XXIX.

- (2) **The Pathology of Isle of Wight Disease in Hive Bees.** By P. Bruce White, B.Sc., Bacteriologist to the Bee Disease Investigation, University of Aberdeen and N. of Scotland College of Agriculture. *Communicated by* Dr JOHN RENNIE. (With One Plate.)

(From the Department of Pathology, Marischal College, Aberdeen.)

(Read November 1, 1920. MS. received December 7, 1920. Issued separately March 25, 1921.)

Isle of Wight disease is, as we have seen, primarily a disease of the respiratory system, in which the organism remains localised throughout the entire course of the attack.

The effects are, however, far-reaching, and are registered in the disordered functioning of several organs, and in visible pathological changes in some of them.

The parasitic invasion has two aspects.

We have, in the first place, to consider the active injury wrought upon the host by a parasite developing and living at the expense of its body fluids. With this aspect of the question may be coupled the possibility of a definite toxic action on the part of the parasite.

In the second place, we have to consider the passive rôle of the mites in hindering or inhibiting the normal functions of the infected organs.

Before proceeding to consider the various pathological conditions, a few words on the distribution of the parasite within the host is called for.

In infected bees the mites are consistently present in the tracheal system of the thorax, and in a certain number of bees are also to be found in the air-vessels of the head. No mites have, up to the present, been discovered in the abdominal system.

The primary parasitic invasion takes place through one or both of the first pair of spiracular orifices, and apparently through these alone. The whole anterior thoracic system of major tracheæ and air-sacs is liable to infection. The infection may be unilateral or bilateral, and in some cases where the mites have entered on one side only, they may migrate into the vessels of the other side, setting up a secondary bilateral infection. The passage is effected through the roots of the anterior air-sacs, which form a commissure between the large paired vessels supplying the head.

A single mite may enter the bee, or several may enter together or at intervals. Sometimes the pregnant female may advance as far as the secondary tracheæ before depositing eggs, but the primary trachea becomes involved sooner or later in the vast majority of cases.

It is usually only in the later stages of the attack that the mites attain the smaller tracheæ, the thoracic air-sacs, and the vessels of the head. In such cases,

however, the young mites frequently advance as far as the calibre of the vessels permits.

The various pathological conditions which may be encountered in infected bees will now be considered, the various systems being treated of in sequence.

TRACHEAL SYSTEM.

Macroscopical appearances.—The first change visible to the naked eye is an increased opacity of the infected vessels due to the aggregation of ova and the younger forms of the parasite within the lumina. As the disease advances the trachea assumes a brown tint, which gradually deepens and becomes flecked with black. Finally considerable portions of the infected tracheæ may become dead black. This change in colour is accompanied by an increasing hardness and brittleness of the parts, which become rigid. This brittleness results in a phenomenon which is of some use in the field diagnosis of the disease. It is frequently found that upon exerting moderate pressure upon the upper surface of the thorax of bees crawling from the disease, that a drop of fluid—blood—will exude from the first spiracle of one or both sides, the rupture of the trachea at its insertion having thrown the hæmocœle open to the exterior.

Microscopic appearances.—During the earlier stages of the attack, the oval and almost colourless ova and embryos may be seen lying within the lumina of the tracheæ. The parent mites, too, may often be found in the vicinity. The tracheal wall may show here and there a few fragments of brownish matter, the fæces of the invading adults.

This condition is maintained till, with the appearance of the later developmental stages of the parasite and the young adults, the wall becomes encrusted with granules of fæcal matter. These granules, irregular in size and discoid or spherical in shape, become arranged in the interspaces between the tracheal thickenings, forming an irregular series of transverse bands upon the tracheal wall. They are of a brownish or yellowish colour, and when densely aggregated appear black. The colour of the deposit upon the wall therefore varies with the thickness of the crust and the amount of pigment it contains. The pigment may become extracted, leaving the pallid granules behind.

A similar deposit may collect in the lacunæ between the parasites themselves.

A typically affected tracheal tube is shown in fig. 1, while a fragment of the encrusted wall is shown further enlarged in fig. 2.

The fæcal matter may, further, be inhaled and, though the bulk appears to be trapped in the air-sacs and larger vessels, may attain the finer ramifications of the system, sometimes forming small emboli in the tracheoles. This is particularly frequent when the parasites are present in the air-sacs.

Careful study of the tracheal wall for perforations reveals little. In two cases

only has it been possible to observe the long piercing apparatus of the mite actually passing through punctures in the wall. The material damage done in this way is seemingly small.

MUSCULAR SYSTEM.

Visible pathological changes of the muscle fibres occur, but these are apparently restricted to the thoracic muscles of flight. Though a considerable number of fibres, in highly infected crawling bees, may show signs of the atrophic change to be described, the number showing definite degenerative changes is usually small. While no such changes have been noted in non-infected bees of whatever age, they may occur in infected bees which show no outward symptoms of the malady. On the other hand, a percentage of infected crawling bees show no marked muscle changes.

Macroscopic appearances.—Upon teasing out in saline the thoracic muscle mass of a bee crawling from the disease it is usually found that certain fibres—averaging 2–6 in number—contrast markedly with the flaccid, greyish-yellow normal fibres by their opaque white colour, slenderness, brittleness, and rigidity.

Microscopic appearances.—Under the low power of the microscope these white fibres are conspicuous by their slenderness, density, and granular appearance. The ends show an irregular fracture quite unlike the frayed-out ends of the normal fibres. A number of micrometer measurements on these and on healthy fibres of the muscles of flight gave the following values:—

Average width of healthy fibres of muscles of flight = '24 mm.			
„	„	atrophied	„ „ „ = '12 mm.

In fresh preparations examined with the 1/6" objective it was possible to make out the nature of the change which had taken place.

In the normal muscle of the bee the bulk of the fibre is composed of the fibrillæ, upon and between which lie the large flattened sarcosomes or myochondria. These granules mask the transverse, but not the longitudinal, striation of the fibre. When the fibre is teased out the fibrillæ fray out, allowing the diaphanous sarcosomes to escape.

In the case of the atrophied fibres of infected bees the appearances are different. Microscopic examination may show little or nothing of the original fibrillar structure. It is often found that the bulk of the fibre is composed of densely arranged longitudinal columns of closely packed and very coherent sarcosomes which do not escape and float away when the fibre is teased out. Between these granular columns it is found, upon closer examination, that remnants of the fibrillæ persist, though many may be reduced to thread-like vestiges of their original form.

A drawing of the low-power appearances of normal and atrophied fibres is shown in fig. 3, and a piece of atrophied fibre is drawn under the high power in fig. 4,

while in fig. 5 the substance of a highly atrophied fibre (A) is contrasted with two normal fibrillæ and normal sarcosomes (B).

The sarcosomes of the atrophied fibre are much denser and more cubical in shape than the normal granules, and may be of relatively enormous size. In highly degenerate fibres they may form conglomerate masses of considerable size.

When degenerate muscle is treated with dilute acetic acid the fibrillar vestiges swell, forcing the granular columns apart, and a picture closely resembling normal muscle when treated in the same way is achieved.

When freshly obtained normal and atrophied fibres are placed in a drop of dilute eosin or methylene blue, it is found that while the former are only slowly and superficially stained, the latter become rapidly and deeply stained through their entire substance. This would seem to indicate that the degenerate fibres are dead.

Though various fixation and staining methods have been employed, they have added little to the facts derived from the study of fresh material.

These muscle changes may be summarised as—

A general wastage of the fibrillar substance and loss of sarcous fluid, with the condensation of the frequently enlarged sarcosomes in densely arranged longitudinal columns, the process resulting in a shrinkage of the fibre with loss of function.

All stages in this process are, of course, to be encountered.

A further feature of some atrophied fibres, and occasionally of those which do not show the typical signs of wastage, is the development of pigmented spots in their substance. Such spots are represented in fig. 5.

These spots vary in colour from yellow to a deep brown or black, and often appear to bear a definite relationship to the distribution of the tracheoles supplying the muscles.

Careful scrutiny has not entirely elucidated their origin. It seems possible that they may be caused by staining of the muscle by the fæcal dye of the parasite, which has percolated into the final ramifications of the tracheal system.

Other explanations which have been considered are that the discoloration is due to a degenerative process in the muscle or to an accumulation of waste products. This point may perhaps be cleared up by further work.

In some diseased stocks these spots are found in almost every crawling bee; in others, apparently at the same stage of the disease, they are absent.

THE BLOOD.

The blood of the crawling bee is often scanty, though when such bees are warmed and fed they recover their normal complement of body fluid.

No qualitative cytological difference has been noted between the blood of healthy and crawling bees, though the number of cells per unit volume may be increased in the latter. This increase is probably entirely due to loss of plasma and cannot be regarded as a leucocytosis.

THE ALIMENTARY SYSTEM.

As regards the alimentary system, the investigation has but little to add to the observations of former workers. The disordered condition of the alimentary tract has attracted much attention in the search for a clue to the causation of Isle of Wight disease.

The facts may be briefly outlined.

In the majority of crawling bees the hind gut and small intestine are distended to the limit of their capacity with accumulated fæces, and the contents of the lower region of the chyle stomach may contain a large admixture of fæcal matter. The chyle stomach itself may present a rather wasted appearance, and its contents may be of an unusually deep purple colour. These changes are in all probability merely due to a reduction in the fluid contents of the organ.

In the vast majority of crawling bees no lesion is to be found in the alimentary wall either macroscopically or in stained sections. On two occasions only have signs of penetration of the wall by organisms (other than *Nosema*) been noted. In one case there was an infiltration of the wall near the insertion of the Malpighian tubules by a large filamentous bacillus; in the other case fungal hyphæ had invaded the epithelium of the lower portion of the chyle stomach. Such phenomena are to be regarded merely as terminal infections.

The flora of the alimentary tract of the normal bee has been carefully investigated and compared with that of bees crawling from the disease. Very little qualitative difference has been found between the two.

In Isle of Wight disease there is a colonisation of the chyle stomach by the intestinal organisms, and certain organisms, such as coliform bacilli and yeasts, are more frequent and abundant than in healthy bees. Certain streptococci, to be described elsewhere, have also a predilection for the alimentary tract of Isle of Wight bees.

MALPIGHIAN TUBULES.

In a proportion of crawling bees certain of the Malpighian tubules, when mounted in saline, may possess a bright yellow colour due to the presence of large amounts of the excretory pigment. In these coloured areas the excretory granules within the epithelium may be abnormally large and spicular. In fresh preparations the cells appear to be filled with large bacilli. A similar condition may arise in bees after a period of confinement.

NERVOUS SYSTEM.

The examination of the nervous system for pathological changes is as yet very incomplete. The observations of the writer have been mainly restricted to the thoracic ganglia. No changes have been noted in the posterior thoracic ganglionic

mass, and the few sections which have been examined of the anterior thoracic ganglia of sick and healthy bees show no alterations which cannot be accounted for as physiological variations due to senility.

DISCUSSION.

With these facts before us an attempt may be made to discuss the correlation between the action of the parasite, the pathological changes, and the symptomatology of the disease.

We have alluded to the two aspects of the primary effect of the parasite upon the host: the active injury produced by a parasite living upon the host fluids, with the added probability of a toxic action, and the passive obstruction of the respiratory system of the head and anterior thorax.

The pregnant parasites producing many, relatively large ova, the developing brood and the young adults must make considerable demands upon the host. It has been pointed out that the blood of crawling bees is often scanty, but it is improbable that this is in any significant degree directly due to the mites, but arises from the fact that fluid lost by excretion and transpiration is not replaced owing to the inability of the stricken bee to take or to obtain food. As many heavily infected bees continue to forage, though their tracheæ are bronzed and blackened by long sojourn of the mites, it would seem probable that nutritive sapping does not *per se* render the bee effete.

The same uncertainty surrounds the question of a toxic action. One member at least (*T. intectus*) of the genus to which the parasite belongs is known to be venomous, but the exact importance of this factor in the disease must, like the foregoing, remain for the present a matter of surmise.

The passive action of the parasites and their products in partially or completely blocking the infected tracheæ is a factor of which the importance is much more readily estimated.

It is obvious that any obstruction of the tracheal lumen must reduce the efficiency of the respiratory exchange of the organs supplied. In the vast majority of crawling bees the effective lumina of certain of the major tracheæ are obviously very much reduced, and in some all but obliterated. The organs supplied by such tracheæ must be reduced to an acute degree of oxygen starvation, and among the organs of which the respiratory supply is thus endangered are those of the head and the thoracic muscles of flight.

It is clear that the effects must vary from case to case :—

- (a) With the degree of the obstruction.
- (b) With the position of the obstruction.
- (c) According as to whether the obstruction is bilateral or unilateral.

The actual number of parasites distributed through the respiratory system is from

this point of view of secondary importance, a fact which may explain the apparent vigour of many heavily infected bees.

In order to obtain some idea of the effects actually arising from mechanical obstruction of the spiracles, a series of experiments were undertaken upon healthy bees. In these experiments melted paraffin wax was applied to the first spiracle of one or both sides of each bee in such a way as to give, on solidification of the wax, complete closure of the spiracular orifice without impairing the free play of the wings.

Bees treated in this way were maintained in boxes and were examined at intervals. In each experiment ten to twenty experimental bees were employed, and parallel controls were kept under the same conditions.

Upon closure of one spiracle the experimental bees at once lost the power of flight, but remained otherwise active in their movements, running quickly over the bench and beating the air with their wings. Upon the second and third days it was sometimes found that a proportion of the bees were capable of flight—which was, however, usually of very short duration. In these it is probable that the wax had become partially dislodged. The majority of the bees continued to crawl. After the lapse of several days these crawling bees became more sluggish in their movements, sometimes showing a tendency to drag their hind legs, and about the sixth to seventh day, bees were noted which showed a dislocation of the wings similar to that so common among bees crawling from Isle of Wight disease. About this time, too, some of the bees began to die: many were, however, maintained up to the beginning of the third week. During this period also a few of the control bees died, but the remainder retained the power of flight throughout.

At intervals experimental and control bees were killed for examination. Both in the “artificial crawlers” and in those control bees which had not been given opportunity to void their fæces on the wing, the hind gut was found distended with fæcal matter. At the end of the first week of experiment it was found that the thoracic musculature of the experimental bees showed, in many cases, atrophy of exactly the same type as had been found in infected bees. The degree of this atrophy and the number of fibres affected varied with the duration of the experiment. No such changes were noted in the control bees.

In those experiments in which the first spiracles of each side were closed with wax, the phenomena were different. As before, the power of flight was at once lost, but after twenty-four to forty-eight hours the bees had developed a reeling gait and appeared to be continually falling over their own heads. It was seldom that any survived the third day. No muscle atrophy was to be discovered, death having supervened too rapidly for the accomplishment of this change.

From these experiments it may be stated that:—

Through closure of the first spiracle of one side, a condition of crawling is induced which bears a close resemblance in its symptoms to Isle of Wight disease, and that,

further, the procedure may occasion atrophic muscle changes which are only known to occur in that disease. When the first pair of spiracles is closed, a state of complete incapacitation results, ending rapidly in death.

Though too close a parallel must not be drawn with the natural disease, these experiments are illuminating in that they give a basis to the view that the rôle of parasites in partially preventing thoracic respiration is of prime importance in the disease—possibly in itself capable of occasioning all the symptoms by which we are wont to diagnose the disease and the muscle atrophy so often associated with it.

The pathological syndrome of Isle of Wight disease is undoubtedly complex. Apart from the sapping of the host fluids and the probable injection of a venom, the mites may impair the mechanism of the bee either by destroying the respiratory supply of the individual organs or by cutting off that of the nerve centres which control and co-ordinate their activities. It is possible that the indirect effect through the nervous system, possessed as this is of a dual respiratory supply, is particularly acute when there is considerable bilateral obstruction of the tracheal system.

Through the combined influence of these factors the power of flight is lost, and a series of secondary conditions arise.

The faeces normally voided on the wing accumulate, thus increasing the difficulty of locomotion and compressing the abdominal air-sacs—another blow at the respiratory function. Intestinal pressure must hinder the excretory activities of the Malpighian tubules, and this excretory stasis, together with the absorption of toxins from the stagnant gut, must be reflected back upon the body of the insect.

As soon as the power of flight is lost death of the bee becomes imminent, for once it leaves the warmth and stores of the hive, unable to return, it perishes of cold and starvation. Should it elect to remain within the hive it is faced with a prospect of functional stagnation which cannot be indefinitely maintained. It would seem too that in the colder months sick stocks often perish *en masse* through inability to maintain the hive temperature.

It seems that in rare cases individual bees may recover from the attack upon being abandoned by the parasitic brood. Such cases are recognised by their bronzed and blackened tracheæ, which, however, contain no living mites. Bees in this condition have been found foraging for infected stocks.

CONCLUDING REMARKS.

It is somewhat remarkable that the macroscopic changes of the thoracic tracheæ and muscle have so long escaped observation in spite of the detailed examinations of several independent workers.

IMMS (1) held that "the disease is eminently one of the digestive system, and might be described as a condition of enlargement of the hind intestine," while

MALDEN (2) was of opinion that "the disease must be regarded as an infectious one which primarily affects the chyle stomach."

The latter states that in his investigations "no changes were discovered in the salivary glands, brain, fat body, *tracheæ*, *air-sacs*, Malpighian bodies, or honey stomach," but that "the chyle stomach in many cases showed marked changes in section." Of these changes in the chyle stomach the present investigation has seen little or nothing. Two exceptional cases have been noted where the epithelial lining had been definitely invaded.

MALDEN, as a result of his bacteriological work, suggested a "plague-like" bacillus, called by him *B. pestiformis apis*, as the cause of the malady. He, however, made the suggestion with some reserve, and later, when his work had been overshadowed by the "Nosema theory," considered that toxins produced by various species of bacteria played an important secondary rôle in the disease.

There is, however, an underlying truth in his summing up: "The actual cause of death is uncertain, but it is probably brought about by malnutrition, possibly combined with the absorption of a specific poison and of the products of decomposition in the colon, and probably aided to some extent by the imperfect oxygenation of the tissues, owing to the pressure exerted by the distended colon on the abdominal air-sacs."

Imperfect oxygenation, and possibly malnutrition and a toxic condition, are the main factors in the disease as we see them to-day.

In this paper it has been sought to outline those pathological facts which have so far come to light, and to relate them as reasonably as possible to the action of the parasite on the one hand and the symptoms of the disease on the other. Certain points have been merely touched on and others left in doubt, but it is hoped that further details will be soon forthcoming.

There are many to whom my thanks are due. To Dr J. RENNIE, who, as director of the research, has been an inspiring leader throughout, I tender my warmest thanks for help and advice and much personal kindness. My sincere thanks are due to Professor T. SHENNAN and the staff of the Pathology Department, Marischal College, Aberdeen, who have given me every facility for carrying out this work. It was in the Pathology Department that the parasite was independently discovered on the 11th May 1920, and the theory of its significance in the disease formulated. I wish also to express my gratitude to Professor J. ARTHUR THOMSON, Mr A. H. E. Wood, and the members of the Joint Committee of the University of Aberdeen and N. of Scotland College of Agriculture for all their interest and support.

REFERENCES TO LITERATURE.

- (1) IMMS, *J. Board of Agric.*, vol. xiv, No. 3, June 1907.
- (2) MALDEN, *Ibid.*, vol. xv, No. 11, February 1909.

DESCRIPTION OF FIGURES.

Fig. 1. Infected trachea showing typical changes. The black faecal deposit is arranged in t lines. ($\times 70$.)

Fig. 2. Fragment of wall of infected trachea, shown much enlarged. Granular deposits of faecal matter lie between the tracheal thickenings.

Fig. 3. Normal (*n*) and atrophied (*a*) fibres from the thoracic muscle of a crawling bee. Note the density, slenderness, and fractured ends of the atrophied fibres. ($\times 50$.)

Fig. 4. Fragment of an atrophied muscle fibre showing dense longitudinal columns of sarcosomes from between which a few fibrillar remnants (*f*) project. ($\times 500$.)

Fig. 5. A teased-out fragment of an atrophied muscle fibre (A) is contrasted with normal fibrillæ and sarcosomes (B). ($\times 600$.)

Fig. 6. Portion of atrophied muscle fibre showing blackened spots in its substance which follow the distribution of the tracheoles.

WHITE: PATHOLOGY OF ISLE OF WIGHT DISEASE.

1

2

5

4

3

6