MAEDI, A CHRONIC, PROGRESSIVE INFECTION OF SHEEP'S LUNGS

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During the last twelve years or so a lung disease of unknown etiology has been prevalent in sheep in Iceland. Except for a general emaciation, distinctive anatomical changes in this disease are found only in the lungs, which show a chronic pneumonia, and in the tracheobronchial lymph nodes. Although the cause of this disease is not known, all circumstantial evidence suggests that it is contagious, and that it has a very long incubation period—one to three years or more. It was probably introduced into this country in 1933 with some rams of karakul breed which were imported from Germany at that time. Two other diseases which were undoubtedly introduced with these rams have been described earlier (Dungal et al., 1938; Sigurdsson, 1945), so that this seems to be the third disease introduced with this flock.

The disease in question is in Iceland called maedi. It is very prevalent in many districts in Iceland and causes extremely heavy losses to the farmers, as it is not unusual that 20 to 30% of the flock may succumb annually. It is rarely observed in sheep less than two years old, but from that age on losses are often heavy.

In the absence of any other methods of control this disease has in recent years been dealt with under a slaughtering policy in certain districts. All the sheep in a given district have been destroyed, and the lambs from an infected area have been introduced in their place. So far about 170,000 sheep have been destroyed according to this plan, and about 80,000 healthy sheep have been introduced into the same areas again. There remain in infected areas about 130,000 sheep which will have to be destroyed if the slaughtering policy is to be continued.

It is still too early to conclude that these measures have succeeded in eradicating the disease from the areas in question, and any attempts to predict this would be futile, as the mode of spread of maedi is not known. If the disease does not reappear in the next few years in the areas where an exchange of the stock has been made, it is likely that the sheep in the remaining infected areas will also be destroyed.

The present paper will not discuss the epidemiology of maedi, as this aspect has been more fully investigated by others, but we shall instead confine ourselves to some clinical and laboratory studies on this new disease.

CLINICAL STUDIES

The first signs of maedi are usually a slowly advancing listlessness and loss of condition. This often becomes apparent when the weather changes for the worse, or after the sheep have been exposed to some unusual physical strain such as long driving. An early sign is that the sheep become dyspnoeic, and after an exertion the respiration becomes very rapid and shallow. It is not possible to distinguish in the individual case be-
tween maedi and any other kind of afebrile pneumonia, but if the history of the case is known, the protracted course and progressive loss of condition in a flock where maedi has occurred are suggestive. In contradistinction to epizootic adenomatosis (Dungal et al, 1938) maedi does not produce in the lung any appreciable quantity of fluid which could be poured out through the nostrils.

The frequency of respiration is usually above normal and sometimes excessively high (80 to 120 per minute at rest). As the disease progresses the respiration becomes very difficult or labored, the nostrils are dilated and flank breathing or pumping is commonly observed, even while the animal is at rest. In some cases coughing with more or less nasal discharge has been observed.

The body temperature in uncomplicated cases is within the normal range, although possibly close to the upper limit. This has been observed repeatedly over a number of years. The same may be said of the pulse rate, although this is rather difficult to determine in sheep, as the handling involved tends to influence the rate of the pulse.

As the disease advances and the sheep lose condition the hemoglobin content of the blood decreases. This is not an early sign, but values as low as 7 to 8 g hemoglobin per 100 ml blood are sometimes found in late stages. The normal value for hemoglobin in sheep in Iceland is from 12 to 14 g per 100 ml blood.

The number of red cells also decreases but proportionately less than the hemoglobin content, so that the anemia is of the hypochromic type. The mean corpuscular hemoglobin value is normally from 11 to 13, but in cases of anemia due to maedi it is usually between 10 and 11.

The number of white cells in the blood of maedi cases varies generally from 6 to 12 thousand per mm³, averaging about 8.5 thousand per mm³. This is slightly above normal as the normal figures found here are usually from 4 to 6 thousand per mm³.

Nonprotein nitrogen of blood plasma was found to be normal. The average and standard error for eight diseased sheep was 31.5 ± 1.7 mg per 100 ml plasma, and for a control group of seven clinically healthy sheep 31.4 ± 1.3. These determinations were carried out according to Koch and McMeekin (1924) after deproteinization of the plasma with tungstic acid.

Total protein was determined in plasma from 19 diseased sheep. The average for these determinations was 6.05 ± 0.12 g protein per 100 ml of plasma. A control group of 20 clinically healthy animals gave the average 6.50 ± 0.08 g protein per 100 ml of plasma. These determinations were carried out by a micro-Kjeldahl method with direct nesslerization (Koch and McMeekin, 1924; Miller and Miller, 1948). A correction for nonprotein nitrogen was introduced. The nitrogen content of the protein was assumed to be 16%. The lowest of the values determined, 4.7 and 4.8 g protein per 100 ml plasma, were found in the plasma from two of the diseased animals. Both of these sheep, and only these two, showed hepatitis upon autopsy. The highest value in the diseased group was 7.2. The probability, P, that the difference in the two averages is caused by chance alone, was calculated to be far below 0.01, whether the two sheep suffering from hepatitis are included or not. The average total protein in plasma in the diseased group thus turns out to be significantly lower than the average for the control group.

Preliminary analysis of the albumin-content of plasma after fractionation with sodium sulfate indicates that the disease is accompanied by hypoalbumi-
nemia, which would account for the low total protein of the plasma while the globulin is near its normal value. The low values for total protein are possibly an indication of the general wasting of the organism encountered in advanced cases of maedi. This may be compared with the emaciation found in malignant disease and chronic infections.

Fibrinogen was determined in plasma from 19 diseased sheep. The average found was $0.359 \pm 0.022$ g fibrinogen per 100 ml of plasma. A control group of 10 sheep of approximately the same age distribution as the former, gave an average of $0.297 \pm 0.014$ g fibrinogen per 100 ml of plasma. For the difference in these two averages $P$ was calculated to lie between 0.10 and 0.05. The difference thus cannot be considered as significant. It was found essential to take into account the age factor, since 10 healthy yearlings were found to have significantly lower fibrinogen than the control group, i.e., $0.216 \pm 0.013$ g fibrinogen per 100 ml plasma. The determinations were carried out by the same micro-Kjeldahl method as was used for the determination of total protein. Fibrinogen was isolated as fibrin (Cullen and Van Slyke, 1920) and brought into solution with a minimum amount of 1% solution of sodium hydroxide.

Reducing sugars in blood plasma were found to be normal. The average for 6 diseased sheep was found to be $62.3 \pm 1.9$ mg per 100 ml of plasma, calculated as glucose. The average for five control sheep was $63.8 \pm 1.1$ mg per 100 ml of plasma. The determinations were done on protein-free plasma filtrate according to Benedict (1931). Venous blood was drawn 14 hours after the last meal and the plasma was deproteinized with tungstic acid.

The carbon dioxide capacity of blood plasma was determined in plasma from 16 diseased sheep. The average for these determinations was $70.0 \pm 1.2$ volumes percent. A control group of 15 sheep gave the average $64.2 \pm 0.9$ volumes percent. These determinations were done manometrically in the Van Slyke and Neill manometric gas analysis apparatus, after saturation of the plasma with alveolar air. A calculation of the significance of this difference gives $P$ much lower than 0.01, and this increase in carbon dioxide capacity is therefore considered significant.

The increased carbon dioxide capacity of the plasma suggests in this case acidosis of respiratory origin. The lung damages may inhibit the normal release of carbon dioxide from the blood and give an increased carbon dioxide tension in the plasma. This would stimulate the compensatory mechanisms and lead to an increased carbon dioxide capacity.

It is doubtful that recoveries ever occur in maedi, and they are certainly rare. General practice among the farmers is to destroy all cases encountered in the fall and until mid-winter. Those that occur after March or so they let live in the hope that they will be able to rear a lamb, which they sometimes do. Under natural conditions cases of maedi very often succumb to a terminal acute pneumonia, usually showing large numbers of the bacilli described by Dungal (1931). If the sheep are not killed and if they meet with no particular hardships they may usually be expected to survive for three to six months after the first symptoms are noticed. In certain cases symptoms last much longer, even for years.

FINDINGS AT AUTOPSY

Macroscopic findings. — Uncomplicated cases of maedi show no typical pathological macroscopic changes outside the thoracic cavity. The carcass is usually thin, and there may be some
serous fluid in the abdomen. When the thorax is opened the lungs collapse less than normally, and in a minority of cases fibrous adhesions are found covering a portion of the surface of one, or occasionally both, lungs.

The most striking fact about the diseased lungs is their size. Instead of the normal weight of from 300 to 500 g the maedi lungs weigh from two to five times that much. The tracheobronchial lymph nodes are also greatly enlarged. Table 1 gives the weight of the lungs and of the tracheobronchial lymph nodes in 10 consecutive cases of maedi autopsied in our laboratory.

The color of the maedi lungs differs considerably from normal lung color. It varies from greyish yellow to greyish blue. To the touch the diseased lungs are diffusely thickened and feel somewhat like a rubber sponge, although less elastic. The whole lungs are usually remarkably uniform in color and consistency, i.e., the pathological changes seem to progress evenly in the whole organ with no normal lung tissue visible anywhere. This is in contrast to other lung lesions with which we are familiar, where diseased and normal parts may be easily distinguished in the same pair of lungs. On the cut surface the tissue is rather dry, and it is homogenous as to color and consistency there also. The larger bronchi often contain some mucus. It should be emphasized that the consistency of the lung tissue in maedi is always very much softer than in ordinary pneumonic hepatization.

**Microscopic findings.**—The main characteristic of the histological picture in maedi is a proliferation of mesenchymal tissue with a thickening of the alveolar septa, and at times numerous macrophages in the alveoli (figs. 1, 2, and 3). Large solid areas are usually not seen in the slides, but in advanced cases the air spaces sometimes appear as round or oval openings in the lung tissue, which has to a great extent lost its characteristic structure and is transformed into strands of mesenchymal cellular tissue. In such cases much more area in a given field is covered by the solid tissue than by the air spaces. Presumably the alveolar septa have given rise to the bulk of the pathological tissue that is seen in the microscope. This would be concluded from the appearance of the tissue in young cases rather than from the structure of the lung in advanced cases, where it is no longer possible to recognize many of the mesenchymal strands as septa. In young cases the alveolar septa seem hyperemic, but later in the disease there is a considerable increase in fibrous tissue and the smooth muscle normally found at the level of the alveolar ducts appears in many cases hypertrophic.

In some cases of maedi microscopically visible accumulations of lymphoid tissue are found scattered through the lung. These accumulations are at times very conspicuous and are particularly to be found close to a bronchus or a bronchiolus. It is, however, difficult to assess the significance of this finding, as in our experience accumulations of lymphoid tissue are frequently found in normal sheep's lungs, although we
FIGS. 1–3.—A typical maedi lung. For a description of the lesion see the text. Fig. 1, X14. Fig. 2, X130. Fig. 3, X480.
believe it is particularly conspicuous in maedi.

The cellular exudate in the alveoli is composed mainly of the common alveolar macrophage. A few lymphocytes are also seen, but few if any segmented leucocytes appear, except in cases where an acute pneumonia has superimposed, when they are, of course, extremely numerous.

In Giemsa-stained smears from maedi lungs we find almost always large mononuclear cells with protoplasmic inclusions of a rather typical appearance (fig. 4). They are almost perfectly round, varying in size from 1 to 3 μ, and they stain with a soft greyish blue color that is rather characteristic. They are seen either single or multiple within a cell. If multiple they may vary considerably in size. We have not seen these inclusions in normal lungs, but we have not searched for them in a sufficient number of other pathologically changed lungs to be able to maintain that these inclusions are pathognomonic for maedi, although with the evidence at hand this would seem to be so.

The bronchial tree is in general remarkably free of lesions, the epithelium usually being intact and the lumina free of exudate. It is a common finding, however, to see small epithelial formations outside the bronchi in the form of irregular cavities lined with cubical or cylindrical epithelial cells. These epithelial formations are never very extensive and remind one of the findings in influenza as reviewed and extended by Loosli (1949).

**Fig. 4.—Giemsa-stained cells in smear preparations from a maedi lung. X1575.**

The enlarged tracheobronchial and mediastinal lymph nodes show a generalized hyperplasia and chronic inflammatory changes.

Inflammatory changes in the livers have been found in several advanced cases of maedi. These appear as small, scattered infiltrates of lymphocytes and plasma cells without apparent connection with the vessels of the liver.

Supplementing our historical observations we determined the amount of connective tissue in four maedi lungs and four normal lungs by digesting in 0.1 N sodium hydroxide (Warren and Wahi, 1947) and drying and weighing
the insoluble, fibrous residue. This method is admittedly rather rough, but the results shown in table 2 indicate that on a dry matter basis the amount of fibrous tissue is approximately the same in infected and in healthy lungs, the average of fibrous tissue being 14% and 12%, respectively. The table also shows that there is about the same amount of dry matter per unit weight of wet substance in the diseased lungs as in the normal lungs.

**Table 2.** Amount of fibrous tissue and percentage of dry matter in four maedi lungs and four control lungs.

<table>
<thead>
<tr>
<th>Lungs no.</th>
<th>Wet weight in grams</th>
<th>Dry weight</th>
<th>Put in digestion (mg)</th>
<th>Fibrous tissue remaining after digestion</th>
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<tbody>
<tr>
<td></td>
<td>Before digestion</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Wet weight</td>
<td>Dry weight</td>
<td>Percent of dry weight</td>
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<tr>
<td></td>
<td>Grams</td>
<td>Grams</td>
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<tr>
<td>Maedi-infected sheep</td>
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<td></td>
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<tr>
<td>73</td>
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<td>Average</td>
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<td>23.94</td>
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</tr>
<tr>
<td>Control sheep</td>
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<td>Average</td>
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<td>1.040</td>
<td>20.77</td>
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</table>

**ETIOLOGICAL STUDIES**

The experience in the field seems to indicate that maedi is transmitted from sick to healthy sheep by contact. Our experimental results which will not be reported here also indicate this.

We have not, however, been able to demonstrate any etiological agent responsible for this infection. The lungs from uncomplicated cases of maedi are commonly found to be sterile when inoculated on the ordinary plain or enriched bacteriological mediums (blood-agar, made with horse blood, chocolate agar, tryptose agar) and incubated aerobically or anaerobically. Ten consecutive cases of maedi which were recently autopsied were studied by grinding pieces of lung tissue in a glass grinder with sterile distilled water to make a 20% suspension. This suspension was then inoculated on plates of plain agar, blood agar and chocolate agar, two drops on each plate. Five days later an average of three colonies was found on each plate, but these colonies were the common contaminants.

Attempts to cultivate fungi on Sabouraud's medium have also given negative results.

Dr. C. E. Roach of the Lilly Research Laboratories, Indianapolis, Indiana, kindly furnished samples of histoplasmin, blastomycin, toxoplasmin and torula antigen for intradermal tests. Dr. Charles E. Smith of Stanford University School of Medicine, San Francisco, California similarly furnished samples of coccidioidin. Each antigen was tested intradermally on from 11 to 25 proven or suspected cases of maedi with negative results. This would seem to exclude fungi and the tortula yeast as factors of etiological significance in maedi.

Serums from six cases of maedi were subjected to complement fixation tests with antigens specific for psittacosis and Q fever. Five normal serums were tested in the same way at the same time as controls. The tests for psittacosis were negative. Some of the serums showed a slight fixation with the Q fever antigen, but this was true of both the maedi serums and the controls.
Lungs from cases of maedi are generally no more heavily infected with lung worms than are lungs of other sheep in Iceland, nor are there other known reasons to suspect that metazoa may play a role in the etiology of this disease.

If an insect vector for the hypothetical infectious agent were to be considered, it may be pointed out that the sheep ked (Melophagus ovinus) is almost universally present on Icelandic sheep.

As no bacteria or fungi have been found that could be suspected of causing maedi, we have attempted to demonstrate a virus in the infected lungs. These attempts will be reported later.

We have also attempted to demonstrate a specific antigen in lungs of infected sheep. For this purpose we have prepared in various ways numerous extracts from infected lungs and used them in the complement fixation test with serums from natural cases of maedi. We have not succeeded in demonstrating conclusively the presence of a specific antigen in maedi tissue.

We have tested the effect of aureomycin* on five cases of maedi. The drug was administered intravenously as follows: The initial dose was 5 mg per lb of body weight. After that 2.5 mg per lb of body weight were administered every 12 hours for four days. A second course of exactly the same treatment was given again ten days later. Five similar cases of maedi were kept as controls. The treated animals showed no clinical signs of recovery. The frequency of respiration continued to increase in both groups, the hemoglobin values of the blood fell at about the same rate in both groups, etc.

The sheep were slaughtered and autopsied about two months after the treatment was begun. They were all found to be typical cases of maedi, and no striking differences between the weight or general appearance of the lungs in the two groups were observed.

Although the infectious nature of maedi seems beyond question, we have considered the possibility that a nutritional deficiency might be involved. We have no evidence that such is the case. In particular we have considered the possibility that a deficiency of the microelements, copper and cobalt, might be involved. Several serums of sheep on an affected farm were analyzed for copper. The values found varied from 0.09 to 0.12 mg copper per 100 cc of blood, which is within the normal range.

Samples of soil and herbage from two affected districts were analyzed for cobalt. The average values from the first district were 2.07 parts per million cobalt in the soil and 0.24 p.p.m. in the herbage, and from the second district 7.8 p.p.m. in the soil and 0.29 p.p.m. in the herbage. A nutritional deficiency of cobalt would not be expected to occur in animals grazing on land showing these values for cobalt.

Extensive feeding experiments with copper and cobalt did not indicate that these microelements had a beneficial effect on clinical cases of maedi, nor did they prevent the occurrence of new cases in flocks where these minerals were being fed.

DISCUSSION AND SUMMARY

A chronic pneumonia of sheep is prevalent in many districts of Iceland. The epidemiological behavior of the disease, which in Icelandic is called maedi, definitely indicates that it is an infectious process. The cause of the disease is

* Obtained through the courtesy of Lederle Laboratories Division, American Cyanamide Company, Pearl River, N. Y.
not known, but it is believed that microscopically visible organisms have been ruled out as a possible cause. The disease is characterized by an incubation period of about two years and a protracted course. The lungs of a typical case weigh from 800 to 2000 g instead of the normal weight of 300 to 500 g. Anatomically the disease consists principally of a diffuse mesenchymal proliferation in the lung. The bronchial and mediastinal lymph nodes are also enlarged.

Other clinical and laboratory characteristics of this disease are discussed in the present paper. No indication was obtained in these studies that the disease is primarily a metabolic or nutritional disorder.

The etiology of maedi is not known, although a virus infection is suspected. No serological tests are available to distinguish it from or identify it with other diseases of sheep lungs.

In the absence of such definite etiological information it seems futile to discuss at the present time the possible relationship of maedi to other lung diseases of sheep. We want to point out, however, that typical cases of infectious adenomatosis or jaagziekte, which is a well known disease of sheep lungs occurring in this country and elsewhere, are distinctly different from maedi both anatomically and as regards the clinical picture.

REFERENCES