopneumonia. There is also present in this case an old ossified subpleural lesion showing no evidence of flaring up.

CONCLUSIONS

That irrigation was responsible for the development of the tuberculous mastitis in the cows examined by us is clearly shown by the pathological examinations of these cases. The fact that primary tuberculous mastitis developed in each of seven

irrigated quarters is clearly significant.

Further confirmatory facts can be given. The stirks born in the spring of 1942 received bulked milk from the herd and, apart from the three oldest animals, which later ran with the irrigated beasts, none developed tuberculosis. Since these calves were fed on bulked milk, it may be taken that tuberculosis of the mammary gland was absent from the herd during the summer of 1942, since Stamp (1943) has shown that all cases of mammary tuberculosis excrete tubercle bacilli in the milk. On the other hand, the 14 calves born in the spring of 1943, feeding on bulked milk from the herd after irrigation, were, with one exception, all positive reactors. This indicates that infection of the udder took place between the late summer of 1942 and the spring of 1943. Finally, there is the history that clinical symptoms rapidly followed the irrigation of the udders.

It may be argued that irrigation merely caused a flare up of a pre-existing mammary tuberculosis. This could not have been so because (1) in the six cases examined by us no tuberculosis was seen which could have been primary to the udder lesion, (2) in the seven irrigated animals killed previously no obvious lesions of tuberculosis were noted by the meat inspector. Finally, as pointed out above, none of the non-contact 1942 stirks fed on bulked milk from the herd in 1942 were positive reactors.

Another remote possibility is that tuberculosis existed in the irrigated animals elsewhere in the body than in the udder and that irrigation caused a flare-up with localisation in the udder. Pathological examination, however, showed in every case that the mammary lesion was the oldest and most advanced in the body.

From the above it is clear that in six of the animals the irrigation caused tuberculous mastitis by the introduction of tubercle bacilli into the udder, either on the syphon or in contaminated irrigation fluid. It is highly probable that the ten irrigated cattle not seen by us were similarly affected and in that case the 21 quarters of the 16 irrigated cows were inoculated with viable tubercle bacilli and developed tuberculous mastitis. As regards the source of infection, it is almost certain that the tubercle bacilli were introduced into the herd by the irrigating apparatus. As we were too late to examine all the irrigated cows, this point cannot be completely clarified, since it is within the realms of possibility that an open case of tuberculosis, presumably of the udder, existed in the herd and that this udder was the first to be irrigated. The arguments against such an occurrence are very strong and have been given above.

DISCUSSION

There are several important features which are worth emphasising. The rapid spread of tuberculosis

in an attested herd is generally known and accepted. It is very well illustrated in this instance. Subsequent to the irrigation and development of tuberculous mastitis every beast in the milking herd and the bull, i.e., 100 per cent. of in-contact animals, became infected, all within a period of six months. Udder tuberculosis, whether primary or secondary, from its "open" nature and the usual methods of milking, is probably the most dangerous form of tuberculosis to have in a herd. Tuberculous mastitis readily gives rise to tuberculosis of young stock, not only by their feeding on the tuberculous milk, but also from the habit of recently weaned animals sucking cows, whether in lactation or not. It is clearly recognised by competent clinicians that diagnosis of mammary tuberculosis by clinical examination is in a great many instances impossible. This difficulty was well shown in the present instance. The cows suffering from massive udder tuberculosis were examined routinely and terminally by a number of competent and experienced men, none of whom suspected tuberculosis until the tuberculin test was found to be positive; this is not an isolated incident. Stamp (1943) has pointed out that there are several types of tuberculous mastitis which could only be diagnosed during life by means of a biological examination or by means of a direct smear, and, in addition, that there are two types which would escape all but a most thorough post-

mortem examination. The probability that a contaminated irrigating apparatus infected every quarter on which it was used indicates that the number of tubercle bacilli required to set up lesions in a dry quarter with a mild mastitis must be very low.

In view of the widespread use of irrigation in the treatment of mastitis, the ease with which the udder may be infected by tuberculosis and the difficulties of diagnosis of this condition, we feel that the strongest emphasis should be placed on the necessity for using a sterile teat syphon for every quarter irrigated and for taking the greatest care that the apparatus and fluid in use does not become infected by regurgitation or other means.

SUMMARY

(1) It has been shown that irrigation was responsible for the introduction of tubercle bacilli and subsequent development of tuberculous mastitis in each of the seven irrigated quarters of six cows in a single herd. Fourteen additional quarters in the same herd, irrigated with the same apparatus and on the same day, developed a clinical condition similar to and probably identical with those examined by us. This is the first time that udder irrigation has been shown to cause infection of the cow with tuberculosis.

(2) Every animal in contact with those

irrigated developed tuberculosis, as shown by the tuberculin test. Thirteen of 14 calves fed on bulked milk from this herd developed a positive tuberculin reaction. Three stirks running with the cows when dry gave positive tuberculin reactions, probably from sucking the irrigated udders.

(3) The ease of infection of the bovine mammary gland with M. tuberculosis is pointed out and the great danger of mammary tuberculosis to the "in contacts" is emphasised.

(4) The frequent impossibility of clinical diagnosis of tuberculosis of the bovine mammary gland is stressed.

(5) The necessity for taking adequate precautions when irrigating udders is emphasised.

REFERENCES

Baum, H., (1912) Das lymphgefass system des Rindes. Hirschwald, Berlin.

Behring, E. von, (1903) Dtsch. med. Wschr., 29, 689.

Blacklock, J.W.S., (1932) Tuberculosis Disease in Children. Medical Research Council Special Report, Serial No. 172. London.

Cadeac, (1909) Vet. J., 16, 487.

Calmette, A., and Guerin, C., (1905) Ann. Inst. Past., 19, 601; (1906) Ann. Inst. Past., 20, 353 and 609.

Calmette, A., (1923) Tubercle Bacillus Infection in Man and Animals. Baltimore.

Editorial (1908) Vet. Rec., 21, 129.

Editorial (1910) Vet. Rec., 22, 724.

Edwards, S.J., (1937) J. Comp. Path., 50, 377.

Friedberger and Frohner, (1895) Pathology and Therapeutics of Domestic Animals, Vol. 11. Philadelphia.

Ghon, A., (1916) The primary lung focus of tuberculosis in children. Trans. by King, D.B., pp. 172. London.

Glass A., (1934) Amer. J. Roentgenol, 31, 328.

Glover, R.E., (1941) Vet. Rec., 53, 746.

Gofton, A., (1922) Vet. J., 29, 411-416.

Gofton, A., (1926) J. R. Sanit. Inst., 46, 384; (1931) Congr. R. Sanit. Inst., p. 5.

Gofton, A., (1931) Congr. R. Sanit. Inst., p. 5.

Gofton, A., (1937) Edin. Med. J., 44, 333.

Innes, J.R.M., (1937) Vet. Rec., 49, 783.

Innes, J.R.M., (1940) Vet. J., 46, 391.

Koch, R., (1901) British Congress on Tuberculosis. London.

Krause, A.K., (1925) Amer. Rev. Tuberc., 11, 303.

Laithwood, J., (1896) Vet. Rec., 8, 585.

Law, (1912) Text Book of Veterinary Medicine. Ithaca, New York.

McFadyean, J., (1891) Vet. J., 33, 407.

McFadyean, J., (1898) Rep. Roy. Comm. Tuberc. Cd. 8824.

McFadyean, J., (1910) J. Comp. Path., 23, 239-250, 289-303.

McFadyean, J., (1917) J. Comp. Path, 30, 57 and 139.

McFarlane, D., Garside, J.S., Watts, P.S., Stamp, J.T., Vet. Rec., 1944, 56, 369-373.

McKay, W.M., (1943) Vet. J., 99, 47-53.

Medlar, E.M., (1940) Amer. Rev. Tuberc., 41, 283-306.

Mettam, A.E., (1906) Vet. J., 13, 551.

Millar, N.S., (1937) The Lung. Thomas, Springfield.

Mullar, O., (1906) J. Comp. Path., 19, 19.

Nieberle, K., (1929) Arch. prakt. Tierhlk., 60, 291.

Nieberle, K., (1930) Arch. prakt. Tierhlk., 61, 81.

Nieberle, K., (1930) Arch. wiss. prakt. Tierhlk., 61, 277.

Nieberle, K., (1931) Ergeb. allg. Path. path. Anat., 25, 631.

Nieberle, K., (1937) Arch. prakt. Tierhlk., 71, 323, 332, 347, 356.

Nieberle, K., (1941) Dtsch. tierartzl. Wschr., 49, 69.

Nocard, E., (1895) The animal tuberculoses. Trans. by Scurfield, H. London.

Ostertag, (1905) J. Comp. Path., 18, 84-86.

Parrot, (1876) C. R. Soc. Biol. Paris, 308.

Ranke, K.E., (1916) Dtsch. Arckiv. Klin. Med., 119, 201.

Rich, A., (1944) The Pathogenesis of Tuberculosis. Springfield, Illinois.

Savage, W.G., (1929) Brit. Med. J., 11, 492.

Savage, W.G., (1929) Prevention of Human Tuberculosis of Bovine Origin. MacMillan, London.

Schroeder, E.C., and Cotton, W.E., (1906) U.S. Dept. Agric. Bureau. Anim. Indust., No. 93.

Schulz, G., (1937) Beitrage zur Pathogenese der Kalber tuberkulose. Inaug. Diss., Berlin.

Smith, T., (1894) U.S. Dept. Agric. Bureau. Anim. Indust., No. 7.

Spartz, L., (1934) Bull. Acad. Vet. France.

Stamp, J.T., (1943) J. Comp. Path. and Therapeutics, 53, 3, 220-229.

Stamp, J.T., and Wilson, A., (1946) Vet. Rec., 58, 2, 11-15.

Stamp, J.T., (1948) J. Comp. Path. and Therapeutics, 58, 1, 1-8.

Stamp, J.T., (1948) J. Comp. Path. and Therapeutics, 58, 1, 9-20.

Theives, B., (1939) Inaug. Diss., Leipzig.

Udall, D.H., (1939) Practice of Veterinary Medicine. Ithaca, New York.

Udall, D.H., (1943) Practice of Veterinary Medicine. Ithaca, New York.

Vallee, M.H., (1905) Ann. Inst. Pasteur.

Villemin, J.A., (1868) Etudes sur la tuberculose, pp. 640. Paris.

Wallis, Hoare E., (1913) A System of Veterinary Medicine. London.

Whipham, T., (1909) Tuberculosis Conference, Oxford.

White, E.G., and Minett, F.C., (1941) Brit. J. Tuberc., 35, 69.

Yamagiwa and Jasikawa, (1930) Selected contributions from the Mukden Institute for Infectious Diseases of Animals, Vol. 1, 215.

ABORTION OF SHEEP

In Britain although abortion in the ewe is not one of the most serious diseases in an overall picture of sheep diseases, it is well known that it may cause considerable economic loss and this is especially so in certain districts of which the Border area of England and Scotland is one. Abortion in sheep has been widely described throughout the world, the causal factor being frequently bacterial. Vibrio foetus, Salmonella abortus ovis, Brucella abortus, Brucella melitensis and Bacterium coli are only a few of the organisms which have been incriminated. In this country Vibrio foetus (McFadyean and Stockman, 1913) and Salmonella abortus ovis (Bosworth and Glover, 1925) are the only organisms causing abortion. In many cases no organism can be isolated to account for the abortion.

Abortion due to Vibrio foetus

This organism is fairly widely distributed among sheep in England but it has never been isolated in Scotland. The majority of outbreaks of abortion due to this organism are sporadic and it is likely that outbreaks in clean flocks are due to carrier animals. This type of abortion is rarely experienced in the same flock in successive seasons which suggests that a lasting immunity is developed.

The abortions may commence at a fairly early stage in the gestation period and continue over a

considerable time. The lambs are generally fresh when aborted although McFadyean and Stockman in their original investigation found that the foetuses were decomposed when aborted.

Examination of the aborted lamb shows sub-cutaneous oedema and a considerable excess of blood stained serous or seri-fibrinous fluid in the body cavities. The liver may be considerably enlarged and show a number of pale necrotic areas.

The most reliable method of diagnosis is to demonstrate the causal organism either in the stomach of the foetus or in its membranes. This can be done either by staining a smear with dilute carbol-fuchsin or by cultural methods. If such material is not available the agglutination test might be useful if blood samples were taken from a number of animals in an affected flock.

Abortion due to Salmonella abortus ovis

This organism appears to have a very limited distribution being confined almost entirely to the South East of England. At one time it was an important source of loss in this area (Bosworth, 1936) but during recent years it has not been of great importance. Carrier sheep are probably the chief means of spread.

The flock appears perfectly healthy until about six weeks before lambing is due to begin when abortions commence suddenly and in considerable

numbers. The aborted lambs are always very fresh.

Diagnosis depends mainly upon the isolation of the organism from the foetus. The agglutination test can be used but the results are frequently difficult to interpret.

Enzootic abortion

Greig (1936) described abortion in sheep as being widespread on the farms of South East Scotland and stated that the disease was extending in its geographical distribution. Regarding the causal factor Greig was unable to determine any causal organism and considered that the evidence suggested that a deficiency in the diet was the cause. Experimental data however failed to confirm that deficiencies in phosphorous, Vitamins A or E or protein had any bearing on the cause of the abortion. Greig gave the name Enzootic abortion to the condition he had described.

ORIGINAL OBSERVATIONS

My field investigations since 1945 have amply confirmed the presence of abortion in the sheep of South East Scotland but it appears that at least two types of abortion are present, one occurring in sheep on the arable farms and the other type in sheep on hill farms (Stamp, 1946), both types being classed as Enzootic abortion by Greig. A considerable number of both types of outbreaks have been investigated. Detailed post mortem,

histological and bacteriological examinations were carried out on aborted ewes and their foetuses. In hill sheep it was determined that tick borne fever was commonly present in the aborting ewe. As a result of the observations on hill sheep a field experiment was carried out to determine whether tick borne fever in the pregnant ewe would cause abortion. The results gave definite evidence that tick borne fever can cause abortion. A natural sequel to this was an investigation into the immunity of sheep to tick borne fever on several tick infested farms where abortion is known to occur and on tick infested farms where abortion does not occur. The differences in the immune reactions of the sheep on the two types of farm give supporting evidence that tick borne fever is a cause of the hill sheep type of abortion. The immune reactions of artificially infected sheep were also followed. The results of the above observations have been published (Stamp and Watt, 1950). In the low ground type of abortion, like many other previous workers I was unable to find a bacterium as a causal agent but nevertheless the epidemiology of the disease, especially the observation that immunity appeared to develop after abortion convinced me that the disease was infectious and probably had a virus aetiology (Stamp, 1948). A small transmission experiment in the field gave me positive evidence that the disease could be transmitted experimentally. As a result of

my findings I asked Dr. Greig of Moredun Institute to co-operate in a large scale transmission experiment since to do this alone was beyond the physical capacity of my laboratory. As a result of this experiment I found a psittacosis like organism to be the cause of the low ground type of abortion (Stamp, McEwen, Watt and Nisbet, 1950). Since my original observation, work has been carried out at several centres including Moredun Institute on different aspects of the disease, my own contribution being the pathology of the disease, the typing of the organism (Stamp, 1951) and the development of a Complement Fixation Test (Stamp, Watt and Cockburn, 1952). In this work I have been ably aided by my assistants, Messrs Watt, Nisbet and Cockburn, who have helped me to carry out the considerable routine field and laboratory work.

ABORTION IN SHEEP

REFERENCES

Bosworth, T.J., and Glover, R.E., (1925) Vet. Jn., 81, 319.

Bosworth, T.J., (1936) Vet. Rec., 48, 42.

Greig, J.R., (1936) Vet. Rec., 48, 42.

McEwen, A.D., Stamp, J.T., and Littlejohn, A., (1951) Vet. Rec., 63, 197.

McFadyean, J., and Stockman, J., (1913) Report of a departmental committee - Board of Agriculture and Fisheries to enquire into epizootic abortion (Part 3).

Stamp, J.T., (1946) Annual report of the Edinburgh and East of Scotland College of Agriculture.

Stamp, J.T., (1948) Annual report of the Edinburgh and East of Scotland College of Agriculture.

Stamp, J.T., McEwen, A.D., Watt, J.A., and Nisbet, D.I., (1950) Vet. Rec., 62, 17.

Stamp, J.T., and Watt, J.A., (1950) Vet. Rec., 62, 465.

Stamp, J.T., (1951) Jn. Comp. Pathology and Therapeutics, 61, 3, 215.

Stamp, J.T., Watt, J.A., Cockburn, R., (1952) Jn. Comp. Pathology and Therapeutics, 62, 1.

Reprinted from *The Veterinary Record*, 62. 251.

ENZOOTIC ABORTION IN EWES

I. Transmission of the Disease

BY

J. T. STAMP,

EAST OF SCOTLAND COLLEGE OF AGRICULTURE, EDINBURGH,

A. D. McEWEN,

ANIMAL DISEASES RESEARCH ASSOCIATION, MOREDUN INSTITUTE, EDINBURGH,

J. A. A. WATT AND D. I. NISBET,

EAST OF SCOTLAND COLLEGE OF AGRICULTURE, EDINBURGH

Abortion and premature lambing occur in many flocks on tick-free hill pastures and arable farms in the south-east of Scotland. Abortions also occur on tick infested pastures and may sometimes be the consequence of tick transmitted disease. The information given in this paper has been obtained from investigations made on flocks free from tick infestation. Numerous aborted foetuses and premature lambs from these flocks have been examined culturally for evidence of a bacterial infection but none has been found and no micro-organism has been recognised in smears made from foetal stomach and intestinal contents, nor have inclusion bodies been found on histological examination of foetal organs.

FIELD EVIDENCE

In flocks in which the disease has recurred for several years the incidence of abortion is usually reported to be approximately 5 per cent. but occasionally and particularly among first and second lamb ewes the incidence may be four or five times higher. When the disease appears in a hitherto clean flock the incidence may be high for two or three years, up to 25 per cent. or even 30 per cent. of ewes of all ages aborting or lambing prematurely.

There is general agreement among owners who have kept records that ewes having once aborted or lambed prematurely, lamb normally at subsequent pregnancies.

It is often impossible to find a plausible explanation for the first appearance of the disease in a flock, but at other times it is reported as following on the introduction of bought in non-pregnant ewes or even gimmers (virgin ewes).

Abortion and premature lambing, the only striking clinical signs, generally take place late in pregnancy, most frequently during the last two or three weeks of the normal gestation period, but some abortions may occur even six weeks before full term. Premature lambs may be weak and die. In multiple pregnancies one foetus may be dead, while one or more live lambs may be born showing various degrees of strength and vigour, and from the appearance of the dead foetus it may be obvious that death occurred some days or weeks before the gestation ended.

Ewes that abort lambs which appear to have been dead *in utero* for some weeks are generally in poor condition, and it is not uncommon for such animals to die within a few days of aborting. When the lambs have died shortly before parturition or are born alive before or at full term, there is generally less disturbance in the ewe's health, and the ewes may appear normal. Retention of the foetal membranes, however, may occur, resulting, in some cases, in a loss of condition and even in death. Farmers agree that with rare exceptions ewes that have aborted or lambed prematurely conceive without difficulty, and that sterility is not a sequel to the disease.

The epidemiology indicates that the disease is caused by an infection and that ewes that have passed through the phase of aborting or lambing prematurely have acquired the ability to carry their lambs to full term.

EXPERIMENTAL

In the spring and summer of 1949 an experiment was carried out from which it was hoped that information on the aetiology of the disease would be obtained.

From February 9th to March 10th 38 aborted foetuses and premature dead lambs were received from outbreaks of abortion in East Lothian and Berwickshire. The spleen, kidneys, liver, lungs and stomach contents of each foetus or lamb were cultivated for bacteria but apart from an occasional obviously contaminating bacterium the organs and stomach contents were bacteriologically sterile.

Portions of these organs and stomach contents were stored at —70° C. At the end of February bacteriologically sterile materials from nine foetuses belonging to four flocks and consisting of 35 grams of spleen, 115 grams of kidney, 320 grams of liver, 173 grams of lung tissue and 160 grams of stomach contents were mixed and ground to a smooth paste in a " Premier Paste Mill." A 20 per cent. suspension of the paste was made in saline serum broth (10 per cent. horse serum). A part of the suspension was centrifuged in an angle centrifuge at low speed for ten minutes to deposit coarse particles, and the remainder, after more prolonged centrifuging at higher speed, was filtered through kieselguhr and finally through a 0·4 μ gradacol membrane. The suspension and filtrate were inoculated into pregnant ewes on March 1st.

Later another suspension and filtrate were prepared in the same way from materials collected from 12 foetuses belonging to seven flocks. This suspension and filtrate were inoculated into pregnant ewes on March 15th. On both March 1st and 15th control ewes were injected with saline serum broth. The experimental ewes were all young sheep (gimmers), from an area believed to be free from abortion, and they ran with the tups from the middle of December to the end of January. A plan of the experiment is shown in Table I.

TABLE I

Plan of Infection Experiment

Each ewe received 5 c.c. intravenously and 5 c.c. subcutaneously.

Material	*Date*	*No. of Ewes*
Suspension	1.3.49	28
Filtrate	„	28
Saline Serum Broth (Control)	„	27
Suspension	15.3.49	27
Filtrate	„	27
Saline Serum Broth (Control)	„	27

A record of the service dates was kept, and on March 1st the ewes were apportioned to the above groups, a similar number of ewes at approximately the same stage of gestation being placed in each group.

All the ewes were run together as a flock throughout the experiment. From the beginning of April they were kept under observation during daylight so that, should any abortions occur, the dead lambs and membranes might be collected for examination. Abortions commenced on April 21st and continued until near the end of the lambing season. In the earlier abortions the foetuses were obviously premature ; later, premature live lambs were born that died despite careful nursing. The abortions and premature lambings were confined to those ewes inoculated with the unfiltered suspensions. As lambing proceeded an effort was made to collect and examine the foetal membranes from all the ewes, the membrane in some cases being removed manually. The results of the experiment are recorded in Table II.

TABLE II

Showing the results of the inoculation of suspensions Batch 1 and Batch 2

	28 *Ewes Inoculated* 1.3.49	27 *Ewes Inoculated* 15.3.49
Aborted, infected foetal membranes ...	10	18
Premature (born alive but died) infected foetal membranes	2	2
Normal birth (lambs lived), infected foetal membranes	1	1
Normal birth, no infection of foetal membranes	2	0
Normal birth, no foetal membranes examined	7	1
Barren	4	2
Slaughtered before lambing, infected foetal membranes	2	2
Slaughtered before lambing, non-infected foetal membranes	0	1
	28	27

None of the foetal membranes from the ewes inoculated with filtrates or from the control ewes injected with saline serum broth were infected, and none of the ewes aborted or lambed prematurely.

The aborted foetuses from experimental ewes showed abnormalities similar to those encountered in field cases, namely varying degrees of blood-tinged oedema in the subcutaneous and intramuscular tissues and blood tinged transudates in the large serous cavities. The foetal membranes of aborted foetuses, of most premature lambs and of one apparently normal full term lamb showed gross abnormalities which varied in degree and extent from case to case and which consisted in necrosis of the cotyledons and chorion and accumulations of a reddish to a dirty pink coloured discharge. The affected cotyledons had lost the dark purplish colour of normal tissue and ranged from a dark red to a dull clay colour. They had a matted appearance and a more solid consistency than normal. The exudate mentioned above tended to accumulate round and to adhere to the interstices of the cotyledons. The chorion presented irregular areas of thickening which ranged from an oedematous to a dry leather-like thickening of the tissues (Fig. 1). The extent of the thickened areas varied greatly: in some membranes almost the whole chorion was involved, whereas in others a small area only was affected. Diseased areas frequently had a mottled appearance on account of variation in the thickness and the colour of the affected parts. The gross appearance of the membranes was similar to that of bovine foetal membranes infected with *Brucella abortus*. In contrast to the foetal membranes the uterus was apparently normal, the uterine mucosa and cotyledons showing no evidence of an inflammatory reaction.

Smears made from the diseased cotyledons and chorion and from the exudate were stained with dilute Ziehl-Neelsen carbol fuchsin (1 : 10) for ten minutes ; they were then differentiated very rapidly with dilute acetic acid and lightly counterstained with dilute methylene blue. Very large numbers of small red-stained micro-organisms were present in these smears, occurring singly and in clusters. Some of the more closely packed clusters were intracellular. These minute organisms were coccal in shape and unless present in clusters were easily overlooked (Fig. 2). They stained by Macchiavello and Castaneda stains for rickettsiae and also by Giemsa stain. For the routine examination of smears the modified Ziehl-Neelsen stain was the most satisfactory, and when smears stained by this method were examined by dark field illumination the organisms showed as bright pale green round bodies (Fig. 3). These bodies will from now on be referred to as elementary bodies.

The normal chorion of the sheep consists of a thin layer of connective tissue covered by a single layer of epithelial cells which are in intimate contact with the uterine mucosa in the intercotyledonary areas of the uterus. This layer of epithelial cells is continuous with the epithelium covering the villi of the foetal cotyledons and in the sheep remains present throughout gestation. The epithelium of the uterine mucosa, on the other hand, is not continuous over the villi of the maternal cotyledon, which consists only of tufts of blood vessels set in a slight connective tissue matrix. The essential pathological changes found consisted of a subacute inflammatory thickening of the subepithelial tissues of the chorion with considerable round cell infiltration and with some necrosis of the lining epithelium (Fig. 4 and 5). The foetal cotyledons showed varying degrees of abnormality. Some cotyledons were greatly necrosed, the epithelium, connective tissue core and vessels being completely destroyed and replaced by inflammatory exudate (Fig. 6, 8 and 9) while other cotyledons were only partially destroyed (Fig. 5). The uterine mucosa apart from a very slight round cell infiltration was normal while the maternal cotyledon generally showed only slight superficial necrosis and very little infiltration or exudation (Fig. 7.) The location of the elementary bodies was best seen in sections stained by Giemsa. In the areas of necrosis considerable numbers of elementary bodies, single and in small groups, were seen, but there was little doubt that the characteristic habitat of the organism was in the cells of the epithelium of the chorion and foetal cotyledon. These cells either

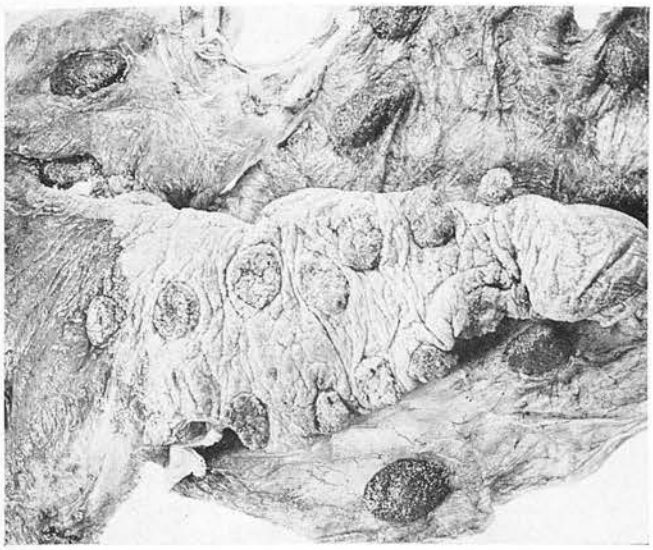

FIG. 1.
Foetal membrane of aborted foetus showing an area of thickened chorion and necrotic cotyledons contrasted with the appearance of a more normal area behind.

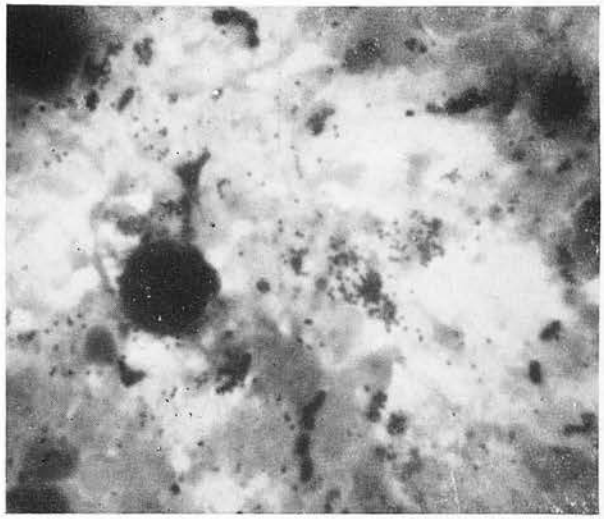

FIG. 2.
Smear made from a diseased foetal cotyledon showing small coccal shaped elementary bodies both singly and in groups. Stain modified Ziehl Neelson. X1500.

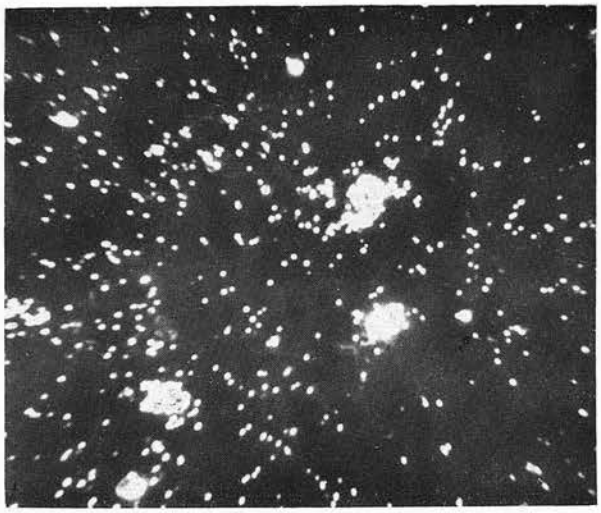

FIG. 3.
Same smear as in Fig. 2. Dark field illumination. X1000.

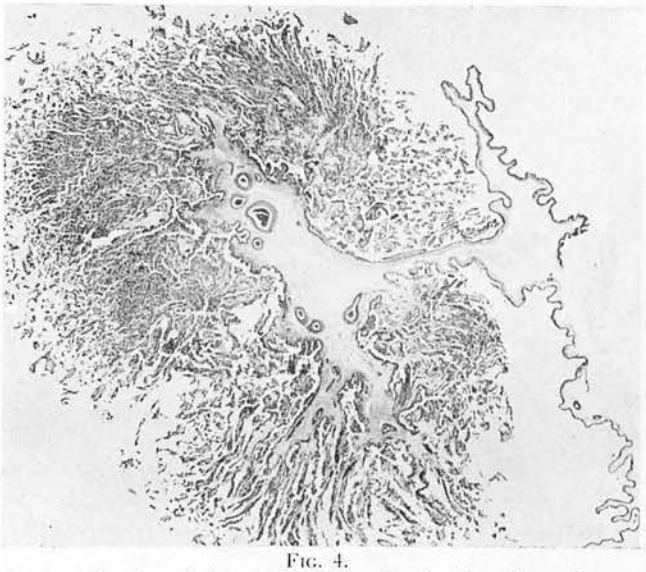

FIG. 4.
Normal foetal cotyledon and chorion showing breakdown but no necrosis. Haematoxylin and Eosin. X5.

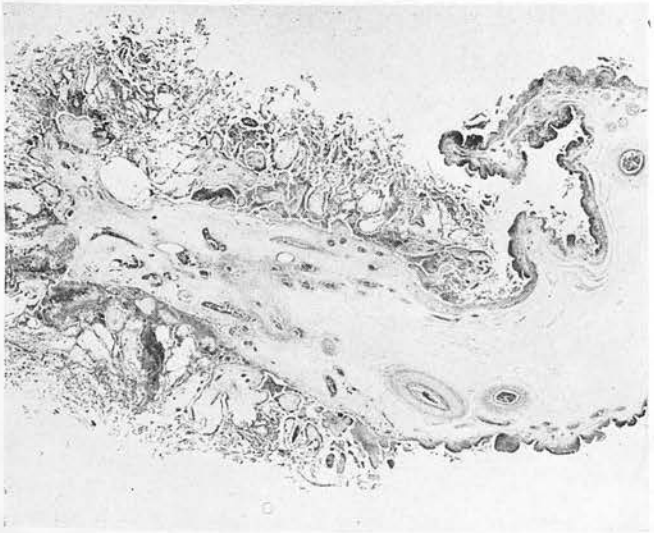

FIG. 5.
Infected foetal cotyledon showing areas of necrosis and chorion thickened with inflammatory granulation tissue. Haematoxylin and Eosin. X5.

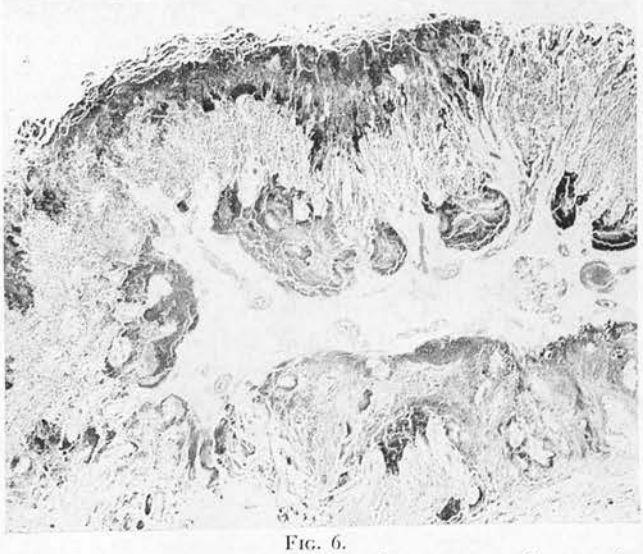

FIG. 6.
Infected foetal cotyledon showing large areas of necrosis. Haematoxylin and Eosin. X5.

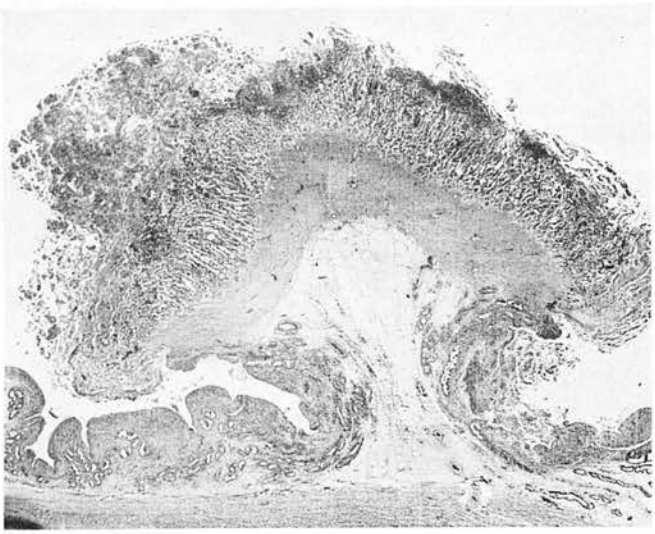

FIG. 7.
Maternal cotyledon showing slight superficial necrosis with some necrotic foetal cotyledon still attached. Haematoxylin and Eosin. X5.

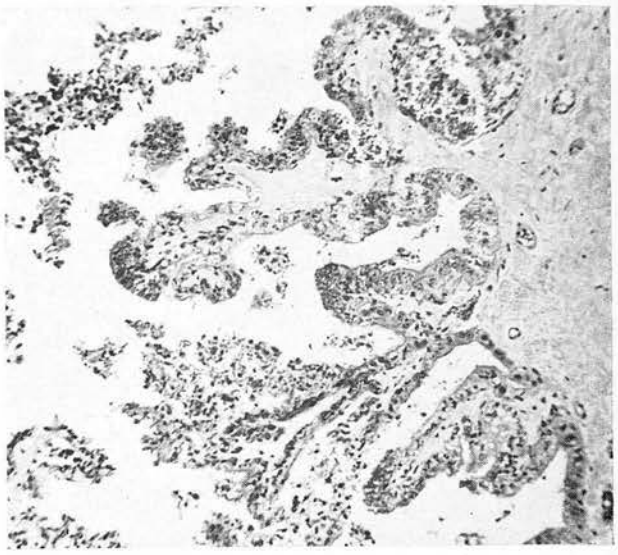

FIG. 8.
Normal foetal cotyledon showing villi and lining epithelial cells. Haematoxylin and Eosin. X100.

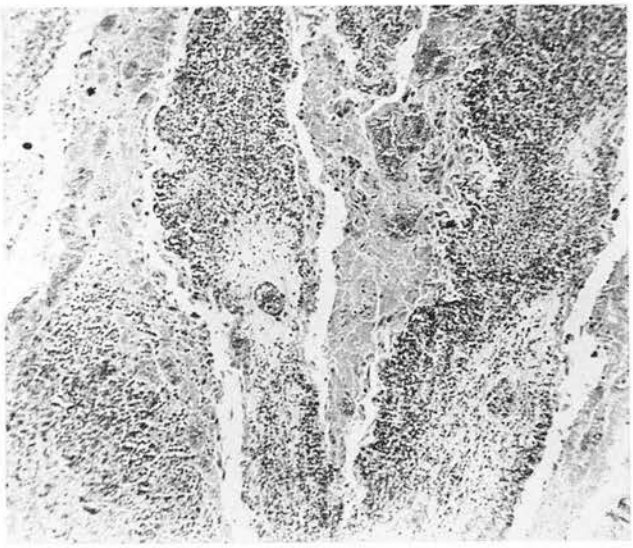

FIG. 9.
Necrosis of villi of foetal cotyledon. Haematoxylin and Eosin. X100.

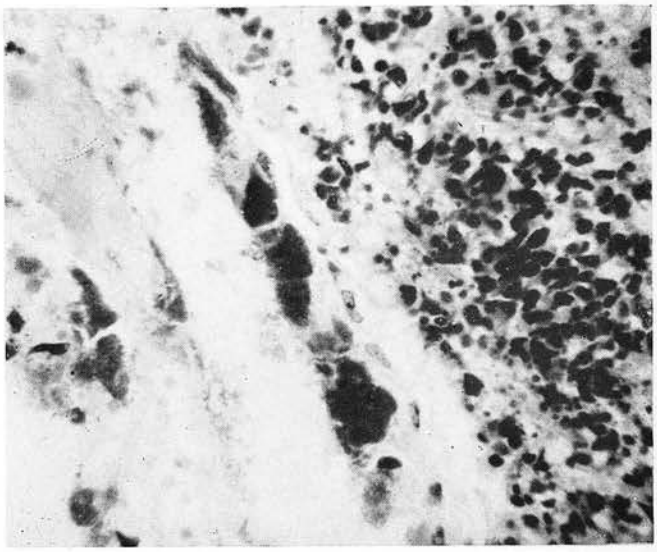

FIG. 10.
Lining epithelial cells of the villi of an infected foetal cotyledon packed with elementary bodies. The normal connective tissue is infiltrated with inflammatory cells. Giemsa. X600.

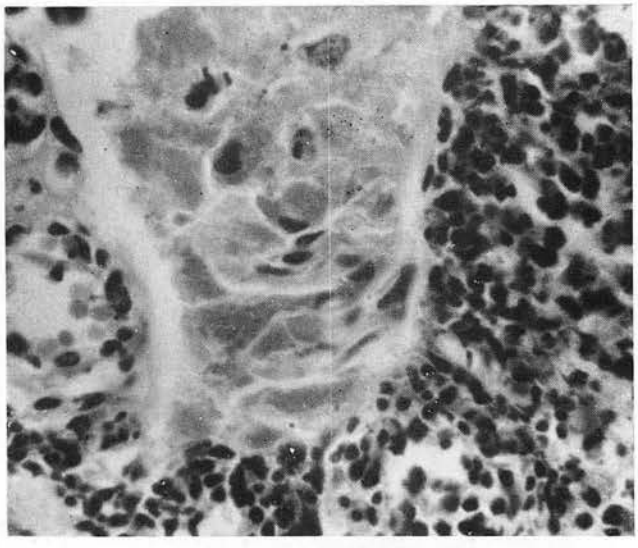

FIG. 11.
Group of desquamated necrosed epithelial cells, the cytoplasm of the cells being packed with elementary bodies. Haematoxylin and Eosin. X800.

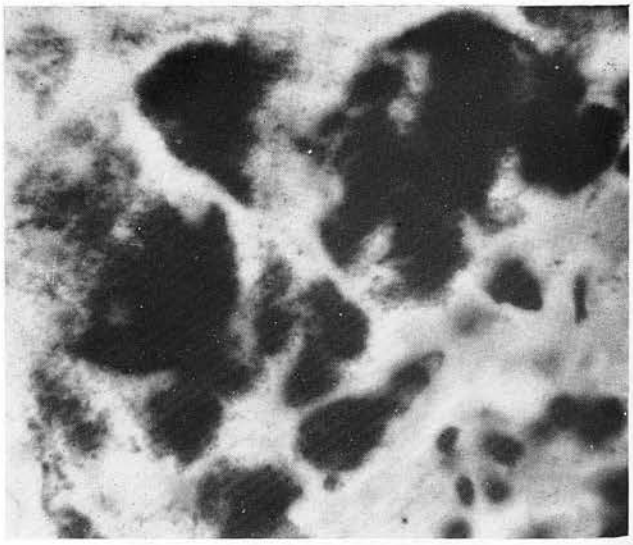

FIG. 12.
Same field as in Fig. 12 showing packing of cells with elementary bodies. Giemsa. X1500.

singly or more commonly in groups, were densely packed with the elementary bodies. The cells varied considerably in appearance and situation. Some were greatly enlarged and the nucleus was pushed to one side while others were obviously degenerating and becoming broken. In an occasional cell the centre of a dense mass of elementary bodies was vacuolated. Some cells were still in continuity with the rest of the epithelium (Fig. 10) while others were detached but still in groups (Fig. 11 and 12) and yet others were found scattered here and there amidst large areas of necrosis. The epithelial cells of the uterine mucosa were only occasionally parasitised and never necrosed and desquamated. The uterine cotyledon being free of epithelium was free of elementary bodies except where the necrotic foetal cotyledon was still attached.

No bacteria were cultivated from the internal organs of the aborted foetuses and smears prepared from foetal stomach contents showed no micro-organisms.

It was apparent that necrosis of the foetal membranes had been produced by the inoculation of the suspension of foetal material obtained from the field outbreaks of abortion and that the lesions in the membranes were constantly associated with a heavy infection of elementary bodies.

A supply of elementary bodies, free from contaminating bacteria, was obtained from the uteri of pregnant infected ewes slaughtered before the uterine seal had broken. The elementary bodies were readily cultivated on inoculation into the yolk sac of a six or seven day chick embryo. The embryos died on the fourth to ninth day after inoculation when large numbers of elementary bodies were found in the yolk sac. Passage from yolk sac to yolk sac presented no difficulty but no enhancement of pathogenicity for the embryo has been observed. Infective materials stored at temperatures of —20° C. and —70° C. have remained viable for several months.

The stomach contents of aborted foetuses, and suspensions of tissues from lungs, liver, spleen and kidney of the foetuses have not been found infective for chick embryos. Colostrum from infected ewes and suspensions of the internal iliac lymph glands of infected ewes slaughtered before aborting or lambing have not been found infective for the chick embryo.

Broth dilutions of bacteriologically sterile foetal fluid containing large numbers of elementary bodies were prepared. The undiluted fluid and fluid diluted 10^{-1}, 10^{-2} and 10^{-3} were respectively inoculated subcutaneously in 1 c.c. quantities into each of four sheep. The one sheep inoculated with the 10^{-3} dilution remained normal ; the other three sheep all showed a rise in temperature three days after inoculation; the temperatures returned to normal by the eighth day. The maximum temperatures ranged from 106·4 to 107° F.

An infected yolk sac was emulsified and suspended in 10 c.c. of broth. Two sheep were each inoculated intravenously with 3 c.c., two were inoculated subcutaneously with 1 c.c. and two were inoculated intradermally with 0·1 c.c. The following day all the sheep showed a rise in temperature ; the temperature remained high for three days and then returned to normal. The maximum temperatures shown by individual sheep varied from 106·4° to 108° F. Two weeks after the first inoculations had been given the sheep were each reinoculated as before but with double the previous dose. On the following day each sheep showed a rise in temperature of from 2° to 4° F. By the the second or third day the temperature had returned to normal. Apart from the thermal reactions and some loss of appetite when the temperature was high all the sheep remained in normal health. Control sheep injected with suspensions of normal yolk sac showed no rise in temperature.

A suspension of infected yolk sac dropped into the conjunctival sac of sheep and inoculated into the prepuce of sheep caused neither a local nor a systematic reaction.

Rabbits and guinea pigs inoculated subcutaneously and intraperitoneally with suspensions of infective yolk sacs showed, on the first and second days after inoculation, a rise in temperature of 1° to 2° F., but four days after inoculation their temperature had returned to normal and remained normal until recording stopped three weeks later.

Male guinea-pigs inoculated subcutaneously and intraperitoneally with in some cases infective material from ovine foetal membranes and in others with infective yolk material, were killed and examined at varying intervals after inoculation. No abnormalities were found and impression smears made from the surface of the visceral peritoneum and the peritoneum of the scrotal sac never showed elementary bodies.

Pregnant guinea-pigs inoculated subcutaneously and intraperitoneally with infective material and killed before gestation ended sometimes showed elementary bodies, in very variable numbers, in the placenta.

The intracerebral inoculation of guinea-pigs with infective material produced no results.

Mice and rats inoculated by the subcutaneous, intraperitoneal and intracerebral routes remained well. Infection has not been observed in mice inoculated while pregnant.

One cow in the sixth month of gestation was inoculated intravenously with a suspension of ovine infected foetal membranes. Ten weeks after inoculation the cow aborted. The foetal membranes were recovered two to three hours later and were grossly diseased. The macroscopic lesions were indistinguishable from those caused by a *Br. abortus* infection, and histologically the membranes showed necrosis of the cotyledons and chorion, elementary bodies being present in the epithelial cells. Smears prepared from the foetal membranes showed elementary bodies singly and in clusters but less abundant than in the necrotic foetal membranes from sheep. The foetus was examined culturally but no bacterium was isolated. Smears made from the foetal stomach contents showed no elementary bodies.

Mice inoculated intranasally with heavily infected suspensions of yolk sac died five to seven days later. Their lungs showed areas of pneumonia containing numerous elementary bodies. The infection was transmitted in series from mouse to mouse.

Rats inoculated intranasally did not develop an infection; and the elementary bodies were not propagated in the lungs of calves and sheep inoculated intratracheally with infective material.

Discussion

Necrosis of the foetal membranes of sheep together with abortion and premature lambing has been produced by the inoculation of pregnant ewes with material from aborted lambs from flocks where outbreaks of abortion were occurring. The constant presence of large numbers of elementary bodies in the foetal membranes and discharges in the experimentally produced disease suggests that they are the causal microbe-organisms or the visible stage of a virus responsible for the disease. In their morphology and staining reactions the elementary bodies resemble the rickettsiae and the elementary bodies of the psittacosis-lymphogranuloma group of infective agents. Knowledge of the manner of transmission of the disease in the field might assist in the

classification of the infective agent. At present we do not have this knowledge; all that can be stated is that ticks, the vectors of some rickettsial diseases, are not implicated.

The failure to find the elementary bodies in the body of the foetus was surprising. In the bacterial infections that cause abortion the causal organisms multiply in the stomach contents where they are found in great numbers. Presumably the elementary bodies are obligate tissue parasites and failure to multiply in the stomach contents might account for the inability to recognise them in smears. The failure to infect the chick yolk sacs by the inoculation of stomach contents or of tissues from the internal organs of aborted foetuses suggests either that the elementary bodies were present in very small numbers and therefore incapable of setting up infection in the yolk sac, or that the elementary bodies had been destroyed in the stomach contents and possibly in the foetal tissues.

The elementary bodies or their precursors must have been present in the infective material used for the inoculation of the experimental sheep. Whether they were present in the stomach contents or in the foetal tissues or in both is a matter of conjecture. Another source is possible, however, namely contamination from the lambs' fleece wet with infected foetal fluids. Most of the lambs were examined without preliminary immersion in or wetting with disinfectant and attempts to avoid contamination when opening the peritoneal and thoracic cavities by the repeated sterilisation of instruments in boiling water, may not have been successful. We were probably fortunate in reproducing the disease with the type of material used. Obviously the materials most certain to transmit infection are suspensions of diseased foetal membranes and infected foetal discharges or fluids.

The foetal membranes of the sheep are vulnerable to attack by the elementary bodies or the infective agent, as also are the foetal membranes of the cow, and the placenta of the guinea-pig is to some extent susceptible. The somatic tissues of the ovine and bovine foetuses appear to be immune to attack, and, with the exception of mouse lung, the somatic tissues of all animals exposed to infection have been found to be resistant. The thermal response evoked by the inoculation of material containing elementary bodies may arise from the disposal of these bodies, or of a toxin associated with them, by the tissues, and not from any reaction to the invasion of host cells by the elementary bodies.

Whether most of the outbreaks of abortion and premature lambing in the south-east of Scotland are attributable to the same type of infection that caused disease in the experimental sheep is not known and the answer to this question must await further investigation.

Greig (1936) described an enzootic abortion of sheep in the Border counties of Roxburgh, Selkirk and Berwick and in other countries in Scotland. The information gathered by us on abortion in flocks in the south-east of Scotland, while different in some respects from that given by Greig, no doubt concerns the same disease or group of diseases. The only important discrepancy in the two descriptions occurs over the future behaviour of sheep that have aborted. According to Greig the same sheep may abort repeatedly whereas our information shows that after abortion they may be expected to lamb normally. Repeated abortion would suggest that the disease is not caused by an infective agent and this was the opinion held by Greig who though suspecting a dietary deficiency was unable to obtain experimental evidence that a deficiency was at play. An acquired resistance to abortion would suggest on the other hand, an acquired resistance to an infective agent.

The wide distribution of the disease reported by Greig shows the necessity of extending investigations in Scotland beyond the south-eastern areas.

Summary

A disease of the foetal membranes of the lamb caused by infection with an agent which in its morphology and in its staining reactions resembles the rickettsiae and the psittacosis-lymphogranuloma group of infective agents is described.

Ewes with infected foetuses may abort, give birth to premature weak lambs, or to apparently normal lambs. The abortions usually occur late in gestation.

The disease is probably widespread in sheep flocks in the south-east and other areas of Scotland. Definite information regarding the extent and the distribution of the disease must await further investigation. The disease occurs in flocks on tick free pastures.

The disease can be diagnosed by demonstrating the infective agent, elementary bodies, infected foetal membranes and discharges.

Experiments on the pathogenicity of the infective agent are described.

Acknowledgment.—We are indebted to and thank Dr. D. R. Wilson who kindly prepared the filtrates used in these experiments.

Reference

Greig, J. R. (1936.) *Vet. Rec.* **48.** 1225.

Erratum.—In caption below heading Fig. 12, *for* Fig. 12 *read* Fig. 11. We regret that early printing of plate precluded alteration.

Printed by H. R. Grubb, Ltd., Croydon.

Reprinted from
The Journal of Comparative Pathology and Therapeutics,
1951, Vol. 61, No. 3

EDINBURGH AND EAST OF SCOTLAND COLLEGE OF AGRICULTURE
Miscellaneous Publication No. 70

DEVELOPMENTAL FORMS OF THE VIRUS OF OVINE ENZOOTIC ABORTION

J. T. STAMP.
The Edinburgh and East of Scotland College of Agriculture, Edinburgh.

Printed by C. Tinling & Co. Ltd., Liverpool, London and Prescot
and published by the University Press of Liverpool

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DEVELOPMENTAL FORMS OF THE VIRUS OF OVINE ENZOOTIC ABORTION

By
J. T. STAMP
The Edinburgh and East of Scotland College of Agriculture, Edinburgh.

INTRODUCTION

Stamp, McEwen, Watt and Nisbet (1950) described a placentitis of the ewe caused by a virus showing morphological similarities to the elementary bodies of the psittacosis-lymphogranuloma group of organisms. Cultures of the virus obtained fairly easily in the yolk sac of a six or seven day chick embryo which is killed in 5 to 8 days. The virus also grows quite readily on the chorio-allantoic membrane, but in this case the embryo remains viable. In recent years investigations by a number of workers have shown that there is a group of viral agents which is morphologically similar to the viruses of psittacosis and lymphogranuloma venereum in that they form elementary bodies and appear to undergo a definite life cycle showing various developmental forms. The proper classification of the virus under study as a member of this group is thus, in part, dependent upon the demonstration of similar developmental forms.

MATERIAL AND METHOD

In order to demonstrate the possible presence of these developmental forms 0·25 cc. of a 10 per cent. broth suspension of a heavily infected yolk sac was inoculated on to the chorio-allantoic membrane of thirteen-day-old chick embryos using the technique described by Beveridge and Burnet (1946). In the first instance two eggs were killed and examined every two hours after inoculation but later it became obvious that the period 34 to 48 hours after inoculation was the most informative period so that eggs were examined more frequently during these hours of growth. Impression preparations of the infected membrane were made by cutting about one square cm. of membrane and laying the cut piece, ectodermal surface down, on to a clean dry slide. The cut membrane was then blotted and the slide warmed in the paraffin oven. When almost dry the blotting paper was lifted off, carrying most of the membrane with it, but leaving behind on the slide a good deal of ectoderm. The impression smear was then fixed in methyl alcohol, stained by prolonged Giemsa and afterwards differentiated in alcohol or on occasion acetone. Other pieces of the membrane were taken for histological examination, fixed in Regaud's fixative and sections stained by Giemsa.

Examination of the Chorio-allantoic membrane

The most characteristic lesions are seen in eggs opened 3 to 5

days after inoculation, although the macroscopic lesions are never very significant. The membrane may be thickened by oedema and show a varying degree of general opacity, there may be flecks but sharply defined lesions are not seen. The histological appearance of such a membrane is characteristic in that on the surface of the epithelium are darkly staining virus colonies packed with virus bodies. These colonies are surrounded by a degenerating zone of epithelium staining a homogeneous pink colour. The epithelial cells proliferate and are vacuolated to some degree, but the changes in the mesodermal and ectodermal layers are insignificant unless the membrane has undergone mechanical trauma (Fig. 1 and 2).

Growth behaviour of the virus

Some 36 hours after inoculation there appear virus bodies 0·5 to 0·7 μ in diameter staining deeply with the basic dyes and generally spherical in shape (Fig. 3). Within a short time these bodies grow in number and in size to become large intensely staining structureless bodies of 6 to 8 μ in diameter. Even after prolonged differentiation the structureless nature of the matrix remains except that at the periphery of some plaques it was possible to make out collections of darker staining bodies almost hidden by the matrix (Fig. 4). At slightly later stages the plaques begin to lose some of their affinity for the basic dye especially at the periphery and in the paler matrix one can now see the development of elementary bodies (Figs. 5 and 6). A number of plaques appear to become granular early in their formation and differentiate rapidly and evenly into colonies of elementary bodies without the prior formation of the large deeply staining bodies within the matrix of the plaque (Fig. 7).

A further method of development is, however, seen alongside the formation of the plaques. The initial virus bodies which appear at about 36 hours, instead of developing into plaques, may proliferate as such to form colonies of large forms of virus without any definite matrix; they are circular, oval or angular with flattened sides. These large forms do not stain well with the basic dye and not infrequently the bodies take on different depths of colour in the same colony. These virus colonies grow considerably in size, the large forms multiplying in number but at the same time becoming smaller in size to form colonies of intermediate forms (Figs. 8, 9, 10, 11, 12, 13 and 14). Finally by 72 hours the bodies have decreased in size to such a degree that the colonies are now packed only with elementary bodies.

It would also appear that the elementary bodies can divide as such to form colonies.

DISCUSSION

The virus of ovine enzootic abortion would appear to have a developmental cycle similar in many ways to that of the viruses of psittacosis and lymphogranuloma venereum. For descriptive pur-

poses the development of the virus falls into several phases but these go on side by side in the same eggs and at the same time intervals. The phases may be classified as follows:

1. Homogeneous plaques which may develop into large forms and then into colonies of elementary bodies.
2. Smaller plaques which develop rapidly and directly into colonies of elementary bodies.
3. Colonies of large and intermediate forms without the prior formation of a plaque. These develop into colonies of elementary bodies.
4. Colonies of elementary bodies multiplying as such.

The present work adds nothing further to the previous interpretations of the virus plaque (Bedson and Bland, 1934; Bland and Canti, 1935; Levinthal, 1935; Yanamura and Meyer, 1941; Rake and Jones, 1942). The findings are very similar to those previously described in that it would appear that the homogeneous plaque is a colony of large forms obscured by an extremely dense matrix. The origin of these forms in the plaque cannot be determined since the smaller plaques remain completely homogeneous even after very prolonged differentiation. The second type of plaque which differentiates early into colonies of elementary bodies suggests very strongly that the elementary bodies have been merely hidden by a matrix and that the plaque in this case is more in the nature of an inclusion body. The two methods of development of the plaque appear very different.

The colonies of large forms on the other hand are very definite having no confusing matrix. The evidence here is very clear that the large forms develop directly and multiply as large forms, becoming in turn intermediate forms and finally finishing as a virus colony of elementary bodies. In this form there is no confusion with matrix and the appearance of the colonies suggests that the large forms are virus material and not inclusion bodies of cytoplasmic matrix.

SUMMARY

The development of the virus of ovine enzootic abortion on the chorio-allantoic membrane of the chick embryo is described.

The findings suggest that this virus belongs to the psittacosis-lymphogranuloma group.

ACKNOWLEDGMENT

I am grateful to Mr. R. Cockburn for his help in the preparation of the material and the photographs.

REFERENCES

Bedson, S. P., and Bland, J. O. W. (1934). *Brit. J. exp. Path.*, **15,** 243.
Beveridge, W. I. B., and Burnet, F. M. (1946). *Med. Res. Counc. Spec. Rep.,* No. 256.

Bland, J. O. W., and Canti, R. G. (1935). *J. Path. Bact.*, **40,** 231.
Levinthal, W. (1935). *Lancet.*, 1207.
Rake, G., and Jones, H. P. (1942). *J. exp. Med.*, **75,** 323.
Stamp, J. T., McEwen, A. D., Watt, J. A. A., and Nisbet, D. I. (1950). *Vet. Rec.*, **62,** 251.
Yanamura, H. Y., and Meyer, K. F. (1941). *J. inf. Dis.*, **68,** 1.

[*Received for publication, February 10th,* 1951]

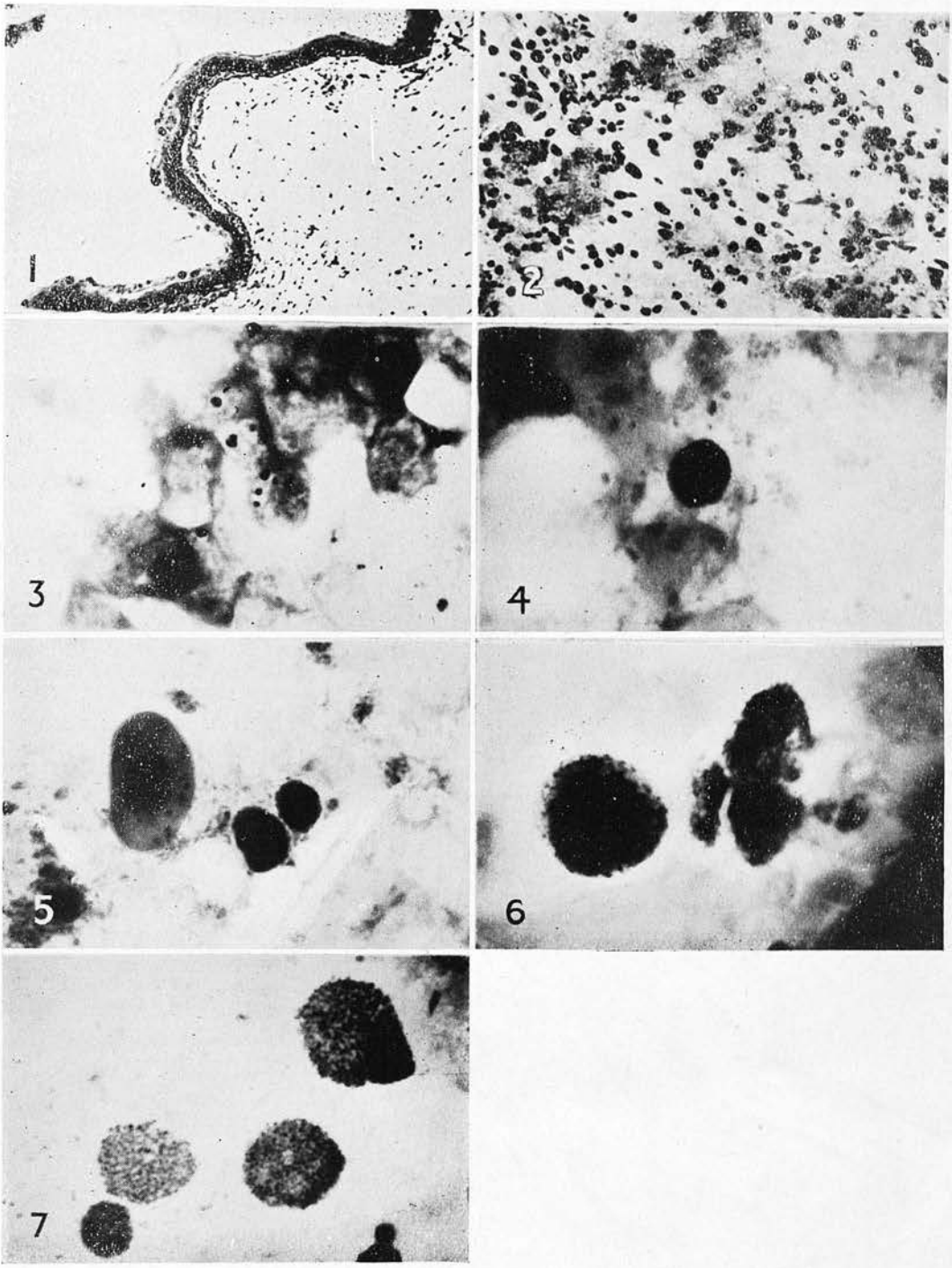
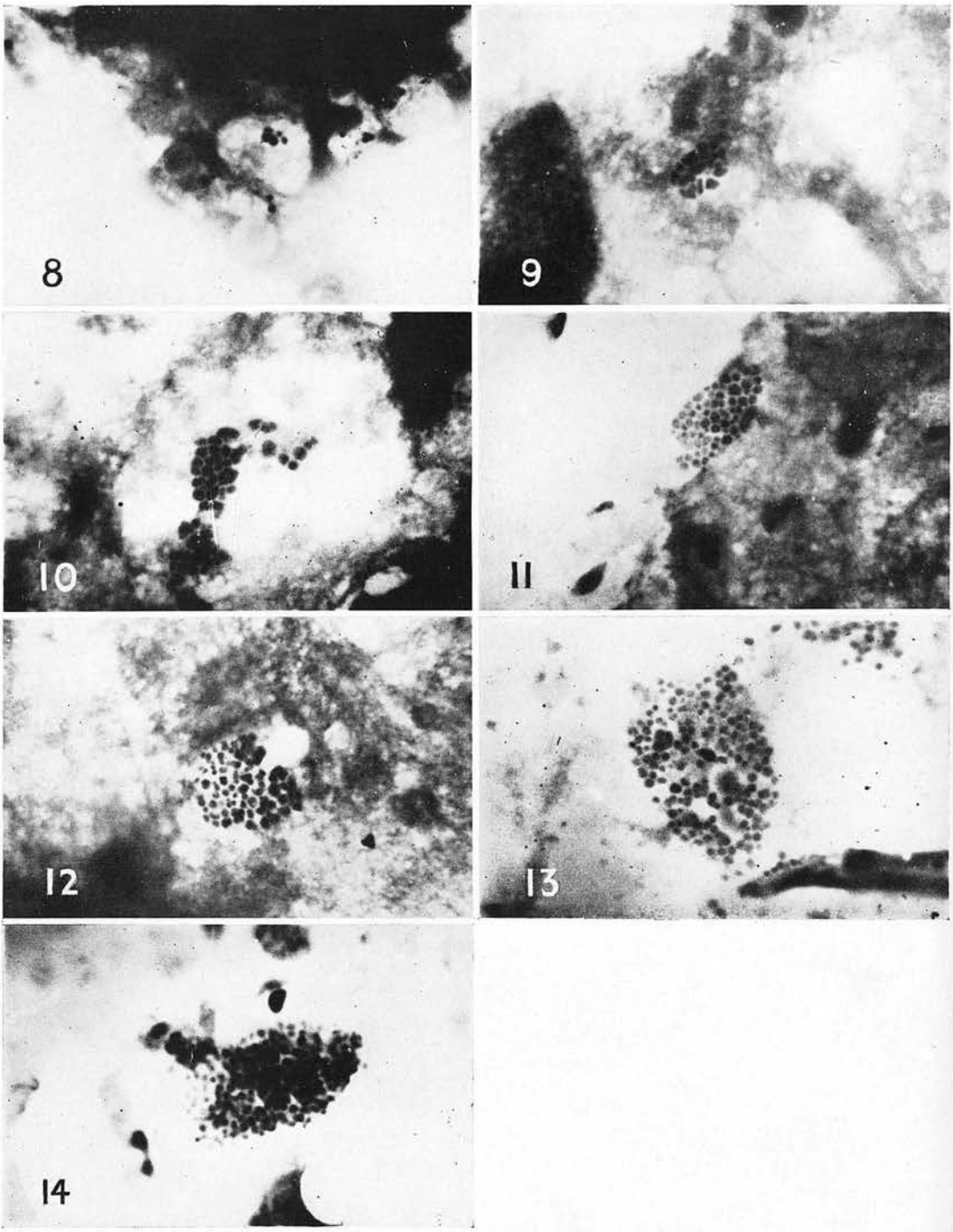


FIG. 1. Section of chorio-allantoic membrane showing the virus colonies of elementary bodies on the surface. Giemsa × 100.

FIG. 2. Impression smear of chorio-allantoic membrane showing large numbers of virus colonies packed with elementary bodies. Giemsa × 100.

FIG. 3. Impression smear of chorio-allontoic membrane showing the first virus bodies to be seen 36 hours after infection. Giemsa × 1,500.

FIGS. 4, 5, 6 & 7. Impression smears of chorio-allantoic membranes showing successive stages in the development of elementary bodies from the structureless plaque. Giemsa × 1,500.



FIGS, 8, 9, 10 & 11. Impression smears of chorio-allantoic membranes showing various stages in the development of colonies of large forms of virus. Giemsa × 1,500.

FIGS. 12, 13 & 14. Impression smears of chorio-allantoic membranes showing the formation of colonies of intermediate forms and elementary bodies arising from the colonies of large forms of virus. Giemsa × 1,500.

Reprinted from *The Veterinary Record*, 63. 197. March 17, 1951.

ENZOOTIC ABORTION IN EWES

II. Immunisation and Infection Experiments

BY

A. D. McEWEN,

Animal Diseases Research Association,
Moredun Institute, Edinburgh,

J. T. STAMP,

East of Scotland College of Agriculture, Edinburgh,

AND

ANNIE I. LITTLEJOHN,

Animal Diseases Research Association,
Moredun Institute, Edinburgh.

Stamp, McEwen, Watt and Nisbet, 1950, described a placentitis of the ewe caused by a virus showing similarities to those of the psittacosis lymphogranuloma group, and Littlejohn, 1950, found the disease to be widespread among "halfbred" (Border Leicester x Cheviot) and Cheviot ewes on lowland and marginal farms in an area of the south east of Scotland. The disease has been diagnosed during the past season in Aberdeenshire (Jamieson, 1950), in Westmorland (Heath, 1950) and in Shropshire (Shaw, 1950), and we have found it in Fife, Angus and Dumfriesshire.

Although abortion is a feature of the disease, lambs with infected membranes may be born prematurely or at full term and sometimes they are normally vigorous. Clinical manifestations are, therefore, of limited value in the interpretation of tests relating to infection or immunity in groups of experimental ewes. The most reliable data are obtained from an examination of the foetal membranes. As the foetal membranes may be lost it was found advisable in some of the experiments described in this paper to slaughter the experimental sheep shortly before the expected lambing date and examine the foetal membranes. In those cases where the ewe aborted or lambed and the foetal membranes could not be found, a diagnosis was based on the presence or absence of elementary bodies in smears made from vaginal swabs. The swabs were taken on the day of parturition and if the result was negative swabbing was repeated on the second and third day.

Yolk Sac Vaccine.—Three different batches of yolk sac vaccine were prepared. One batch was used in Experiment I and in a field vaccination trial. A second batch was used in part 1 of Experiment II and a third batch in part 2 of the same experiment. All these batches were used soon after preparation.

Infective material, containing virus that had been passaged two to four times through the chick embryo, was inoculated into six-day chick embryos and incubation was continued. Embryos dying during the first three days after inoculation were discarded. On and after the fourth day the embryos were examined twice daily and the yolk sacs harvested from those that died between the fourth and eighth day after inoculation. Not more than six yolk sacs at a time were pooled and ground in a Griffith tube; the emulsion was examined microscopically and if rich in "elementary bodies" and free from bacteria it was used for vaccine. The ground yolk sacs were emulsified in a fluid consisting of one part of M/15 phosphate *p*H 7·0 and four parts of physiological saline solution to which formalin had been added to give a final concentration of 0·4 per cent. 10 c.c. of the fluid was used for each yolk sac and the crude emulsion constituted the vaccine which was stored in the cold room for not less than two weeks after which, having been shown to be bacteriologically sterile, it was ready for use.

Foetal Membrane Vaccine.—A ewe in the third month of gestation was inoculated intravenously with infective material. Forty-five days later the animal was slaughtered and bled. The uterus was removed and the foetal membranes collected. The membranes, which showed extensive lesions and were heavily infected with "elementary bodies," were minced, ground in a Premier paste mill and made into a 10 per cent. suspension in the formalinised phosphate saline already described. The suspension was passed through a fine mesh sieve and stored in the cold. Before use it was shown to be bacteriologically sterile. Only one batch of foetal membrane vaccine was prepared.

On microscopical examination the yolk sac vaccines and the foetal membrane vaccine all appeared equally rich in "elementary bodies."

EXPERIMENT I

Yolk Sac Vaccine.—Twelve Dorset Horn ewes in approximately mid-pregnancy were each injected subcutaneously on two occasions and at an interval of 14 days with 5 c.c. of vaccine. On September 9th, 14 days after the second injection, the ewes were divided into two groups, A and B. Six ewes in group A and six control ewes were inoculated subcutaneously with 1 c.c. of a 1:1,000 broth dilution of a third passage infected yolk sac; the six ewes in group B and six control ewes were each inoculated subcutaneously with 1 c.c. of a 1:100,000 dilution of the same yolk sac.

The relevant data on the experiment are summarised in Table I (over-leaf.) Vaccination produced an immunity clearly seen in the group that received the smaller infective dose.

The sheep used in the subsequent experiments, except Experiment III, were young Blackface ewes purchased as unserved animals on October 20th and November 3rd, 1949, from an area where enzootic abortion is not known to occur. The rams were run with the ewes from December 5th, 1949. Later it was found that a number of the sheep had conceived to a service before purchase, but this was not discovered until after the animals had been allotted to different experimental groups, thus necessitating the removal of some sheep from the designed experiments.

TABLE I

IMMUNISATION OF THE PREGNANT EWE BY THE SUBCUTANEOUS INJECTION OF YOLK SAC VACCINE

Group A. Immunity challenged on the 9.9.49 by the subcutaneous inoculation of a " large " infective dose

Vaccinated				Controls			
		Membranes				Membranes	
Sheep	Date of parturition	Macroscopic appearance	Microscopic examination	Sheep	Date of parturition	Macroscopic appearance	Microscopic examination
1	19.10.49	+	+	1	*Killed 24.10.49	—	—
2	28.10.49	+	+	2	26.10.49	+	+
3	3.11.49	—	—	3	29.10.49	Retained	+
4	3.11.49	—	—	4	30.10.49	+	+
5	7.11.49	—	—	5	30.10.49	Retained	+
6	12.11.49	+	+	6	5.11.49	„	+
Total number of sheep 6 Number infected 3				Total number of sheep 6 Number infected 5			

Group B. Immunity challenged on the 9.9.49 by the subcutaneous inoculation of an infective dose 100 times smaller than the " large " infective dose

Vaccinated				Controls			
		Membranes				Membranes	
Sheep	Date of parturition	Macroscopic appearance	Microscopic examination	Sheep	Date of parturition	Macroscopic appearance	Microscopic examination
1	31.10.49	—	—	1	18.10.49	+	+
2	13.11.49	—	—	2	24.10.49	Retained	+
3	13.11.49	—	—	3	26.10.49	+	+
4	24.11.49	—	—	4	3.11.49	+	+
5	27.11.49	—	—	5	5.11.49	+	+
6	3.1.50	—	—	6	13.11.49	+	+
Total number of sheep 6 Number infected 0				Total number of sheep 6 Number infected 6			

+ Under " Macroscopic appearance " = Macroscopic lesions present.

+ Under " Microscopic examination " = Elementary bodies found.

* This ewe had a prolapsed vagina and was, therefore, slaughtered before lambing.

EXPERIMENT II

A COMPARISON OF THE YOLK SAC VACCINE WITH FOETAL MEMBRANE VACCINE

PART 1.—Two groups of ewes (1 and 2) were vaccinated, the one with yolk sac vaccine and the other with foetal membrane vaccine. Each sheep received two subcutaneous injections of vaccine at an interval of 14 days, the second injection being given on November 29th, 1949, seven days before the rams were run with the ewes. Later two further groups of ewes (3 and 4) were vaccinated in the same way, the second injections being given on February 16th, ten to eleven weeks after the rams were first allowed to run with the ewes. The immunity of the vaccinated groups was challenged by the subcutaneous inoculation of a " large " infective dose on March 4th, 1950, 14 weeks and three weeks respectively after vaccination.

Table II, part 1, shows that all four groups of vaccinated animals possessed an immunity but degrees of immunity between the different groups were not demonstrated.

PART 2.—A group of ewes was injected with yolk sac vaccine and another group with foetal membrane vaccine as groups 1 and 2 of part 1 of the experiment. On March 4th, 1950, the immunity of the sheep in these groups was challenged by the subcutaneous inoculation of an infective dose 100 times more dilute than the challenge dose used in part 1. From Table II, part 2, it will again be seen that vaccination produced an effective immunity.

EXPERIMENT III

THE IMMUNITY OF EWES THAT ABORTED OR LAMBED WITH DISEASED FOETAL MEMBRANES IN THE PREVIOUS SEASON (1949)

Ewes found infected at the 1949 lambing season and now carrying their second lamb were divided into two groups. The immunity of one group was challenged during pregnancy by a " large " infective dose. Nothing was done to the other group. The control sheep in Experiment II, part 1, acted also as controls to Experiment III, which experiment is presented in Table III. This experiment indicates that in the pregnancy following an abortion the foetal membranes are not infected; and that previously infected sheep possess a considerable degree of resistance to reinfection. These results are in accordance with field experience (Stamp *et al.*, 1950; Littlejohn, 1950).

TABLE III

EWES THAT HAD HAD LAMBS WITH INFECTED FOETAL MEMBRANES IN PREVIOUS PREGNANCY

Previously infected ewes, not challenged					Previously infected ewes challenged on the 4.3.50 by the subcutaneous inoculation of a " large " infective dose. For controls see Table II, Part 1				
			Membranes					Membranes	
Sheep	Lambed or killed	and date	Mac. app.	Mic. ex.	Sheep	Lambed, died or killed	and date	Mac. app.	Mic. ex.
1	L	25.4.50	Lost	—	1	L	23.4.50	—	—
2	L	26.4.50	—	—	2	D	25.4.50	—	—
3	K	3.5.50	—	—	3	K	3.5.50	—	—
4	K	„	—	—	4	K	„	—	—
5	K	„	—	—	5	K	„	—	—
6	K	„	—	—	6	K	„	—	—
7	K	„	—	—	7	K	„	—	—
8	K	5.5.50	—	—	8	K	„	+	+
					9	K	„	—	—
Number of sheep in group 8 Number infected 0					Number of sheep in group 9 Number infected 1				

EXPERIMENT IV

THE INFECTIVITY OF THE VIRUS WHEN ADMINISTERED PARENTERALLY BEFORE SERVICE

The virus of ewe abortion being of low pathogenicity for non-pregnant sheep and laboratory animals (Stamp *et al.*, 1950) it was thought probable that inoculation even a short time before service might not result in infection of the foetal membranes. If this were so the inoculated animals might be resistant to infection when they became pregnant.

Table IV, part 1, shows the results obtained from the subcutaneous, intravenous and intradermal inoculation of a " large " dose of virus three weeks before the rams were

TABLE II—PART 1

COMPARISON BETWEEN YOLK SAC VACCINE AND FOETAL MEMBRANE VACCINE. IMMUNITY CHALLENGED ON 4.3.50 BY THE SUBCUTANEOUS INOCULATION OF A "LARGE" INFECTIVE DOSE

1 Yolk sac vaccine before service. Second injection 29.11.49					2 Foetal membrane vaccine before service. Second injection 29.11.49					3 Yolk sac vaccine after service. Second injection 16.2.50					4 Foetal membrane vaccine after service. Second injection 16.2.50					Controls				
Sheep	Lambed aborted or killed and date		Membranes Mac. app.	Mic. ex.	Sheep	Lambed aborted or killed and date		Membranes Mac. app.	Mic. ex.	Sheep	Lambed aborted or killed and date		Membranes Mac. app.	Mic. ex.	Sheep	Lambed aborted or killed and date		Membranes Mac. app.	Mic. ex.	Sheep	Lambed aborted or killed and date		Membranes Mac. app.	Mic. ex.
1	K	26.4.50	—	—	1	L	26.4.50	—	—	1	K	26.4.50	—	—	1	K	26.4.50	—	—	1	K	14.3.50	—	—
2	K	„	—	—	2	K	„	—	—	2	K	„	—	—	2	K	27.4.50	—	—	2	A	16.4.50	+	+
3	K	27.4.50	—	—	3	K	„	—	—	3	K	27.4.50	—	—	3	K	„	—	—	3	A	„	+	+
4	K	„	—	—	4	K	27.4.50	—	—	4	K	„	—	—	4	K	29.4.50	—	—	4	A	17.4.50	+	+
5	L	„	Lost	—	5	K	„	—	—	5	K	1.5.50	—	—	5	K	1.5.50	—	—	5	K	18.4.50	—	—
6	K	1.5.50	—	—	6	K	1.5.50	—	—	6	K	„	—	—	6	K	„	—	—	6	K	„	—	—
7	L	„	Lost	—	7	K	„	+	+	7	K	5.5.50	—	—	7	K	„	—	—	7	K	„	—	—
8	K	„	—	—	8	K	„	—	—	8	K	12.6.50	—	—	8	K	„	—	—	8	A	19.4.50	+	+
9	K	„	—	—	9	K	5.5.50	—	—	9	K	„	—	—	9	K	„	—	—	9	A	21.4.50	+	+
10	K	„	—	—	10	K	„	—	—											10	K	1.5.50	—	—
11	L	11.6.50	—	—																11	K	„	+	+
No. of sheep in group 11					No. of sheep in group 10					No. of sheep in group 9					No. of sheep in group 9					No of sheep in group 11			
No. infected 0					No. infected 1					No. infected 0					No. infected 0					No. infected 6			
										1a	L	7.3.50	—	—	1a	L	18.3.50	Lost	—					
															2a	L	24.3.50	—	—					
															3a	L	17.3.50	—	—					

Sheep 1a, 2a, etc., were in lamb before purchase.

TABLE II—PART 2

COMPARISON BETWEEN YOLK SAC VACCINE AND FOETAL MEMBRANE VACCINE. IMMUNITY CHALLENGED ON THE 4.3.50 BY THE SUBCUTANEOUS INOCULATION OF AN INFECTIVE DOSE 100 TIMES SMALLER THAN THE INFECTIVE DOSE USED IN PART 1

Yolk sac vaccine before service. Second injection on 29.11.49					Foetal membrane vaccine before service. Second injection on 29.11.49					Controls				
Sheep	Lambed aborted or killed and date		Membranes Mac. app.	Mic. ex.	Sheep	Lambed aborted or killed and date		Membranes Mac. app.	Mic. ex.	Sheep	Lambed aborted or killed and date		Membranes Mac. app.	Mic. ex.
1	L	28.4.50	Lost	—	1	L	30.4.50	—	—	1	A	16.4.50	+	+
2	L	29.4.50	—	—	2	L	„	—	—	2	K	20.4.50	+	+
3	L	2.5.50	Lost	—	3	L	1.5.50	+	+	3	K	„	+	+
4	L	3.5.50	—	—	4	L	4.5.50	—	—	4	K	21.4.50	+	+
5	L	7.5.50	—	—	5	L	5.5.50	—	—	5	L	29.4.50	+	+
6	L	8.5.50	—	—	6	L	7.5.50	—	—	6	L	4.5.50	+	+
7	L	10.5.50	—	—	7	L	9.5.50	—	—	7	L	7.5.50	+	+
8	L	12.5.50	Lost	+	8	L	10.5.50	—	—	8	L	9.5.50	—	—
9	L	„	—	—	9	L	20.5.50	—	—	9	L	10.5.50	—	—
10	L	18.5.50	Lost	—	10	L	23.5.50	—	—	10	L	„	+	+
										11	L	12.5.50	+	+
Total number of sheep 10					Total number of sheep 10					Total number of sheep 11				
Number infected 1					Number infected 1					Number infected 9				
1a	L	16.3.50	—	—	1a	L	15.3.50	Lost	—					

The sheep 1a were in lamb before purchase.

run with the ewes. In every group, infection of the foetal membranes was produced in some of the animals.

In part 2 of Table IV are records of sheep inoculated subcutaneously before service with a " large " infective dose and again inoculated with a " large " infective dose after service. For the latter inoculation the material used was the same as that given to the controls in Experiment II. None of the eight sheep inoculated with virus before and again after service showed any sign of infection. In view of the proved infectivity of both inoculations this result was unexpected.

that the disease found in the experimental groups grazed on pastures previously used by infected sheep originated in the intentional administration of infective material.

Field Vaccination Trial

Ewes on 14 farms in Berwickshire and Roxburghshire were vaccinated during September, 1949, each ewe receiving two injections of yolk sac vaccine, an approximately equal number of similar sheep being left as controls. The shepherds on the farms were asked to keep a daily record of the abortions occurring in the flocks, *i.e.*, the expulsion of dead or non-viable foetuses about one to three weeks

Table IV

Infectivity of the Virus by Parenteral and by Oral Administration

Part 1															Part 2						Part 3					
Inoculation of " large " dose of virus before service															Subcutaneous inoculation of " large " dose of virus before service, followed by inoculation after service of " large " dose of same virus as used in Experiment II, Part 1						Feeding of 50 grammes of infected membranes 2-3 months after service					
Subcutaneous					Intravenous					Intradermal					Lambed aborted or killed and date		Membranes Mac. app.		Mic. ex.		Lambed aborted or killed and date		Membranes Mac. app.		Mic. ex.
Sheep	Lambed aborted or killed and date		Membranes Mac. app.		Mic. ex.	Sheep	Lambed aborted or killed and date		Membranes Mac. app.		Mic. ex.	Sheep	Lambed aborted or killed and date		Membranes Mac. app.		Mic. ex.																							
1	L	27.4.50	—	—	1	A	13.4.50	—	—	1	A	28.4.50	+	+	1	A	17.4.50	—	—	1	A	17.4.50	+	+
2	K	28.4.50	—	—	2	K	29.4.50	+	+	2	K	29.4.50	—	—	2	K	26.4.50	—	—	2	K	18.4.50	—	—
3	A	,,	+	+	3	K	,,	—	—	3	K	,,	—	—	3	K	,,	—	—	3	A	23.4.50	+	+
4	A	,,	+	+	4	K	,,	+	+	4	K	,,	—	—	4	K	27.4.50	—	—	4	K	24.4.50	+	+
5	L	,,	—	—	5	K	,,	+	+	5	K	1.5.50	+	+	5	K	,,	—	—	5	K	,,	—	—
6	K	29.4.50	—	—	6	K	,,	+	+	6	K	5.5.50	—	—	6	K	1.5.50	—	—	6	K	,,	—	—
7	K	,,	—	—	7	K	,,	+	+						7	K	5.5.50	—	—	7	K	,,	+	+
8	K	,,	+	+	8	K	1.5.50	—	—						8	K	,,	—	—	8	K	,,	+	+
9	K	,,	—	—	9	K	,,	+	+											9	K	1.5.50	—	—
10	K	23.5.50	+	+	10	K	,,	—	—											10	K	16.5.50	—	—
Total No. of sheep 10 Number infected 4					Total No. of sheep 10 Number infected 6					Total No. of sheep 6 Number infected 2					Total No. of sheep 8 Number infected 0					Total No. of sheep 10 Number infected 5					

EXPERIMENT V

Infection by Ingestion

An experiment was made to determine whether pregnant ewes exposed to infection by ingestion contracted the disease. The dose of infective material given to the ewes was probably greatly in excess of what may be ingested under field conditions. The experiment, summarised in Table IV, part 3, demonstrates that infection can be produced by ingestion.

Controls

The sheep in experiments II and IV were kept from the time of purchase in the autumn of 1949 to March 21st, 1950, on pastures where experimentally infected ewes had aborted or lambed in April and May of 1949, and after March 21st they were placed on pastures used by infected sheep that had lambed in the previous autumn.

It was necessary to consider the possibility that the experimental sheep using these pastures might acquire a natural infection. Accordingly, a group of sheep was left as normal controls. Sixteen pregnant sheep in this group were killed at different dates from April 25th to May 16th. All were in a very late stage of gestation and in no case were the membranes found to be infected. It is concluded before the expected date of lambing. At a routine weekly visit the losses from abortion were recorded and the data were later compared with and if necessary incorporated in the shepherds' written records of abortions to give the figures in Table V.

Table V

Field Vaccination Trial

Farm No.	No. of vaccinated animals	Aborted	% Abortion	No. of controls	Aborted	% Abortion
1	94	0	0	90	6	6·6
2	53	0	0	53	7	13
3	171	2	1·1	170	19	11·2
4	69	1	1·4	68	2	3
5	60	5	8·3	70	12	17
6	49	2	4·1	49	7	14·3
7	109	3	2·8	176	11	6·2
8	60	2	3·3	63	1	1·6
9	58	3	5·2	63	2	3·2
10	150	2	1·3	201	15	7·5
11	70	2	2·8	70	1	1·4
12	45	0	0	51	3	6
13	36	0	0	37	4	10·8
14	160	7	4·4	140	12	8·5
Totals	1,184	29	2·4	1,301	102	7·8

Results

(*a*) Farms where the incidence of abortion in controls was more than 10 per cent.:—

	Vaccinated	Control	
Aborted	9	49	58
Not aborted	360	330	690
	369	379	748

Percentage abortion for control sheep = 15.
Percentage abortion for vaccinated sheep = 2·5.
$x^2 = 28{\cdot}9$ so that $P < {\cdot}000001$.
Result is very highly significant.

(*b*) Farms where the incidence of abortion in controls was less than 10 per cent.

	Vaccinated	Control	
Aborted	20	53	73
Not aborted	795	869	1,664
	815	922	1,737

Percentage abortion for control sheep = 5·7.
Percentage abortion for vaccinated sheep = 2·4.
$x^2 = 11{\cdot}63$ so that $P < {\cdot}002$.
Result is significant.

In both groups vaccination produced a significant reduction in the abortion percentage, the results being highly significant when the incidence of abortion on the farm as judged by the controls was high. The incidence of abortion in vaccinated animals was about 2½ per cent. in both groups.

Summary

Sheep inoculated either before or after service with formalinised killed vaccine developed an effective resistance to an experimental infection.

Vaccines prepared from yolk sac and ovine foetal membranes appeared equally effective.

The foetal membrane vaccine did not lose its immunising properties on storage for four months, the longest period tested.

Sheep that produced foetuses or lambs with diseased membranes in 1949 lambed normally the following year and were not infected. Similar sheep possessed an effective resistance to experimental reinfection.

The parenteral inoculation of sheep before service produced infection in a number of the animals when later they became pregnant.

The feeding of material from diseased foetal membranes to pregnant ewes reproduced the disease in some of the animals.

A group of normal sheep grazing shortly before and during pregnancy on pastures where abortions had occurred the previous lambing season all lambed normally without infection.

The evidence obtained from a small-scale field vaccination trial showed that vaccination produced a significant reduction in the abortion rate.

References

Heath, G. B. S. (1950.) Personal communication.
Jamieson, S. (1950.) Personal communication.
Littlejohn, A. I. (1950.) *Vet. Rec.*, **62.** 571.
Shaw, J. G. (1950.) Personal communication.
Stamp, J. T., McEwen, A. D., Watt, J. A. A., & Nisbet, D. I. (1950.) *Vet. Rec.*, **62.** 251.

Printed by H. R. Grubb, Ltd., Croydon

REPRINT

ENZOOTIC ABORTION IN EWES

COMPLEMENT FIXATION TEST

by

J.T. STAMP, J.A.A. WATT and R.B. COCKBURN

Veterinary Investigation Department

The Edinburgh and East of Scotland
College of Agriculture

(Jn. Comp. Path. & Therap., 1952, 62, 1)

Stamp, McEwen, Watt and Nisbet (1950) described a placentitis of the ewe caused by a virus of the psittacosis lymphogranuloma group (Barwell and Bishop, 1951; Stamp, 1951). The infection gives rise to abortion and premature lambing amongst ewes in the South East of Scotland, a condition which has been prevalent for many years in this area. Although abortion and premature lambing is a feature of the disease, it is known that infected sheep may give birth to normal healthy lambs so that clinical signs are insufficient for assessing the incidence of infection in a flock (McEwen, Stamp and Littlejohn, 1951). It was considered that a laboratory diagnostic test would give more satisfactory information and since Bedson, Barwell, King and Bishop (1949) had shown the complement fixation test to be accurate in the diagnosis of lymphogranuloma venereum, and Barwell and Bishop (1951) had used the

same method for the classification of the present virus into the psittacosis lymphogranuloma group, it was decided to try to evolve a similar method for field investigation.

Technique

Egg Antigen

This antigen was prepared in a manner similar to that described by Bedson, et al (1949) from the yolk sac of eggs inoculated on the fifth day of incubation and used 5 to 6 days later, only yolk sacs very rich in the organism being used. The growth of the virus in the yolk sac of the developing egg has been previously described by Stamp, et al (1949). The yolk sacs were ground in Griffiths tubes and suspended in 2 c.c. of phosphate buffer pH 7.6, per yolk sac. After sedimentation in the refrigerator for 24 hours the supernatant fluid was centrifuged for 2 hours on an angle centrifuge at 5,000 r.p.m., the deposit being suspended in half the original volume of saline. When smears of this suspension, stained by the modified Ziehl Neelson technique, showed a good virus content, the suspension was steamed for 20 minutes and then sodium azide was added to a concentration of 0.3 per cent to act as a preservative.

Foetal membrane antigen

During the lambing season of 1951 considerable

numbers of infected foetal membranes were sent to the laboratory for diagnosis of enzootic abortion. Some of these membranes were extremely contaminated but a number were received in a fresh state and only slightly contaminated with bacteria. Microscopical examination of smears made from diseased portions of such membranes showed enormous numbers of virus elementary bodies and it was considered that this material might be made into an antigen more easily than infected yolk sacs. The obviously contaminated portions of the membrane were carefully removed and discarded, while the epithelial surface of the remaining portions was scraped and washed with phosphate buffer. Many of the membranes were thickly encrusted with dry, yellow caseous masses which on microscopical examination were seen to be composed almost entirely of virus elementary bodies and along with the more fluid portions of the membrane were carefully preserved for use. The whole of the collected material was broken down in phosphate buffer made up to 400 c.c. per foetal membrane by shaking with small glass beads for a period of one hour. A preliminary centrifugation was then carried out on a large horizontal centrifuge for 10 minutes at 2,000 r.p.m. to get rid of gross particles. The supernatant fluid was next centrifuged for 1½ hours at 5,000 r.p.m. on an angle centrifuge, the deposit being resuspended in 100 c.c.

of saline. If an examination of smears showed that the suspension was rich in virus and relatively free from bacteria, it was treated in the same manner as the egg antigen. Finally all the different batches of membrane antigen were bulked and stored in the refrigerator at 4°C.

The Complement Fixation Test

Preserved guinea pig serum (Burroughs Wellcome) was used throughout, a dose of 2 MHD being calculated by the titration of complement in the presence of the standard amount of antigen. The amount of antigen to be used was determined by finding that dilution of antigen which when used in 0.1 ml. doses was non anticomplementary and yet had a high antigenic value. The most satisfactory dilution was found to be one in four since the bulked antigen itself was anticomplementary and a dilution of more than one in six lost some of its antigenic value. Dilutions of test sera inactivated at 56°C. for 30 minutes were made from 1 in 2 to 1 in 1024 doubling each time, 0.1 ml. of each dilution being used. Fixation was for 20 minutes at room temperature followed by 30 minutes at 37°C. in the water bath. Overnight fixation in the refrigerator was not satisfactory since the anti-complementary action of the antigen was increased out of all proportion to the increased fixation achieved. The haemolytic system comprised 0.2 ml.

of 5 per cent sheep red cells sensitized with 5 MHD of amboceptor. The quantity of fluid in each tube having been made up to 1 ml. incubation was carried out in the water bath for 30 minutes, readings being taken immediately afterwards. The greatest dilution of serum giving complete fixation of the 2 MHD of complement used throughout the tests was taken as the titre. Suitable controls were included together with pooled sera of known positive and negative titres respectively.

RESULTS

Diagnostic value of the Complement Fixation Test

It was first necessary to determine the complement fixing titres of serum samples taken from sheep known to have aborted their lambs on farms where active infection with the virus of enzootic abortion had recently been demonstrated. In the case of a number of these aborting sheep the virus elementary bodies had been actually demonstrated in the foetal membranes, but in the majority of sheep the evidence of infection was only presumptive, but nevertheless reasonably certain on the grounds of clinical evidence and the known presence of infection in the flock. Serum was used which had been taken six weeks after abortion had occurred. Altogether sera from 146 aborting sheep were examined. In addition, sera from 211 sheep which were born and lived on

various farms known definitely to have no history of clinical abortion were also tested to act as control sera. The results are recorded in Table I.

It will be seen that none of the control sheep sera gave a titre as high as 1/16 whereas 81.3 per cent of the suspected sera gave titres of 1/16 or over. It is probable that some of the suspected sera giving titres of less than 1/16 may in fact be sera from non-infected sheep in that amongst the considerable number of specific abortions, a few may have been caused by agents other than the virus of enzootic abortion. The figure of 81.3 per cent is, therefore, a conservative estimate of the accuracy of the test. From the present investigation it would appear that a titre of 1/16 can be taken as being suggestive of infection and titres over 1/16 as being definite evidence of infection. These tests were duplicated using both egg and membrane antigen and the results in every case were similar. For convenience the membrane antigen was used for the remainder of the work.

Variation in individual titres during a four month period following abortion

For the purposes of determining the diagnostic value of the complement fixation test, sera were taken approximately six weeks after abortion had occurred when it was considered that the optimum antibody response would have taken place. In

TABLE I

Complement fixation titres of aborting sheep on infected farms

and of healthy sheep on clean farms

Number of Sheep	Sheep sera giving complete fixation at dilutions of:-												Positive Complement Fixation 1/16
	0	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{256}$	$\frac{1}{512}$	$\frac{1}{1024}$	$\frac{1}{2048}$	
146 ewes	0	2	12	14	30	24	27	21	11	3	2	0	81.3%
(All having aborted)	(0%)	1.3%	8%	9.4%	20.1%	16%	18%	14.7%	8%	2%	1.3%	0%	
122 (65 hoggs) (57 ewes)	33	39	39	11									Nil
From non-infected farms	27%	32%	32%	9%	-	-	-	-	-	-	-	-	
89 (25 hoggs) (64 ewes)	None gave a titre of 1/16 - lower titres were not recorded												Nil
From non-infected farms													

addition, in the majority of cases samples were also taken two weeks and four months after abortion, to follow variations in the titre and to determine the period during which the titre would remain at a high level. It is proposed to continue the investigation of this point for a longer period. It was found that between the first and second bleedings, i.e. between two and six weeks after abortion, the serum titres remained steady in forty-two cases (37 per cent), showed a slight rise or fall in 58 cases (51 per cent), and changed from a negative to positive reading in 13 cases (12 per cent). In no case did a positive titre become negative during this period. Between the second and third bleedings, i.e. between six weeks and four months after abortion, the titres remained steady in 54 cases (48 per cent), showed a slight rise or fall in 55 cases (49 per cent); in no case did a negative reading change to a positive one. In four cases the readings dropped from a suspicious titre of 1/16 to a negative reading.

The results indicate that immediately after virus abortion, a small number of sheep gave negative complement fixing titres which six weeks later, changed to positive readings. With the exception of four cases which gave readings of 1/16 at the second test and were negative at the first and third tests, all the positive reactors continued to react for a period of four months after abortion.

Determination of incidence of infection in various age groups of sheep on known infected farms

Clinical manifestations of the disease are of very little value in determining the true incidence of infection in a flock since, although abortion is a feature of the disease, it is assumed that lambs may be born at full term when they may be weakly or quite normal and vigorous (McEwen, et al, 1951). In consequence, it was considered that the complement fixation test might give useful information on the actual incidence of infection in various age groups. Four farms were selected to carry out this investigation. Farm A was chosen since over a number of years the incidence of the clinical disease had been fairly constant and was representative of the disease as it occurs commonly on the Border farms of S.E. Scotland. Farm B was chosen since there had been a high incidence of abortion in the current lambing season but the disease had not previously been recognised. Farm C had had an average incidence of clinical abortion for many years, but during the period immediately preceding the tests only a few abortions had been observed each year. Farm D appeared to have no abortion history, but for the last two years quite a number of "rotten" lambs had been born.

Owing to lack of facilities it was not possible to test every sheep on these farms, but random

selection of about fifty sheep in each group was carried out. The samples were taken about four weeks after lambing time.

The results given in Table 2 are based on the assumption that a complement fixation titre of 1/16 or over is evidence of previous infection. The complement fixation titres are given in Table 3.

The incidence of clinical abortion is low when compared with the incidence of infection as shown by the complement fixation test. This is particularly evident in the gimmers on Farms A and B. It is realised that some of the infection in the gimmers may have developed after lambing time but even if the percentage infection of the hoggs is taken as an indication of the incidence in the gimmers before lambing time, the incidence in the gimmer age group is still considerable. On Farms A, B and D the incidence in the 2nd or 3rd crops is considerably higher than in the younger animals. The amount of clinical abortion is also higher but even in these groups less than 1 in 10 of positive reactors abort. There is also good evidence that a high incidence of infection takes place in young sheep. This may occur within one month of birth; alternatively, hoggs may become infected during lambing time since at this period they are frequently grazing with ewes which have aborted. It is suggested that the very low incidence of clinical abortion in the gimmers,

TABLE 2

Percentage of infected and aborted animals in various age groups on Farms A, B, C, and D

	Farm A		Farm B		Farm C		Farm D	
	Infected	Aborted	Infected	Aborted	Infected	Aborted	Infected	Aborted
Lambs	2%	-	5%	-	0%	-	2%	No abortion but a number of "rotten" lambs born
Hoggs	33%	-	29%	-	25%	-	16%	
Gimmers	44%	1.6%	64%	0.8%	20%	Nil	16%	
2nd Crop	50%	9%	70%	9%	24%	Nil	37%	
3rd Crop	30%	5.2%	72%	13%	26%	Nil	36%	
4th Crop	-	-	60%	7%	16%	Nil	-	

TABLE 3

Complement fixation test in various age groups of sheep on infected farms

	LAMBS						Total Number of Animals
	0 - 1/8	1/8+	1/16	1/16+	1/32	1/64	
Farm A	49	-	1	-	-	-	50
Farm B	36	3	2	-	-	-	41
Farm C	50	-	-	-	-	-	51
Farm D	47	2	-	-	1	-	50

	GIMMERS						Total Number of Animals
	0 - 1/8	1/8+	1/16	1/16+	1/32	1/64	
Farm A	19	9	11	6	3	-	48
Farm B	12	6	8	9	11	4	50
Farm C	31	5	1	8	-	-	45
Farm D	35	7	-	3	3	2	50

	3rd CROP						
	0 - 1/8	1/8+	1/16	1/16+	1/32	1/64	
Farm A	15	15	6	1	5	1	43
Farm B	6	6	7	9	9	7	44
Farm C	26	11	2	3	3	5	50
Farm D	30	2	2	3	12	1	50

	HOGGS						Total Number of Animals
	0 - 1/8	1/8+	1/16	1/16+	1/32	1/64	
Farm A	23	9	16	-	-	-	48
Farm B	20	15	10	4	-	-	49
Farm C	21	14	5	7	-	-	47
Farm D	37	5	2	6	-	-	51

	2nd CROP						
	0 - 1/8	1/8+	1/16	1/16+	1/32	1/64	
Farm A	16	10	6	9	6	5	52
Farm B	8	7	7	9	12	7	50
Farm C	21	16	4	4	3	1	49
Farm D	35	3	5	5	10	3	61

	4th CROP						
	0 - 1/8	1/8+	1/16	1/16+	1/32	1/64	
Farm A	No 4th Crop sheep kept						
Farm B	14	6	5	7	13	5	50
Farm C	36	5	2	4	2	-	49
Farm D	No 4th Crop sheep kept						

(+ is where complete fixation occurs at the given titre but higher dilutions give partial fixation.)

even when the number of positive reactors is considerable, may be due in some way to the relatively high incidence of reactors amongst the hoggs. It may be that sufficient immunity is developed to overcome the majority of infections before the first pregnancy and it is only when infection occurs for the first time at a later age that a higher incidence of clinical abortion occurs. The findings on Farm C support this hypothesis to some extent. Further field survey work is, however, being carried out in an attempt to clarify such problems of epidemiology.

Lambs reared on ewes which had aborted just previous to becoming foster mothers

The sera of twelve ewe lambs, suckling ewes which had previously aborted, failed to give positive reactions before four months of age. This finding would suggest that complement fixing antibodies are not passively transferred in the colostrum and that, if infection is transmitted from the aborted ewe to the offspring during suckling, complement fixing antibodies do not develop during this period.

Sera from other sources

Rams

Sera from fourteen rams which had been used extensively and for a varying number of years on Farm B gave no positive reactions to the complement fixation test.

Laboratory Staff

Sera from four members of staff who had worked continuously with the virus of ewe abortion over several years, (at times perhaps not too carefully) were subjected to the C.F.T. with negative results.

SUMMARY

Antigen suitable for the complement fixation test was prepared both from infected yolk sacs of the developing chick embryo and also from infected membranes of the sheep.

The efficiency of the complement fixation test was assessed by testing blood sera from known infected sheep and from presumably non-infected sheep. A titre of 1/16 would appear to be suggestive of infection and titres over 1/16 are evidence of infection.

Complement-fixing antibody is present in the majority of cases within two weeks of abortion having taken place and is detectable for at least four months.

The complement fixation test was used to determine the incidence of infection on four farms. There appeared to be a high rate of infection especially in the 2nd and 3rd crop ewes: moreover, lambs and virgin sheep may give a positive reaction.

The sera of fourteen rams were negative.

REFERENCES

Barwell, C.F., and Bishop, L.W.J., (1951), Nature, 167, 998.

Bedson, S.P., Barwell, C.F., King, E.J., and Bishop, L.W.J., (1949), J. Clin. Path., 2, 241.

McEwen, A.D., Stamp, J.T., and Littlejohn, Annie I., (1951), Vet. Rec., 63, 197.

Stamp, J.T., McEwen, A.D., Watt, J.A.A., and Nisbet, D.I., (1950), Vet. Rec., 62, 251.

Stamp, J.T., (1951), J. Comp. Path., 61.

Reprinted from *The Veterinary Record*, 62, 465

Edinburgh and East of Scotland College of Agriculture.
Miscellaneous Publication No. 58.

TICK-BORNE FEVER AS A CAUSE OF ABORTION IN SHEEP—PART I

BY

J. T. STAMP

AND

J. A. WATT

THE EDINBURGH AND EAST OF SCOTLAND COLLEGE OF AGRICULTURE, EDINBURGH

Lyle Stewart (1936) suggested that tick-borne fever was a cause of abortion in sheep and Harbour (1945) also considered that this was the case. That this can only be so under certain conditions is obvious since abortion is not present on the majority of tick-infested farms. For example, Harbour (1945) considered that the high abortion rate (30 per cent. and 16 per cent.) observed when non-acclimatised ewes were moved on to tick-infested land was due to tick-borne fever. He also pointed out that in the Borders, hill hoggs (ewe lambs) wintered away on tick-free ground may entirely miss the spring cycle of tick activity and only meet with tick-borne fever infection again as pregnant gimmers when abortion may occur as a result of tick-borne fever. Abortion also occurs on farms where the tick infestation is comparatively light and again it is suggested that tick-borne fever is the cause. It is assumed in all these cases that the aborting sheep have either lost their "immunity" to tick-borne fever or have never obtained any immunity. It also follows that sheep on tick-infested farms where there is only a spring tick cycle are assumed to retain their "immunity" from year to year.

The present investigation was carried out in an endeavour to clarify the position regarding these assumptions.

THE EXPERIMENTAL PRODUCTION OF ABORTION IN SHEEP BY TICK-BORNE FEVER

BOGHALL EXPERIMENTAL FARM

The object of the following experiment was to determine the effect with special reference to abortion, of inoculating tick-borne fever infective blood in (*a*) ten Black-face susceptible pregnant gimmers; (*b*) ten Black-face ewes of mixed ages which had been born and reared on a tick-infested farm in Aberdeenshire. For this period of the experiment both groups were grazed on a tick-free pasture, having been tupped so that lambing would commence at the end of April.

On March 23rd all 20 pregnant sheep were inoculated subcutaneously with 10 c.c. citrated infective blood. The strain of tick-borne fever used was that freshly isolated from sheep on a Border farm. It was passaged twice through susceptible lambs which gave the typical febrile reaction, tick-borne fever being confirmed by microscopical examination of blood smears. The blood used as inoculum was taken from the second passaged lamb at the height of the temperature reaction (107·5°). Temperatures of all sheep were taken before inoculation (maximum 102·7°), and thereafter daily for 14 days. Blood films were made from all sheep on the fourth, fifth and sixth days and examined for tick-borne fever bodies.

Results. (See Table I.) (For tables see pages 466-468.)

Susceptible Sheep.—All sheep in this group showed tick-borne fever bodies in their blood and all but one showed typical temperature reactions. They were noticeably ill for several days. Five of them aborted within the period seven to ten days after inoculation, four lambed normally and one was barren. Examination of the aborted lambs was negative for pathogenic bacteria.

Acclimatised Ewes.—Apart from one animal which died of blackquarter following inoculation, these sheep showed no temperature reaction, no illness and no tick-borne fever bodies. Eight out of the nine surviving animals lambed normally, one being barren.

IMMUNITY TO TICK-BORNE FEVER

Gordon *et al.* (1932) state that a specific immunity to tick-borne fever can be produced in sheep after either one or on occasion several injections of infected blood. Jamieson (1947), however, considers that immunity is probably dependent upon constant repeated infection and that removal of sheep to tick-free pastures for a period of six months results in a marked lowering of immunity to the disease. Since immunity to tick-borne fever would appear to play a part in the prevention of abortion in sheep it was decided to carry out a small scale experiment on the persistence of immunity.

The experimental sheep used were the gimmers and ewes with their surviving lambs which had already been used at Boghall for the production of abortion by tick-borne fever. As already stated, these ewes were inoculated with virulent T.B.F. blood on March 23rd; the Aberdeen sheep being already immune gave no or very little reaction, while the local sheep gave a T.B.F. reaction.

Every two months after this date the immunity of two of these sheep was challenged by inoculation of 10 c.c. of virulent T.B.F. blood given subcutaneously. The strain of T.B.F. used was the same as that used in the initial experiment and kept going by repeated passage through susceptible lambs. The experiment extended over a period of 12 months from the date of the original inoculation. The results are given in Table II, and with one exception the challenging dose caused no significant temperature reaction nor the presence of T.B.F. bodies in the blood over the 12 months' period. Control susceptible sheep gave a very definite tick-borne fever reaction in every case.

A similar experiment was carried out on the lambs of the Boghall abortion experiment. The lambs when three months old were inoculated subcutaneously with 10 c.c. of the same strain of T.B.F. as given above. All the lambs gave the usual signs of tick-borne fever and this was confirmed by demonstrating T.B.F. bodies in every case. Thereafter two were challenged with 10 c.c. of virulent T.B.F. blood approximately every two months for a period of eleven months. (It was intended to extend over 12 months, but this was not possible.)

The results are given in Table III in which it will be seen that the majority of lambs gave no significant temperature reaction; two lambs, however, did have a small

temperature rise. In no case could tick-borne fever bodies be demonstrated on daily examination.

It has been observed for some time that in the Border counties of Scotland certain farms, where tick infestation was only slight or where tick infestation was comparatively recent, had an annual incidence of abortion, especially amongst the gimmers and to a less extent amongst the second crop ewes. On some farms the abortion was confined to certain hirsels. The incidence varied from farm to farm, but was never very high, being about 5 to 10 per cent. of the affected age groups. In contrast, abortion was not a feature of the more heavily tick-infested farms. It was considered a possibility that on such farms immunity to tick-borne fever was not sufficiently widespread in the younger age groups to give an over-all immunity to the fever and that resulting abortion would occur at least in some of the gimmers and second crop ewes.

It was decided that the immunity of hoggs and gimmers to tick-borne fever might be estimated on two tick-infested farms, one farm (Farm B) having few ticks and a 5 to 10 per cent. incidence of abortion amongst the gimmers and second crop ewes, the other farm (Farm G) in the same area having a relatively high tick infestation and no abortion. It had previously been demonstrated in a number of cases of abortion on Farm B that tick-borne fever bodies were present in the blood immediately after the abortion. Louping-ill on Farm B was unknown but common on Farm G. On both farms the immunity to tick-borne fever of ten hoggs and ten gimmers was challenged by the inoculation of 10 c.c. of T.B.F. blood subcutaneously during the autumn period (November). The results showed that on Farm G the immunity to tick-borne fever was complete, whereas on Farm B eight of the ten hoggs and four of the ten gimmers were not immune.

TABLE I

GROUP A SUSCEPTIBLE		GROUP B IMMUNE	
B2	Lambed normally	41	Tup Eild
B2 CMR	Aborted 2-4-46	79	Lambed normally
B5	Aborted 2 4 46	54	Lambed normally
B5 CMR	Aborted 2·4·46	14	Lambed normally
B2 CML	Lambed normally	36	Lambed normally
H R H	Aborted 31·3·46	47	Lambed normally
T 5	Lambed normally	1	Dead
T5 CMR	Aborted 30·3·46	21	Lambed normally
T5 CML	Tup Eild	38	Lambed normally
T5 CMB	Lambed normally	31	Lambed normally

Field "Vaccination" Experiment

Since the above observations suggested that a degree of immunity could be obtained to tick-borne fever it was considered that "vaccination" of hoggs and gimmers on Farm B with a strain of tick-borne fever just before tupping might prevent the onset of natural tick-borne fever in the following spring, and if this disease was the cause of the abortion then the vaccination should prevent this also. For this experiment 20 hoggs and 20 gimmers were inoculated subcutaneously with 10 c.c. of virulent tick-borne fever blood several weeks before tupping. They were then allowed on the hill. This inoculation caused a very definite check in a number of the thriving sheep. A similar number of animals in each group were left as controls. Adequate marking of both groups was carried out. The abortion rates in the two groups in the following spring were:—

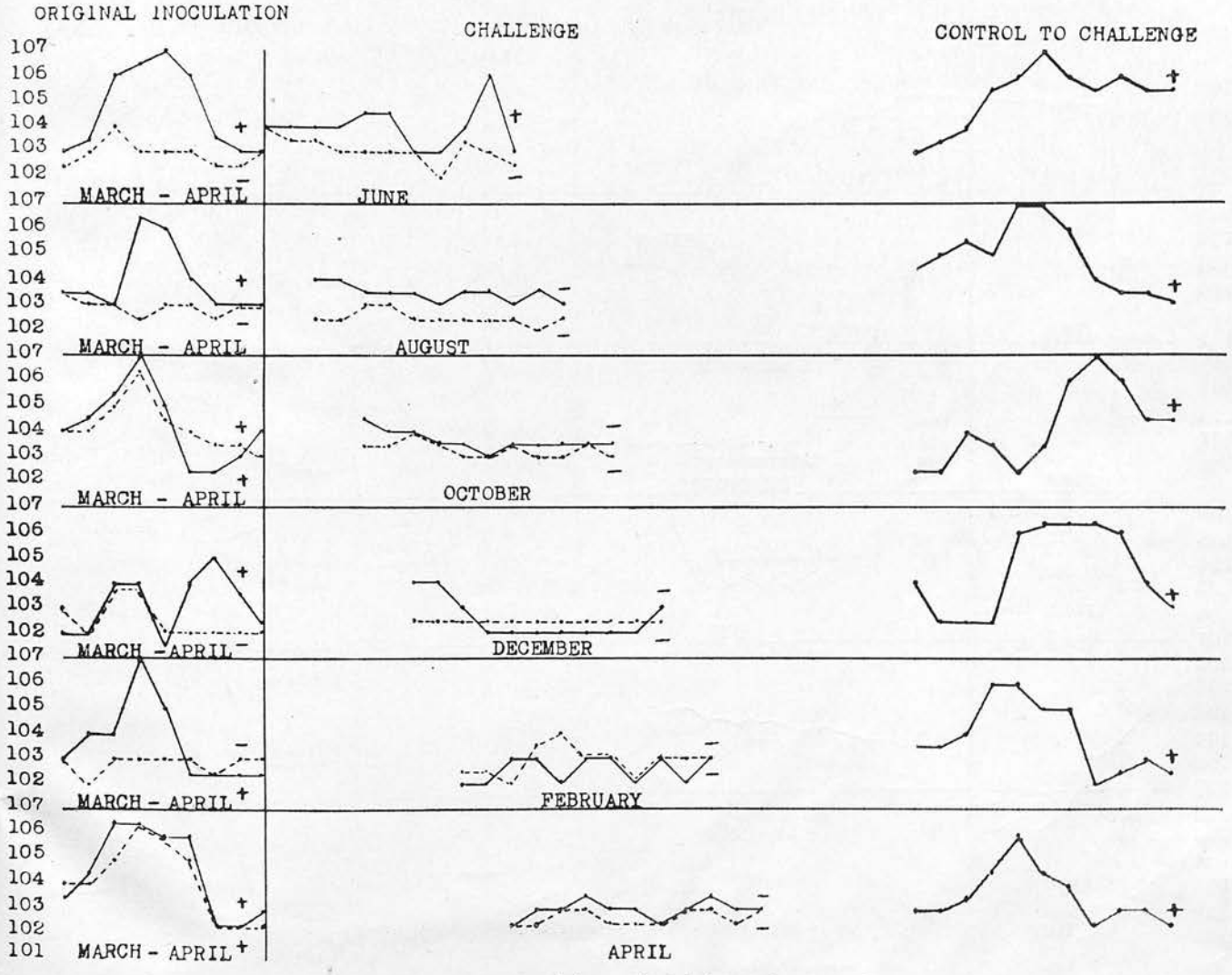
40 sheep in inoculated group—no abortions.
40 sheep in control group—five abortions.

Discussion

There is little doubt from the above results that tick-borne fever is capable of causing abortion when pregnant sheep are affected near the lambing time, while immune control sheep showing no evidence of tick-borne fever after inoculation, lambed normally several weeks later.

The whole question of immunity to tick-borne fever is complicated, but the present results suggest that one inoculation of 10 c.c. of virulent T.B.F. blood is sufficient to give a considerable degree of immunity to a similar challenge dose for a period of 12 months. In the present experiment this applied to both lambs and their mothers, the lambs receiving their first inoculation at three months. Our findings are in agreement with those of Gordon *et al.* (1932), who state that a specific immunity can be produced after either one or on occasion several injections of infected blood. A similar lasting immunity in gimmers to such a challenge dose was also seen on the heavily tick-infested Border Farm G for a period of at least eight months. These findings are not in agreement with those of Jamieson (1947), who stated that immunity under field conditions is dependent upon constant repeated infections and gave field evidence to support this. On Farm B where tick infestation was only slight the immunity of hoggs, gimmers and second crop ewes was by no means over-all, and this suggests that many of these sheep did not become infected with tick-borne fever in their earlier years or that the immunity was insufficient to carry over the tick-free periods

Table II



from spring to spring. On the present experimental evidence the former would appear to be the more likely.

From the experimental results the following deductions can be made regarding the occurrence of T.B.F. in pregnant gimmers and ewes and the consequent risk of abortion.

(1) Adult sheep born and reared on heavily tick-infested farms are immune to tick-borne fever and therefore to this cause of abortion.

(2) Susceptible sheep introduced to tick-borne fever when pregnant will abort in considerable numbers. This may occur when sheep are brought on to tick-infested pastures and introduced to T.B.F. for the first time before lambing or when sheep are reared on very lightly tick-infested pastures not obtaining an immunity to T.B.F. before pregnancy.

References

Gordon, *et al.* (1932.) *J. comp. Path.* **45.** 301-306.
Harbour, H. E. (1945.) *Trans. Highl. agric. Soc. Scot.* **57.** 16-40.
Jamieson, S. (1947.) *Vet. Rec.* **59.** 201-202.
Lyle Stewart, W. (1936.) *Ibid.* **48.** 1225-1232.
———. (1936.) Second Rep. Duke's Fund. Sheep and Lamb Diseases.

PART II

BY

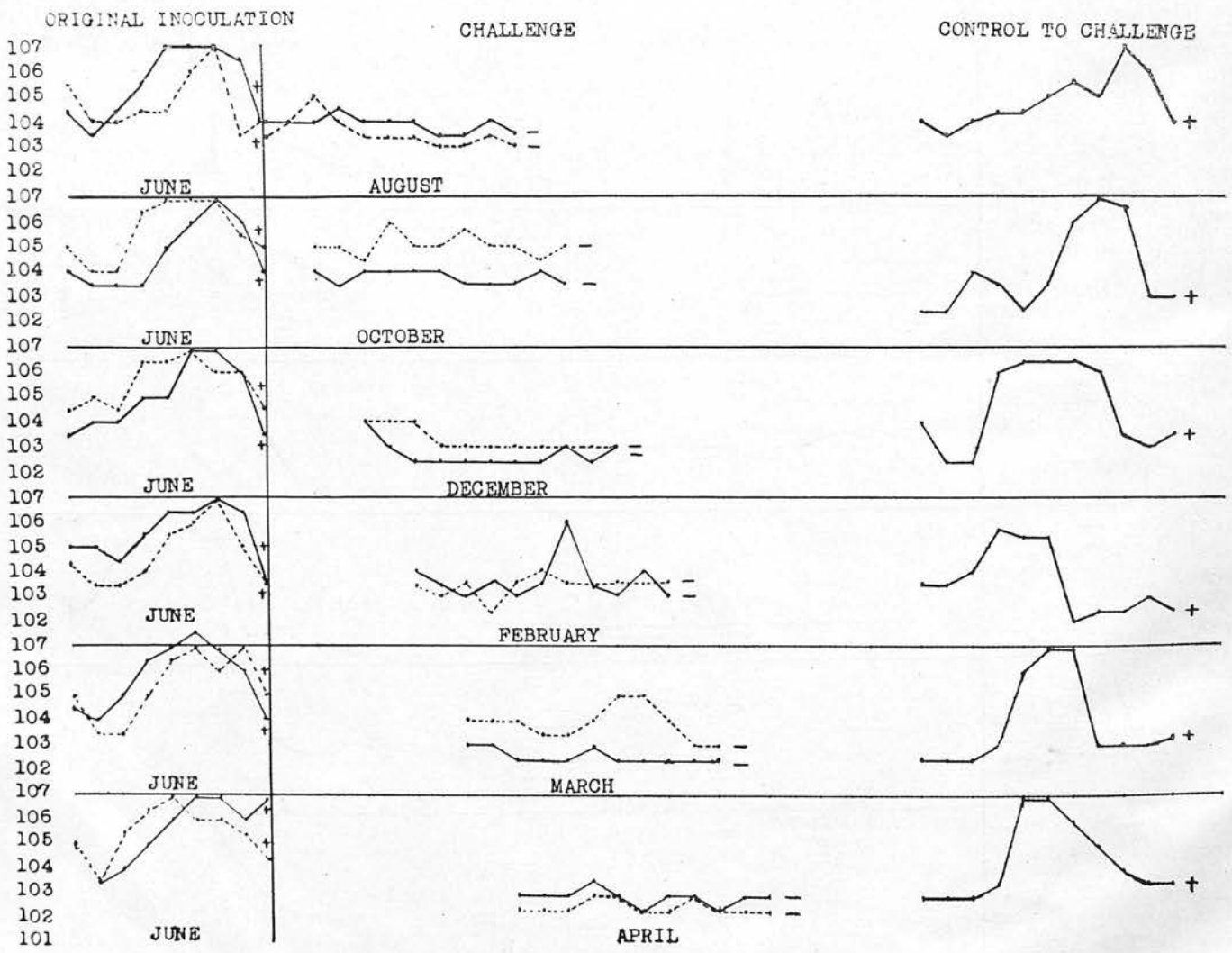
S. JAMIESON,

Veterinary Laboratories, Mill of Craibstone, Bucksburn, Aberdeenshire

Field Evidence

Farm G. Kincardineshire.—This is a farm known to be heavily tick-infested. In October, 1944, 68 gimmers were purchased from a farm free from ticks and placed on hill pasture on farm G. In January and February, 1945, 23 of the gimmers aborted and eleven died. The sheep were vaccinated with louping-ill vaccine before their introduction to the farm. It was possible to examine thoroughly only four gimmers, two dead, two alive and three foetuses. No evidence of bacterial infection was found. Tick-borne fever bodies were recognised easily in the blood of the two gimmers alive. The temperatures of these animals were 105·6° F. and 106·2° F. respectively the day following abortion.

Table III



Farm F. Inverness-shire.—Eighty gimmers were purchased in October, 1946, and introduced to this tick-infested farm. In February and March, 1947, 41 aborted, 16 of which died. Tick-borne fever was diagnosed in 18 of the gimmers. It was subsequently found that the native farm of the gimmers was free from ticks.

In view of these cases and cases with a similar history reported to me by Harbour (1946) it was decided to test experimentally the effect of tick-borne fever infection on pregnant susceptible ewes and on pregnant immune ewes.

Experimental

Glensaugh Hill Farm Research Station was chosen because it is well known to be tick-infested and louping-ill and tick-borne fever are known to occur. In introducing susceptible sheep it was, therefore, necessary to inoculate with louping-ill vaccine prior to the start of the experiment. Unfortunately, owing to the unavoidable delays in obtaining the susceptible sheep, it was not possible to inoculate with louping-ill vaccine until the day of commencement of the trial, and sheep were obtained so late that lambing in the controls started soon after the commencement of the experiment.

Ten Black-face gimmers were purchased from a farm known to be tick-free, and these constitute Group B. Group A consisted of ten Glensaugh-bred ewes of five to six years of age. Both groups were inoculated on April 1st, 1946, each with 10 ml. of infective tick-borne fever blood and 5 ml. of louping-ill vaccine. The tick-borne fever blood was that used by Stamp and Watt in their experiments. After inoculation all sheep were put on to a tick-infested paddock and temperatures recorded daily.

Results are shown in Table I.

It will be observed that in Group A all the ewes lambed full-term lambs except No. 147 which lambed twins alive, but probably about one week premature. Ewe 125 lambed on April 11th. Her lamb was dead and had probably been dead at least two days before lambing.

The ewes in this group had no apparent setback, although some showed a rise of temperature.

In Group B, on the other hand, all the gimmers were very dull and sick two days after inoculation, and were found scattered all over the paddock. It was impossible to gather them with a dog. With one exception (No. 194) all animals in this group lambed prematurely. All lambs were born dead or lived only a short time and only four

TABLE I EWES & GIMMERS INOCULATED T.B.F. 1/4/46 GLENSAUCH

GROUP A Home bred ewes	LAMB	DAYS AFTER INOCULATION	EWE	GROUP B Purchased gimmers	LAMB	DAYS AFTER INOCULATION	EWE
1·8	Normal	9th	Alive	4 187	Aborted Dead	9th	Alive
1·25	Normal Dead	11th	Alive	4 188	Aborted Dead	8th	Dead
1·29	Normal	6th	Alive	4 189	Aborted Dead	8th	Dead
1·39	Normal	3rd	Alive	4 190	Aborted Twins dead 1 alive ½ a day	6th	Dead
1·40	Normal	6th	Alive	4 191	Aborted Dead	20th	Alive
1·46	Normal	4th	Alive	4 192	Aborted Dead	6th	Dead
1·47	TWINS 1 WEEK premature	11th	Alive	4 193	Aborted Dead	7th	Dead
1·52	Normal	4th	Alive	4 194	Still	carrying lamb	23/4/46
7·25	Normal	7th	Alive	4 195	Aborted Dead	11th	Alive
7·49	Normal	17th	Alive	4 196	Aborted Dead	8th	Dead

of the ten gimmers survived. The brains of all dead ewes were examined for louping-ill, but results were negative.

Ticks were counted in botn groups on April 11th. Tick numbers averaged 83 per ewe in group B and 52·4 per ewe in group A.

Discussion

The interpretation of the effects of tick-borne fever infection in field outbreaks will remain complicated so long as the criteria of diagnosis are only temperature reactions and the demonstration of tick-borne fever bodies. Experimentally, interpretation is severely restricted, due to the techniques available. This is especially so when the experimental design involves immunity studies. For instance, Gordon *et al.* (1932) report specific immunity to tick-borne fever in sheep following injections of infected blood. This is now confirmed by Stamp and Watt (1950). Observations in the field suggest that immunity would appear to be dependent upon the size and duration of the dose (Jamieson, 1947). Until, however, the infecting agent is isolated and variations in strains and the quality and quantity of " an immunising dose " are determined, and a technique evolved to produce graduated infecting inocula, interpretation of results must remain restricted.

There appears little doubt, however, that pregnant sheep from farms free from tick infestation abort when introduced to tick-infested farms. It is not possible at this stage to assess whether abortion is due to specific qualities of the tick-borne fever infecting agent or is a simple reaction to the febrile state.

Deaths following tick-borne fever infection are not in keeping with the observations of Gordon *et al.* (1932), or Brownlee (1935). At Glensaugh station, however, deaths from tick-borne fever have been observed previously (Jamieson, 1947).

In this experiment six out of ten sheep in the non-immune group died, apparently as a result of tick-borne fever infection. It is difficult to correlate these findings with those obtained by Stamp and Watt (1950), where no deaths occurred, although the infective material was from the same source. It may be significant that the only apparent difference between the experiments at Boghall and Glensaugh is that the former was tick-free and the latter tick-infested.

Acknowledgments.—I am indebted to Mr. G. Jamieson, farm manager at Glensaugh Experimental Station, and Mr. J. J. Thompson for their help in the field.

References

Brownlee, A. (1935.) *Agric. Prog.* **12.** 118.
Gordon, W. S., Brownlee, A., Wilson, D. R., & Macleod, J. (1932.) *J. comp. Path.* **45.** 106.
Harbour, H. E. (1946.) Personal communications.
Jamieson, S. (1947.) *Vet. Rec.* **59.** 198.
Stamp, J. T., & Watt, J. A. (1950.) *Vide supra.*

Printed by H. R. Grubb, Ltd., Croydon.

Edinburgh and East of Scotland College of Agriculture, Miscellaneous Publication No. 15

BOVINE TUBERCULOSIS—INCORPORATING A REVIEW OF JOHN FRANCIS' BOOK

BY

J. N. RITCHIE, B.Sc., M.R.C.V.S., D.V.S.M. and J. T. STAMP, B.Sc.

[*Reprinted from* "TUBERCLE," *Vol. XXIX, Nos.* 2 *and* 3, *February pp.* 34-40; *and March,* 1948, *pp.* 61-66.]

Bovine Tuberculosis—Incorporating a Review of John Francis' Book*

By J. N. RITCHIE, and J. T. STAMP

There is no doubt that John Francis' Monograph has collated for the first time an enormous amount of information regarding bovine tuberculosis, including as it does chapters on the incidence of tuberculosis, pathogenesis, tuberculosis of bovine origin in man, detection of tuberculous cattle, vaccination against tuberculosis, and the control of bovine tuberculosis. As is made plain in the foreword by Dr J. R. M. Innes, the greater part of the Monograph is not based on original work but the author, quite rightly, is not content with merely collecting published information; he has sifted the literature and from the evidence obtained he has expressed many opinions. Such a work calls for a careful and detailed review for it is felt that many of the opinions expressed are open to criticism. One of us (J. T. S.) has dealt with pathogenesis and the other (J. N. R.) with incidence and control.

This book will form a useful reference on all matters connected with bovine tuberculosis and is in a form to which reference can readily be made.

The Incidence of Tuberculosis

Francis' references to the early spread of tuberculosis in cattle indicate that tuberculosis is a disease of great antiquity. He suggests that infection may have been introduced into Britain with importations of cattle made by the Romans. Further infection may have come from Holland with cattle imported from that country in the seventeenth century. The Dutch cattle helped to form the Holderness or Durham Shorthorns, later used to improve English cattle which were in their turn exported to many other countries into which they may have had a share in introducing infection. The assumption that tuberculosis became common among British cattle with the introduction of town dairies to supply the manufacturing towns from 1750 onwards is a very reasonable one. The description of the cowsheds in the middle nineteenth century indicates that the conditions of close contact and bad ventilation clearly provided the greatest possible opportunity for the infection to spread rapidly through a herd. It was not until 1843 that tuberculosis was described among domestic animals in this country, and even then reports more frequently referred to horses. Francis' assumption that many instances of the disease amongst horses can be taken as evidence that the disease was common amongst cattle at this time is one which can readily be accepted. He mentions that tuberculosis was included with rinderpest, pleuropneumonia and foot-and-mouth disease as one of the four 'bovine scourges.' It may be of some interest to mention that in 1888, at the first meeting of the Metropolitan Veterinary Society of Edinburgh, two diseases of prime importance to the veterinary profession at that time were mentioned—contagious bovine pleuropneumonia and tuberculosis. Tuberculosis in cattle was also discussed at the meeting in February, 1889, and a resolution was passed at the end of the meeting 'that this Society, thoroughly believing tuberculosis to be a systematic and contagious disease, urge upon the Government—(1) to stop the sale of milk from animals suspected of being affected with tuberculosis; (2) to suppress the consumption of meat from tuberculous animals; and (3) to give compensation for a limited number of years.' It is noteworthy that the recommendations of this resolution might well have been the basis for the earliest legislative control of the disease in this country, although that was not introduced until 1913 and was suspended from 1914 until 1925.

Tuberculin Surveys

The only method of accurate assessment of the incidence of tuberculosis in cattle in any country is by tuberculin surveys on an extensive scale but this could not be justified merely to obtain this evidence. Estimates can be accepted with more confidence from countries in which good progress has been made towards eradication or in localities where similar progress has been made. It is, however, of considerable interest to have a

* Bovine Tuberculosis. By John Francis. Pp. 220. Tables 21. Illus. 36. Staples Press Ltd. 25/-.

comprehensive view of the evidence available from so many countries.

An estimate of the incidence in this country as a whole is of value in order to assist in assessing the losses which can be attributed to the disease, but more particularly to indicate the size of the job to be tackled in eradicating bovine tuberculosis. If the herds throughout the country carried an equal weight of infection the problem would be more difficult but it is evident that the incidence in herds or in localities varies within wide limits depending on the type of herd, the system of herd management and the traditional cattle trade in the district. It is therefore of greater practical importance to know the incidence of disease in smaller communities of stock than in the country as a whole.

In the smallest unit, the herd of cattle, the method of control must be decided in the light of the number of reactors disclosed at the first test in each group of animals; in a district the first herds from which the disease is to be eradicated may be chosen in the light of their tuberculosis history in the past; and in the country as a whole the first areas to be dealt with will be those showing the lowest incidence of disease. Francis has accepted as the most reliable estimate 20 per cent of affected cattle in England, 7·5 per cent in Wales and 14 per cent in Scotland; and 17 to 18 per cent in Great Britain with an overall incidence of 30 to 35 per cent in cows. As a result of recent more rapid progress towards eradication this estimate is likely to be out of date very soon, but it will be of considerable value if it is accepted in reference to tuberculosis in the cattle of Great Britain in place of the earlier estimates which have been so often quoted and misquoted. Because they have been so often misquoted, it may be valuable to repeat the earlier estimates more fully. The People's League of Health Inc. appointed a special committee to make a survey of tuberculosis of bovine origin in Great Britain and it reported in October 1931. Among the conclusions were:—

'(*a*) It is estimated, on the basis of the tuberculin reaction, that at least 40 per cent of the cows in this country are infected with the tubercle bacillus, though only a minority of them are in an actively infective condition;

(*b*) About 0·2 per cent of all cows (or 1 in 500) are suffering from tuberculosis of the udder, and are therefore probably excreting living tubercle bacilli in the milk; and

(*c*) About 40 per cent of cows slaughtered in the public abattoirs show naked-eye lesions of tuberculosis.'

In 1934 the Economic Advisory Council, Committee on Cattle Diseases, included in its report this paragraph on the incidence of bovine tuberculosis:—

'The incidence of bovine tuberculosis among cattle is probably as high in Great Britain as anywhere else in the world. A number of witnesses before us have expressed the view that 40 per cent of the cattle in this country are infected with it in such degree that they will react to the tuberculin test. This estimate is based first upon the percentage infection found in herds tested with tuberculin for the first time, and, secondly, upon the results of the inspection of carcases of cows slaughtered. For example, the results of tests in 144 herds which had not previously been tested for tuberculosis were quoted in various memoranda submitted to us, and showed that of 5,199 cows 2,233, or 43 per cent, gave a "positive" reaction. It was also recorded in a report drawn up by the People's League of Health that 39·5 per cent of 55,318 cows slaughtered at various centres were found on post mortem to be infected. In considering this latter percentage it must be remembered that many cows which are infected with tuberculosis do not reach the slaughterhouse, as they die upon the farm, or are sent to kennels or knackers. It may therefore be unduly low. In the light of such figures as these the generally accepted conclusion that at least 40 per cent of cows in dairy herds are infected with tuberculosis does not appear exaggerated. This percentage of infection may be compared with a percentage of something over four in the United States, and of approximately twelve in Canada, before steps were taken against the disease. We have no evidence to show whether the disease is increasing or diminishing in Great Britain, but the widespread neglect of adequate precautions against its increase makes the latter improbable.'

There need have been no confusion in these estimates. Both clearly relate in their conclusions to 'cows' and the only reference to 'cattle' in the 1934 report is an expression of the views of a number of witnesses before the Committee.

The Economic Loss Due to Tuberculosis

As Francis states, any estimate of losses due to bovine tuberculosis can only be very approximate, but it is well to draw attention to the

serious loss which does arise. There are direct losses due to death or slaughter of clinically-affected animals and through condemnation of carcases and offals in the slaughterhouse; there are indirect losses due to the fact that tuberculous cattle are less efficient as milkers, breeders or feeders, and the losses must include the costs of control and eradication of the disease. These losses only relate to economic loss to the farming industry and ignore the Public Health aspect of the problem.

Pathogenesis

It is perhaps a pity considering that the book will be widely read that the disease process in man was not more fully described. As it is presented it is neither clear nor critical. It is felt that in the first place it would have been desirable to describe briefly the various forms of the disease which occur commonly in human tuberculosis. Without description of the classifications to which reference is made, Francis' outline may be a little confusing. Thus human tuberculosis can be divided into:—

(1) Primary complex, active, healing or progressive, associated frequently with haematogenous disseminations.

(2) Bronchogenic phthisis.

The Primary Complex

In the past it was widely debated whether infection of the human body took place by inhalation or by ingestion. In the latter case it was argued that the tubercle bacillus might enter the body by the digestive tract but not produce foci until it was transmitted to the lungs. A large volume of experimental work, aimed at producing good evidence of the route of infection, was carried out but on the whole the results are inconclusive. Undoubtedly, it is the description of the primary complex which has given the greatest evidence as to the common route of infection in the human so that it is now widely, but by no means unanimously, thought that the primary complex is not only evidence of primary infection but also indicates the portal of infection. On this evidence the portal of infection in the human is predominatingly respiratory. Thus, when the tubercle bacilli enter the lung a small exudative caseous bronchopneumonic focus develops which is known as the primary focus, or Ghon's focus, and this in turn is quickly followed by a similar lesion in the regional lymph glands so forming the primary complex of Ranke. In the great majority of primary infections the lesions of the primary complex are arrested and become encapsulated, calcified and even ossified while still very small. A relatively small number of primary complexes do not heal but progress and, especially in the case of young children, the primary focus becomes an enlarging area of destructive caseating tuberculous bronchopneumonia giving rise to further bronchial dissemination, while in some cases the lymph gland lesion ruptures into a bronchus giving an aspiration pneumonia. Intimately associated with the primary complex is the development of haematogenous disseminations of tubercle bacilli to all parts of the body. These occur frequently and produce lesions varying from fatal massive miliary tuberculosis to isolated foci in various parts of the body. When these isolated foci occur in the lung they are sometimes known as Simon foci and they show a marked tendency to regression. This stage is frequently called the stage of early post primary haematogenous dissemination or Ranke's second stage.

Phthisis

In the majority of people who develop phthisis primary infection as described above has occurred years previously. Phthisis usually begins as a small focus of caseous pneumonia which, once formed, has all the potentialities of developing into the many forms of phthisis. This pulmonary focus, unlike the primary one, does not cause marked caseous lesions in the regional lymph glands. The origin of these lesions is much debated. Whether they represent an exogenous superinfection, or whether they arise by exacerbation of part of the primary complex, or from foci originating during the early post primary haematogenous dissemination is still far from clear. Thus primary infection followed by a considerable period of latency ending in progressive phthisis, appears to be the usual development of human tuberculosis. The marked differences between primary infection and phthisis have long been known and since the primary complex is most commonly seen in children it is frequently called 'childhood type' while phthisis is called 'adult type.'

Ranke

It is not doubted that these differences occur in 'childhood' and 'adult' tuberculosis but it is the explanation of these differences which has led to so much argument. It is especially Ranke who

has given the most interesting theory. He divided the disease into three stages, corresponding to first infection, dissemination and phthisis. Thus the first and second stages cover the period of primary complex and dissemination and the third stage is the localization of the disease to a single organ, such as occurs in phthisis. Immunologically Ranke classified the first stage as occurring in normal tissue where only native resistance played a part. The second stage occurs with the development of allergy, while the third stage is associated with acquired immunity. Ranke made no attempt to demonstrate his theory experimentally; it was based purely on morbid anatomical and histological findings. Francis, quoting Rich, criticizes Ranke's views that phthisis develops not because of acquired resistance but rather on account of an age determined native resistance. There is little doubt that Ranke's theory does explain the usual type of development in human tuberculosis. The main criticisms of Ranke's theory are based upon cases which do not follow the usual development of tuberculosis. It is thought worthwhile to list these criticisms concisely so that comparison can be made with the disease in the bovine. First, it is doubted whether all cases of phthisis have, in fact, previously shown a primary complex; secondly, there is some evidence that primary infection, when it occurs in the adult, does not lead to a typical primary complex but in fact gives rise to typical phthisis; and thirdly, there is experimental evidence that typical phthisis can be produced by inherited elevated native resistance. Francis having gone so far as to quote Rich might also have quoted Pagel's critical review of Rich and also the evidence of Pinner.

Route of Infection in Cattle

As Francis points out there has never been any doubt that bronchial mediastinal lymph gland tuberculosis is the most frequently occurring form of tuberculosis in cattle. The great difference of opinion arises over the origin of these lesions, whether the portal of entry of the tubercle bacilli is intestinal or respiratory. Francis quotes the evidence under several headings. Many references of experimental evidence are given. None of the evidence, however, is new and the present position is still inconclusive.

As stated previously it is undoubtedly the descriptions of the primary complex in human tuberculosis which has given the greatest evidence as to the common route of infection in the human. It is felt that it must be stressed at the outset that no equivalent description has ever been given in bovine tuberculosis. Francis, in evidence of inhalation infection, quotes a number of references which he considers indicate the frequency of pulmonary lesions. These extensive figures are nearly all based on meat inspection records where only lymph gland lesions are recorded. The figures given, in fact, only support a long acknowledged fact that bronchial mediastinal lymph gland tuberculosis is the most common form of tuberculosis in the bovine. There is no published evidence that these thoracic lymph gland lesions are part of a pulmonary primary complex; there is, indeed, some evidence that they are not (MacFadyean, 1891; Freidbergen and Frohner, 1895; Nocard, 1895; Laithwood, 1896; McFadyean, 1898; Law, 1912; Wallis Hoare, 1913; Yamagiwa and Jasikawa, 1930). The work of Nieberle (1929, 1931, 1937 and 1941) indicating primary complexes in cattle was done almost entirely on calves and most certainly, as will be shown later, cannot be used as evidence of what occurs in the more commonly infected adult animal. Some recent work has been done on the frequency of primary complexes which would indicate that, in fact, primary pulmonary complexes do frequently occur in adult cattle (in press, Stamp, 1948). The primary pulmonary lesion (Ghon focus) and the primary complex (Ranke), on which the evidence as to the portal of entry of tubercle bacilli in the human is based, have not been adequately described in the bovine, so that the evidence of the portal of entry of tubercle bacilli in the bovine still rests on an inconclusive experimental basis. The fact that the writer of the foreword to Francis' Monograph stresses the dangers of transferring the concepts of human pathology to animal pathology suggests that he agrees.

Francis clearly points out that, for every year of life, there is an increase of about 7 per cent in the number of infected animals. The incidence of infection in calves is quoted at about 2 per cent while about 40 per cent of dairy cows which have reached the age of 5 to 6 years are affected. In other words, primary infection occurs at all ages in the bovine. The incidence in calves is based largely on abattoir statistics and is not representative of calfhood infection if by the latter is understood calves up to six months old;

for instance it is known that the 2 per cent of infected calves recorded by Gofton represent chiefly very young calves (only a few days old), and cannot be taken to represent infection as it occurs in older calves so few of which are sent for slaughter. The assessment of the route of infection in calves several days old has always been difficult. There is no doubt that congenital infection is commonly recognized, but as Francis points out, there is considerable doubt whether many of the so-called intestinal infections, accepted as post-natal, are not indeed also congenital infections. It was in fact a change of opinion over this question that altered Nieberle's contention that intestinal infection in calves was more important than inhalation. Other criticisms of evidence based on such material are—firstly, several cases which on morbid anatomical grounds would most certainly have been classified as respiratory infections have been seen where most certainly infection occurred prenatally. Secondly, many cases showing bronchial gland caseation failed to show primary lung lesions. As to the route of infection in older calves, there is practically no published evidence based on morbid anatomical findings, but it is a common experience to find a large proportion of calves reacting to the tuberculin test in herds where the milk of the herd is infected. Such calves from dairy herds would probably survive to adult life and therefore cannot be recorded as infections of calves judged by slaughterhouse returns.

'Childhood' and 'Adult' Disease in Cattle

Francis contends that, contrary to the findings of Nieberle, bovine tuberculosis cannot be divided into calfhood and adult types similar to 'childhood' and 'adult' types in the human. It is considered that this statement must be qualified and amplified considerably more than has been done by Francis. As already stated the differences between the two types depend upon the fact that in 'childhood' tuberculosis there is a primary complex along with early dissemination by all possible methods of propagation—lymphatic, haematogenous, intracanalicular and by contiguity, the immunological state now being one of hypersensitivity (Pinner, 1946). In 'adult' tuberculosis the disease is confined usually to the lungs in the form of phthisis when fibrosis is prominent; caseation of regional lymph glands is not conspicuous and metastatic haematogenous lesions are not common but intracanalicular dissemination is usual. Francis justifies his statement that calfhood and adult types of the disease cannot be recognized in the bovine by the following arguments, that the majority of bovine infections occur after maturity, that congenitally infected calves do not die and that, excluding congenitally infected animals where infection is often directly into the blood stream, haematogenous dissemination is not more frequent in calves than in adults. This evidence is not convincing. In the first place, the mere fact that infection does not occur as commonly in the calf as in the adult in no way prevents the disease following a different course when infection of the calf does happen to take place. Secondly, the evidence regarding haematogenous dissemination in the calf rests on the evidence of Stamp and Wilson (1946) which is obtained directly from abattoir material and therefore is subject to the same limitations as given previously, in that the calves were mostly a few days old when slaughtered. There is no available evidence as to what might have occurred if these animals had been allowed to survive, nor is there any evidence of what occurs during the primary infection period of the older calf. Lastly, the mere fact that calves do not die is no proof of the original contention. The vast majority of primary infected children do not die and in addition there is no evidence that the susceptibility of the calf is similar to the child, therefore it is incorrect and irrelevant to compare the mortality of the two.

Nevertheless the terms 'calfhood' and 'adult' are objectionable in that they suggest differences in the course of the disease dependent upon age immunity. A similar objection has been raised to the terms 'childhood' and 'adult' in the human disease, particularly because, in recent years, primary infection is by no means confined to childhood. As previously stated there is doubt whether the adult type of the disease is due to acquired resistance or to age immunity. Some evidence has been produced (Stamp, 1948) that in cattle there are no differences in the disease process due to differences of immunity caused by age. This evidence is based on morbid anatomical studies and, like similar studies in the human, it appears that primary infection gives a fairly distinct morbid anatomical picture which is in no way influenced by age. The anatomical picture of primary infection in the bovine is somewhat different from the human, but

nevertheless it only varies in detail and not in its main features. It is undoubted, however, that a form of chronic pulmonary tuberculosis follows primary infection. Thus in the bovine it is considered that there are stages in the disease similar to the classical stages of Ranke and these are independent of age. Similar conclusions, contrary to the quoted views of Rich, are held by many workers in the human field.

Francis states that one would not expect any striking difference between tuberculosis in the calf and the adult. It would be better to say that one would perhaps not expect any striking differences between primary infection in the calf and primary infection in the adult, for there are considerable differences between primary infection lesions and the chronic pulmonary tuberculosis of the post primary period which is so commonly seen in adult bovines. The fact given by Francis that the percentage of haematogenous disseminations is not greater in the disease of the calf than in the adult is to be expected as the dissemination is probably part of the process of primary infection in both cases.

To summarize these views one could say that it is unfortunate that the terms 'calfhood' and 'adult' were used since it confuses the issue of age determined differences of immunity and acquired immunity. This confusion did not exist in Ranke's original theory of stages since the evidence was convincing that all adults had been primary infected in childhood. It is probable that Nieberle used the terms 'calfhood' and 'adult' as representing Ranke's stages. This further confirms the fact that confusion arises when concepts of human pathology are used indiscriminately in animal pathology. It would have prevented the present confusion if Nieberle had used Ranke's more precise terminology for he most certainly meant to apply Ranke's theory of stages rather than any question of age immunity.

In discussing the question of chronic pulmonary tuberculosis Francis considers that Nieberle's statement that lymph-gland changes are minimal or absent in this stage of the disease is the very reverse of the truth and gives his own protocols and figures as evidence of his contention. Several of his figures (15, 17 and 24), however, clearly indicate that the massive caseation of the lymph gland is in fact part of a primary infection process. It is agreed that chronic pulmonary tuberculosis does in many cases give rise to caseation of the regional glands but, in comparison with the degree of caseation associated with the primary infection, it is indeed frequently minimal. In addition, there is often continuous lymphatic dissemination throughout the lymph-gland system in the bovine arising from the primary infection which has not healed. A similar process occurs in human tuberculosis.

Failure of Primary Lesion to Heal

Francis describes the formation of a primary complex in the lung as generally recognized in the human and as described by Nieberle in the calf. Regarding the anatomical distribution of primary foci in the lung the work of Medlar is quoted but it should be made clear that Medlar's findings were not confined by any means to primary infection lesions. It has been found that there is no predilection site for the primary focus, indeed, primary foci have been seen in all portions of the lung. Nevertheless, such lesions were more frequently found in the dorsal part of the diaphragmatic lobes (Stamp, 1948). It is agreed fully that there are differences between primary foci in man and cattle. As Francis states the great majority of primary lesions in children 'heal' and give rise to the familiar non-progressive globular little lesions of the primary complex. In cattle complete healing is rare and the lesions remain open. Only in a minority of cases is the primary lesion even promptly arrested let alone completely healed. Much more commonly the primary lesion becomes large and caseous reaching in many cases to 5 and 6 cm. in diameter. It is, therefore, suggested that in cattle the primary focus progresses considerably further than is the rule in the human. Nevertheless, these large caseous lesions do eventually become arrested and encapsulated but the localization is only partially successful, for further bronchial dissemination, giving rise to chronic bronchopneumonia, is common. For these reasons it is doubtful whether the term 'primary complex' should be used for cattle since to those who recognize its morbid anatomical features in human tuberculosis, it conveys a wrong picture. With reference to the developing chronic bronchopneumonia mentioned above, it is not agreed that this is similar to the development of progressive bronchopneumonia in children, as Francis states. The pathology and underlying immunological principles are quite different. In the case of the child the pneumonia progresses rapidly and without evidence of immunity,

whereas in cattle the pneumonia is chronic and can be compared with phthisis in the human disease.

Francis quite rightly emphasizes that exogenous and endogenous reinfections have received much less attention in the bovine than in man. It should also have been emphasized that chronic bronchopneumonia in the bovine and phthisis in the human have quite different pthisiogenetic principles. Thus in the human there is usually a very long period between primary infection and the development of phthisis. This is not usually so in cattle. Chronic tuberculous pneumonia in the bovine frequently arises not by endogenous reinfection or by exacerbation but by bronchogenic progression of the primary lung focus.

Does Exogenous Reinfection Occur?

It is appreciated that it is extremely difficult to determine whether true exogenous reinfection occurs in the bovine since primary lesions as a rule progress. As Francis states, however, Stamp considers that he has some evidence that this occurs. It is fully agreed that in cattle the regional lymph nodes show caseation in post primary pulmonary infection, but it is an exaggeration to say that this caseation is massive if one compares it with the caseation of the primary infection lesions. The morbid anatomical picture of chronic pulmonary tuberculosis is very varied but fundamentally similar to that so adequately described in the human subject. The differences between the lesions in the human and bovine are dependent upon the differences in the structure of the lungs in the two species. In the bovine lung the interlobular septum is very definite so that individual lesions are restricted, and confluence of neighbouring lobule lesions is not common. This, in turn, greatly restricts the size of cavities although small cavities are common in bovine tuberculosis.

As Francis points out, intestinal infections occur in the bovine and it is also agreed that caseation of the mesenteric nodes is not confined to primary infection. These post primary lesions, however, can usually be distinguished from the lesions of the primary complex. Francis quite rightly leaves the pathogenesis of the frequently occurring retropharyngeal lymph-gland lesions *sub judice*. One fact, however, which is clear is that caseation of these glands is more frequently associated with bronchial mediastinal lymph-gland caseation than with caseation of mesenteric lymph glands.

Francis stresses the fact that lymph gland caseation is frequent in post primary tuberculosis of cattle, a rarity in man. This undoubtedly is a clear indication that the immunological reactions of the bovine are different from those of the human. Such differences are also obvious in the formation and progression of the primary infection.

Uterine Tuberculosis

There is no doubt that tuberculosis of the uterus in cattle is much more frequent than is generally appreciated and that primary infection of the uterus is by no means rare. Both these points are adequately made by Francis. He also gives good evidence that apart from primary infection the uterus is generally infected during haematogenous dissemination. This is contrary to the generally accepted theory that the uterus is infected by descending tubal infection from a tuberculous peritoneum. The monograph gives good evidence that, in fact, the peritoneum is infected by ascending tubal infection from a tuberculous uterus. The importance of tuberculosis of the uterus as a cause of abortion is given adequately. It is also a factor in the causation of infertility.

The only criticism of Francis's views of congenital tuberculosis is that an aspiration type of congenital tuberculosis does occur in the bovine.

Little can be added to the excellent description of udder tuberculosis given by Francis. It must be made perfectly clear, however, that there has been little correlation of the types of udder tuberculosis with the stage of development of the disease in the rest of the body. Nieberle contends that Types 1 and 2 are associated with early post-primary haematogenous dissemination while Type 3, probably the commonest type, is classified as a form of isolated organ tuberculosis.

Francis makes no mention of these fundamental aspects which is perhaps an important omission. There is no evidence to support Nieberle in his contention that chronic diffuse granulomatous tuberculous mastitis is a form of isolated organ tuberculosis. It is considered that until some further evidence is presented on these questions an important aspect of the immunological principles of bovine tuberculosis remains obscure.

Meat Inspection

The regulations in Scotland and the memoranda in England which guide the meat inspector in his judgment of the carcases of bovines for

human food are apparently framed with a view to condemnation of the whole carcase when there is evidence of widespread haematogenous dissemination. It is apparent, however, in the light of the more modern conception of this phase of the disease, that some cases of haematogenous dissemination may be overlooked. Thus when only pulmonary and broncho-mediastinal lymph-gland lesions are observed, according to the practice of meat inspection, there is no obligation to examine carcase lymph glands, whereas complete examination may show carcase lymph gland lesions in an appreciable number of such cases. Otherwise the guidance, if properly followed, allows of a high standard of meat inspection.

Slaughterhouse returns of tuberculosis are often of value in tracing tuberculous infection in a herd, notably by tracing the dams of calves congenitally infected. They will be of increasing value as a check on the accuracy of tuberculin tests as eradication progresses.

Tuberculosis of Bovine Origin in Man

In the past there has been much discussion regarding infection in man by the bovine type of the tubercle bacillus. The bovine organism is certainly a danger to human health, especially in regard to the infection of children from cows' milk. The only solution is complete eradication of the disease from cattle, but in the meantime efficient pasteurization is a reliable safeguard where it is practicable.

The Detection of Tuberculous Cattle

The detection of tuberculous cattle is the most important question to be considered in formulating any method of control or eradication of the disease. At routine herd inspections, required under Milk and Dairies Regulations, clinical examinations are made by veterinary surgeons in an effort to identify the affected animals. Clinical cases are removed in an effort to reduce the weight of infection in the milk supply and this is, of course, of value from the public health point of view. Removal of such dangerous animals is of importance if the farmer is proposing to clean up his herd, but unless other active steps are taken it will not make any significant contribution to the reduction of infection in the herd, since the majority of infected animals are infective long before they can be recognized as clinical cases of tuberculosis. It is true that infected animals even with extensive disease may show no recognizable symptoms calling for closer examination and that some of these, even if closer examination is carried out, cannot be detected by the methods available to the veterinary surgeon; but there are many cases which can well be diagnosed by an experienced clinician.

Clinical Signs

The veterinary surgeon carrying out herd examinations may decide to make a thorough examination of an animal which has shown a rapid decline in condition following calving, such indefinite symptoms as general unthriftiness, listlessness, some loss of milk or a degree of infertility; with more obvious symptoms such as accelerated respiration, frequent cough, or induration with or without enlargement of a quarter of the udder. There are also infrequent cases when tuberculosis of the eye is a prominent symptom. Closer examination will always include auscultation of the lungs, which will very often provide the necessary evidence. It is sometimes an advantage to drive the animal out of the cow shed and force her to exercise before the examination is made. As additional aids to diagnosis, samples of milk, sputum, vaginal or uterine discharge and urine may be examined microscopically; and particularly in the case of milk may, if negative, be submitted to biological test. It is, therefore, possible to diagnose the disease when there is considerable or active lung involvement; infection of the udder; infection of the uterus and occasionally the kidneys; disease in lymph glands which are palpable, notably the retropharyngeal glands.

In connexion with the microscopical examination of milk, Francis rightly stresses the value of searching for the organisms in the characteristic cell groups which can be detected by examination with low magnification. This undoubtedly has brought about much greater accuracy in the diagnosis of tuberculosis of the udder, as no positive diagnosis can possibly be made without demonstration of the organism in the milk. This method, however, is not nearly so accurate as the biological test.

Slaughter

Certain of the animals clinically affected with tuberculosis may be slaughtered in the terms of the Tuberculosis Order. They are—'a cow which is affected with tuberculosis of the udder or is giving tuberculous milk, or a bovine animal

which is affected with tuberculous emaciation or is affected with a chronic cough and showing definite clinical signs of tuberculosis, or is excreting or discharging tuberculous material.' The terms of the Orders are quoted because Francis has mentioned in connexion with the Tuberculosis Orders those animals which the farmer is obliged to report as suspicious and not those which may be slaughtered.

It must be quite clearly understood that the authority to slaughter animals dangerous to the public health is the real value of the Order; this becomes apparent when it is realized that before the introduction of the Order such animals could only be *removed* from dairy herds and their ultimate destination was not under control and might, in fact, be another dairy herd. It is essentially a public health measure and it was not expected to bring about any marked reduction in the incidence of bovine tuberculosis. The Annual Report of the Proceedings Under the Diseases of Animals Acts for the Year 1926 states 'It is desirable at the outset of this Report to correct a prevailing and erroneous impression that the Order (The Tuberculosis Order of 1925) has in view the eradication of bovine tuberculosis.'

The only method of detecting tuberculous cattle in the control or eradication of the disease is the tuberculin test; the other methods which have been described have only historical interest and have no place in modern schemes of eradication.

Value of Vaccination

In heavily infected herds vaccination of calves might possibly be used to protect them for a period during which the reacting stock is gradually removed from the herd, allowing of two stages of eradication; first, the establishment of a herd of immunized cattle and then the gradual establishment of tubercle-free cattle from calves born in the immunized herd. The most heavily infected herds are flying herds and do not retain the calves; in the most heavily infected breeding herds the calves may be infected at an early age, particularly if a cow giving tuberculous milk is in the herd and vaccination can be of no value in these circumstances. Less heavily infected herds, so long as the young stock shows a low incidence of infection, can far more easily be cleared by immediate removal, or removal after isolation, of reactors found at the first test. The vaccine does interfere with the tuberculin test and if the animals must eventually be judged free of infection by the test this is a serious drawback. It is also essential that the vaccine should give complete protection and not merely retard the progress of the disease in the animal. It is suggested that vaccination of calves has no place in the tuberculosis eradication programme in this country.

Value of Tuberculin Testing

A great deal of the evidence of the results of tuberculin tests is of doubtful value because there is no precise knowledge of the amount of tuberculoprotein in the tuberculins used: the potency of the earlier tuberculins used varied within wide limits.

The test was sometimes wrongly interpreted because of the fairly generally accepted principle that oedema must be appreciated in a reaction, otherwise it must not be classed as positive. This, along with the difficulty of delayed reactions (reactions which appeared only after the normal final assessment had been made), was responsible for failure to identify a proportion of infected cattle.

There were diagnosed cases of avian tuberculosis and of 'skin tuberculosis' among animals removed as reactors from tubercle-free herds tested only with mammalian tuberculin, although only a small percentage of these reactors became available for post-mortem examination. As an example, twenty reactors from herds being dealt with under the Attested Herds Scheme in Scotland from November 1936 to January 1938 (when a concentrated (precipitated) synthetic medium tuberculin was in use) were submitted to complete post-mortem examination at Moredun Institute, and five of them were shown to be infected with avian tuberculosis and two were probably so infected. The comparative test has proved that many animals showing sensitivity to mammalian tuberculin are not infected with bovine tuberculosis (see later where 88 such animals were found among 545 animals slaughtered). This supports the belief that, when mammalian tuberculin was used alone, many of the reactors were not in fact infected with bovine tuberculosis.

In spite of the early handicaps there is good evidence that herds were maintained free of infection with the earlier tests and tuberculins.

The comparative test using mammalian and avian tuberculins of a known high potency has

gone so far to meet these difficulties that greater accuracy of diagnosis has been achieved. The test is admittedly cumbersome but since the introduction of the single intradermal test, in which only one post-injection observation is necessary, a considerable amount of time in testing is now saved. The importance of the time factor increases as more herds become attested.

Single Intradermal Comparative Test

The single intradermal comparative test has been used in Great Britain since June 1947. This test was introduced following an extensive field trial in which animals were tested on one side of the neck by the double intradermal comparative test and on the other side with the single intradermal comparative test. The trial was not designed to prove the efficiency of either test separately, but to compare the two; it resulted in agreement between the two tests in 99·12 per cent of 7,247 animals in attested and 'T.T.' herds, and in 96·57 per cent of 2,224 animals in herds believed to be heavily infected (non-designated herds). Attested herds were selected which had shown a poorer history in general than the average attested herd; 'T.T.' herds were included because they usually reveal a higher percentage of reactors for removal than attested herds. The incidence of reactors in these herds was 1·75 per cent which is more than three and a half times as high as the average in all attested herds. The non-designated herds were selected in an effort to reveal a large number of reactors and, in fact, over 40 per cent of animals were judged by the tests to be infected with bovine tuberculosis.

It is difficult to prove the value of the comparative test except on such general grounds as the continued freedom from tuberculosis of the tubercle-free herds (when a number of reactors is found, a source of infection arising since the previous test, is usually disclosed); the absence of numerous reports from slaughterhouses of tuberculous lesions in cows from attested herds; the absence of lesions in such animals when opportunity for post-mortem examination presents itself. For example, six attested herds comprising 545 animals were slaughtered on account of foot-and-mouth disease and no lesions of tuberculosis were found, although 88 of the animals had reacted to mammalian tuberculin at the last test of the herd but had been considered free of bovine tuberculosis in the light of the comparative test result.

With the comparative test it is impossible to distinguish between bovine and human type infections but they can readily be distinguished from infections with the avian type or with the Johne's disease organism. 'Skin tuberculosis' presents considerable difficulty because, in the first place, the reaction produced is variable and because lesions of 'skin tuberculosis' have been found in animals also affected with bovine type tuberculosis. The comparative test must be interpreted on a herd basis and all relevant facts, besides the immediate result of the tests of each animal, must be taken into account. The general picture of the type of infection revealed at the herd test, the past history of the herd, and the results of clinical examinations including an examination for the presence of 'skin tuberculosis', must also be considered. When properly interpreted on a herd basis the comparative test is probably the most accurate test available; it is of much less value when applied to individual animals. It is often necessary to retest animals which do not give a clear-cut result at the herd test. Animals with lesions of 'skin tuberculosis,' even if the first test suggests bovine type infection, are usually retested. Retests are done not less than a month after the herd test when, in general, if the mammalian tuberculin shows a proportionately less response, the animal can be accepted as free from bovine type tuberculosis.

It is essential with the comparative test to inject the two tuberculins in comparable sites in the neck for it has been found that sites nearer the head and high on the neck are more sensitive. It has therefore been accepted as good practice to inject the two tuberculins some four inches apart on a line parallel with the crest of the scapula.

Every effort must be made to improve the accuracy and if possible to simplify the comparative test. The possible lines of approach lie in the modification of the tuberculoprotein by chemical means to achieve greater specificity, and in the substitution of tuberculin produced from the bovine organism in place of the human organism. Suitable growths of the bovine organism can now be produced for tuberculin production. The relative potency of the mammalian and avian tuberculins may be altered to allow of a simple method of interpretation of the test.

Herd Eradication

The incidence in the herd depends broadly on whether it is 'self-contained' or 'flying.' Flying

herds are usually heavily infected as the replacements for them are drawn from the open market. In a truly self-contained herd sufficient heifers are reared to provide replacements of the milking or breeding herd, and only bulls or an occasional choice female are bought. Such herds usually contain few reactors. Other herds which are not entirely self-supporting will usually show an incidence of reactors bearing some relation to the number of replacements required. It is common to find only a small number of reactors, particularly among the young stock, as it is generally found that the young stock do not closely contact the adult herd. If a cow has been giving tuberculous milk at least one leet of heifers will show almost 100 per cent infection.

If only a few reactors are found they are removed, and short interval tests are made until infection is eliminated. If there are too many reactors to allow the farmer to remove them immediately he may separate the reactors from the non-reactors, and, by transferring the calves born from the infected portion of the herd to the clean portion, gradually build up his clean herd while he disposes of the reactors as opportunity offers. Calves must be transferred as soon as possible after birth. This system is not satisfactory unless the farm buildings are suitable for housing the two lots of cattle separately and safely without undue disturbance of farm routine, or, of course, when two farms are available. It is often much less expensive in the long run to remove all the cows if there is a high incidence of infection among them and replace them with attested cattle. The young stock are retained except for the few reactors found among them. The buildings previously occupied by the cows must be thoroughly cleansed and disinfected, manure made by them must be removed to arable land, and grazings which may be infected must be kept free of stock for about a month. When these precautions are taken the results of subsequent tests of these herds have been very satisfactory and much better than the results in herds cleaned by the more gradual methods. The number of tubercle-free herds can be increased rapidly by this method; it can only be used when there is a plentiful supply of tubercle-free replacement stock but it has the great advantage of providing a means of directing a goodly number of tubercle-free cattle, surplus to the requirements of their breeders, into herds where they will still be maintained free of tuberculosis. Flying herds can also be cleaned in this way and it is becoming easier for the owner of a flying herd to purchase replacements from tubercle-free sources allowing him to continue his normal method of herd management. In Scotland, where a large number of tubercle-free replacements are available, an increasing number of herds are becoming attested by this system. Of 2,276 herds which became attested in Scotland in the year to October 1946, 392 or 17·5 per cent qualified in this way and similarly in the following year of 2,048 herds 549 or 26 per cent qualified.

The greatest risk of reinfection of tubercle-free herds is from the introduction of infected stock and from contact with infected stock on neighbouring farms. Rules guarding against these risks must be observed in attested herds. In recent years a number of herds have been reinfected when udder irrigation is used as a treatment for mastitis and less frequently at the time of uterine treatment for sterility. This risk is, of course, greatly reduced in districts where a large number of the herds are attested and would probably be non-existent in a large tubercle-free area.

Success of Methods

Rapid progress has been made since work under the Tuberculosis (Attested Herds) Scheme was started in 1935. Under this Scheme the herd has been the unit of freedom and up to the end of October 1947, 29,552 herds comprising 1,171,822 cattle have qualified for the attested herds register. This represents 13·4 per cent of the cattle in Great Britain (30·1 per cent in Scotland, 24·5 per cent in Wales and 7·9 per cent in England). In addition, there are probably about 250,000 cattle in herds licensed to produce 'T.T.' milk which are not attested. If these figures are compared with those quoted by Francis for 1945, it will be seen that eradication is now proceeding rapidly. The position is much more favourable, however, in some localities. Certain counties have a large proportion of their cattle population in attested herds—Shetland 92·9 per cent, Bute 83·7 per cent, Ayr 78·9 per cent, Cardigan 70·4 per cent, Carmarthen 60·9 per cent; eight counties in South-West Scotland (containing over 520,000 cattle) 61 per cent; and seven counties in South-West Wales (containing over 515,000 cattle) 39 per cent.

Routine tuberculin tests are carried out at

intervals of ten to fourteen months, and if reactors are found herd tests are repeated until the herd is again free of infection. The movement of cattle into attested herds is controlled by permits which are issued for direct movement between herds, and to and from markets specially authorized for attested stock; animals moved from herds which are not attested are required to be isolated and tested before addition to the herd. Farmers have also to take precautions to prevent the introduction of infection, for example, by erecting double fences against neighbouring untested stock. All these precautions are necessary to guard against reinfection. The risk of reinfection would largely disappear in an area in which all the cattle were attested and in consequence less frequent routine tests could be practised; movement of stock could be uncontrolled except from herds in which reactors had recently been found. This would give a welcome reduction in the amount of work required to maintain the large number of established attested herds free of infection.

Area Eradication

Area eradication is the next step contemplated in this country. The Agriculture Act of 1937 authorizes the declaration of 'eradication' and 'attested' areas, the former an area in which 'a substantial majority of the cattle are free from any particular disease' and the latter an area in which 'any particular disease of cattle is for practical purposes non-existent.' The Act also provides for the slaughter of reactors. Up to the present reactors have not been slaughtered. They are prevented from entering tubercle-free herds and their addition to infected herds does not appreciably alter the disease position in those herds, nevertheless it is obvious that at some stage in the process of eradication the disposal of reactors must be controlled. It is appropriate that they should be slaughtered in 'eradication' and 'attested' areas.

We have evidence from the U.S.A. that the 'area eradication plan' is practicable. There, areas were declared 'accredited' when the incidence of reactors had been reduced to 0·5 per cent. Although the general incidence in the country was low, there were counties, particularly in New York State, which had an incidence comparable to some of the most heavily infected counties in Great Britain. Slaughter of reactors was part of the plan from its inception, and was practicable because sufficient replacement cattle were available from localities in which the incidence was low, and because, in the country as a whole, there was usually a surplus of cattle. All the States are now 'accredited areas.' This favourable position is maintained by routine testing at intervals of three to six years, and only inter-State movement of cattle is controlled.

There is, therefore, good reason for the belief that tuberculosis can be eradicated from the cattle of Great Britain.

Printed by Staples Press Limited at their Great Titchfield Street, London, establishment.

Reprinted from THE VETERINARY RECORD, 60. 335.

Edinburgh and East of Scotland College of Agriculture. Miscellaneous Publications, No. 23.

A Field Trial of M 42 (DDT) Dip in the Control of Sheep Myiasis *

By

J. T. STAMP, J. A. WATT AND I. S. BEATTIE

EDINBURGH AND EAST OF SCOTLAND COLLEGE OF AGRICULTURE

INTRODUCTION

In this country there is some published evidence that DDT is of considerable value in the control of sheep blowfly (Cragg, 1945 ; Harbour and Watt, 1945 ; Cragg, 1946 ; Hughes, Jenkins and Jones, 1946 ; Cragg, 1947, and Hughes *et al.*, 1947). Hughes *et al.* (1946) state that arsenic when used alone or in combination with crutching to reduce faecal contamination in the region of the tail has not proved satisfactory because the period of protection it gives is too short to be of value. In discussing their results (1945) they state that the general impression may be gained that DDT and 666 are not of much greater value than arsenic but that the figures for strike in the control sheep are lower than normally experienced and that some factor has apparently reduced strike below the normal range expected. They suggest as possible factors that the presence of DDT-dipped sheep may have prevented *L. sericata* from striking the controls or that DDT may have been transferred to the controls by contact. The same reasons are suggested for the relatively long mean time of striking in the control groups and they also suggest that DDT will not protect lambs which are scouring but they give very few figures for comparison. Cragg (1947) states that in the Agricultural Research Council trials the sheep available on each farm were divided into approximately equal groups and the experiment lasted six weeks. In this experiment the incidence of blowfly strikes in the commercial dip controls was 3 per cent. and in the DDT group 1 per cent. Cragg states that at most centres records were kept of the positions at which strikes were found. Of 115 strikes on the controls, 98 occurred on the tail region, with 27 out of 30 as the corresponding figures for the DDT-dipped sheep. In other words, 86 per cent. of the strikes where position was recorded was due to faecal contamination in the tail region. In the discussion it is stated that the most noteworthy feature is the very low incidence of strike recorded in 1945, but that from the results it is evident that DDT saved approximately two-thirds of the sheep that would otherwise have been struck (*i.e.*, 66 sheep out of 5,600 sheep). Cragg, like Hughes *et al.*, suggests that it is not certain whether the low incidence of myiasis on the experimental farms was due entirely to unfavourable season, location and good hygiene or whether the fact that controls and DDT sheep were grazing together significantly altered the total incidence of strike. Whether this effect is small or large Cragg considers that it must be taken into consideration in future trials despite the practical difficulty of selecting pastures of equal "probable strike incidence." In the papers both of Hughes *et al.* (1946) and of Cragg (1947) it is noted that strike was reputed to be much greater on adjacent farms than on the experimental ones. Hughes *et al.* (1947) during the summer of 1946 carried out a field trial to assess the relative values of DDT and benzene hexachloride, using arsenic as a control dip. They obtained results which they claim show DDT as the most efficient dip tested. Again it is shown that scouring plays a significant part in blow-fly control and they state that insecticidal treatment is unlikely to give effective control unless it is supplemented by crutching to minimise soiling of the breech ; out of a total of 152 strikes, 104, or 69 per cent., were breech strikes. The effect applies to the degree as well as the duration of protection. These authors, contrary to Cragg (1947), consider that their results show DDT dipping to be of economic value. It is again suggested that the presence of DDT-dipped sheep influences the fly population, depressing the strike figures in the control sheep.

METHODS

The present experiment was designed in order to overcome the possible influence of DDT-dipped sheep upon the blowfly population of an area, which it has been suggested affected the number of strikes in arsenical-dipped control sheep in previous experiments. In this experiment an endeavour was made to select groups of neighbouring farms with equal "probable strike incidence." Altogethe four groups were chosen ; one in Roxburghshire comprising two large arable farms (Group III—1,480 sheep) ; one in Clackmannanshire, comprising three arable farms (Group I—1,820 sheep) ; one in Forfar comprising three feeding farms (Group II—417 sheep) ; and one in Perthshire comprising two large hill farms (Group IV—3,000 sheep). In each group the sheep on one farm were dipped in M 42 giving a bath concentration of 0·5 per cent. DDT, immersion time 30 seconds. The sheep from the other farm or farms were dipped in a commercial arsenical dip. All experiments lasted six weeks, the sheep in each group being dipped on the same day or the following days. The time of dipping the various groups, however, varied between the beginning of July and August. The actual recording of blowfly strikes was made by the shepherds who recorded the date of strike, whether on lamb or ewe, position of strike, whether on clean or dirty wool and the intensity of strike.

The DDT dip was easy and pleasant to use and showed no evidence of breakdown of emulsion.

RESULTS

In Group IV over 3,000 sheep were dipped in DDT and arsenic but the experiment was completely nullified in that the arsenical-dipped sheep were re-dipped after a three weeks interval. No reason could be ascertained except that it was general practice to do this. Up to the date of re-dipping myiasis was negligible in both groups.

In Groups I, II and III 161 strikes occurred n 3,717 sheep. Of these, 84 per cent. occurred on lambs and 16 per cent. on ewes. Of the 136 strikes on lambs 121, or 89 per cent., occurred on the tail region of scouring animals while of the 25 strikes on the ewes, nine, or 36 per cent., occurred on dirty tails. Comparison of the arsenic with the DDT-dipped sheep in each group shows that in Groups II and III DDT gives a small but significantly greater protection than does arsenic for clean sheep providing the experiment is in fact dealing with groups of farms of equal strike incidence. Thus in Group II 1·3 per cent. of clean sheep dipped in DDT are struck compared with 4·3 per cent. of clean sheep dipped in arsenic, while in Group III equivalent figures are 0·0 per cent. and 2·0 per cent. The duration of protection is 41 to 30·6 days in Group II and 42 to 38 days in Group III. Group I does not show a similar advantage of DDT over arsenic : 0·6 per cent. clean sheep dipped in DDT against 0·1 per cent. dipped in arsenic. Consideration of the strikes occurring on dirty sheep (73 per cent. of all strikes) shows that no assessment of the relative protective values of DDT and arsenic can be made, since it is obvious that the degree of faecal contamination is an uncontrolled variable from farm to farm, so making the results obtained of no comparable value. Group III (7·5 per cent. dirty strike in DDT group), however, shows very clearly that DDT will not give even useful protection to lambs which are scouring and it is therefore unlikely to give effective control unless the scouring factor is also controlled.

DISCUSSION

The present results and the results of previously published work show that the question of faecal contamination or scour is of the greatest importance in the control of sheep blowfly. In our experiments the percentage of strikes occurring on dirty sheep was 73 per cent. of the total, so that here the majority of strikes cannot be used in the assessment of the relative efficiency of DDT and arsenic, since the degree of scouring probably varies considerably from farm to farm in each group of farms. In other words, the DDT-dipped groups of sheep probably differ considerably from the arsenic-dipped groups in a character which is relevant to the issues to be determined. Further, in experiments such as the present series it is probably not feasible to determine this variation from farm to farm, nor is it possible to measure the varying degrees of attractiveness to the blowfly population of scour. It would appear, therefore, that the present type of experiment is not of great value for determining the efficiency of DDT.

Similar objection can be taken to previously published results of experiments, since in these, 77 per cent. of all recorded strikes occurred on dirty sheep. In these experiments the sheep on individual farms were divided into two or more groups but no indication is given in the text that true random sampling was carried out or that the grazing of the two samples was identical in every way, an important feature for the presence or absence of scouring. Even if these difficul-

* This work was carried out under the direction of the Sheep Dips Sub-Committee of the Scottish Hill Farm Research Committee.

ties had been met the number of sheep (especially lambs) on each farm was relatively small, so that it is quite likely that random allocation would not necessarily have equalised the groups regarding the factor of scour. In any case no successful attempt could be made to verify if equality of scour did occur and if our suggestion is correct this is probably impracticable. It seems necessary that in all future experiments the results must be deliberately sorted regarding scour and the experiments be carefully planned to this end. It is doubtful whether this can be done in field experiments of the type under discussion.

Further consideration of previous results raises other doubts regarding the validity of the claims. For instance, in the work of Hughes *et al.* (1946) the figures for percentage struck are derived from the summated totals of strikes for each group, notwithstanding the very important fact that both the sheep populations at risk and the fly populations may have varied considerably from farm to farm and from control groups to DDT groups. In fact on one farm, where by far the largest number of strikes occurred, there were no sheep dipped in either arsenic or gammexane. Results obtained from such summations may be quite misleading. The authors would, however, have been justified in comparing the results on each farm separately, if the question of scour be ignored. Had this been done using the " fourfold " table of the Chi Square test and the additive characteristic of χ^2 in doubtful cases, the following results would have been obtained.

Significant Differences

Hughes et al., 1946

Farm No. 1.—DDT ——→ Nothing
Gammexane ——→ Nothing
Arsenic ——→ Nothing
Farm No. 2.—DDT ——→ Nothing
No arsenic used
Farm No. 3.—DDT ——→ Nothing
No arsenic used
Farm No. 4.—DDT ——→ Arsenic
(DDT is not better than nothing)
Nothing ——→ Arsenic
Farm No. 5.—Negative
Farm No. 6.—Negative
Farm No. 7.—Negative.

(——→ = better than)

The analysis indicates that DDT, gammexane or arsenic may be better than nothing. There is, however, no evidence that DDT is better than arsenic except on Farm No. 4, but the evidence from this farm is confusing since the figures given show that undipped sheep are better protected than those dipped in arsenic, and that DDT gives no greater protection than leaving sheep undipped.

Thus the results of Hughes, Jenkins and Jones are certainly not conclusive that DDT is a better dip than gammexane or arsenic.

Some of these criticisms also apply to the results of Cragg (1946); there is no justification for assuming equal division of scouring sheep nor for the summation of totals of strikes. If, however, one ignores the question of scouring and uses the Chi Square test regarding significance on individual farms, one finds that the results on nine of the 38 farms, five of these being definitely significant, indicate that DDT is a better dip than arsenic. On 25 farms there was a suggestion that DDT was the better dip, but individual farm results were not significant. If on these farms, however, χ^2 is summated the results do show a significant difference between DDT and arsenic.

The work of Hughes, Pollard, Field & Jones (1947) again ignores the question of unequal division of scouring sheep but their results taken at their face value show that DDT is a better dip than arsenic on four out of eight farms, but that gammexane is of no greater value than arsenic.

It will be seen that consideration has been given only to strike incidence and that the question of duration of protection has not been discussed. This is because evidence published was insufficient to support statistical analysis of the results quoted, and because scour must play a major part in this question. It is possible that control of scour in lambs might even be a better preventative of strike than dipping.

Conclusion

The present experiment was unsatisfactory owing to the interference of scouring sheep, an uncontrolled variable. This factor must be considered in all future experiments. DDT appeared to have some advantage over arsenic on clean sheep, but it is certain that DDT is not capable of efficient protection of dirty sheep.

Previous experimental evidence is not convincing since scour, which played a major part in the results, was not assessed. Random division is not sufficient since the number of sheep used was relatively small on each farm. It is difficult to know how to overcome this disturbing factor in field experiments.

Many of the previously published conclusions are based upon summation of the number of strikes from several farms. This is not justified, since both numbers of sheep and strike incidence vary and in such circumstances summation could lead to erroneous conclusions.

Our conclusion is that convincing experimental evidence of the greater efficiency of DDT compared with arsenic as an antiblowfly dip has still to be obtained. The evidence so far is far from convincing, the most that can be said being that, in the absence of scouring, it appears to have a slight advantage.

References

Cragg, J. B. (1945.) *Nature*, Lond. **155.** 394.
Harbour, H. E., & Watt, J. A. (1945.) *Vet. Rec.* **57.** 685.
Cragg, J. B. (1946.) *Ann. Appl. Biol.* **33.** 127.
Hughes, L. E., Jenkins, J. R. W., & Jones, J. M. (1946.) *Vet. Rec.* **58.** 251.
Cragg, J. B. (1947.) *Vet. J.* **103.** 117.
Hughes, L. E., Pollard, E., Field, H. I., & Jones J. M. (1947.) *Ibid.* **103.** 265.

Printed in Great Britain by H. R. Grubb, Ltd., Croydon

[*Reprinted from* SCOTTISH AGRICULTURE, *Vol. XXVIII., No. 2 Autumn* 1948]

EDINBURGH AND EAST OF SCOTLAND COLLEGE OF AGRICULTURE

Miscellaneous Publication No. 25

Disease Survey of Sourhope Farm

J. T. STAMP and J. A. WATT

Veterinary Investigation Officers, Edinburgh and East of Scotland College of Agriculture

AT Martinmas 1946, the Edinburgh and East of Scotland College of Agriculture as tenants took over responsibility for the hill sheep farm of Sourhope. The farm is in the heart of the Cheviot hills at the head of Sourhope Water, a tributary of Bowmont Water. It is one of three situated in various parts of Scotland which are to function under the supervision of the Hill Farm Research Committee as research stations—field laboratories — for investigation into problems affecting hill sheep farming. Work on this particular farm, representative as it is of conditions on both sides of the Border, will involve work on problems as important to farmers in the north of England as to those in the south-east of Scotland. English workers and Scottish workers will thus find opportunities for close collaboration on common problems.

The farm is on the Border. The steading is some 750 feet above sea level. The highest point on the farm reaches 1985 feet. Its 2340 acres are nearly all hill grazing and are divided into three hirsels. The sheep stock, both Blackface and Cheviot is not bound to the ground, most of the sheep having been bought on since 1942. In recent years it has not been the practice to winter ewe hoggs away. For a number of years it has been known that sheep farming in the Bowmont Water area has been very unprofitable, so much so that it became impossible to obtain tenants for the farms and the complete removal of all sheep stock from the hills had been contemplated. The farm, besides providing field facilities for research, itself presents many of the problems which have made Border sheep farming so difficult in recent years. It is hoped that, in the course of time and as research develops, the answer to some at least of these problems will be found.

Before initiating a programme of research it was decided that the first task was to undertake a scientific survey of the farm as it was when taken over—the present condition of the stock in relation to disease, the general distribution and composition of the herbage, the condition of the soil and other factors. This is essential in order to define precisely the problems of the farm and to provide a datum line against which to measure the results of future research. This survey is in progress, but various

practical difficulties have so far delayed its completion in some aspects. So far an appreciable amount of survey work has been done on the condition and health of the sheep stock, and the results to date are summarised below. As a report it is unfortunately far from complete, since the survey has been restricted by a lack of adequate laboratory facilities at Sourhope and the impossibility of stationing a veterinary officer there, because of absence of accommodation. The report does, however, bring out some points of general interest.

The particulars of the sheep stock, taken over at Valuation at Martinmas 1946, are :—

Blackface ewes and gimmers	833
Blackface hoggs - - -	186
Blackface tups - - -	21
Blackface tup hoggs - -	9
Total B.F. stock - -	1049
Cheviot ewes and gimmers -	274
Cheviot hoggs - - -	285
Cheviot tups - - -	8
Total Cheviot stock - -	567
Total sheep stock - -	1616

Unfortunately, immediately after valuation, one of the worst snowstorms in memory occurred and the stock suffered very severe losses. For about ten weeks it was impossible to reach the farm on account of blocked roads. The effects of the storm were reflected in the number of stock which remained at the July 1947 clipping count, the associated losses being as follows :—

Blackface ewes and gimmers	142
Blackface ewe hoggs - -	51
Blackface tups - - -	3
Cheviot ewes and gimmers -	33
Cheviot ewe hoggs - -	160
Total - - - -	389

The 1947 lambing season was extremely bad owing to debilitated ewes, cold wet weather, scarcity of grass, weakly lambs and ewes with little or no milk. These conditions, common to so many hill farms, complicated the assessment of the first year's lambing season.

INTERNAL PARASITES

While parasitic gastro-enteritis has long been recognised as a major disease of intensive sheep farming, until recently it has not been realised that parasitic helminths are important in hill sheep. Recent work made it obvious that an estimation of the worm burdens of the Sourhope sheep would be an essential part of any disease survey. The clinical condition of the hoggs in the Spring of 1947 clearly suggested that parasitic gastro-enteritis was very prevalent and so in April 1947 faecal samples of all hoggs and representative samples of the ewe stock were taken for worm egg counts :—

	Number of Sheep Examined	*Average number of eggs per gramme of faeces*
Hairney Law		
Hoggs - - -	123	2300
Ewes - - -	19	480
Park Law		
Hoggs - - -	47	1400
Ewes - - -	24	496
Schil		
Hoggs - - -	53	800
Ewes - - -	55	509
Southside		
Hoggs - - -	77	1400
Ewes - - -	73	500

In addition a number of hoggs which died or were slaughtered *in extremis* were examined post-mortem. The carcases showed

emaciation, with very large numbers of worms in stomach and intestine (i.e. 5000-20,000). There is little doubt that the severe winter, along with the overcrowding and faecal contamination of ground which inevitably is associated with hay net feeding of hoggs, had given rise to an epidemic of parasitic gastro-enteritis, the severity of which can easily be judged by the number of hoggs lost. Since these losses were much greater on Hairney Law, Park Law and Southside and were fewer on the Schil, this corresponded closely with the severity of the parasitic infestations.

The losses were such that after the immediate cause of death had been ascertained, treatment of all hoggs with 20 gms. of phenothiazine was carried out. This was followed by a spectacular and sustained drop in worm egg counts and mortality rates. It would appear that had such treatment been carried out earlier losses of hoggs would have been reduced considerably. A controlled experiment was carried out on the surviving hoggs during the summer of 1947 when a group of hoggs on each hirsel was given monthly doses of 20 gms. phenothiazine, the rest being left as controls. Monthly weighings and worm egg counts were made. No difference was seen between treated and untreated sheep except on the Park Law hirsel, where there was a significant increase in the weights of the treated hoggs compared with the untreated. This suggests either that the worm burdens when removed by the initial dosing were not replaced, or that the improvement in condition of the hoggs during the summer was sufficient enough to overcome further parasitism by worms.

TICK INFESTATIONS AND TICK-BORNE DISEASES

In the latter part of April and in May 1947, tick counts were made on sheep from the several hirsels of Sourhope. Tick Counts on sheep from Park Law, Hairney Law and Southside were extremely low, while the Schil sheep were lightly infested—the highest count per sheep being five engorged females. Louping ill has been confirmed in several sheep but this disease is of minor importance as a cause of loss. Tick pyaemia is, however, prevalent in lambs on the Schil hirsel. It has not so far been practicable to arrange a survey of the occurrence of tick-borne fever.

COBALT DEFICIENCY

The view has been widely published that cobalt pining is prevalent in the Borders, especially in the Cheviot Hills area, but since there are no data recording deficiency of cobalt in the soil of Sourhope a soil survey will require to be undertaken. Cobalt pine occurs in lambs shortly after weaning, when a progressive debility appears accompanied by anaemia and emaciation, the lambs presenting a stunted unthrifty appearance. All degrees of intensity of these symptoms may occur. In the past it has been the practice at Sourhope to dose the sheep twice a year with tablets containing cobalt sulphate to prevent "pine." In order to determine whether cobalt deficient pine is in fact present a controlled dosing experiment is being carried out.

Stock ewe lambs on the Southside and Schil hirsels were divided into two equal groups, one group on each hirsel being dosed every two weeks with 60 mgms. of cobalt sulphate, and all lambs were weighed monthly. The experiment commenced in September 1947 and is still continuing. Unfortunately there were not enough lambs on the Hairney Law hirsel to justify their inclusion in the experiment. It is, however, the lambs of the Southside hirsel which are reported to show cobalt deficiency. Worm egg counts which are being carried out monthly show no significant variation between the control and experimental lambs.

As can be seen from the graphs on next page, there is no evidence of a cobalt deficient pine on either the Schil or Southside hirsels. In fact the hoggs in both control and experimental groups are in good, thriving condition and show no evidence even of sub-clinical "pine." A more extensive experiment is being carried out on the 1948 lamb crop.

BARREN EWES (TUP EILD) AND ABORTION

Barren ewes should be rare under conditions of good husbandry. But under less favourable conditions sterility is common and it is therefore economically important. Sheep which abort early in pregnancy are a further cause of small lamb crops. At lambing time such sheep are usually classed as barren since it is then difficult to distinguish between the two conditions. Enzootic abortion in sheep is of very considerable economic importance in Scotland. This type of abortion usually occurs late in pregnancy, often only one or two weeks before the lambing date. Lambs born alive may be too weak to survive. In other cases all that is noticed is that the aborted ewes have a slight swelling of the vulva with some blood stained discharge. While on many hill farms the greater proportion of abortions occurs in the gimmers the disease is by no means confined to this class and its cause is unknown.

In a disease survey of this kind the efficiency of the breeding stock must be known. While the 1947 lambing was upset by the blizzard, no such complication arose in 1948 and a clear picture was obtained:—

HAIRNEY LAW
(Total breeding stock 217)

Tup Eild and Aborted Sheep.

Gimmers	-	51	46% of gimmers
2nd Crop	-	7	20% of 2nd Crop
3rd Crop	-	11	16% of 3rd Crop
Total	-	69	31.5% of breeding stock

Of these 70% aborted near lambing time.

PARK LAW
(Total breeding stock 171)

Tup Eild and Aborted Sheep.

Gimmers	-	21	47% of gimmers
2nd Crop	-	6	13% of 2nd Crop
3rd Crop	-	14	17% of 3rd Crop
Total	-	41	24% of breeding stock

Of these 62% aborted near lambing time.

SOUTHSIDE
(Total breeding stock 440)

Tup Eild and Aborted Sheep.

Gimmers	-	23	30% of gimmers
2nd Crop	-	26	28% of 2nd Crop
3rd Crop	-	23	22% of 3rd Crop
4th Crop	-	10	11.5% of 4th Crop
5th Crop	-	12	21% of 5th Crop
Total	-	94	20.7% of breeding stock

Of these 49% aborted near lambing time.

SCHIL
(Total breeding stock 298)

Tup Eild and Aborted Sheep.

Gimmers	-	14	23.5% of gimmers
2nd Crop	-	7	11% of 2nd Crop
3rd Crop	-	13	18% of 3rd Crop
4th Crop	-	4	6.6% of 4th Crop
5th Crop	-	5	13% of 5th Crop
Total	-	43	14.4% of breeding stock

Of these 63% aborted near lambing time.

Thus over the whole farm about 22% of the breeding stock did not produce a surviving lamb.

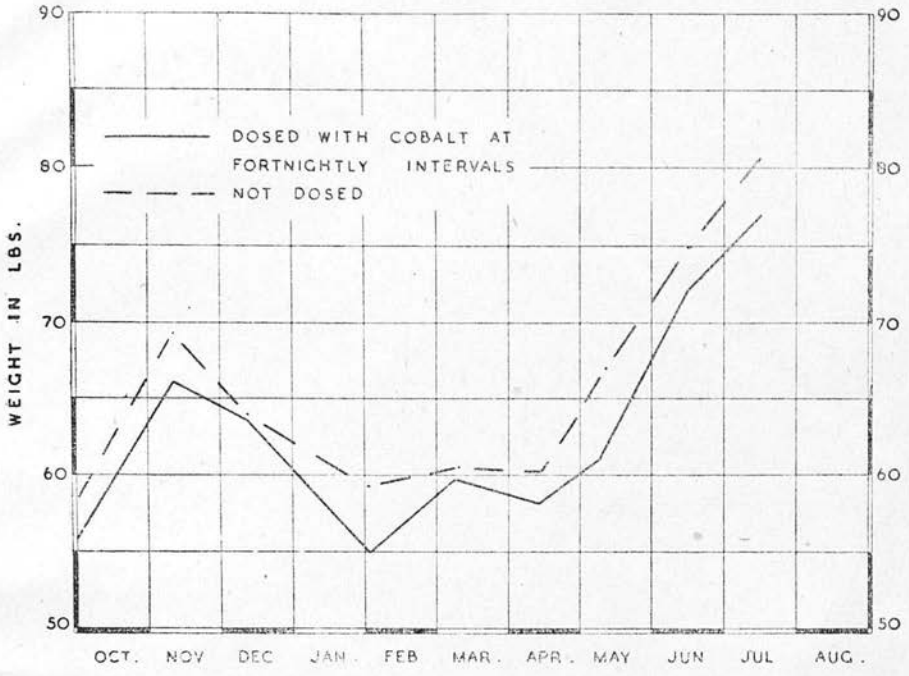
OTHER DISEASES

Joint ill, lamb dysentery and pulpy kidney disease have been diagnosed, but losses from these diseases are small. Contagious pustular dermatitis (Orf) is not serious. One case of scrapie and one case of double scalp have been seen.

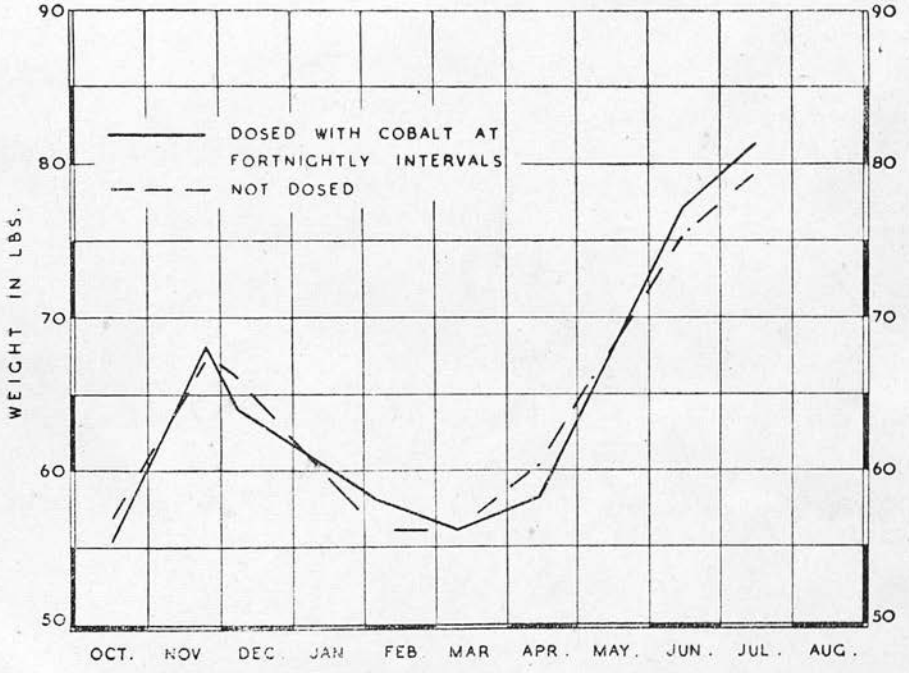
SUMMARY

Up to the present, abortion, barrenness and helminthiasis have been established as the important disease problems of Sourhope. No evidence so far has been obtained that pine due to cobalt deficiency is present.

SOUTHSIDE HIRSEL



SCHIL HIRSEL



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