XXIX.—ISLE OF WIGHT DISEASE IN HIVE BEES.

(1) The Etiology of the Disease. By **John Rennie**, D.Sc.; **Philip Bruce White**, B.Sc.; and **Elsie J. Harvey**. (With One Plate.)

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Introductory.

Isle of Wight Bee Disease has been known in this country certainly since 1904, when it was first recognised in the island from which it derives its popular name. According to Imms it was probably present in Derbyshire in 1902, and was also known in Cornwall and other districts in 1904. Prior to these dates periodic losses of bees of a serious character are on record, dating as far back as the middle of the eighteenth century. Bullamore and Malden (1912) have summarised fully these outbreaks in historical series in their report in *Journal of Board of Agriculture*, Supplement 8, xix. From a study of the records which they have brought together and from personal inquiries which we have made at various bee-keepers of wide experience, it would appear that none of these earlier outbreaks attained the general distribution throughout the country which we know in Isle of Wight Disease at the present date, nor did any of them remain established over such an extensive period of years as that which has continued without interruption from 1902 until the present time.

Another striking characteristic, and one which has an important bearing in any investigation which seeks to trace the source of this malady, is the fact that no disease of such a permanent and extensive nature has so far been recognised unmistakably outside the British Isles. All the facts we are at present aware of suggest its definitely localised and insular character.

Since 1907 investigations into the cause of this disease have been carried out by a series of workers who have from time to time reported upon the subject: Imms (1907), Malden (1909), Graham Smith, Fantham, and others (1912 and 1913) in England; and Anderson (1916), Anderson and Rennie (1916), Rennie and Harvey, No. 1 (1919) and No. 2 (1919), in Scotland.

The net result of these investigations has been that the English workers (Graham Smith *et alii*, 1912 and 1913) put forward as the causal organism the protozoan, *Nosema apis*. It is due to Anderson amongst the Scottish workers to state that he was the first to call this conclusion in question, and we think that the later work referred to above (Anderson and Rennie, and Rennie and Harvey, 1919) has succeeded in establishing (1) that Isle of Wight disease and *Nosema* infection are not coincident, and (2) that there exists a distinct disease due to *Nosema apis*, which

exhibits, however, totally different external symptoms and a distinct pathology in the individual bee. Collateral work by G. F. WHITE (1918) in America supports this latter conclusion. The problem of the cause of Isle of Wight disease until now has thus been left unsolved.

CHARACTERISTICS OF THE DISEASE AS HITHERTO OBSERVED IN THE COLONY AS A WHOLE.

The diagnosis of Isle of Wight disease from "symptoms" has always been a more or less unsatisfactory procedure. Hitherto the presence of the disease in a colony has not been recognised until infection has been well advanced in a high proportion of the bees. At this stage of disability, the most usual features recognisable by the bee-keeper are inability to fly, accompanied sometimes with imperfect folding of the wings. In fine weather a proportion of the affected bees may leave the hive and crawl around, climbing grasses, etc. Later, in the cooler part of the day, they commonly collect in small clusters. Such bees are lost to the colony, since they do not return to the hive, and in any case are useless as workers at this stage. Sometimes large numbers come out and loiter on the alighting board in the sun, returning to the hive when the sun has gone. Associated with the incapacity for flight there is usually a congested condition of the colon. In certain circumstances dysentery may be present as a complication. Most of these symptoms may be present in other disorders of a more temporary kind, and we have been accustomed to regard as true Isle of Wight disease only those cases where such visible conditions, once commenced, continued in the stock, affecting succeeding broods of bees. There is a continuous mortality from the disease. BULLAMORE and MALDEN regard no single symptom as characteristic, and state that "the only essential feature is the death of large numbers of bees."

The association of the causative organism now to be considered will henceforth afford an exact means of diagnosing the disease, which we suggest should now be designated Acarine disease.

DISCOVERY OF THE CAUSAL AGENT OF ISLE OF WIGHT DISEASE.

The present and following papers announce the discovery of a parasitic organism invading the respiratory system of the adult bee, which after exhaustive investigation we now bring forward as the causal agent in this disease. This parasite is a hitherto undescribed mite, identified by one of us (J. R.) as belonging to the genus *Tarsonemus*. It was first observed by one of us (E. H.) in December 1919, when a single example was found in a portion of trachea present in a preparation, permanently preserved, of the thoracic glands (fig. 1).* It was significant of the fuller knowledge of the disease, soon to be attained, that the bee in which it occurred was

* This find was followed up at the time by a systematic search for mites in hives, upon frames, bees, etc., which resulted in the finding of no fewer than five different species in definite association with bees, dead and alive. (J. R.)

"healthy" in the sense that it belonged to a colony which had no history of disease and was regarded as free from such. In the following May, Mr WHITE made the further and independent discovery that mites in all stages of development occurred in certain of the major thoracic tracheæ of "crawling" bees. In reporting this discovery he stated that he had found this condition in at least 150 sick bees, representative of several diseased stocks, and also that he had failed to find mites in 95 per cent. of apparently healthy bees.* On this occasion he expressed the view that the parasites seen by him bore a definite etiological relationship to the disease.

That this discovery was one of very great significance was obvious, and the senior author immediately proceeded to its further verification. The first stock of bees examined was one in a highly prosperous condition. The bees were occupying twenty frames in the latter half of May; they were working splendidly and a source of great satisfaction to the owner. Twelve flying bees were captured as they entered the hive. They were taken to the laboratory and the first bee opened was found to contain the parasite in limited numbers. All the twelve were examined, and of the remaining eleven, one other was found also to be harbouring the mite in question.

These facts presented, as in a nutshell, the problem confronting us. It was evident that the distribution was not limited to those bees or stocks hitherto regarded as "sick," and as the result of the extensive investigations which followed and which are now recorded, we are able to announce that notwithstanding variations in the course of the disease in different stocks, we regard it as established that there is an invariable association of the parasite with diseased stocks, and that there is a definite pathology in relation to infection in the individual bee.

INCIDENCE OF INFECTION WITHIN THE COLONY.

Brood.—Except in one doubtful case we have not found infection with *Tarsonemus* in brood of any stage, nor has it been found amongst the very youngest of adult bees.

Workers.—Amongst workers the infection is most marked in the older bee, and in any case it has not been found except in incipient stages amongst nurse bees whose adult life has been brief.

Drones.—We have found that drones of affected stocks suffer equally along with the workers.

Queens.—The important and interesting question of the relation of the queen in this connection has been investigated by one of us (J. R.) in a limited number of cases. Of fifteen queens examined of stocks known to be affected with this disease, ten were found to be free, whilst the remaining five harboured this organism. That queens may undergo infection is specially interesting, in view of the familiar fact that the

* The systematic examination which followed showed the number of apparently healthy stocks harbouring the parasite to be as high as 36 per cent. (J. R.)

queen of an infected Isle of Wight diseased stock survives usually until the colony is extinct. That her survival is in a measure due to the fact that she remains within the hive, is supported by the knowledge we already possess that workers affected with the disease may live for months after they are incapable of flight, and are thus useless to the colony.

Region of Infection within the Bee.

The mite, *Tarsonemus*, occupies a very restricted region in that part of the tracheal system which has its origin at the anterior thoracic spiracle. In a well-established case of infection it will be found that, extending inward from this spiracle on either side indifferently, parasites in all stages of development may be present in any part of this portion of the respiratory system, whilst the ill effects of their presence may be seen not only in the region of occupation, but in the muscular tissue to which these extend. It is not an infrequent occurrence in advanced cases of the disease for these wider tracheæ to be occupied with mites in closely packed formation. All stages of development occur; *e.g.* ova, larvæ, nymphs, and adults may be found together (figs. 2 and 5). In the smaller branches frequently these are occupied as far as their diameter will permit, when a single individual may be found practically blocking the tube, and sometimes a linear succession of individuals may be seen in such a position.

The facts which have led us to the conclusion that the occurrence of this organism in the position indicated is to be regarded as causally related to this disease, are to be found not alone in the presence of *Tarsonemus* in the respiratory system of the bee. There is the universal coincidence of its occurrence in diseased bees. Further, we have been able to trace the development of the disease within bee colonies from the earliest stages of infection to its complete manifestation in crawling and other definite symptoms. We have observed that the total effects resulting from its development, feeding upon the bee and life generally within it, renders it useless as a working unit, disorganises the social system and eventually shortens the bee's life. Further, these vital effects are accompanied by visible pathological conditions in the tissues. The most obvious of these is a browning or blackening and thickening of the tracheal wall (figs. 6 and 7). The thickened tracheæ become progressively hardened and brittle in texture, and certain muscle fibres become atrophied. This latter aspect of the problem is the subject of separate detailed treatment in the paper which follows by Mr White.

These pathological appearances in an infected bee may be present on both sides of the anterior tracheal system. What we have described is the condition in a well-established instance where breeding has been in progress for some time, but as has been mentioned early stages of infection have been frequently witnessed in which the number of parasites present have been observed to be as few as a single mite and no abnormal condition apparent.

Cumulative Evidence that Tarsonemus is Causally Related to this Disease.

In the course of our investigation we have searched over three thousand individual bees representing 250 separate stocks scattered throughout Great Britain. These examinations covered over 110 stocks reported to us by reliable bee-keepers or certified by ourselves as suffering from Isle of Wight disease. The parasite was present in every one of those stocks. A striking result of this part of the inquiry, which involved the examination individually of 700 bees at least, was the discovery that in every case showing the familiar symptoms of Isle of Wight disease the parasite was present. No exception has been found. There is apparently an invariable and clear association of this organism with all bees suffering from Isle of Wight disease.

These examinations applied not only to bees obtained during 1920, but included samples representative of all seasons of the year, and dating back as far as September 1916. These observations relating to the earlier dated bees were made upon diseased bees which had been preserved by Rennie and Harvey on the dates mentioned (see p. 752).

Reputed Healthy Stocks.

Amongst the 250 stocks above mentioned there were about 50 which were reported to us as healthy and in which we found the parasite *Tarsonemus* to be present. That is to say, of 140 stocks believed by the owners to be healthy, 50, or nearly 36 per cent., harboured this parasite. Concurrent with such discoveries we ascertained by direct examination ourselves of flying bees (1) which were members of colonies in which the disease was definitely established and (2) which were taken from colonies believed to be healthy and showing no indications otherwise, that amongst these were to be found considerable numbers harbouring the parasite. This was further complicated by the fact that in those infected flying bees certain of those pathological conditions—*e.g.* the blackening and hardening of the tracheal tubes—were very marked. As an example it may be quoted that this condition was found in bees entering the hive carrying pollen or nectar, both belonging to stocks in which crawling and other symptoms were well established, and also to those reputed healthy stocks.

A Particular Case.

As an illustration of this aspect of the disease we may quote the following :—

At the door of the hive of a sick stock showing habitual crawling in fine weather and steadily declining from the disease, we captured as they alighted 27 foraging bees in the course of a single afternoon. *Tarsonemus* was found in every one of these bees, all stages of development being represented. In a number of the cases, soiling and destruction of the tracheal tubes was very marked, quite as bad as anything we have observed in bees crawling from the disease.

In several other stocks showing the disease in an advanced stage, every bee taken over a period extending to weeks, including drones, flying and crawling workers, was found on examination to be infected. The flying workers were frequently more heavily parasitised than were the bees of the same stock which were unable to fly.

These facts have shown us that "crawling" is only one of the phases of the disease and that it cannot be dependent exclusively upon the intensity of the infection, as shown by numbers of parasites; it may be incidental, in part at any rate, to a critical position of certain of the mites, so that oxygen starvation of groups of fibres of the muscles of flight results. Evidence in support of this is brought forward in Mr WHITE's paper which follows, where also other possible factors are considered.

The following Table summarises the typical results of our examinations of bees for the presence of *Tarsonemus*. H. indicates that the stock from which the bees were obtained was showing no indications of disease, and was described by the owners as *Healthy*.

S. stands for *Sick* stock, and invariably indicates that the ordinary symptoms of Isle of Wight disease known to bee-keepers were present. The figures quoted under the heading *Tarsonemus* indicate the number of bees in which *Tarsonemus* was found.

TABLE I.

Ref. No.	Date.	Locality.	Condition of Stock.	Numbers Examined.	Tarsonemus.	Remarks.
1	22 May	Stonehaven	H.	12	2	Developed the disease subsequently.
6	26 „	Edinburgh	H.	20	0	
7		Rubislaw	H.	2	1	No. 44 in record.
10	14 „	Aberdeen	S.	13	11	Requeened. Reduced to 1 frame, 30th October.
18	22 „	Kintore	S.	12	12	Crawling marked.
22	27 „	Northumberland	S.	5	5	Stock died out.
37	4 June	Copford	H.	31	0	Infected artificially later. Became queenless. Died out subsequently.
48	6 „	Shipton	H.	3	0	
57	8 „	Cults	H.	12	2	Died out from disease and queenlessness.
55	9 „	Banchory	S.	3	3	
62	14 „	Kirriemuir	H.	9	0	
70	17 „	Huntly	H.	5	5	Early stage of infection. Disease very prevalent in immediate neighbourhood and robbing noticeable.
74	17 „	Glasgow	H.	34	3	No disease signs so far (30/12/20).
75	18 „	Drumlithie	S.	6	6	A stock dwindling from the disease. Minor tracheæ were blocked.
78	20 „	Cawdor	H.	30	0	(4 stocks.)
82	20 „	Aboyne	H.	6	6	Last stock of 6,—5 of which have already died of disease.
86	22 „	Laurencekirk	S.	20	17	An ordinary case.
92	21 „	Dingwall	H.	6	6	Stock much reduced and in bad condition, but no crawling had been seen.
94	21 „	Aberdour, Fife	H.	20	0	No record of disease in this apiary.

TABLE I.—*continued.*

Ref. No.	Date.	Locality.	Condition of Stock.	Numbers Examined.	Tarsonemus.	Remarks.
97*a*	26 June	Bristol	S.	6	6	Heavily infected from a cast which up till a few days previously appear healthy. Crawling sudden and extensive.
99	28 ,,	Banchory	S.	6	6	An ordinary advanced case.
100	26 ,,	Rubislaw	H.	30	0	See record, No. 60.
102	29 ,,	Edinburgh	S.	13	10	Has shown none of usual signs of Isle of Wight disease, but has dwindled since spring. Queen found free of infection.
103	29 ,,	Northumberland	S.	12	12	
105	30 ,,	Turriff	S.?	7	5	Intermittent signs of disease shown. These bees not known to have the disease.
108	2 July	Ellon	S.	8	6	Reported 17th September:—"Now very healthy and closely packed down on ten standard frames. Gave one and a half crates shallow frames surplus."
111	2 ,,	Ellon	H.	13	0	Same apiary as No. 108.
112	2 ,,	Ellon	H.	7	1	Strong stock all along, swarmed twice. Gave two and a half crates shallow frames surplus. Never showed any signs of disease.
115	3 ,,	Port Elphinstone	H.	30	0	See record, Re No. 1.
116	3 ,,	Warrilow, Sussex	H.	7	0	
118	5 ,,	Cluny	S.	6	6	Intermittent disease history from previous September. Died out about this date.
119	5 ,,	Nairn	H.	5	0	
120	5 ,,	Cults	S.	13	13	An ordinary advanced case.
124	3 ,,	Inverurie	S.	6	6	Do.
126	8 ,,	Glasgow	H.	30	3	There have been no signs of the disease.
128	8 ,,	Keig	S.	6	6	
299	8 ,,	Witney, Oxon.	S.	6	6	Slight crawling only, at this stock.
129	8 ,,	Inverness	S.	6	6	Crawling observed for months previously. Infection very heavy.
134	8 ,,	Rubislaw	H.	30	5	Died subsequently of the disease.
140	9 ,,	Rubislaw	S.	19	19	From 4th June onward this stock by repeated examination (4 times in one month) showed practically every bee infected. It appeared to be doing moderately well. Mass crawling was never observed, but the stock became queenless and died out before the end of July.
141	10 ,,	Inverurie	S.	10	10	
146	11 ,,	Boat of Garten	H.	3	3	Stock believed healthy; from apiary where other four stocks had died from the disease.
148	13 ,,	Park	S.	4	4	Stock crawling. Drones were included in this examination.
149	14 ,,	Boat of Garten	H.	14	10	Not known to be sick, but some bees seem to have dislocated wings.
165	15 ,,	Coull	S.	5	5	Yielded a good top swarm, and crawling appeared subsequently. Tracheæ were closely packed with mites in all cases.

TABLE I.—*continued.*

Ref. No.	Date.	Locality.	Condition of Stock.	Numbers Examined.	Tarsonemus.	Remarks.
166	15 July	Rubislaw	H.	20	3	Infection slight. This stock died out in the course of the summer.
167	16 ,,	Rubislaw	H.	20	0	This stock later developed an infection. It is still in existence.
168	16 ,,	Dingwall	H.	8	8	
169	16 ,,	Italy	H.	22	0	Two samples. Attendants accompanying queens. Both lots infected with Nosema.
170	16 ,,	Bandon (Ireland)	H.	26	3	
153	11 ,,	Kinaldie		7	7	Roof stock. All these bees were crawlers.
154	11 ,,	Kinaldie		30	8	Same stock. All these bees were fliers.
175	18 ,,	Nairn	H.	11	0	
180	19 ,,	Rubislaw	H.	30	0	Standing in an apiary beside diseased stocks. It has since become infected, but there are no external indications.
190	21 ,,	Dorridge	S.	6	4	A strong stock of Italian hybrids with a 1919 queen.
191	21 ,,	Dorridge	H.	28	0	An Italian nucleus with a 1920 queen.
195	29 ,,	Fyvie	S.	7	7	Stock reported crawling for weeks previously.
199	6 Augt.	Norfolk	H.	12	3	Stock remains apparently healthy.
201	14 ,,	Norfolk	H.	3	2	A light infection.
202	14 ,,	Old Meldrum	S.	6	6	Died out from the disease.
216	18 ,,	Dingwall	S.	10	10	A bad case.
217	18 ,,	Birkenhead	H.	10	0	
219	20 ,,	Birkenhead	S.	10	10	From 2 stocks, examined separately; both infected.
222	20 ,,	Lucerne	H.	29	0	From 3 stocks.
230	26 ,,	Lugano	H.	44	0	From 2 stocks.
235	28 ,,	Wick	S.	10	10	A suspected stock. A definite case of infection in an area only recently affected by the disease.
239	30 ,,	Washington, U.S.A.	H.	37	0	
243	1 Sept.	Beauly	H.	9	0	
263	22 ,,	Bucksburn	S.	10	5	
264	23 ,,	Washington, U.S.A.	H.	23	0	
268	27 ,,	Eddieston	H.	21	0	Maintained in an area free from disease.
269	25 ,,	Weston-s.-Mare	S.	11	11	Two stocks; examined separately, both infected.
273	28 ,,	Austria	H.	20	0	
276	28 ,,	Stoke-under-Ham	H.	4	0	
277	28 ,,	Stoke-under-Ham	S.	4	4	Not known by owner to be sick, but crawling bees found.
283	17 Oct.	New Machar	H.	9	0	
287	17 ,,	New Machar	S.	10	10	Tracheæ badly blackened.
290	28 ,,	Rubislaw	H.	25	4	In infected apiary; no external signs of disease.
302	30 ,,	Bungay	H.	30	27	Some crawling about middle of month. Put up for winter.
303	30 ,,	Bungay	H.	28	23	No sign of crawling. Put up for winter.

TABLE II.

The following illustrates the progress of infection as observed in the periodic examination of 4 stocks of the same apiary :—

Date.	Locality.	Condition of Stock.	Number Examined.	Tarsonemus.
20 May	Fintry No. 1	H.	6	0 } Flying bees.
1 July	,,	H.	3	1 }
3 Aug.	,,	S.	7	7 Crawling bees.
,,	,,	S.	5	4 Fliers.
20 May	Fintry No. 2	H.	5	0 Fliers.
8 June	,,	H.	8	2 Fliers.
3 Aug.	,,	S.	4	4 Crawlers.
20 May	Fintry No. 3	H.	24	0 Fliers.
8 June	,,	H.	16	1 Fliers.
1 July	,,	S.	10	3 (8 fliers, 2 crawlers).
3 Aug.	,,	S.	22	22 Crawlers.
20 May	Fintry No. 4	H.	15	0 Fliers.
3 Aug.	,,	S.	6	6 Crawlers.

The data set forth in the foregoing Tables are thoroughly representative of our results as a whole. Examination of the figures quoted will show :—

1. That in every case of a "Sick" stock, *Tarsonemus* was present in the stock, and in a high proportion of cases it was found in every bee examined.
2. That in the majority of stocks marked "Healthy," *Tarsonemus* was not found.
3. That in a proportion (36 per cent.) of supposed "healthy" stocks, *Tarsonemus* was found. Of these within the period of observation (five months at most) a proportion eventually developed the usual symptoms, and died out from the disease. A proportion died out without having shown the symptom of "mass crawling," and a few remain apparently healthy.

Regarding the admittedly diseased stocks in which *Tarsonemus* has been found, we deem further illustration unnecessary. Concerning those other cases, some of which may appear to present difficulties, we consider it important that details should be submitted, and of these we now quote typical examples.

For ease of comparison the main facts regarding each are summarised at the end of the series.

Stock Records.

R. No. 1.—This stock at the end of May was covering fully twenty frames and was in very good condition. On the 22nd of this month twelve bees taken entering the hive were examined for the presence of *Tarsonemus*. Two of the twelve were found affected at the initial stage. The parasites were few and the tracheæ were perfectly clean. As stated, there were no signs of disease. About a month afterwards the stock, which meantime had worked well and shown no signs of disease, was again examined. On this occasion fifty-two bees were searched, and of these forty-three contained the parasite. In most cases the tracheæ were heavily infected but the tubes were comparatively clean. A further sample was obtained upon the 6th July, and at this time twenty-five bees out of a total of twenty-eight taken were infected. It should be stated that these bees were taken at random by shaking off a frame into a box placed below. A number of these showed a bronzing of the tubes, especially at the forks. By this time the bees were showing some listlessness and not working so well. The owner made an artificial swarm, removing the old queen and supplying the main stock with a virgin Italian queen. Twenty-three pounds of drained honey were obtained at this time. The two stocks were subsequently placed side by side. On 21st July, in a sample of thirty-five bees, twenty-eight contained the parasite. About the third week of August, after a period of cold weather, crawling became evident in both stock and swarm. About the end of August a sample of twenty-eight bees was supplied from the parent stock, and of these twenty-six were badly parasitised. Both stocks continued to crawl in large numbers, and as robbing by other bees was going on, the owner destroyed them about the end of September.

No. 44.—This stock was obtained upon 11th April from an apiary which has been in existence for many years, and in which Isle of Wight disease has never been known. It was placed on the date mentioned in a new hive upon its own frames in an experimental apiary in which there were stocks suffering from Isle of Wight disease. The stock progressed normally throughout the summer and by the middle of June the bees were working in a super. On the 26th May an examination made on two bees taken from the stock showed one to be infected with *Tarsonemus*. Two days later one out of eight was found similarly affected. Subsequently, periodic examinations as follows were made on the dates mentioned.

Date.	No. Examined.	Result.	
		Infected.	Not Infected.
12 June	31	3	17
22 July	10	5	5
31 Aug.	33	32	1

Although during the whole of this period crawling symptoms never were in evidence and the bees appeared to be working normally, the numbers did not increase, nor were stores accumulated. In the later part of July and August the decline in numbers was rapid, and crawling developed towards the end. The stock was robbed actively and became extinct towards the middle of September. The apiary from which the stock was obtained remains clear of the disease.

No. 61.—On the 20th May a stock of Italian bees infected with *Nosema apis* was obtained from Glassel. It built up rapidly in the course of the following weeks and by the middle of July the bees were covering fifteen frames.

On the 30th May four bees were taken entering the doorway; these were active and inclined to sting; two of them were found infected with *Tarsonemus* and two were clear. On 7th July thirty bees were taken and of these five were infected. Again, on 14th July twenty bees were taken; ten were infected with *Tarsonemus* and ten were free. Up to this date there were no external signs of disease in the stock. The season being poor there were no surplus stores, but, as already stated, the stock was strong in bees.

On 30th August thirty-four bees were examined and of these twenty-seven were found infected, and about this date crawling amongst the bees was observed for the first time. By this date the stock was reduced to about seven frames of bees, and robbing by other bees was being persistently attempted. It eventually died out at the end of September.

No. A. Ch.—In the month of May a stock was obtained from an apiary in Dyce, where there had been no disease for many years. It was a swarm of the previous year, and after transference was isolated from other stocks. On 17th July six bees taken from the stock, which appeared perfectly healthy, were all found infected, but to a slight degree. The infection appeared to be recent. A fortnight later crawling became evident in the stock, and six crawling bees supplied were found to be all infected and more extensively than in the previous sample. By the 1st September the stock had declined to about four frames of bees. There were no stores. Nine flying bees were taken; these were all found infected and having their tracheal tubes much blackened. The owner at this date destroyed the bees.

No. Glasgow, I.—On the 8th July a sample of thirty bees was taken from a stock of Dutch bees obtained from West of Scotland College of Agriculture on 22nd March. The stock at the time the sample was taken appeared perfectly healthy and was doing well. Of the thirty bees, three were found infected with *Tarsonemus*. The stock swarmed, and the swarm for a time appeared strong and healthy. At the beginning of August the parent stock covered eight frames, with stores and brood on six. No loiterers or crawlers have been observed at either the parent or swarm stocks. Thirty-five bees of the parent stock were examined at this date, and of these three were found infected with *Tarsonemus*.

It remained so at the end of October, although at this date it showed a proportion of infected bees of about 27 per cent. (33 bees, 9 infected).

At the present time (30th October) this stock is strong in numbers, without visible signs of disease. The owner has united it with one half of the swarm.

No. Glasgow, P.—This stock at the beginning of June was strong and working well. The bees covered ten frames, six of which were very well filled with brood. Brood was also present upon the other four and there were plenty of stores. The stock swarmed at this date but the swarm, secured with difficulty and with a loss of bees, was returned to the parent stock. On 17th June a sample of thirty-four bees was examined, and of these three were found infected with *Tarsonemus*. There were no external signs of disease and matters appeared normal with the stock. During the next three months the owner paid little attention to it, and in September the bees were reduced to four frames with brood and were without food. None of the usual signs of Isle of Wight disease had ever been seen about the stock, which was now being fed. A sample of twenty-nine bees was taken on 7th September, and of these two only were infected. This shows a slight decrease as judged by the samples. A later sample supplied at the end of October, however, showed an increase in proportion of infected bees. The stock as a whole does not appear affected by the presence of the parasite, but it is not particularly strong in numbers.

No. 62.—Early in May of this year a nucleus of three frames of bees with queen and brood was obtained.

On 27th May fifteen bees, and again on 3rd June four bees, were examined for *Tarsonemus*, with a negative result. The bees were standing in an infected apiary and at this time were working well and rapidly increasing in numbers.

On 14th June twenty-five bees were examined, and of these twenty-two were found clear of the parasite; of the remaining three, two contained several parasites and one a single adult female. The bees multiplied rapidly and swarmed twice in the course of the summer.

On 15th September thirty-three bees were examined, and of these nineteen proved infected.

At this date there were no signs of disease as far as behaviour of the bees was concerned. The numbers were well maintained and the stores sufficient.

At the end of October, forty bees were taken and all except three were found infected. The pathological features were not marked. The stock is apparently in a strong condition as regards numbers at this date.

No. 60.—On 20th May a small lot of bees covering three frames was obtained, which on examination was found to be harbouring the parasite *Nosema apis*. Apart from this there were no external signs of disease about the stock, and it built up moderately well. By 2nd August the bees covered over nine frames, with brood upon seven. The season was poor and stores were short.

At the middle of September the stock appeared well, apart from a shortage of stores. Further at this date *Nosema* was still present.

In the course of the summer, bees of this stock were periodically examined between 17th June and 27th September for the presence of *Tarsonemus*. In all one hundred and fifty-six bees were tested and on only two occasions, namely upon 20th August and 27th September, was *Tarsonemus* found. In each case only one bee was found infected. This and a preceding stock (No. 61) were obtained from the same apiary and have stood together, but a little way apart from the other stocks, during the period of observation. Several of these other stocks in the same apiary were at this time suffering from Isle of Wight disease. At the end of October a sample of thirty-five bees was taken off the frames, and all were found free from *Tarsonemus*.

W. No. 2.—This stock, on 4th August, headed by a young queen, appeared normal and in good condition. There were no visible grounds for suspecting infection. Of twelve bees taken at this date, three were found harbouring *Tarsonemus*.

On 6th September the bees were covering most of the frames, and there was a good amount of sealed brood and eggs in the inner frames. There were no signs of disease. At 20th September one crate of sections honey was obtained and about 20 lbs. of stores were left in the hive. At this date fourteen bees were taken at random, and of these twelve showed infection with *Tarsonemus* and two were clear.

At the end of October, of twenty-eight bees supplied, twenty-three were found infected.

No. 24.—This was a nucleus of five frames of Italian bees obtained upon 11th June.

Twenty-two bees were examined at this date and these were found to be free from *Tarsonemus* infection. A fortnight later thirty-two bees were examined and one bee only was found containing this parasite; everything appeared normal with the stock. Throughout the summer the stock prospered only moderately well. In the first week of September twenty-three bees were taken at random, and of these one only contained *Tarsonemus*. The stock has yielded no surplus stores and there has been no indications of disease. *Nosema apis* is not present in the stock and the apparent weakness cannot be attributed to it or to *Tarsonemus*.

Twenty-two bees were examined, and all found negative, on 29th October 1920. It would, therefore, appear that although *Tarsonemus* was present in the stock as early as the 25th June, there was no apparent increase in the incidence of infection as late as the end of October.

Re. No. 1.—This stock was brought to Aberdeenshire in the month of June from Caithness-shire. The bees, headed by a 1919 queen, were bred in this district in an apiary which had existed for many years and has had no experience of the disease. Thirty bees were examined on 3rd July and all were found free from *Tarsonemus* infection. The bees were placed in an area which has not been free

from Isle of Wight disease for a long time. On the 9th September the owner reported "so far the bees have done well and the stock is strong, but I have made no attempt to take honey; indeed, I have been feeding a little recently just to keep the queen breeding so as to supply young bees for winter."

At this date a second sample, consisting of twenty-two bees, was supplied, and of these, one bee was found harbouring *Tarsonemus*. The infection was localised just inside the spiracle of one side, and was limited to one adult and a few ova.

It is practically certain that infection of the stock in this case was effected within three months and probably not much earlier. In other words the stock stood in a highly infectious area for over two months without contracting the disease.

At the end of October, the owner reported the stock as "specially strong." A sample of six bees was received, and of these, one was found harbouring *Tarsonemus*, the other five being free.

Re. No. 2.—A second stock of similar origin, and with queen of same age, was obtained and placed alongside No. 1, just described, upon the 9th July. Exactly two months afterwards a sample of fifteen bees was taken and found free from infection. The stock is strong and is receiving similar treatment to the other.

Of a sample of twenty-one bees of this stock examined at the end of October, twenty were free from infection and one showed an initial infection, consisting of a few mites near the spiracle on one side. The owner reported it as "lively, and taking in pollen. There have been no signs of crawling about this hive. There is plenty disease in the neighbourhood."

R. No. 2.—Early in August a presumed healthy stock of bees was placed alongside two stocks both at the crawling stage of Isle of Wight disease. At the end of August thirteen bees were taken from amongst the foragers as they entered the hive. Ten were clear of parasites and the remaining three were affected, all of them slightly. Two of the infected cases showed only one or two adults and a few ova just within the spiracle of one side. Infection had evidently taken place during the period the stock was upon this site and not before. Upon advice given, the stock was removed at the end of August some distance from the others referred to above. At the end of October the owner reported: "It has filled up fairly well on the heather, is very lively and seems all right. To-day they are gathering in pollen and I send you a sample from those that were flying out and in."

The sample contained thirty bees, and of these twenty-nine were infected with *Tarsonemus*. There was a fair amount of bronzing of the tracheæ, all stages of development were present, and in a number of cases the mites were densely packed in the outer tubes.

W. No. 3.—This stock, requeened in the middle of July, was normal and in good condition on 14th August, when, out of a small sample of three bees, two were found to be infected. On the 6th September the stock was examined and found to have bees covering nearly ten frames, with plenty brood and eggs. This stock has

yielded four crates of sections and had fifteen pounds of stores left in the hive. The owner writes, "I am pleased to state there are no signs of any trouble." Seven bees were supplied from the stock, and of these five were harbouring *Tarsonemus* at the same date.

About the middle of October a slight amount of crawling was observed in this stock, but weather conditions have prevented further observations. At the end of October a sample of bees supplied was found heavily infected. The stock continues under observation.

Notes on Stock Records.

R. No. 1.—In about six weeks after the infection was first discovered, but not until the incidence of infection had risen to over 89 per cent., did visible signs of the presence of the disease appear.

No. 44.—This stock certainly developed an infection of *Tarsonemus* within the period of 11th April to 26th May, *i.e.* about six weeks. The examinations showed a rapid spread of *Tarsonemus* within the colony, so that in a little over four months from the arrival of the stock the incidence of infection was 97 per cent. And yet crawling was never in evidence until near the end.

No. 61.—This stock shows a striking parallel to the previous. Within the three months from 30th May to 30th August, the infection rose to about 80 per cent., and only now did crawling symptoms appear, although meantime the stock had visibly declined in numbers.

No. A. Ch.—Examination of bees from the original apiary in September, which were showing suspicious signs, showed that they too were infected with *Tarsonemus.* It appears probable that this stock was infected before leaving the original apiary, and from the fact that on 17th July the percentage of infection was so very high it would appear that the distribution of *Tarsonemus* was well established, though probably of recent origin.

Glasgow, I.—This stock was known to have a definite infection on the 8th July, which rose at the end of October to 27 per cent., without disease signs appearing. This is a case in which the spread of the disease within the stock is progressing with relative slowness.

Glasgow, P.—This is a similar case to the foregoing in that the spread of infection has been slow, and in which the ordinary disease symptoms have never appeared.

No. 62.—Nearly six weeks in an infected apiary elapsed before this clean stock was found to be harbouring *Tarsonemus.* After four and a half months from the time the presence of *Tarsonemus* was first discovered the incidence had increased to over 92 per cent., and no disease symptoms have ever been seen and the stock appears in a prosperous condition.

No. 60.—This is a stock which, although found to have *Tarsonemus* present in August, appears to have lost the infection.

W. No. 2.—*Tarsonemus* has been known to be present in this stock since the beginning of August. It is now wintering, and no signs of disease have ever been observed.

No. 24.—Infection from near the end of June to the beginning of September and not found later. Stock has not prospered.

Re. No. 1.—Slow progress of infection. No signs of disease; stock wintering.

Re. No. 2.—Infection took place within three months, and the progress very slow.

R. No. 2.—Proximity to two heavily infected stocks is a feature of this case. Infection probable within a month, rose in two months about 97 per cent., and the stock has dwindled to very small dimensions.

W. No. 3.—Known to be infected for two months before any suspicious signs appeared, and these only slight in character. The stock has done well, but its possible survival till spring is doubtful.

TABLE III.

Date.	Locality.	Condition. of Stock.	Numbers Examined.	Tarso-nemus.	Remarks.
24 Sept. 1916	Aberdeen	S.	5	4	Crawling bees.
24 Apr. 1917	Rubislaw	S.	1	1	Development stages of parasite.
26 ,, 1917	Glassel	S.	9	8	Stock crawling.
5 July 1917	Aberdeen	S.	1	1	All stages of development of parasite.
1 Feb. 1918	Stoneywood	S.	3	3	,, ,,
18 July 1918	Glassel	S.	1	1	,, ,,
11 Jan. 1919	Aberdeen	S.	7	7	,, ,,
8 Dec. 1919	Aberdeen	S.	3	3	Stock crawling.

The bees referred to in the foregoing table were all obtained from stocks recognised as suffering from the disease. The bees had been preserved on the dates mentioned. The results confirm the presence of *Tarsonemus* in diseased bees over the four years 1916–20, and also make clear the important fact borne out by the 1920 examinations that the breeding of *Tarsonemus* goes on throughout the whole year.

GENERAL CONSIDERATIONS.

For a sound appreciation of the foregoing records, and particularly of their diversity in detail, it is necessary that the various factors likely to be present affecting the course and culmination of the epizootic within the bee colony should be clearly set forth.

A colony of bees consists normally of a population which particularly in summer is undergoing a continuous and relatively rapid change, both as regards its constituent members and also as regards the total numbers. Daily during the working season there is both a steady mortality and a steady increase. This latter depends

upon the age and fertility of the queen. Also, for normal prosperity, there must be maintained a definite proportion of nurse bees and foragers.

In a colony affected with disease of any kind, which significantly affects the normal mortality rate, the age incidence of the mortality amongst the workers, and the productivity of the queen, are characters of the highest importance as affecting the maintenance of the colony as an effective and prosperous unit. These two opposing factors struggle with each other—losses from idleness and crawling, and mortality due to disease, added to the normal wastage on the one hand, and gains from the production of new bees on the other. A young and prolific queen by sheer production of new bees may so keep down the proportion of infected and hence more or less ineffective members as to render such a colony to some degree profitable.

Disease may be maintained within a colony in two important ways. It may be instituted by the infection of a few members of the colony by contact with a single bee carrying mites, which has mingled in the cluster. This may be a stray bee from another colony or a member of the stock which has been robbing a diseased colony, and such infection may constitute the only one from the outside. In this case we may expect that progress will be slow, if indeed the infection does not die out. The attacked bees may be old and die away from the hive before transmitting *Tarsonemus* to other members of the colony, or the infection may be so swamped by normal increase as to be practically ineffective. Whether a stock once infected is doomed sooner or later in every instance we have not sufficient evidence as yet to say. Some of the cases quoted, if the samples of bees taken may be regarded as representative, appear to have lost the infection. And we do have some evidence that extinction may be delayed for a long time. Rennie and Harvey, No. 1 (1919), have already directed attention to cases where the source and time of infection of a stock was known in autumn and the usual symptoms did not become evident until the following year.

A second and highly important factor, however, which we are satisfied is very frequently in operation, is repeated or multiple infections continued from the outside over a considerable period of time. We then have the disease spreading from many foci. The drifting of bees into strange hives is common. Once the disease has gained some ground, the social instinct of the colony is weakened, both by the disturbance of the normal balance of worker types and by the illness of a high proportion of bees. Robbing may now take place, and amongst the robbers there may be infected bees which will intensify the trouble. This robbing, at first resisted, is eventually allowed to become rife, and when this is established we have noticed that extinction is practically inevitable.

Other factors which may tell against the stock are the presence of an indifferent queen whose production may be poor, and from whose low racial vigour shorter-lived bees result.

The varying character of the factors shows that a uniform course of spreading of

infection within a stock is not likely to occur. Yet apart from any special combination of adverse factors, it appears to be common for the disease to steadily gain ground within a stock once it is established. It is, however, clear that no sound argument against the view that *Tarsonemus* is the cause can be built up from cases where, in the presence of *Tarsonemus*, disease did not spread within the stock and destroy it as a whole, so long as it can be shown that there is an associated pathology which in due course renders the infected bees ineffective members of the colony, and all the time causes loss of bees by crawling or directly by death.

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EXPLANATION OF FIGURES.

All the figures are photographed with Watson Service Microscope.

Fig. 1. *Tarsonemus* in trachea of hive bee. The first specimen observed. A. *Tarsonemus*. B. trachea. C. thoracic glands. $\frac{1}{12}$ in. oil imm. obj., ocular No. 2.

Fig. 2. Teased preparation of infected trachea showing various stages of *Tarsonemus*. $\frac{2}{3}$ in. obj., ocular No. 2.

Figs. 3, 4, and 5. Tracheæ containing *Tarsonemus*.

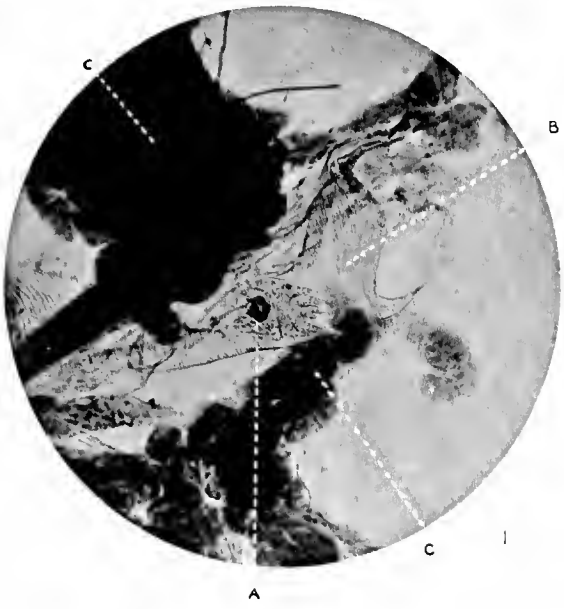
Fig. 3 shows the blackening of the tubes.

Fig. 5 shows blocking of wider tube with larval stages. $\frac{1}{6}$ in. obj., ocular No. 4. Fig. 5, ocular No. 4.

Fig. 6. Section of infested trachea wall. A. not badly affected. Mites seen in section. $\frac{1}{6}$ in. obj., ocular No. 4.

Fig. 7. Do., showing A. thickening of wall of tube. B. Two ovigerous females *in situ*. $\frac{1}{6}$ in. obj., ocular No. 2.

RENNIE, WHITE, AND HARVEY: ETIOLOGY OF ISLE OF WIGHT DISEASE.



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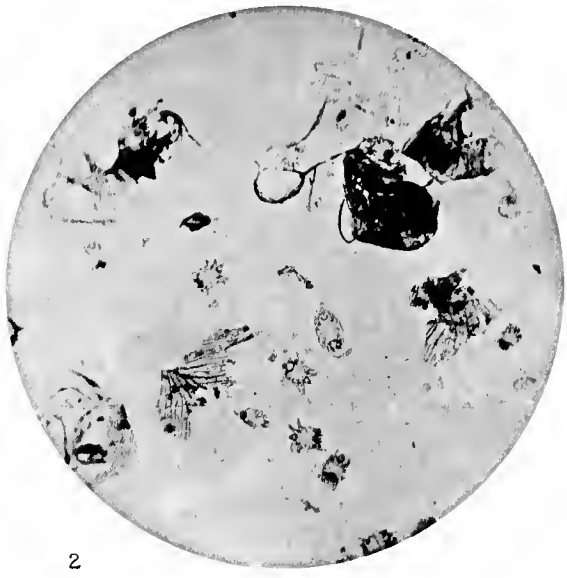
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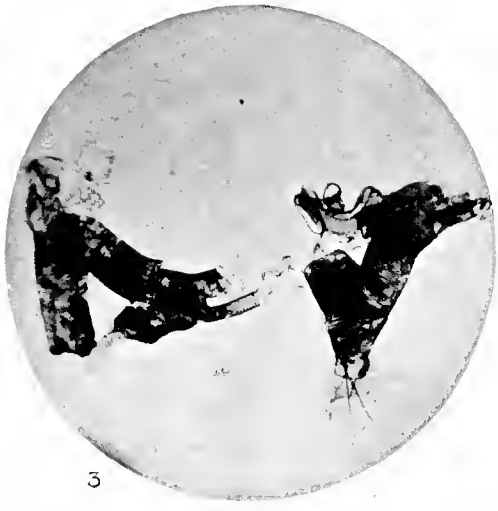
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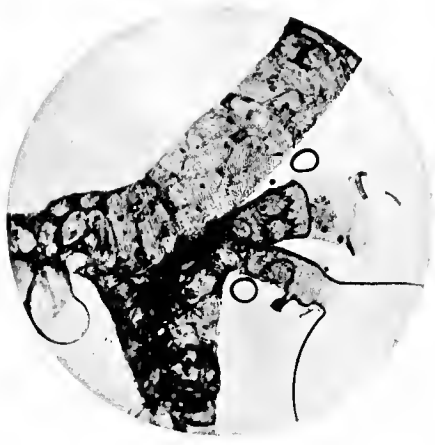
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XXIX.

(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.)

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(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 fæces 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 fæcal deposit is arranged in transverse lines. (× 70.)

Fig. 2. Fragment of wall of infected trachea, shown much enlarged. Granular deposits of fæcal 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. (× 50.)

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

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

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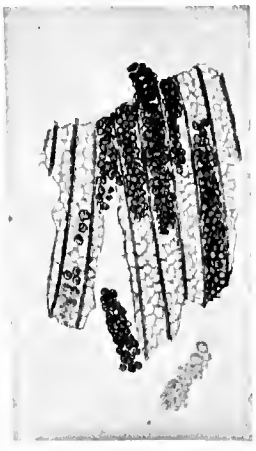

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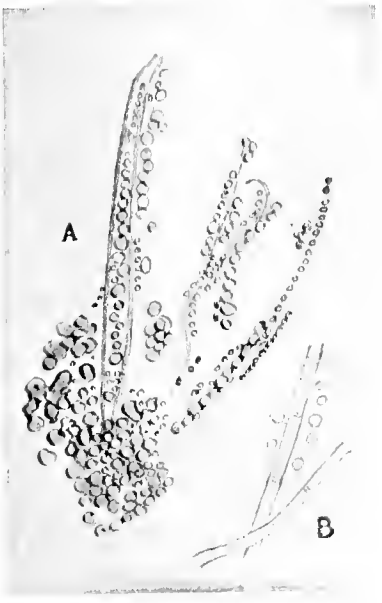

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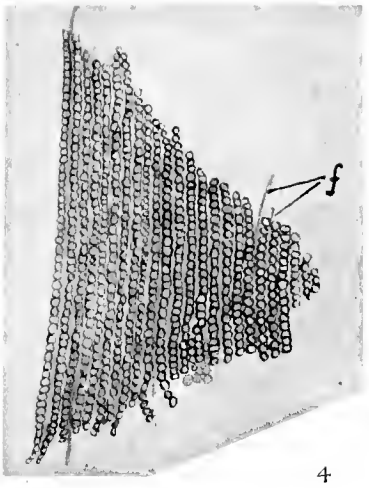

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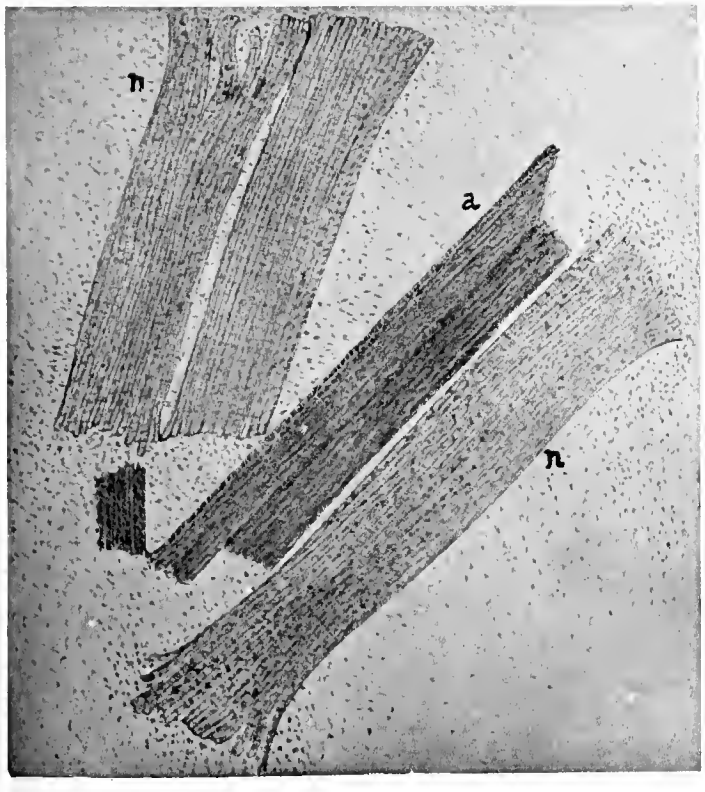

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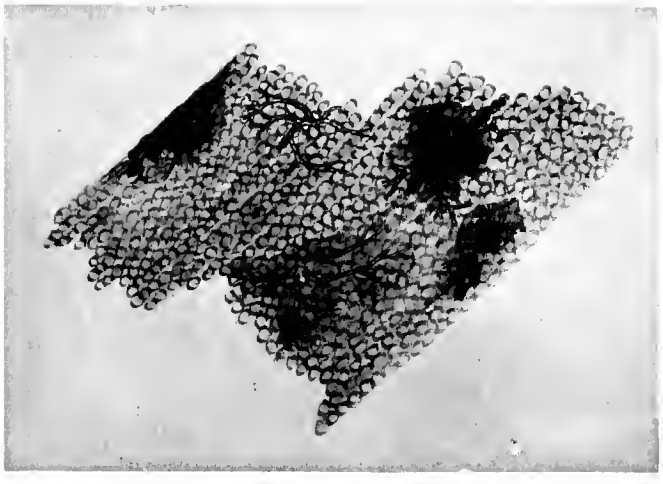

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XXIX.

(3) Isle of Wight Disease in Hive Bees—Experiments on Infection with Tarsonemus woodi, n. sp. By Elsie J. Harvey. *Communicated by* Dr John Rennie.

(Read November 1, 1920. MS. received November 27, 1920. Issued separately March 25, 1921.)

Introductory.

The following experiments and observations have been undertaken with a view to discovering the means by which *Tarsonemus* is transmitted from one bee to another. It is obvious that a stage of the parasite exists outside the bee, and that there are also several possibilities (which may occur). One is the passage from bee to bee within the hive either directly or through the medium of frames or combs. Another, also within the hive, where the mites may in wandering upon the frames enter the cells and invade the body of the developing larvæ or pupæ, and in this way be present in the bee when it hatches. A third possibility is that whereby foraging infected bees may leave the mite upon flowers, vegetation generally, drinking grounds, or other situations, to be picked up later by other bees chancing to visit these. Crawling or dead bees may in a similar manner prove to be a source of infection through the contamination of the ground about the hive or of the actual hive itself.

Exhaustive investigation of this last possibility is a somewhat difficult matter, for which no opportunity has yet been found, and owing to the short time at my disposal it has been set aside in favour of the more promising one of infection by direct contact between bee and bee.

Review of Former Experiments.

The conclusions arrived at from former experiments published before the organism was discovered, pointed to the probability of the disease being of an infectious character. This was shown when, *e.g.*, a frame of infected bees, say of a black colour, were placed in a healthy stock of Italians, and in due course the disease became evident in the yellow bees. It is admitted, of course, that only a probability is indicated in such an experiment.

What evidence we have from experiments with brood, in which frames of sealed brood of bees of one colour from an infected hive have been placed in the hive of a healthy stock of a different colour, points to the disease being an affection of adult bees only. Many experiments of this nature have been tried, and the results have been on the whole uniformly in favour of the view that brood hatched out under such circumstances was free from disease.

Experiments.

1. *To Discover whether Bees become infected before Emergence from Cells.*

Early in the spring of this year a few frames of brood from a badly affected stock were placed in an incubator; 155 of the bees which hatched out were examined, with the result that only the tube on one side in one bee was found infected with the parasite. This evidence, as far as it goes, therefore, does not exclude the possibility that mites may enter the cells and invade a bee's body before it emerges from the cells, but its rarity, as shown here, would seem to suggest that at best it is only an incidental occurrence, and is not one of the regular ways in which infection is conveyed.

2. *To Discover the Stage or Stages which occur normally outside the Bee.*

(*a*) By examination of the individuals in the tubes, in cases where the infection had only newly commenced, it is often found that an ovigerous female with a few eggs in different stages of development are the only parasites present. This would suggest that the migratory stage of the parasite is the fertilised female. In such a case migration of the male does not seem to be necessary, although, as is seen from the results of the experiments recorded, they leave the body of the dead bee.

(*b*) Equal numbers of living and of dead bees were placed in separate petri dishes, and these were kept as nearly as possible at the temperature of the hive. The dishes were examined microscopically at intervals of from two to forty-eight hours. Fifteen such experiments were carried out, with the result that only 10 mites, all of which were females, were recovered from the living bees: one of these was alive and active. As many as 75 (62 female and 13 male) were found in the dishes containing the dead bees. Of the 62 female mites, 8 were alive and active, while all the 13 males appeared dead. None of the females seen outside the bee were carrying fully developed ova. It would appear from these experiments that migration of both sexes takes place from the dead bee.

3. *An Endeavour to produce Infection artificially.*

(*a*) By contact with living sick bees.

These experiments were carried out in small queen cages. The bees were fed with soft candy and kept as far as possible at the temperature of the hive. The infective bees were in five cases crawlers picked up from in front of the hive; in two cases the bees were caught on entering the hive of a stock which was known to have a high per cent. of infection, and in the remaining three, the bees were taken from the frames of a sick stock. Virgin queens headed six of the experiments.

In these experiments the healthy bees were maintained in contact with sick bees for periods extending from four to seventeen days. In only one case was a positive

result obtained. On the fourteenth day of the experiment one bee was found to contain one ovigerous female at the entrance of the tube.

(*b*) By contact with dead bees.

Before commencing these experiments, observations were made to discover how long the parasites lived after the death of the host. It was found that a few female mites were still capable of feeble movement on the fifth and sixth days.

Seven of these experiments were put up in the same way as the foregoing, substituting newly killed bees for the living sick bees. The result in this group was negative.

(*c*) By placing tracheal tubes containing the parasite on the thorax of healthy bees.

Preliminary observations were made on the behaviour of the mites when the tubes had been dissected out of the bee. These were placed in welled slides, and both sexes of the mites were seen to emerge within an hour. These wandered about actively, and were occasionally seen to re-enter the tubes. In most cases the mites became inert and passive within twenty-four hours of leaving the tubes. It cannot be said with certainty that in any of these experiments the mites are really dead.

Bees were now taken from a stock known to be free from infection, and tubes containing the parasite were placed on the thorax near the first spiracle. Twenty-four bees were treated in this manner, and were examined after twenty-four hours. It was found that no infection had taken place.

Examination of the stocks from which the experimental bees were taken, for the presence or absence of the parasite, were being regularly carried out.

It is to be clearly understood that the whole of the foregoing experiments are provisional in character, and all of them, as well as others, are being repeated upon a larger scale.

From the results obtained, as far as they go, however, it appears that experimental infection with the parasite *Tarsonemus* is difficult to effect; it must be taken into consideration that any deviation from the normal habits of the bee host such as is involved in these experiments may have a corresponding effect upon those of the parasite.

I wish to express my sincere thanks to Dr Rennie, both for his help and advice in the preparation of this paper, and for his kindness personally whilst I have worked under him.

XXIX.

(4) Isle of Wight Disease in Hive Bees—Acarine Disease: The Organism associated with the Disease — Tarsonemus woodi, n. sp. By John Rennie, D.Sc. (With One Plate and Two Figures in the Text.)

(Read November 1, 1920. MS. received November 27, 1920. Issued separately March 25, 1921.)

The organism which has been found living in the anterior tracheal system of hive bees, and whose presence is associated with Isle of Wight disease, I have identified as a hitherto undescribed species of the genus *Tarsonemus*. This genus was founded in 1876 by CANESTRINI and FANZAGO, and since then a moderate number only of species has been established. The true systematic position of these Acarines has been much in doubt, and their position in the order has from time to time been revised. CANESTRINI (1888) constituted the Tarsonemes the type of a special family, the Tarsonemini; they have been associated with the Oribatidæ by BERLESE, and with the Cheyletidæ by TROUESSART (1892). BANKS (1904) regarded them as showing resemblances to the Tyroglyphidæ, and placed them in a super-family Sarcoptoidea. An important character of the Tarsonemes is the existence of a tracheal system in the adult female, which is not found in the male nor in any pre-adult stage of either sex. This feature was adopted by BERLESE (1897) as the basis of his sub-order, Heterostigmata, and by OUDEMANS (1906) in his division Trachelostigmata. This super-family includes two families—Tarsonemidæ and Scutacaridæ* (OUDEMANS, 1916). This last is the Disparipedidæ of BERLESE.

The Tarsonemidæ are a small family of soft-bodied mites, the females of which are tracheate, and which usually exhibit prominent hairs upon the tarsi of the last pair of legs. The body is more or less clearly segmented dorsally. The mandibles are needle-like, the palps slender and minute. The females possess in most instances, between the first and second pair of legs, a pair of delicate rounded or club-shaped organs which have been designated pseudostigmata by OUDEMANS. The legs are short, with six or fewer joints. They are bedecked with a limited number of stout hairs, and terminate in claws. The tarsi of the first pair possesses a single claw, the second and third, two. The fourth tarsus varies in the different genera. Suckers are frequent. There may be distinct sex dimorphism, especially in the genus *Tarsonemus*.

THE GENUS TARSONEMUS.

CANESTRINI's original description defining the genus is as follows:—

"Rostro normale e libero. Zampe del quarto pajo nella femmina poco sviluppate, sfornite di uncini e di ventosa e terminate da duo setole; zampe del primo pajo,

* I desire here to gratefully acknowledge the courtesy of Dr OUDEMANS in guiding me to the literature of the Tarsonemidæ and Acarina generally.

pure nelle femmine, normali, conformate come quelle del secondo e terzo pajo, colla differenza che hanno un' unghia sola. Zampe del quarto pajo nel maschio robuste, constituenti insieme unachela, terminate da un' unghia robusta. Epimeri del terzo e quarto pajo nel maschio assai lunghi e forti e convergenti insieme verso l'avanti. Scudo dorsale diviso in segmenti. Animali viventi su piante."

The adult female of the species to be described conforms well to the generic characters given above, and cannot, in my opinion, be separated from the genus *Tarsonemus*. The only morphological character upon which such a separation could be based is the conformation of the fourth pair of legs, and possibly the absence of pseudostigmata (not included in the original generic description). The male is undoubtedly more specialised in the fourth leg characters, but to constitute a new genus upon this fact, or upon the parasitic habit with which this specialised feature appears associated, seems to me inadmissible, at any rate in the present state of our knowledge.

A distinctive feature of the genus *Tarsonemus* is the fourth pair of legs, which in the female are slender, terminate in two hairs, and are devoid of claws. In the male, in the gall-inhabiting and free-living species, the last pair of legs is robust and terminates in a claw-like segment, usually incurved and frequently strongly developed. In some of the species recorded as endoparasitic, these characters in the male appear less well marked, and in the main show a reduction in size of this pair of appendages. In the species to be described the hind legs in both sexes present, especially in the male, features which I regard as related to the parasitic mode of life and restricted habitat of the insect's tracheal system.

Tarsonemus woodi, n. sp.

I propose to designate this species, which is parasitic in the anterior thoracic tracheal system of the hive bee, *Apis mellifica*, and which does not appear to have been described before, by the name of *Tarsonemus woodi*, n. sp. The adult ovigerous female measures from ·14 to ·19 mm. in length, the male about ·11 to ·15 mm. (fig. 1). Viewed with reflected light, these mites are more or less bean-shaped in form, greyish in colour, and scantily bedecked with hairs. When removed from the tracheæ of the bee they progress slowly upon glass, but when seen within the tube, although continued observation has not revealed much progression, a good deal of active and vigorous leg movement may be observed.

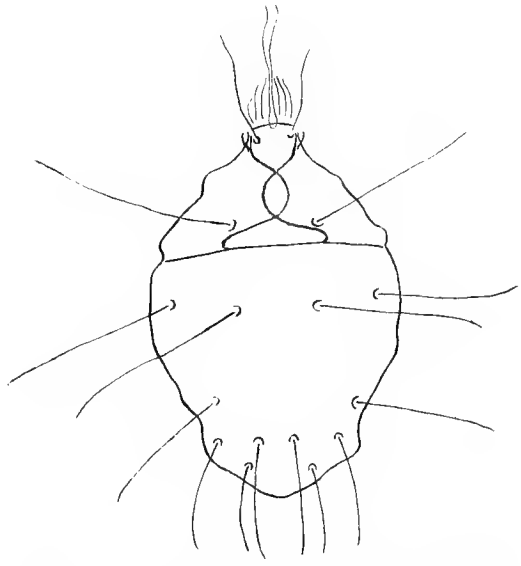
Ovigerous Female.—Seen from above, the body presents a somewhat oval form, broadest in the neighbourhood of the second pair of legs. The following are typical dimensions for a fully grown adult:—

Total length from tip of gnathosoma to hinder end of body, ·19 mm.

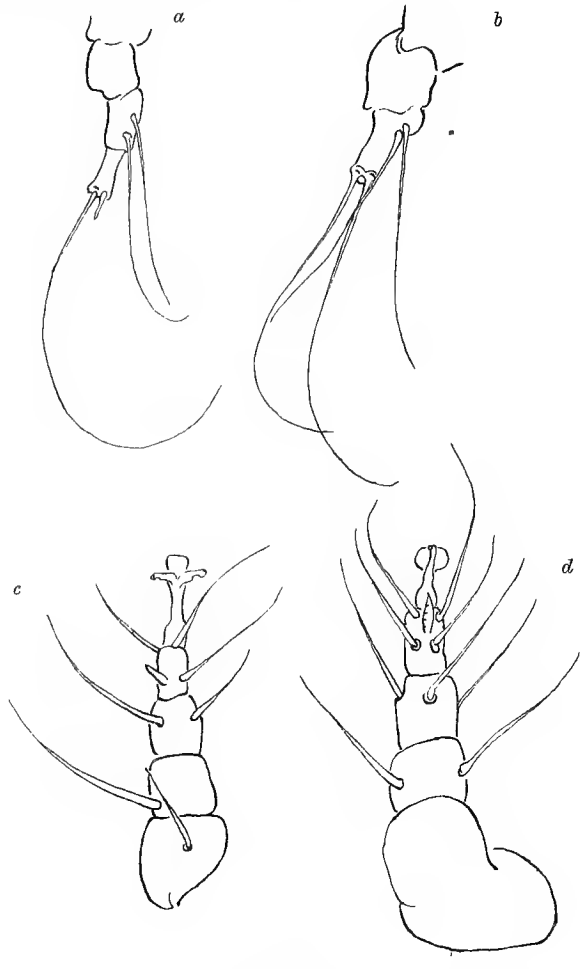
Total length from tip of gnathosoma to tip of longest hair of fourth pair of legs, ·25 mm.

Width at broadest part of body, ·10 mm.
Length of gnathosoma, ·03 mm.
Length of first, second, or third legs, ·05 mm.
Dorsally the body shows definite segmentation.

On the ventral side there is a distinct transverse furrow behind the second pair of legs. The gnathosoma is bluntly triangular and freely movable, and in



Text-fig. 1.—Dorsal view of body of immature female, showing distribution of hairs. The anterior portion of the two main tracheæ are shown.



Text-fig. 2.
a, Fourth leg of male ; *b*, fourth leg of female ; *c*, second leg of male ; *d*, first leg of male.

the middle line in front the mandibles are frequently to be seen protruded as a pair of slender curved needles. The two anterior pairs of legs are directed forward or outwards, and some distance apart from the third and fourth pairs.

Body Hairs.—On the dorsal surface there are eight pairs of hairs fairly evenly distributed along the body (text-fig. 1). There is a short pair anteriorly, directed forward close to and upon each side of the gnathosoma, arising on the medial side of the stigmata. A second pair arises opposite the second pair of legs,

about one-third of the body width from the margin, and slightly in front of the dorsal furrow. Behind these are two pairs at about the middle of the body, one pair near the margin and the other in a nearly direct line behind the second pair. A pair arises nearly opposite the origin of the third pair of legs; behind these are two pairs constituting an almost straight transverse row, closely followed by a final pair close to the hinder end of the body, and separated by about a third of the width.

Tracheal System.—A pair of stigmata opening dorsally occurs anteriorly close to the base of the gnathosoma. From these the more dorsal and anterior portions of the tracheal system pass backward as a pair of curving, slender tubes, which cross each other at the level of the anterior limbs. These pass ventralwards curving inwards toward each other, and again outwards towards the bases of the posterior limbs.

Legs (text-fig. 2).—The anterior pair is jointed, and terminates in a single hook, with circular sucker in addition. On the penultimate segment upon the dorsal side there is a sensory spine-like organ—"riech-haar" of OUDEMANS. The second pair also carries a similar spine and ends in a double claw and sucker. The third pair of legs resembles the second, but a "riech-haar" is absent. These limbs arise near the margin and are directed outward and backward, and usually lie clear of the body for the greater part of their length. The fourth pair of legs, which arises closely behind the third pair, exhibits distinctive features. In contrast to what is usual in other species, instead of being long and slender, this limb is moderately stout at its basal segment and slender beyond. But the whole limb consists of only two segments and is much reduced in length. Upon it are four hairs, two of which are terminal and of considerable length. One of the remaining two arises nearer the base, but is usually seen along with the terminal hairs, forming a group of three projecting beyond the body (text-fig. 2, *b*).

IMMATURE FEMALE.—This differs in size and general shape, being more truncated behind (fig. 9).

Length of body from ·13 to ·15 mm.
Width ,, ,, ·08 to ·09 mm.

ADULT MALE.—The adult male is smaller than the female and more truncated in form behind.

Total body length ·11 to ·15 mm.
,, width ·06 to ·08 mm.

Dorsally, it appears to consist of three segments, besides the gnathosoma. Only five pairs of hairs are present, all placed slightly inward from the lateral margin, and nearly equidistant along the side. On the ventral side the following features are observable. The transverse furrow behind the second pair of legs is well marked. There are no longitudinal epimeral grooves such as are prominent in most free-living

species. The external genitalia consist of two rounded lobes, projecting posteriorly on each side of a tapering triangular penial sheath.

Legs.—Special features regarding the limbs are: On the penultimate segment of the second pair of legs there is a prominent sensory organ. The fourth pair of legs is relatively slender, and presents the most distinctive characteristic of the species in this sex. There are three joints, all of which are comparatively weak; the terminal one bears the very long, stout, and finely curved hair distinctive of the genus. Just within this, and almost at the tip of the last segment, is a small straight spine, which occupies the position of the incurved terminal claw, characteristic of free-living species. Close examination has suggested that this structure is of the same character as that on the second pair of legs of the males, and on the female limbs also, and that it is probably sensory in function (fig. 7).

Larva.—The larva is large, being about ·2 mm. in length by about ·08 mm. in width at its broadest part. The mouth parts resemble those of the later stages. There are three pairs of short legs; the first terminates in a double claw (fig. 4).

Ovum.—The egg is large, being about ·14 mm. long by ·06 mm. broad, and slightly concave along one side (fig. 2).

All the Heterostigmata appear to be parasitic, some on plants, others upon insects, and doubtfully on warm-blooded vertebrates. The majority of the described species of *Tarsonemus* are found upon plants; the others are from the bodies of vertebrate animals, in which a number have been found associated with malignant growths. *T. woodi*, n. sp., appears to be the only species known to occur in insects. Its discovery as an endoparasite within the hive bee therefore constitutes a significant advance in our knowledge of these mites, and of the general importance of the genus, apart from the far-reaching suggestiveness of its causal relationship to a disease in hive bees which has baffled inquiry for the last sixteen years.

As giving a more detailed indication of the habits of the genus, the following brief references to the best-known species are submitted. In view of the importance of habit and habitat in the case of *T. woodi*, I have preferred to refer to these, not in the order of their original discovery or description, but to group them from this point of view.

Gall-forming Species.

T. floricolus Cn. and F., 1876.—This species is described as occurring on the flowers of *Verbascum*, forming galls in foliage of *Vitis vinifera*, *Coryllus avellana*, *Salix alba*, etc., in putrefying stuff, and in frass of bacon beetle. *T.* (*Cheylurus*) *socialis*, according to Berlese, is of the same species. It is described from the skin and base of the feathers of birds of very diverse species, both terrestrial and aquatic.

T. buxi Canestr. and B., 1884.—Occurs as an inquiline in Phytoptus galls and in Diplosis galls. See Canestrini (1886), pp. 320–1.

T. canestrini Massal., 1897.—Forms small rounded galls on several European grasses.

T. phragmitidis Schlechtendal, 1897.—A species resembling *T. canestrini*, which occurs as an inquiline in phragmitid galls.

T. contubernalis Reuter, 1906.—An inquiline in galls upon *Galium verum.*

T. latus Banks, 1904.—Causes galls on the main shoots of the mango plant.

T. intectus Karpelles, 1885.—In barley; producing severe irritation on hands of workers in the Danube region in Hungary and Russia.

T. spirifex Marchal, 1902.—On grasses; causes elongated swellings on oat. Occurs in colonies.

Described as doing Damage to Plants but not Apparently Associated with Galls.

T. oryzæ Targ., 1878.—Infests the ears of the rice plant.

T. culmicolus Reuter, 1900.—From spikes of meadow grass; produces "silver top" in grasses in Finland, where it is found in the leaf sheaf above the uppermost node.

T. anasæ Tryon.—Described as causing injury to pine apples in Australia.

T. fragaria Zimmerm., 1904.—Has been found on strawberries.

T. graminis Kramer, 1886.—So named by Kramer because it occurs in abnormally rolled-up grass leaves.

T. bancrofti Michael.—Has been described as causing damage to sugar-cane in Queensland and in Barbados.

Described from Animals.

T. floricolus.—Already quoted above as occurring in the bases of birds' feathers.

T. soricola Oudms., 1903.—Found on *Sorex vulgaris.*

Regarding the placing of these species here, it may be mentioned that Oudemans is of opinion that when forms occur on animals these are probably no more than transporting agents.

T. hominis Dahl, 1910.—From human ovary in carcinoma and fibroma, and from bladder in cystitis (Blanc and Rollet, 1910).

T. sauli Dahl, 1910 (*T. equi; T. muris; T. canis*).—From tumours in mammals.

T. woodi, n. sp., Rennie, 1920.—In thoracic tracheæ of *Apis mellifica.*

In this connection reference should also be made to a form described by Myake and Scriba (1893) from the urine of the human subject as *Nephrophages sanguinarius.* According to Oudemans this form is a *Tarsonemus.* It is tracheate, blind, has needle-formed manibles, and the pear-shaped pseudostigmatic organ has been mistaken for eyes. According to him the mouth parts are not described or figured correctly.

The group of species which have been obtained from mammalian tissues presents

some features of interest in relation to *T. woodi*, and it seems worth while considering these in some detail. DAHL has described a species termed by him *T. hominis*, which was obtained by E. SAUL, the female from a fibroma of the human ovary, and the male from a carcinoma of the same organ. Later in the same year, SAUL published micro-photographs of *T. hominis* and of others obtained from a cancer of the mouse, a papilloma of a horse, and a sarcoma of a dog. Following the publication of SAUL'S photographs, BLANC and ROLLET (1910) published a statement that they had in their possession an acarid obtained in 1909 from the urine of a patient suffering from a refractory cystitis. They describe the specimen in detail and recognise it as a male of *T. hominis*.

T. hominis is distinguished, according to DAHL, from all previously described species by the following. In the female the fourth pair of legs is more shortened than in other species. Except for the end bristles it does not reach to the hinder end of the body. The third pair has a longer, thinner, two-segmented end part sharply marked off from the basal segment by the greater width of the latter. The two bristles at the hind end of the body are wider apart than is the case in other species. The male is distinguished from all other known males by the size and thickness of the long bristle at the end of the last pair of legs, and by the presence of a thick, club-shaped appendage on the second pair of legs (riech-haar of OUDEMANS). Both sexes are further differentiated by the course of the epimeral grooves on the posterior ventral surface.

DAHL groups all the forms from mouse, horse, and dog tumours as *T. sauli*. Amongst these there are two males, distinguished from *T. hominis* in that, of the five longitudinal furrows, the three innermost are united by a well-developed transverse furrow, and the sensory organ on the second pair of legs is not more developed than in the first pair. In the females constant distinguishing characters could not be made out. He states that the same difficulty applies to the females from gall-inhabiting species.

Mr STANLEY HIRST has kindly directed my attention to the fact that the conclusions of DAHL have been severely criticised by REUTER (1910) both as regards the probable accidental introduction of the mites in question to the preserved tissues from which the preparations were made, and as to the identity of the species. It appears to me that DAHL has not shown sufficient care in differentiating the forms found from species already described.

AFFINITIES OF TARSONEMUS WOODI, n. sp.

DAHL (1910) regards the genus *Tarsonemus* as representing a transitional stage between the gall-forming mites, Eriophyidæ or Phytoptidæ, and other mite families. He bases his conclusions largely upon the characters of the fourth pair of legs. In the species which are not endoparasitic in animals, but lead a life in relatively free space and where mating may be effected in the open, the fourth pair of legs in the

male is relatively large and of robust build, and terminates in a stout curved spine. These features are regarded as of value in mating. In the male of *T. hominis* this limb appears definitely smaller in size and general build in proportion to the other parts as compared with other species. DAHL interprets this as related to an endoparasitic life. In view, however, of our very slight knowledge of this species, and especially on account of the doubt which exists as to its normal habitat, the conclusion must be received with reserve.

If we apply such a comparison to *T. muris* and *T. equi* (*T. sauli* Dahl), these occupy an intermediate position between such a species, *e.g.*, as *T. floricolus* and *T. hominis*, and so far affords some support to DAHL's view.

Including *T. woodi* in this comparison, we regard its place as undoubtedly at the end of the series. *T. woodi* agrees with *T. hominis* in general appearance in both sexes, but in detail more closely in the male than in the female. In the male they agree in possessing on the second pair of legs a sensory organ (riech-haar of OUDEMANS) of relatively large size as compared to the one on the first pair. The reduction in size of the last pair of legs is also a common feature, although in *T. woodi* the whole limb is markedly slighter in build than in *T. hominis*. With respect to the terminal claw also the comparison is interesting. In *T. hominis*, though showing the inward curvature characteristic of the genus, this is smaller than is the case in all the hitherto known species. In *T. woodi* the limb appears to terminate in a straight, sharp spine. The mite has been seen carrying this spine directed inwards at right angles to the limb. Under a high power it exhibits an appearance similar to that of the sensory organ upon the anterior limbs, and there is doubt as to its homology with the terminal claw present in other species. Whatever view we take regarding the reality of the endoparasitism in *T. hominis*, etc., I incline strongly to the view that these special features in *T. woodi* are to be interpreted in relation to the fact that the habitat of the male is probably limited to the tracheæ of the host, and also that mating takes place in this confined space. These conditions largely obviate the necessity for specialised clasping limbs. At the same time a sensory organ on the limb would obviously be of value.

Another feature worthy of notice is found in the nature of the ventral surface, which is devoid of the five longitudinal epimeral grooves which are prominent in most species, including some of those which are regarded as endoparasitic in mammalia, *e.g. T. hominis*.

In the female of *T. woodi* fewer points of comparison can be laid hold of. The most noteworthy are to be observed in the two hinder pairs of legs. These in *T. hominis* and in *T. sauli* are comparatively weak and slender. In both cases the fourth pair terminates in the usual two long bristles. In *T. woodi* the fourth pair is not slender, but is reduced to three segments, is somewhat stumpy, and its two bristles are long and sweeping. Pseudostigmata have not been described in any of the so-called endoparasitic species, and they have not been observed in *T. woodi*.

Reviewing the main features of *T. woodi*, it appears that there are good grounds for regarding this as a species of specialised structure in relation to the particular habitat in which it lives.

Biological Considerations.

I now propose briefly to consider the biological problem presented by *T. woodi* in relation to Isle of Wight disease.

For the final acceptance of the thesis that *T. woodi* is exclusively responsible for the condition known as Isle of Wight disease, careful consideration must be paid to the biological aspect of the problem.

I. Although the numbers of bees examined from outside Great Britain in relation to those from within have been comparatively few, yet considerable numbers have been tested. Through the assistance of the Ministry of Agriculture, bees arriving in this country accompanying queens from Italy have been obtained in a number of cases for examination.

In all, several hundreds of bees were obtained from this source. These, along with others obtained direct from Italy, were searched for the presence of *Tarsonemus*. The result of these examinations was that the bees were found entirely free from the parasite. The evidence is so far satisfactory that it may be accepted that *Tarsonemus* is not being introduced to this country in Italian bees. Smaller numbers of Dutch bees so imported have also yielded on examination a similar result. Bees in limited numbers have also been obtained from Switzerland and from North America, all of which were also free from this parasite. The evidence is not complete by any means, but, as far as it goes, it is of one kind. Since this disease has never been clearly demonstrated to exist outside the British Isles, nor certainly any epizootic approaching in any way the dimensions of Isle of Wight disease in the British Isles, and further, since all such evidence as we possess points to a causal relation between *Tarsonemus* and Isle of Wight disease in bees, this coincidence in distribution is noteworthy. If a geographical distribution limited to Britain should be established in the hive bee—and to do this is a mere matter of time and favourable opportunity—in my opinion it would point to a relatively recent invasion of the bee, although the opposite finding would not be against such a view.

It may be noted that Zander (1911), who has paid particular attention to the recording of pests found in hives and upon hive bees in Germany, makes no reference to Acarids of any kind. In the course of our investigations we have found in hives or upon combs, dead or live bees, at least five different species, including one other species of *Tarsonemus*.* These mites will be dealt with in a subsequent publication.

* In *C. R. Acad. Paris*, t. 62, 1866, M. Emil Duchemin records the occurrence of a microscopic *Acarus* on diseased hive bees. He gives no description nor figure. This is clearly not an endoparasite, since M. Duchemin found that it bred upon sunflowers protected from the bees.

II. Morphology, development, and habits all point to the fact that this is a parasitic organism which must have been related to the tracheal system of some host for an indefinite period. The habit is not new. If *T. woodi* has been a parasite of bees for ages, it seems improbable that the disease phenomena which accompany its presence, and such as we are now familiar with, could have escaped notice. On the other hand, it may be that, although the parasitic relationship is not new, the pathology is. But such is not very probable.

It is true that, as far as bee records go, there have been in the past periods of epizootic disease in bees from time to time, but there is no evidence that a continuous epizootic extending from sixteen to eighteen years has taken place.

III. It may be suggested that earlier methods of bee-keeping, whereby destruction of bees was annually resorted to, kept down this parasite. This would certainly have been the case, if the parasite were present, and the method should be applied to all existing diseased stocks before winter. But surely the disease would then, as now, have manifested itself constantly in the working season to a degree sufficient to attract attention. And it must be remembered that modern methods of bee-keeping are not confined to Great Britain and Ireland.

IV. May it not be that *Tarsonemus*, owing to some unknown change in the normal balance of inter-relations, is at present undergoing one of those periods of undue increase such as occurs from time to time in various animal forms. We must recognise that it may be a parasite of bees which normally does not attain such an incidence as to attract special notice, and that in recent times there has been some change in the "balance of nature" which has led to its excessive increase. Bee-keeping has increased in Britain within the last twenty-five years; can it be said that, apart from the ravages of this disease, our Islands are overstocked? This again is unlikely.

V. It has been suggested that British bees of the present time are of a deteriorated breed, and have lost resisting power, so that *Tarsonemus*, a relatively non-pathogenic parasite ordinarily, is able to breed excessively. My provisional answer is that other racial forms are similarly affected. For example, Egyptian, Dutch, Punic, and Italian bees can be readily infected, and in these *Tarsonemus* multiplies with disastrous results, as in British bees. But the question of the ability of a stock to survive a prolonged period of *Tarsonemus* infection is not a simple one; amongst other factors, it involves the question of relative fertility of particular queens, as well as that of individual tolerance of the parasite (p. 753).

VI. *Tarsonemus* may be relatively new to hive bees and normal to some other insect.

There remains the possibility that *Tarsonemus* exists normally in some wild insect—possibly a hymenopteron—related to the hive bee, and that invasion of the bee is recently established. In such a case, the unknown normal host will remain a potential reservoir of the parasite.

It is noteworthy that many species occur on plants, but, as has been shown, these, as far as known, do not possess the specialised characters of *T. woodi*, and for this reason it seems improbable that bees have recently become infected from flower-haunting forms. It is more probable that this took place through contact with other insects, possibly robbing visitors to hives.

It may be remarked that along with my colleagues I have examined numbers of wasps, humble bees, earwigs, wax moths, *Braula cæca*, and although other mites were readily found upon the exterior, particularly upon the earwigs, the thoracic tracheæ in all cases were found clear.

The importance of finding answers to all of these questions raised is fully recognised by the Investigation I have the honour to direct, and the work is continuing with unabated vigour. I desire to take this opportunity of thanking my colleagues for the skill and ability with which they have aided me in the work so far accomplished, and particularly Miss Elsie Harvey, my personal assistant, whose loyalty and diligence have been noteworthy.

The foregoing researches have been carried out under the Joint-Committee upon Animal Nutrition of the University of Aberdeen and the North of Scotland College of Agriculture, with the aid of grants from the Development Commission, together with the generous financial help of A. H. E. Wood, Esq., of Glassel. To all of these, for their valuable assistance, and to the Local Advisory Committee, under Professor John Arthur Thomson, whose encouraging advice has been unfailing throughout, I desire to express the cordial thanks of my colleagues and myself. I also desire to thank the Carnegie Trust for help in connection with this research.

Parasitology Laboratory,
University of Aberdeen.

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EXPLANATION OF FIGURES.

Tarsonemus woodi, n. sp. Microphotographs.

(All the preparations are photographed with a Watson Service Microscope.)

Fig. 1. Adult female, ventral view. Obj. $\frac{1}{6}$ in. Ocular No. 4.

Fig. 2. A, young tracheate female; focussed on ventral side to show tracheæ. B, ovum. C, a male. Obj. $\frac{2}{3}$ in. Ocular No. 4.

Fig. 3. Adult male. Obj. $\frac{2}{3}$ in. Ocular No. 4.

Fig. 4. Larva. Focussed to show double claw on first pair of legs. Obj. $\frac{1}{6}$ in. Ocular No. 4.

Fig. 5. Larva containing nymph. Focussed to show the latter: a female. Out of focus: A, posterior end of larva. B, first pair of legs of larva. C, gnathosoma of larva. Obj. $\frac{1}{6}$ in. Ocular No. 4.

Fig. 6. Male. Focussed to show sensory organ, A, on second pair of legs. Shows also spine on fourth pair of legs. Obj. $\frac{1}{6}$ in. Ocular No. 2.

Fig. 7. Posterior end of male, showing last pair of legs and external genitalia. A, spine. B, genital lobes. Obj. $\frac{1}{6}$ in. Ocular No. 4.

Fig. 8. Teased trachea showing: A, adult female. B, immature female (tracheate). C, two males. Obj. $\frac{2}{3}$ in. Ocular No. 4.

Fig. 9. Female, showing tracheæ. Obj. $\frac{1}{12}$ in. Ocular No. 4.

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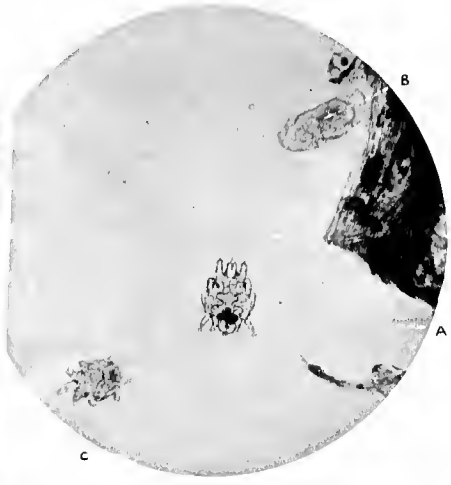

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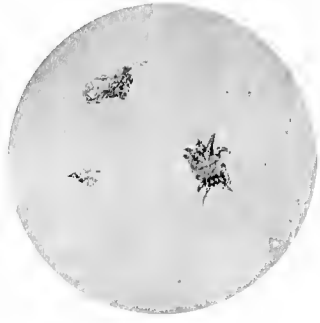

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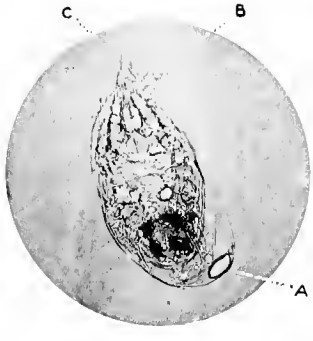

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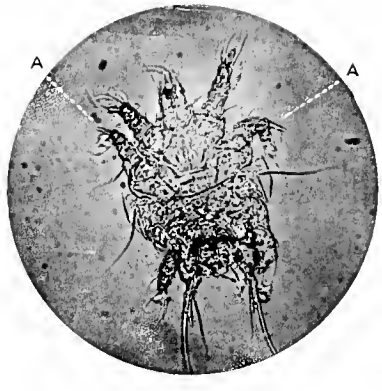

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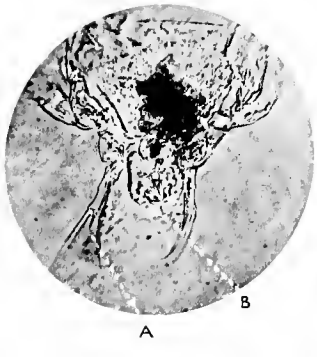

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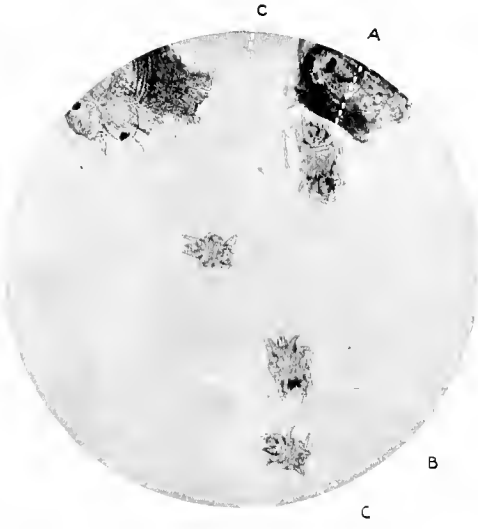

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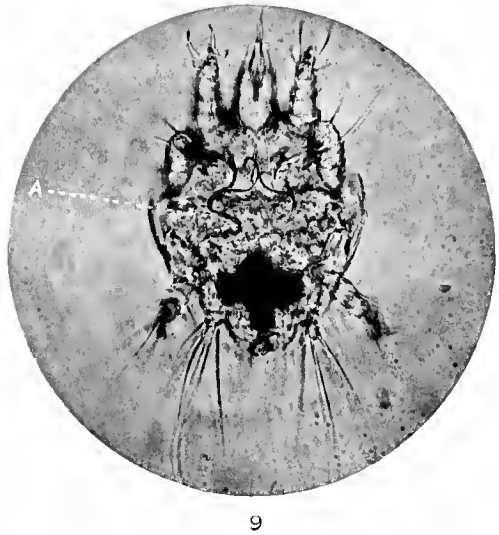

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