STUDIES ON THE ETIOLOGY OF JAGZIEKTE.

I. THE PRIMARY LESIONS.*

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(From the Laboratories of The Rockefeller Institute for Medical Research.)

Plates 14 to 16.

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Jagziekte is usually regarded as a specific chronic catarrhal pneumonia of sheep which is peculiar to South Africa. The name is derived from the Dutch jagt, to drive, and ziekte, a sickness, the combination of which is intended to indicate that the initial symptoms become first noticeable when the animals are driven for some distance. It is thought to be contagious and causes severe economic losses. An interesting feature of the disease consists of an extensive overgrowth of the pulmonary epithelium, but it is not known whether this proliferation constitutes the primary lesion nor has its tumor-like structure been studied. In view of the general similarity between this overgrowth and the proliferation of bile ducts in coccidiosis, Sir Arnold Theiler has suggested the possibility of the local action of some epithelial parasite susceptible of microscopic demonstration.

It was only upon my return from South Africa after comparing the lungs of jagziekte animals with the presumably normal ones of American sheep not affected by jagziekte, that evidence was found tending to show that the proliferations of epithelium are secondary to changes in the interalveolar tissue. It is proposed to describe briefly these primary changes and to give, in a second paper, a description of the origin and tumor-like character of the epithelial proliferations and of the subsequent changes.

* Fourth contribution by the South African Expedition of The Rockefeller Institute for Medical Research.

The observations were made in the laboratory of the Department of Agriculture at Onderstepoort and at New York. Cordial thanks are due to the Government of the Union of South Africa, to Sir Arnold Theiler, and to the members of his staff for the many courtesies extended.
Preliminary to this description a short summary of our knowledge of the disease, as contributed by Hutcheon, Robertson, and particularly by Mitchell, may be helpful.

Etiology.—Hutcheon considered jagziekte to be infectious "under certain climatic conditions" and Robertson described peculiar crescent-shaped bodies with rounded ends in smears made from the lesions. These he thought to be of protozoan nature and suggested a resemblance to the crescents of subtertian malaria. But Mitchell, with much more extensive material at his disposal, failed to observe them and laid aside the parasitic theory, concluding from his experiments "that the conditions of transmission point to a specific virus." Experimental proof of the existence of a virus in animals suffering from the disease is lacking. It has never been possible even to transmit jagziekte to healthy sheep by the injection or inhalation of blood and diseased tissue.

Pathology.—According to Mitchell, jagziekte is essentially a chronic catarrhal pneumonia which progresses in the affected lung by continuity and contiguity. It is characterized by the presence of lymphoid nodules which show a marked tendency to infiltrate the surrounding tissue. These may develop in connection with the bronchi or in the interalveolar tissue. There is also bronchopneumonia with typical exudation of catarrhal products into the alveoli, interstitial fibroid changes, bronchitis and peribronchitis, and thickening of the arterial walls. As a result a general fibrosis of the affected portions ensues which renders the lesions hard and dense. Compensatory changes occur in the remaining tissue; viz., emphysema, atelectasis, and pleuritis.

Mitchell found considerable variability in the occurrence of "proliferation of the bronchi." In his total of fifteen cases he described the proliferation as very marked in two, well marked in three, present in two, not marked in two, not apparent in one, absent in two, while in the remaining three he made no comment regarding its presence or absence. Moreover, in his general summary, which has been abbreviated in the above paragraph, he did not apparently consider the proliferation of bronchi worthy of mention.

Epidemiology.—Jagziekte has been recognized as a specific and very destructive disease of sheep since about 1893, although it probably occurred earlier. It is prevalent in many districts of the Cape province, Orange Free State, and the Transvaal, but has not been reported in the more humid parts of Natal. There is evidence that the character of the grazing does not determine the incidence of the disease because it is found on grass, Karoo, bush veld, and mixed veld.

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1 Hutcheon, D., Diseases of stock in South Africa, Pretoria.
2 Robertson, W., J. Comp. Path. and Therap., 1904, xvi, 221.
4 Mitchell, p. 600.
water supply of infected farms is equally variable, being obtained in some cases
from above dams, in others from rivers or bore holes. Mitchell states that "the
greatest mortality from the disease occurs in particularly wet years and [that]
more cases develop in the flock during the rainy season than during the dry season." All breeds of sheep appear to be equally susceptible, males as well as females. The disease occurs most frequently in sheep about 3 years old. It has never been
reported in lambs. Mitchell estimates the average mortality in the infected areas
at 1.6 per cent per annum. This percentage does not vary greatly from year to
year. Cases usually occur singly and no instance is on record in which large
numbers of sheep in the same flock were affected simultaneously, although un-
usually severe outbreaks were reported in 1911-12.

The general belief that jagziekte is transmitted by contact is based upon field
observation and direct experimentation. It is said that the disease spreads to
clean areas by the importation of infected sheep and that kraals, in which sick
animals have been kept, retain the infection and are able to transmit it to healthy
sheep. The disease is known to be insidious and may be passed on to other sheep
before any symptoms become apparent in the newcomers which carry it. It is
this fact that makes laboratory experimentation so difficult and inconclusive
because of the possibility that seemingly healthy animals have already contracted
the disease before being placed in contact with sick sheep. The lesions appear
from 3 to 5 days after contact (Mitchell). Under natural conditions death occurs
in from 2 to 8 months.

Course.—There is a progressively increasing respiratory distress, followed by
catarrhal discharge from the nose, cough, moist bronchial rales, loss of appetite
and of weight, progressive muscular weakness, anemia, extreme emaciation, and
death, with little or no fever at any time.

Differential Diagnosis.—The disease may be distinguished from the caseous
lymphadenitis of Nocard by the above mentioned increased respiratory movements
on exercise, cough, nasal discharge, and bronchial rales. Similar clinical manifesta-
tions may be produced by extensive infection of the lungs with the cysts of
Tenaia echinococcus but these may always be definitely identified post mortem.

Prognosis.—Death is apparently inevitable although life may be slightly pro-
longed by keeping the animals under good conditions and on a nutritious diet.

Prophylaxis.—The immediate slaughter of all cases is recommended.

Material and Methods.

Abundant material was placed at my disposal by Sir Arnold Theiler,
and the members of his staff, which may be classified as follows:

1. Tissues from sixteen autopsies made on jagziekte sheep by the officers on
duty.
2. Portions of the lungs from thirteen sheep, preserved in formalin, sent to the
laboratory for examination, and diagnosed as jagziekte.
3. Parts of the lungs and bronchial lymph glands from thirty-eight sheep which died from bleeding in the preparation of blue-tongue vaccine. These sheep came from a flock in which cases of jagziekte were occurring from time to time. Four of them exhibited early lesions, the existence of which was not suspected during the life of the animals.

4. For controls, the lungs of six sheep which suffered from heartwater, and of three sheep which were carefully examined by Dr. Steck before death, and pronounced to be apparently normal.

5. As additional controls clearly free from even the earliest of jagziekte lesions, the lungs of thirty-eight sheep obtained in New York where they were sent from the Ozark region of the Middle West.

In the case of the first three autopsies, the heart, spleen, liver, kidneys, suprarenals, and gastrointestinal tract were examined in addition to the lungs merely to afford material for verification of the statements of Mitchell that the significant lesions of the disease are restricted to the lungs. Subsequently the lungs alone were studied, together with the bronchial lymph glands when it seemed desirable.

For routine purposes the tissues were fixed in Zenker’s fluid or in 10 per cent formalin and were stained by Giemsa’s method, or with hematoxylin and eosin. Special methods were used in the identification of cell types and in the search for microorganisms, including Levaditi’s silver method for spirochetes. A total of 504 samples of tissue was thus examined in sections, to which may be added 13 blocks of tissue which were cut into serial sections occupying from 41 to 91 slides each. These latter were used in order to reconstruct roughly the topography of typical proliferations. Smears prepared in several ways were also studied and living tissues were examined in physiological saline solution by direct and oblique illumination.

**The Primary Lesions.**

The difficulty, or impossibility, of distinguishing clinically between animals in the early stages of jagziekte and other actually healthy animals offered an obstacle in the determination of the primary lesions.

At the time that tissues were being collected (July, 1924, to January, 1925) the disease was appearing with considerable frequency among the sheep kept at Onderstepoort. A thorough examination was accordingly made of the lungs and mediastinal lymph glands of thirty-eight sheep which before death did not exhibit noticeable symptoms of jagziekte, although some of them were not specially

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5 Blue-tongue is a kind of catarrhal fever of sheep peculiar to South Africa.

6 Heartwater is a specific febrile disease which affects sheep, goats, and cattle in South Africa and is caused by *Rickettsia ruminantium* transmitted by the tick *Amblyomma hebraeum* (Cowdry, E. V., *J. Exp. Med.*, 1925, xliii, 231, 253).
scrutinized for the disease. Four of these at autopsy showed distinct lesions characterized by variable degrees of epithelial proliferation and pneumonia, while others seemed to differ in less noticeable respects from the normal.

In order to ascertain the actual status of these suspected differences the lungs of an equal number of apparently normal sheep shipped to New York from the Ozark region of the Middle West (prairie country) were examined by the same methods. Very distinct differences were noted between the pulmonary tissue from these sheep, which were free from jagziekte, and that from the South African sheep, them-

TABLE I.

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<th>American sheep</th>
<th>South African sheep</th>
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<tr>
<td>Accumulation of macrophages and lymphocytes in interalveolar tissue.</td>
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<td>Penetration of macrophages into alveolar lumen.</td>
<td>Present in 5.</td>
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<td>Accumulation of polymorphonuclear leukocytes in interalveolar tissue.</td>
<td>Absent.</td>
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<tr>
<td>Penetration of polymorphonuclear leukocytes into alveolar lumen,</td>
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<td>Peribronchial lymphocytic infiltration.</td>
<td>Present in 30.</td>
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<tr>
<td>Epithelial proliferation.</td>
<td>Absent.</td>
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<td>Vascular congestion.</td>
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<td>Occurrence of foreign material.</td>
<td>Present in 3.</td>
<td>“”</td>
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<td>“” bacteria.</td>
<td>Absent.</td>
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selves apparently free from the disease but coming from flocks in which the disease was certainly endemic. A glance at Table I shows that the latter exhibited a wider variety of lesions which were also of greater severity. That this circumstance is not due to the elimination of diseased animals by the inspector in New York may be accepted, because the tissues were removed before inspection took place. Both sets of animals were killed by bleeding.

The changes in the lungs of the South African sheep, which were never noticed in the American sheep and which may therefore be of significance in the etiology of jagziekte, center in the interalveolar tissue. This is noticeably thickened in thirteen out of thirty-eight
animals; that is to say, in about 33 per cent. The extent of these first alterations may be estimated by comparing Figs. 4 to 7 with Figs. 1 to 3, which latter represent approximately the normal appearance of the interalveolar tissue in American sheep.

The thickenings do not take place evenly throughout the lungs but are first seen in scattered foci 1 to 15 mm. in diameter. These may be directly beneath the pleura or may occur more deeply in the pulmonary tissue, but no definite relationship to the bronchioles or to the interlobular septa was observed.

They are caused by the accumulations of large phagocytic ameboid cells possessed of abundant cytoplasm and simple spherical nuclei which have been variously called macrophages (Metchnikoff), polyblasts (Maximow), endothelial leucocytes (Mallory), and mononuclear leucocytes (Ehrlich). They are accompanied by many lymphocytes of variable size, the larger ones of which resemble them closely, and by a few polymorphonuclear leucocytes. None of these cells exhibit signs of mitotic division.

No evidence was found that the macrophages are produced through the multiplication of the endothelial cells of the alveolar capillaries as described by Permar. Probably they are chiefly of hematogenous origin. In some instances they were seen within the capillary lumina in contact with erythrocytes and free from the lining endothelium (Fig. 9). In all likelihood they may also arise from cells resident in the interalveolar connective tissue, in other words from the histiocytes of Kiyono, or the rhagiocrine cells of Renaut (to employ only a few of the available synonyms). That they may be derived from the lymphocytes which are likewise present in the interalveolar tissue is to be considered as a third possibility in view of Maximow's observation that freshly emigrated blood lymphocytes are capable of transformation into large phagocytic ameboid cells; that is to say, in this case, into macrophages. The lymphocytes, in turn, like most of the macrophages themselves, probably emigrate from the alveolar capillaries, since, although we have no specific information regarding the pulmonary lymphatics of sheep, Miller has been unable to find in

man any lymphatics in the walls of the air sacs beyond the ductuli alveolares. Proximal to this point there are no unusual accumulations of lymphocytes in these early stages in the lesions of the South African sheep. Indeed the American sheep show much more extensive peribronchiolar lymphocytic infiltrations than they do. A comparison of the walls of the bronchioles and of the bronchi of the two sets of animals did not reveal any instructive differences between them which might suggest the spread of infection by the lymphatic route. 10, 11

Coincident or antecedent to this infiltration of the interalveolar tissue a variable degree of similarly localized dilatation of the alveolar capillaries is to be noted (see Figs. 10, 12, and 13) which conforms with Mitchell's statement that the primary lesions of jagziekte are hyperemic. At the time when this dilatation is present the macrophages commence to migrate into the alveolar lumina in numbers which exceed the normal as seen in American sheep. The exudate at this time contains very few polymorphonuclear leucocytes. One macrophage in the act of entry into an alveolus is represented in Fig. 10. Its typically rounded nucleus has assumed a kind of hour-glass shape, half inside and half outside of the alveolus, and has been preserved in this state by the fixative.

In this new location the macrophages accumulate in large numbers where they have been frequently reported in diseases of the lungs. They are the cells so often referred to as mononuclear phagocytes of the lungs and as epithelioid cells. 12 They may be distinguished from desquamated epithelial cells, with which some investigators have identified them, 13-18 by their characteristic structure. Their nuclei are now less spherical and more kidney-shaped. By staining with iron-hematoxylin a centrosome, or a diplosome, is revealed on the side of the nuclear concavity. These cell organs are always centrally

12 I am indebted to Dr. H. S. Willis for the details of a special method for identification of epithelioid cells.
13 Briscoe, J. C., J. Path. and Bact., 1908, xii, 66.
14 Sewell, W. T., J. Path. and Bact., 1918-19, xxi, 40.
placed and are surrounded by a radial arrangement of cytoplasmic granules. Coloration with fuchsin and methyl green after fixation in Regaud's fluid brings to light numerous rod-like mitochondria in the peripheral cytoplasm in company with droplets of neutral fat which may be stained in frozen sections with Scharlach R. These epithelial cells, which may still with propriety be termed macrophages, differ only from the typical macrophages, or large mononuclears (monocytes) from which they arise, by changes incident to the amount, character, and condition of the materials which they have ingested, as has been clearly shown in the case of the epithelioid cells of tuberculous lesions by Lewis, Willis, and Lewis. And furthermore, like those in tuberculous lesions, they also give rise to multinucleate giant cells (Fig. 14) which often attain considerable dimensions, particularly in the later stages of jagziekte.

It is in hyperemic areas of this kind with interalveolar infiltrations of macrophages, some of which penetrate into the alveolar lumina that the earliest epithelial proliferations, which are so characteristic of jagziekte, commence (Fig. 8). That these infiltrative and exudative changes constitute in reality the primary lesions of the disease may be accepted from the following observations:

1. That they were found in thirteen out of thirty-eight sheep which before death (in the preparation of blue-tongue vaccine) failed to exhibit noticeable symptoms of jagziekte although the disease was appearing from time to time in sheep belonging to the same flock; whereas they were absent in the lungs of an equal number of American sheep not subject to jagziekte.

2. That in all of the thirty-three cases of pronounced jagziekte examined in which the disease was readily detected clinically, the epithelial proliferations without exception arose in areas of the lungs thus modified.

3. That no epithelial lesions were found, although exhaustive search was made, in the lungs of thirty-four South African sheep which did not contain these preliminary infiltrative and exudative alterations.

An interesting deduction follows; namely, that since these primary

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lesions occur in the absence of typical signs of jagziekte it is possible that some, perhaps many, animals in the early and thus unrecognizable stages of the disease do recover, although after the development of positive symptoms a fatal termination may be inevitable, as is generally supposed.

On a purely morphological basis no satisfactory explanation could be found for the prevalence of these primary lesions in the lungs of sheep of a flock subject to jagziekte, but brief reference may be made to the incidence of foreign material and of bacteria detectable microscopically.

The foreign material was usually observed within the peribronchial lymphatics and less frequently within macrophages in the thickened interalveolar tissue (Fig. 11). It was identified in the lungs of sixteen sheep and in the mediastinal lymph glands of eleven others, from which we may infer its presence in a high percentage of the flock. It was found to occur both during the humid rainy weather of midsummer (January) and also during the driest and most dusty period of winter (August), as well as in sheep from different localities.

The material differs from that noted in the lungs of three of the thirty-eight American sheep. It is readily identified in sections stained by Giemsa's method in which it assumes an intense, rosy red color. It remains unmodified in color after treatment with hematoxylin and eosin. Its properties were studied in unstained sections by methods advised by Mavrogordato on the basis of his experiments upon the effect of foreign materials upon the lungs. It was found to occur in the form of biconvex lens-shaped bodies with sharp edges from 3 to 15 microns in diameter and 1 to 5 microns in thickness. These do not offer sufficient resistance to the knife to cause tears in the sections. When the bodies are broken, as was often the case, the lines of fracture are straight and not rounded. No definite crystalline facets were noted. The bodies are translucent and faintly yellow in color. They are not dissolved by the action for 1 hour at 60–65°C of the following reagents: (1) acetone, (2) 95 per cent alcohol, (3) 95 per cent alcohol plus 1 per cent of hydrochloric acid, (4) half absolute alcohol and chloroform, and (5) 0.5 per cent aqueous hydrochloric acid. Evidently the bodies do not consist of cholesterol or

17 Mavrogordato, A., J. Hyg., 1918, xvii, 439.
some other material likely to be produced locally through pathologic changes. On the contrary, their properties and the fact that they are actively engulfed by macrophages indicate clearly that they are of exogenous origin.

In addition to this substance, irregular fragments of vegetable tissue were sometimes observed, which were likewise absent from the lungs of American sheep; but their occurrence was not sufficiently constant to indicate the advisability of further study. They are mentioned only because it is important to note that the lungs of South African sheep are subject to the inhalation of an unusual variety of substances which may have an irritating action.

Though it is known that the lungs normally possess a varied bacterial flora the observation is interesting that the lungs of six of the South African sheep contained bacteria demonstrable histologically, whereas none were seen in the lungs of American sheep not subject to the disease. At least two kinds were noted: a Gram-negative bacillus, about 5 by 1 microns, which was colored faintly by Giemsa's method, and a Gram-positive diplococcus, about 1 by 2 microns, which was stained deep blue by Giemsa's technique. Both were often associated with the above mentioned foreign material and perhaps gained entrance with it.

SUMMARY.

The lungs of South African sheep, in a district in which jagziekte is appearing sporadically, differ from those of normal American sheep. Their structure is in general variable. They are more subject to the action of bacteria, demonstrable histologically, and to the effect of inhaled foreign material. The most significant difference, however, centers in the interalveolar tissue, which, in about 33 per cent of the animals, is definitely thickened beyond the range of variation in this direction observed in American sheep. The thickenings occur in localized areas several millimeters in diameter. They begin with engorgement of the alveolar capillaries and accumulation of macrophages and of lymphocytes. Many of the macrophages pass into the

19 Spray, R. S., J. Infect. Dis., 1922, xxxi, 10.
alveolar lumina and assume the appearance of typical epithelioid cells. These infiltrative and exudative changes are primary to the epithelial proliferations which always arise in tissues modified in this way and which it is proposed to describe in a second paper.  

EXPLANATION OF PLATES.

PLATE 14.

Fig. 1 to 3. The normal range of development of the interalveolar tissue as seen in American sheep not subject to jaagsiekte. × 120.

Fig. 4. Beginning infiltration of interalveolar tissue in a South African sheep. × 120.

PLATE 15.

Figs. 5 to 7. Progressive stages in the infiltration of the interalveolar tissue with macrophages and lymphocytes. × 120.

Fig. 8. Early alveolar proliferations in an area thus modified. × 120.

PLATE 16.

Fig. 9. A macrophage within an alveolar capillary. × 1,000.

Fig. 10. Another macrophage in the act of emigration is seen in the center. × 1,000.

Fig. 11. Foreign material undergoing phagocytosis by macrophages in the thickened interalveolar tissue. × 1,000.

Fig. 12. Macrophages and lymphocytes of variable size in the interalveolar tissue. × 1,200.

Fig. 13. Macrophages (epithelioid cells) within the lumen of an alveolus. Note the congestion of the alveolar capillaries. × 1,200.

Fig. 14. A small multinucleated phagocytic group cell within the lumen of an alveolus. × 1,200.

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(Cowdry: Etiology of jaagsiekte. I.)