Aberdeen University
Studies : No. 90

Studies in Parasitology

University of Aberdeen.

UNIVERSITY STUDIES.

General Editor: P. J. ANDERSON, LL.B., Librarian to the University.

1900-1913. Nos. 1-63.

1914. No. 64.—*Zoological Studies.* Professor Thomson and others. Ser. VIII.
,, No. 65.—*Highland Host of* 1678. J. R. Elder, D.Litt.
,, No. 66.—*Concise Bibliography of Aberdeen, Banff, and Kincardine.* J. F. Kellas Johnstone.
,, No. 67.—*Bishop Burnet as Educationist.* John Clarke, M.A.
1915. No. 68.—*Territorial Soldiering in N.E. Scotland.* J. M. Bulloch, M.A.
,, No. 69.—*Proceedings of the Anatomical and Anthropological Society*, 1908-14.
,, No. 70.—*Zoological Studies* Professor Thomson and others. Ser. IX.
,, No. 71.—*Aberdeen University Library Bulletin.* Vol. II.
1916. No. 72—*Physiological Studies.* Professor MacWilliam, F.R.S., and others. Ser. I.
1917. No. 73.—*Concise Bibliography of Inverness-shire.* P. J. Anderson.
,, No. 74.—*The Idea of God.* Professor Pringle-Pattison. (Gifford Lectures, 1912-13.)
,, No. 75.—*Interamna Borealis.* W. Keith Leask, M.A.
,, No. 76.—*Roll of Medical Service of British Army.* Col. W. Johnston, C.B., LL.D.
1918. No. 77.—*Aberdeen University Library Bulletin.* Vol. III.
,, No. 78.—*Moral Values and the Idea of God.* W. R. Sorley, Litt.D. (Gifford Lect., 1914-15.)
1919. No. 79.—*God and Personality.* C. C. J. Webb, M.A. (Gifford Lect., 1918.)
1920. No. 80.—*Divine Personality and Human Life.* C. C. J. Webb. (Gifford Lect, 1919.)
No. 81.—*Bulletins of College of Agriculture.* Nos. 15-27.
1921. No. 82.—*Subject Catalogue of Cruickshank Science Library.*
,, No. 83.—*Physical Geology of the Don Basin.* Alexander Bremner, D.Sc.
,, No. 84.—*Roll of Service*, 1914-19. M. D. Allardyce.
,, No. 85.—*Catalogue of Taylor Collection.*
1922. No. 86.—*Aberdeen University Library Bulletin.* Vol. IV.
,, No. 87.—*Records of Banffshire.* James Grant, LL.B.
1923. No. 88.—*Viri Illustres Universitatum Abredonensium.* W. E. McCulloch, M.B.
,, No. 89.—*Domain of Natural Science.* E. W. Hobson, F.R.S. (Gifford Lect., 1921-22.)
,, No. 90.—*Studies in Parasitology.* John Rennie, D.Sc., and others.

Studies in Parasitology and General Zoology

By

John Rennie, D.Sc.

Lecturer in Parasitology and Experimental Zoology

Alexander Reid, M.A.

John Alexander Innes, B.Sc., M.B.

Alexander Elmslie Campbell, M.A., B.Sc., M.B.

John Anderson, M.A., B.Sc.

Elsie J. Harvey

Christina Hunter Sutherland, M.A., B.Sc.

Philip Bruce White, B.Sc.

Printed for the University of Aberdeen

1923

CONTENTS.

[Reprinted from "The Annals of Scottish Natural History," October 1907.]

CENTROLOPHUS NIGER, GMELIN, ON THE SCOTTISH COAST, WITH A NOTE ON ONE OR TWO POINTS IN ITS STRUCTURE.

By John Rennie, D.Sc.

In the month of June in the present year I received from Mr. Ewen, schoolmaster, Cullen, a fish regarding which he supplied me with the following note: "It was caught in a net among herring. The locality was a herring area called by fishermen 'Smith Bank,' a sandy tract about 18 to 25 miles N.E. of Cullen. The skipper thinks it had been feeding on young herring. None of them has ever seen such a fish before." This interesting find on examination proves to be a specimen of a somewhat rare visitor to northern waters, viz. *Centrolophus niger*, Gmelin, regarding which notes have already been made in these "Annals." It is generally regarded as a Mediterranean fish; Regan ("Ann. and Mag. Nat. Hist.," 1902, p. 195) gives its distribution as "Mediterranean and N. Atlantic." According to Traquair, it has also occurred in Ireland, and Jordan and Evermann record it from Dennis, Mass., although Traquair throws doubt upon the identity of the species in this latter case. The present specimen is the fourth recorded from the east coast of Scotland; three of these have been found between Lossiemouth and Aberdeen, the fourth occurred in Largo Bay (see notes in the "Annals" for 1902 by Dr. Traquair and Mr. George Sim). The earliest record is by Dr. Gordon in 1841.

In Dr. Traquair's communication some particulars are given as to dimensions, in which he notes in the proportions of his specimen some differences from the current descriptions and figures. Since the Cullen example proves on the whole intermediate it may be of some value to record particularly its more important characteristics.

Along the dorsal region the fish is a deep blue-black colour; ventrally it is lighter, being of a greyish slaty blue. This lighter colour is in part, however, due to the fish having been immersed in spirit. The total length is 14.7 inches; the greatest depth, just in front of the anus, is about 3 inches. It is thus contained slightly less than five times in the total length. Traquair's specimen is stated to be in body depth "slightly over four times in the total." Regan gives the depth of the body as about four times. From snout to posterior margin of operculum this latest example measures 2.9 inches, almost exactly one-fifth of the total length. This accords with the proportion given by both Gunther and Day, viz. one in five. Traquair's measurement is "rather less than one in five and a half." Regan gives this dimension as four and a third to five times, hence Traquair's figure extends the range of variation of this dimension. The pectoral fin measures 1.6 inches to the tip of the longest ray, giving a proportion of rather more than half the length of the head.

Traquair's figure for this dimension is one-half, while Day's is two-thirds. There are 20 rays in the left fin and 21 in the right. The caudal fin is 2.5 inches long, and is thus practically one-sixth of the total. This is Traquair's proportion; it is less than that of Cuvier and Valenciennes or of Day. There are 38 dorsal and 22 ventral fin rays, but no other specially distinctive external characters.

Mr. Sim in his note comments upon the œsophageal pouches and speculates as to their function. These structures appear to be characteristic of the Families Stromateidæ and Tetragonuridæ, to the former of which *Centrolophus* belongs. They are carefully described by Sim, who, however, omits to note that the ridges which divide the pouches into compartments are simply continuations of the œsophageal ridges. They are here deeper, bridging over the cavity of the pouch, and are fibrous in character. In their anterior parts these ridges are very large, and project prominently into the lumen of the gullet as expanded plates. The edges of the ridges as well as of these plates are beset with numerous recurved setiform spines, which, as Sim points out, are simple and not barbed as stated by Gunther. As to the function of these pouches, it may be of interest to note that I found their cavities filled with a soft, creamy, pulpy substance similar to the contents of the stomach and pyloric cæca. The only difference observable was that the material in the latter organs was in a more fluid condition; it was, in fact, in a further advanced stage of digestion. Remarkable as it undoubtedly is, the facts seem to suggest that these fishes regurgitate their food; and as these pouches are so very thoroughly supplied with spines it seems possible that some sort of rumination is indulged in. Certainly the substance in the pouches had no resemblance to recently swallowed material unacted upon. Boulenger states that the Stromateidæ feed on crustaceans, medusæ, and the fry of other fish; the circumstances under which this example was caught suggest the last named at the time of capture, yet there were no recognisable traces of herring fry, either in the pouches or in the stomach.

Natural History Department,
The University, Aberdeen.

5

On the Relation of the Islets of Langerhans to the alveoli of the Pancreas

by

John Rennie.
D. Sc. Natural History Department, University of Aberdeen.

Of the various views which have from time to time been put forward concerning the morphological and functional nature of the so called Islets of Langerhans, recent investigations have brought into prominence two opinions which are generally regarded as fundamentally opposed to each other. On the one hand, these islets are declared, chiefly on experimental grounds to constitute a tissue *sui generis*, by Schulze [*6*], Helly [*4*], and Tschassownikow [*7*]. Schulze and Tschassownikow have independently found that as a result of occluding the pancreatic ducts in mammals, degeneration of the alveoli results, while the islets remain unaffected. Helly has studied their development and finds that the two tissues are interdependent throughout life. The morphological relations as investigated by Diamare [*3*], in all the vertebrate classes, and by Rennie [*5*] in Teleostean fishes, are in agreement with this conclusion. On the other hand the opposite opinion, viz. that these two tissues, islet and alveolus, are interdependent has been put forward by several of the earlier workers, whose views are criticised in detail in several of the aforesaid works.

In recent years Dale [*2*], and Vincent and Thompson [*8*] have revived the latter view in the form that the islets are modified masses of ordinary secreting tissue. Dale regards them as simply a phase, dependent for their existence upon the physiological condition of the pancreas, and that in particular they have a function in exhaustion which it is assumed is performed by internal secretion. This opinion is also based on experimental results, and some of Dale's conclusions may be quoted. "Change from the secreting to the islet condition is greatly accelerated, both in mammals and amphibians by exhaustion of the gland by means of secretin. True exhaustion of the mammalian gland was not found possible unless the animal was also bled. This suggests that secretin stimulates both anabolic and katabolic activity of the pancreatic cells, and that anabolism must be otherwise depressed if the exhaustion effect is to be produced . . . The proportion of islet tissue to secreting tissue is also increased by prolonged fasting. In other words disappearance of the stored material of the secretory cell, whether by discharge into the duct, to produce the secretion, or by absorption into the blood and lymph, when the nutrition of the body fails, is attended by increased formation of islets from secretory alveoli . . . Occlusion of the duct causes disappearance of most of the pancreatic tissue in the course of a few weeks. That which escapes destruction assumes a form resembling the islets, but the already existing islets exhibit no special immunity from the destructive effects of the operation." It is Dale's opinion that this change is a "reversion to an embryonic type". Vincent and Thompson have still more recently practically repeated the work of Diamare, Rennie, and Dale. They confirm the morphological results of the two former authors, including the existence in bony fishes of the permanent "principal islet" described by Rennie. On the physiological side they fully confirm the results of Dale, and on this account, coupled with the fact that they find apparently abundant evidence of transformations of tissue in progress apart altogether from experiment in the normal pancreas, they adopt the view that there is a functional interdependence of the two tissues. Their more important conclusions are: "The islet columns are frequently in complete anatomical continuity with the surrounding zymogenous tubules, and all kinds of transition forms are common throughout vertebrates . . . In mammals (dogs and cats), birds (pigeons), and amphibians (frogs) the effect of inanition is to markedly increase the amount of the leptochrome islet tissue, at the expense of the zymogenous tissue. In this condition direct continuity and transition forms are even more marked than in the normal animal. If after a period of inanition, an animal be restored to its normal condition, the pancreas likewise returns to the normal, and the presumption is that alveoli are reconstructed from islets. An increase in the amount of islet tissue may be induced by exhausting the pancreas with secretin . . . Although the islets of Langerhans cannot be regarded as in any sense a tissue *sui generis,* their abundant capillary supply and the nature of their protoplasm suggest that they may have an internal secretion, though we do not consider the evidence on this point to be conclusive."

These views are highly interesting, and based as they are upon the work of experienced investigators demand careful consideration. That the results obtained by these observers involve the general conclusion that the islets "cannot be regarded as in any sense a tissue *sui generis*" appears to me however, premature, and *ex parte* in the sense that such a conclusion does not allow for certain established facts in the morphology of these structures.

I propose therefore to deal briefly with some of the difficulties which appear to me to lie in the way of a full acceptance of this conclusion.

In the first place it is to be noted that the evidence as to the effect of occlusion of the duct is contradictory. Tschassownikow is the latest experimenter in this line, and he finds that the islets are undoubtedly preserved, although they may be altered in size and form through the growth of connective tissue. Dale found as already quoted that in the course of a few weeks most of the pancreatic tissue disappeared, and that "that which escapes destruction assumes a form resembling the islets, but the already existing islets exhibit no special immunity from the destructive effects of the operation". We may be excused if we express no surprise at this last mentioned result after an operation lasting a few weeks one effect of which is to induce an abundant growth of connective tissue. It may be suggested as reconciling the results of these two observers that under the conditions of this experiment degenerative changes of the pancreatic tissue simulating islets do occur, or that the pancreas reverts to "an embryonic condition", and that if the experiment is not too prolonged, the true islets will be found to persist. It is not proved that their disappearance in Dale's experiment is not traceable to secondary causes. In connection with these and analogon experiments there is further the important difficulty of estimating the effect of the experiment, owing to our ignorance of the amount of islet tissue in any individual animal at the commencement of observations. There are admitted individual differences in respect to the amount of islet tissue present, and if it be the case (as held by Dale, and Vincent and Thompson) that this amount is, to express it mathematically, a function of the physiological condition of this individual, then the difficulty of estimating it at the commencement of an experiment is considerable. Dale recognises that the effects produced must be such that they extend beyond the known limits of variation under normal conditions. *This means that abnormal conditions are required to prove his case.* Consequently when he interprets a transformation as possibly a "reversion to an embryonic type" he is probably quite right. It seems abundantly clear from the experiments of Vincent and Thompson that in the experiments with secretin rapid changes do occur, and it is possible that prolonged fasting may indeed produce degenerative changes in which the alveoli revert to embryonic conditions, and that it is possible to detect transition stages of this degeneration. It is of course rather unlikely that these supposed degenerate patches should be absolutely indistinguishable from true islets, and this Vincent and Thompson assert to be the case with regard to the transformed tissue observed by them. We must not however lose sight of the fact that the conditions are abnormal, and that the results afford no proof that such transformations take place under normal conditions in nature. Consequently, so far as the experiments with secretin are concerned we do not think they prove a function in exhaustion for the islets. Vincent and Thompson indeed state that in their experiments with mammals injection of secretin was followed by "a decided increase in the amount of islet tissue" and that this change "which can occur within a few hours can scarcely be interpreted as a morphological one, but must, on the other hand, be considered as representing a phase of physiological activity". This argument is very plausible; it is certainly not conclusive, for the morphological aspect cannot be put aside in this way and the force of it is further greatly weakened by Dale's statement that "exhaustion of the mammalian gland (with secretin) was not found possible unless the animal was also bled". Regarding the results obtained in fasting experiments it is not beyond possibility that the changes observed are degenerative, or as Dale expresses it "reversions to an embryonic type" and that a function in exhaustion is not therein proved, nor need be assumed. That such changes do not invariably accompany even prolonged fasting is proved in the instance quoted in a subsequent part of this paper. One of the cases given by Vincent and Thompson is that of winter frogs. Since hibernation is a natural phenomenon the evidence obtained during this condition ought to be specially valuable, and we are interested to learn that they find" in the pancreas of these animals killed towards the end of the winter a marked increas in leptochrome islet tissue over those killed at the beginning". If such could be found to be the case generally in hibernating animals it would constitute very good evidence for the argument of interchange of these tissues dependent on functional state, particularly in fasting. We find that Carlier (*1*) who has made a detailed histological study of the pancreas of the hibernating hedgehog has not discovered either transitional forms or as far as one can judge unusual proportions of islet tissue. In one instance I obtained a like negative result. To quote Carlier; in a large animal in a profound state of hibernation he found that the pancreas was in an "active condition and that the islets are numerous though small and contain many large cells filled with fine eosinophile granules". p. 344.

In connection with this aspect of the subject we must at this stage refer to a point which much to our surprise has not apparently attracted the attention of other observers.[1]) While arguing in favour of the oneness of the two tissues, Dale, and Vincent and Thompson are all the time perfectly well aware that these islets are very generally more numerous at the splenic end of the pancreas than elsewhere. In fact all of them state quite naïvely, that they took their material for the investigation of this problem from this region, and as the evidence seems to show from no other. But if it is the case that the presence of islets is a question of the physiological condition of the pancreas as a whole, what reason can be given for their appearing more numerously at one part, and that always the same part, rather

[1]) We find that Laguesse has in point of fact emphasised the existence of a permanent juxta-splenic islet in Ophidians. See Vincent and Thompson, p. 90.

than any other? Should they not be found more or less evenly distributed throughout the whole gland? It is abundantly proved that they are not. In certain types, at least, they are habitually found to be unequally distributed in respect that they are aggregated mostly at the splenic end. A group which appears to illustrate this peculiarity well is the Ophidia (See Oppel, Lehrbuch Vergleich. Anatomie p. 805), and it is not without interest in this connection to recall the presence of the relatively large and constantly occurring juxta-splenic "principal islet" of many Teleostean fishes. It has occurred to us to look more particularly for the effects of fasting, not in the part of the pancreas where islets are known to occur more or less numerously at all times, but in a region where if any marked change has taken place as a consequence of inanition such changes will be indisputable. In this connection we record here a case which tests the matter in this particular way.

The Case of a Grass Snake (Tropidonotus natrix).

A grass snake, which in the month of April last year (1907) was placed in a glass jar amongst some dry moss, was through an oversight forgotten. It was discovered in the month of October, having been five and a half months without nourishment, and that at a time when it is accustomed to feed. It had been purchased from a dealer and the date of its last meal was not known. Indeed it is questionable if the animal had broken its fast of the previous winter. When discovered in October it was very feeble, inert, and was evidently at the point of death. It was immediately killed with chloroform, the pancreas was removed along with the spleen to which it closely adheres, cut in two and fixed in corrosive-acetic mixture. After some hours it was washed, dehydrated, embedded and the entire pancreas cut in serial sections of from 10 to 15 microns in thickness. In the anterior half, with the exception of two very small patches which on the first survey were overlooked, the whole series consisted of typical pancreatic acini. In the posterior half of the pancreas no islets were encountered until near the end, where they were found to be numerous, of various sizes, and sometimes very irregular in form. There are parts which taken by themselves exhibit appearances which most strongly suggest transformations in progress. It is undoubted that the two tissues, islets and acini, are frequently in intimate contact; there are places where an acinus appears to pass directly into an islet, where a secreting cell lies adjacent to an islet one. It was doubtless such appearances as these which led Giannelli and Giacomini[1]), Vincent and Thompson, and Dale to conclude that they represented transitions in progress. While prepared to accept these appearances as in general corroborative of the results of Vincent and Thompson's and of Dale's fasting experiments we have important reservations to make. Firstly, with regard to *Tropidonotus* our admission is made rather hesitatingly on account of the fact that not only alveolus and islet of the pancreas are intermingled in a way suggestive of transformation but pancreatic and splenic tissue are to be found in the region where they are in contact to be equally closely associated. It is quite clear that alongside a single column of pancreatic cells are to be seen undoubted islet cells, and on this account taken with the more extensive observations of the forementioned investigators we may assume that transformations in one direction at least take place under certain conditions. We cannot see in this however any argument for the denial of the independence of the two tissues under natural conditions. Prolonged fasting, it appears to us has not been shown to induce transformation in the region of the pancreas where islets normally are rare; it has on the other hand been shown that prolonged fasting (in the case of the snake) fails to do so. What these results suggest to us is that at most a transformation (reversionary or degenerative) of alveolar tissue *in the vicinity of the islets* and possibly proceeding outwards from these takes place. Transformations have not been proved to occur where islets are originally absent.

The transformation theory as held by these workers involves various difficulties. Some of these have been already indicated in the present paper, but one or two points remain still to be dealt with. These relate specially to morphological questions. Vincent and Thompson state that transformations in experiments with secretin take place so

[1]) Quoted by Vincent and Thompson.

rapidly that the change "can scarcely be interpreted as a morphological one". It is a morphological fact whose importance depends entirely upon whether the change indicates normal functional change or whether it is a degenerative one. The former is assumed by the observers and the structural changes in the size of the cell and of the nucleus are thereby accounted for. This implies in particular very rapid reorganisation of the nuclear mechanism with resulting change of function; the change in fact taking place in the course of a few hours. Such an occurrence we have difficulty in realizing and the authors themselves are evidently in the end not satisfied, for on page 95 they state. "As to whether the temporary modification into islet tissue corresponds to a specialisation of function, the evidence is at present inconclusive." One other remarkable fact upon which Vincent and Thompson are silent is that of the blood supply of the pancreatic islets. Obsérvers are agreed that in normal islets there is a rich and distinctive capillary supply. Can it be shown that these transformations into islet tissue of secreting alveoli in fasting, in hibernation, and under the influence of secretin involve a reorganisation of the capillary system in the region of the transformed cells? Such would be welcome and convincing evidence that transformations are of normal occurrence and that the transformed islets of experiments are functionally similar to those occurring naturally. But we find no reference to such a morphological agreement and if it occurs, it ought certainly to be emphasised.

In conclusion it may be observed that all modern investigators are at one that these islets are neither effete nor otherwise useless structures, but ascribe to them an internal secretion. Whether they are normally dependent upon the zymogenous tissue for their existence (which seems unlikely) or not, they are always present, and there seems to be little doubt that they fulfil an important role in vertebrate metabolism. That their function is the control of the amount of sugar in the blood has been the one most frequently suggested, and some experimental work has been put forward in support of this (Rennie & Fraser, Biochemical Journal, II 1906, pp. 7—19), but it cannot as yet be said to have been satisfactorily established.

13

References.

1. Carlier, Journ. Anat. and Physiol. Vol. 30. pp. 334—346. 1896.
2. Dale, Phil. Trans. Roy. Soc. Series B. Vol. 197. pp. 25—46. 1904.
3. Diamare, Intern. Monatsschr. f. Anat. u. Phys. Vol. XVI. S. 155—209. 1899.
4. Helly, Arch. Mikr. Anat. Vol. LXVII. S. 124. 1905.
5. Rennie, Quart. Journ. Micr. Sci. Vol. 48. Part III. pp. 379—405. Nov. 1904.
6. Schulze, Arch. Mikr. Anat. Bd. 56. S. 491. 1900.
7. Tschassownikow, Arch. Mikr. Anat. Bd. 59. S. 286. 1902.
8. Vincent and Thompson, Internat. Monatsschr. f. Anat. u. Phys. Bd. XXIV. Heft 1/3. S. 61—102. 1907.

Note upon a Tachinid Parasite (*Bucentes geniculatus*, D. G.) of Tipula sp. By John Rennie, D.Sc.

(Read 23rd October 1911. Received 23rd October 1911.)

In a collection of Tipula larvæ undergoing examination for Protozoan parasites a dipterous larva was found in the body cavity of a small number of specimens. It was thought worth while to examine the remaining specimens in hand for external signs which might serve to distinguish infected from uninfected individuals. In a certain number of cases it was noted that a yellowish patch was visible beneath the skin, more or less sharply defined, and this was presumed to be the maggot within. Those suspected were sacrificed, and the majority were found to be infected. In the remaining cases the fat body had served to mislead.

At this stage, which was in the month of February of the present year, an attempt was made to keep the extracted maggots alive upon Tipula flesh in salt solution, but this effort was not successful.

With a view to ascertaining whether the parasite occurred widely, Tipula larvæ were obtained from two other sources, and in each case the parasite was found in small numbers. The three localities from which infected larvæ were obtained were Old Meldrum and Clova in Aberdeenshire, and Cornhill in Banffshire. From this it may be presumed that the parasite is generally distributed in Tipula, in the north-eastern counties at all events. From the known habits of the group (Tachinidæ) to which the parasite belongs, it is possible that other insect hosts exist.

The Tipula larvæ were now kept in soil in protected flower-pots, upon which loose, fresh turf was periodically placed and in which oats were also sown. This in due course germinated. From time to time the earth in the pots was turned out and the larvæ examined. Most continued in a healthy condition—firm skinned and curling readily when handled. Some were found in the act of moulting. On 22nd April out of over fifty larvæ, one dead and four very inert specimens were found. These living ones appeared certainly infected; one was sacrificed and from it a maggot obtained. This was preserved alive in a petri dish upon the host tissue. No parasite was found within the dead grub nor within another live suspected one. The remaining suspected larvæ, which were much smaller than the others (about half an inch long), together with some other small examples selected from the general collection, were now placed in soil in a small glass observational jar $3\frac{1}{2} \times 3 \times \frac{1}{2}$ ins. in size, and protected by a muslin cover. The confining of the larvæ to a small quantity of soil served two purposes. It enabled their movements in the soil to be observed through the glass, and it limited the amount of soil to be searched in the event of the maggots leaving their hosts on pupation.

On the following morning (23rd April) it was found that the maggot removed and preserved in the petri dish had pupated. The pupa is reddish in colour, barrel-shaped, about 4 mm. long.

On the 26th April the contents of the glass vessel were examined, and this time only apparently healthy larvæ were found living. The decomposing remains of two larvæ were also found, which were manifestly two of the originally infected forms. In addition, *lying free in the soil were three pupæ* identical with the one obtained from a liberated larva a few days previously.

During the last few days of April and beginning of May, the soil in the flower-pots containing the rest of the larvæ was carefully and systematically searched with the aid of a large hand magnifier, and no pupæ were obtained. Nor did any of the larvæ living show signs externally of containing a maggot.

The four pupæ were preserved in a shallow glass vessel in a thin layer of loose soil, and covered by a glass plate. The soil was kept very slightly moist and exposed to the warmth of the sun from time to time.

On 23rd May, exactly one month after the first pupa was obtained, a fly hatched out, and on the following day the hatching of other two took place. These were placed alive in a wide test-tube along with some grass and a slice of potato. They are exceeding active flies. They flew energetically about the tube, ran up and down upon the grass, brushed their wings and heads with their feet, and generally groomed themselves in a perfectly healthy manner. Meantime their structural features were noted and the species identified as *Bucentes geniculatus*, De Geer. It was hoped that the flies might live for some time, and that it would be possible to observe their habits. One, however, died on the 24th. Meantime endeavours were maintained to provide suitable food for the others, and as the species is known as a frequent visitor to a considerable number of flowers, various flowers rich both in pollen and nectar were placed beside the flies, but while they ran about upon these, they were never seen to feed, although a good deal of time was spent watching their movements closely.

One of the most striking features about these flies is their proboscis, which is very long and bent upon itself like the blade and handle of a clasp-knife. This they were frequently seen to unfold and stretch in front of the head, touching things with it, but they were never seen to probe into flowers, or, as far as could be judged, use it in feeding. Up till the evening of 26th May both flies were lively and apparently well, but during the following day one died, and on the succeeding morning the last one was found also dead.

Description of the Fly.

As already noted the fly has been identified as *Bucentes geniculatus*, De Geer, a member of the Family Tachinidæ. This family is a very large one, comprising an enormous number of species whose larvæ live as parasites upon other insects, chiefly caterpillars.

The fly is blackish looking, rather smaller than an ordinary housefly, with numerous bristles on the body, particularly upon the abdomen, and with large, pale squamæ above the halteres. A distinctive generic character is the long, slender filiform proboscis which is geniculated at the base and at the middle. The free extremity is bent downward and directed backward when not in use. In the act of extension, it is bent first downwards and then upward and forward. The first and second joints of the antennæ are tawny in colour, and the arista bears very fine and extremely short hairs. The legs are tawny with black tarsi. The wing characters are as figured. The fly is very common, but the only account I have been able to find regarding its habits is the reference given in Knuth's *Handbook of Flower Pollination*, where it is reported as a visitor to numerous flowers. As these include early and late flowering species (*Viola lutea* and *Mentha aquatica*) the imaginal period probably extends through the whole summer. The latter plant does not flower in this country until August. From the character of its proboscis, the fly is likely to collect nectar from moderately sized flowers with partially concealed nectar. Theobald mentions (*Second Report on Economic Zoology*) having found a Tachinid in a cage in which Tipulæ were being reared, but from this indefinite reference one can only regard the identity of the species as probable.[1]

The Larva.

The internal structure of the larva has not been examined. The largest examples found measured 7 mm. in length, by 1¾ mm. at the broadest part of the body. At each segment the body is encircled by four rows of

[1] The following records have since been found in Brauer und Bergenstamm: Die Zweiflüger des Kaiserlichen Museums Zu Wien. Denk. Akad. Wiss. lxi. Bd., 1894, p. 537.

Siphona (Bucentes) geniculata (Fll. Rdi. Ins. p.) in *Mamestra brassicæ* L.

Siphona (Bucentes) cristata (Beling. Verh. K. K. Zool. bot. G. 1886, 171) in *Tipula gigantea*, Schrk.

minute hooks. There are two sets of spiracles (amphipneustic). The pair at the hinder end of the body is shortly bilobed, the anterior pair, in line with the mouth, is fan shaped and eight-lobed. An interesting fact is that in a number of cases the larva, when found, was holding on firmly to one of the larger tracheal trunks of the host, by means of its hooks. Unfortunately so far the exact relations to the tracheal system have not been made out, and the fact just recorded may have no particular significance. But it may be recalled here how, in the case of *Ugimyia sericaria*, a Tachinid parasite of the silkworm caterpillar in Japan, the maggot early "enters the tracheal system, boring into a tube near a stigmatic orifice of the silkworm, where it forms a chamber for itself by biting portions of the tissues and fastening them together with saliva. In this it completes its growth, feeding on the interior of the silkworm with its anterior part, and breathing through the stigmatic orifice of its host" (Sasaki: quoted from Sharp, Camb. Nat. Hist.). The maggot in the Tipula larva was never found near the stigmata, which are terminal, but was always situated in the middle region of the body. It may also be mentioned here that in several cases two maggots were found.

The hook apparatus of the maggot is moderately complex and may be briefly described. There is a large basal plate bearing a pair of wings or lateral plates with rounded posterior margins. In front of the median plate lies the hook apparatus proper. This consists of a ladder-like arrangement of cross bars bearing at its anterior end a pair of hooks. These hooks have a pair of rounded extensions at their base directed both forward and backward.

(*Issued separately*, 22*nd January* 1912.)

(From *Proc. Royal Physical Society, Edinburgh*, vol. xviii., pt. 4, no. *xxiv.*; the original pagination is without parenthesis.)

The Cestoda of the Scottish National Antarctic Expedition. By John Rennie, D.Sc., and Alexander Reid, M.A., University of Aberdeen. (With Two Plates.)

(MS. received May 6, 1912. Read June 17, 1912. Issued separately September 6, 1912.)

The Cestode material obtained by the *Scotia* Expedition consisted of eight adult and three larvæ or immature forms. Of these, one (*Anchistrocephalus microcephalus*, Rud.) is not Antarctic, having been obtained from the Sunfish (*Orthagoriscus mola*), at Station 107, in 39° 12′ S., 53° 44′ W., on January 1, 1903.

Of the others, only two appear to have been previously described, viz. *Dibothriocephalus antarcticus*, Baird, and *Dibothriocephalus wilsoni*, Shipley. The hosts from which the Cestoda of the Antarctic and sub-Antarctic regions were obtained are, with the exception of the Bonito,* from which a larval *Tetrarhynchus* was obtained, Seals and Penguins. In view of this fact, the number of forms obtained may be regarded as relatively large. A study of the species on record from Arctic Pinnipedia suggests the interesting fact that the two Cestode faunas are quite distinct. Eight species of *Dibothriocephalus* are on record from Pinnipedia of the Arctic regions, none of which have so far been obtained in the Antarctic. The adult forms found, however, with one exception all belong to this genus.

A noteworthy feature is the relatively large proportion of very small and delicate species of Cestoda occurring in the Pinnipedia of the Antarctic. Indeed, none of the forms obtained can be described as large; the maximum size is that of *D. pygoscelis*, viz. 29 cm.

Shipley has suggested with regard to the Cestoda of Ross's Seal that, in view of the feebleness and variability of its dentition, it probably feeds on soft substances, and expresses the opinion that the plerocercoid stage probably occurs in the tissues of Cephalopods. Jellyfish are also mentioned, and these form part of the food of this Seal.

With regard to Seal Cestodes in general, we note that although the parasites are small the infection is generally heavy, and from this it may be argued as probable that the intermediate hosts become infected without much difficulty. The embryos are extraordinarily minute, and if dissipated in the waters would probably infect drifting organisms, *e.g.* Jellyfish or Ctenophora, more readily than others, *e.g.* Fishes. On the other hand, Crustacea and similar organisms of scavenging habits, feeding on the fæces of the Seals, have an even better chance of being infected, and these may provide the intermediate host. Beyond this it is scarcely profitable to speculate further.

* This fish (*Thynnus pelamys* Linn.) it appears was found at Station 31—some distance south of the Cape Verde Islands—on 4th December 1902, and its parasite therefore cannot be described as Antarctic or sub-Antarctic.

(REPRINTED FROM THE TRANSACTIONS OF THE ROYAL SOCIETY OF EDINBURGH, VOL. XLVIII., PP. 441–453.)

Description of Species.

Ariola (1) divides the family Bothriocephalidæ as under :—

Sub-families :

Diplogoninæ. Two sets of gonads to each segment.

Mesogoninæ. A single set of gonads ; genital apertures on surface, All the Bothriocephalidæ found, with one exception, belong to this group.

Pleurogoninæ. Marginal genital apertures.

One of the species found, *Anchistrocephalus microcephalus*, Rud., belongs to this group.

Order PSEUDOPHYLLIDEA, Carus.

Family Dibothriocephalidæ, Lühe.

Sub-family mesogoninæ (Ariola).

Genus *Dibothriocephalus* (Lühe).

Dibothriocephalus scoticus, n. sp. (Pl. I. figs. 1 and 2.)

This form occurred in the intestine of a Sea-leopard (*Stenorhynchus leptonyx*).

The maximum dimensions are: strobila, length 13·3 cm., breadth 6·8 mm. ; scolex, 2·5 mm. by 1·5 mm. The scolex when fully extended shows a pair of dorso-ventral suckers widely gaping posteriorly and tapering towards the tip, which is rather sharply conical. The sucker lips are rather thin, almost weak.

No distinct neck is present. The proglottides are fairly broad, with well-marked backwardly directed flanged margins. Anteriorly they are roughly rectangular, much shorter than broad ; in the mature parts of the strobila they become relatively longer.

The cuticle is rather thin, and beneath it an extremely fine circular layer of muscle can be made out with difficulty. Next this is a longitudinal layer, also slightly developed. This longitudinal layer lies between the narrowed ends of the cellular subcuticula, whose elements form a clearly defined band. Following on this is the layer of the yolk follicles, which, in the mature segments, except at the level of the uterus and cirrus sac, forms a practically continuous band. Within this occur two muscle layers, a well-defined longitudinal layer outermost ; while, within, a thin circular band separates the peripheral area from the central.

The longitudinal nerve cords are placed about one-fourth of the transverse diameter from the margin.

In the mature segments there are about nine testes follicles external and about six internal to the nerve cord on each side.

Central longitudinal excretory canals were not observed, but there are numerous small peripheral canals in the subcuticula just external to the yolk follicles.

The yolk cells are extremely variable in form and size, and may be described as amœboid in appearance. The largest measure ·017 mm ; the smallest noted, ·012 mm. There is a well-marked yolk reservoir of pear shape between the two lobes of the ovary.

The ovary in transverse section appears as an elongated band, becoming shorter and thicker in its more anterior parts. The larger cells measure ·014 mm. in diameter.

The uterus consists of a winding tube of about four loops, the limbs of which in transverse section are seen to wind both dorsally and ventrally. There is a large dilated space just at the opening. On external view, the uterus in mature segments appears clustered in a rounded mass posteriorly, the winding portion being distinct only in its more anterior region. The shelled ova vary in size; the smallest measure ·070 × ·043 mm., and the largest ·082 × ·048 mm. They are operculate.

The testis is very large, consisting of numerous follicles occupying the greater part of the medullary region. The follicles are more or less spherical in form, and lie in close-set rows extending across the whole width of the proglottis, being separated from each other by the dorso-ventral muscles, which are correspondingly numerous. A common size of a follicle in a mature proglottis is ·069 to ·087 mm. in maximum diameter.

The cirrus sac is thick-walled, and oval in transverse section, presenting no distinctive peculiarities.

From the foregoing description, it appears that this species has not been previously observed. From the same host, von Linstow (3) has described *D. quadratus*, and with his account a careful comparison has been made. The scolex in the two species is very similar in general form. In *D. quadratus* it measures 1·3 mm. by ·71 mm., or about half the dimensions of the present species. The strobila is 22·5 cm. long, and 3·5 mm. broad at its widest part; the proportions of the present species are, it will be observed, altogether different. The longitudinal dimension of the ova given by von Linstow is ·055 mm., which is considerably less than the smallest measurement observed in *D. scoticus*. The shelled ova are in *D. quadratus* described as non-operculate; in the present species they are clearly operculate. Further, the appearance of the yolk follicles is quite different in the two species.

A comparison has also been made with other *Dibothriocephalus* species recorded in Pinnipedia, with like negative results.

In honour of the Scottish Expedition, we have named this new species *Dibothriocephalus scoticus.*

Dibothriocephalus coatsi, n. sp. (Pl. I. figs. 5 and 6.)

In *Stenorhynchus leptonyx* there occurred along with *Bothriocephalus scoticus* a number of specimens of a small, hitherto unrecorded Cestode.

The total length of strobila of the examples found is from 42 to 80 mm. In a specimen of 42 mm. the width at the broadest part, which is 23 mm. from the anterior end, is 1·8 mm.

The scolex is of distinctive appearance, being long, blunt, and of almost uniform width, measuring 2 mm. by ·75 mm. in extent. There is a pair of shallow, widely gaping suckers, dorso-ventrally placed, extending the whole length, and open at both ends (fig. 5).

The mature segments are rectangular in form, with slightly undulating margin. In the specimen 42 mm. long, the largest, which were terminal, measured ·61 mm. long by 1·04 mm. broad. They are also relatively thick, measuring in section ·61 mm. dorso-ventrally.

The cuticula and sub-cuticula are of typical appearance. Beneath the sub-cuticula are the yolk follicles. These are very numerous, and in many sections, *e.g.* those at the level of the ovary, they form a practically continuous band. The individual yolk cells, which vary in form, measure on an average about ·014 mm. by ·017 mm.

The shape of the ovary presents no unusual features. In a section at the level of its junction with the yolk ducts it has the form of a transverse band. Posteriorly to this it appears as a pair of detached, more or less rounded, and thicker masses. The ovarian cells measure ·017 mm. by ·01 mm.

The uterus consists of a few close coils which wind dorso-ventrally, so that in section it usually has the appearance of an almost complete circle. The shelled ova measure ·052 mm. by ·041 mm.

The testis follicles, which occupy the greater part of the central area of the proglottis, measure in their greatest dimensions ·034 mm. by ·052 mm.

There is a well-developed inner layer of longitudinal muscles; the dorso-ventral muscles are also well marked.

The longitudinal nerve cords are extremely ill-defined and weak, although relatively large. They are placed slightly less than one-fourth of the width of the proglottis from the margin, and are slightly nearer to the ventral than the dorsal surface.

The central longitudinal excretory canals can be made out only in places. They are placed at the extreme lateral margin of the central layer, next to the longitudinal muscles, but, as they frequently cannot be traced in serial sections, they probably anastomose a good deal. Peripheral canals are present just exterior to the yolk follicles. These are most clearly visible at the lateral margins, where two or three frequently occur close together.

This form differs in most particulars from all the hitherto described species of the group to which it belongs, and we have therefore classed it as new, naming it *Dibothriocephalus coatsi.* It is an interesting fact that two new species should have been obtained from *Stenorhynchus* by the Scottish Expedition, and that *D. quadratus*, the only form hitherto described from this host, should not have been found.

Dibothriocephalus antarcticus.

Bothriocephalus antarcticus, Baird, 1853.

About a dozen or more examples of this species were obtained from the stomach of a Ross's Seal, *Ommatophoca rossi*. These were all smaller than BAIRD's specimens, which were about 9 inches long. The *Scotia* examples range from 132 to 29 mm., but most measure about 100 mm. BAIRD's (2) description is rather brief and confined to externals, but from this, together with his excellent figure, there is no mistaking the identity of the *Scotia* specimens with his type.

This species was also found by the *Discovery* Expedition, and the specimens are described by SHIPLEY, to whose account reference is made below.

Dibothriocephalus antarcticus, Baird (2), is a slender-bodied worm, with a conical scolex and with fairly sharp tapering point. The two suckers are long and comparatively deep. According to BAIRD, there are "two small rounded projecting lobes" at their posterior margins, but these in the *Scotia* examples are only occasionally present, and appear to be dependent upon the state of contraction of the animal. There is no neck; the anterior part of the body for some distance behind the scolex is rounded, resembling an annelid in form; in the more posterior part the form is thick and flattened, being here elliptical in section. The colour is reddish yellow. The segments, even in the mature part of the animal, are very short; they are deeply constricted off from each other, and as the free margins are directed backward the segments appear to overlap more than they actually do. The only dimensions given by BAIRD are: "length, about nine inches; greatest breadth of body, about three lines," and although the *Scotia* examples are very much smaller, the proportions agree well. The *Discovery* specimens come much nearer in length to the *Scotia* examples, although there is a very distinct discrepancy as regards width. SHIPLEY reports that most of the *Discovery* examples "were just under 10 cm.," and that "the greatest breadth is 7 mm. in the largest specimen." The longest *Scotia* worm is 13·2 cm., and its greatest breadth is 4·5 mm.; most of the specimens are about 4 mm. in width. Again, as regards scolex dimensions, SHIPLEY gives "3 mm. in length and 3 mm. in breadth posteriorly." In none of the *Scotia* specimens is the greatest breadth equal to the length of the head; they measure from 3 to 3·5 mm. long by 2 mm. wide. The actual differences here, however, are slight.

A general account of the anatomy is given by SHIPLEY (4). He mentions that, besides the two longitudinal canals of the excretory system, "there are also small canals which lie close under the surface at the edges of the proglottides, usually two at each side, but they also break up from time to time into twisting branchlets." These canals appear to be very numerous; from 42 to 45 may be present in a section, while at each lateral margin a group of four can usually be made out.

The testes which occupy the central layer lie mostly towards the dorsal surface. There are from 18 to 20 follicles in a transverse section.

Dibothriocephalus wilsoni, Shipley. (Pl. I. fig. 4.)

This small tapeworm, which Shipley (4) has already referred to as "very attractive" in appearance, was also found by the *Scotia* investigators, although not in the same host. These were obtained in the intestine of Weddell's Seal along with numbers of *Bothriocephalus mobilis*, n. sp. The *Discovery* specimens occurred in Ross's Seal (*Ommatophoca rossi*).

It is a small, semi-translucent, delicate-looking Cestode, not undeserving of Shipley's epithet. The scolex is short and conical in the contracted state, as appears in Shipley's figure. In more extended specimens, however, it is more rounded at the free end, as well as longer. An interesting point is the early appearance of mature segments; the first of these may be but the fifth behind the head. Shipley's dimensions for this species are: length, 4 to 5·5 mm.; greatest breadth, 1 mm.; 9 to 13 proglottides; scolex, ·5 mm. Some of the *Scotia* specimens are quite 10 mm. in length, and have 18 segments; one which measured less than 4 mm. contained 8 segments, none of which were mature, but in 5 of which the outline of the developing uterus and other sex ducts could be traced in a surface view.

The only other point made out with regard to which Shipley's account may be supplemented refers to the dimensions of the ova. His figures are ·042 by ·035 mm., and these he gives as about the average. We find the ova do vary in size, and while we have not struck an average figure, we think that on the whole the dimensions we have to quote are fairly common and typical. These are ·069 by ·037 mm.

The general appearance of this Cestode is given in fig. 4.

Dibothriocephalus mobilis, n. sp. (Pl. II. figs. 7 to 10.)

This is an extremely pretty little Cestode, highly translucent, which was found in the intestine of Weddell's Seal, where it occurred in considerable numbers. It measures from 12 to 20 mm. in length, and is about 2 mm. at its widest part. The scolex is broad at its free end, narrowing towards its junction with the strobila. It measures ·5 mm. in diameter. The suckers are lateral in position, deep and widely gaping the whole length of the scolex, and having extremely mobile lips. They are capable of extension backward, showing in such a case large posteriorly directed flaps. Owing to the extreme mobility of the scolex, it is rather variable in form, although its general appearance remains characteristic (figs. 7 and 8).

No neck is present. The segments are rectangular, at first narrow, being about twice as broad as long, lastly becoming practically square at the posterior end. The number varies from about 16 to 25; they are mature about the 7th or 8th segment. On a surface view the genital pores are seen to lie together close to the anterior border of the proglottis.

The uterine pores are placed for the most part alternately right and left of the middle line in successive segments. The uterus in the immature segments shows three loops to each side; in the mature parts it appears as a rounded mass filled with the shelled ova. The yolk follicles are exceedingly numerous in the mature segments, lying closely over the whole of the inner part of the peripheral layer and visible externally. They form morula-like masses, more or less irregular in shape. In section they are mainly circular, and measure ·052 mm. in diameter. The individual yolk cells are large, measuring when fully grown about ·016 mm.

The ovary consists of a pair of flattened circular masses, which in their maximum development measure ·174 mm. in diameter, connected by a narrow band in the middle. The shelled ova measure ·051 mm. × ·034 mm. They are not operculate.

The testes follicles, which occupy the central area, occur in the planes between those occupied by the yolk follicles. Where the yolk follicles occur the central area is narrowed, and only the extreme ends of the individual testes appear here. The individual cells of the testes are extremely small. The cirrus sac is highly muscular, circular in form; the short protrusible penis is relatively thick.

The cuticle is remarkably thick, measuring ·014 mm.; the sub-cuticle consists of fairly large cells of irregular shape, amongst which the small excretory canals occur. These are fairly numerous, viz. between 30 and 40. The rest of the body consists largely of a thin and loosely packed parenchyma.

This form is clearly differentiated from all the other small Bothriocephalids in the laterally placed suckers and distinctive form of the scolex, size and general shape of the proglottides, nature of the yolk follicles, and characteristics of the ova.

We propose for it the name of *Dibothriocephalus mobilis.*

Dibothriocephalus pygoscelis, n. sp. (Pl. II. figs. 11 and 12.)

A small quantity of Cestode material, undated, and labelled, "Adult tapeworms from some species of *Pygoscelis*, probably *P. antarctica* or *P. adelia*; possibly, though not likely, *P. papua*," was found to consist of a number of extremely brittle fragments of a *Dibothriocephalus.** Only one or two head pieces could be found, the larger of which measured 29 cm. Fragments up to 21 cm. in length occur in the collection.

The scolex measures 1·8 mm. in length, is of almost uniform breadth, slightly broader at the posterior border, where it measures ·7 mm. in diameter. The suckers are long and shallow, forming a pair of dorso-ventral grooves, extending nearly the whole length of the scolex.

There is a short neck; the anterior proglottides are markedly flanged, and at least four times as broad as long. In the broadest part of the worm they reach 9 mm. in breadth and about 1·5 mm. in width. The common genital pore can be seen upon the ventral surface as a rather broad crescentic slit, a little way behind the anterior border, while the uterine pore is placed slightly behind in the middle line.

* This was found by Dr PIRIE lying on the snow near the beach at Scotia Bay, South Orkneys, where a number of penguins had been congregated—chiefly *P. antarctica* and *P. adelia*,—January 11, 1904. See *Zoological Log*, p. 95, including footnote.

The following additional points have been made out.

The cuticula and sub-cuticula are well developed. Peripheral excretory canals are numerous. The yolk follicles are very numerous and large. In longitudinal section they appear as closely arranged, long, narrow bands, sometimes spindle-shaped, extending from the sub-cuticula to the longitudinal muscle layer, which is well marked.

The uterus has four or more turns, winding dorsally and ventrally in a spiral manner (fig. 12). The shelled ova vary in size. A common dimension is: length ·073 mm., breadth ·051 mm. But there is a small proportion of long and narrow eggs measuring ·100 mm. by ·041 mm. The eggs are operculate.

The species appears to be unrecorded previously. No *Dibothriocephalus* species have hitherto been described from either Arctic or Antarctic birds. It resembles generally the scolex of *D. quadratus* in form and dimensions, but the proglottides are smaller and the ova dimensions are dissimilar; it resembles *D. cordatus* in the dimensions of the eggs, but disagrees in other features. *D. lanceolatus* is a much smaller form. In general features *D. pygoscelis* resembles *D. romeri*, but is on the whole larger, and again the egg dimensions are greater. In particular, the specially large size of the shelled ova and form of the scolex differentiate it from all other described Arctic or Antarctic species occurring in either birds or Pinnipedia.

We propose to name it *Dibothriocephalus pygoscelis.*

Sub-family PLEUROGONINÆ (Ariola).

Genus *Anchistrocephalus*, Monticelli, 1890.

SYNONYMS.

Tænia, Auctorum.
Bothriocephalus, Rudolphi, 1808.
Dibothrium, Diesing, 1850.
Polyonchobothrium, Diesing, 1850.
Anchistrocephalus, Monticelli, 1890.

Anchistrocephalus microcephalus (Rud.), 1819. (Pl. I. fig. 3.)

This tapeworm was found in very large numbers in the intestine of the Sunfish, *Orthagoriscus mola*, in a mass weighing several pounds, and almost completely blocking the intestine. *O. mola* was captured at Station 107.

It was first described by RUDOLPHI, in 1810, and its occurrence has since been noted and its anatomy described by other investigators. It is a readily recognisable species, and does not appear to have been recorded in any host other than the Sunfish. The scolex has a pair of rather deep, open, thick-margined, square-looking suckers topped by a hemispherical rostellum, the base of which is encircled by several close-set rows of small hooks (fig. 3 (*a*)).

The genital pores are marginal in position (fig. 3 (*b*)).

The appearance of the scolex varies with the state of contraction, and the rostellum may be retracted so as to be concealed below the anterior margins of the suckers, and thus appear to be absent. Similarly, the anterior proglottides, which in the extended condition are rather long, with thick, overlapping posterior margins (described by ARIOLA as "campanulate"), in the contracted condition become rectangular, short, and relatively very broad. The maximum size occurring in the *Scotia* specimens is 40 cm. by 5·5 mm., which is considerably less than that given by ARIOLA, viz. 66 cm. by 7·5 mm. This, which appears surprising in view of the large number of examples in the collections, is probably due to breakages. The specimens occurred very closely matted together, and there are numerous fragments without scolices. ARIOLA (1) has given a summary of the chief features of this species.

The following additional points have been made out in transverse sections.

The longitudinal nerve cords, which are large and well defined, are situated about one-fifth of the transverse diameter from the margin, external to the longitudinal excretory canals.

The central excretory canals are six in number, three each, right and left of the middle line.

Order CYCLOPHYLLIDEA, van Ben.

Family TÆNIIDÆ, Ludw.

Hymenolepis, sp. (?).

The Cestode here described was found in the intestine of the Ringed Penguin, *Pygoscelis antarctica*—locality, South Orkneys. It occurred in groups of from four to twelve, having their heads within a small swelling upon the intestine of about the size of a pea. The swelling, which had brownish granular contents, projected upon the outer side of the intestine. The heads appeared, as far as could be made out, to lie freely in the cavity formed by the swelling or cyst. This opened to the intestinal cavity by a very narrow aperture through which the closely grouped necks of the worms passed.

The "heads" are of very irregular and variable form. This anterior region is best described as a "pseudo-scolex." The "neck" is very long, and in most cases is at one part enlarged in a long oval form. The segmented portion is nearly cylindrical—not flattened—and, apart from colour, has quite an annelid appearance. The following measurements were made :—

Length of "neck," 6–12 mm.
Width of "neck" at broadest part, ·93 mm. to 1·13 mm.
Length of segmented region, about 1 cm.
Number of segments, about 40.
Diameter of broadest segment, 1·21 mm.

As already stated, the "heads" are very irregular in form. In the neck region calcareous corpuscles are very numerous.

The oldest proglottides are sexually immature. Only the testes are developed; they lie in the middle layer, occupying the area between the excretory vessels. There are from 16 to 19 follicles in a cross-section through their region of greatest development. The follicles are oval in section and measure from ·019 mm. to ·038 mm. along their longer axis.

Calcareous corpuscles are extremely abundant, especially in the cortical area; they are oval or circular in form, and measure from ·0063 mm. to ·0095 mm.

There is a pair of longitudinal excretory vessels on each side, placed dorsal and ventral, but quite near to each other; only the larger pair appears to be connected by transverse vessels. Both pairs have thick walls.

The longitudinal nerve cords, which lie outside but near to the excretory canals, are very ill-defined.

The question whether this type is normal is somewhat difficult to determine. The ill-defined nature of the scolex region is rather against such a view. MEGNIN (quoted by BRAUN) considers that the pseudo-scolex condition is characteristic of the very old stages of worms, but in the present case the worms are immature. Again, this condition may be a case of retarded development. This is not altogether impossible, in view of the marked pathological condition set up in the intestine at the point of attachment, and the occurrence of the parasites in groups within a single cyst, both of which conditions are unusual in other cases of Cestode fixation. On the other hand, their occurrence in this way in several different specimens suggests that the features described are usual with this species.

What positive structural data are available are not sufficient to permit of exact classification, but the type may provisionally be placed near the genus *Hymenolepis* on account of the shape of the segments, the character of the neck, and the limited number of the testes.

Order TETRAPHYLLIDEA, Carus.

Family PHYLLOBOTHRIIDÆ, Braun.

Phyllobothrium, sp. (Pl. II. figs. 3 and 4.)

From the areolar tissue under the blubber of Weddell's Seal there were found on two occasions examples of a bladder-worm whose features, especially those of the scolex. are characteristic of the genus *Phyllobothrium*. One of the specimens is incomplete.

The complete specimen consists of a scolex having four much-plaited or folded bothria. Accessory suckers are absent. Behind the scolex is a neck piece slightly flattened, 17 mm. long and about 2 mm. broad. Behind the neck is a long oval bladder, creased or wrinkled upon the surface, thick-walled and hollow, with terminal pore or slightly inverted posterior end. The bladder measures 32 mm. in length, and at its widest part is 10 mm. in diameter.

The incomplete specimen is of interest in so far as it shows a portion of the neck invaginated within the bladder. Since this is the condition in which cysticerci usually occur in the tissues of their host, the existence of another specimen in the fully extended condition in such a situation is worthy of special note.

The presence of these larval Cestodes in the subcutaneous tissue of an animal such as Weddell's Seal is of particular interest. The hosts of adult *Phyllobothria* are, as far as known, mostly Selachians.

With regard to the question as to the probable host of the adult worm, Dr BRUCE has made the interesting suggestion that this may be the Grampus. He informs me that *Stenorhynchus leptonyx* and *Lobodon carcinophaga* are frequently seen with large gashes upon their sides, which he is of opinion may be due to the attacks of a Grampus (*Orca*, sp. ?). He considers it likely that Weddell's Seal is liable to similar attacks, and in fact that the whole seal may at times be eaten. The following birds are fond of blubber, and devour the carcases of seals, viz. the Giant Petrel (*Ossifraga gigantea*), Sheathbill (*Chinois alba*), and Skuas (*Magalestris MacCormicki* and *M. antarctica*). Such habits render them liable to infection with the bladder-worm in question, and it is possible that the normal host of the adult occurs amongst these.

Order TETRARHYNCHA, v. Ben.

Family TETRARHYNCHIDÆ.

Tetrarhynchus, sp. (Pl. II. figs. 15 to 18.)

From the muscles of the Bonito (*Thynnus pelamys* Linn.) caught at Station 31, a small number of cysticercoids of a *Tetrarhynchus*-like organism were found. These were not enclosed in a bladder, but lay quite free in the muscles, the proboscides being in a number of instances partially extruded. They were not in any instance fully extended.

The specimens measure about 6 mm. in length and $1\frac{1}{2}$ to $1\frac{3}{4}$ mm. in width. There is a thick, firm, slightly wrinkled, glistening cuticle upon the exterior. A distinctive feature is the entire absence of suckers at the anterior end. There are four slender retractile proboscides bearing about sixteen longitudinal rows of closely set, recurved hooks. The proboscides are connected with four well-developed muscular bulbs, such as are characteristic of this group.

At the posterior end there is a small spherical bulb which is retractile within a cavity. In most examples the bulb is within, but in one or two instances it occurred exserted, the body of the cysticercoid being constricted closely around its base (fig. 15).

Transverse sections of the bulb show it to contain a deeply staining connective tissue in which there is a transverse row of ten or twelve excretory canals (fig. 18). These merge in each other, converging to a terminal pore. Longitudinal sections show the branches of the canals to be very numerous.

The body of the cysticercoid consists of a peripheral and a central portion. The former is limited by a well-defined, thick cuticle, contains numerous excretory vessels (about 60 in transverse section) and a loose parenchyma. The central region contains the muscular bulbs of the proboscides, and around these a well-developed mass of longitudinal muscles (fig. 16). The central area at its posterior end merges into the protrusible bulb (fig. 17).

The question of the more exact identification of the species to which the form belongs must be left undecided.

G. R. Wagener (5) has described a similar form from *Phycis mediterranea*.

LITERATURE.

(1) Ariola, V., "Revisione della famiglia Bothriocephalidæ s. Str.," *Arch. Parasitol.*, iii. No. 3, 1900.

(2) Baird, *Proc. Zool. Soc. London*, 1853.

(3) von Linstow, *Jahrb. Hamb. Wissensch. Anst.*, ix. Jahrg., 1891.

(4) Shipley, A. E., *National Antarctic Expedition Reports*, 1907, "Cestoda," vol. iii.

(5) Wagener, G. R., *Verhdlgn. (Nov. Act.) d. K. Leop.-Carol. Acad. d. Naturf.*, Bd. xxiv. Suppl., Breslau, 1854.

(6) Zschokke, *Fauna Arctica*, "Die Arktischen Cestoden," Bd. iii., Lieferung i., 1903.

REFERENCES TO FIGURES.

c. = cuticula.
c.s. = cirrus sac.
exc.c. = excretory canals.
exc.b. = excretory bulb.
l.m. = longitudinal musculature.
n.c. = nerve cord.
o. = ovary.
per.a. = peripheral area.
r.m. = retractor muscles of proboscides.
s.c. = sub-cuticula.
sh.ov. = shelled ova.
t.f. = testes follicles.
ut. = uterus.
v. = vagina.
y.c. = yolk cells.

EXPLANATION OF PLATES.

Plate I.

Fig. 1. Transverse section of *Dibothriocephalus scoticus*, n. sp., at the level of the ovary.

Fig. 2. (*a*) Entire specimen of *D. scoticus*; (*b*) scolex of *D. scoticus*.

Fig. 3. (*a*) Anterior end of *Anchistrocephalus microcephalus*; (*b*) immature proglottis of *Anchistrocephalus microcephalus*.

Fig. 4. Four specimens of *Dibothriocephalus wilsoni*, Shipley.

Fig. 5. Scolex of *Dibothriocephalus coatsi*, n. sp.

Fig. 6. Transverse section of *D. coatsi*.

PLATE II.

Fig. 7. Entire specimen of *Dibothriocephalus mobilis*, n. sp.

Fig. 8. Scolices of *D. mobilis*, n. sp.

Fig. 9. Transverse section of proglottis of *D. mobilis*.

Fig. 10. „ „ through uterus and cirrus sac of *D. mobilis*.

Fig. 11. Proglottis of *Dibothriocephalus pygoscelis*, n. sp.

Fig. 12. Diagrammatic longitudinal section of proglottides of *D. pygoscelis*, showing position of sex openings and uterine coils.

Fig. 13. Metacestode of *Phyllobothrium* sp., from blubber of Weddell's Seal.

Fig. 14. Scolex of *Phyllobothrium* sp.

Fig. 15. Larval *Tetrarhynchus* from the muscles of the Bonito.

Fig. 16. Transverse section of larval *Tetrarhynchus* through retractor muscles of proboscides.

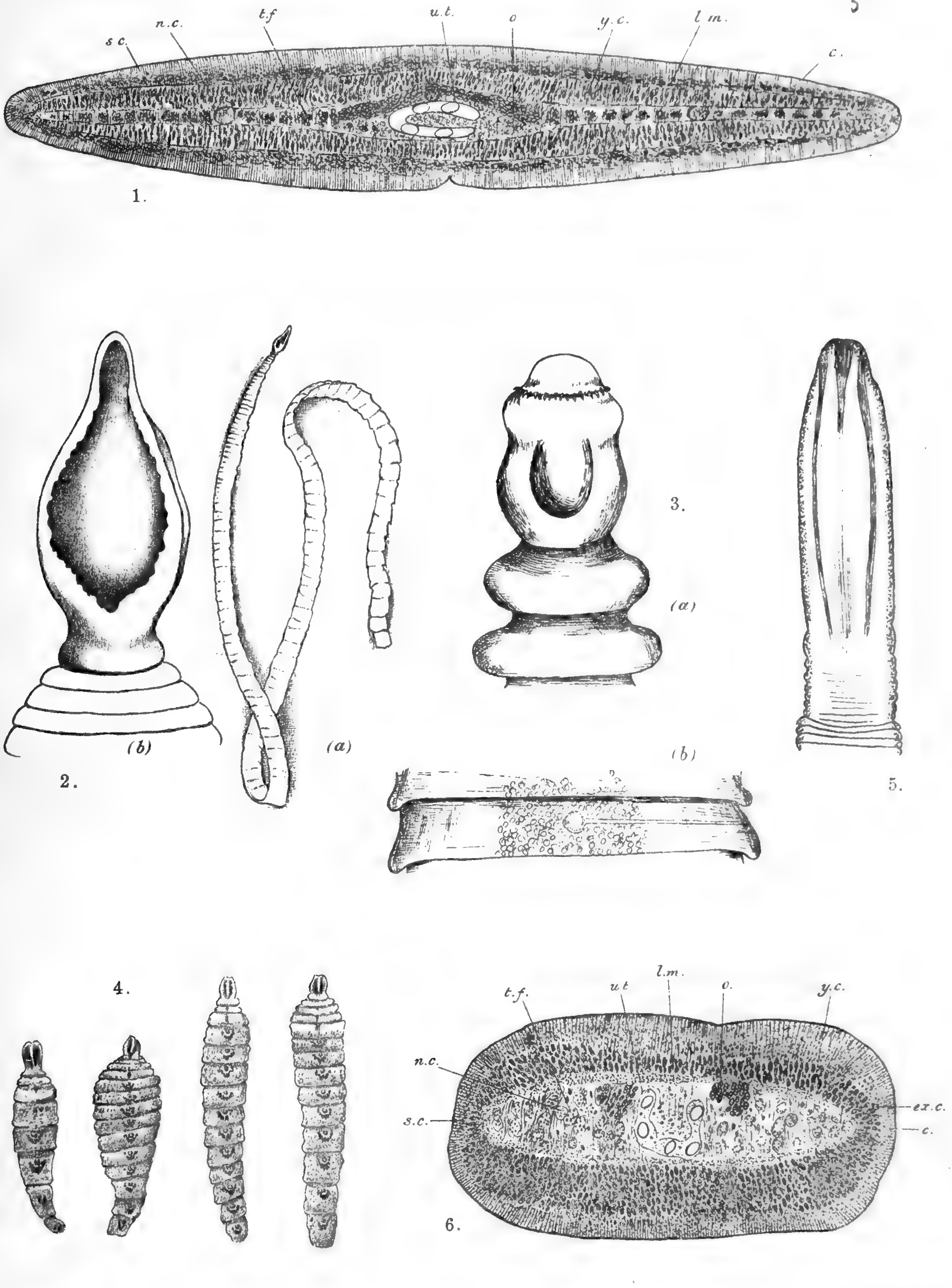
Fig. 17. Diagram of posterior end of larval *Tetrarhynchus* showing excretory bulb retracted.

Fig. 18. Transverse section of larval *Tetrarhynchus* through retracted bulb, showing row of excretory vessels.

32

RENNIE AND REID: "SCOTIA" CESTODA—PLATE I.

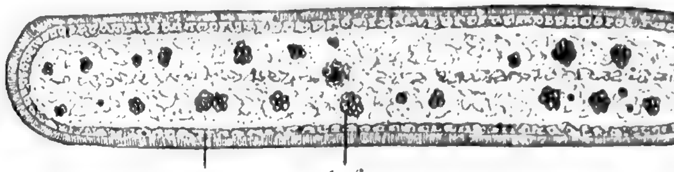
32



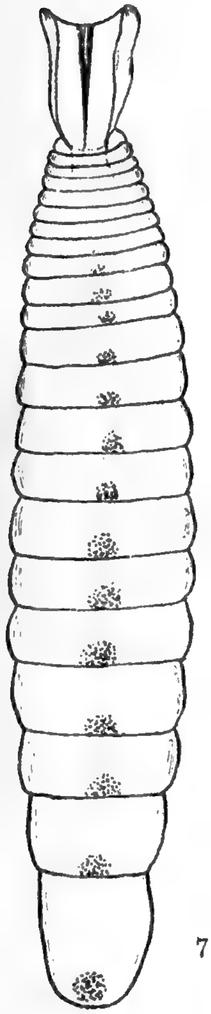
Del. A. R.

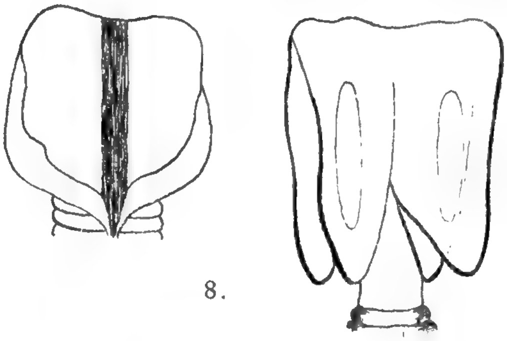
McFarlane & Erskine Lith. Edin.

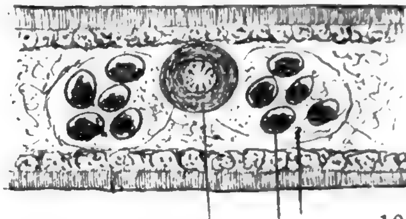
RENNIE AND REID: "SCOTIA" CESTODA—PLATE II.



9.


7.

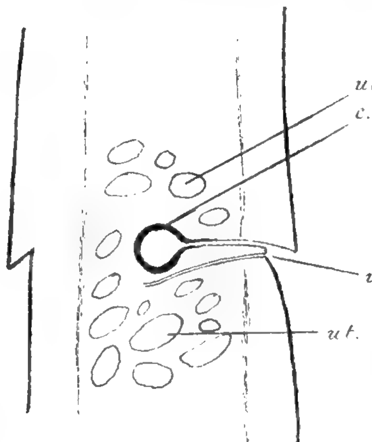

8.



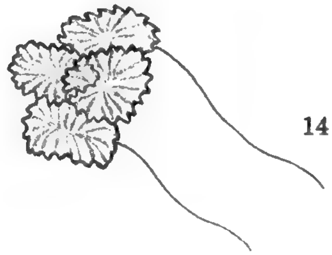
10.

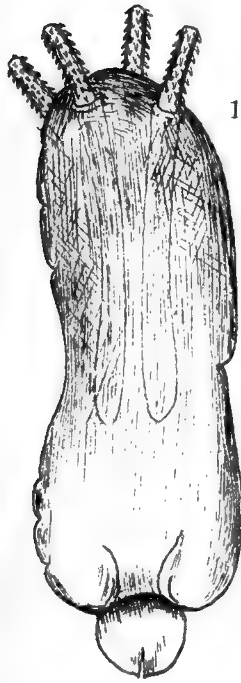

11.

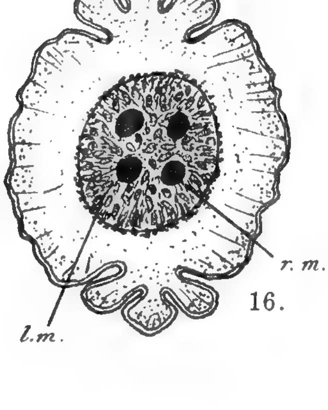

13.



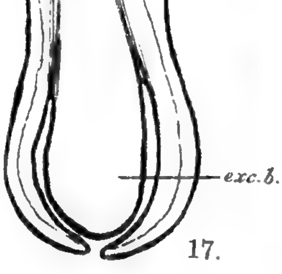
12.


14.

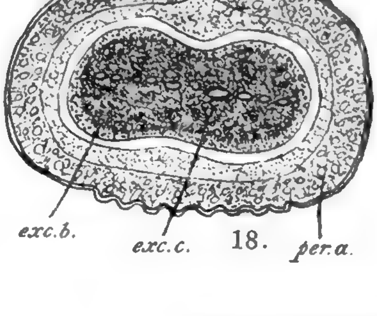

15.



16.



17.



18.

Del. A. R.

McFarlane & Erskine, Lith. Edin.

XV.—Egg Coloration in the Cuckoo, *Cuculus canorus*, and its bearing upon the theory of Cuckoo Sub-species. By John Rennie,

D.Sc., F.R.S.E., University of Aberdeen.[1]

(Read 27th October 1913. MS. received 12th November 1913.)

THROUGH the generosity of Mr R. Hay Fenton, Aberdeen University possesses one of the finest collections of clutches of birds' eggs in the country. This collection includes 300 Cuckoos' eggs together with the foster clutches with which they were found. The value of Mr Fenton's gift is further enhanced by the fact that he has along with it supplied much important data regarding the eggs, and, in particular, has given a full list of localities and dates of their taking.

The material and information thus available have made possible the use of the collection for scientific investigation, and I wish here to express my indebtedness to Mr Fenton for calling my attention to the particular facts submitted and discussed in this paper dealing with egg coloration in *Cuculus canorus* and its bearing upon Newton's theory of Cuckoo "gentes." This theory has recently obtained some support from biometric evidence supplied by Latter (3).

It is well known that *Cuculus canorus* practises the parasitic habit of foisting the duty of incubation and rearing of its offspring upon a large number of different birds. Sharpe puts the number of species at 119. In the Fenton collection we have evidence of about 60 species in Britain.

It is generally held that individual Cuckoos in the main parasitise upon particular species of fosters during the whole of their lives, transmitting this bias to their offspring, and Newton has suggested that by this means several "gentes" of Cuckoos have thereby become established. The main evidence for this suggestion is found in the coloration and size of the egg. Latter investigated the problem biometrically, taking length and breadth dimensions in 1572 eggs, and claims that the balance of evidence is "decidedly in favour of there being distinct sets of Cuckoos." He finds evidence, in particular, of races of Robin-cuckoos, Wren-cuckoos, Whitethroat-cuckoos, and Hedge-sparrow-cuckoos, and says, "It now seems fairly certain that Prof. A. Newton's suggestion is correct, and that there are certain 'gentes' of Cuckoos whose members being closely related lay eggs of somewhat similar dimensions, and in the main confine their attentions, generation after generation, each to its own particular variety of foster parent. In other words, the evidence here adduced points to the conclusion that the species

[1] Reprinted from *Proceedings Royal Physical Society of Edinburgh*, Vol. xix., No. 5, pp. 97-107, Feb. 1914.

Cuculus canorus is tending to break up into a number of sub-species, each with its own particular habits in respect of selecting a foster parent" (p. 363).

It is not my intention in the present paper to criticise this conclusion on the evidence submitted in Mr Latter's paper, further than to remark that there appears to be an arithmetical slip in his determination of the significance of the difference between the mean lengths of the total Cuckoo eggs treated of and the mean length for the "Sedge-warbler-cuckoo" group. Besides, the standard deviation of sampling used is not that of the difference between a general mean and a local mean, which is the difference Latter considers. Even when the correct formula is used it does not render distinctly significant the difference in the "Sedge-warbler-cuckoo."

The proposition here submitted, based upon the coloration of the Cuckoo's egg, is that the assumed habit on the part of the Cuckoo of adhering to a particular species of foster bird is not in general agreement with fact. Further, we have here confirmed two generally recognised facts:—

(1) A considerable variability of colour in Cuckoos' eggs in general;
(2) A marked uniformity of colour of the egg of the individual bird.

An examination of the 300 Cuckoo eggs in the Fenton collection reveals the fact that in this species there is a wide range of variation as regards egg coloration. This agrees with the observation of other investigators, *e.g.* Newton (4), Baldamus (1), Herrick (2). Herrick states: "When taken at random they are very variable in colour, ranging from blue, or blue-green, through speckled blue, brown, mottled or marbled brown, and grey to nearly plain white." There is also general agreement upon the question of the uniformity in coloration of the eggs of the individual Cuckoo. Newton writes: "It is unquestionable that whatever variation there may be among the eggs laid by different individuals of the same species, there is a strong family likeness between the eggs laid by the same individual, even at the interval of many years" (D. of B., p. 123). Herrick states: "The same Cuckoo always lays eggs of similar colour, colour pattern, size and form in a single season, and probably during life. According to Baldamus this has been proved to hold true in one case for three successive years. If two or three Cuckoos' eggs are found in the same nest, they are supposed to belong to different birds, and no case is known where such eggs were similarly coloured."

An examination of the Cuckoos' eggs in the Fenton Collection, grouped according to locality and date of taking, shows the presence, in numerous instances, of sets of eggs of strikingly uniform coloration. No other conclusion can be drawn as to their origin, so remarkably similar are the eggs, than that they are the product of the same bird. In most cases the groups of eggs, arranged on the basis of locality and near dates, show distinctly more agreement as regards size and colour than do the foster eggs within the individual clutches. The following are illustrations :—

I.—*Two eggs taken at Budworth Mere, Cheshire.*

Museum No.	Foster.	Date when taken.
A 6/2	Reed Warbler	29th May 1910
A 6/3	Reed Warbler	5th June 1910

These eggs are of a bluish-grey colour, with dull brownish blotching; there are abundant fine dots and also numerous larger specks of varying shape and size. The eggs are not noticeably darker in colour at the broad end. The two eggs cannot be distinguished. There is one foster type only.

II.—*Four eggs taken at Budworth, Cheshire.*

Museum No.	Foster.	Date when taken.
G 15/1	Sedge Warbler	8th June 1910
G 6/8	Reed Warbler	10th June 1910
G 15/2	Sedge Warbler	13th June 1910
G 6/9	Reed Warbler	18th June 1910

These are pale grey eggs with finely grained brown markings. There are numerous sharply defined dark brown spots. The resemblance is most marked in all four eggs. There are two types of foster in this case. Taken in conjunction with the foregoing set two Cuckoos are indicated in this area.

III.—*Set of two eggs, presumably of the same bird, taken near Lancaster.*

Museum No.	Foster.	Date when taken.
D 11/3	Yellow Bunting	22nd May 1912
D 13/2	Hedge Sparrow	27th May 1912

This is a greyish egg with a good deal of brownish pigment in fairly large irregular patches well distributed evenly over the whole egg, but more deeply at the broad end. There is a brownish speckling over the whole end area in both eggs—not in the form of a ring. On both eggs there are a very few quite dark brown well-defined marks. The egg in the Yellow Bunting's nest resembles well the eggs of the foster, but is a little darker. That in the nest of the Hedge Sparrow is, of course, conspicuous. There are two foster species.

IV.—*Two eggs, apparently laid by the same bird, taken at Delamere, Cheshire.*

Museum No.	Foster.	Date when taken.
F 15/3	Sedge Warbler	2nd June 1912
F 29/1	Lesser Whitethroat	6th June 1912

These are pale grey eggs with brown spots. The general impression is that of a very uniform lightish coloration, *i.e.* the speckling is very evenly distributed. The two eggs are remarkably alike and cannot be distinguished. There are two kinds of foster in this case also.

V.—*Three eggs, apparently laid by the same bird, taken at Wigglesworth, Yorkshire.*

Museum No.	Foster.	Date when taken.
B 16/2	Willow Wren	23rd May 1910
B 7/2	Meadow Pipit	27th May 1910
B 18/2	Spotted Flycatcher	28th May 1910

These show a most marked resemblance to each other. Colour is greyish-blue with brown speckling evenly distributed. There are a few darker spots of brown, very small but sharply defined. The three eggs cannot be distinguished save by these spots, which are of course not identical in size or numbers. It is noteworthy, notwithstanding the probability of their being the product of the same bird, that each occurred in the nest of a separate species of foster. The collector states that "all of the three eggs were found close to where water was in abundance, both stagnant pools and running."

VI.—*Six eggs, taken by the banks of the River Ouse, in Huntingdonshire, within a range of half a mile.*

Museum Mark.	Foster.	Date when taken.
C 11	Sedge Warbler	11th June 1909
C 1	Sedge Warbler	16th June 1909
C c	Reed Warbler	17th June 1909
C x	Lesser Whitethroat	18th June 1909
C w	Whitethroat	18th June 1909
C h	Hedge Sparrow	14th July 1909

The ground hue of these eggs is of a bluish-grey, with brown speckling fairly thickly distributed. The colour contrasts quite noticeably with that of the next set, which was taken in the same region but over a wider area, and all, with one exception, in the following year. With the exception that in egg Cx there is a suggestion of a dark band at the broad end, it is impossible by ordinary observation to distinguish these eggs from each other. The nests of five foster species are represented by these six eggs.

VII.—*Eleven eggs, believed to be laid by the same bird, taken by the River Ouse, Huntingdonshire.*

Museum Mark.	Foster.	Date when taken.
E v	Reed Warbler	18th June 1909
E c	Reed Bunting	24th May 1910
E 11	Sedge Warbler	4th June 1910
E °	Whitethroat	4th June 1910
E 5-1	Sedge Warbler	6th June 1910
E w	Whitethroat	8th June 1910
E/o	Sedge Warbler	8th June 1910
E v¹	Sedge Warbler	8th June 1910
E iii	Reed Bunting	11th June 1910
E x	Reed Warbler	11th June 1910
E o/	Reed Warbler	15th June 1910

Notes on the individual Eggs of this Group.

E v. This egg resembles the Reed Warbler's in appearance, but is lighter, the speckling is more uniform in size all over, not run into blotches but more thickly laid down at the broad end, forming an irregular ring not unlike a Blackbird's egg.

E c. The above description holds good for this egg.

E 11. This egg cannot be distinguished from the foregoing.

E °. On this egg are two end-spots sharply defined; otherwise as above.

E 5-1. Quite similar to the others already mentioned. The markings are not so intense as those of the Sedge Warbler in this nest, nor so greenish, but except for size the egg is remarkably like the foster eggs.

E w. A marked general resemblance to others of this set.

E/o. Like the rest. The resemblance here to the foster eggs is remarkable. The Sedge Warbler's eggs here are darker than those of *E 11*; they vary more amongst themselves than do the Cuckoos' in this set.

E v¹. This egg is slightly paler than the others on one side, towards the narrow end.

E iii. Like the others generally.

E x. There are some larger spots towards the narrow end, but general appearance is as the others.

E o/. Like the others generally.

An unbiassed examination of these eggs and their foster clutches suggests the following observations:—

(*a*) There is a resemblance in coloration both as regards intensity and distribution which is remarkable.

(*b*) It is not surpassed nor even equalled in the coloration of the eggs composing the individual clutches in which the eggs have been found. There is noticeable variation in the different clutches of the same kind of bird's eggs, *e.g.* Sedge Warbler's in this collection which contrasts with the uniformity of appearance of the Cuckoo's eggs of the series.

We have here either the eggs of two or more birds, in which case a marvellous coincidence of colour resemblance extending to eleven eggs occurs; or the eggs are those of a single bird.

If the latter alternative be correct, two facts of interest are to be noted. One is the confirmation of the observations of Baldamus and others that the resemblance in the eggs of the individual Cuckoo extends from one year to another. The second is that a single Cuckoo may lay as many as ten eggs in a season, and that within so short a period as twenty-two days.[1]

Further, it will be noted there are four foster species involved; these however all nest in similar situations. The actual distance limit of this Cuckoo's ovipositing operations here considered was about two miles.

VIII.—*Three eggs, taken in Huntingdonshire.*

Museum No.	Foster.	Date when taken.
K 1	Robin	24th May 1913
K 2	Yellow Hammer	24th May 1913
K 3	Sedge Warbler	26th May 1913

These are pale greenish eggs with brown blotching, mostly at the broad end. These eggs were all taken in the same area. Three foster species are involved.

IX.—*Three eggs, taken in Huntingdonshire.*

Museum No.	Foster.	Date when taken.
L 4	Sedge Warbler	26th May 1913
L 6	Robin	29th May 1913
L 13	Reed Warbler	3rd June 1913

[1] Six to seven is the usual number of eggs reputed to be the product of a single Cuckoo in a season. I do not know upon what evidence this is based, but considering the contingencies in the matter of rearing there might well be a compensating factor in this species in the direction of an increased egg production.

These are grey eggs with a moderate amount of brownish speckling. The small brown dots are fairly numerous and quite noticeable upon these eggs. A comparison of the eggs of this set with those in the previous one reveals a difference in ground colour and in size and amount of superimposed blotching. Although the two sets are from the same area, they are probably the product of two different Cuckoos. Again, three foster species are involved.

X.—*Seven eggs, taken in the same district as the two preceding sets.*

Museum No.	Foster.	Date when taken.
H 7	Reed Warbler	3rd June 1913
H 8	Lesser Whitethroat	5th June 1913
H 10	Reed Warbler	12th June 1913
H 11	Reed Warbler	16th June 1913
H 12	Sedge Warbler	23rd June 1913
H 14	Reed Warbler	24th June 1913
H 15	Whitethroat	29th June 1913

These eggs again differ from the two previous sets, but amongst themselves constitute a very uniform group. The ground colour is a pale bluish-green of a deeper tint than set K. The latter is a yellowish-green; this set, when placed alongside, looks quite different. The grain of brown speckling is finer and does not run into large blotches as in set K. Four foster species are involved.

XI.—*Two eggs, taken at West Clitheroe.*

Museum No.	Foster.	Date when taken.
I 7/12	Meadow Pipit	19th May 1913
I 4/10	Tree Pipit	22nd May 1913

This is a pair of beautiful eggs of a general pale reddish hue, with reddish-brown speckling thickly distributed, especially at the broad end. The eggs are quite different in appearance from any of the other sets described in this paper, whilst they are practically indistinguishable from each other. There are two fosters in this case.

XII.—*Three eggs, taken near Huntingdon.*

Museum No.	Foster.	Date when taken.
5	Hedge Sparrow	28th May 1913
9	Whitethroat	5th June 1913
16	Reed Warbler	1st July 1913

This is a specially interesting series. Nos. 5 and 9 are greyish in colour, moderately well speckled with brown. No. 5 is slightly darker than No. 9 at the broad end, but they fit in well as a pair with the general theory of common parentage. No. 16 is an egg of a different type, smaller in size and pale bluish-green with regularly distributed small grained brownish speckling. It is probably the egg of a separate bird. On comparing it with the eggs of series X., from the same area and of same year's taking, and particularly with **H** 14 and **H** 15 which were found about the same time (24th and 29th June), we still find this egg distinctive. Whilst it approaches these two more nearly in appearance, its ground colour is of a deeper blue and the amount of brown speckling is rather greater. It is also a smaller egg.

XIII.—*Two eggs, taken at West Drayton (Bank of Colne) Middlesex.*

Museum No.	Foster.	Date when taken.
M 6/14 . . .	Reed Warbler . . .	1st July 1912
M 15/5 . . .	Sedge Warbler . . .	11th July 1912

These eggs are of a pale, sandy colour, with light brown speckling running in places into fairly large blotches. The brown colouring is moderately sparse, generally, and absent at the narrow end of the egg which is rather pointed looking. The resemblance of the two eggs is rather striking, and they are probably the offspring of the same bird. Two foster species are involved.

When single eggs of the foregoing thirteen series taken at random are placed alongside, the interesting fact becomes evident that with one exception no two resemble each other, even approximately, as the members of the sets themselves do. Further, a comparison of sets from the same locality, by the method of taking an egg at random from each group, yields eggs readily distinguished by their colour in each case. There are two sets from Budworth Mere, Cheshire, of the same season, and these are quite distinct when laid alongside, the difference being more marked than any verbal description can bring out.

There are six sets from Huntingdonshire, representing three different years' takings. Sets IX. and XII. are rather alike, but their resemblances are not so great as the members of any of the sets considered in this paper. They constitute the one exception quoted above. The other examples can all be distinguished at a glance from these and from each other.

What conclusions can be drawn from these facts?

The following are possible explanations :—

(*a*) The resemblance between the eggs of each of the "sets" is coincidence merely, the eggs of a "set" being the product of two or more birds.

(*b*) The eggs of each set are the product of a single bird.

Now, should the former view be accepted and the theory of Cuckoo "gentes" be also adhered to, we have the following facts to face. We must hold, generally, that in each of the supposed "sets" there are as many Cuckoos represented as there are foster species. We need not postulate more, although there might be more.

For example:

Set I.	Two eggs . . .	One	foster species.
Set II.	Four eggs . . .	Two	"
Set III.	Two eggs . . .	Two	"
Set IV.	Two eggs . . .	Two	"
Set V.	Three eggs . . .	Three	"
Set VI.	Six eggs . . .	Five	"
Set VII.	Eleven eggs . . .	Four	"
Set VIII.	Three eggs . . .	Three	"
Set IX.	Three eggs . . .	Three	"
Set X.	Seven eggs . . .	Four	"
Set XI.	Two eggs . . .	Two	"
Set XII.	Three eggs . . .	Three	"
Set XIII.	Two eggs . . .	Two	"

What is the probability, we may pertinently ask, of *five distinct birds* (Set VI.) in the same district all laying in the same season, each in a *separate foster-species nest*, eggs so resembling each other in ground hue, depth and distribution of superimposed pigment that they cannot be distinguished from each other? Such is not likely to be the case; the probability is very remote.

A similar argument applies to the other sets. Take Set VII. Here also it is highly improbable that four birds will, amongst them, lay ten eggs which in colour cannot be distinguished from each other. And so with the other groups.

Let us consider the other alternative. The eggs of the groups or "sets" discussed in this paper may be regarded as the product of a single bird. To most observers who may examine these eggs, this will probably be the more acceptable alternative. It involves fewer difficulties.

Close resemblances between the offspring of a single bird are more to be expected than between those of several. From such a conclusion it follows:—

Cuckoos laying in Sedge Warblers', Reed Warblers', Whitethroats,' Lesser Whitethroats', Reed Buntings', Robins', Hedge Sparrows', and other named birds' nests do not in England form separate gentes, "each with its own particular habits in respect of selecting a foster parent."

It may, of course, be pointed out that it is not held that individuals of a supposed "gens" adhere in every case to the gens type of foster nest, but only "in the main." This means, however, that the departure from the "gens" habit is exceptional, which cannot be claimed with regard to the cases under consideration. In all these the foster species are multiple, except one—(I.) a case of two eggs in Reed Warblers' nests.

An important factor bearing upon the problem of the evolution of Cuckoo "gentes" exhibiting specialised egg characters is the polyandric habit of the Cuckoo. If we allow that the female exhibits a hereditary bias in the selection of foster parents for her offspring which is evolving along with a definite morphological differentiation in egg dimensional characters, we exclude the male parent, *who may be of a different gens*, from exercising any hereditary influence upon the "foster-bias" of the offspring, with its accompanying egg differentiation. To put the matter concretely: if a female bird bred from a "Robin-cuckoo" gens mates successively with a male of "Hedge-sparrow-cuckoo" stock and with a male of, say "Wren-cuckoo" stock (assuming that such stocks exist), will the offspring of these matings be "Robin-cuckoos" only or even mainly? The "gens-theorists" must have an answer to this problem.

For the view of a possible evolution of "gentes" going on, it may be stated: Cuckoos may come back not only to the locality but to the particular hedge or reed bank in which they were reared, in search of a foster parent for their offspring. It is possible that they are predisposed—some more, some less—to observe more readily, and so to find the species and the nest of the type which incubated and reared them. There may be *pari passu* with this, a process of Natural Selection going on whereby an increasing number of Cuckoos reared by foster species of a specially assiduous and faithful type are surviving, and in turn a majority of these, reminiscent of and let us hope grateful for past privileges, which tend to seek a similar foster parent for their own offspring. In some such way physiological sub-species may be in process of forming.

This theory is plausible enough, and there may be some support for it in the frequency with which in some districts a particular type of foster is common, *e.g.* Meadow Pipit. But it must be admitted that this view is open to criticism similar to that suggested above. As regards the foster species considered in this paper, all that the evidence may safely be claimed to show is that while a bias towards a particular haunt, *e.g.* a river bank, may be exhibited by a Cuckoo, the choice of species for foster parents within that haunt would appear to be determined more by the frequency of occurrence of its nests, or the facility with which the nests are discovered by the Cuckoo, than by any hereditary bias towards a particular foster.

Literature Cited.

1. Baldamus, A. C. E. *Das Leben der europäischen Kuckucke*, Berlin, 1892.
2. Herrick, Francis H. *Jour. Exp. Zool.*, 9, 1910, p. 169.
3. Latter, O. H. *Biometrika*, vol. 4.
4. Newton, A. *Dictionary of Birds*.

(*Issued separately*, 14*th February* 1914.)

u5

[Reprinted from PARASITOLOGY, Vol. VII, No. 2, pp. 189–200, June 19, 1914]

THE OCCURRENCE OF *OXYURIS VERMICULARIS* IN THE HUMAN VERMIFORM APPENDIX.

By J. ALEXANDER INNES, B.Sc.,
and A. ELMSLIE CAMPBELL, M.A., B.Sc., M.B., Ch.B.

(*From the Parasitology Laboratory, University of Aberdeen.*)

This investigation was commenced by one of us (J. A. I.) in 1910, and carried on for three and a half years. Our purpose was to determine as accurately as possible the percentage of appendices operated on for appendicitis which contained intestinal parasites. The results of a study of 100 unselected cases are given below.

Previous records.

Isolated cases of intestinal parasites in the appendix have been reported from time to time during the past years, but these have served no useful purpose except to corroborate the fact that the appendix is a not infrequent habitat of certain parasitic worms. In 1634, Fabricius ab Aquapendente mentions that he occasionally found a worm in the appendix, but as to the species of worm he makes no reference. Santorini, in 1724, made the same observation. Birch-Hirshfeld, in 1871, records an extremely rare condition of *echinococcus* of the appendix alone, without *echinococcus* of the liver.

Still (1899) was the first to investigate, with any precision, the parasitology of the appendix. Working with both normal and pathological appendices of children, he came to certain conclusions: of these, the following are the more important:

(1) That the appendix is a common habitat of *O. vermicularis* in children.

(2) That the appendix may serve, in some cases, as a breeding place for the worms.

(3) That the presence of threadworms may cause a catarrhal condition therein.

(4) That infection of the appendix with threadworms causes pain which may simulate that of appendicitis.

He states that 19 % of healthy appendices of children harbour *O. vermicularis*. Metchnikoff (1901) records a case of Trichocephalids in the appendix, and the same author describes three cases of appendicitis which he holds were due to the presence of *Ascaris lumbricoides*. Girard (1901) also reports a case of appendicitis in which Trichocephalids were found attached to the mucosa of the appendix. Hamilton Russell (1901) records an interesting case of appendicular colic which he holds was due to the presence of *O. vermicularis* in large numbers. The lumen of the appendix was dilated, the mucosa thickened and congested, and there was a considerable excess of lymphoid tissue. On inquiry, it was ascertained that all the children of the family were infected with threadworms. Von Moty (1902) attempts to make a distinction between the characters of the lesions excited by the different varieties of parasites, and finds that *Ascaris lumbricoides* seems to be more often associated with gangrenous appendicitis, while *Oxyuris* and *Trichocephalus* lead to chronic inflammatory changes.

Erdmann (1904) found threadworms in 4 out of 250 cases of appendicitis in children. In this case, however, it is probable that a thorough examination of the contents of the appendix under the microscope would have revealed a much higher percentage.

Hoepfl (1904) in Germany found that cases of appendicitis showed 21 % of the removed appendices to contain *Oxyuris*.

Patterson (1906) found *O. vermicularis* in 8 cases, and *A. lumbricoides* in 10 cases of diseased appendices. Sprengel (1906) gives Oppe's figures —threadworms found six times in 60 cases of appendicitis—and those of Rostewsew, who found *O. vermicularis* three times in the examination of 163 normal appendices.

Still (1909) records a case where 111 threadworms were found in a single appendix. In children between the ages of 2–12 years he found that 32 % were infected with *Oxyuris*, and that two-thirds of these worms were found in the appendix.

Brumpt (1910), who is an authority on this subject, goes very fully into the question in his *Précis de Parasitologie*. He examined 800 appendices obtained from autopsies at Paris, and found *Oxyuris* infection present in 3–5 % of the appendices. In the appendices of children the percentage was about four times as great, or roughly twice out of every thirteen cases. From his examination of appendices removed for appendicitis, he found 10 cases out of 27 infected with *Oxyuris* (37 %).

In the same way Railliet (1911) found the worm 14 times in 33 cases of appendicitis (42 %), and also 58 times in a series of 119 cases (48 %); Schloss (1910) found the threadworm present in 7 % of healthy children living on the east side of New York City. Russell and Bulkley (1912), from a study of 148 unselected appendices from appendicectomies of children between the ages of two and fifteen, state that of these 19 were normal, and of the remaining 129, parasites were found in 19, of which 17 had *Oxyuris* infection, and two had *Trichocephalus* infection. The number of worms present varied from one to thirty-five. In eight cases the males outnumbered the females. Out of 19 normal appendices, parasites (*Oxyuris*) were found in three. Four adult cases of *Oxyuris* appendicitis were also examined. They classify their cases in the following manner:

	Oxyuris	*Trichoceph.*	No infection	Total
1. Acute catarrhal appendicitis,				
(*a*) Children	14	1	5	20
(*b*) Adults	4	0	0	4
2. Chronic appendicitis	0	0	20	20
3. Acute suppurative	0	0	25	25
4. Acute gangrenous	3	1	60	64
5. Normal appendices	3	0	16	19
	24	2	126	152

Ney (1912) cites two cases of appendicitis in which there were in the one case, two, and in the other, five *Oxyuris* in the appendices. He also reports that in a series of 100 appendices removed at the Hebrew Hospital, only three contained threadworms.

In the Clinical Records of the *Edinburgh Medical Journal* (1913) details are given of 14 cases in which various nematodes were found in the appendix. These cases may be summarised:

A. *Ten Cases of Appendicitis.*

No.	Sex of patient	Age (years)	No. of *Oxyuris* found	Type of appendicitis
1	M	8	Large number	Acute
2	M	14	20–30	,,
3	M	8	Large number	Recurrent
4	M	38	Several worms	,,
5	F	15	7–8	,,
6	M	9	Numerous worms	,,
7	M	18	Large number	Chronic
8	F	15	,,	,,
9	M	19	Numerous worms	,, ?
10	F	21	,,	,,

B. Case of a female, age $5\frac{1}{2}$, operated upon for intussusception, and in whose appendix several *Oxyuris* were found.

C. Case of a female, aged 7, operated upon for appendicitis, appendix found to be normal, but was packed with threadworms.

D. Case of a female, aged 32, operated upon for recurrent appendicitis. The appendix was ulcerated at one point, and contained four *Ascaris lumbricoides*.

E. Case of chronic appendicitis (no clinical history given) operated upon and the appendix found to contain one *Trichocephalus trichiurus*. The appendix was also the site of a primary carcinoma.

These selected cases, although of much interest individually, do not help towards forming an estimate of the percentage of infected appendices, but they bear out the suggestion of von Moty (1902) that different varieties of parasites may cause different lesions of the appendix. Von Moty suggests that *Ascaris lumbricoides* causes an acute inflammation going on to gangrene, and that *O. vermicularis* and *T. trichiurus* lead to a more chronic inflammatory condition. Our own cases confirm his suggestion regarding the inflammatory changes produced by *Oxyuris*.

With regard to the records of isolated cases of appendicitis in which *Oxyuris* have been found in the appendix, it will be sufficient if we group these together. A bibliography of these cases has been appended for further completeness. Wakefield (1908), Wagener (1906), Walther (1905), report single cases, but no description of the gross or microscopic lesions is produced. Culhane (1910), Martin (1907), von Moty (1902), Pabeuf and Dubois (1908), Ashhurst (1909), Bégouin (1902), report clinical cases and describe the histological changes found. They fail to establish any relationship between the parasites and the changes described. Winkler (1910), Hippius and Lewinson (1907), Weinberg (1907), Romanovitch (1911), Brumpt and Lecène (1910), Galli-Valerio (1903), report single cases of *Oxyuris* appendicitis in which the parasite is shown to be the actual cause of the disease. Wilson (1912), Burgess (1912), Grippen (1912), Macdonald (1912), etc. also report cases of *Oxyuris* in the appendix. Unterberger (1908) records two cases of *Oxyuris* appendicitis accidentally discovered at autopsies.

Trichocephalus trichiurus in the Appendix.

In view of the fact that *T. trichiurus* was not found present in any of our cases, we give below some of the statistics compiled by other investigators with regard to this worm. Brumpt (1910) quotes the following :

"On 1600 medico-legal autopsies performed at Chicago, Mitchell never found a single helminth." Lejar (1897), Guinard (1900) and Girard (1901) had cases of *T. trichiurus* in the appendix. Ménétrier (1909) published several cases: Brumpt found the worm in 4 % of cases (autopsies), Railliet only once found the nematode in 119 cases, but the ova were often present in the appendix. In a table compiled by Blanchard and Braun in 1880, they give the percentage of infected caeca at Greenwich as 68 % to 75 %, so that in all probability the appendix infection must have been correspondingly high.

Scope of Present Investigation.

This comes under five heads:

To determine (1) The percentage of infected cases.
(2) The relationship of age to infection.
(3) The nature of the infecting parasite.
(4) The relationship of the parasite to appendicitis.
(5) The probable mode of infection.

Methods:

We have examined over 150 appendices representative of all types of appendicitis, but for the sake of accuracy the first 50 or 60 appendices examined were rejected. The chief reason for this was that we might become thoroughly accustomed to make a reliable examination, to identify quickly and definitely any worms, if these were present, and to attain some degree of precision in the enumeration of the parasites. It also gave us an opportunity of testing various methods for the examination of appendices. Ultimately the following procedure was adopted, and the next series of 100 appendices was examined in this manner. Every appendix was examined within two to four hours after its removal from the body, and the routine examination was made in almost all cases in the laboratory.

The appendix was slit open along the attachment of the meso-appendix, from the proximal end. Great care was taken in making the first cut at the proximal end, as we have often found the worms to be most numerous at that point. In many cases, where single worms were found, they were found in that region. Having laid the appendix open, we transferred it carefully to a large Petri dish half filled with normal salt solution. If worms were present, the females stood out clearly, and these were removed and counted. The mucosa of the appendix was then scraped off, and left in the salt solution, while the appendix was removed. The Petri dish with the saline and mucous scrapings was examined microscopically under a low power, and immature females and males were "spotted," and counted as they were removed. In many cases, to make more certain, we examined the contents of the Petri dish, part by part in a watch glass.

The method recommended by Brumpt (1910) of placing the mucous scrapings between two glass plates, we did not find very satisfactory, as it was liable to crush the worms, and to introduce errors in counting. It is, however, an excellent method for determining the fact of the presence of worms in the appendix.

The material was obtained from appendicectomies performed at the Royal Infirmary, and in two cases from the Royal Hospital for Sick Children. All types of appendicitis were examined, but normal appendices removed during the course of other operations, such as laparatomies, although investigated, have not been used in compiling the results. In no case did we find worm infection in a healthy appendix.

Results of Investigation.

The types of appendicitis studied were classified under five groups, special attention being given to the "*recurrent*" type, as this was found to show the highest percentage of infected appendices.

The term "*recurrent appendicitis*" has been applied to that type which has a history of a year's duration or more, and in which there have been several more or less severe attacks of appendicitis at varying intervals.

The number of cases in each group was as follows :

(*a*)	Acute catarrhal appendicitis	30
(*b*)	Subacute catarrhal appendicitis	22
(*c*)	Chronic appendicitis	14
(*d*)	Gangrenous appendicitis ..	8
(*e*)	Recurrent appendicitis ..	26
	Total	100

Out of these cases, 17 were found to harbour *O. vermicularis*, while in no case were trichocephalids or other worm parasites found. The ova of *Oxyuris* were found in two of these cases, but as in each there was also a ruptured mature female worm, no stress can be laid on this fact. The ova of *T. trichiurus*, or of other nematodes, were not seen.

The details of the infected appendices are :

No.	Sex of patient	Age	No. of *O. vermicularis* Males	Females	Total	Type of appendicitis
1	F	22	0	1	1	Recurrent
2	F	7	10	10	20	Acute catarrhal
3	M	22	5	14	19	Recurrent
4	F	15	1	3	4	Recurrent
5	M	12	21	21	42	Recurrent
6	F	19	1	0	1	Subacute catarrhal
7	F	17	0	1	1	Recurrent
8	F	24	0	1	1	Recurrent
9	F	23	0	1	1	Recurrent
10	M	23	1	1	2	Subacute catarrhal
11	M	11	1	11	12	Recurrent
12	F	6	0	4	4	Acute catarrhal
13	F	17	1	1	2	Recurrent
14	M	21	0	2	2	Recurrent
15	F	12	2	4	6	Recurrent
16	F	23	0	1	1	Subacute catarrhal
17	M	18	0	2	2	Recurrent

It is a matter of some importance to note the various sizes of worms found, in connection with the degree of maturity attained by them. The length of the female worm is given by authorities as from 9 to 12 mm. and that of the male worm as from 2 to 5 mm. Out of the 121 *Oxyuris* measured, no female worm exceeded 9 mm. in length, and only one specimen gave that measurement. The average length of the female was from 5 to 6 mm., and 3 mm. in the case of the male.

Judged by size, the worms found in the appendix were not mature, but the males had reached a further state of development, on an average, than the females.

Still (1909) discusses this point of immaturity, and holds that this is a good reason for believing the appendix to act as a breeding ground for *Oxyuris*. He doubts if infection is kept up solely by the repeated swallowing of ova.

Percentage of Infected Cases.

The percentage of infection, viz., 17 % in all cases of appendicitis, is undoubtedly lower than what it would be if the appendices had been examined under conditions which excluded any possible source of error. In spite of every means being taken to attain the greatest accuracy, it is certain that many male *Oxyuris* have been missed and not enumerated, especially in cases where no female worms were present to definitely establish the fact of the presence of worms in the appendix. We could devise no plan which might ensure *every* worm being counted. Again, we could not interfere with the preparation of the patients for operation. This preparation included the routine administration of purgatives, and also, in most cases, of a turpentine enema, given a few hours before operation. Both these measures—vermifugal in their action—could quite easily dislodge any of, or all, the worms in the appendix, and in some cases our examination was negative when it might have shown the presence of worms if no preparatory measures had been taken.

These facts must be noted in considering the percentage.

With regard to the comparison of statistics available for *O. vermicularis*, we tabulate below, and compare with our results the findings, as far as they have been obtainable, of other investigators.

Railliet	42–48 %	in appendicitis
Brumpt	37–40 %	,, ,,
,,	15 %	,, normal appendices (children)
,,	3–5 %	,, normal appendices (adults)
Hoepfl	21 %	,, appendicitis
Still (1909)	20 %	,, appendicitis of children
,, (1899)	19 %	,, normal appendices (children)
Innes and Campbell	17 %	,, appendicitis
Russell and Bulkley	15 %	,, appendicitis of children (including two *trichocephalids*)
Oppe	6 %	,, appendicitis
Ney	3 %	,, appendicitis
Rostewsew	1·8 %	,, normal appendices (adults)
Erdmann	1 %	,, appendicitis of children

As previously stated, Erdmann's figure is unreliable, because a thorough examination was not made in every case.

One must also bear in mind that many of the percentages in the above table were arrived at from a study of a small number of appendices, and therefore the figures are not strictly accurate in every case.

It will be seen that the figures given vary greatly from exceedingly low percentages to correspondingly high ones, and whether this has to do with climatic conditions it is not yet possible to say, but in every instance it seems that the percentage of worms is higher in cases of appendicitis than in those of normal appendices. More statistics, however, must be available before anything definite can be put forward.

Relationship of Age to Infection.

Our series of cases was mainly composed of adults, whose ages ranged from 15 to 53 years, and the commonest age of infection with *Oxyuris* in the appendix seems to be between the 21st and 24th years. At present it would be difficult to say with certainty that children show a higher percentage of infection, but probably such is the case. The figure given by Still (1909) of 20 % is higher than the average figure for adults in this country, but Brumpt (1910) and Railliet (1911) both give much higher figures for adults in France. This question, therefore, still remains open.

Nature of Infecting Parasite.

In the course of this investigation, only one species of parasite was met with, viz. : *O. vermicularis.* We understand, however, that at the Royal Infirmary one or two cases have been found of *Trichocephalus trichiurus* in the appendix. Undoubtedly the occurrence of this worm in cases of appendicitis is very rare in this part of the country.

Other observers note that *T. trichiurus* is much less frequently found than *Oxyuris*, but it seems strange that in 1880 Blanchard and Braun should have found the worm in the caecum 11 times out of 16 cases examined by them at Greenwich. No record of *Ascaris lumbricoides* having been found in the appendix could be obtained.

Relationship of the Parasite to Appendicitis.

This opens up a much disputed question, and as it lends itself to wide discussion, we propose to deal with this in a separate contribution which will be published shortly by one of us (J. A. I.). It will be sufficient to remark here that we think it very probable—in view of collected clinical data—that worm infection of the appendix gives rise, in many cases, to certain symptoms and to a definite type of history which are of diagnostic importance. From the table of cases investigated by us, it will be evident how largely recurrent appendicitis bulks in the total series, and also that of the infected cases, 65 % were of the recurrent type.

Gangrenous appendices never showed the presence of any worm parasites, but we corroborate the fact already pointed out by von Moyt (1902) that the subacute and chronic inflammatory changes are more characteristic of *Oxyuris* infection.

An interesting condition of the infected appendix was the occurrence at definite areas of numerous small, discrete, punctiform haemorrhages, which suggested the points of attachment of the nematodes.

Probable Mode of Infection.

This was the least satisfactory part of the whole inquiry, for in most cases nothing definite could be elicited from the patient regarding even his or her own opinion as to how worm infection took place. In two cases there was a certain history of worm infection, which had probably been continued through auto-infection. In one of these cases there was a family history of infection, and it is interesting to note that in this family three girls had appendicitis. Information as to the exact contents of the appendices was available only in the infected case. Still (1909) remarks, "not very rarely more than one child in a family is suffering from threadworms; but this does not happen with sufficient constancy to justify the assumption that such a source (of infection) is the usual or even a common one."

In many of the cases there was a history of chronic nail-biting, and this could quite easily give rise to a fresh infection or continue an already existing one, but as Still says: "The number of ova on the fingers must be very large and easily demonstrable." He found, however, that in an experiment carried out on five children who were passing large numbers of threadworms that the dirt on the finger-ends and under the nails showed only one solitary ovum in the case of one child out of five.

One infected patient had a great passion for eating raw fruit, and infection may have resulted from eating contaminated oranges or apples, etc.

Another patient employed at a farm house was very fond of animals, and in her own words: "held a lot of work with cats and dogs." In this case it is possible that infection was carried by these animals from outside sources.

Conclusions.

1. That the percentage of appendices from cases of appendicitis infected with *O. vermicularis* is a fairly high one. In Aberdeen 17 % were found to be infected.
2. That normal appendices show a much lower percentage of infection.

3. That the appendices of children probably show a higher percentage of infection than those of adults.

4. That the recurrent type of appendicitis is most frequently associated with *Oxyuris* infection.

5. That there is probably a clinical type of *Oxyuris* appendicitis.

6. That the suppurative type of appendicitis has practically no relationship to *Oxyuris* infection.

7. That *Trichocephalus trichiurus* is not commonly found in the appendix.

We have to record our indebtedness to the staff of the Aberdeen Royal Infirmary, and of the Aberdeen Royal Hospital for Sick Children for help given towards the collection of material for this investigation, and also to Dr John Rennie for many valuable suggestions.

REFERENCES.

ASHHURST (1909). *Amer. Journ. of Med. Sci.* CXXXVIII. 583.
BÉGOUIN (1902). *Gaz. Hébd. Soc. Méd. de Bourdeaux,* XXIII. 363.
BIRCH-HIRSHFELD (1871). *Archiv f. Heilkunde,* 191.
BRUMPT (1910). *Précis de Parasitologie,* 355–358.
BURGESS (1912). *Brit. Med. Journ.* I. 415.
Clinical Records (1913). *Edinburgh Med. Journ.* I. 437.
CULHANE (1910). *Journ. Amer. Med. Assoc.* LIV. 48.
ERDMANN (1904). *New York Med. Journ.* LXXIX. 537.
GALLI-VALERIO (1903). *Centralbl. f. Bakteriol.* XXXIV. 350.
GIRARD (1901). *Ann. Inst. Pasteur,* XV. 440.
GRIPPEN (1912). *Brit. Med. Journ.* I. 1072.
HIPPIUS and LEWINSON (1907). *Deutsche med. Wochenschrift.* XXXIII. 1780–1782
HOEPFL (1904). *Archiv f. klin. Chir.* LXXIII. 100.
MACDONALD (1912). *Brit. Med. Journ.* I. 485.
MARTIN (1907). *Montpel. Méd.* XXIV. 531–537.
METCHNIKOFF (1901). *Journ. des Praticiens,* XV. 185.
—— (1901). *Bull. Acad. Méd.* XIV. 301.
MOTY, VON (1902). *Echo Méd. du Nord,* VI. 217.
—— Quoted in *Lancet* (1902), II. 1211.
NEY (1912). *Johns Hopkins Hosp. Bull.* XXIII. 123.
PABEUF and DUBOIS (1908). *Bull. Méd.* XXII. 153.
PATTERSON (1906) *Amer. Journ. Med. Sci.* CXXXI. 859.
RAILLIET (1911). *Compt. rend. Soc. de Biol.* LXX. 310.
ROMANOVITCH (1911). *Compt. rend. Soc. de Biol.* LXX. 122.
RUSSELL (1901). *Internat. Med. Journ. of Australia,* Dec. 1901
RUSSELL and BULKLEY (1912). *Journ. of Exper. Med.* XV. 225
SCHLOSS (1910). *Amer. Journ. Med. Sci.* CXXXIX. 675.

SPRENGEL (1906). *Deutsche Chir.* Stuttgart, S. 182.
STILL (1899). *Brit. Med. Journ.* I. 898.
—— (1909). *Common Disorders and Diseases of Childhood*, London, 267, 271.
UNTERBERGER (1908). *Centralbl. f. Bakteriol.* Abt. 1, XLVII. 495.
WAGENER (1906). *Virchow's Arch.* CLXXXII. 145.
WAKEFIELD (1908). *Jour. Amer. Med. Assoc.* L. 1904.
WALTHER (1905). *Bull. et Mém. Soc. de Chir.* XXXI. 355.
WEINBERG (1907). *Ann. Inst. Pasteur*, XXI. 417.
WILSON (1912). *Brit. Med. Journ.* I. 829.
WINKLER (1910). *Die Erkrankungen des Blinddarmenganges*, Jena, 234.

From THE ANNALS OF APPLIED BIOLOGY, Vol. II, No. 4, April 1916.]

ON THE BIOLOGY AND ECONOMIC SIGNIFICANCE OF *TIPULA PALUDOSA*.

By JOHN RENNIE, D.Sc., F.R.S.E.

(*North of Scotland College of Agriculture.*)

Part I. Mating and Oviposition.

(With Plate XXXVI.)

In the course of the summers of 1913 and 1914 the mating and oviposition of *Tipula paludosa* formed the subject of special study both in the field and in laboratory experiments. As far as the writer knows no account of the sexual behaviour of this species is on record, at all events in detail. The points of special interest which have been made out are summarised in the present paper; the observations of the first season have been confirmed in the second.

Mating.

The earliest occasion in a season upon which mating was observed in progress was 20th June, 1914. This was in a field cage in which the flies had hatched out naturally. In the previous year, when a closer watch was kept, and the flies were reared in captivity and probably hatched earlier than in the field, the date was actually later, viz. 4th July. It may here be mentioned that, as detailed in a subsequent paper, there is only one generation of this species in the year in the north of Scotland. In the south of Britain it is held that two generations occur.

The flies experimented with were reared from larvae which had been kept in field and laboratory cages. The pupae were collected and transferred to small vessels about the size of ordinary flower pots

The work recorded in this and subsequent papers has been carried out with the aid of grants from the Board of Agriculture for Scotland.

containing soil covered with small pieces of turf. On the top of the turf was placed a glass cylinder about 9 inches high and 3½ inches in diameter. In some instances, lamp chimneys with nearly twice this width at their base were used. These were about 12 inches high but narrowed to about 2 inches diameter at the top. The vessel was kept closed by a piece of light cardboard placed upon the opening at the top. There were never more than two or three adult insects confined in these simple cages; they appeared quite comfortable and lived in most instances about a week, in some cases as long as eleven days.

The following observations are typical of the general results obtained and will serve as descriptive of the mating habits of these insects under the conditions which have been specified.

On the 5th of July, 1913 at 11.15 a.m. a pair of crane flies of the species *T. paludosa*, here referred to as M 1 and F 1, was found *in coitu* in one of the larvae rearing cages. They were removed without being separated to one of the glass observation vessels and watched until 1 p.m. at which time they still remained united. At 1.45 p.m. they were found to have separated. They were the sole occupants of this particular cage. This is referred to as Mating $(M1 + F1)1$.

At 2.30 p.m. on the same day they again spontaneously effected coitus, remaining attached until 3.30 p.m. Mating $(M1 + F1)2$.

At 4.15 p.m. on the same day mating was again accomplished between this pair. Coitus lasted until 5.23 p.m. Mating $(M1 + F1)3$.

The same evening these flies were placed together upon a small enclosed grass plot. For a short time they remained indifferent to each other, and the female commenced oviposition.

A second female, F 2, newly hatched, was placed beside them and almost immediately the male flew towards her and, after a short struggle in which some of the legs of the female were broken off, coitus was effected. Mating $(M1 + F2)1$.

At 10.20 a.m. on the following day a newly hatched male, M 2, was introduced to the vessel beside F 1, who had as already noted been ovipositing. Union was immediately effected. At 12 noon they still remained *in coitu*, and at this hour they were taken apart and placed in different vessels. Mating $(M2 + F1)1$.

At 12.15 p.m. M 1 was once more placed beside F 1. At 2.40 they had taken no notice of each other. They were now disturbed and made to fly about the cage. Upon coming into proximity they mated. Mating $(M1 + F1)4$.

At 4.30 they were found to have separated. They were left together overnight.

On the morning of the 7th, this female *F* 1 was removed to another vessel beside a male, *M* 5, hatched overnight. He flew at her instantly on her arrival and in a few seconds mating was effected, 9.55 a.m. At 11.20 a.m. they were found to have separated. Mating (*M* 5 + *F* 1) 1.

F 1 was now placed in a small glass vessel over some moist cotton wool. Up till 6.35 oviposition had not taken place under these conditions. At this hour she was placed over soil to see if this would now supply the stimulus. The result was negative. This female, however, was known to have oviposited previously, and at this stage she had lost the full-bodied appearance of the unmated female and was quite slender in form.

On the 9th, *F* 1 was placed with another male, *M* 6, but no union took place. From this date she was kept in the company of a male until she died. This occurred upon the 16th and meantime no further mating had been effected in her case. Reviewing the facts regarding her, it is noted that between the morning of the 5th and the morning of the 7th she mated at least six times, and with three different males. She lived 11 days in captivity.

The male *M* 1, as has already been recorded in the account of the behaviour of *F* 1, mated with her four times in the course of two days. He also mated with another, *F* 2, within a short time of her hatching. This took place at 7.10 p.m. on the 6th July. At 9.15 they were still mated, and on the following morning they were again found *in coitu.*

At 10.35 upon the morning of the 6th this male was introduced to a vessel in which there were already three females and one male. He at once flew amongst these and in a few seconds became coupled to a hitherto unmated female *F* 3. They remained *in coitu* until 12.15 when they separated. Subsequently as recorded for the female, *F* 1, he paired with her at 2.40 p.m. of the same day.

No further opportunities for mating were given to this male until the 9th (four days after he was first found mated), on which day he was placed with a newly hatched female *F* 9 at 11 a.m. They were left together until 3.20 p.m. but coitus did not take place. He mated at least seven times in the course of two days. He died upon the 12th, having lived seven days.

A number of similar experiments were performed and always with consistent results. Recently hatched insects paired most readily, and most were ready upon opportunity to pair a number of times. The following are additional notes.

F 12 placed in vessel with males, *M* 11, *M* 12, and *M* 13. *M* 11 attempted coitus and failed. *M* 12 succeeded. *M* 12 subsequently mated with *F* 13.

F 16, *F* 17, *F* 18 placed in a vessel containing a crowd of males. The males immediately flew to them and in a few seconds all three females were mated.

F 20 and *M* 17, both hatched on the morning of 18th July. They mated at 10.30 a.m. During the day she oviposited. *F* 21 and *M* 18, hatched later in the day, both mated. *F* 21 had defective wings. These four insects were placed together. Both pairs mated again in the evening. On the following evening *F* 20 mated once more with either *M* 17 or *M* 18. By this time she had become quite slender, and had evidently oviposited freely.

F 25, hatched on 28th July. Placed with *M* 24. Mated and oviposited. On the 30th she was placed with 14 males captured in the open. There was much commotion on her arrival and in a short time one of the males succeeded in mating with her.

F 27, hatched 6th August. Dropped into vessel containing *M* 24, *M* 25, and others. An immediate contest for her took place; mating successful.

F 28. Placed with the foregoing, with same result. The males immediately surrounded her, and mating was effected in the course of a few seconds.

In seeking to effect coitus, the male alights above the female and prevents her escape, should she attempt it, by intermingling of their limbs. Meantime his abdomen, which is now markedly upturned at the tip, is passed below that of his mate. The widely gaping claspers seize her on the thickened basal part of the ovipositor and the hold having been made secure the pair rest a few moments in this position. The male, now releasing his hold by the limbs, turns round so as to face in the opposite direction from the female. This is the position maintained until separation takes place. Sometimes the wings are folded, but more usually in both sexes they are extended. During coitus the antennae of the male continue in active backward and forward quivering motion. In his case too, the halteres quiver periodically in spasms of about a second's duration and at frequent but irregular intervals. This last feature was constantly to be observed and appeared to be directly associated with the sexual activity in progress. After attachment contractile movements are observable at the tips of both abdomens for a short period; subsequently the female rests absolutely passive. Cf. Plate XXXVI.

Oviposition.

This may take place quite early after mating. *F* 1 after having mated three times upon the 5th July commenced oviposition in the evening. *F* 2 mated on the 5th was watched in the process of egg laying on the morning of the 6th. Another female, *F* 5, hatched at 9.50 a.m. upon the 7th and mated immediately afterwards. She mated again with the same male *M* 12 in the afternoon. They separated at 4.20 and at 5.27 she was watched engaged in oviposition, and her eggs were collected when she had moved away.

Curtis (*Farm Insects*, p. 445) says: "the eggs are laid by the females as they fly or when they rest among the herbage and are propelled as from a pop-gun." So far I have not been able to witness this propulsion of the eggs while the insects were on the wing, but it will be understood that the conditions of my earlier experiments were not favourable to this method.

I have repeatedly watched the process amongst grasses. The female stands in a vertical position with the ovipositor pushed well down and into the soil if she can reach it. Sometimes a backward and forward screwing motion of the body is indulged in so that the ovipositor is bored well into the ground. Spasmodic jerks of the hinder part of the abdomen indicate the expulsion of the eggs one by one. After a few minutes she moves along a little way and the process is repeated. About half-a-dozen eggs may be deposited at the same spot, frequently fewer. In one case I was able on lifting a female, not actually ovipositing but exhibiting spasmodic movements of the ovipositor, to get her to lay an egg upon a card in my hand.

Newly hatched females are bulky in appearance at the anterior end of the abdomen. After oviposition they become slender. In this way one can tell whether a female has oviposited or not, though not generally whether the process is completed or not. The eggs in the newly hatched female mostly show black in colour through the skin and are shelled before fertilization. Advanced female pupae have the abdomen filled with the ovaries which are of a pale salmon pink colour. Dissection of females some time after oviposition shows that all the ova are not mature at hatching but that there is at least a second batch of eggs. Females captured in the open which are slender bodied, *i.e.* which show signs of having oviposited, have small pear-shaped ovaries occupying the posterior part of the abdominal cavity. The eggs in these may be well-developed and of the typical shape as when mature, but of the pale salmon colour. This suggests that females having oviposited may continue to live and to produce a fresh race of ova. This second lot of ova is not merely mature at hatching but without shells: they increase in size during the adult period. A female, ***F*** 22, hatched on the evening of the 20th July, and which had been mated and had oviposited, died on the 26th. Dissection showed that all the black shelled ova had been laid, and that the ovaries were small in size and confined to the hinder part of the abdomen. In this case, however, the individual ova were much smaller and had much less yolk than was found in the fly captured in the open and which was therefore presumably older. The question of the length of life of individual crane flies has not so far been settled.

Experiments were performed to test the degree of stimulus needed for the act of oviposition. Flies which had been mated and were placed upon cotton wool did not oviposit. Also when the wool was placed upon a layer of soil, they still failed to respond. Only in a few cases were eggs laid upon bare soil, whilst amongst herbage they were deposited readily. Although crane flies are known to oviposit usually in grass, it was found that they may do so in standing corn also.

The flies kept in captivity were not fed. They usually had access to growing grasses, and to soil, both of which were watered. They were observed licking at moist soil and at the wet sides of the glass vessels in which they were confined.

EXPLANATION OF PLATE XXXVI.

View of *Tipula paludosa* in coitu. The halteres are not visible owing to their vibratory movements.

Mating of *Tipula paludosa*

[103]

[FROM THE ANNALS OF APPLIED BIOLOGY, VOL. III, Nos. 2 and 3, JANUARY 1917.]

ON THE BIOLOGY AND ECONOMIC SIGNIFICANCE OF *TIPULA PALUDOSA*.

BY JOHN RENNIE, D.Sc., F.R.S.E.
(*North of Scotland College of Agriculture.*)

PART II. HATCHING, GROWTH AND HABITS OF THE LARVA[1].

(With Plates XVIII—XX and 3 Text-figures.)

THE most common species of Crane-fly larva occurring in grass and corn land in the north-east of Scotland is *Tipula paludosa*. *Tipula oleracea* occurs also, but is much less frequently met with. Along with these, there has also been found in comparatively small numbers in fields the larval stage of *Pachyrhina histrio*, but this species appears to occur more frequently in garden ground. The following Tipulidae in addition have been found in the winged stage in the district surrounding Aberdeen:

Tipula varipennis, common and generally distributed.
T. gigantea, in small numbers.
T. lutescens, in small numbers.
Pedicia rivosa, L. widely distributed in the northern area, but not common.

The Egg.

Hatching of the flies goes on during the months of June, July, August and September, and as already recorded (Part I) the first mating and oviposition may take place within a very short period. In captivity, hatching mating and oviposition have all occurred within a few hours. A newly hatched female contains considerably over 400 shelled ova. In two such taken at random the actual numbers were found to be 448 and 490. A third female captured out of doors *in coitu* contained 255 black shelled ova together with a quite small number—about 12—of pale coloured immature shelled examples. The form of

[1] The work recorded in this series of papers has been carried out with the aid of Grants from the Board of Agriculture for Scotland.

this female when found indicated that oviposition had previously taken place. A female *Pachyrhina histrio* taken in the open contained 259 black shelled ova.

The egg measures 1·1 mm. × ·4 mm.; it is black in colour, with a dark purplish metallic lustre. As development proceeds this lustre diminishes, and finally before hatching the shell is of a dull black colour. The covering of the egg is a strong tough membrane, which is completely formed around the egg before copulation takes place. I have been unable to detect a micropyle, but this may be present. It is possible that the membrane before coming in contact with the air is permeable to the spermatozoon. The somewhat remote possibility of parthenogenesis taking place with the first brood of eggs has not been overlooked, and females have been kept apart from males from the period of their hatching until death, but oviposition never took place under these conditions.

The Early Larva.

The emergence of the larvae takes place in about 14 days after the eggs have been laid. They are then of a pale reddish sandy colour, and about 2·7 mm. in length, Plate XIX (*b*). When fully extended, thirteen body segments can be made out. On each of these from the third to the twelfth there is a small tuft of laterally placed, moderately strong bristles. The thirteenth segment which bears the spiracles and terminal papillae, has a pair of tufts of relatively stronger and longer curved bristles, borne alongside the large lateral conical para-anal papillae. These tufts constitute the most striking difference between the early larva and the later form.

Through the skin the two longitudinal tracheal trunks are visible, and also the alimentary canal with its four anteriorly and one posteriorly placed diverticula. The masticatory apparatus is well developed, both mandibles and first maxillae being strongly toothed.

In a short time the segmental bristles tend to become very short or worn away, and so also do the anal tufts. Traces of the lateral bristles persist even in the fully formed "grub," but the anal tufts disappear completely. These changes appear to come about by attrition. In about 12 to 13 days from the time of hatching the larvae are 4—5 mm. in length when fully extended, and already resemble the older and more familiar "leather jacket." By about three weeks they have attained a size of 6 mm. They feed from the first day onward.

The fully grown Larva: External features.

Owing to the difficulty of keeping alive recently emerged larvae which were prevented from burrowing into the soil, it has not been possible so far to follow the changes in external form effected at the various moults. The larva when fully grown attains at full extension a length of about 40 mm. It is now of a brownish-grey earthy colour interspersed with irregularly placed blackish dots. Frequently the longitudinal tracheal trunks, two in number, may be seen through the skin. The shape is cylindrical, slightly narrowed anteriorly, and expanded posteriorly into a peristigmatic papillae-bearing area. The skin, which is generally tense in healthy larvae, exhibits the following characteristics:—along each side there is a moderately wide strip which on the animal contracting folds outward, forming a pair of blunt keel-like longitudinal ridges. Besides numerous transverse wrinklings, there are slight but definite transverse furrows marking off distinct segments. Eleven of these can generally be counted. Each segment bears on its ventral surface four very minute bristles, two lateral and two near the middle line slightly in front of the lateral pair. On the first four segments behind the head dorsally, there is a row of bristles, and a pair of dorso-lateral bristles on succeeding segments. The head bears a pair of short jointed antennae; there is a very strong chitinous and highly complex mouth armature which includes massive toothed mandibles with palps, a pair of serrated first maxillae, notched united second maxillae and elaborately folded and bristled labrum (Plate XIX (*a*)). The whole set of structures is based upon a large strong bivalved chitinous support which surrounds the gullet.

The anus, which is sub-terminal, is surrounded by large fleshy lobes and a pair of large retractile laterally placed conical papillae. Beling regards these papillae as characteristic for this species.

On the somewhat truncated terminal region there is a pair of large brown coloured circular stigmata, each with a lighter glistening dull golden marginal ring. This stigmatic area is expanded on its border into six conical papillae, of definite form and arrangement. There is a ventral pair whose tips are black with a clear central area. This pair appears to have a sensory function, and may be seen at times in the living animal apposed to the stigmata above. Below each of these ventral papillae there is a pair of small pigmented spots which are sometimes united to form a short streak on each side. The remaining four papillae project dorsally in two pairs. These bear on their exposed surface numerous fine hairs which follow the boundary of an elongated slightly raised conical area; the outer pair is tipped in black. Fig. 1.

(*a*) (*b*)

Fig. 1. Stigmatic area in *Tipula paludosa*, (*a*) showing papillae with hairs, stigmata, and pigment spots; (*b*) showing anus and fleshy lobes, together with para-anal papillæ.

DP. Dorsal papillæ; LP. Lateral papillæ;
VP. Ventral papillae; AP. Para-anal papillæ, outside stigmatic area; A. Anus.

Duration of Larval Period.

In the N.E. of Scotland the adult flies may be seen frequenting cultivated land from early in June to the beginning of October. It appears to be the accepted opinion in England (Theobald, *Agricultural Zoology*, 1913, p. 228) that there are two generations of these flies, *T. paludosa*, and *T. oleracea*, in the course of the year. Our observations have shown that in this area, probably owing to the higher latitude and more rigorous climate, there is only one.

The following observations made upon a small collection of flies reared from eggs which hatched in September, 1913, are typical of the results obtained in rearing during several years. The parent flies had hatched out within small laboratory cages or had been caught upon the college farm and placed in these. The cages had wooden roof and base, wire gauze sides and glass front. They contained a bed of turf two to three inches deep. Mating and oviposition readily took place, and on the turf being broken up later a considerable number of recently hatched larvae was found. A later search in the month of October, however, showed the mortality to have been considerable. On 1st November, the turf was again broken up and the surviving grubs collected and measured. The lengths were taken by allowing the larvae to crawl upon a sheet of paper and pricking this at the moment of their maximum extension. About one half the number of larvae was found to be under 16 mm. in length, and the remainder from 30 to 35 mm. It is remarkable that the larvae may attain to nearly their maximum length quite early, but it must be noted that they are relatively slender at this period. Subsequent growth takes place in the direction of thickness. By the end of the month the disparity in size was not so great. They all conformed to Beling's description of *Tipula paludosa* larva. In November clover was sown in the cages; this for a time afforded opportunities for feeding but was allowed to die out during winter. These larvae lived throughout the greater part of the following summer in soil containing decaying vegetable matter only, and duly pupated and emerged as adults in July and the early days of August.

As a control upon the above, portions of second year's grass upon the farm were dug up in October, the turf was disintegrated, shaken up in sieves, and the soil searched. Larvae were found and measured in a similar manner, and the commonest size at this date was found to range from 20 to 25 mm. These also were identified as *T. paludosa*.

Larvae collected out of doors during November and the first half of December showed a maximum size of 20 mm. These were relatively slender at this degree of extension, and contracted when handled to much smaller bulk than the laboratory reared specimens.

The larvae collected out of doors on 1st November, and which were separately caged, but kept under similar conditions, when fully extended measured 36 mm. in December, but in this state were distinctly more slender in the body. Examination of the contents of the alimentary canal showed that they had fed upon the decaying rootlets in the soil. As the season progressed it was found that there was eventually no significant difference in size between those reared indoors and those living a natural life outside. The records of soil temperature taken on the farm showed that there had been little frost during this experiment. The winter was a mild one, and the facts suggest that some feeding at least had taken place amongst the larvae out of doors. In a subsequent season caged larvae kept short of food were found in February to measure from 20 to 30 mm. Generally, there has been found out of doors great variation in the size of larvae at the end of winter in the same district and even upon the same field.

Tipula paludosa has been kept under direct observation throughout its whole life cycle, and owing to variations in the length of the larval stage pupation and consequently hatching of adults is spread over a considerable period, viz., in this district, June to September. (Rarely, I have found adults in the cages in May.) Under experimental conditions of limited food supplies larvae have been kept alive and been continuously under observation for fifteen months. The minimum duration of the larval period has been found to be about nine months—September to June. Before all the larvae of a season have pupated the next season's larvae may have appeared, so that there may be larvae present in the soil all the year round. There is a possibility that this fact may have led to the view that there are two generations of flies in the year. I have had under observation in breeding cages in the month of July, larvae, pupae, and adults of one generation, together with developing eggs and emerged larvae of the next generation—all alive at the same time; and in the variable climate of the region under observation such occurrences are not improbable in the field.

Bionomics of the Larva.

The newly emerged larvae are very susceptible to drought, and when kept in dry soil were found to die off quickly. Strong sunlight, even when the soil is moist, was also fatal in a short time. Artificially reared larvae require to be kept moist and sheltered from direct sunlight, otherwise the mortality in the early days is very great. Larvae which are hatched from eggs which have been placed upon the surface of the ground immediately burrow into the soil, avoiding the light. A large proportion of larvae reared from eggs in 1913 died in the course of the first eight weeks, especially towards the end of this period, notwithstanding all attempts to reproduce natural conditions, and only a comparatively limited number of flies have been reared from many thousands of eggs laid in the laboratory cages.

In view of the fact that very large numbers of eggs are laid, and of the probability that the adults only rarely approximate to these numbers, there must be a considerable mortality in the course of the life-history, due, of course, to various factors. Our experience suggests that the first few weeks of life constitute a period in which the insect is particularly susceptible to the prevailing physical conditions. While difficulty was experienced in rearing large numbers of larvae from the egg to the adult stage, no such difficulty was met with in rearing flies under the same laboratory conditions from larvae collected in the field in late winter. It may be suggested for example that wet weather in the end of summer, and early autumn months, will favour the survival—apart from natural enemies—of greater numbers of larvae, and that conversely, prolonged drought will tend to kill off numbers of those hatched about this time. In this connection it may be worth while to quote the opinion held by some farmers in this area that a wet summer and autumn foreshadows a plentiful supply of crane-fly in the following year.

The Larva on Farm Lands.

Published references to the activities of the larva as far as I have been able to trace them deal exclusively with instances of serious or even excessive damage effected by these insects upon grass or corn crops. But in the course of the present investigation it has become clear that Tipula larvae are very commonly present upon farm lands, sometimes in considerable numbers, without their presence becoming apparent. Cases of excessive damage have also been experienced, but the following instance may be taken as an average experience under the conditions named. It is quoted in full because it illustrates a number of features related to the larval habits.

These observations were made in the spring and summer of 1913, upon the College farm at Craibstone. Search was made for the presence of Tipula larvae in the end of March and beginning of April. The weather was cold at the time, and the searches were not very fruitful. The Woodlands field (Fig. 3) which was in grass at this time was selected for enquiry, samples being dug up at a number of places, and the turf thoroughly examined, but no Tipula larvae were obtained. Grey slugs were particularly plentiful. This was on 3rd April. On the 19th, ploughing was in progress and the plough was followed, samples of the furrow were taken, disintegrated, and searched, but no larvae were found in this way. Further search by two observers resulted in four larvae being found. These were found under stones at the surface, on the part not touched by the plough. After the field had been sown and rolled it was again examined on the 29th, and larvae were now found to be very numerous under the turf clods upon the surface. In the interval between the times of examination there had been a good deal of rain.

On the 3rd of May the field was visited at 6 a.m. The two previous days had been dry and sunny, but in the end of April there had been much wet. The morning was fine, and at 6 a.m. the sun's rays had reached the western end of the field only. The eastern end was still in the shadow of the trees.

A search was commenced at the eastern end where there was some frost in the ground. In about 45 minutes 94 larvae had been collected. In the southern hollow where the sun had now reached, 42 were obtained in about 10 minutes; on the crest of the field at the west end (in sun) 75 were found in 20 minutes, and on a low part (N.W. corner), in the sun, 15 in 15 minutes. Two collectors were at work. In all in about 1½ hours 226 larvae were obtained. In some cases from six to a dozen were found in a single piece of turf. The smallest number appeared to be present in the shade at the highest level of the field (E. end). The larvae were found mostly under the turf clods, and largely in the "mids"; sometimes they were lying on the soil below, and sometimes embedded in the turf with heads well buried amongst the roots. They were not seen distributed generally amongst the sown grain.

The field was again examined on the 10th of May. There had been continuous and heavy rain for several days previously, and the ground was very wet. The oats had "brairded" early in the week, but the wet weather had rendered rolling impossible. At the time of search rain was not falling, but there was a mist close down to the ground. Larvae were frequently found beneath the loose turfs upon the surface, and they were also to be seen crawling freely on the ground. Some trouble was taken to find them in the act of attacking the young crop, but with no success. The ground in a number of places was scraped with a toothed digger and the plants turned over. A few larvae were found in the ground in this situation, i.e., free from turf clods and amongst the soil in which the oats were growing, but none was seen actually attacking any part of the crop. The oats were scarcely an inch above ground. In many places no corn could be seen and here the ground was turned with the digger. A few larvae were found in this way, but it could not be said that they were more numerous than in other situations. It was found that mostly the seed in these places lay deeper and was growing all right.

During the following week the weather improved and there had been several warm and dry days. On the 17th the field was again examined. It had been rolled during the previous day. The crop looked well, and there were no indications of Tipula attacks. Larvae were again found in the most usual situation, viz., below the turf clods; 40 were collected in a few minutes. Search amongst the growing oat plants resulted in only one larva being found; none was seen upon the surface, and none detected attacking the crop.

On the 21st another examination was made. The weather in the interval had been showery, but not very cold. The day was warm and there was some wind. The field was carefully searched, particularly for traces of larvae moving freely in the soil or actually attacking the crop. They were found in the usual places below or burrowing into loose "foggage" upon the surface. In a few cases they were found below flat stones at the surface. Only a very few were obtained by searching the open soil around the oat plants. In this search the soil was turned over with a digger and the oat plants uprooted. Sometimes the ground was scraped and stirred. The examination ought to have discovered larvae if they were present in the soil in proximity to the roots of the oat crop, and it is concluded they were absent in this situation at the time of search, viz., between 10 a.m. and 12 noon. The weather at the time was warm and showery, and larvae could always be found amongst the decaying turf. The crop at this date, notwithstanding the undoubted presence of Tipula larvae in large numbers, showed no bad effects. Up to the beginning of July, when the last search for larvae was made upon this field, there was no apparent effect of the presence of Tipula upon the crop. On this occasion the search was effected by cutting the crop over certain areas, and sifting the soil by spade and sieve. Larvae were obtained, but no pupae were seen.

It should be mentioned that in view of the presence of Tipula in appreciable numbers and the possibility of an attack ensuing, a plot experiment was early arranged upon the field with the object of testing the effect of rolling and of some common manurial substances. The experiment, though negative in its results as far as its original purpose is concerned, is given here because it confirms the conclusion that Tipula was not visibly damaging the crop. The field, which is surrounded by trees, was rich in humic matter. Below is given a diagram of its situation, and of the experimental plots together with the report of Mr W. Findlay, N.D.A., Superintendent of Field Experiments.

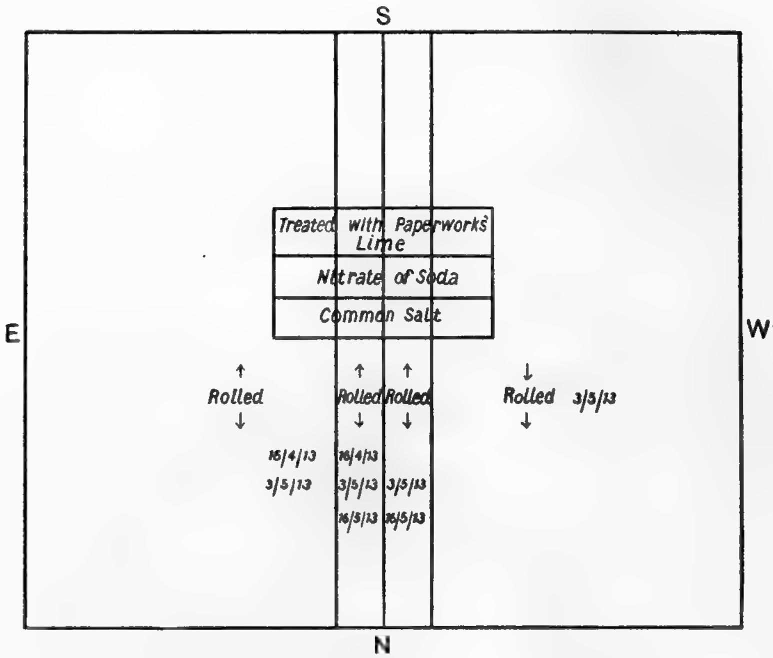


Fig. 2. Diagram of Experimental Area of Field.

Report. The Woodlands field at Craibstone was ploughed out of Lea during the second week of April, 1913, and immediately sown with Sandy oats at the rate of six bushels per acre. A heavy rainfall prevented the whole of the field being rolled at that time, and an interval of about two and a half weeks elapsed before the soil was dry enough to finish the operation. One strip the whole length of the field was rolled three times, and other two strips were rolled twice.

On the 21st May three plots were laid off and treated as follows:

1. 4 cwts. Paperworks' Lime per acre.
2. 1½ „ Nitrate of Soda per acre.
3. 2 „ Common Salt per acre.

The accompanying plan will show the scheme of the different treatments and cultivations.

At no time was there any difference either in the thickness or strength of the crop between the parts rolled at different times.

The plot to which Nitrate of Soda was applied showed an increased crop of about 20 per cent., but those to which Lime and Salt were applied could not be distinguished from the rest of the field.

WM. M. FINDLAY.

The experience here recorded has been general for a series of years; large numbers of Tipula larvae have been regularly obtained from lea fields upon farms on Deeside and elsewhere in the neighbourhood, which during the periods tested had no crop losses due to their attacks. In a good many such cases the numbers obtained from single fields were considerable. A case where serious damage was effected is given upon page 132.

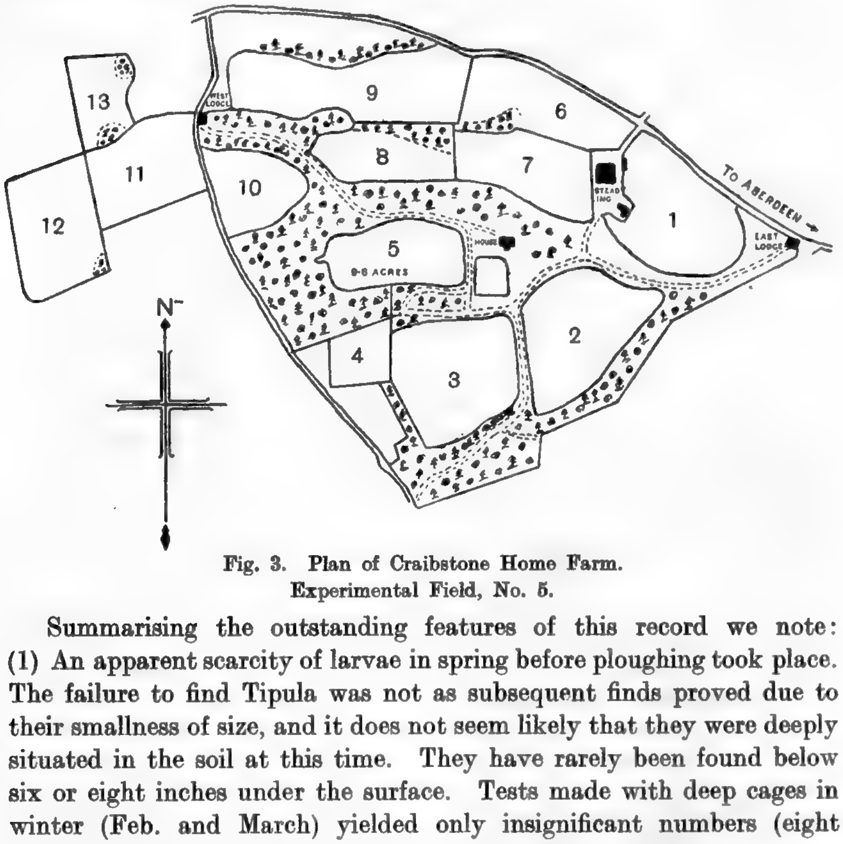


Fig. 3. Plan of Craibstone Home Farm.
Experimental Field, No. 5.

Summarising the outstanding features of this record we note: (1) An apparent scarcity of larvae in spring before ploughing took place. The failure to find Tipula was not as subsequent finds proved due to their smallness of size, and it does not seem likely that they were deeply situated in the soil at this time. They have rarely been found below six or eight inches under the surface. Tests made with deep cages in winter (Feb. and March) yielded only insignificant numbers (eight per cent.) below six inches from the surface.

(2) When known to be present in the field they could not be found in the act of attacking the crop. The suggestion that they feed at night is plausible, but observations on larvae in cages have shown that they feed readily at all times. The fact that there was an abundance of humic matter in the soil is probably not without significance in this connection (see pp. 127—8).

(3) The occurrence of most of the larvae at the surface beneath loosened decaying turf. Their presence here in the spring is general; they occur both loosely below, and also very frequently deeply embedded in the turf. The disturbance of the soil in ploughing and harrowing is probably the cause of their gathering in these situations, and their presence is probably primarily due to the need for shelter and moisture.

(4) The absence of harmful effect upon the crop notwithstanding their presence in considerable numbers throughout the spring and summer. This feature is considered in connection with the further data given below.

Further consideration of feeding habits.

With a view to rendering clearer the feeding habits of the larvae and to throw light upon the circumstances under which they attack growing crops numerous experiments were made of which the following are illustrations.

I. A small lot of larvae reared from eggs laid in September was kept in ordinary field soil covered with loose turf in a small laboratory cage. No crop was sown in the soil, but it was watered from time to time. They wintered under these circumstances and continued throughout the following summer. The larvae pupated in July and the last of them emerged as flies on the 4th August and mated on the same day. The life cycle in this case occupied about eleven months.

II. A collection of larvae was kept out of doors in small boxes containing ordinary garden soil without growing vegetation during the months of May, June, and July. They survived this treatment, but were undersized. Some managed to pupate, but others died in the larval stage. One imago was observed to fail in the act of emerging from the pupal case. The larvae of this group did not on the whole do so well as those of lot I, and the mortality towards the end of the experiment was high. Dissections showed the presence of vegetable fibres in the intestine, and a considerable amount of gritty material. It may be mentioned that this latter is normally present in the intestine.

III. A collection of larvae was kept in small cages with no growing plants, and a limited amount of decaying vegetable matter amongst the soil. These conditions were maintained during the months of May and June of the present year. At the end of June all were alive and healthy looking, and some were well grown. The cages were set in a large field rearing box containing washed sea sand, amongst which the metal cages containing the larvae were sunk. A considerable number of larvae wandered from the cages and were subsequently recovered amongst the sand, alive and to all appearances quite healthy. Adult flies were later seen emerging from the cages in the laboratory to which they were removed during July. The larvae of this and Experiment II were collected in the fields and had wintered out of doors.

The foregoing, together with other experiments and observations of a like nature have shown clearly that the Tipula larva may subsist in the soil and complete its development independent of the presence or absence of a growing crop upon the ground. The results here obtained have led to the institution of further and more exact experiments dealing with the nutrition of soil larvae including Tipula. These are at present in progress.

IV. A number of larvae caged in the autumn of 1915 were kept in soil without growing vegetation except for a short period when a small quantity of corn sown in the cage germinated. In February they were found to have reached a fair size. Several killed and examined on the 19th were found to contain soil particles and fragments of vegetable fibre. At this date they were found mostly in compact earthen cells formed against the sides at the bottom of the cage. This habit has been frequently observed in winter and suggests a quiescent period under the adverse conditions of cold, confinement and restricted food supply.

In order to determine more clearly the circumstances inducing destructive attacks upon crops the following type of experiment was resorted to:

Groups of larvae were put up in large cylindrical glass cages of about 10 inches diameter, in prepared soil, in which the visible amount of vegetable matter was very slight. This soil was, further, mixed with well washed sea sand. Around the cylinders between the glass and the soil, oat seed was introduced. The cages were kept at room temperature and were examined daily. After the corn had germinated, the larvae were kept under close and continuous observation for prolonged periods at a time. The larvae appeared sluggish, and not much movement was seen in the day time, although their burrows soon became very numerous between the soil and the glass (Plate XX). They could be seen lying in these, and after the corn had germinated, or even before this, they could be seen attacking it, gnawing at the husk, radicle and plumule. They were also seen eating the blades which had come above the ground. The glass cylinders had removable ends of perforated zinc of fine mesh. A few larvae passed through the perforations at the bottom although these were small Within the cage they tunnelled freely to a depth of six or seven inches. After a week, when all the corn appeared to have germinated, and both radicle and plumule were of some length, the cylinders were removed and the state of the seedlings ascertained. These were separated out carefully and placed in water. The soil was removed as far as possible by gentle washing, and each seedling examined in turn. Care had been taken that no other creatures were present in the soil capable of damaging the oats. There were usually about 25 larvae present. A typical result is given:

34 seedlings apparently sound.
11 had radicle or rootlets more or less eaten away.
39 had plumule cut through or bitten into. This includes a number attacked in the blade, above ground.
8 seeds were attacked. In some cases this had been effected before germination, in others, afterwards. In some of these the food store was completely eaten out, in others only partially so.

92

Equal to 63 per cent. of the plants damaged.

The factors contributing to such a result appear to be:

(1) The absence of decaying or other vegetable matter, with consequent restriction to the crop for food supplies.

(2) The large proportion of larvae to the cubic content of soil—two dozen to about $1\frac{1}{2}$ cubic feet of soil.

(3) The confinement of the larvae to a limited amount of space; it was not possible for them to leave the cages.

A further factor to be noted is the favourable conditions of warmth and suitable moisture, inducing rapid germination. This would be of some importance in the field in favour of the crop "growing away" in a proportion of cases before the damage done became irremediable.

In other experiments of this nature with germinating oats, clover, or timothy grass, the numbers of larvae used were very much greater, with correspondingly serious damage. It was found subsequently in examples of very severe attacks upon crops in the field, that the numbers used in these experiments were in excess, and that serious loss may ensue where the numbers, in average samples of an affected area, are about 10 to 15 per square foot of surface (see p. 133).

Migrations.

The use of large numbers of larvae upon experimental plots or open shallow cages brought to light the fact that scarcity of food in spring due to over stocking results in the larvae migrating. The first proof of this was obtained from a lot of 120 larvae contained in a small box in soil in which clover had germinated. As the food supply became exhausted the larvae left the box in large numbers and were found crawling about the laboratory. Two other instances of this occurred in connection with field plot experiments at Craibstone. In these experiments, small plots of 2½ feet by 6 feet were walled off with wooden boards sunk six inches in the ground and standing about three inches above the surface of the soil. It had been established in experiments in a previous winter that these larvae even in very cold weather rarely went below this depth of six or seven inches, and it is not considered likely that they burrowed beneath the limiting boards of the plots. The plots were stocked with large numbers of larvae; in one instance three adjacent contained 900 larvae each and in other three plots, there were 700 each. When it was discovered that the crops in these had failed, at the end of June the soil in each was sifted for larvae. The hatching season had just commenced, but only a very few flies had been seen; of the 2100 larvae in the second group of three plots only 295 were recovered, together with a small number of pupae and two or three empty pupal skins.

Another plot was not disturbed. It had stood exposed in the same way as its neighbour, for some weeks, but later a large field cage was fitted upon it. Here there had been placed originally 2700 larvae, and now to these were added the 295 recovered from the other plot. In due course flies hatched out and appeared in the cage, but the numbers up to the end came very far short of those of the grubs introduced. The soil was searched and no trace in the form of empty pupal cases or dead larvae was found.

In a duplicate experiment on Holm Farm, in Lewis, carried out at the same time, designed to test the relative resisting or recovering power of selected oat varieties, it is probable that a similar migration took place. This experiment consisted of plots of Hamilton, Sandy and Potato oats, into each of which 900 larvae were placed. There was also a control plot of Hamilton oats, which received no larvae. The oats were sown on 18th May, and "brairded" on 3rd June.

The plots were under continuous observation till the end of June, during which time no difference was observed in the growth of the different varieties. At this date the plots were searched and only a few larvae could be found. The local observer states that he is confident birds did not interfere with the plots, and the only conclusion that can be drawn is that the larvae migrated from the plots before the crop had germinated. Had they remained and continued their development, pupae and empty pupal cases would have been found in the plots. As a matter of fact these were examined before adult flies had begun to appear. Had the larvae died within the plots it is certain that the remains of some at least would have been found, when the soil was passed through the sieve. But nothing of the kind was found.

The question remains—do they migrate in appreciable numbers normally, in search of food? On the fields under observation I have found them wandering upon the surface, but so far only occasionally. With regard to the plots, it is to be borne in mind that the larvae had been placed in excessive numbers on soil which did not contain any growing plants (except the ungerminated corn), while around there was abundant green vegetation. Their power to climb up even a few inches of a vertical board was not expected; that they did so was also evidenced by their being found in the control plots in which none had been placed.

A further interesting case, bearing on the point, is recorded in an editorial note in the *Scottish Naturalist*, 1915, p. 1.

The reference is brief and may be quoted in full:

"A few weeks ago it was reported to us that the inhabitants of a certain district in Perthshire had been seriously alarmed by an invasion —not of Germans—but of an immense number of small worm-like creatures which crawled over the road near the houses in such numbers as to make walking decidedly unpleasant. Examples of the creatures were brought to us, and were recognised as 'leather jackets'—otherwise the larvae of *Tipula oleracea*, the commonest species of crane fly."

As bearing further on this subject, the Editorial note proceeds to quote from an article by P. Desol, in *Compt. Rend. Soc. Biol.*, Paris, LXXVII. No. 21, June, 1914, pp. 126—7.

The summary given is quoted from the October 1914 issue of the *Review of Applied Entomology* (Series A). As the conclusions here are at variance with my own on *T. paludosa* and with the occurrences just described they are reproduced. The observations were made in the meadows of Avesnois. "In the spring of 1914, circular patches of from 15 to 60 feet in diameter, or the entire areas of meadows, were to be seen covered with yellow and dead grass the roots of which were found to be full of the earth-coloured larvae of *Tipula oleracea.* Grasses and clovers are chiefly attacked while plants with hard or thick roots are not affected. Where the infested zone borders on a furrow the larvae fall into it during the night and being incapable of climbing out may be collected in large numbers. They are not migratory, so that healthy meadows may be found close to infected ones. Infection is due to chance circumstances bringing fertilised Tipulids to the ground." I am satisfied that the statement that the larvae are not migratory is erroneous and that the existence of healthy meadows near them may easily be otherwise explained.

The conclusion to the experiments just described—unsatisfactory as regards the object for which they were planned—was due to the excessive numbers of larvae used on the plots. The large numbers were decided on with a view to avoiding an indifferent result, and this was supported by field experience, where large numbers had been seen and which had not proved harmful. In its result, however, it is useful and the possibility of migrations from waysides, waste land, or grass fields to germinated corn crops cannot be neglected. The experience recorded on p. 122 may be recalled, in which a field examined in April, and found almost negative as regards the presence of Tipula larvae, was later found to have an abundant supply. This problem is under continued investigation.

Various field experiments and observations have been made with the view of testing the effect of manurial agents and various insecticides upon the larvae. One of these is quoted below, but in general the results obtained were not consistent, and in cases of some well-known insecticides were not corroborated by direct tests upon larvae in confinement. The experiments were useful mainly in bringing into prominence some factors which are contributing elements affecting the degree of attack upon the crop, and which are considered in the succeeding section.

Field Experiment: Holm, Stornoway. Oat Crop.

In the previous season the field was grazed by cattle right on to the end of November, and with sheep and cattle on to January. Ploughing began early in January, and was finished by the end of February.

Weather.

From the beginning of October to the middle of March it was exceptionally wet and boisterous. There was practically no frost and the temperature was normally mild. The Farm is worked on the five shift system; the present tenant came to the farm in 1910, and previous to this the farm had been much neglected, especially as regards drainage and weeds. Oats were sown in the last days of March. After sowing, the field was immediately harrowed—one single and two double. It was not rolled till 23rd May.

The oats "brairded" in about a fortnight from sowing time and looked strong and healthy for a week. About 20th April the crop began to look sickly in parts and by the end of another week the presence and activities of Tipula larvae became apparent. The weather for some weeks after sowing was warm and sunny.

The state of the crop was reported to the College authorities in May, and on 24th May arrangements were made to treat the field experimentally. At this time, it showed parts fair, parts very thin, and fairly large tracts were quite blank. All over the field leather jackets could be readily found, and on the bare parts they were present in very large numbers; scores could be found near the surface in a square foot of soil. A part of the field including the worst portions was plotted as under:

Rolled twice	Plot	Plot	Plot	Plot	Plot
Rolled once	No. 5	No. 4	No. 3	No. 2	No. 1
Unrolled	No manure	4 cwts. common salt	1 cwt. Nitrate of Soda	2 cwts. salt	1 cwt. Nitrate of Soda

The manures were applied on the 6th of June, and two-thirds of the total area rolled early the following morning. On the 14th June one half of that part was rolled a second time. The other third was not rolled.

On June 26th the larvae were still to be seen, and active. Ten trial counts were made on square foot samples taken at random. These were dug to a depth of six inches and the larvae counted. The following are the numbers found: 4, 5, 4, 4, 5, 4, 12, 14, 14, 13. No sample tested was found blank. The last four lots were from Plot 5, unrolled part. Plots 1, 3, and 4, showed improvement on the crop at this date.

On July 14th the results showed as follows:

Plot 1. Originally the thinnest. Recovery good.
Plot 2. Originally 2nd thinnest. Now same as Plot 1.
Plot 3. Originally the best. Much ahead of all.
Plot 4. Originally only medium. Second best.
Plot 5. Originally 2nd best. Worst of all.

There was no very obvious difference between the once and twice rolled parts of the plots, but the unrolled parts were decidedly poorer. Larvae were still active in the field at this date.

On 15th September the field was visited and the crop as a whole found to be in a very good condition, it having tillered remarkably well. The variety of oat was Hamilton. Signs of the larvae having been present were scarcely apparent, any patching being extremely slight. The nitrate plots did extremely well, the salt ones also were very good. From the appearance of the crop in May, it would have been impossible to have foreseen such an excellent recovery. Local circumstances did not allow of the estimation of the yield of the separate plots by weight per plot.

Factors favouring the Larva.

It may be regarded as well established that Tipula larvae are generally distributed and ordinarily present in the soil, though not necessarily only upon farm lands. Even upon these they have been found to be present in appreciable numbers, when no recognisable loss in crops resulted. The question of importance is,—what are the circumstances determining a destructive attack upon a crop?

Naturally the first condition of importance is the presence of excessive numbers. The various factors contributing to the periodical appearance of large numbers of any species of insect have not hitherto been appreciated in advance, and indeed cannot be said to be well understood. In general with regard to Tipula they may be held to include:

(1) favourable weather conditions for the survival of the large numbers of young which are generally produced:

(2) unfavourable conditions for competitors:

(3) ready supply of food:

(4) absence or diminution of numbers of natural enemies.

As regards the influence of the weather in affecting numbers it has been already noted that the newly hatched larvae are very susceptible to drought and that the mortality in laboratory reared larvae which are not kept moist is high. There is a fairly general popular impression that severe winter with plenty of frost kills the larvae, but our experience so far does not bear this out. Larvae were left in the open exposed upon stones during a night of severe frost, and were found alive the following morning. The apparent beneficial result following frosts seems to be due to the improved tilth favouring a more rapid primary growth of the crop in spring. The environmental factors are the subject of continued investigation. Unfortunately, owing to the presence of the larvae on cultivated land, the food supply is adequate. Food is not ordinarily scarce, and as already indicated a high percentage of larvae kept in captivity complete their development upon ordinary turf in the soil.

The question of natural enemies is dealt with in a succeeding paper.

Tipula attacks on Oats.

Conditions unfavourable to the oat crop may render it susceptible, and Tipula, even when the numbers are not excessive, may work havoc. Apart from the question of manuring and general farm practice which are not considered here, the weather in spring has been found to be a significant factor. These observations have been carried on during a period of seven years; along with these the experience of over 130 farmers on a wide area has been collated and there is a universal testimony to the fact that a cold late spring in which the primary growth of the plant is delayed constitutes one of the most certain conditions for crop failure due to Tipula attack. It is during the early days of growth from the time of sowing until the adventitious root system is established that Tipula attack results in the destruction of the individual plant. After this period the plant may be regarded as out of danger; it may be weakened but probably it will not be killed outright by subsequent attack. Any cause therefore tending to extend the period of germination or immediately subsequent growth increases the liability of the crop to loss from Tipula attack. In this area the time between sowing and brairding for oats in an average season is 10 to 14 days. In a series of seven cases which came under observation in one season in which failure of parts of the oat crop, attributed to Tipula attack, took place, this period ranged from 16 to 21 days. There was one case in which, owing to severe drought, it lasted six weeks. The actual proportions of crop failure were stated thus:

Period between sowing and germination	Proportion of crop lost
16 days	one-fourth of whole crop
6 weeks	one-third of whole crop
17 days	one-tenth of whole crop
3 weeks	loss incurred; proportion not estimated
16–18 days	almost one acre destroyed
3 weeks	one-third of crop
3 weeks	one-third of crop; it recovered later

On this account early sowing, especially in our north-eastern climate, is attended with a certain amount of risk. The same risk would apply to all late districts in seasons when fine weather early in spring tempts the farmer to sow early.

Tipula and Oat Varieties.

The question of the relative resisting or recovery power of different oat varieties in relation to Tipula attack has been investigated both in the field and in plot experiment. The experience of the farming community within the College area has been obtained and discussed, and the general view is given below. There exists some opinion that the larva exercises a selective capacity and prefers some varieties to others, as is evidenced by the following statements:

"They (i.e., the Tipula larvae) like Potato oats better than Sandy."
"Potato oats are more liable to attack than Red Oats or Sandwich."
"They are more fond of 'Potato' varieties."
"Waverley suffers from attack more than Potato or Sandy."
"'Banner' oats suffer more than Potato."

All the evidence, however, goes to show that this is not the correct interpretation. It is not the larvae which "prefer" particular varieties, but particular varieties which show better power of recovery from their attack. Some of the writers expressed their experiences more carefully:

"Of the 'Waverley' and 'Hamilton' oats which were sown side by side, the 'Waverley' had to be resown; 'Hamilton' oats were only slightly thinned."

"The 'Red Oats' resisted poorly, 'Providence' and 'Potato' did better."

Taken collectively the views of over a hundred farmers who have had experience of Tipula attack is contradictory with regard to the relative merits of particular varieties. This is likely to be the case so long as other factors such as quality and source of seed, type of soil, particular agricultural practice, manuring, and so on, are not considered. But there is general agreement that the newer varieties of oats have distinctly less power of recovery, because they do not tiller well. This is well borne out by wide experience.

Another practice which has been found of service in overcoming Tipula is to effect a "change of seed." Seed grown on the coast and sown in an inland locality, and seed grown in the Lothians and sown in Aberdeenshire have in both instances proved more resistant where larvae were at work, than native grown oats. Further, seed from an early district and sown in a late one, also from a light soil to a heavy one, have in both instances been proved satisfactory measures against losses from Tipula attack.

The subject of preventive agricultural practice and remedial measures in relation to Tipula are dealt with in a further paper.

REFERENCES TO PLATES.

Plate XVIII. *Tipula paludosa.* Larvae. 3 times natural size.

Plate XIX. *a.* Head armature of *Tipula paludosa.* (A) antenna; (B) mandibles; (C) first maxillae; (D) fused second maxillae; (E) labrum; (F) internal basal support of appendages.

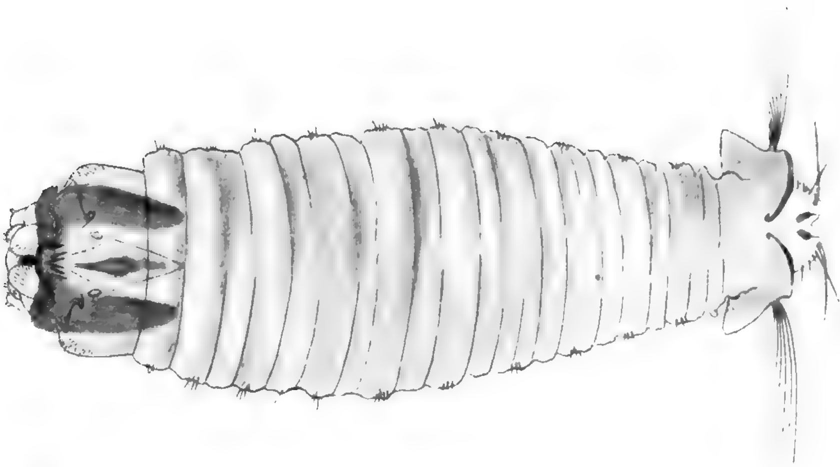
b. Newly hatched larva of *Tipula paludosa.*

Plate XX. View of larvae in soil within glass rearing cage, showing their burrows and damaged oats, which were grown between the glass and the soil. Sketched from nature.

REFERENCE.

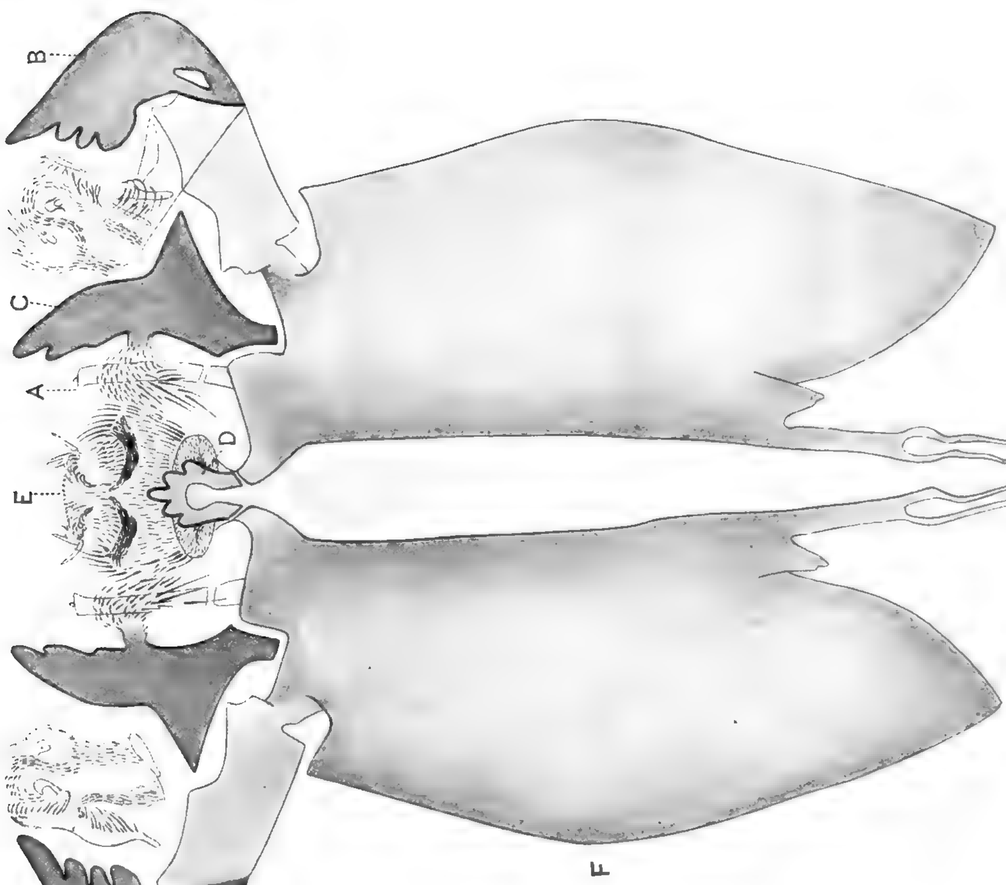
Beling, Th. Beitrag zur Naturgeschichte verschiedener Arten aus der Familie der Tipuliden. *Zool. Bot. Gesellsch. in Wien,* xxiii, Bd. 1873, pp. 575–592.

124



(b)

Del. B. Simpson



(a)

Del. E. Harvey

Del. B. Simpson

The Connection of *Nosema apis* with Isle of Wight Disease in Hive Bees. Remarks on the evidence submitted in the Board of Agriculture Reports of 1912 and 1913. By John Anderson, M.A., B.Sc., Lecturer in Bee-keeping to the North of Scotland College of Agriculture.

(Read 24th January 1916. MS. received 5th February 1916.)

I. Historical and Introductory.

Isle of Wight Bee Disease was reported from the island after which it is named in the year 1904, and it was at first stated that it did not appear on the mainland till 1909. It is now, however, believed that "the trouble was not unknown on the mainland prior to the Isle of Wight outbreak." [1]

It was in 1906 that bee-keepers became really alarmed, and Mr A. D. Imms, B.A., M.Sc., was requested by the Board of Agriculture to proceed to the Isle of Wight to investigate. He found the disease prevalent over practically the whole of the Island, and he collected much information from the bee-keepers as to the symptoms and course of the disease, the ways in which they believed that infection was conveyed, and the remedies they had experimented with. He dissected a number of bees, and made smears of the gut contents, fixing and searching these for bacteria.

Imms' description of the symptoms is as follows: "The earliest noticeable symptom of the disease is the inability of the affected bees to fly more than a few yards without alighting. As the disease progresses the bees can only fly a few feet from the hive, and then drop and crawl about aimlessly over the ground. They are often to be seen crawling up grass stems, or up the supports of the hive, where they remain until they fall back to the earth from sheer weakness, and soon afterwards die. In a badly infected stock great numbers of bees are to be seen crawling over the ground in front of the hive, frequently massed together in little clusters, while others remain on the alighting board. . . . Affected stocks examined in early spring show symptoms similar to those of dysentery. The bees discharge their excrement over the combs and on the sides, floor, and alighting board of the hive. . . . The bee-keepers state that this condition is only present after the winter confinement within the hive. . . . After the winter is over and the bees are all on the wing, no dysentery is noticeable, and all the diseased bees that have been dissected showed the opposite condition of distension of the gut. . . . The colon and adjacent part of the rectum are enormously distended

[1] Board of Agriculture Report, 1912, p. 13.

[*Reprinted from the "Proceedings of the Royal Physical Society," Vol. xx., pp. 16–22.*]

with a congested mass of material, consisting primarily of pollen grains. . . . Some amount of a dark-coloured fluid is present very often in the chyle-stomach, but it is not distended with it."

Mr Imms suggested no theory to account for the disease, but he recommended destruction of the affected stocks, with thorough disinfection of the hives and appliances.

Dr Malden's researches were carried out during 1908, and his report was published in February 1909. He made a systematic bacteriological examination of the alimentary canal of the bee. All bacteria observed were separately investigated, and it was found that no one type was characteristic of the disease. With the exception of one form, all the bacteria found in diseased bees were found also in healthy bees, and just as commonly. A type, called by Malden *Bacillus pestiformis apis*, was, however, present in 60 per cent. of the affected bees, so he made pure cultures and fed these to healthy bees. The bacillus was transmitted but the disease was not produced, so Dr Malden concluded that *Bacillus pestiformis apis* could not be the cause of the trouble.

The third and largest report, issued in May 1912, was the joint production of five workers. This report extends to 143 pages, is very comprehensive, and claims to contain everything of value in the earlier reports of Imms and Malden. From the point of view of this paper the most important sections are IV., V. and VI., because these deal with the alleged connection between Isle of Wight Bee Disease and the parasite *Nosema apis*. The succeeding sections of the report lose a considerable part of their significance if it can be shown that *Nosema apis* is not responsible for the disease.

It was in 1906 that Drs Fantham and Porter discovered in diseased bees from the Isle of Wight a protozoan parasite which has been stated to be invariably present in bees suffering from Isle of Wight Bee Disease. In 1907, E. Zander in Bavaria had independently discovered the same parasite, had named it *Nosema apis*, and had declared that it produced in bees a disease which he called "malignant dysentery." (Imms states, however, that dysentery is not a characteristic symptom in Isle of Wight Disease.)

Drs Fantham and Porter have worked out the life-history of *Nosema apis* with much care and in very minute detail. Their monograph, contained in Section V. of the report, is illustrated by many drawings on a large scale. The organism produces a characteristic spore, oval, highly refractive, easily distinguished, being 2 to 4 μ in diameter, and 4 to 6 μ in length. In badly infected specimens almost every cell in the chyle-stomach of the bee appears to be a mere bag of spores. The ripe spores escape into the colon, are discharged by the bee in flight, and are ingested by other bees when they sip the dew on the grass. When the chyle-stomach of the new host is reached, an amœba escapes from the spore, penetrates one of the cells of the gut-lining, and starts to grow at the expense of the cell. Two or more parasites may enter the same cell, but even one by repeated subdivision may fill the cell with spores.

II. Some Experiences in Lewis, Outer Hebrides.

Specimens were first sent to Cambridge from Lewis in October 1911, at the time of the second outbreak of disease in the Island. The specimens were from two stocks, and these are Nos. 54*a* and 54*b* in the table on page 47 of the 1912 report. Dr Graham-Smith examined these himself and reported "many young stages" of *Nosema*. Destruction of both stocks was advised, but they were watched for a few days longer, and only one was killed. The other, a colony of American Golden Bees, completely recovered, wintered in excellent order, and never again displayed crawling symptoms. A stock which "crawled" during the following January was found by Dr Graham-Smith to contain *Nosema* in all stages. It was allowed to die out, and became extinct on 29th February. In the summer of 1912 another stock displayed "crawling" in a very marked degree. Specimens were sent to Dr Graham-Smith, and finally, in July, the remnant of the stock, with combs, was despatched to Cambridge. They were kept for some time there, and in those which died Dr Graham-Smith found "nothing very special."

In June 1913, there was in Stornoway a very typical case of Isle of Wight Disease. The stock affected was at full strength, crowding ten standard combs, and fully occupying a section crate of the usual size. The crawling bees could be found all over the garden on a fine day, and the other symptoms of the disease were very marked. A number of the "crawlers" were examined for *Nosema*, but no spores were found. Specimens ("crawlers") were sent as usual to Dr Graham-Smith. Dr Porter, who had now been entrusted with the work, made the following report with regard to the specimens sent: "The bees examined contained some young stages of the parasite. One had a very few spores. There is no doubt that *Nosema* exists in them, at any rate in some, which act as parasite carriers."

I was much interested to learn from this report that *Nosema* could not always be found in "crawling" bees, because this agreed with my experience in Lewis. By this time I had examined a large number of bees for spores of *Nosema*, and the result had been surprising. I had no difficulty in getting the spores, but these were found in bees which appeared perfectly healthy rather than in bees which were displaying symptoms of Isle of

Wight Disease. There were two stocks in particular from which I could procure spores at almost any time, yet these stocks showed only the normal death-rate, and could not at any time be said to display symptoms of disease. The theory that *Nosema* was the cause of Isle of Wight Disease did not seem to fit the facts as observed in Lewis. It might, therefore, be profitable to examine the results of the Cambridge investigation to see how far the main conclusion is warranted.

III.—Criticism of the Conclusion that Nosema is the Cause of Isle of Wight Disease in Bees.

1. If *Nosema* were the cause of the disease then *Nosema* ought to be demonstrable in every bee suffering from the disease. But Dr Porter was unable to find any stage of *Nosema* in some of the "crawling" bees which I sent her in June 1913. On page 41 of the 1912 report is an account of 66 cases investigated jointly by Drs Graham-Smith, Fantham and Porter. *Nosema* was not found at all in 13 cases, spores were found in only 29 cases, and so-called young stages in other 24 cases. The authors discount three cases in which the bees arrived dead, though it is quite easy to find spores in bees that have been dead for months. They claim, therefore, to have found *Nosema* in 53 out of 63 cases, or in 84 per cent. of the cases examined.

If *Nosema* is present in every case of Isle of Wight Disease, it is somewhat surprising that it was not noticed by Imms or Malden in 1907 and 1908. Both were searching for bacteria, and Dr Malden in particular claims to have exhausted this field. *Nosema* as compared with the average bacterium is relatively large, and one has difficulty in believing that Malden could have failed to notice it if it were actually present.

2. If *Nosema* be the cause of Isle of Wight Disease, the presence of the parasite, at any rate in quantity, should always produce the disease. But there is much evidence to show that *Nosema* may be present without producing the characteristic symptoms of Isle of Wight Disease. Two such cases in Stornoway have been mentioned above. We still have one of these stocks under observation, and we can get spores of *Nosema* from it almost at any time. I have frequently picked up bees entering this hive with pollen on their legs, or nectar in their honey-sacs, yet on examination they displayed heavy infection of *Nosema* in the spore stage. On page 50 of the 1912 report we read: "Spores in small numbers were found, however, in specimens from several stocks in one apiary in Scotland. No symptoms of the disease had previously been noticed, and none have appeared since."

Maassen, writing in 1911,[1] states that *Nosema* can be found in most

[1] *Mitteil. a. d. K. biolog. Aust. f. Land. u. Fortswistschaft*, 1911, p. 50.

stocks in Germany, and that stocks containing the parasite winter well, and display no signs of dysentery or May-pest. He admits that *Nosema* may be pathogenic, but believes that disease makes its appearance only when the stocks are weakened by unfavourable conditions. In the season 1909–10, he carried out an experiment with 30 stocks of bees all strongly infested with *Nosema.* In the spring three colonies showed dysentery; two had a heavy loss of bees; one died outright, apparently from starvation. The three dysenteric colonies recovered. The remaining 24 stocks wintered well and developed normally in spring, except that some of the colonies displayed "*Maikrankheit*" without great loss. It was easy to prove the presence of *Nosema* in all the colonies, and at times the parasite was present in enormous numbers. Only the bees newly hatched were at all times free from infection. The stocks with May disease got over it and became prosperous in summer. Heberle, discussing this experiment in the *American Bee Journal* of May 1914, remarks that "Dr Maassen's experiment tends to show that a *Nosema* infection is not necessarily very disastrous, since even the 24 colonies that were taken through the winter did not show an unusual number of dead bees. They wintered well, and developed normally in spring. Only 10 per cent. of the *Nosema*-infested colonies developed dysentery, and even these got over it, and became useful colonies."

In Australia, Canada, and the United States there is a disease of adult bees, known as "paralysis," with very much the same symptoms as Isle of Wight Disease. On the continent a similar disease is known as "Maikrankheit" or May Disease. In Australia the disease has long been studied by Mr F. R. Beuhne, B.Sc., who is quoted on page 51 of the report for 1912. He had stated that *Nosema* had been proved to occur all over Victoria, and that "many specimens of paralysis of the most pronounced type were entirely free from *Nosema.*" In *Gleanings in Bee Culture* for December last, he writes again: "You can find hundreds of colonies with the *Nosema* parasite and no symptoms; and any number of others with all the symptoms of paralysis but no *Nosema* parasites in the bees." The reply of the Cambridge workers is that by "*Nosema*" Beuhne means "spores of *Nosema*" (page 52). They suggest that in all cases of disease in which he could not find spores, *Nosema* must have been present in the "young stage." In reply to the statement that "you can find hundreds of colonies with *Nosema*, and no symptoms," the Cambridge workers suggest that Victoria must contain a large percentage of stocks which consist of parasite carriers. But to assume that there are hundreds of stocks composed mainly of parasite carriers seems to be putting somewhat of a strain upon the term.

3. If *Nosema* be the cause of Isle of Wight Disease, it ought to be possible to produce the disease by a pure infection of *Nosema* spores. The Cambridge

experimenters made numerous attempts to infect healthy bees with *Nosema*, and these were usually very successful so far as transmitting *Nosema* and killing the bees were concerned. But the bees had never any real opportunity of displaying the characteristic symptoms of Isle of Wight Disease. The bees experimented on were either small lots confined in cages or bell-jars, or full stocks kept in confinement. Those kept in cages and fed on fresh spores died very rapidly indeed, usually in little more than a week after infection. A heavy spore infection was noted as early as the fifth day. When confined merely to an infected cage, or when fed on spores some months old, the bees lived longer (27 days), but were believed to have died of *Nosema* in the end. Controls were kept alive as long as 21 days! In two cases the bees died rapidly, but *Nosema* was not found. The fact is that it is not easy to understand the behaviour of bees confined in cages. I have kept a queen and her attendants for weeks at a time in my pocket without any signs of trouble. On other occasions when the conditions were apparently similar the bees died in a day or two, sometimes within a few hours. Queenless bees in a cage would probably worry themselves to death in quite a short time.

The first experiments on full stocks were carried out in 1910 in Scotland. A hut was divided with double partitions of muslin into four compartments, 9 feet by 3½ feet, and a healthy stock placed in each compartment in July. The stocks in I., II. and III. each received an addition of diseased bees, while IV. was kept as a control. The bees in I. were all dead by 11th March, and the dead bees contained numerous spores of *Nosema*. But this stock had shown "no obvious signs of disease"! (p. 87). No. II. died off by the end of March after suffering from dysentery, and "spores of *Nosema apis* were found in moderate numbers." No. III. was a stock of hybrids, and the bees remained well and active, till they died of starvation about the end of March. No spores were found. The control stock in No. IV. remained "strong and well till the middle of March, after which they gradually died off." No spores were found in the bodies. Two things are noteworthy: (1) there is no proof that any of the stocks suffered from Isle of Wight Disease, and (2) the infected bees survived practically as long as the control bees.

Similar experiments were carried out at Cambridge in 1911 by Graham-Smith and Bullamore. Compartments 9½ feet by 3½ feet were used, and six stocks of bees were placed in these on 18th May. I. and VI. were control stocks. The former died out on 17th July, and the latter on 17th October, a difference of exactly three months. The fact that of two similar stocks, one survives just twice as long as the other under confinement, indicates how little reliance can be placed on these experiments. Neither stock gave spores of *Nosema*. No. II. was given sealed stores from a badly infected stock, and died out early in October without yielding any stage of *Nosema*. No. III. got a frame of sealed brood from a badly infected stock, and survived till 30th October (longer than either of the controls). A small proportion of the bees gave "young stages" of *Nosema*. No. IV. was fed on syrup containing numerous old spores. The bees were doing well on 8th December, but were dead by the middle of February. The hive was much soiled with dysenteric material in which *Nosema* spores were plentiful. Bees from an infected hive were introduced into No. V., and this stock died out on 12th October, without any evidence of infection with *Nosema*. These experiments were quite inconclusive. We have no proof that any of the bees suffered from Isle of Wight Disease at all, and it is again apparent that the *Nosema* infection did not appreciably hasten the death of the bees. Indeed the stock shown to have the heaviest *Nosema* infection lived longest of all.

The difficulties of the observers are obvious. They were afraid to infect a stock living under natural conditions lest they should spread the disease, so they were compelled to experiment on bees in confinement. We get little glimpses of the behaviour of the bees, and these indicate the very unnatural condition to which they were reduced: "The bees roamed round the windows during the first day or two, and many died. . . . The queen gradually ceased to lay. . . . The bees became listless, and many failed to return to the hive at night. . . . The bees did not appear to be interested in anything." In 1912 further experiments on full stocks of confined bees were attempted, but with equally disappointing results, and the account concludes with the remark: "It was found that even in winter bees cannot be kept satisfactorily in such compartments" (Report of 1913, p. 25).

In view of this difficulty, it occurred to me that in Lewis we had a very convenient area for further experiments on bee disease. There were no hive bees on the Island when I brought in the first stock in May 1909, and the history of each stock subsequently introduced was known to me. Further, it would be easy to carry out experiments on full colonies of bees living under natural conditions, and yet so far apart that no cross-infection could occur. In the spring of 1914 a Government grant for the investigation of bee disease became available through the Natural History Department of the University of Aberdeen. From that time I have been associated with Dr Rennie of the Natural History Department in carrying out further observations and experiments in Lewis and elsewhere. The results of these joint researches, along with many observations previously made by me in Lewis, of which a careful journal was kept, are dealt with in the paper by Dr Rennie and myself in the present volume of the *Proceedings*.

Observations and Experiments bearing on "Isle of Wight" Disease in Hive Bees.[1]

By **John Anderson**, M.A., B.Sc., Lecturer in Bee-keeping, North of Scotland College of Agriculture, and **John Rennie**, D.Sc., F.R.S.E., Lecturer in Parasitology, University of Aberdeen.

(*With Plate.*)

(Read 24th January 1916. MS. received 11th February 1916.)

CONTENTS.

I.—INTRODUCTION.

THESE observations were commenced in 1909 by one of us (J.A.), whose journal contains a detailed record of the history of each stock of bees in the Island of Lewis. In the autumn of 1913 *J.R.* paid two visits to the Nicolson Apiary at Stornoway, and subsequently arrangements were made, through Professor J. Arthur Thomson, for continuing the research in association with the Natural History Department of Aberdeen University.

The work here reported on was carried out with the aid of grants from the Development Fund and the University of Aberdeen through the Joint Committee on Research in Animal Nutrition of the University of Aberdeen and North of Scotland College of Agriculture. In the work we have been assisted by Mr John Innes, B.Sc., M.B., who has carried out most of the examinations of bees for the presence of *Nosema*, and

[1] This constitutes a preliminary report, indicating the trend of the results so far accomplished. Although the number of experiments is not large, stress is laid upon the fact that they are the only ones on record in this country which have been made upon full stocks of bees living under natural conditions. Further experiments are in progress, the results of which will be recorded in a subsequent report.

[*Reprinted from the "Proceedings of the Royal Physical Society of Edinburgh," Vol. xx., pp. 23–61.*]

after his retiral on taking up military service, by Miss Beatrice Simpson, M.A., who continued this work. Mr Robert Ewen, M.A., Nicolson Institute, Stornoway, has acted as local supervisor of the hives in Lewis since May 1915. We have also had the valued co-operation, in connection with the Deeside epizootic, of Mr A. H. E. Wood, of Glassel House, and of Miss Nancy Robinson, both of whom are bee experts of the British Bee-Keepers' Association. The latter has provided a detailed record of the Deeside outbreak, which has proved of value as a basis for scientific study.

We are indebted also to various local observers in Lewis and elsewhere who have taken charge of experimental hives, forwarded diseased bees, and otherwise supplied helpful information regarding the disease.

II.—THE COURSE OF THE DISEASE, AS OBSERVED IN THREE DISTINCT LOCALITIES.

(a) Lewis Observations.

Prior to the 20th May 1909, all hive bees in Lewis had become extinct, but on that date one stock was brought to Stornoway and placed in the grounds of the Nicolson Institute. These were black bees obtained from Wormit, Fife, and their previous history is unknown. In autumn of the same year a second stock of black bees was imported from Duirinish, and placed at Bayhead, Stornoway. Since 1909, other bees have been introduced at various dates, as stocks or swarms, and some queens have also been imported. At the present time there are bees in Stornoway, Lurebost, Sandwick, Bayble, Tong, Tolsta, Barvas, Shawbost, Carloway, Breasclete, Achmore, Marybank, Laxdale. The races include Blacks, Italians (Ligurians), Cyprians, Carniolans, and certain hybrids.

First Appearance of Isle of Wight Disease.—On the 20th September 1910, two lots of driven bees arrived in Stornoway from Sussex. They had come from cottagers' skeps, but had travelled to Lewis in swarm boxes on standard combs containing some honey. These standard combs probably came from the apiary of the bee-master who "drove" the bees, and it is possible that the disease was derived from these combs. One box was sent unopened to Bayble, 5½ miles from Stornoway, and the other lot was retained in Stornoway. The bees in both cases were fed on syrup, settled down quietly, bred freely, and gathered pollen up to 4th November 1910.

On 31st January 1911, bees were observed crawling in front of this stock, apiary number, A9. Next day a letter was received from Bayble stating that the other stock of driven bees had begun to crawl on the same day. The symptoms in both cases were those of Isle of Wight disease; bees emerged on the alighting board and did not fly, some eventually fell off the board on to the ground where they collected in little clusters, finally dying of cold and hunger. Bees of this stock continued to die in this way at Stornoway till 2nd March. On this date the survivors were killed and the hive and ground disinfected. Caustic soda was spread upon the soil, which was then turned over, and all the combs and quilts were burned. The Bayble stock was also killed off and the hive disinfected.

Second Attack. Two Stocks affected, B14, C13.—On the 6th October 1911, two other stocks in Stornoway Apiary began to crawl. On this occasion, samples of living bees were sent for diagnosis to Dr Graham-Smith, Cambridge, who reported "young stages of *Nosema apis* in both stocks." Spores were apparently not observed, and it was not stated whether the examples seen included intracellular forms. One of these stocks (B14) consisted of American Golden Bees, and in their case crawling ceased after a few days; on 14th October they were busily engaged once more carrying in pollen. They wintered in excellent condition, built up rapidly in spring, and never showed any further sign of Isle of Wight disease. They were not again tested for *Nosema*. Unfortunately the queen died of old age, 15th June 1912, and the strain became extinct. The noteworthy feature in the case of this stock is that the symptoms, although associated with the presence of *Nosema*, disappeared in a few days.

The other stock (C13) which consisted of black bees, became rapidly worse, and, on 12th October 1911, it was destroyed. These two stocks stood side by side.

D1	G9	B14	C13

Arrangement of Stocks, October 1911.

Third Attack. One Stock, No. D1.—Crawling bees were first observed in front of this stock, the position of which is indicated above, on 20th January 1912. The bees were a black stock, numerous, and having abundance of natural stores. It had wintered in excellent condition. Samples were again sent to Cambridge, and Dr Graham-Smith reported the presence of all stages of *Nosema*.

This stock was allowed to die out naturally, but the hive had been removed to another site, and the original stance disinfected. A small remnant was found stiff and motionless on one comb, on 29th February 1912. Thorough disinfection of the site was effected by spraying crude carbolic acid, of such strength that the worms came up and died, and the grass also was killed. All inside fittings of the hive were burned, paraffin was also poured on the hive site, and burned off.

Fourth Attack. Stock D4.—This stock, a strong one, crowded with bees and brood, along with two others, came from Exeter on 21st May 1912, where as yet the disease was unknown. It was placed on the stance of D1, referred to above. Isle of Wight disease symptoms appeared on 28th May, exactly one week after the arrival of the stock upon the island, and large numbers of crawling bees were observed in front of this hive on every fine day. On 8th June, all the stores were exhausted and all the brood was dead. Syrup was supplied, and the queen laid more eggs. On the 13th June there were still numerous bees, eggs, a few grubs, and food. At this date samples were sent to Cambridge, and Dr Graham-Smith's report upon them is as follows:—

"The bees you sent all arrived dead. No spores were found by microscopic examination, and it was impossible to say for certain whether young stages of *Nosema* were present or not. I tried to infect some healthy bees, but this experiment has turned out negative. 2/7/12."

On the 17th bees were lying about in large numbers on the alighting board and on the grass around, and dying off rapidly.

On the 11th July, the remaining bees, along with queen, were sent to Dr Graham-Smith, who kept them for some time. His report regarding them is:—

"The bees you sent have been doing moderately well, and nothing very special has yet been found in those which have died."

This must mean that *Nosema* was not found, yet the behaviour of the bees, and the high mortality clearly indicated Isle of Wight disease.

In the case of this stock no disinfecting measures were adopted, nor in any subsequent instance in the Nicolson Apiary, Stornoway.

Fifth Attack. One Stock (K.M.).—This stock arrived in Stornoway from the South of England on 31st May 1912. Its ten combs were crowded on 11th June and a super was added. The super was quite full of bees on 22nd June, and some sections were ready for sealing on 13th July. Later a second super was required, and some excellent sections were removed. Crawling began on 10th September, and was very typical when observed on the following day. On 12th October, all the bees were dead except the queen and a handful of workers. This stock was situated about 200 yards distant from the Nicolson Apiary.

Sixth Attack. Stock E14.—This stock consisted of Swiss pedigreed bees (blacks), and was imported from Exeter on 4th June 1913. When a super was added on 9th June the hive was crowded with bees and brood. When examined nine days later the super was crowded with bees, the foundation had been drawn out, and some honey stored in the cells. The stock had been placed in a garden which had not previously been used for bee-keeping, a short distance from the Nicolson Apiary.

On the 19th, bees were observed crawling in front of the hive, exactly a fortnight after their arrival in Lewis. Numerous bees were examined on the spot, their intestinal contents being searched microscopically. *Nosema* spores were not found. Sick bees were sent to Cambridge, and regarding these Dr Annie Porter reported: "A very few spores in one bee, young stages in some of the others, the rest negative." Notwithstanding the apparent paucity of *Nosema*-infected bees, the mortality amongst members of this stock continued, and at this period was exceedingly heavy. On 7th July, the Swiss queen was replaced by a Cyprian imported from Nicosia. The Swiss queen was caged with some Cyprian bees, and died of injuries received from them. She was sent to Dr Annie Porter, Cambridge, for examination, but no report was obtained regarding her condition. By this time, not many blacks were left alive; by September the stock was weak in numbers, but apparently healthy, and very few blacks were present. On 16th September, one of these was found to contain spores in abundance, but no spores were found in the young Cyprians. A few of these were observed to "crawl" on fine days, but the stock proved too weak to winter, and was allowed to die out.

Seventh Attack. Two Stocks affected, Gp. and Gs.—A stock of black English bees had been brought to Stornoway from the mainland of Ross (Duirinish) in the autumn of 1909. It prospered normally during 1910, 1911, and 1912, producing several swarms, and giving surplus honey. In 1913 it swarmed on 25th July. "Crawling" began in the swarm three days after it was hived, and was well marked and typical when observed on 16th August. On this date a few crawlers were observed in front of the parent stock. On 6th September, when the apiary was again visited no crawlers were found in front of the swarm, but a large number was seen in front of the parent stock. Both hives were examined comb by comb on this occasion, and found to have queens and brood in all stages. The swarm was small in number, but lively, and apparently quite healthy. The parent stock was more numerous, but a considerable number of bees showed dislocation of the wings, an indication which, however, has little importance as a symptom of the disease. On the 12th September, sample bees were taken from the parent stock. These were picked off the combs, bees with dislocated wings being specially selected. Owing to unfavourable weather conditions there were no bees out of doors at this date. Microscopic examination showed no recognisable traces of *Nosema*.

The hives in which the bees were housed had no previous history of Isle of Wight disease. The bees were too few to keep warm in the hives during winter, and, on this account alone, survival was not possible.

Eighth Attack. One Stock, F14.—Without any disinfecting measures, Hive No. 14, which housed the previously mentioned infected stock (E14), was used for a lot of Welsh driven bees procured in the late autumn of 1913. The infected combs were also retained with contained honey. In early spring, 1914, heaps of dead were found on the floor; later, on fine days many "crawlers" were observed. At the beginning of April, the bees covered only a single comb, but they were carrying pollen freely, and there was a patch of brood. Crawling had now ceased, the stock built up during the summer of 1914, wintered on natural stores, and was quite strong in the spring of 1915. On 17th June 1915, J. R. examined six live bees, active after journey (posted 14th), all parts normal, no *Nosema* observed. Other samples of bees which were sent to Aberdeen for examination arrived dead, and were not satisfactory for Isle of Wight disease diagnosis. On the other hand, it can be stated positively that *Nosema* spores were not present in the bees examined. Bees were examined individually, and also pulped in groups. The colons contained masses of pollen, but *Nosema* was not found. A stained smear was also made of one bee, and this gave a negative result.

In August 1915, the stock was found weak and queenless. In October it was quite extinct, and the local observer (Mr Gibson) reported that crawling had been very marked.

In October the hive was restocked with a new lot of driven bees from Wales, the old combs and stores being retained. Efforts were made to put this stock in good condition for wintering, but the weather was rather cold while feeding was going on.

This is now the third stock housed in the same hive in which two previous stocks had died of Isle of Wight disease. This new stock and hive is named G14.

No further cases of Isle of Wight disease have arisen in the Nicolson Apiary, although, as the records below show, *Nosema* could be obtained from bees there at almost any time.

Outbreaks of Isle of Wight Disease in Lewis.

Date of Introduction of Stock	Date of Outbreak	Stock	*Nosema* x, o, or y.s.	How terminated	If Site, etc., disinfected
29/9/10 from Sussex	31/1/11	Blacks A9 and Bayble	—	Destroyed 2/3/11	Yes
	6/10/11	American Goldens B14	y.s.	Recovered	—
	6/10/11	Blacks C13	y.s.	Destroyed 12/10/11	Yes
	20/1/12	Blacks D1	x	Allowed to die out 29/2/12	Yes
21/5/12 from Exeter	28/5/12	Blacks D4	?	Remainder sent to G-S., Cambridge	No
31/5/12 from S. Eng.	10/9/12	Blacks KM	—	Died out 12/10/12	Yes
4/6/13 from Exeter	18/6/13	Swiss pedigreed E14	Very few spores in 1; numerous in Sept.	Died out before Winter	No
Autumn 1909 from Duirinish	28/7/13	Blacks Gp	o	Died out Winter 1913-14	No
Autumn 1913 from Wales	Early Spring 1914	Welsh F14	o	Recovery in 1914 Relapse in 1915	No
11/7/14 from Exeter	12/7/14	Black (at Bayble)	o	Blacks died out in Autumn; remnant of Yellows survived Winter	—

of
founc
on fine
die out.

ʼhe number of stocks in the apiary varied from 4 to 14 during this period.

Seventh A x—spores present. o—spores absent. y.s.—young stages.

English bees h
(Duirinish) in the' *of Characteristics of Isle of Wight Disease as seen in*
1911, and 1912, pro *Lewis Outbreaks.*
1913 it swarmed on 25 *-ea beyond the Island.*
after it was hived, and w ous outbreak of two cases 5½ miles apart of stocks intro-
August. On this date a t same source (Sussex), 4 months previously. A9 and
stock. On 6th September, .

(*b*) D4. Disease appeared 1 week after stock arrived from Exeter.
E14. „ 2 weeks „ „ „
KM. „ 3½ months „ „ S. England.
F14. „ 6 months after introduction from Wales.

See also under (2).

(2) *In relation to other affected stocks in Lewis.*

B14. } More than 7 months after the termination of the previous
C13. } outbreak.

D1. Three months after B14 and C13.

F14. Six months after introduction, upon which it had been placed in hive in which a stock, E14, had just died out from Isle of Wight disease.

(3) *Duration, where not interfered with.*

B14. A few days, and recovered.
D1. 40 days, and died out.
KM. 32 days, and died out.
E14. June to beginning of winter, and died out.
Gp. July to winter, died out.
F14. Few weeks—recovery—recurrence in the following year, lasting August to September 1915.

(4) *Relation to Nosema apis.*

1. Spores found in only two cases coincident with Isle of Wight disease symptoms.
2. Young stages were found in two other affected stocks, one of which completely recovered.
3. Healthy stocks known to have *Nosema* all the time in which no outbreak of Isle of Wight disease occurred.
4. A particular case, E14, in detail.
   - Placed on a new site when introduced.
   - Strong stock.
   - Mortality heavy.
   - Extreme paucity of *Nosema* in bees while this mortality in progress.
   - *Nosema* fluctuations at different stages.

(5) *Effect on Apiary as a whole.* No more than two stocks ever displayed symptoms at one time or in succession. During this period the apiary contained various races and strains of bees.

(*b*) Example of Naturally Occurring Outbreak of Isle of Wight Disease, showing Spontaneous Recovery.

Craibstone Apiary.

On 4th June 1915, three strong stocks of bees imported from Devonshire were placed in this newly constituted apiary and housed in perfectly fresh hives. During the fine weather of June they were very prosperous, each stock swarming once and one of them twice. The swarms were hived separately. Three races were represented, viz. pure Italian, pure Carniolan (queen imported from Austria in 1914), and ordinary black bees.

Symptoms of Isle of Wight disease made their appearance on 30th June, and continued, with fluctuations, till near the end of August. All the stocks and swarms were affected more or less, but the heaviest mortality occurred in the blacks (D4). Three of the seven stocks died out owing to their failure to mate the virgin queens. In all three cases drone breeders were developed. On 22nd September four stocks survived and no symptoms of Isle of Wight disease were apparent, either in the behaviour of the bees, which was perfectly normal, or in the condition of their internal organs. At no stage of the disease were spores of *Nosema* found, although repeated examinations of the contents of the alimentary canal were made.

While the disease was at its height the number of affected bees was very great. Their behaviour was of the usual character, and the walk leading from the hives was littered with crawling bees to a distance of at least 25 yards. Heaps lay on the flower-beds, and a low box-hedge was full of bees incapable of flight.

The mortality was, in consequence, very considerable, and the weather conditions were exceedingly unfavourable to bee life. The recovery is therefore all the more notable.

On 22nd September one of the stocks (D4) was decidedly weak. It was headed by a young queen, had brood on two combs and plenty of store for the number of bees. This is the stock which had the heaviest mortality during the crawling period. Two other stocks (A1 and C3) were in excellent condition for wintering, headed by young queens, populous as to bees, and with plenty of stores. The fourth stock (B2) is the only one now that is not headed by a queen of this year. Possibly on this account, and also on account of heavy losses during the crawling period, this stock is also rather short of bees. The queens in D4 and B2 are black, C3 has a young Carniolan queen. A1, originally pure Italian, is now filled with dark bees, the offspring of a young queen.

On 23rd September, a small lot (3 lbs.) of driven bees was united to D4, and a new stock, E5, consisting of a small lot of Welsh driven bees with their own queen, was placed between C3 and B2. On 10th October, an Italian queen, supplied by Penna, was caged over D4, the original queen having been removed, and this Italian queen was duly accepted. On the same date it was observed that B2 (the weakest stock in the apiary) had been entirely robbed out. D4 and E5 were fed with sugar syrup and duly wintered down.

On 28th December, the four Craibstone stocks were examined. Except at A1 there were scarcely any dead bees to be seen, either on the ground on the alighting boards, or on the floors of the hives. Even at A1 the number of dead bees was not remarkable.

D4 was opened, and the bees were found active and in good condition generally.

During the mild days in January 1916 the bees were moving freely and even carrying pollen. The behaviour of C3, E5 and D4 was normal, but A1 showed very definite "crawling" symptoms and a high death-rate.

(c) Isle of Wight Disease on Deeside.

1.—History of Outbreak.

By Nancy M. Robinson, First-Class Expert, B.B.K.A.

So far as can be ascertained, Isle of Wight disease was not recognised anywhere on Deeside until 1913, when it appears to have broken out in several distinct districts.

(1) On *Lower Deeside*, in *Culter* and the surrounding districts, the disease was at its worst in 1914.

A.—A's apiary was the first affected. He had bought bees from the South of England in 1912. These stocks did well early in 1913, but they had died out by 1914 after showing all the usual symptoms of Isle of Wight disease.

In 1913 these stocks had been taken to the heather, east of the Hill of Fare, in the Echt district, and several apiaries there, including two large ones, were badly affected by the same disease in 1914 and 1915.

During 1913 and 1914 all bee-keepers within a radius of two miles or more from A's apiary found their bees affected or dying off rapidly, and by the end of 1914 there were no bees left in that area.

Some apiaries were started again but with little or no success, owing to the recurrence of the disease.

In 1915 cases of the disease appeared still farther afield and embracing a far wider area.

Drumoak.—In the parish of Drumoak, on the north side of the Dee, a case of Isle of Wight disease occurred in 1913.

B.—B had sent to England for driven bees in 1912, and in the spring of 1913 the disease first began to show in his apiary.

During this year his six stocks gradually dwindled and died out. The hives were cleaned up and disinfected.

As far as is known no further cases occurred in this parish in 1913, unless it might be at H's, which is mentioned later. But in 1914 all the apiaries in B's vicinity became affected.

C.—C lived about a quarter of a mile north of B. In June 1914, C reported that his bees were not working, although the weather was good and there was plenty of nectar to be had. The apiary, consisting of two hives and a ruskie, was found to be in the final stages of the disease, and in July there were very few bees left and less honey than in June. The owner then destroyed his bees and disinfected his hives.

D.—D was another neighbour, living about three-eighths of a mile north-east of B. He had only one stock, which was found to be very weak in June 1914. The stock was left until August, when it was so much reduced that the owner destroyed the few bees that were left and disinfected his hive.

E.—E's apiary, situated three-quarters of a mile north-east of B, was attacked in August 1914. The bees may have been suffering earlier, but no record of this exists. E had sent samples of bees to the *British Bee Journal*, which reported them to be suffering from Isle of Wight disease. Incidentally, it may be mentioned that bees from the same source sent to Aberdeen University investigators were reported as free from *Nosema.* Only two stocks appeared to be infected, but in September 1914 the remainder showed marked signs of the same trouble. In October three out of four stocks were seriously affected, and between this time and April 1915 these three stocks had died off, and the remaining one was so weak that there was merely a handful of bees on the comb. These the owner treated with candy and quinine without success, and the stock died out before the end of the month.

F.—F lived about a mile north-east of B and owned four stocks. In August 1914 there were slight indications of Isle of Wight disease. In September one stock became badly affected, and the three others all showed symptoms. The worst of the affected stocks was removed to Aberdeen for experimental purposes (60 DD. of this report). The *British Bee Journal* reported on samples of bees sent: "It is Isle of Wight disease." Dr Rennie reported: "*Nosema* can not be found in this stock."

The remaining stocks were not destroyed; but all died out except one before April 1915. This stock was then very weak and was destroyed, and the hives and appliances were disinfected.

G.—G kept two stocks of bees, about three-eighths of a mile east of B. In June 1914 both were very strong; but at the end of June, drones were being thrown out in great numbers, and later in the year the bees were suffering from Isle of Wight disease. Before April 1915 both stocks were dead.

H.—H's house was quite close to G. In the spring of 1914 H had an empty hive in his garden; it was filled with old combs and had been left uncared for since the loss of a stock during the previous winter. (The symptoms accompanying the loss of this stock were unmistakably those of Isle of Wight disease. *J.R.*) In June a swarm came and took possession of this empty hive and flourished remarkably. 1914 proved a good season for honey, and this stock came through the winter, 1914-1915, very well. When visited in April 1915, the bees were found working vigorously and very strong for that time of year.

A swarm came off at 9.30 A.M. on 22nd May (the earliest swarm known on Deeside that year). H succeeded in skepping it, but unfortunately it rose later and was lost.

In due time a second swarm came off, which was skepped successfully, and stock and swarm both appeared to thrive.

Later, this swarm showed distinct signs of Isle of Wight disease during two or three days, and then it seemed to recover. At the end of the honey season, the two stocks were united. For further history of the united stock, see p. 59 of this report.

J.—J, three-quarters of a mile south-east of B, kept two stocks of bees, No. 1 and No. 2. When visited in June 1914 both stocks were found very strong, but No. 1 was especially good and was working well in the supers. In July large numbers of drones were being thrown out of the hive, in spite of fine weather and a good honey flow.

In August No. 1 showed marked signs of the disease, and J then destroyed the bees. This stock had given him 126 sections.

No. 2 swarmed, and the old queen having been killed the swarm was returned to the parent hive. A young queen mated successfully, and this stock did very well in 1914, but died out during the winter 1914-1915.

It may be mentioned here that J had earlier in the season assisted some of his neighbours in destroying their infected bees and cleaning and disinfect, ing their hives.

In 1915 J resumed bee-keeping. He obtained a swarm and placed it in one of his disinfected hives. This stock built up steadily at first, later it began to show some symptoms of the trouble, and then partially recovered.

K.—K lived a mile south-west of B. He had only one stock, which became seriously affected with Isle of Wight disease during the summer of 1914. It was destroyed in September 1914.

In 1915 numerous outbreaks occurred farther afield from B's apiary. There were several cases about $1\frac{1}{2}$ to 2 miles north of B. Many of the stocks had died out during the winter, and not from starvation as there was plenty of honey in the combs. Those stocks which survived soon began to show the ordinary symptoms of Isle of Wight disease, and all died out. B's stocks, it may be noted, were cleaned out and disinfection effected in 1913.

On the south side of the Dee, opposite Drumoak, two cases were noted in 1915.

L.—L lived rather over 2 miles south-east of B's apiary, and owned six hives and two ruskies. In June 1914, all were strong and apparently well, but owing to the prevalence of Isle of Wight disease in the district, L was warned to watch his bees carefully. No symptoms were recognised until June 1915; all the stocks were then strong, but later they became seriously affected and eventually died out before the end of the year. L dealt a good deal in so-called "remedies."

M.—M's apiary was 2 miles south of B. In May 1915, several of his stocks were found to be affected. M was inclined to think the disease was derived from some bees from Culter, which had been brought to the heather near by, in August 1914, and which had died out while there.

(2) *Banchory-Ternan District.*

In this district the distance between the outbreaks has been greater than in the others.

N.—A case occurred at Crathes in June 1914. N's apiary lies about three miles east of B. There were three stocks, Nos. 1, 2 and 3. The following observations were made:—

13th June.—A warm, bright day. There were numerous dead bees outside the hives, and a great many unable to fly and crawling on the ground all over the garden. No clustering was seen, and no old bees observed crawling. The wings and abdomens appeared to be normal, but the bees seemed weak. Stocks all seemed strong.

15th June.—A fine day. Only a few bees were seen crawling, and very few dead bees in front of the hives. Birds were seen to pick some up, and possibly numbers may have disappeared in this way.

16th June.—A fine day. Bees appeared normal. No. 2 looks like swarming. No crawling bees seen.

21st June.—A dull, rather cold day. Bees were clustering on the ground near all three hives in small groups. No. 2 appeared to be the only normal stock, and was strong. The bees from No. 1 and No. 3, both inside and outside the hives, appeared weak and dormant and unable to sting. There were very few dislocated wings. The abdomens and general appearance of the bees, with a few exceptions, seemed normal. It is doubtful whether any bees from No. 2 were clustering, although there were clusters under that hive.

22nd June.—A fine day. The symptoms just as previous day. No. 1 and No. 3 stocks were destroyed and the hives disinfected.

24th June.—No 2 was working well and looked like swarming. The ground was limed.

26th June.—No. 2 working well.

29th June.—Swarm came off, but got away and was lost.

9th July.—Bees working well, but some few drones observed crawling.

15th August.—No. 2 working well and seems strong. Supers nearly full.

This stock died out during the winter 1914-1915. All stocks belonging to three bee-keepers, who were near neighbours of N, were lost also.

O.—O's apiary was 2 miles north of N. O had six stocks, and in July 1914 these were all found to be suffering from Isle of Wight disease. Only one stock survived till 1915, and that eventually died out.

Several cases were reported at Raemoir in 1914 which were not verified as the stocks had been destroyed before notice was received.

In 1915, there were two outbreaks, three-quarters of a mile distant from each other, at this place.

P.—P had five stocks in good hives, clean and well kept. The stocks were all strong. All appeared healthy in June 1915, but in July Isle of Wight disease was very marked. Numerous bees were crawling on the ground and clustering; others were climbing up grass and leaves; many had dislocated wings and distended abdomens. In those examined the colon was very much enlarged and clogged. There were many dead lying about.

Three of the hives contained parent stocks, while the other two held swarms. The parent stocks seemed to be more severely attacked. P destroyed all his bees at the end of July and cleaned and disinfected his hives and appliances.

Q.—Q's apiary was not so well kept, and his stocks were weak, consequently the outbreak did not appear so marked, and the disease lingered on for a long time. I have frequently noticed that when a really strong (*i.e.* numerous) stock becomes affected, disease progresses much more rapidly than in the case of weaker stocks.

R.—A serious outbreak occurred in July 1915, in R's apiary, situated about a mile and a half south-east of N's apiary. R owned one hive and five ruskies, and by the end of 1915 nearly all his bees were dead.

S.—This brings us within a mile of Banchory village where a case broke out in July 1915 in S's apiary. This was on the east side of the village and within a mile of the last outbreak at R's.

S owned five hives and a ruskie. In May three stocks were very weak. Numerous dead bees lay near the hives, but this might have been the result of robbing.

In July, Isle of Wight disease was marked in a swarm in this apiary. In August all the other stocks but one began to show the same symptoms, and S destroyed them. The one left was strong and gave two crates of sections, which was good for a poor season.

At this date no cases farther west of Banchory and Raemoir have come under notice until Dinnet and Tarland are reached—15 miles away.

(3) *Dinnet Area.*

T.—In 1913, two bee-keepers at Dinnet, very near neighbours, found their bees suffering from some trouble they were unable to recognise. They consulted the North of Scotland College of Agriculture bee expert (Mr Manson), who found the bees to be suffering from Isle of Wight disease. His opinion was confirmed by the *British Bee Journal* expert, to whom samples of bees were sent. The owners were recommended to destroy their stocks in order to prevent the spread of infection. They not only did this, but burnt up bees, hives, and all appliances. One bee-keeper, T, owned fourteen stocks. Before the disease was recognised a swarm had come off and settled in the roof of a cottage close at hand. This swarm seemed to be strong and working well; and owing to the difficulty of removing the bees, it was left alone. T had brought bees from England not very long before the outbreak, and the disease was attributed locally to these imported bees.

In 1914, T started keeping bees again. He had made himself new hives and bought two stocks from a bee-keeper west of Ballater. In July 1914, he had six hives and three ruskies; all were very strong and doing well in the supers, and there was no appearance of disease.

In October 1914, the stocks seemed in splendid condition, and T was also feeding two stocks of driven bees. These had been obtained locally.

In the spring of 1915 he reported that several stocks had shown marked signs of dysentery, but there were no crawlers. T disinfected his hives after the attacks of dysentery.

In the spring of 1915 the swarm of bees already mentioned, which had settled in the roof of the cottage in 1913, showed marked symptoms of the disease. T, with the help of neighbours, promptly removed the bees, disinfected the place, and blocked up the entrance.

In June 1915, T's stocks were strong and working well.

In August 1915, one stock showed very slight signs; there were very few crawlers, and it is understood that T meant to destroy it.

U.—U's apiary is situated about half a mile due east from T.

In 1914, he owned seven hives and five ruskies.

In October 1914, there were no signs of Isle of Wight disease; but robbing was going on very vigorously. On this account some stocks were probably weak.

During the following winter, 1914-1915, five stocks died out, and all showed signs of dysentery in the early spring.

In June the remaining stocks were fairly strong, and all combs were badly marked with dysentery. When visited the weather was dull and no crawlers were seen.

The following day was bright at intervals, and a few crawlers were then observed. These were found to have the colon very much dilated and clogged.

In August 1915 only one stock remained, viz. the one which had appeared the weakest in June. It showed slight but distinct traces of the trouble, and U decided to destroy the bees and clean up and disinfect his hives. He did so in order to prevent the disease spreading among his neighbours.

No other cases in the immediate neighbourhood have been reported, although there are several apiaries near by.

V.—V's apiary is situated about five miles north-east of T. It consisted of ten hives and twenty-five ruskies.

In August 1915 all seemed very strong and well cared for, but examination showed the ground covered with bees unable to fly, with distended abdomens, dislocated wings, and all the usual appearances of Isle of Wight disease. I could not locate any special stocks as being the affected ones, for the bees were distributed all over the grass, and not in front of any particular hives. Samples of bees were sent to Aberdeen University investigators as usual, who reported that the appearances were those associated with the disease. The bees sent did not contain *Nosema.*

A week later, I found only three hives and one ruskie left. The rest had been destroyed on receipt of the above-mentioned report.

The surviving stocks were examined, but no symptoms of Isle of Wight disease were apparent. The owner, as a precaution, however, destroyed them.

X.—X, situated about one mile north of V, had originally come from Strathdon, and his bees had never flourished, but had been continually dying out. V had been down to work among them several times in 1915, and used his own gloves, smoker, and veil.

Drones had been thrown out of one of X's stocks abnormally early, and V had come in order to advise. Later, a first swarm came off this stock, and died out very shortly afterwards. A second swarm then came off, and these bees also died off.

When the stock was visited in August 1915, there was merely a handful of bees left. X then destroyed these, his last stock, and cleaned and disinfected his hives.

Y.—In 1914 X had sold an old hive to a neighbour Y, who lived about two miles south of his apiary. The stock which was placed in this hive died, and another stock beside it was reported dying out also.

No other cases within a radius of five miles are known.

2.—Extracts from Diary of Visits of *J. R.* to Deeside Bee-Keepers.

23rd August 1915. Visited Anguston Apiary.—Found twenty-one hives in small garden all affected with Isle of Wight disease. Ground littered with dead bees so that it was not possible to walk in garden without treading on them. Some had been treated with a syrup of sugar and jalap, and were reported to be improved, but this was not permanent. Sample taken; pulped. Negative result as regards *Nosema* spores.

Visited R's Bees at Drumoak.—Both stock and swarm active. Swarm had required an eke. There were a few crawling bees about, but not many. This is a case where intermittent crawling, moderate only, has occurred. Is this an aspect of the resistance of this stock where the infection has laid hold of a few, but has not overcome the majority of the stock?

D——, Durris.—When last seen, one stock had Isle of Wight disease; now, four or five have it. He has been spraying ground with Izal, and feeding an advertised preparation to the diseased stock, but apparently without effect. The disease has spread. Bees examined did not contain *Nosema* spores. When visited again in January 1916, all the stocks were extinct.

24th August. Visited Cullerlie, K's Apiary.—Isle of Wight disease prevalent in most stocks. No. 10 has got it. But the Italians, Nos. 25 and 18, are both going on well with no signs. Hybrids also are well, and these have swarmed lately. This swarm has settled in the top of a hive at Woodside which has got Isle of Wight disease very badly. I saw both stocks working (above and below) from the same hive. Took sample from No. 10. Result—failure to find *Nosema*.

H——, Finnercy.—Has Isle of Wight disease in most of his stocks. Took a sample from the worst, but did not find *Nosema* spores. Examined all K's sample, also H's. All had congested colons with pollen in a pasty condition. Most had chyle-stomachs, purplish; some were quite dark.

Main Features of Deeside Epizootic.

This report deals with twenty-three apiaries extending from Culter, near Aberdeen, to Dinnet, a stretch of about 30 miles along the Dee valley, and covering 3 or 4 miles on either side.

I. The epizootic was observed first in 1913, in particular at Culter and Dinnet, and in stocks introduced from the South of England. In the Culter case one year at least elapsed between the time of arrival and the commencement of the outbreak. The disease is still prevalent in this district.

II. Within the area there has been a very considerable amount of destruction of bees and appliances, of disinfecting generally, and also of experimenting by the bee-keepers with advertised remedies. All these efforts have failed to cure the disease or to affect recognisably its continuance.

III. There are instances of stocks "passed over" within apiaries, though in close proximity to sick stocks. In this area at least one example is known of a stock prospering in a non-disinfected hive in which a stock had died out of the disease.

IV. Bees, in a number of instances, were sent by bee-keepers to the editors of the *British Bee Journal*, and were reported to be suffering from Isle of Wight disease. We have been unable to find *Nosema* in the bees of Deeside generally.

III.—INVESTIGATIONS BEARING ON THE RELATION OF *NOSEMA APIS* TO ISLE OF WIGHT DISEASE.

Methods of Examination of Bees for the Presence of Nosema apis.

As carried out by John Innes, B.Sc., M.B., Captain R.A.M.C.

Each bee was examined in a routine manner, and the following notes were made:—

(1) Activity, as determined by movements in the postal cage—usually a match-box.

(2) Power of flight on exposure in open space.

(3) Dislocation of wings; if present or absent.

(4) Gait; whether co-ordinated or paretic.

(5) Response to stimuli, such as handling, etc.

The chyle-stomach and colon of each bee were examined systematically, and the removal of these parts from the body was carried out in the following manner. The head of the bee was firmly crushed between fairly stout dissecting forceps, and while held thus, the last segment of the abdomen was grasped with straight fine-pointed forceps, and gentle traction applied. In this way the colon was carefully pulled out and the chyle-stomach also came away with it. These organs were placed on a slide, separated at their anatomical junction, and teased. Cover-glasses were then applied, and the preparation examined microscopically in the wet, unstained condition. If the spores of *Nosema apis* were present they were readily identified by their shape, size, and refractility. Young, intracellular stages and planonts were also observed in like manner.

Stained preparations from smears of the colon, and of the chyle-stomach, were made from every sample of bees examined, and the stain which was almost exclusively used was the iron-hæmatoxylin of Heideinhain. Although requiring longer time for staining, this was found to give the most reliable and most definite results. The fixative used was a hot alcoholic solution of corrosive sublimate.

Various other stains were tried, such as Giemsa, Romanowsky, Erhlich's triacid, etc., but the varying results led to their abandonment in favour of the more stable hæmatoxylin preparation.

No stain, however, was found which would show up the spores clearly. Various experiments in this connection were tried, but with no success. Heat had very little effect in helping the stain to penetrate the resistant spore capsule. For its identification in stained preparations we could rely only upon the surrounding structures being stained and the spore, left untouched, appearing as a clear structure.

The number of bees examined from each stock was, on an average, about eight. Where more than eight bees were available from a stock, then these were also examined.

The amount of infection was reported as slight, heavy, or very heavy, according to the number of spores found.

Where young stages were found, these were recorded as such, but it was rare to find a heavy *young stage* infection without the presence of a fairly heavy infection with spores.

The routine here described was that followed by Dr Innes in the laboratory, in all cases of bees which were sent for *Nosema* diagnosis from the Lewis stocks, from apiaries on Deeside, and several other districts. Examinations of bees for *Nosema* by the other workers was mostly, though not exclusively, confined to the searching for spores. These were obtained by examining bees in the fresh state and by the pulping method described later. Miss Simpson and Dr Rennie also prepared a number of sections of the alimentary canals of *Nosema*-infected bees (apparently normal in behaviour), of Isle of Wight diseased bees, and of healthy bees, some of which are utilised in illustration of the present work.

Diagnosis of Nosema Infection in Dead Bees.

Graham-Smith, Fantham and Porter, in their report upon *Nosema* as the cause of Isle of Wight disease, express the opinion that dead bees are unsuitable for *Nosema* diagnosis (p. 41). This opinion is reflected in the Board of Agriculture Leaflet, where it is stated that "it is not possible as a rule to express any opinion from an examination of dead bees." Leaving out of the question altogether the problem of Isle of Wight disease, and considering only that of *Nosema* infection, this statement has not been borne out by our experience. Undoubtedly, the most reliable means of diagnosis of *Nosema* infection is the recognition of the spores, and our experience has been that these are recoverable from bees at least as long as seven months after their death. We have in our possession at the present time (January 1916), a number of dead bees which were artificially infected with *Nosema* early in June 1915, in which we are still able to obtain spores, not only from numbers pulped together, but readily from individual bees.

Graham-Smith, Fantham and Porter are of opinion that spores are not often formed in the warmer months of the year. They state: "Undoubtedly the most certain evidence of the presence of *Nosema apis* is afforded by finding the characteristic spores. Unfortunately these are not always present. . . . Our observations show that during the warmer months the parasites often do not reach the spore stage, but seem to kill the affected bees before this stage is reached" (p. 48 of 1913 report).

Our evidence is unfortunately rather contradictory, as we have been able to find abundance of spores in the months of May, June, July, August and September as readily as in other months. Our illustration of a section of a chyle-stomach in which every visible cell is heavily infected with spores (Pl. I. fig. 2) is from a summer bee. And we are a little doubtful of any heavy mortality resulting entirely from infection in the period of the bees' maximum vigour, at an early stage of the parasites' growth. This is especially so since we have found, throughout the summer, living bees without any obvious signs of disease, which proved to be very heavily infected with spores. Further it is claimed that the chyle-stomach of the bee is, even under normal conditions, constantly shedding and renewing its secreting epithelium.

Pulping Experiments.

The routine of examining individual bees for *Nosema* diagnosis, although valuable and most reliable as regards the single bee in question, has several serious disadvantages. The first of these is due to the amount of time required to examine thoroughly even one bee, and the second, which depends upon this fact, is that one is led to base conclusions regarding a whole stock upon the results obtained from the examination of relatively insignificant numbers. A small sample of bees taken at random from a stock does not correspond, say, to a sample of a homogeneous fluid or solid substance.

This difficulty has been largely overcome in our later work by the introduction of the method of pulping collections of bees designed for examination for the presence of *Nosema* spores. Our method is simply to cut off the abdomens of the bees, letting them drop into an ordinary mortar. They are then gently pulped in sufficient water to render the contents fluid. Sometimes we have removed the alimentary canals from behind the honey sac and teased these collectively in a little water in a large watch-glass. Samples of the resulting fluid in either case are taken up with a pipette, and if *Nosema* spores are present in the pulped preparation, they are usually found in the first drop examined. Our first experiment of this nature was as follows.

Four samples of dead bees were taken. Two of these were from collections of bees which had been infected by feeding with *Nosema* spores nearly three months previously, and the remaining two consisted of bees from Isle of Wight diseased stocks.

(*a*) Pulp of twenty-six *Nosema*-infected bees yielded, on examination, numerous spores in the first and every succeeding drop of fluid taken from the mass.

(*b*) Pulp of a second (separate) lot of *Nosema*-infected bees—about a dozen bees. Result as in case of (*a*).

(*c*) Pulp of bees, "crawlers," from two stocks—different apiaries on Deeside—same stocks as were used for infection in second Shawbost experiment (p. 54)—about twenty bees. Result: No *Nosema* spores observed after repeated examinations.

(*d*) Pulp made of about two thousand bees from a stock which had died out from Isle of Wight disease at Banchory. The fluid, after draining off from the solid residue, was tested extensively (twenty separate examinations), and *Nosema* spores were not found.

Other similar tests are given below:

1. Thirty "crawlers" from DES1 stock (p. 57) were picked up on 10th September. They were allowed to die in a small cage, and on 14th were pulped in a little water in the usual way. In this stock there had been found previously an unidentified organism. The first field examined in the first small drop contained numerous spores of this organism. *Nosema* spores were not seen.

2. Sample from Anguston, consisting of nineteen "crawlers," had their abdomens pulped and examined for *Nosema* spores. Negative result.

3. On 11th September 1915, visited Anguston and found nineteen of the stocks had succumbed to Isle of Wight disease since last visit. Took a sample of dead bees from the site of one of the dead stocks.

On 20th September these bees, sixty in number, were pulped, and examined. Nineteen samples were dealt with and no *Nosema* found.

4. On 25th September 1915, seven bees which had died in transit from Glenhouse, Stornoway, were pulped. Negative result as regards *Nosema* spores. These were "crawlers" from the front of the hive, but we have no other record of Isle of Wight disease in connection with this stock. Seven live ones which survived the journey were similarly treated. Result: Negative as regards *Nosema*.

5. Six bees which remained of the lot of Carniolans (No. 7) from Stornoway were pulped on 25th September. No *Nosema* was found.

6. Pulped 27 chyle-stomachs of Welsh driven bees, which were hived to-day in Desswood apiary (29th September 1915), and examined twelve drops for *Nosema*. Result: Negative.

Numerous further tests of this nature have been made, and in every case where *Nosema* was known from previous experience to be present in the collection from which the bees were taken, *Nosema* spores were found.

As early as 1913 we had become doubtful of the conclusion that *Nosema apis* stood in direct causal relation to Isle of Wight disease. From February of that year we regularly examined bees for *Nosema* spores, especially any that crawled or seemed sickly. We found such spores very frequently, particularly in old bees and not necessarily in association with unusual mortality in a stock. We have found bees with a heavy spore infection able to fly perfectly and even to carry loads of pollen and nectar. We have had under observation for three years, 1913-1915, a stock of Ligurians, G9 (Nicolson Apiary), which has stood in proximity to several of the forementioned stocks throughout their periods of crawling. This stock has never displayed any noticeable crawling symptoms. Yet all the time *Nosema* has been present in it, sometimes in enormous quantities (see Pl. I. fig. 4—a microphotograph, taken in the present year, of the colon contents of an apparently healthy bee from this stock).

It is significant that, while G9 contained *Nosema* over a prolonged period, it failed in any marked way to infect the apiary generally. We have not found *Nosema* distributed in the bees of all the stocks, but only in a few stocks at any one time, and where it occurred, Isle of Wight disease was not present.

History of certain Stocks in Lewis—with special reference to the alleged connection between Nosema apis and Isle of Wight Disease.

We visited Bayble on 7th May 1914, and took samples of a stock of bees which had no history of crawling. Spores of *Nosema* were found in several of the bees, the infection being very heavy in a number of cases. On 4th September, when again visited and examined, this stock was in a highly prosperous condition; it filled the brood box of ten combs and crowded also a super of ten shallow frames filled with honey. On 14th August 1915, this stock was still prosperous and had never shown any crawling symptoms. Sample bees from this stock were taken in September, and these were found to be free from spores. Twenty bees were examined: these were lively, vigorous, and flew readily after two days in transit. The chyle-stomachs of five examined individually were of a healthy appearance; the colons were not markedly congested, and contained watery material consisting almost entirely of indigestible pollen residues. The abdominal parts of the remaining bees were pulped, and drops of the fluid examined as offering the most reliable means of detecting the presence of *Nosema* spores. This stock remained healthy alongside the next-mentioned.

Meanwhile there had been in the same apiary, and in close proximity to this stock, an independent outbreak of Isle of Wight disease. A swarm of black bees arrived from the South of England on Saturday, 11th July 1914, and was duly hived. The bees began to crawl next day, and the symptoms were very marked when we visited the apiary on 13th July. Then, nearly half of the stock was to be seen crawling or dead upon the ground.[1]

Bees taken on this occasion were examined in Stornoway by J. A. and J. R. with negative results, and stained preparations were afterwards made without young stages being found. This swarm was headed by an Italian queen, which had been supplied just before the stock was despatched to Lewis. A little patch of brood was hatched, and the yellow bees did not "crawl." The blacks were all dead in autumn and the yellows were not strong enough to winter.

Stock G9: Nicolson Apiary.—This stock has no history of Isle of Wight disease and no abnormal death-rate. A few "crawlers" have occasionally been observed, but nothing to indicate disease, yet this stock is known to have harboured *Nosema* for at least three years. The original queen was a pure Ligurian imported from Bologna (apiary of Enrico Penna), in the autumn of 1911. A black stock on each side of this hive (the stocks already referred to as C13 and D1) died of Isle of Wight disease during that autumn and winter, but the Ligurians showed no signs of trouble. These extinct stocks were replaced in 1912 by two other black stocks (D4 and H12) which died out—D4 certainly, and H12 probably, from Isle of Wight disease before the following winter. But still the Ligurians showed no signs of this disease.

1st September 1912 was very windy, and the hive of G9 was blown right over, the combs being scattered. The bees were gathered up and the queen found uninjured, but this accident handicapped the bees for getting into condition for wintering. The spring of 1913 found them very weak and hardly any of the young bees were able to fly.

Examination for *Nosema* spores gave negative results at this stage. The queen eventually ceased laying, and she was transferred to a queenless black stock. In due course brood appeared, and in this case most of the young bees

[1] Bees from this stock were used in Tolsta experiment (see p. 52).

could fly. A number of those which "crawled" were sent to Dr Annie Porter, together with a description of the symptoms, and she reported as follows:—"Every bee contained spores of *Nosema.*" This was in July 1913. The crawling referred to here was not typical of Isle of Wight disease, and lasted for quite a short time. Also the number involved was very limited, and consisted entirely of young Italian bees. At intervals during June, weak and aged bees, both blacks and Italians, had been picked up in front of this hive and examined on the spot, by one of us (J.A.). These were full of spores of *Nosema*, but none were found in the young Italians examined at the same time.

A neighbouring queenless black stock was now united with this Ligurian stock, and soon there was a prosperous colony with plenty of brood, worker and drone. On 12th September 1913, bees taken at random off the frames were examined and no spores of *Nosema* were seen. During the winter of 1913-14 frequent samples were taken, and a large proportion of the bees examined were found heavily infected with spores. Yet those bees were quite active and healthy and could fly perfectly.

On 19th December 1913, the old queen died and stock G9 continued queenless and broodless for over three months. On 28th March 1914, we added a small lot of Italians which had been found starving in another hive. The queen was a Penna Italian imported in 1913. Bees of this stock were found to be heavily infected with *Nosema* on 2nd June.

When we examined the stock on 20th June 1914, it was quite prosperous with a good show of brood. The weather was wet and cold, and diligent search in front of the hive resulted in the finding of eight benumbed bees. Seven of these were full of spores of *Nosema.* Eight bees were also picked off the combs at random, and three of these contained spores of *Nosema.* Again, on 13th July, one bee out of six examined contained an abundance of spores. This stock is still alive at the present time.

Nosema Infection Experiments.

(*a*) *Infection of a full Stock of Bees with Nosema apis.*

On 11th July 1914, a small lot of Cyprian bees which were known previously to harbour *Nosema* were placed in confinement. On 28th July most had died, and the remainder appeared weak. Four were examined, two live and two dead. All contained *Nosema* spores in quantity. The whole lot of bees, ninety-one in number, were mashed with honey, and fed to a full stock of bees at Achmore, Lewis. These were ordinary black bees imported as a swarm from the South of England in June 1914. They had been examined on 30th June, and again on 13th July, and found negative as regards *Nosema* spores and young stages. There were no other hive bees within a radius of 5¼ miles.

On 19th of August, the stock was visited and examined. It covered nine combs, and clustered on the dummy. Not a "crawler" nor dead bee was to be seen in front of the hive, although the weather was very favourable for this. Ten bees, taken at random, were examined for *Nosema*, and spores were found in four, two of these showing a very heavy infection.

On 7th September, a second sample consisting of twelve bees was examined, again taken at random from within the hive, and five were found infected, three of them heavily. The day was fine, and there were no indications of Isle of Wight disease.

On 10th October, the bees were again visited and samples taken, from inside the hive as formerly. It was a fine October day, and the behaviour of the bees was normal.

On 13th October nine of the sample bees were examined. They were active, flying readily, and their intestines were normal in appearance. No spores of *Nosema* were found. On the 14th, eight more bees were examined, again with negative results. On the 22nd, three bees of another sample were taken. These arrived alive and active. On examination they were found to have the intestine normal, and to contain no spores of *Nosema*.

From the foregoing it appears certain that this stock of bees was definitely infected with *Nosema*, but this infection failed to produce Isle of Wight disease.

The following are illustrations of numerous artificial infections with *Nosema* carried out on small lots of bees confined in cages.

(b) *Laboratory Experiments.*

I. *On 14th June 1915.—Nosema* spores derived from bees of Stornoway, stock No. 7 (Carniolans), were used to infect some bees taken from an observation hive at Marischal College, Aberdeen.

On the 17th, Nosema spores were found in plenty in five bees taken at random from the infected lot. These spores were in alimentary canal; no intra-cellular forms were seen. Our view is that those seen were the spores ingested.

19th June 1915.—Numerous spores were found in the colon of one dying bee, and a few in the chyle-stomach. Young stages were observed in cells of chyle-stomach (J. A.).

21st June.—A dead bee from infected lot was examined; spores were found in colon, but not in chyle-stomach. Many cells of the chyle-stomach showed intra-cellular stages (J. A. and J. R.).

1st July 1915.—On 30th June, two bees were found dead. When examined on this date a few spores were found in both, and a great many young stages were seen, especially in one bee. A stained smear showed many meronts within the cells, there being frequently four parasites in one cell (J. R. and J. A.).

This infection experiment, through pressure of other duties, was allowed to come to an end about this time. But the dead bees were preserved, and in September they were found to contain *Nosema* spores in great abundance. At this latter date, however, several attempts to produce infection by means of these spores failed.

II. *20th September 1915.*—On this date thirty bees from *Craibstone No. A1* (a stock originally Italian, but now consisting entirely of black bees) were confined in a small cage, and fed on spore-infected material mixed with honey. The spore material was obtained from the 14th June experiment. Five bees were killed and their chyle-stomachs pulped and examined, before infection, for *Nosema* spores. Result—negative (J. A. and J. R.).

On 21st, the bees were again fed with the spore-laden material. Up to 25th the behaviour of the bees was normal; they fed freely, and were quite active. There had been no mortality except in the case of three, which died on the 21st. In one of these ingested spores were found.

On 26th, one bee died and one was killed. No spores were found in either of these.

On 28th September, two died and two were in a weakly state. No spores were found in any of these.

On 29th, one died and no spores were found. The bees were fed on Scholtz candy.

The intestine in the last examined bee was whitish, and not unlike the appearance of a *Nosema* infection, but, as stated, spores were not observed.

On 7th October, fourteen bees which had died were pulped. These were black bees: no *Nosema* spores were found.

It is clear from the foregoing that the spores used in this experiment failed to infect.

III. *On 30th September*, a small lot of Welsh bees were caged and fed on *Nosema*-infected material of 15th June. Spores were present in the material fed. A similar number of bees were caged as a control, and fed on clean candy. Feeding with the infected material, in the case of the first lot, was continued on the 1st and 2nd October, after which clean candy was given.

On 7th October, it was found that sixteen bees of the infected lot had died. These were pulped (abdomens) and examined for *Nosema* spores. Free spores in moderate numbers were found; two or three in every field examined of the first drop taken. One live bee examined gave a negative result. In the control, two bees had died. These were pulped (abdomens only), but no spores were found.

16th October 1915.—Five bees dead. These were pulped on 18th, and *Nosema* spores were found. Other three were examined, and spores found in all. Other six were pulped, and spores were again found. All the control bees had died at this date.

From these results it seems probable that no infection had taken place.

IV. *4th January 1916.*—On this date all the Desswood stocks were in flight. The opportunity was taken to procure a number for experiment, and samples were captured on the alighting board of the "Kelly" hive. These were fed with candy, which on the same day was well smeared with pulped matter from bees of the June experiments. This matter was found on preliminary tests to contain *Nosema* spores in plenty.

The bees were kept in a small wooden cage with glass cover. By the 6th several had died. Nine were removed at this date, but of these, two were dead shortly after caging, having probably been hurt.

6th January 1916.—Two were allowed out of the cage, and these flew quite strongly, and one of them defecated.

8th January 1916.—Other six bees were found dead. One released proved unable to fly, although it was quite active. When lifted it used its sting. This bee had chyle-stomach, pale in colour, and colon dilated with a pasty mass of pollen. Search in the dead bee and in the surviving one failed to find any trace of *Nosema* spores. Another live bee very active was taken, killed and examined. This bee had a pale chyle-stomach and inflated watery colon. Numerous free spores were found in the latter, but a very thorough search failed to discover any trace of infection in the chyle-stomach.

12th Januarg 1916.—On the 12th all the bees had died. These (thirteen) were pulped, and examination showed free *Nosema* spores in moderate numbers.

V. *24th June.*—Infected two boxes of "Kelly" bees with *Nosema* material from No. 7 Stornoway hive.

1st July.—Examined two bees which had died. Planonts seen in both bees and intra-cellular forms in stained film. No free spores seen.

8th July.—On this date the chyle-stomach of a bee of this experiment, taken alive, was found on examination to contain enormous numbers of spores and planonts. Spore clusters common.

VI. *20th June.*—Fresh spores of *Nosema* were, on this date, mixed with honey and fed to a number of bees in a small cage. On the 26th several of the bees were killed, and an examination was made of the alimentary canal. Planonts were seen in very large numbers. A further examination on the 30th revealed the presence of both planonts and meronts. Spores were not seen.

In connection with the foregoing experiments control lots of bees were in most cases kept. Further we have had a very considerable experience with caged bees under the same conditions as those of the experiments. We have not been able to detect significant differences in mortality amongst such caged bees. No conclusions are therefore drawn with reference to the cause of death. Our experience is that captivity in small cages without a queen is eventually fatal to the healthiest of bees.

With regard to the behaviour of the bees while under experiment, it was not possible to decide whether Isle of Wight was present. The condition of the intestine which we have learned to associate with Isle of Wight disease, and which we find has been described by Imms (*Jour. B. of A.*, xiv. pp. 133-4), has never been observed in those caged bees.

The experiments numbered II.-IV. were made in September and January with spores obtained from bees which died in June. These dead bees were kept in a laboratory cupboard under ordinary conditions, and the results would suggest that in some instances at least the vitality of the spore is brief.

Conclusions regarding Relation between Nosema apis and Isle of Wight Disease.

Our main conclusion regarding *Nosema apis* is that so far we have been unable to recognise any causal relation between the presence of this parasite and the disease. We have found it to be present over prolonged periods in healthy stocks, while we were unable to find it in other stocks in the apiary, nor did Isle of Wight disease spread under these conditions although various races of bees were present. Deliberate infection of a stock with *Nosema* did not produce the disease. It is well established also that the disease occurs where the parasite cannot be found. We have numerous instances of this on Deeside.

From known facts regarding *Nosema* in other countries, *e.g.* in Germany and in Victoria, Australia, it appears that *Nosema apis* is just as common in healthy bees as in diseased. They are practically all "parasite carriers."

We recognise that *Nosema* may be a contributing weakening factor favouring in certain cases the development of this disease. But we have not found that it is an essential factor.

IV.—Observations and Experiments bearing on the Infectivity of Isle of Wight Disease. As distinguished from Microsporidiosis.

(*a*) Attempts to Produce Isle of Wight Disease by Artificial Infection.

No. 1. Tolsta Experiment. July 1914.

The stock utilised in this experiment was a small swarm of unknown race, originally imported from the South of England. It was transferred for the purpose of this experiment from the Nicolson apiary to Tolsta which is six miles from the nearest hive of bees. A control examination gave a negative result as regards *Nosema.*

On 18th July 1914, three large lots of "crawling" bees, numbering hundreds, were taken from the stock at Bayble suffering from Isle of Wight disease (see p. 46). These were beaten into a compost with honey and spread over the tops of the combs of the Tolsta bees. When the hive was visited on the 27th July everything had been cleaned up by the bees. There was no trace of the honey nor of the mashed-up bodies. The bees were multiplying and required extra combs.

On 19th August, they covered fully eleven combs and had gathered considerable stores of honey. Not a "crawler" was visible, nor even a single dead bee to be seen in front of the hive. Samples at this date were examined by Innes who reported:—"Bees alive, active. No *Nosema* spores found."

On 12th September, the bees were found to fully occupy twelve combs and not a dead bee was to be seen. There were no indications of any kind suggestive of Isle of Wight disease.

On 17th October, one fully sealed outside comb of honey was removed leaving eleven combs loaded with honey. The bees were in excellent order.

On 7th November, the Tolsta bees were again visited. It was a fine mild day and the bees were flying freely. Several drones were observed in flight, but these were being attacked by the workers. The presence of drones so late in the season probably indicated that the queen was failing. There was no crawling or other symptoms of Isle of Wight disease.

On 2nd January 1915, the stock was visited and found normal.

On 13th March, the stock was found lively and vigorous.

On 28th April, the bees were found numerous and lively; there was a patch of drone brood in worker cells suggesting the presence of a drone-laying queen. Probably the aged queen had died during the winter leaving eggs and young brood, from which the bees were able to raise a young queen. This queen could not be mated, however, at this season. This drone breeder was actually found on 3rd May and removed. After some difficulty a fertile queen was again established in the hive and the stock continued its prosperous career, giving, in July 1915, a natural swarm, which was lost.

On 8th August, the swarmed stock was found queenless and broodless and much reduced in numbers, but lively and vigorous, and with much stored honey. The stock was re-queened on 10th August with a young American Golden queen bred in Stornoway.

This stock, in spite of a heavy contamination with fresh Isle of Wight material, remains free from the disease to this date, January 1916.

No. 2. Shawbost. First Experiment. October 1914.

The bees which were the subject of this experiment were Carniolans, and the stock had swarmed in July 1914. It was headed, therefore, by a young queen, and occupied seven combs.

On 1st October, two ordinary match boxes of "crawlers" from an infected stock (60 DD, see p. 56) were sent to Lewis from Aberdeen. The crawlers were taken from a lot which came out in great numbers on a sunny afternoon when the "Isle of Wight" signs were most typical. They were picked up after having been out over night, and despatched by post alive, with food. These crawlers were mashed up on arrival, mixed with honey, and fed to the stock at Shawbost, on 3rd October.

On the 10th October, the bees were reported to be in fine order with brood on two combs. All infected material had been cleaned up by the bees, and not a dead bee was to be seen.

On 24th October, the local observer reported: "No sign of any disease."

On 20th February 1915, the stock on examination was found to be in good condition, and no dead were visible.

On 27th April, the stock was reported to be in fine condition, the bees were lively and "vindictive," and there was a good deal of brood.

At the beginning of June 1915, the stock was reported to be rather weak in numbers but it built up rapidly, and on 12th August the report was as follows: "The seven original combs are now quite inadequate, and the bees have passed behind a double dummy and have built a considerable mass of new comb which is filled with honey. This is a highly prosperous stock."

On 4th September, the stock was still in excellent condition. At this date eleven months had elapsed since the Isle of Wight disease material was served up to the bees, and throughout the whole of that period the bees had been perfectly healthy, and no infection had resulted from the experiment.

No. 3. Shawbost. Second Experiment. September 1915.

On 24th August 1915, the colons and chyle-stomachs of twenty-five crawling bees were taken from front of hives No. 10 of K and the worst stock of H, Finnercy. Both stocks were very bad with Isle of Wight disease. The day was warm and sunny, and there were many hundreds of "crawlers" about in both cases. The intestines of all the bees (twenty-five in number) were drawn out, and in every case the colon was congested with pollen in a pasty condition. Most of the chyle-stomachs were purplish. These were laid out in a row and allowed to dry—and on the following day heaped together.

The above-mentioned bee-material was mixed in a little water and twelve samples examined. It consisted chiefly of pollen grains, a good many of which were digested. A few epithelial cells of the chyle-stomach in various stages of disintegration, some still showing the zymogen granules present, were seen, also some which seemed intact. None of these showed any sign of *Nosema* infection, and no free spores were observed.

Bacilli, long and short, and also cocci were seen in the preparations. This material was mixed with candy and sent to Lewis the same day, viz. 30th August. The local observer (Ewen) reported that on 4th September he fed this to Shawbost bees, and describes the bees at this date thus:—"I placed the compost on the top of the frames. The day was fine and the bees were very active. The stock in the beginning of June was very weak in numbers, but has done very well during the summer." This stock is still under observation, January 1916, and apparently healthy.

No. 4. Back Bees, Stornoway. June 1915.

In this experiment old combs and hives were used. The bees originally occupied only one of two hives, the other being left open in the hope that any swarm might hive itself. The nearest bees to Back are at Tong, about 2 miles away, and the Tong bees have always been quite healthy.

At June 1915, the Back bees were so prosperous that they were supered. On 28th June, the super was removed and the tops of the frames smeared with a paste made by mashing diseased bees with honey. These bees were crawlers picked off the ground in front of an infected Deeside stock. The bees were sent alive from Aberdeen and pulped in Stornoway.

On 8th August the stock was visited, and it was found that the bees and the two hives had been shifted from the original site to a less suitable one in a very unskilful manner, by one who was not a bee-keeper. The bees had previously swarmed, and the swarm had occupied the vacant hive as had been expected. The combs and quilts had been disarranged, so that the swarm got a very poor chance. The jar used in infection was still on the parent stock and some comb had been built in it, but there was no trace of the Isle of Wight material.

On 14th August, both lots of bees had to be hurriedly shifted to still another site at a distance of about 400 yards, and during the process a considerable number of bees was lost. There was no brood in either hive; presumably both were headed by virgin queens. This absence of fertile queens, the two disastrous removals, and the swarming constituted a combination of factors which could not fail to weaken and discourage the bees.

On 19th June, the bees had been examined microscopically; no spores of *Nosema* were found.

On 11th September, bees from Back arrived in Aberdeen. They were active, and some of them survived until 16th. On this date live bees were examined individually for *Nosema* with negative results. The dead bees were pulped, and samples examined were also found free from *Nosema* spores.

On 20th October, bees were received which had been dispatched on the 12th, with the following report:—"Symptoms same as No. E14 in Nicolson Apiary. The stock is almost exterminated. The bees sent represent 'crawlers' and remnants in the hive."

Of these fifteen dead bees were pulped, and drops examined for *Nosema* with negative results.

On 28th October, Back was visited and both stocks were found almost extinct. A small knot of bees remained in each hive on the combs. The queen of one was warmed into activity, and lived for a few hours. There was plenty of food. The local observer (Morrison) reported that both lots had displayed the typical "crawling" symptoms.

It is worth noting that while this experiment has culminated after four months in the bees apparently contracting Isle of Wight disease, which did not occur in other two similar experiments, it is not clear that this was due to the artificial infection. There were specially adverse circumstances in the present case. In the other two instances of attempted infection, the bees were maintained in good general external conditions. It should be noted further that *Nosema* was not found at any time during the experiment.

The foregoing experiments in artificial infection were made in Lewis upon isolated stocks. The following were carried out in Aberdeen.

No. 5. 60 DD. May 1915.

This stock, which stood in an apiary in a district suffering badly from Isle of Wight disease, was found, on 24th September 1914, to have large numbers of bees on the alighting board, and groups of "crawlers" on the grass around. The weather conditions were fine and sunny. The same thing was observed on the following day, and the hive and bees were removed to an observation area in Aberdeen. For a few days after this the stock seemed to be doing well enough and a good deal of pollen was being taken in. On fine sunny days, however, "crawling" became very evident, and on some occasions it was very extensive. As recorded elsewhere a sample of the "crawling" bees was utilised in an infection experiment (p. 53).

On 7th October, which was a fairly good day with a little sunshine, "crawling" was again in evidence, but pollen was still being gathered. About this time it was reported that three other stocks from the same apiary were suffering from the disease. All these eventually perished (p. 34).

Up to 17th October, bees "crawled" every fine day, but the last of them perished in the early winter, leaving abundance of stores in the hive.

On 19th May 1915, a fresh stock arrived from the South of England, Italian hybrids, fully covering ten combs crowded with brood. The queen had apparently been recently lost, but there were numerous queen cells on the combs. The new stock was put in the hive in which the above-mentioned stock, 60 DD, had been housed. There was no disinfection of any kind, but the dead bees and the old combs were removed. The honey was drained from some of those old combs and fed to the new stock.

On 3rd June, a swarm came off and was duly hived in a perfectly new hive on frames with full sheets of foundation. This swarm was also fed on honey drained from the old combs left by stock 60 DD.

On the afternoon of the following day a second swarm came off and was hived in a straw skep. This second swarm received none of the honey from the old combs, but was fed on a little honey bought in the open market.

Eggs were found in the parent stock (DEP) on 14th June, and the queen of the first swarm (DES 1) began to lay on the 15th.

The queen in the second swarm was long in being mated, and was balled by the bees on 28th June. A black virgin queen was introduced on the 30th June, and she began to lay in due course. The other two queens were as yellow as pure Italians, and the young bees were also light coloured.

The population of the parent stock was much depleted by the double swarming, and further, on 23rd June, foul brood was found to be present. This was a variety which attacks the larvæ before sealing, and attempts were made to treat the infected cells with dilute formalin. This treatment was successful so far in that the disease appeared to be checked, but unfortunately the queen was lost either during the manipulation, or poisoned by the formalin vapour. Queen cells were made from the brood in the hive and a black queen was produced, which ultimately began to lay. The stock was now very weak in numbers but was active in raising brood, in defending the hive against robbers, and in carrying pollen.

Up to 4th September it could not be said that there were any symptoms in the apiary of Isle of Wight disease. From the nature of the experiment here being recorded, a specially close watch was kept for indications of its appearing. Only at one period were suspicions aroused. The month of July was very wet and must have been very trying to the bees. On the 25th, a few "crawling" bees were seen in front of the parent stock and also the first swarm, as well as in front of another stock of black bees not related to the bees of the present experiment. And again, on the 31st, it is recorded that there were a few "crawlers," most in front of the first swarm (DES 1). August was also a wet month, but nothing of a suspicious nature was noticed. In spite of the very adverse circumstances above narrated, all three stocks remained perfectly healthy, and up to this date (20th September) the parent stock, which is housed in the presumably infected hive, was fed with honey from this hive, and was later given some of the original combs, is healthy and, as far as the eye can judge, growing in prosperity.

On 30th September, a lot of driven bees from which the queen had been removed was imported from North Wales and added to the little stock (DEP). The bees were fed on sugar syrup boiled with vinegar and were packed down for winter.

On 14th October, the first fine day since the addition of the Welsh bees, large numbers of bees were seen out upon the alighting board. This was probably related to robbing, which was being actively attempted at several hives in the apiary.

On the 28th December, a mild day, with a few bees offering to fly, the hive was opened, and the quilts were found in a sodden condition owing to a defective roof. The bees, however, were in good condition with plenty of stores.

It has already been mentioned that "crawlers" were observed on two occasions in July in front of the hive occupied by the first swarm. From that date until the beginning of September there were no definite symptoms. August, like July, was mostly wet, and the Italians with their usual improvidence had converted practically the whole of their stores into brood. From 4th September onward this stock, however, now showed undoubted signs of the disease. The first half of this month was uniformly fine, with a great deal of sunshine, and on every occasion the alighting board and the grass in front was covered with numbers of "crawling" bees. Some of these were fairly active and could perform short flights but not sustained ones. Those which left the board were, as far as we could see, unable to return to the hive and crawled about, sometimes as far as twelve yards at least from the hive, ultimately dying on the ground. Those near the hive tended to form small clusters as the day advanced. The alighting board was always cleared in the course of the afternoon. All this is of course typical of Isle of Wight disease. Further, the internal appearance was that characteristic of this disease, although all our microscopical examinations failed to reveal the presence of *Nosema*. The appearance within the hive during these days was practically normal, pollen was being carried in, and there were no indications that the disease was making rapid progress. From 4th September sugar syrup was supplied to all the stocks, and this was freely appropriated by the bees and stored.

The question naturally arises here, Had this outbreak any connection with the proximity of the infected hive or the fact that this swarm came from it? In view of the prosperity of the parent stock which had continuously occupied the hive for four months, and the length of time, viz. three months, since the swarm constituting this stock left the hive, it does not seem likely. But we must admit the possibility of infection having been transferred and remaining latent until conditions favourable for its development within the bees had arisen.

No further "crawling" symptoms have been observed since the beginning of October. On 20th October, a large lot of Welsh bees (driven, queenless) was added to this stock. It is probable that these killed the queen, for on 1st November several dead yellow queens were found on the floor of the hive, and there were queen cells on one of the combs. No free virgin queen was observed, and another queen was caged over the stock. This queen was found on the alighting board a few days later. This stock, therefore, has a virgin queen (grand-daughter of the original English queen).

The second swarm, standing alongside the first, has never displayed any symptoms of the disease.

(b) Natural Occupation of Isle of Wight Affected Site.

The Hive and its Contents as Possible Sources of Infection.

The following case is related to the foregoing experiment in respect that it is a naturally occurring instance in which the same factors, viz. hive and combs of an extinct Isle of Wight diseased stock, are involved.

Bees which occupied a frame hive began to exhibit "crawling" and other symptoms of Isle of Wight disease in the autumn of 1913, and the last survivors perished in the spring of 1914. The hive was not disinfected nor the honey and dead bees removed, but the door was closed. In June 1914, a vagrant swarm alighted beside the hive and was allowed to enter. No particular attention was paid to them, for it was not expected they would survive. But it was observed that the new-comers were very diligent in removing the dead bees and getting the hive in order. The bees wintered in good order, and in May 1915 they swarmed. This top swarm was unfortunately lost, though followed for some distance, but a second swarm on 7th June was duly hived in a skep and placed alongside the parent stock. Both stocks were visited and examined for eggs on 12th, 15th, and 21st June, but none was observed. Up till this time both stocks appeared perfectly healthy and were working normally, except that the parent stock had only a small number of bees, and drones were unusually numerous. Thus it will be seen that in the case of the parent stock a full year had elapsed since the bees took possession of a hive and combs with a recent history of Isle of Wight disease.

On the 26th of June "crawling" symptoms were developed in the bees of the swarm, and these were very marked when the stocks were visited on 28th June. The garden in front of the skep for a considerable distance was covered with crawling bees, with characteristic grouping very noticeable. The internal appearance of the crawling bees was quite characteristic of Isle of Wight disease, and we satisfied ourselves that this disease was present. It should be added here that both in 1914 and 1915 the whole surrounding district had been swept by the disease, and there were very few stocks surviving in it. Examination of the combs showed that both queens were laying, and there was a good deal of sealed brood in the skep.

The crawling symptoms above noted became less noticeable from day to day. There were, however, fluctuations, till on 23rd August both stock and swarm appeared to be free of the disease and were working normally. The skep being overcrowded had been provided with an "eke." On the 11th September, both stocks were found apparently in perfect health. The bees in the skep had extended their combs down into the eke.

All this time there had been no symptoms of Isle of Wight disease in the parent stock.

On 6th October, the parent stock in the bar-frame hive was found rather weak in numbers, and the bees in the skep were driven and united with it. The queen of the skep was removed and introduced to a stock in Desswood apiary. The combs of the skep were cut out and piled over the frames in the wooden hive.

On 29th December, the bottom of the hive was found to be thickly covered with dead bees, but there were bees still alive amongst the piled combs just under the quilt.

It may be noted that to date bees have been living in this hive on the old "Isle of Wight" combs for over 18 months without any symptoms of the disease appearing, and the conclusion is legitimate that the appearance of Isle of Wight disease in the swarm is to be traced to a separate source.

There has also come under our observation the case of a bee-keeper near Perth, who had lost several stocks from the disease, but had several remaining which were apparently in excellent condition, although he had fed them on the stores left by the diseased stocks. One in particular, through an accident, had been soaked with water during last winter (1914-15), and by spring the bees had been reduced to a handful. We examined this stock in October 1915, and found it in fine condition. The bees were black, but the owner declared they were Italians because they had had an Italian queen three years previously. Some of the surviving stocks displayed yellow bands, and the bee-keeper attributed their resistance to the admixture of Italian blood.

Of direct interest in connection with our failure to produce Isle of Wight disease by means of presumably infected hives and combs is the experience of Beuhne, of Victoria, as given in *The Australian Bee-keeper*, 15th August 1915. He says: "When a very heavy mortality occurred amongst the bees in the Grampians in 1909-10, and microscopic examination showed the parasite in the bees of the apiaries concerned, the question arose whether bee-keepers should follow the directions of the British Board of Agriculture, and destroy all the remaining bees as well as all the combs, or give the survivors a chance, and take a risk with the combs of the defunct colonies.

"My personal experience with this kind of mortality was in 1900-01, when nothing was known of *Nosema*, and therefore I was unaware of any risk, and used the combs from which the bees had disappeared, when restocking the apiary with purchased bees, and 'nothing happened.'"

Beuhne is of opinion that "under ordinary conditions, that is, conditions favourable to bees, the parasite is merely a casual inhabitant of the intestine of the bee."

General Conclusion Regarding Infectivity.

The general conclusion to which the foregoing facts point is that Isle of Wight disease, although probably an infectious disease, is one which requires the coincidence of other and presently unknown external factors (besides a specific organism) before the disease develops. The disease is not necessarily conveyed by mere contact with contaminated hives or combs, or by feeding upon contaminated stores.

Explanation of Plate.

Fig. 1. Section of the chyle-stomach of a bee displaying symptoms of Isle of Wight disease. Spores of *Nosema* are not present, and no young forms were found in this outbreak. × 180 (Iron-hæmatoxylin).

Fig. 2. Section of the chyle-stomach of a bee infected with *Nosema*, but not suffering from Isle of Wight disease. The stained spore contents came out as black dots in the photograph. × 180 (Iron-hæmatoxylin).

Fig. 3. Portion of the teased chyle-stomach of a bee, showing detached cells, some containing spores of *Nosema*. Fresh preparation. × 180.

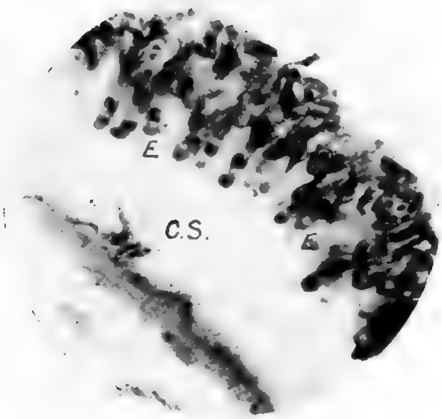
Fig. 4. Fresh unstained preparation, showing *Nosema* spores lying free in the gut. × 180.

Fig. 5. This shows the behaviour of a stock (E14) suffering from Isle of Wight disease. Note the bees, incapable of flight, clustering on the hive front. Large numbers on the grass cannot be seen, but some can be observed climbing up the leg of the hive.

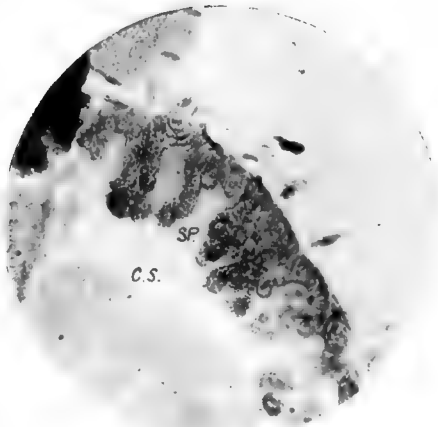
c.s. Chyle-stomach of bee.
e. Epithelium lining chyle-stomach.
sp. Spores and epithelium of chyle-stomach.

5

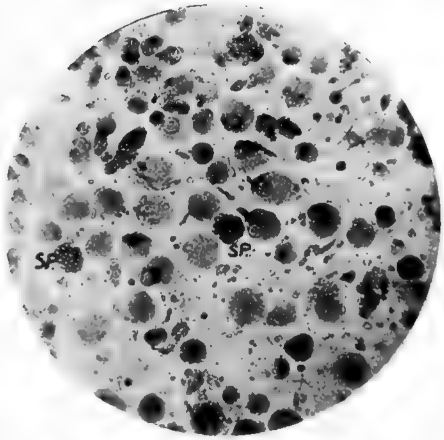
1



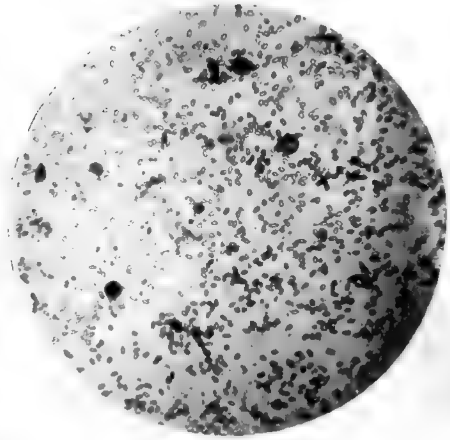
2



3



4



J.A. photo.]

"ISLE OF WIGHT" BEE DISEASE.

ISLE OF WIGHT DISEASE IN HIVE BEES.

JOHN RENNIE, D.Sc., University of Aberdeen, and
ELSIE J. HARVEY, *Research Assistant.*

[*Reprinted from "The Scottish Journal of Agriculture," Vol. II., No. 2, April* 1919.]

IN a former paper issued under the present investigation[1] the failure to produce Isle of Wight disease in hive bees within a working season by means of direct contact with frames and hives and by feeding upon stores of bees suffering from this disease, was established in a limited number of cases.

Further experiments and observations of a like kind have been continued, and in six other instances the result within the time specified was the same, *i.e.* disease did not develop amongst the bees which fed upon the stores and lived upon the frames of sick bees during the whole of the experimental period, which in each case extended over the whole of the working season. It would certainly appear that normally the disease is not readily communicated by such means.

In addition to the foregoing we now desire to record further experiments in which sick worker bees, brood and queens from diseased stocks have been separately utilised.[2]

Experiments in Infection by means of Frames, Stores, and Sick Bees.—SUMMER EXPERIMENTS.—NO. I. A stock, whose earlier history is given in the communication referred to above, p. 56, wintered (1915-16) successfully. In March flying had commenced and pollen was being taken in. During this month an adjacent stock, a swarm of the previous summer, developed very definite signs of Isle of Wight disease, and large numbers of bees crawled when the weather admitted of their coming out. Bees of both stocks were repeatedly examined for *Nosema apis*, and no stage of the parasite was found.

On the 11th April the queen of the diseased stock was removed and the two were united. The union was accomplished with comparatively slight mortality. During May the weather was good and the disease symptoms disappeared. The stock was seen working on every fine day. It now appeared prosperous, and there were large numbers of eggs, larvæ, young and old workers and a good supply of stores.

[1] *Proc. Roy. Phys. Soc. Edin.*, 1916.

[2] These experiments have been carried out concurrently with an investigation upon the relation of the parasite *Nosema apis* to Isle of Wight disease. This is in course of publication in a separate paper. We conclude that the causal organism of Isle of Wight disease has not yet been found.

The researches have been conducted with the aid of Grants provided by the Development Commissioners through the Board of Agriculture for Scotland.

(5.) 50. 5/19. J.S.&Co.,LTD.

After a period of prolonged rain the morning of 1st July was bright and sunny. Many of the bees were active and vicious, but large numbers appeared on the alighting board and many clustered on the ground; others crawled around. Drones were turned out. The appearances were characteristic of the Isle of Wight disease. The weather continued to be very wet. On the 10th the stock was examined; there were few dead bees within, but there were some slight indications of dysentery on the alighting board. On the 17th these signs were very much increased, and there were many dead bees, including drones, on the ground all around. At this date the stock was active and working well; it was rather short of stores and syrup was given.

On 29th July the stock was found in the following condition. The bees were active, gathering nectar and pollen. They covered about six frames, and three of these had sealed brood in plenty. Eggs and unsealed larvæ were also present. There was a moderate amount of stores, both honey and pollen, and two new sheets of foundation which had been put in about 12 days previously were well drawn out. Outside the hive there were numerous dead bees. Besides these, there were a few crawling bees—not more than a score or so were seen—some hanging to grasses, others collected in small clusters and remaining motionless, others making short flights—but all were definitely incapacitated. All that were seen were examined. In every case the usual condition associated with the Isle of Wight disease was found present.

The number of affected bees was relatively small, and there did not appear to be many on the floor of the hive within. Generally the impression obtained at this date was that the stock contained only a small number of sick bees and was building up.

On the 5th August the stock was working well, and a large amount of sealed brood covering 3 frames—also eggs and larvæ—were present. The conditions were similar to those observed on 29th July, except that the numbers of eggs and younger larvæ were very much increased. Stores were short and syrup was given. The weather at this time was very warm. Sick bees continued to crawl and climb upon the grass.

It may be regarded as certain that the crawling bees observed in the later weeks of July and in the beginning of August were not the diseased bees introduced upon the 11th April, which were bees of the previous autumn. The sick bees were hatched in the hive subsequent to the introduction of the disease, and had been in immediate association with diseased inmates of the hive for a longer or shorter period.

A further development of this case is described in the next experiment.

No. 2. About the middle of June 1916 a frame of bees from the aforementioned experimental stock was placed in an observation hive, and to these an Italian queen was successfully introduced. Owing to bad weather there were few opportunities for flying, but when these occurred the bees gathered both nectar and pollen. The observation hive stood near the parent stock, but few losses by return to it could have taken place, as there was no appreciable diminution of bees. On the date that crawling in the parent stock appeared (July 1st) the observation hive was removed to a new situation 22 miles distant. On the 2nd and 3rd, dysentery became very marked in the observation stock, which owing to the bad weather had to be fed continuously. The bees were given both syrup and candy. Very few flew, even when the weather was favourable. They fed readily, but mostly did not leave the comb. Crawling bees were in evidence in moderate numbers, and eventually the queen ceased to lay. The mortality within the hive was not excessive, but owing to the crawling losses the numbers steadily dwindled. The fæces of the bees were thin and watery, later they became thicker and ropy looking. The appearance of the bees when out of the hive, crawling, making short hopping flights, as well as the condition of the alimentary canal with congested colons, were not distinguishable from those seen in Isle of Wight disease, and we concluded that the disease was present. After the queen ceased laying, and the numbers had become much reduced, a recovery seemed to take place, the dysentery ceased and the bees ceased to crawl, but they were too few to build up. With warming indoors and feeding, this lot of bees improved in condition and became much more active. The weather also improved at this time, and opportunities were thus given the bees for flight. They still crawled, but in fewer numbers, and there was less dysentery. They did not collect pollen, and it is doubtful if any honey was gathered. The flying seemed to be about the hive simply.

The improvement continued, and on the 15th dysentery was absent and only a few crawlers were seen. A greater proportion flew. They fed readily from supplies given, but did not seem to be gathering stores; so far the queen had not recommenced to lay.

The significance of the aforementioned facts lies in the practically simultaneous appearance of disease symptoms in the parent stock and in the bees which had been isolated from it at least a fortnight previously. During the period from the 11th April until 1st July there were no observed indications of the disease, and when it did appear upon the latter date, very large numbers showed the symptoms suddenly. The sudden outbreak in the two lots which lived together for two months, but which had been widely separated for a fortnight, strengthens the view that the disease had a common source in both, and adds to the probability that that source was the diseased element introduced upon the 11th April.

No. 3. On 14th May 1917 a stock of Dutch bees occupying a new hive and with new frames and foundation was found to be actively robbing a small stock rich in stores which was suffering from Isle of Wight disease. Contamination having thus been established, and the robbed stock being reduced to the limits of one frame, it was added with the bulk of its stores to the Dutch stock.

Up till the beginning of July this stock prospered in a normal manner, and no signs of disease ever became apparent. At this date preparations for swarming developed. The bees covered ten frames thickly and there was brood upon nine. We made an artificial swarm, but discovering later that the parent stock was queenless, we re-united the two with the original queen on the 23rd of July, making a stock of over seventeen frames of bees.

During summer the stock continued in good strength, and no sign of disease was ever observed. In September one frame of bees was removed for experimental purpose, and on the 27th a clearing board was introduced and the surplus honey removed, and the bees were made up for winter in the lower box. Two pounds of Bacterol candy were left on the frames. On various occasions throughout the experimental period bees were examined in numbers. *Nosema apis* was not found, and no signs of Isle of Wight disease were ever observed.

No. 4. On the 17th August 1918, to a stock of bees[1] covering about six frames and headed by a young queen (Italian bred, mated in Aberdeen) of the current year we added a lot of bees together with brood and stores upon four frames, the remnant of a stock reduced to this condition by Isle of Wight disease. Its previous history is as follows:—One week after swarming, viz. 7th July, indications of Isle of Wight disease were observed, and during that month the stock was repeatedly treated with flavine, notwithstanding which the bees continued to crawl until the stock was reduced to the condition stated upon the date mentioned. The union of the two lots was effected without loss of bees, and the condition of the increased stock was ascertained upon 29th August to be as follows:—The bees covered ten frames fully, and there was brood upon six, including eggs. The queen was seen upon the frames, and there were no signs of disease outside the hive. Stores were short. The weather was changeable, cold to mild.

During the following month the weather was mostly wet, and there were few indications of disease outside the hive. On 20th September it was ascertained that there was brood upon one frame only, and the bees were covering nine frames. No crawling amongst the Italian bees was observed throughout the autumn, and the bees were put up for winter. The mortality up till the end of February was slight. The bees were flying at this date in bright but cold weather. This stock has wintered successfully, and in fine weather the bees have been seen gathering pollen.

WINTER EXPERIMENTS.—No. 5. In the autumn of 1916 a stock of bees which had been showing the usual Isle of Wight disease symptoms intermittently over a considerable period was treated with Bacterol both by feeding and spraying. This had no permanent effect.

At the beginning of October crawling was much in evidence. There was sealed brood upon two frames, but the stock was greatly diminished in numbers. In the endeavour to maintain the stock throughout the winter, we added a lot of driven bees obtained in Wales at the beginning of November. The united stock was ascertained a week afterwards to have clustered over six frames. On fine days the bees were flying, and signs of the disease were in abeyance. At the end of December on a fine day bees from this stock were flying and removal of dead was observed. Again on

[1] These bees had an earlier history of *Nosema* infection and of contact with brood of Isle of Wight diseased stock.

22nd January 1917 we observed dead bees being carried out. February 23rd was sunny and warm; the bees were active, and large numbers flew on this date. During March the bees flew on every fine day. Pollen gathering and cleaning up was in evidence.

The stock continued normally throughout the spring and summer, and it is clear that the driven bees added in November did not contract the disease, although sick and healthy had been in close association throughout the winter.

The disease re-appeared in this stock in the following July.

In addition to the foregoing experiment, four other cases of stocks affected with the disease were united in the autumn with lots of healthy bees. These healthy bees in each case covered from five to six frames. In two instances the queens of the healthy stocks were retained, and in the remaining two the queens of the diseased stocks were left to head the combined colonies. All the stocks were wintered with ample stores. In the following spring it was found that two of these united stocks had succumbed, whilst two survive. Of the survivors, one is headed by a healthy queen and the other by a queen of a diseased stock.

In another apiary nine stocks suffering from Isle of Wight disease were strengthened by giving them driven bees in September. The majority succumbed in December and January, and only one remains alive. In these cases failure was not due to shortage of healthy bees, as in each case the numbers used were sufficient in themselves for wintering.

In the following record further illustrations of the readiness with which sick bees may communicate the disease to other bees will be found, and also in some of the cases evidence that susceptibility is not merely racial, *i.e.* that the bees are not suffering from the disease in the first instance merely because they are all the offspring of a particular queen.

Results of Experiments in Re-Queening.—To a one frame lot of bees, an Isle of Wight diseased remnant, a healthy queen obtained from Wales with driven bees, which had wintered at Aberdeen, was introduced upon the 28th March 1917. Honey was fed from an Isle of Wight diseased stock, and the frames in the hive (two) were both from Isle of Wight diseased stocks.

Up till the 2nd of May, exactly five weeks, this lot continued to exhibit symptoms of the disease, but after this date these disappeared, and for four weeks the stock appeared perfectly healthy. We regarded this as the period in which the old lot of infected bees were gradually dying out, while meantime the young bees of the new queen exhibited no signs of infection.

On the 31st May two frames of bees from another stock were added. From this time onward throughout the summer the stock gradually increased in numbers, and at no time could it be said positively that Isle of Wight disease was present. At the beginning of August the strength had reached ten frames with brood on eight. A rack of sections was placed on the frames. This month, however, was very unfavourable to the bees, and on the 13th crawlers were seen in front of the stock, and this was observed up to the 4th September. This symptom passed off, and subsequent to this date was not again observed. The stock, which was short of stores, was fed with Pascall candy and put up for winter.

On 23rd January it was found to be much reduced in numbers, but to have plenty stores. It was still in existence on the 5th February, but on the 10th it had died out. The queen was found apparently dead also, but on removal and warming she revived.[1]

On more than one occasion bees were removed from this stock for experimental purposes in the late autumn. The cause of its dying out is not clear. Our impression on reviewing the whole history was that the new stock built up by the Welsh queen introduced to unhealthy bees never contracted the disease.

In one of the apiaries, Isle of Wight disease developed generally during the working season, 1917. A number of the stocks were re-queened. The season proved highly successful for honey, although the disease continued. The stocks were wintered, but before spring all were dead.

Season 1918 in the same apiary. On August 23rd, a sick stock was re-queened with a young imported Italian queen. Six weeks afterwards there were Italian bees, offspring of this queen, amongst the crawling diseased bees. A duplicate experiment also yielded the same result, offspring of this second Italian queen being found amongst the crawling bees at the end of six weeks.

Our conclusions regarding re-queening are that, whilst it may be useful temporarily in the early part of the working season, it is not successful in ridding a stock of the disease.

Experiments with Brood of Diseased Stock.—With a view to obtaining information as to the possible infectivity of the brood in relation to this disease a number of experiments have been performed, of which the following are typical examples.

No. 1. This stock was obtained in the spring of 1918. Samples of the bees were microscopically examined on several occasions. At the end of May two bees were found heavily infected with *Nosema apis*, and again this parasite was found in two bees at the end of June. These occurrences were exceptional, as in numerous other instances such examinations yielded negative results, and the parasite was not found after the latter date specified above. It is certain there was no general infection with this parasite.

The stock about the middle of May was covering six frames and had young brood and eggs upon five, besides sealed brood. Increase was rapid, and before the 18th of June the stock had yielded four frames of bees for the formation of nuclei or strengthening of other stocks.

On the 10th June one frame of brood from each of two Isle of Wight diseased stocks was introduced. Again on the 18th a third frame of brood, also from a similarly diseased stock, was introduced. This brood had all hatched by 6th July, and the frames were in ordinary routine utilised by the queen in egg-laying. The stock steadily built up, and by the middle of June was thickly covering

[1] The queen of this stock was subsequently given to the remainder of an Isle of Wight diseased stock, and later to one frame experiment of date February 28th, 1918. She later headed a *Nosema* experiment in May.

twenty frames. There was a large quantity of surplus honey, while the second brood box of ten frames also contained considerable stores.

No signs of Isle of Wight disease have ever been observed in this stock, which on 1st November remained strong in numbers and healthy. During the whole season it has stood in an experimental apiary into which Isle of Wight diseased stocks have been introduced as a matter of routine throughout the summer and autumn. Further, it actively robbed several of these stocks early in August. The presence of *Nosema apis* amongst the bees has already been recorded.

From the foregoing it may be gathered that the whole of the bees introduced as brood or eggs from the diseased stock was hatched by the 9th of July. Since no signs of disease appeared amongst the bees from that date onward until the bees wintered, it appears that the brood was healthy although bred and a good proportion of it (sealed brood) was nurtured in the affected hive.

No. 2. On 15th May 1918 an Italian queen was introduced to a two frame nucleus of queenless black bees. *Nosema apis* had been found in the attendant bees which accompanied this queen from Italy. On the 24th the queen was seen upon the frames, and there were larvæ and eggs in the brood nest.

On the 27th May one frame with brood in all stages, but without bees taken from a stock suffering from Isle of Wight disease, was introduced. Another similar frame was given upon the 31st, and another upon the 11th June. Upon the 1st of July three additional frames containing brood in all stages taken from another Isle of Wight diseased stock were placed in the hive, making six frames in all. The patches of brood in all cases were of moderate size. The introduced brood duly hatched, and the nucleus continued to prosper in a normal manner. On the 1st August the stock was making up well, the bees were covering nine frames and the brood nest extended to seven. This represents the maximum strength so far as was observed.

It will be noted that all the brood introduced from the diseased stocks had hatched out by 22nd July. These bees were recognizable by their difference in colour from those bred by the queen. During August they represented a fair proportion of the bees present.

During the whole course of the experiment the stock was watched for indications of the presence of disease, and nothing in the appearance or behaviour of the bees, either black or yellow, suggestive of the disease was ever seen. The stock has wintered successfully.

No. 3. On 27th May 1918 a stock of Punic bees covering five frames which had wintered in Aberdeen was given a frame of brood from an Isle of Wight diseased stock. Again on 31st another frame was given from the same source. On 11th June a third frame of similar brood from another diseased stock was added. The stock increased in numbers rapidly to the middle of July, when the queen was found to be missing and many queen cells were being made. These were removed and a queen introduced, but on 20th July the stock swarmed, and on 1st August the parent stock was covering twenty frames and storing well, but no surplus has been obtained. The last bee from the Isle of Wight diseased stock was due to hatch on 2nd July. Such bees were present in the stock from 27th May onward. On no occasion have any indications of Isle of Wight disease been observed in the stock, which has wintered successfully.

No. 4. On 4th July two frames of brood from a stock suffering from Isle of Wight disease were introduced to a small swarm of Irish bees covering five frames. The stock continued to work normally during the summer and at the beginning of August was covering nine frames with brood upon eight. There were plenty stores. The whole of the brood introduced from the diseased stock was due to be hatched upon the 25th July. There have been no signs of Isle of Wight disease in any of the bees, and the stock has wintered successfully.

No. 5. On 4th July a swarm of bees from Tipperary covering five frames was given two frames of brood from a stock suffering from Isle of Wight disease. The stock built up well during the summer, and in the middle of August had a good supply of stores. There were ten frames of bees, nine of which were carrying brood. The last bee from the Isle of Wight brood was due to hatch on 25th July. No indications of disease have ever been observed in this stock, which wintered successfully and has become the subject of another experiment.

No. 6. On 14th July 1918 twenty frames of brood belonging to an Isle of Wight diseased stock, originally of thirty frames, were placed with a healthy stock, very strong in bees. This stock was isolated in a heather area at the beginning of August. It did well, and is now wintering in good condition, having yielded a large surplus. No signs of disease have ever been seen.

In the selection of the brood in the foregoing experiments the endeavour was always made to utilise the maximum amount of sealed brood. In this way bees were taken which had had the full opportunity for receiving infection from attendant bees.

The results have been uniform throughout, and it would appear that bees reared in a stock suffering from Isle of Wight disease are not susceptible to the disease in the brood stage. Further, we may add, dead brood has never been found to be a feature of the disease.

Incidental to these experiments we have found that whilst as recorded the brood removed to a healthy stock did not develop the disease, that which was left with the sick parent stock in due course succumbed to it as adult bees.

Experiments with Queens of Diseased Stocks.—With a view to ascertaining whether bees associated with a queen from an Isle of Wight diseased stock or bred from her in healthy surroundings develop the disease, the following type of experiment was carried out.

Experiment No. 1.—On the 11th April 1917 we introduced to a small stock of queenless Welsh bees, covering two frames, which had been wintered in Aberdeen, a queen which survived from a stock recently defunct and exhibiting Isle of Wight disease symptoms. Up to the middle of May progress appeared to be slow, and the queen did not lay well. We removed this queen, and replaced her by another queen surviving from a badly affected Isle of Wight stock. During the following months the stock progressed normally. No signs of disease could be observed, and at the 23rd July the bees were covering ten frames with brood on eight. August was very unfavourable to the bees generally. No surplus stores were obtained, and feeding had to be resorted to before winter. On the 24th January 1918 the stock was found to be alive and was supplied with Bacterol candy. On the 9th February the bees were on six frames with brood sealed and unsealed on one. At the end of February this stock was disturbed by workmen, and losses appear to have been incurred through the bees flying out in too cold weather. In the middle of March the hive was found empty save for a few dead bees, including the queen. There were very good indications that this stock was about to continue satisfactorily in the forthcoming spring, and the failure appeared undoubtedly due to the external cause indicated. We regard this experiment as successful in showing that a queen surviving from an Isle of Wight diseased stock may continue for a satisfactory period (10 months) without communicating the disease.

Experiment No. 2.—This stock consisted of bees obtained from Wales in the autumn of 1916. It was wintered upon frames from an Isle of Wight stock, and in February the bees were fed upon Bacterol medicated candy, and meantime the Isle of Wight stock frames were removed and replaced by others. At the end of March the strength was low, amounting to about two frames. On the 28th of this month the Welsh queen was removed and a queen from an Isle of Wight stock was introduced. During April and May the stock did moderately well, and at the end of May covered about five frames; the bees worked well, and at no time were there any signs to indicate that Isle of Wight disease was developing. In the beginning of May the bees robbed stores from an empty hive containing the frames of a stock defunct from Isle of Wight disease. The hive was closed, but the bees were making use of the escape funnel at the top.

Throughout the summer matters progressed well on the whole, and on the 10th July the stock consisted of ten frames good with brood on nine. There was also a considerable amount of stores, the brood nest being rather restricted. A super was put on on the 23rd, but the bees did not make use of it. The month of August was extremely wet, and the bees had few opportunities for flight. At the beginning of September the stock was still a crowded one; there was brood on eight frames, but the stores were running short. Disease was constantly watched for, but no indications were ever observed. The stock continued well and was fed with Bacterol candy with a view to wintering. At the beginning of November it was concluded that the queen, having been with the stock for over seven months and no disease resulting, had failed to infect the colony, and the experiment was regarded as concluded. At this date the stock was divided and the bees utilised in other experiments.

Experiment No. 3.—This stock commenced with a nucleus of one frame of bees, to which was given a queen from an Isle of Wight diseased stock on 4th June. This queen headed the stock up to 13th July, at which time it was strengthened with bees from other sources, and at this date covered eight frames. At the time of one of these additions the queen was lost. She was replaced by another of similar history. This latter queen remained with the stock, which at the beginning of August had a strength of nine frames with brood upon seven, and was working very well.

On 12th September stock No. 22, a similar experiment, was united without its queen to this stock. The combined colony, originally covering about thirteen frames, is now in one brood box, with dummy excluder between. On 23rd the queen was seen upon the frames. *There were crawling bees on the plot in front of this stock at this date,* and subsequently the same feature was observed on several occasions.

The combined stock was found in January to have died out.

Experiment No. 4.—On 15th March 1918 a queen surviving from a stock depleted with Isle of Wight disease was placed upon a frame with small cluster of healthy bees. The bees were kept in an observation hive and fed with pure candy. Water was given from time to time. They were also given candy mixed with crocus pollen, and on other occasions with candy made up with pea flour. The hive was situated in the heart of the town and opportunities for obtaining stores were limited. At the end of two months there was a small cluster of young bees on the frames and brood on the combs.

This nucleus survived and in fact increased during May and earlier part of June.

On 12th June this lot was removed to another apiary and strengthened with one frame of Punic bees.

On 10th July, having maintained itself normal, this nucleus was added to another which had been headed by a queen of similar Isle of Wight disease history from 1st June, but which was at this date found to have lost its queen. The strength of the united stocks was six frames at this date, and from then onward until the end of August the increase amounted to little more than one additional frame. The queen was not a young one, which accounts in part for the failure of the stock to increase, but at no time has any evidence been obtained that Isle of Wight disease had developed even in a minor degree. The nucleus was headed by a queen surviving from an affected stock for a period of six months. On 12th September this stock was united to No. 21, a similar experiment. At the time of union the queen was not observed, and subsequent search has shown that she is lost. There were losses owing to the union, but these were small compared with the total number of bees, and the joint stock was wintered up. As recorded under experiment No. 3, disease developed here, and the stock was found to have died out during the winter.

Experiment No. 5.—On 15th July 1918 we removed the queen of a strong stock of Punic bees with brood upon nine frames and substituted a queen taken from a stock suffering from Isle of Wight disease. On the 4th of August the stock swarmed, and the swarm was successfully hived. Up till the 20th of the month the queen with the parent colony had not begun to lay, and upon the 10th September this stock was found to be broodless. On this date the swarm of 4th August was placed on top of parent stock, each with its own queen. The two boxes were separated by excluder. On the 25th September the strength was ascertained to be clustered on eight frames in the parent stock, and on five in the swarm.

During the whole course of this experiment, lasting over a period of three months, from the introduction of the experimental queen until the bees were wintered, no traces of disease were ever observed. The stock was found in January to have numerous dead. There were signs of dysentery upon the frames, and the colony died out in February.

Experiment No. 6.—This nucleus consisted on the 18th of March 1918 of five frames of bees with brood upon two. On the 20th a queen surviving from an Isle of Wight diseased stock was introduced. The stock progressed normally throughout the summer, and at the beginning of July was of full strength, having brood on nine frames. On the 7th July a large swarm came off. The queen cells in the parent stock were destroyed and the queen of the swarm was returned. The stock continued to build up, and at the beginning of September, although it had yielded no surplus stores, was large and healthy, with sufficient stores for wintering. At no period of its history have any signs of disease been observed.

In the beginning of March 1919 this stock was found to have wintered successfully.

Experiment No. 7.—On 4th May 1918 a nucleus consisting of two frames of healthy bees having two small patches of brood was given a queen of above named history which survived from a stock of Isle of Wight diseased bees. The queen was an old one, and on 7th June was withdrawn from this stock, which at this date covered three frames, and was replaced on the 11th by a younger (1917) queen of similar history. The stock progressed with no visible signs of disease until upon the 17th July it covered eight frames and had brood upon six. From this date onward until the beginning of September the strength remained stationary. The amount of sealed stores was moderate, and the stock required feeding before winter. No sign of Isle of Wight disease was ever observed in this stock.

In the beginning of March this stock was found to have wintered successfully.

Communication of the Disease.—As recorded above, attempts to convey the disease by combs and stores have in general been failures, while contact experiments of presumed healthy with sick bees have yielded a proportion of positive results. We incline strongly to the view that the sick worker bee is the chief agent in communicating the disease to other bees.

Notwithstanding the variation in the result as shown in the other experiments, the evidence as a whole derived from a very wide experience in dealing with the disease in the North of

Scotland particularly, leaves practically no doubt in our minds as to the infective nature of this disease. The results recorded show that extinction after known contact with the disease is more frequent in winter than in summer. This is doubtless because in large measure loss in summer is compensated by production of bees; at the same time the closer association as a cluster in winter probably fosters the disease. Also, as the ventilation of the hive in winter is usually much more restricted, this has probably the same effect.

With regard to those cases in which the disease did not develop after contact within the working period, *e.g.* No. 5 (p. 128), but in which at a later season it appeared, we have observed that occurrences of this nature are not infrequent. We have in common with various bee-keepers of experience found that in stocks which have been associated in a particular area with sick bees, *e.g.* those taken to heather in summer, the disease often appears in the following working season. In these cases infection was not conveyed to the bees which were actually in contact with the disease, for with the exception of the queen these had completed their normal life without showing any signs of the disease before it appeared in the stock. And these bees which actually developed the disease were never in the infected area at all.

The readiest explanation in such cases, and one which must hold the field in the meantime, is that in these instances we have an infection from an independent source, and that original contamination was merely assumed. We acknowledge, however, that cases of this nature are fairly numerous, and that we cannot set aside entirely the possibility of another explanation. In the experiment quoted above, No. 5, contamination did take place, and no disease followed in the bees in the hive at the time, whilst it developed in the summer following. The assumption of a latent non-infective period for the causal organism would explain all such cases; so also would the absence in the first season of the conditions necessary for its development in the bees. These views imply a prolonged vitality for the organism outside the bee.

From the results recorded under brood experiments it would appear that the queen from an Isle of Wight diseased stock does not transmit the disease hereditarily. This is corroborated by the general results obtained in the above described cases where such queens were transferred from their sick stocks to healthy, in which they produced bees over prolonged periods covering usually full working seasons without the appearance of disease.

We have further obtained some evidence that such queens do not produce bees which are specially susceptible, *i.e.* of inferior racial quality. Brood from such queens removed from the infected stock and transferred to hives in the immediate neighbourhood did not develop the disease on reaching the adult stage, although the opportunities for infection were very favourable.

In none of our experiments, with the possible exception of No. 3 (p. 134), did the queen convey the disease by contagion.

From the fact that the queen in a diseased stock invariably survives to the last, it is generally assumed that she is immune to this dsease. Such an assumption is not altogether warranted. It must be borne in mind that she is constitutionally adapted to a much longer life than the worker bee and to live a different kind of life. It may be that the queen does contract the disease, although she does not die before or along with her worker offspring. In this connection one feature has impressed us in reviewing our results from experiments with queens. We are not satisfied that the production of queens from Isle of Wight diseased stocks is as good as that of queens of similar age from healthy stocks. This problem is the subject of investigation at present.

General Conclusions.—In the course of our enquiries we have had ample opportunities of studying this disease in all its aspects. The following statements are based entirely upon our own observations and experiments, some of which are not given in detail in the foregoing paper.

The presence of this disease in a stock is manifested by the inability of the worker bee to fly. In fine weather during the working season stricken bees leave the hive and loiter upon the alighting board, or crawl upon the ground or grass in front. They frequently climb the stems of grasses or other plants. They tend, especially as the day advances and the weather becomes cooler, to gather in clusters upon the ground, where they remain almost motionless, except for occasional trembling of the wings and body, until they die. When the weather is not too cold they may survive a night's exposure, and if warmed become more active. But individual bees, once they are stricken with the disease, do not recover, and this independently of whether they have suffered from exposure outside the hive or not. Notwithstanding the fatal nature of the disease, we have been able to keep alive for two months in the autumn a remnant of an affected stock in which practically every bee was unable to fly and showing all the ordinary indications of the disease. Within the hive, as we have noted in sick stock kept in observation hives, the smitten bees do little work, they feed little, and frequently show so great weakness that they fall from the frames. Not uncommonly they immediately attempt to regain their position by climbing back again. They may show their weakness further in lack of co-ordination of the fore and hind wing, the latter frequently projecting at right angles to the body. Very usually the hind gut (colon or rectum) is dilated with undischarged fæces. We regard this as largely due to the incapacity of the bee for flight, defæcation normally taking place only on the wing. The fæces are usually at least semi-solid in character, but when the temperature of the hive is high and there is liquid food (syrup or honey) the condition may become dysenteric.

These familiar symptoms indicate the final phase of the disease, and we have proved its presence in bees while still capable of flight. Our results suggest that infection takes place most readily through contact with sick bees, and that this may occur in the early phase of the adult condition, before the bee has commenced to forage. It is not certain that drones contract the disease, although they may suffer indirectly through the disorganisation of the hive economy when the disease is widespread within it. We have already indicated our results regarding the relation of the disease to brood and to the queen. A hive population is continuously undergoing renewal by loss of older bees and the production of new bees. Recovery in a stock can be attained only by the failure of the disease to spread amongst the young as the older die out. The rate of spread varies in relation to factors at present unknown, and, as far as appearances go, is further obscured by the rate of production of new bees by the queen. When she is young and prolific she may far outstrip the losses from disease, and the colony may maintain itself so as to be profitable to the owner for a time. When new bees cease to be produced the disease gains upon the colony, and in this way the failure of stocks from the disease is more common in autumn and in winter than at other times.

The ordinary symptoms described above are not specific to the conditions known as Isle of Wight disease, but appear to be usual when adult bees are incapacitated from various causes. Hence there are instances in which the bees of a stock may appear to be suffering from Isle of Wight disease, and losses from crawling may continue for several days or even a longer period. But in such cases the non-infective nature of the trouble becomes apparent when the later produced bees are seen to be quite healthy. The "disease" is then usually spoken of as having "passed off," and the bees are said to have "recovered" from the disease. In Isle of Wight disease in our experience there have been no recoveries of the sick bees nor of affected stocks, although the rate of progress has been very varied. The temporary sickness here referred to we have observed on a number of occasions, sometimes widespread in a district and affecting practically all the stocks. In such cases it would appear to be due to some disturbing factor in the nectar or pollen available at that time. We consider that May sickness, which occurs in other months as well, is of the nature here described. In other instances we have concluded that the failing of wintered bees in spring has assumed the appearance of Isle of Wight disease. Bees which have travelled long distances with ready facilities for feeding sometimes show symptoms similar when eventually released. Our later experiences have confirmed us in the view that Isle of Wight disease is an infectious disease, but we have not found *Nosema apis* to be causally related thereto. Experiments dealing with this aspect of the problem are described in a succeeding paper.

NOSEMA APIS IN HIVE BEES.[1]

JOHN RENNIE, D.Sc., University of Aberdeen, and
ELSIE J. HARVEY, *Research Assistant.*

[*Reprinted from " The Scottish Journal of Agriculture," Vol. II., No.* 4, *October* 1919.]

WHILST there are numerous records of disease amongst adult bees, only a very few references exist in which the disease has been shown to be definitely associated with a causal organism. Dönhoff and Leuckhart (3) in a paper published in 1857 described a disease associated with an affection of the chyle stomach, which they showed to be infectious by feeding healthy colonies with syrup contaminated with the macerated stomachs of diseased individuals. They found in the chyle stomach numerous oval corpuscles which they regarded as the spores of a fungus. In the following year Higgins (5), in England, described the loss of a stock of bees associated with the presence of minute oval spore-like bodies in the dead bees. In both of these cases the spores were probably those of *Nosema apis.* In 1912 Graham-Smith, Fantham, Porter and Bullamore (4) in examining bees from the Isle of Wight claimed that a minute protozoan parasite found in the alimentary canal, and previously observed by Fantham and Porter in 1906, was the cause of the condition popularly known as Isle-of-Wight disease. Incidentally it may be mentioned that for reasons in part already given (Anderson and Rennie, 1) and (Rennie and Harvey, 7), and also from evidence here submitted, we dissent from this view. In 1907, Zander (9) described a disease of adult bees in Bavaria with which he associated as a causal agent the protozoan referred to, discovering it independently and naming it *Nosema apis.* In 1909 (10) he published an account of the main facts in the life cycle of this parasite. In 1912 Fantham and Porter (4) also described the development and life history of the same organism. So far as we know, this is the only organism as yet identified in association with disease in adult bees.

The parasite *Nosema apis* has a wide geographical distribution. It is well known in the British Isles. It occurs in Germany, where, according to White (8), "it has been encountered by a number of investigators"; it is known in Switzerland, and we have on a large number of occasions obtained it in bees forwarded from Italy. In these last cases it has never been associated with epizootic disease. In Victoria, Australia, it is well known, and it has there been the subject of extensive study (Beuhne, 2). White, in the paper already referred to, has found it " in samples of bees received from

[1] This article was received by the Board on 2nd June 1919.

(886.) 50. 11/19. J.S.&Co.,LTD.

twenty-seven different states in the U.S.A., and in two samples of adult bees from Canada."

M'Cray and White, 1918 (6), report with reference to United States that "the disease weakens and even kills colonies. . . . The exact losses from it are not known, but in America they are less than has been attributed to it in some other countries."

Considerable doubt has hitherto existed as to what degree, and under what conditions, *Nosema apis* is pathogenic in bee colonies. In the accounts of the occurrence of *Nosema* given by Zander, he associates as a common feature the presence of dysentery in the affected bees accompanied by a high mortality. The bees die in large numbers within the hive, and also in many cases they leave the hive, dying outside. He attributes the condition known as "May sickness" to *Nosema apis*, but claims also that there may be a mortality from its presence at any time of the year. In April and May it is mainly young bees which are affected.

White, in the communication quoted, mentions that in spring especially "many weak colonies show upon examination a high percentage of *Nosema* infected bees. Quite often indeed in the examinations that have been made of such colonies, 50 to 90 per cent. of the bees in samples taken from them were found to be infected with the parasite. It is an interesting and important fact that a very large number of colonies which are strong and apparently doing well are found upon examination to contain at least a small percentage of *Nosema* infected bees." Anderson and Rennie (1) "found it to be present over prolonged periods in healthy stocks," and we have in recent years amply confirmed the observations upon which this conclusion was based.

Beuhne, 1916 (2) records a series of important experiments with stocks harbouring *Nosema*, the result of which showed that in Victoria, Australia, the parasite had not the virulent character described by Zander. *Nosema apis* in Australia is described as comparatively harmless during normal seasons. It was found to be present in almost every apiary in Victoria, and even wild bees in trees were affected.

With a view to obtaining further light upon the conditions under which *Nosema apis* is pathogenic to hive bees, to what extent and under what conditions the parasite is distributed, we have during a series of years made many systematic observations and experiments.[1] Typical examples of these are here recorded.

The Course of Nosema Infection in Observed Cases.

No. 1. **History of a Stock Artificially Infected with Nosema apis.**—On 4th May 1917, a stock of Dutch bees (No. 31)[2] was placed in a new hive upon a site not previously occupied by bees. This stock covered five frames, and there was a good supply of brood. Five additional new frames with new foundation were placed in the hive. On the same day thirty-two bees were caged and fed on candy contaminated with fresh *Nosema* spores. Two of these

[1] These researches are being conducted with the aid of grants provided by the Development Commissioners through the Board of Agriculture for Scotland.

[2] The numbers are apiary references only.

bees were examined on the 8th, and ingested spores were found in their chyle stomachs.

On the 7th forty-six bees were taken from the stock and their alimentary canals pulped. Six smears were examined with the object of determining the presence or absence of *Nosema* in the stock. The result of the search was negative.

On the 9th the *Nosema* infected lot numbering thirty bees was placed on the frames of the main stock in an open cage.

During May the stock continued normally, and on the 1st of June it covered six frames, and again on this date bees fed on candy contaminated with *Nosema* were introduced. At this time the stock was doing very well, and on the 14th it was found covering ten frames full.

At this date several queen cells were seen and destroyed ; three frames of brood and bees were removed for other experiments, and two frames of foundation were placed in the brood nest. Again on the 29th queen cells were found and destroyed. The bees, notwithstanding the loss of those removed a fortnight previously, were covering 9½ frames with brood in all stages on 8½ frames.

Examination of bees taken at the end of June showed that a Nosema infection was established in the stock. On the 4th July the stock swarmed.[1] On the 6th all the frames were gone over; fifteen queen cells were found and of these thirteen were destroyed. On the 25th a queen was seen upon the frames and the bees active, strong, well, and drawing out foundation. During August the weather was very wet, but the strength was well maintained, and at the beginning of September the bees covered nine frames.

On the assumption that the *Nosema* infection had largely been shed in swarming a third infection was made.

On the 22nd September, and again on 2nd October, *Nosema* spores were freely mixed with candy upon slides, and these were placed within the entrance of the hive and upon the top of the frames. The weather at this time was good and the bees were active. During the whole period the stock has been under observation there has been no dysentery and no other signs of disease have been observed.

On the 22nd October bees were taken at random off the frames and were examined for the presence of *Nosema*. The following are the results.—Six proved negative, one showed infection with intracellular stages, and spores were found in three, two of which were heavily infected.

On the 29th October three flying bees were taken from this stock at the entrance ; all contained spores, and one meronts also, of *Nosema*. Four dead bees found in front of the hive all contained spores.

On the 3rd November two live bees were taken and spores found in both. The stock appeared reduced in numbers, but there were no signs of dysentery or other trouble. Nor were there indications of Isle-of-Wight disease, such as crawling in front of hive, clustering, or climbing on grasses, etc.

[1] Unfortunately this swarm was lost.

On the 23rd of this month spores of *Nosema* were again found in bees taken for examination. During the previous weeks the stock had been well supplied with pure unmedicated candy and packed up for winter.

On the 23rd January 1918 the stock was looked at, and three live bees were taken and examined. No stage of *Nosema* could be found, and it was concluded that these were free from infection with this parasite.

During the winter the mortality was fairly high.

In the beginning of February the stock was found to be covering about five frames and to have plenty stores. Good progress was made in spring, and at the end of May it had a strength of ten frames, with brood on nine.

During June the strength was maintained and the bees were storing in a super. As there were signs of preparations for swarming the stock was divided, and a nucleus of four frames with the queen was made on the 18th of this month. The original stock now consisted of seven frames of bees with three sealed queen cells and plenty stores.

On 6th July the stock swarmed, and the swarm was returned after the removal of two virgin queens and two queen cells. On the following day the queen was seen upon the frames.

During August and September the stock carried on normally, and at the end of the month there were eight frames of bees, including two of sealed brood. There were abundant stores for winter, and a small surplus (2 sections) had also been yielded.

During this second year bees from the stock were regularly taken and examined with the results shown in the accompanying table.

The following are the results of examinations made from January 1918 onward:—

23.1.18.	3 bees examined.	No stage of the parasite found.
5.2.18.	20 ,, ,,	A very few spores seen.
22.2.18.	11 ,, ,,	Eight contained the parasite.
13.3.18.	9 ,, ,,	All contained spores.
8.4.18.	11 ,, ,,	No stage of the parasite found.
22.4.18.	12 ,, ,,	Three contained spores.
10.5.18.	9 ,, ,,	Do.
21.5.18.	5 ,, ,,	All contained spores.
10.6.18.	5 ,, ,,	No stage of the parasite found.
26.6.18.	9 ,, ,,	Do.
12.7.18.	18 ,, ,, (Pulped: 6 smears examined).	Do.
28.8.18.	13 bees examined.	Do.
18.9.18.	58 ,, ,, (2 examined separately. 10 teased collectively, 4 examinations. 46 ,, ,, 4 ,,).	No stage of the parasite found.

On various occasions larvæ and pupæ were examined for *Nosema*, but we were never able to observe any traces of it in these stages of the bee.

Summary.—Reviewing the foregoing record we note that the stock in the course of two seasons yielded two nuclei and one natural swarm (the second natural swarm was returned). It maintained itself and yielded a small surplus of stores. The stock being an experimental one of necessity was subject to a considerable amount of interference, and further was infected with the parasite *Nosema apis*.

With regard to *Nosema*, it is to be noted that in the first year the infection was noticeably heavy, yet during the breeding season the strength of the stock was not affected sufficiently to prevent natural swarming or wintering in normal numbers. While certain of the bees undoubtedly died under heavy infection with the parasite others were found in full working activity, also heavily infected. In the second year, although the parasite survived in the stock over winter, it was never found in very large numbers; eventually its presence could not be demonstrated after the middle of June, and we are forced to conclude that if the *Nosema* infection has not effectually died out it has fallen to a low, and for the time being at least, non-significant, level. As already indicated no signs of disease, *e.g.* dysentery, crawling, etc., have been associated with the stock during the course of the experiment, and it is doubtful whether the presence of *Nosema* has proved an adverse feature of much practical importance in this instance. In June 1919 the stock was of full strength and appeared to be prospering well.

No. 2. **History of a Stock Naturally Infected.**—A small stock of bees (No. 32) covering less than four frames was obtained in the end of March and placed in a new situation alongside a stock (No 31) harbouring *Nosema apis*. Sample bees were taken and searched for *Nosema* at the date of deposition and the result was negative.

A month afterwards eleven bees were captured on the frames of this stock and examined individually; one bee was found to have *Nosema apis* in the spore stage. Three days later twelve more bees were taken, five of which were found to be infected with *Nosema apis*.

At the end of May the stock was covering ten frames and had a large amount of brood. *Nosema* was still present, and spores could be found in the dead bees in the front of the hive. Of five living bees taken, one was found to be infected. Unfortunately at this date the queen appears to have been injured. She was found dead outside the hive. On examination she was proved free from *Nosema* infection. Queen cells were formed, but no queen was seen on frames up till end of June. On 5th July a frame with brood and queen cells was introduced from No. 31 (Dutch bees). A queen was hatched and was seen laying on 22nd July.

At the end of June a few bees were taken for examination and *Nosema* was not found.

On 6th July five bees were taken and these were also found to be free from *Nosema*. Meantime the stock swarmed.

During July and August the parent colony, which it may be noted was now headed by a queen bred in the adjacent *Nosema* infected stock, increased satisfactorily, and in the beginning of August covered nearly ten frames with abundance of stores. Examinations for *Nosema* were made in August—two bees were tested separately and yielded a negative result, ten were pulped and six smears examined, again the result was negative.

On 18th September the strength of the stock consisted of about six frames of bees with brood upon one and a half; there were sufficient stores for wintering. Throughout the whole period of observation and experiment no signs of disease have ever been noted, and the infection with *Nosema* appears to have disappeared about the time of swarming.

Summary.—Infection with *Nosema* appears to have occurred naturally from an adjacent stock (No. 31, p. 16) within the period of one month, and to have been maintained for two months; subsequent to this date parasites were not found, and it should be noted that in the adjacent stock at this time the same feature occurred. The infection while present was not accompanied by dysentery or any other disease symptoms, and during its presence increase of the stock was maintained. The infection while it lasted could not be regarded as materially affecting the prosperity of the colony. The success of the stock was not absolute, since only a limited amount of surplus stores was obtained, but we cannot believe that the presence of *Nosema* was more than a minor cause of this; even in relatively unfavourable circumstances the parasite failed to spread within the stock.

No. 3. **Natural Infection of a Nucleus.**—In February 1918 a small one frame nucleus of bees was established with a view to experiment. They were tested for the presence of *Nosema*, five bees being examined individually by microscopic search and eighteen by having their chyle stomachs pulped and searched. No trace of the parasite was observed.

Prior to any experimental use of this nucleus, the bees, during a short period in the middle of the day upon 22nd March, obtained access to a hive containing *Nosema* infected frames, left open temporarily by an attendant. The source of the infection was forthwith removed.

On 22nd April eight bees of the nucleus were examined, and all were found to be infected with *Nosema*. Again on 16th May five young bees were tested and three found infected. Again on 11th June seven dead bees from this stock were examined and *Nosema* was found in two. On 5th July three live bees were examined and no *Nosema* found.

Towards the end of May the queen was lost through the introduction of a small lot of bees intended to strengthen the nucleus. She was replaced by a queen surviving from an Isle-of-Wight diseased stock. Notwithstanding the presence of the parasite the nucleus continued to build up, so that in the beginning of August the bees were covering nine frames with brood upon seven. During the season, from March till August, whilst *Nosema* was being demonstrated in the stock, there never were any signs of dysentery nor of Isle-of-Wight disease. This small colony became the subject of another experiment in which there was no further history of *Nosema*.

No. 4. **Failure to infect by Means of Bees from Colonies previously harbouring Nosema Apis.**—On the 18th June a nucleus (No. 14) consisting of four frames of bees, including the queen, was taken from stock No. 31 and a frame of brood and stores from No. 32. These were placed in a newly disinfected and painted hive situated two miles distant. As already described, both parent stocks had a recent history of contamination with *Nosema apis*.

On 2nd July it was ascertained that there were three frames of fully sealed brood and bees covering six frames, but the queen was missing. A frame of brood with one sealed queen cell was introduced from No. 11, a colony with a disease-free history. On the 12th a queen was seen upon the frames, and on the 19th she had begun to lay. At this date fifteen bees were taken and examined for *Nosema*. No parasites were found.

On 2nd August two bees were examined and scattered spores of *Nosema* found in one. No developmental stages were seen.

In the beginning of September there were five frames of brood, mostly sealed. The bees were covering eight frames and there was a good supply of stores. When examined in December they were found clustered on 4½ frames.

It is to be noted that this stock, which originated in two others known immediately prior to its institution to harbour *Nosema apis*, has so far not developed recognisable infection with this parasite. This is in keeping with the concurrent history of the parent stocks, in which the parasite could not be found during the same period. In this experiment an opportunity was afforded the parasite for development in bees of Dutch race and in ordinary British bees from two separate sources.

No. 5. **Nosema infected Bees with Queen having Isle-of-Wight Disease History.**—On 3rd July 1918 a nucleus (No. 13) consisting of about three frames of Italian bees from a stock known to be heavily infected with *Nosema apis* was given a 1917 queen, transferred from a stock definitely affected with Isle-of-Wight disease. The nucleus during the succeeding two months maintained itself only fairly. The strength attained at the beginning of August was about six frames of bees; during August this did not increase. It was kept under close observation until the beginning of September, during which period there were no signs of disease nor of any undue mortality in the neighbourhood of the hive. The queen was young, and her production was not equal to reasonable expectation. It is to be remembered that she survived from an Isle-of-Wight diseased stock.

Nosema apis was looked for on a number of occasions, and was found only upon one of these. So scarce did the parasite appear to be that it would not be safe to attribute the weakness of the stock to this cause alone.

Summary.—Although the original bees forming this nucleus were abstracted from a stock harbouring *Nosema*, this parasite was never found in significant numbers of the bees. There were two kinds of bees present, viz.:—Italian and Blacks, during the period of observation, and neither appeared to be materially affected. Infection never spread beyond the casual stage during the three months of the experiment (to 1st October).

The stock was alive in January 1919. A large sample of bees was examined at this date and *Nosema* was not found.

No. 6. **Appearance of Nosema in an Isolated Stock.**—The following is a continuation of the history of the stock, whose initiation as an experiment is recorded in a previous paper. (*Proc. Roy. Phys. Soc.*, 1916, p. 57.)

On the 20th February 1916 this stock was found reduced in numbers and short of food. There were signs of dysentery about the frames. Bees examined were found to be infected with *Nosema*. Instructions were given for close observations to be made on the stock.

During March the weather was cold, but the stock seemed to be doing fairly well and dysentery was not so marked.

On the 3rd April three boxes of bees from it were sent to Aberdeen. Nine bees were alive on arrival and were kept under a bell jar in a sunny position till the last bee died on the 11th. All the dead bees were examined and found to contain spores in great numbers. The living bees also were found to be infected, and one of those which flew upon release showed a complete infection of the chyle stomach, not a single healthy cell being visible. At the end of the month the stock was reduced to four frames of bees.

On the 7th of May the queen was found dead on the floor of the hive. She was found heavily infected with *Nosema* spores. The stock was very weak in numbers, and samples examined showed an extremely heavy *Nosema* spore infection.

The chief points of note regarding this stock are these. The colony was isolated from all other hive bees; it was presumed to be free from *Nosema* when this took place, although we cannot be certain that this was the case. As already reported (*Op. cit.*, p. 52), it was fed with stores contaminated with Isle-of-Wight diseased bees, and there is no record at any period of its history of Isle-of-Wight disease symptoms. The colony died out three months after the infection with *Nosema* was detected, the bees at the end showing typical appearances in the intestine of heavy *Nosema* infection. Dysentery was also a feature in this case.

In view of the fact that loss of the queen, as we record later, is an occurrence liable to ensue on *Nosema* infection, it is worth recalling that twice during the history of this stock, viz., in the winter 1914-15 and in the summer of 1915, this stock became queenless. Examinations for *Nosema* were not being made during the period within which these queens were lost.

Further Experiences of Nosema.—In addition to the foregoing, we have on frequent occasions discovered the presence of *Nosema* in stocks of bees under circumstances which prevented us from making continuous studies of the stocks themselves. We have found, *e.g.*, that this parasite is probably more common amongst Italian bees; and further, our observations lead us to the view that such bees are largely tolerant of the parasite under ordinary conditions. In this connection there must be borne in mind the significance of such infected bees in Great Britain as nurseries and disseminators of *Nosema*.

The foregoing account of the course of *Nosema* infection in the main presents a picture of *Nosema apis* as a comparatively harmless associate of bees. This is not, however, invariably the case, as is shown in subsequent records, and our experience has suggested that much depends upon other factors of the environment. In a number of cases in which we kept bees in unfavourable conditions or in which the natural circumstances were adverse, *e.g.* as regards weather, we found that dysentery and a significantly high mortality were associated with the presence of *Nosema apis* in the bees. In particular we have over a series of years kept bees in single frame observation hives, and invariably during winter, when these bees were kept at a living room temperature by day and allowed to feed freely—the temperature falling naturally during the night,—dysentery developed, accompanied by a high mortality. In a number of such cases *Nosema* has been found in a high proportion of the bees. We have no experimental evidence in these cases as to how far the presence of *Nosema* in the unfavourable circumstances here recorded is responsible for the dysentery and rapid mortality, but on the other hand it has been clear to us that once dysentery was present in a *Nosema* infected stock, the numbers of actually infected bees were much greater than in those cases where *Nosema* was present, but dysentery was not observable.

Isle-of-Wight Disease and Nosema Disease.

Records of Concurrent History of Isle-of-Wight Disease and Nosema Disease.—We have now to record cases of *Nosema* disease with which there were associated the symptoms ordinarily regarded as diagnostic of Isle-of-Wight disease, or in which *Nosema* appeared to be affecting the stock in an adverse manner.

No. 7. In the summer of 1916 a frame of black bees removed from an experimental stock suffering from Isle-of-Wight disease was placed in a one frame observation hive, supplied with an Italian queen, and removed to another area over 20 miles away. The parent experimental stock had no history of *Nosema* infection, and was believed to be free of this parasite. At the time of the institution of the experiment now being recorded the weather was unfavourable, and the bees both in the parent stock and in the observation hive were exhibiting dysenteric symptoms and crawling when opportunity offered. The result was that the queen ceased laying. With an improvement in the weather both crawling and dysentery diminished, but the number of bees was now very small. A fresh frame of bees from the same source was introduced to the experimental hive, and after temporary confinement of the queen in a cage, she was released, accepted, and began to lay. The dysentery at this period had almost disappeared.

On the 30th July there was sealed brood on both sides of the frame.

On this date a flying bee taken on the alighting board about to enter the hive was found to be infected with *Nosema*. The chyle stomach was heavily parasitised, and free spore clusters were abundant in the colon. Further examinations were repeatedly made of both live and dead bees at this date, but in only one instance—that of a dead bee, which was very full of spores—was the parasite found.

During this period right up to the middle of September there was no further dysentery, but crawling was intermittent to a limited extent. The record continues as under:—

20th September.—This nucleus has sealed stores on top of both sides of frame. It has been fed with syrup. Bees appear quite healthy.

21st September.—Do. do.

29th September.—All well. A good proportion of sealed stores.

October.—The behaviour of this nucleus has been normal all this month. Welsh bees were added and the nucleus maintained until December, when the bees were returned to the parent stock.

There are two features in the foregoing record which appear to us noteworthy. The dysentery which was present was clearly associated with the bees before the appearance of *Nosema*, and occurred at the time when Isle-of-Wight disease was the prominent feature.

The second point is the failure of *Nosema* to become well established, even while the nucleus was in otherwise unfavourable conditions.

No. 8. As recorded in the previous experiment, a frame of bees having a history of *Nosema* infection was added to a stock suffering from Isle-of-Wight disease. This took place in December.

On the 12th of March following, a sample of bees—30 in number—was taken and examined. The result gave no evidence the presence of *Nosema apis*, nor were there any signs of Isle-of- disease. Up till May no signs of any disease were the stock. The following account gives the subsequent

6th of May bees were carrying out dead and sick. able to crawl but not to fly, was found to be heavily *Nosema apis* in both intracellular stages and free

t of this discovery examinations were made as

bees picked up from the ground in front of the hive d to contain spores and intracellular stages. The d and one live bee taken from the top of the frames. t found in it. Three other dead bees taken from ned spores, a fourth did not.

the weather was favourable and the stock was ely well. Two bees were captured on arrival at the ch had the honey sac full, contained an enormous s; the other, which was carrying pollen, showed a infected cells, but spores were not seen.

aken from the grass in front was full of spores.

er arriving on the alighting board was captured. ney sac full, chyle stomach chalky and completely spores everywhere. The colon was practically

bee taken on arrival had the honey sac full, h chalky with some dark contents,—completely parasitized, all the cells seemed full of spores, the colon was practically empty.

On the 28th five live bees were taken leaving the hive, the chyle stomach was whitish in all. Spores were found in four; one showed early stages of the parasite. The stock was working well all day. There were no "crawlers" and no signs of Isle-of-Wight disease.

During June the weather was mostly favourable and the stock increased to nine frames of bees, but stores were not very plentiful. Microsporidiosis appeared to be on the decrease, but foul brood was suspected on the 8th.

In the beginning of July the stock had increased to ten frames and a super was put on.

Microsporidiosis was not marked, although very numerous spores were found in one bee examined. Experiments with this bee yielded results rich in *Nosema* infection. No difficulty was experienced in maintaining bees alive until the spore stage was reached.

The stock, although as noted was not showing any very marked signs of trouble, did not increase, but probably diminished in numbers in succeeding weeks, although the queen continued to lay. On the older frames the presence of sac brood, which was suspected on the 8th, was definitely confirmed and found to have increased in amount.

Throughout the period that *Nosema* was being readily found in a very high proportion of the bees, there were none of the characteristic signs of Isle-of-Wight disease, and no observer of the stock familiar with this condition could possibly have diagnosed it as present. Nothing to suggest it had been seen since the previous October. It was, however, obvious that the combined presence of *Nosema* and sac brood disease was adversely affecting the stock. About the middle of July the first signs of Isle-of-Wight disease made their appearance. There was an inclination in bright sunshine to crowd in fair numbers on the alighting board, and especially to crowd in large numbers on top of the frames rather than to fly when the hive was opened. Also a limited number of bees when opened were found to have the colons congested and the chyle stomach contents purplish in colour. Further, the number of bees leaving and entering the hive in working hours and in suitable weather was relatively small in proportion to the size of the stock, and to those working from adjacent hives even where these stocks were smaller. At this date, 20th July, the stock was markedly short of stores.

In order to combat the sac brood two of the worst frames with bees upon them were removed to a small hive, and two frames of foundation were placed in the middle of the brood nest. Syrup was fed. Eighteen loiterers were picked off the alighting board, and of these only three showed "Isle-of-Wight" disease, intestinal symptoms; the others appeared normal. Two bees examined in detail showed no trace of *Nosema*, and it may be added here that *Nosema*, although repeatedly looked for, was not again found in this stock.

In the beginning of August sac brood was still present and the stock distinctly reduced in numbers. During August in fine weather crawling symptoms were observed, and samples taken for examination showed the usual symptoms in the intestine in this disease. No traces of *Nosema* were found in either the dead bees examined in pulp or in fresh bees taken individually.

Early in September the strength was reduced to five frames and the stores were very low. The lesser wax moth was present in fair numbers. The queen continued to lay, and eggs and brood in all stages were present upon three frames. The hive floor was cleared of debris and dead bees, Betanaphthol candy was supplied, and the frames with bees were sprayed with Bacterol. During the next week the bees diminished in numbers, and latterly there were few crawling symptoms. Two frames of stores from a healthy stock were supplied. With reference to the dead bees cleared from the floor, it may be stated that these were pulped and tested for *Nosema*. Only a very few spores were observed, and indications were that it could not have been present in a widespread form in this stock during these latter weeks.

The stock dwindled during the next six weeks, although the bees were not showing any signs of disease and the queen laid to the last. The bees were feeding well, being supplied with Bacterol treated candy. Towards the end of October the stock became extinct. One hundred bees were pulped and yielded a negative result for *Nosema*. The queen also was examined for *Nosema* and no trace of this parasite was found.

Notes upon the foregoing history.—Although the *Nosema* infection was accompanied by sac brood disease, it seems clear that this parasite was an essential cause of the failure of the stock to build up in a normal manner.

The disappearance of *Nosema* from a stock, such as occurred here, we have noted on other occasions. From a consideration of all the circumstances the re-appearance of Isle-of-Wight disease must be regarded as an entirely new outbreak. Factors of significance are the weakened condition of the stock from the recent presence of *Nosema* and of sac brood diseases, and also the situation in which the stock was placed. It stood in an apiary set apart for the experimental study of Isle-of-Wight disease in immediate proximity to other stocks suffering from this disease.

Altogether the facts indicate that *Nosema* infection may be quite definitely established in the absence of all symptoms we are familiar with in Isle-of-Wight disease. The fact that when such symptoms eventually appeared in the stock *Nosema* was either in a latent condition or absent suggests that these two conditions are not causally related.

No. 9. **Failure of Nosema Infection with subsequent development of Isle-of-Wight Disease.**—On the 10th, and again upon the 15th May 1918, candy infected with *Nosema apis* spores was fed to a healthy stock of Punic bees (No. 12), previously tested for *Nosema*, with negative results. Examinations of bees for *Nosema* infection were made upon various dates with the results recorded below. Upon the 18th May ingested spores were found in one bee out of five examined. Again, upon the 24th May, spores were found in one bee out of five examined, but no developmental stages were observed. On 11th June the parasite in the spore stage only was again found in a single bee. On 13th July, 25th July, 2nd August and 3rd September examinations were made, all of which yielded negative results.

On the 11th June a few crawling bees were observed in front of this stock (No. 12), and from this date onward this continued until September. The numbers were always small, and the increase of the stock was not markedly impaired. At the same time the indications undoubtedly suggested Isle-of-Wight disease. Most of those which crawled were opened and examined, and in no case were the distinctive appearances of advanced *Nosema* infection ever seen. The stock increased from five frames to nine, with brood upon six, at the beginning of September. At the end of this month the hive was fully crowded with bees on ten frames. Syrup was fed to the bees and the stock put up for winter.

On the 17th December 65 bees were subjected to search, a few individually, and others by pulping their chyle stomachs. In no instance was *Nosema apis* observed.

(10 teased collectively,	3 individually—	3 smears.		
13	„	3	„	
39	„	3	„	All negative.)

By the 10th of January the stock had died out, confirming the suspicion that Isle-of-Wight disease was present.

This record illustrates the failure of *Nosema* to establish itself upon a stock of Punic bees after the stock had been fed with infected material in summer, while meantime Isle-of-Wight disease developed. It is noteworthy that even in these unfavourable circumstances *Nosema* did not spread in the stock.

Experiments showing that Isle-of-Wight Disease is Different from Nosema Disease.—With a view to further establishing the separate nature of the condition known as Isle-of-Wight disease and *Nosema* disease, we carried out the following experiments:—

No. 10. A small lot of bees covering less than one frame was taken from a stock showing the symptoms associated with Isle-of-Wight disease and placed on 31st January 1918 in an observation hive which had recently been occupied by a *Nosema* infected lot of bees. The hive was not disinfected nor cleaned. The Isle-of-Wight diseased stock had been carefully examined for *Nosema* infection previously. No stage of the parasite was ever seen, and the stock was therefore held to be free from this parasite. On the 5th February these bees were fed with candy contaminated with *Nosema* spores from the former occupants of the hive, and on the 8th a queen surviving from a stock which had died out with Isle-of-Wight disease was introduced. The bees at introduction were very dull, most of them showing weakness and falling readily off the frame. Dysentery was present also. During the early days of the experiment they became livelier and improved in appearance, and dysentery was much reduced.

On 22nd February seven live bees were taken for examination and six of them were found heavily infected with spores of *Nosema*.

Two dead bees from the alighting board were examined: one was found infected, the other yielded a negative result.

On the 26th February the bees, which were now much reduced in numbers, were placed along with the queen in a small cage. These survived till the 27th. The queen was found to contain *Nosema* spores in very large numbers, and had thus reached this stage of infection within the period or 22 days, assuming that she was not previously infected. The dysentery which was present, and which we believe was due to secondary causes, such as overheating in confinement with resulting excitement, had practically disappeared in the later stages of the experiment.

The foregoing experiment was designed along with another following to show the distinct character of *Nosema* disease and of Isle-of-Wight disease. The Board of Agriculture investigators in their report concluded that Isle-of-Wight disease is caused by *Nosema apis*, and have explained the failure of observers to find *Nosema* in many cases of the disease as due to the extreme virulence of the parasite in killing off the bees while it is still in the trophic stage. This experiment shows that by definitely introducing *Nosema* into an Isle-of-Wight diseased stock in which we had previously failed to find *Nosema*, we without difficulty obtained it within the period of 17 days from the majority of bees present and from the queen in three weeks.

No. 11. Another frame of bees taken from the same stock as that utilised in the previous experiment was placed on the same day in an adjacent observation hive. The two had a common alighting board. The bees were showing marked Isle-of-Wight disease symptoms. They were feeble, many were frequently falling off the frame and very few could fly. During the first ten days the bees under the new conditions improved in appearance and became more active. The mortality was not excessive. The weather on the whole was rather cold.

On the 12th February a queen which had already been the subject of an experiment with Isle-of-Wight diseased bees was introduced to these bees.

On the 22nd February bees from this lot were taken and examined—the first six were all found infected with *Nosema* in the spore stage, notwithstanding, as explained in the previous experiment, the fact that *Nosema* could not be found in bees of this stock prior to the initiation of this experiment. This result is of special interest and the infection is readily understood. The common alighting board, which is really a full sized window sill, in which the two observation stocks were placed had been previously used by a stock suffering from *Nosema* infection, and was further at this time in use by the bees in the previous experiment. As both lots of bees belonged to the same stock originally it is highly probable that mixing occurred, and that bees from the infection experiment entered the adjacent hive. At all events the infection with *Nosema* in both lots was very complete, and subsequent examination showed the majority of the bees to contain *Nosema* spores in large numbers.

In this experiment we have thus been able not only to adduce evidence of the separate nature of these two diseases, but also to superimpose in the same stock a *Nosema* infection upon Isle-of-Wight disease.

On the 25th the number of bees was reduced greatly and the survivors were put up in a small cage temporarily, and subsequently given a frame of Italian bees to institute another experiment.

Means of Dissemination of Nosema Apis.

With a view to ascertaining the degree of readiness with which infected worker bees and queens may communicate *Nosema* to others, the following experiments were carried out:—

Infection by Contact with other Bees.—*Infection of Workers.*—A small number of bees was given *Nosema* infected candy on 13th September 1918. Infection was confirmed by serial examinations day by day until on the 24th there were only seven survivors. At this date these were caged afresh with a number of Italian bees believed by control examination of samples to be free from infection, and the whole lot supplied with fresh uncontaminated candy.

On the 27th the seven black bees still survived; one of these was released and flew. It was captured later and returned to the cage. The black bees died on 30th September, 17 days after infection. On examination this was found to have been of a gross character.

On 9th October four of the Italian bees were dead. Only in one were stray spores found; the others appeared free. One remaining live bee was killed, examined, and found to be definitely infected, both spores and intra-cellular stages being present.

It would thus appear that the Italian bees underwent infection by contact with the black bees, and that this was definitely established within a period of fifteen days.

Infection of Queens.—*Case* 1. A frame with a moderate number of Dutch bees was placed in an observation hive on 14th September 1917, and two days later a queen from a defunct Isle-of-Wight stock was introduced.

On the 25th, and again on 27th, candy heavily contaminated with spores freshly taken from an Italian bee was supplied. The bees were seen feeding upon this. At this time the bees appeared quiet, but there were no signs of dysentery or other trouble, and it was observed that on fine days the bees were more active and flew. They were supplied with ordinary candy and also with syrup, the latter of which was taken only in part.

On 11th October four bees were taken alive and examined, three contained developmental stages of *Nosema*, and the fourth was heavily infected with spores. On the following day a bee found alive in the doorway lying on its back was taken, and it also was found to contain enormous numbers of *Nosema* spores.

As it appeared from these examinations that this small lot was extensively infected, it was decided to sacrifice the queen with a view to determining whether infection in her case had taken place, and at this date, *i.e.*, seventeen days after the first supply of *Nosema* to this lot of bees, she was taken, killed and examined. A thorough search failed to reveal the presence of any stage of this parasite. It is not without interest to recall the fact that this queen was a survivor of a stock which died out from Isle-of-Wight disease.

On the following day nine dead bees were found at the entrance. These were examined, and two were found containing a very large number of spores; the others appeared to be free.

Case 2. On this date a fresh queen (Italian) was introduced. With a view to strengthening this small lot some Punic bees were introduced ten days afterwards. They attacked the queen and balled her. She was removed and caged, but died on the following day. Fluid expressed from her abdomen was found to contain spores of *Nosema.* This queen had been in contact with the *Nosema* infected bees during a period of eleven days only. There is of course the possibility that she contained *Nosema* prior to this period, but it may be mentioned that the bees which accompanied her from Italy in a small cage were all examined and found free from this parasite.

This small lot of bees was subsequently maintained without a queen, and Italian bees were added on the 9th of November. By the 24th almost all the Dutch bees were dead, and those examined from time to time were in every case found infected with *Nosema* in the spore stage. Although never more than a small lot of bees they survived until the first week in January. *Nosema* infected bees were found to the last.

Case 3. On 31st October a queen from a stock previously tested and found negative for *Nosema* was introduced to a nucleus heavily infected with this parasite.

On 3rd December the queen was isolated with a few of the bees for further experiment. She, however, died two days later, and examination revealed the presence of a limited infection of the chyle stomach.

Further Observations.—The following further results have been obtained in the case of queens heading stocks infected with *Nosema apis.* Of twelve queens examined after death, spores of *Nosema* were found in four. Of four queens taken alive and sacrificed, three showed meronts in the chyle stomach epithelium but no spores. No trace of *Nosema* was found in the fourth.

Infection by means of the Queen.—*Case* 1. *History of a Stock.* On 16th May 1917 an Italian queen was given to a frame of Dutch bees in a nucleus hive situated on a roof 45 feet above street level. The nucleus maintained itself well, and the bees were always active, although, as explained below, the numbers did not increase up to expectations. The food was supplemented during July and August with syrup and with candy medicated with Bacterol, as supplied by Messrs. Pascall, yet on 13th September, when twelve bees were taken for examination, eleven were found infected with *Nosema apis.* No other indications of disease were evident. The bees were active and taking in pollen.

Since these bees occupied an isolated position in a thickly populated part of a large town, and as the parent stock from which the Dutch bees initiating the experiment were taken have never shown signs of the presence of *Nosema*, it is highly probable that the Italian queen was the source of infection. Subsequent discoveries as narrated below tend to confirm this view.

Frequent examinations showed that a very high proportion of the bees of this stock were infected with the parasite. Foragers arriving at the hive bearing pollen were found with cells from end to end of the chyle stomach to all appearances completely filled with *Nosema* spores. In other cases the infection was very heavy but partial, some parts of the chyle stomach being comparatively free from spores, but showing other stages. There were no signs of dysentery. The weather during August was extremely wet, but became finer in September, although very windy. Towards the end of September the nucleus covering about two frames was spirited and busy. The queen was laying and pollen being carried in. Owing to the high situation of the hive, the bees had difficulties in reaching it in a high wind, and eventually it became clear that the stock, which all along had been active, was suffering considerable loss from this cause. There had always been a good proportion of brood and young bees, yet the stock did not increase up to expectations. On the 27th, after several days of boisterous, although dry and sunny weather, a search was made in the neighbourhood of the hive for stray bees which might have been presumed to have been blown away and which were unable to return. The hive was situated at the end of a large concreted roof with a southern exposure. The space in the immediate vicinity of the hive has always been found free from bees except immediately below the alighting board, and this was so on the day named. About 14 to 20 yards distant along the west side of the roof in the shelter of a low parapet a considerable number of bees were found; most were alive and crawling slowly on the ground, and on being collected and warmed in a small cage, they nearly all recovered and flew. To our surprise the queen was found dead about 18 yards distant from the hive. Fortunately no question can arise as to the identity of these bees, including the queen, since, as already noted, this was a solitary stock of Italian bees on an exceptionally high elevation in the heart of the city. Further, no bees are known to be within a very considerable radius of the site. Since it was found on the 1st October that there were two queen cells and also two drone cells on the frame, and further, since eggs had been seen on the 25th, the loss of the queen must have been recent.

On examination the queen proved to be extremely heavily infected with *Nosema*. The chyle stomach cells where not ruptured, were seen to be nothing but sacs packed with spores. It is probable that she left the hive voluntarily or was driven from it by the bees on account of her condition. As already stated, there were present in the hive at this time queen cells showing that she had been laying up to practically the time she left the hive. If the conclusion that the queen introduced *Nosema* to this small lot of bees is correct, then it would appear that she herself lived for a period of at least four months in an infected condition.

On the date on which the queen was found twenty-six dead workers were recovered. These were pulped and examined, but no *Nosema* spores were found. On the floor of the hive a number of dead was collected; six gave a negative result as regards *Nosema*, while three were positive, yielding spores in very large numbers.

The remaining bees survived queenless to the middle of December, and repeated examinations were made for *Nosema*. Infection was found in a high percentage of cases right up till the end.

Case 2. History of a Queen. At the beginning of October two frames of bees taken from a healthy stock were given a young Carniolan queen. Within a limited period in the course of a routine examination the bees of this nucleus were discovered to be generally infected with the parasite *Nosema apis*. Control examination of the parent stock gave negative results for *Nosema*, and the previous history of the experimental hive has no record of *Nosema* contamination. Suspicion thus rested on the queen as the source of this infection. With a view to testing this, the queen was introduced at the beginning of November to a stock of Irish bees in another apiary with a clean history as regards disease. The queen of the stock was removed previously.

Five bees from the Irish stock were examined in the middle of December and one was found infected with *Nosema*.

On the 10th of January nine live bees were taken; these on examination yielded a negative result. Eighteen dead bees were taken from the floor of the hive. Their chyle stomachs were pulped and samples taken for examination. Spores of *Nosema* were numerous. The stock was found to be queenless at this date. The dead queen was not found in the hive nor in its vicinity. The stock died out early in February. Practically every bee had *Nosema* in the spore stage.

Possible Infection of Workers with Nosema apis *from cleaning the Queen's body.*—On the 8th October five bees were confined in a cage sterilised by fire with a queen immediately after she had been isolated and fed on contaminated candy. She had been observed to crawl upon it and her wings were seen to touch it. After the bees had been with her for 24 hours she was removed. A control examination was made of companion bees from the same stock and no *Nosema* was found.

The bees were not examined until on the 17th one was found dead. A search was made, but no trace of *Nosema* was observed.

On the 21st another bee had died, and it also yielded a negative result in a *Nosema* search.

On the 25th the three remaining bees were killed and examined. Two were found to contain *Nosema*, one showing spores in fair numbers and the other with meronts and spores in great abundance. The third bee was found to be free.

Conveyance of Nosema through the alimentary canal of the Queen.—Experiment I. On the 18th October, a queen after being isolated for an hour, was fed with candy which we liberally contaminated with spores of *Nosema apis*. She was observed in the course of a period of four hours to repeatedly feed upon this material. At the end of this time she was removed and placed in a small cage along with a small number of bees. She was left with those over night (24 hours). The queen was next transferred to a cage freshly sterilised with fire containing healthy bees from stock No. 6 (tested *Nosema* free) on 19th October.

25th October. At this date all the bees were alive and active.

30th October. On this date all the bees were still alive. One was taken and examined microscopically. *Nosema apis* was found in the chyle stomach in significant numbers—the stages observed were unopened spores and recently developed planonts. Meronts could not be detected.

31st October. One dead bee was found in the cage at this date. Examination showed it to be free of *Nosema*. As a control we took a live bee and examined it. No parasites in any stage could be found in the contents of the stomach, the cells of which were normal in appearance.

5th November. Two bees which died over night were examined, but no trace of *Nosema* infection could be made out. The queen died on the evening of this date, having survived eighteen days after receiving infection. On the following morning she was examined and found to be extensively infected. Her chyle stomach was whitish in appearance from the presence of myriads of spores, which appeared to be present in practically every cell.

The three remaining bees were now examined, one contained spores, but no cellular infection could be traced, and the other two were negative.

Experiment II. On 10th October a queen which had been fed fifteen days previously with *Nosema* infected candy was again caged and supplied with similar material. She was kept under observation until we satisfied ourselves that she fed upon the contaminated material. As she was seen to tread upon it and to rub her wings and body also against it, she was removed and confined with a number of bees for a period of twenty hours with the object of insuring that all infection adhering to the surface of her body would have a chance of being removed.

At the end of the twenty hours the queen was again transferred to a fresh cage sterilized by fire, and into which there had been introduced a small lot of bees; these were taken from a hive regarded, from the result of extended examinations, as *Nosema* free.

On 12th one bee was removed from the cage and its chyle stomach examined. No *Nosema* spores or other stages were observed.

Later in the day the queen was found dead along with seven other bees, leaving only two alive. One of the newly dead bees was examined and was found to be heavily infected with the parasites in the meront stage. The dead queen on examination was found to be heavily infected with parasites in the spore stage. Infection was thus completed within twenty-two days, and possibly within only seven days.

The remaining bees yielded the following result as regards *Nosema* :—

Dead bees :—2 negative.
3 slightly infected.
1 heavily infected.
1 live bee negative.

From the foregoing it would appear that *Nosema* is readily communicable to the bees by an infected queen. It cannot be said with certainty that the death of the queen under the conditions of this experiment was due to infection with the parasite.

Experiment III. On the 9th October a queen was isolated and observed to feed upon candy contaminated with fresh *Nosema* spores. She was then caged with a number of her own bees with a view to their removing any contamination which may have taken place from the surface of her body.

After twenty hours she was again isolated and transferred to a freshly sterilised cage containing clean candy along with a small number of bees taken from a tested *Nosema* free stock.

On the 12th one bee was removed from the cage and the chyle stomach examined. Only one or two free spores were observed. These we assume were ingested forms.

On the 17th a newly dead bee was examined and a few large meronts were seen.

On the 22nd one live bee was taken and on examination found negative as regards *Nosema.*

On the 25th two bees were killed and examined; in one stray spores were seen but no developing stages. In the other the spores were more numerous, but again infected cells were not observed.

On the evening of this date the queen died. She was examined on the morning of the 26th, and a very heavy infection with *Nosema* was found to have been established. Four attendant bees remained, and these were examined with the following results—two contained scattered spores and intracellular stages, and in the other two meronts only were observed.

The infection in these four bees was of a comparatively slight character and in marked contrast to the condition in the queen.

From the result of the foregoing experiments there seems little doubt that an infected queen under normal conditions will probably be as certain an agent of infection as can exist. Further, the mortality amongst *Nosema* infected queens in our experiments has been significantly high.

Altogether, we feel justified in concluding that in any stock harbouring *Nosema,* there is oonsiderable probability that the queen will sooner or later become infected with this parasite. When this happens, in view of the personal hygienic relations existing between queen and attendant workers, she becomes a centre of infection, and we may expect the disease to make rapid progress. In this connection it may, therefore, be pointed out that an obvious remedial measure, where the presence of *Nosema* is known or even suspected, is to effect a change of queen.

Probably, where the circumstances admit of it, the stock might be left queenless for a time. The significance of a queenless period is that it permits of the dying out of infected bees, and thus increases the possibility of final elimination of the parasite from the stock.

General Conclusions.—In a stock infected with *Nosema apis* the behaviour of the bees has in our experience been in striking contrast to that of the members of a colony afflicted with the condition known as "Isle-of-Wight" disease. It is a common experience to find that bees of an infected colony captured entering the hive carrying pollen or nectar are while doing so harbouring very large numbers of the parasite in the spore stage. Loss of flight power has not been found to be a characteristic of *Nosema* infection until the insect is actually dying. In Isle-of-Wight disease it is usual for this symptom to appear a considerable time before death, if the bees are prevented from sacrificing themselves by crawling and subsequent death from exposure. We have not observed *Nosema* infected bees to loiter in large numbers about the doorway nor to gather in clusters on the ground, as Isle-of-Wight crawlers do. We have seen them come out, when *in extremis*, and falling over on the ground, lie upon their backs with feebly moving or trembling legs. Our observations suggest that the course of *Nosema* disease in a stock may be, first, the initial stage in which workers only are infected. At this stage the disease is not recognisable except on microscopic analysis. The rate of progress appears to depend greatly upon concomitant factors and is not infrequently quite slow. *The loss to the stock is to be measured by a shortening of the life of the individual bee and not by a premature cessation of its activities.* In the summer the natural increase resulting from the normal egg laying of a healthy queen may greatly outstrip the loss from disease and its presence may be overlooked. At other seasons reduction of the stock from the presence of this parasite is to be expected. Sometimes the parasite appears to die out or to persist in a low proportion of the bees. Survival of the stock in this manner does not imply immunity against Isle-of-Wight disease, and such stocks have been known to succumb later to this disease. A factor of great importance in determining the course of the malady is the infection of the queen. When this occurs, the infection may be expected to spread steadily within the colony as long as she survives. We have shown how potent a disseminator of *Nosema* an infected queen becomes. *The frequency with which loss or death of the queen occurs in Nosema disease, is in striking contrast to her survival in Isle-of-Wight disease.*

As regards the occurrence of dysentery in *Nosema* infection, we have been unable to obtain evidence that this is a primary symptom. Our infected stocks have usually maintained themselves for prolonged periods during which dysentery was not present. We have explained the conditions under which we have been able experimentally to introduce it, with a resultingly increased mortality.

Our main conclusion from a study of the presence of *Nosema apis* in bee colonies in this country is that this parasite is always a weakening factor, and in the presence of other adverse conditions favourable to the development of dysentery it may become seriously pathogenic to bee stocks. In ordinary circumstances we have not found it to destroy bee colonies in the rapid and virulent manner common in Isle-of-Wight disease, although at the same time we are satisfied that *Nosema* affected stocks do not yield the same amount of stores as healthy colonies.

REFERENCES.

1. Anderson and Rennie. Observations and Experiments bearing on "Isle-of-Wight Disease in Hive Bees." Proc. Roy. Phys. Soc., Edin., 1916, vol. xx.

2. Beuhne, F. R. "The Australian Bee-Keeper." Vol. xvii., 1915—xviii., 1916, 1917.

3. Donhoff and Leuckhart. "Uber die Fadenpilz im Darm der Biene. Bienen-zeitung (Eichstadt)," vol. xiii., No. 6.

4. Graham-Smith, G. S.; Fantham, H. B.; Porter, A.; Bullamore, G. W. Report on the Isle-of-Wight Disease—Supplement to *The Journal of the Board of Agriculture*, vol. xix., No. 2, May 1912.

5. Higgins, H. *Jour. Proc. Linn. Soc.*, vol. iii. (Zool.) 1858.

6. M'Cray and White. U.S. Dept. of Agric. Bulletin No. 671. "The Diagnosis of Bee Diseases by Laboratory Methods."

7. Rennie and Harvey. "Isle-of-Wight Disease in Hive Bees." *The Scottish Journal of Agriculture*, vol. ii., No. 2, 1919.

8. White, G. F. "Destruction of Germs of infectious Bee Disease by heating." U.S Dept. of Agric. Bulletin No. 92.

9. Zander, E. "Handbuch der Bienenkunde II." Stuttgart, 1910.

10. Zander, E. "Tierische Parasiten als Krantheitserreger bei der Bienen-Munchener Bienen-Zeitung, 1909."

[From PARASITOLOGY, Vol. XII, No. 3, 28 September, 1920.]

16

ON THE LIFE HISTORY OF *BUCENTES* (*SIPHONA*) *GENICULATA* (DIPTERA: TACHINIDAE), PARASITE OF *TIPULA PALUDOSA* (DIPTERA) AND OTHER SPECIES[1].

By JOHN RENNIE, D.Sc.

AND

CHRISTINA H. SUTHERLAND, M.A., B.Sc., Carnegie Scholar.

(*From the Laboratory of Parasitology, University of Aberdeen.*)

(With Plate XIV.)

The Dipterous Family, Tachinidae, comprises a very large number of species whose larvae live as parasites within other insects, particularly in their larval forms.

Bucentes (*Siphona*) *geniculata*, one of the Tachinidae whose larvae are considered in the following paper, is a small ordinary looking fly, blackish in colour and showing a somewhat greyish abdomen, with prominent abdominal bristles. The labium is long and slender, sharply geniculated about the middle of its length, and folded like a clasp knife under the head when not in use[2].

In a former paper (1912) one of us has recorded the occurrence of the larva of this species as a parasite in the body cavity of *Tipula* larvae. It is probably not confined to one host species. In one collection of *Tipula* larvae, a proportion of which yielded the parasite, the majority of the survivors, on hatching, proved to be *Tipula oleracea*. In most instances, however, we have found the infected insects to be *T. paludosa*.

Since the original observation in 1912, we have found this larva regularly every year, and consequently regard it as a normal parasite of *Tipula*. Other observers record it from *Mamestra brassicae* and a related species, *Siphona cristata*, is reported by Roubaud (1906) to occur in *Tipula gigantea*.

OCCURRENCE AND DISTRIBUTION OF THE ADULT FLY.

From an examination of records relating to the adult insect, kindly submitted to us by the entomologists in charge at the British Museum and the Royal Scottish Museum, we find the fly is widely distributed throughout

[1] This work has been carried out with the aid of Grants from the Board of Agriculture for Scotland, to whom we desire to express our thanks.

[2] A full description of the structure and habits of the adult fly is reserved for a further paper

Great Britain. It is recorded from Devon, Hereford, Hants, Kent, Radnor, Middlesex, Hampshire and Inverness-shire. We have found it in Northumberland and Aberdeenshire.

The adults of this fly are recorded over the period from May till September. Schiner (1862) writes regarding the genus: "The flies live in dry places especially heaths. They are also found on Umbelliferae and on waste ground are often to be seen in large crowds on *Daucus carota*." He also states that *B. geniculata* visits *Erica vulgaris*. Walker (1853) reports these flies as "very common."

The following list of flowers known to be visited by *B. geniculata* is compiled from Knuth's *Handbook of Flower Pollination* (1909).

Viola lutea.
Stellaria holostea.
Medicago lupulina.
Potentilla sylvestris.
Potentilla sterilis.
Daucus carota.
Hedera helix.
Asperula cynanchica.
Asperula odorata.
Valerianella olitoria.
Succisa pratensis.
Eupatoria cinnabinum.
Pulicaria dysenterica.
Myosotis sylvatica.
Mentha aquatica.
Origanum vulgare.
Hottonia palustris.

METHODS OF INVESTIGATION.

The Tipula larvae, after being washed free of soil, were examined individually and with a little practice the parasitic maggot could be readily recognised beneath the skin of the host larva as an elongated yellowish patch of definite shape.

The proportions found to be infected varied a good deal in different localities and in different years. In one area, viz. Echt, Aberdeenshire, the numbers reached a significantly high figure. Over a period of one month, during which larvae were examined, a progressive decrease in the percentage of infected individuals was noted. This was found to be due to the fact that as the season advanced, the infected larvae were dying off, and pupae of *Bucentes* were becoming increasingly more numerous in the soil. It must therefore be borne in mind that for the first generation, unless examinations are made not later than about February, any estimate of the degree of infection must be below that actually existing. The following data illustrate this:

Larvae collected in Echt and Lower Deeside areas.

Data for 1918.

Lot	Date	Infected larvae	Healthy larvae	Total	Percentage infected
1.	Feb. 20	170	434	604	28·1
2.	" 27	127	243	370	34·3
3.	" 28	164	524	688	23·8
4.	Mar. 1	73	386	459	16·0
5.	" 6	103	442	545	19·0
6.	" 7	182	819	1001	18·2
7.	" 11	56	345	401	13·7
8.	" 16	42	258	300	14·0
9.	" 23	79	221	300	26·3
Totals		996	3672	4668	21·3

Larvae collected in Northumberland.

For purposes of comparison we obtained a lot of larvae from the north of England. These yielded the following result:

Date	Infected larvae	Healthy larvae	Total	Percentage infected
Mar. 1	51	794	845	6·0

In other areas where larvae were collected in fewer numbers, the parasite was also found and all our observations suggest that it is relatively abundant.

During the season 1919, *Tipula* larvae were again collected and further data concerning the occurrence of *Bucentes* parasites were obtained.

Search was made for *Tipula* larvae in fields on the College of Agriculture experimental farm at Craibstone, Aberdeenshire, from the month of December onwards. During December and January only very small numbers of *Tipula* larvae were found and of these none were infected with *Bucentes*. In February and March the numbers found increased slightly and out of 56 larvae, three were found infected, one of these harbouring four parasitic maggots.

From April onwards appreciable numbers were obtained; the data for the season are shown in the table given below:

Larvae collected in Lower Deeside and Lower Donside.

Data for 1919.

Lot	Date	Infected larvae	Healthy larvae	Total	Percentage infected
Craibstone.					
1.	April 7	25	349	374	6·6
2.	" 11	39	237	276	14·1
3.	" 22	56	429	485	11·5
4.	" 25	46	288	334	13·4
5.	May 8	1	155	156	0·6

No infected larvae obtained here during period May 9 to June 20.

LARVAE COLLECTED IN LOWER DEESIDE AND LOWER DONSIDE (*continued*).

Data for 1919.

Lot	Date	Infected larvae	Healthy larvae	Total	Percentage infected
Craibstone.					
6.	June 21	13	66	79	16·45
7.	„ 25	23 (+7 otherwise infected)	30	60	38·6
8.	„ 30	22 (+6 otherwise infected)	49	77	28·5
9.	July 7	6	9	15	40·0
Anguston, Culter.					
10.	April 26	1158	3467	4625	25·0
11.	May 2	107	456	563	19·0
12.	„ 9	50	903	953	5·2
13.	„ 16	3	807	810	0·37
		1562	7245	8807	17·6

As is indicated in the foregoing table no infected larvae were obtained between May 16th and June 21st. This was the case in other areas in the north of Scotland of which several were examined.

The fall to 0·6 per cent. at the beginning of May, and the complete absence of infected larvae in the soil for nearly five weeks, we attribute to the fact that the *Bucentes* maggots had pupated, and thus the *Tipula* larvae then found were such as had escaped infection by the parasite.

Larvae obtained at Craibstone on June 21st showed an appreciable percentage infected by *Bucentes* larvae, and from that date until July 7th, when the oats had grown too high to allow of further search for grubs, infected larvae continued to be obtained in high percentages, as is shown in the table.

NUMBER OF PARASITIC LARVAE PER HOST.

Observations made in 1918 show that one, two, three, and even four parasitic maggots may occur in one host. This was also the case in specimens found in the early months of 1919. A noticeable feature among the larvae found in the later part of 1919, viz. from June 21st onwards, was that there were seldom fewer than two *Bucentes* maggots in each host larva, 5–6 being common, and in one case as many as nine *Bucentes* maggots were found in a single host.

SIZE OF PARASITIC LARVAE FOUND.

Larvae from 1·5 mm. up to 8·5 mm., which represents the maximum size, have been found.

Even within the same host, the larvae may differ in size. For example, two maggots, 5 mm. in length, were found in the same host along with two others of 3 mm. In one case, where nine parasites occurred together, these were all 3 mm. in length. It seems more probable to us that these differences in size in parasites occurring together are due to variations in the rate of growth than that the same host should have been parasitised on two or more separate occasions.

Duration of Larval Period.

First Generation. In the season 1918, *Bucentes* larvae were obtained as early as February 20th. These larvae were on an average about 4 or 5 mm. in length at this date. In 1919, these larvae were first obtained on February 28th; they were from 3 to 5 mm. in length. This suggests that the parasitic larvae found in February are the product of eggs laid the previous autumn, the larvae having hibernated in the host.

Second Generation. From June 21st onwards, large percentages of the collected host larvae were found to be infected with *Bucentes*, whose maggots were of such a size, viz. 1–3 mm., as to lead to the suspicion that the *Tipula* larvae had been recently infected. From these *Bucentes* maggots a second generation of flies was bred which began to emerge on July 25th. An earlier generation of flies was bred out in May and the early part of June and by June 13th all these flies had died. Moreover, from May 16th to June 16th *Tipula* larvae were collected on four different occasions, to the number of 355, and of these none were found to be infected by *Bucentes*. This evidently indicates that all the parasites had pupated, involving the death of the host larvae, so that the *Tipula* larvae collected between these dates were those which had escaped infection by *Bucentes* during the previous autumn. In view of the foregoing, viz. that there is a period of over four weeks during which no infected *Tipula* larvae can be found in the field, that within this period adult flies appear to die off and that subsequently infected larvae again appear, the conclusion seems obvious that a second generation of *B. geniculata* commences about this time.

Bucentes maggots of the second generation were observed on June 16th, when they measured about 3 mm. The earliest date on which mating was observed was June 2nd, and no imagines survived in the observation cage after June 13th. Oviposition, and the infection of *Tipula* larvae in the field, may therefore be assumed to take place approximately within a corresponding number of days. Pupation of the second generation of larvae was observed to begin in the experimental cage about July 8th. That is to say, the maximum extent of the larval period possible was in this case from June 3rd to July 8th, viz. five weeks. This estimate is only an approximate one, as oviposition has not been observed and the period which elapses between oviposition and the hatching of the larva is unknown.

We have been able to find in the literature dealing with this subject only the scantiest references to this species. Nielsen (1918), in a table showing the life cycle of a number of Tachinidae, includes *Bucentes geniculata* in the form reproduced below, wherein P. signifies Pupa, L. = Larva, I. = Imago.

	April—May	June	July	Aug.	Sept.	Oct.	Nov.—April
Bucentes geniculata	P.I.	L.P.I.	I.	I.L.	I.L.	P.	P.

Unfortunately, there is no evidence in the paper regarding the locality to which the data relate, but it would appear that the species may exist in the pupal condition from October to April.

For comparative purposes we append our data, which show that we are familiar with the life cycle of the parasite during the period from February to August, and that our information would indicate that the winter is spent in the larval condition in our area. Both records agree in showing that two generations occur in the year—a short-lived summer and a longer winter generation.

	April—May	June	July	Aug.	Sept.	Oct.—Nov.	Feb.	March	April
Bucentes geniculata	P.I.	I.L.	L.P.I.	I.	?	?	?L.	L.	L.P.

Pupation.

Observations show that usually the maggot leaves the body of its host and pupation takes place outside in the soil. After having emerged from the host larva, the parasitic maggot may still remain attached, as described later, to the tracheal trunk of the host, but usually the separation from the host, preparatory to pupation, is complete. In one instance, however, pupation was observed to have taken place within the host larva—the latter being still alive.

Within twenty-four hours after the maggot leaves its host, pupation is complete.

Period of Pupation.

First generation. In the season 1918, pupation was first observed on April 1st. The earliest date on which *Bucentes* flies emerged was April 23rd, so that approximately pupation extends over a period of three weeks.

In the season 1919 puparia were first observed on April 24th and flies began to emerge on May 14th. In this instance, the duration of pupation was 20 days.

Second generation. From observations made on pupae obtained from larvae of the second generation in the season 1919, we found pupation to extend from July 8th to 25th, *i.e.* over a period of 17 days.

Emergence of Adults.

In 1918 adult *Bucentes* flies were seen in the observation cage as early as April 23rd. Emergence continued until June 4th, after which date all the flies had died off.

In 1919, when the weather was less favourable, no flies were seen until May 14th. By June 13th all the flies had died.

In 1918, emergence continued therefore over a period of about six weeks. The flies which issued in April were rather small, but those which emerged during May were normal in size.

In 1919, emergence of flies continued only for about four weeks. In the case of the second generation which began to appear on July 25th, the period during which emergence took place was about three weeks.

In order to find out from what depths the adult fly could emerge, the following experiments were set up.

Glass cylinders were filled with earth; in one the soil was loose, in another the soil was moderately compact. Puparia were placed at various depths, these being indicated by strips of gummed paper at the same level on the glass cylinder.

It was found that in loose soil the adult fly emerged from puparia placed at a depth of 2 and 3 inches, but did not emerge from puparia placed 6 inches below the surface. In moderately compact soil, the adult fly emerged from puparia placed at a depth of 3 inches, but not from a depth of 6 inches. The puparium cases were afterwards found at the levels at which they were originally placed. It has been noted that the parasitic maggot, after leaving the dead host, is capable of moving about in the soil, and probably it moves upward near the surface before pupating.

Mating.

Mating took place freely in the cage; in 1918 from May 16th to the end of the month; in 1919, from June 2nd till about the 12th.

Mating *Bucentes* were isolated in a test-tube with one or two daisy blossoms and kept under observation. The flies were found to remain *in coitu* over a period of about two hours. Oviposition was not observed, but flies which had mated were found to survive 4–5 days.

During both seasons, 1918 and 1919, pairs of mating flies were isolated along with *Tipula* larvae, and also in view of their recorded occurrence in *Mamestra*, along with various caterpillars, *e.g. Agrotis exclamationis.* No infection took place under these conditions.

Longevity of the Imago.

Experiments were carried out to ascertain longevity of adult *Bucentes*, wherein newly-emerged flies were isolated in glass cylinders. In the bottom of the cylinder was put about 2 inches of earth in which one or two flowering daisy plants were placed to provide food for the flies. Under these conditions the flies survived 5 to 10 days. Under natural conditions they doubtless live longer than 10 days.

The confined flies are strongly attracted to light, they are exceedingly active and run about freely upon the surface of the soil. This was planted with daisies and dandelions, which were much sought, the flies being seen to introduce their proboscides into the daisy florets and to feed on the pollen.

Summary of Life History.

So far as we have been able to trace the life history of *Bucentes geniculata* it may be summarised as follows:

The winter months are spent as larvae within their hosts, viz. *Tipula* larvae. Pupation may start as early as the beginning of April if the season is good but in a late season, pupation may not begin until nearly the end of this month. After a pupal period of about three weeks the imagines emerge during April and May. By the middle of June the adult *Bucentes* are dying off. A second generation appears in June. After a larval period of about three weeks and a pupal period of about seventeen days the adult flies emerge towards the end of July. Since *Tipula* larvae are found in the winter months parasitised with *Bucentes*, infection probably takes place in the autumn whilst the *Tipula* larvae are comparatively young[1].

Structural relations of the Parasitic Larva to its Host.

Dufour (1827) described the larva of the Tachinid *Ocyptera bicolor*, which inhabits the body cavity of an Hemipteron, *Pentatoma grisea* Latr. He observed a membranous funnel-like structure, equal to about one-third of the length of the larva, extending from the last segment of the body and adhering by means of a pair of horny teeth at the other end to a metathoracic stigma of its host. He noted that this "siphon" could be detached from the larva without injuring it and he also found in other cases, "siphons" adherent to the metathoracic stigmata of the *Pentatoma* in the absence of the parasitic larvae.

Kunkel d'Herculais (1879) described a similar structure in the larva of *Gymnosoma rotundatum* Linn., and regarded the siphon as a product of the larva. In a Tachinid larva of *Carabus*, Cholodkowsky (1884) found a similar structure fixed to a trachea and described it as a chitinous funnel. He interpreted this as a pathological chitinous product produced by the hypodermal layer of the trachea at the point where the larva, having entered by a stigma, perforated the tracheal system to reach the body cavity.

Pantel (1898 and 1909), in *Thrixion Halidayanum*, clearly established the true nature of this funnel as an inflammatory reaction on the part of the host, and has more particularly shown how these anatomical relations are established between the host and parasite by a development of the hypodermal cells of the skin or of the trachea, whichever structure was utilised by the larva as a means of bringing its stigmata into direct relation with the external air. The perforation of the host's tissue at the point where attachment eventually takes place is effected primarily by means of the hooklets or spines which surround the stigmatic area of the parasite.

Roubaud (1906) describes the relations between *Siphona cristata* and its host, *Tipula gigantea*, in the following terms:

"A ce stade relativement jeune, chaque parasite est encore complètement inclus dans un kyste fermé, membraneux, fixé au cordon trachéen par une sorte de calice chitineux dont le fond s'ouvre dans la trachée chez les larves

[1] The summary of the life history as given by Nielsen, is quoted on p. 165

plus âgées, en croissance active, le kyste, détruit antérieurement, n'abrite plus que la région postérieure; le calice chitineux devenu plus épais emboîte étroitement l'extrémité postanale du parasite, allongée en un court siphon respiratoire bisegmenté....La structure histologique de ces organes permet en effet d'affirmer leur nature trachéenne. A la base, les cellules hypodermiques sont abondamment développées et en plusieurs couches. La sécrétion chitineuse ne forme plus, par suite, un simple filament spiral, mais une couche continue, épaisse et noire, de chitine: c'est cette région qui constitue proprement le calice. Antérieurement, l'épaisseur de la paroi kystale s'atténue, comme par étirement de la formation précédente, jusqu'à se réduire à une mince couche chitineuse incolore où l'on ne distingue plus que quelques ilots de cellules hypodermiques, les débris de mues s'ajoutent à l'ensemble."

In *Bucentes geniculata* we have found structural relations between host and parasite similar to those cited above (Pl. XIV, figs. 1, 3, 4, 5, 6, 7 and 8).

The larva of *B. geniculata* lives in the body cavity of *Tipula paludosa*. We have not, so far, found it free in this situation, but always attached to one of the main tracheal trunks of its host by means of a chitinous sheath-like structure similar to that described by the observers quoted.

At its junction with the trachea of the host, and for a short distance along its length, this sheath is thicker and of a dark brown colour. Beyond this, it is membranous in appearance and completely encloses the parasite. Usually, however, and more particularly in the older larvae, the head end of the parasite is extruded from the sheath freely into the body cavity of the host.

Recalling Pantel's description and figures of the genesis of the funnel, in which he shows that the hooks at the posterior end of the parasite are utilised to perforate the skin of the host, it seems probable to us that the attachment between parasite and the tracheal system is established in a similar manner in *Bucentes geniculata*. The body spines of the first-stage larva appear well suited for such a purpose. At first the hind end of the parasite is adherent to the host at the place where the inflammatory reaction is developed, and its skin becomes incorporated with the funnel. On moulting taking place, the larva leaves this portion of its cuticle and retracts away, leaving a clear space in this area. The relation thus set up between parasite and host involves the perforation of the host's tracheal system and establishes a common respiratory system for both. Within the sheath there are always to be found the mouth-parts and spiracles of the previous moults. In this way we have obtained from third-stage larvae two sets of moulted mouth-parts and both anterior and posterior spiracles.

Description of Larva.

First-stage Larva. As in almost all Cyclorraphous Diptera, the larva passes through three stages separated by two moults. *The first-stage larvae* obtained measured about 1·5 mm. Thirteen segments could be distinguished. The head, as is usual in Cyclorraphous Diptera, is divided anteriorly by a deep median groove into two lateral lobes each of which bears a rudimentary bell-shaped antenna.

All the segments bear black chitinous hooks with the points directed backward. On the cephalic and prothoracic segments are several series of hooks, but on the remaining segments, these are smaller and fewer in number. Each of the intersegmental grooves of the abdomen is margined by hooks whose points are oppositely directed on each side of the groove.

The larva is metapneustic at this stage, the post-abdominal spiracles being terminal. These communicate with two lateral tracheal trunks.

The mouth-parts differ considerably from those of the later stages. The cephalo-pharyngeal sclerite is strongly chitinised, its posterior margin being deeply embayed. Anteriorly it is prolonged as a slender bar and terminates in a wedge-shaped vertical plate which protrudes from the mouth. Ventro-posteriorly to this terminal wedge, there lies a free sclerite which is probably homologous with the dentate sclerite of the second and third stage larvae (Pl. XIV, fig. 10).

The Second-stage larva is about 3 mm. long (fig. 3). All the segments bear small chitinous hooks, so directed that each intersegmental area is bounded by two sets of hooks, one set pointing backward, the other forward. These hooks are few in number, each segment bearing only one or two rows. Besides these all the segments bear several series of blunt transparent spines.

The prothoracic spiracles (fig. 25) terminate in five or six papillae. The post-abdominal spiracles are borne on two tubercles, each having three clefts surrounded by peritremes. On the outer border of each peritreme lies a white spot, the opening of the perispiracular gland (figs. 18 and 19).

The bucco-pharyngeal apparatus is similar to that of the third-stage larva but only half the size (fig. 11).

Third-stage Larva. The full-grown larva, when ready to pupate, is about 8·5 mm. long and 1·75 mm. in diameter. There are two cephalic segments, the first bearing rudimentary bell-shaped antennae while the second on its ventral surface bears a patch of backward pointing chitinous hooks. Behind the cephalic segments, eleven segments can be distinguished. Of these three are probably thoracic and eight abdominal. On the ventral surface these segments bear two series of similar hooks, one on the anterior margin pointing backwards, the other on the posterior margin pointing forwards. Each intersegmental groove is therefore bounded by two series of hooks pointing in opposite directions. These hooks are of two kinds—small, sharp and chitinous, and large, blunt and transparent. The small chitinous hooks are borne on the second cephalic segment and on the last three abdominal segments where they are directed forward (fig. 4).

The tracheal system consists of two longitudinal trunks, united posteriorly by a commissure, having along their length branching lateral tracheae.

The prothoracic spiracles, which emerge on the anterior margin of the first thoracic segment, are fan-shaped with from six to eight lobes. In some cases the prothoracic spiracles show a different number of lobes in the same larva, *e.g.* one spiracle has seven, the other eight lobes (figs. 20–24).

The post-abdominal spiracles are borne on two tubercles arising from the terminal segment. These tubercles are surrounded by a chitinous hoop which is open and flattened on the inner side. Each tubercle is again bilobed, each secondary lobe opening to the exterior by two slits. Thus within the chitinous hoop there lie four slits. These slits are surrounded by oval peritremes whose internal border is dentate. The peritremes lie so that their axes converge towards the inner border of the collar. The spiracle tubercle shows two white spots, the openings of perispiracular glands (fig. 17).

The spiracles communicate with the tracheal trunks through a "felt-chamber" which is a short tube filled with a spongy chitinous structure.

On the ventral surface of the last abdominal segment lies the anus in the form of a cleft with a protuberance on each side. The posterior end of the larva moves freely in the chitinous funnel, and, as far as has been observed, the post-abdominal spiracles are never closely adpressed to the host trachea but the air from the host trachea is drawn into the chamber formed by the adhering chitinous funnel where it is at the disposal of the larva.

Bucco-Pharyngeal Apparatus.

The complete masticating apparatus of the mature larva of *Bucentes geniculata* consists of a number of paired sclerites, the members of each side articulating with one another to form a united whole (figs. 12 and 13).

Overhanging the oral aperture is a pair of strong curved hooks, the *mandibular sclerites.* Dorso-posteriorly these have a dentate process, while ventro-laterally they bear a blunt wedge-shaped process. A ventral view shows the mandibular sclerites to be united by a cross bar. Each ventro-lateral process is perforated by a minute pore.

Articulating with the posterior border of the mandibular sclerites are the *hypostomal* sclerites which are united ventrally by a transverse bar. Between the mandibular and hypostomal sclerites there lies ventrally a fused dentate sclerite which is perforate.

The posterior extremities of the hypostomal sclerites articulate one on each side with the *cephalo-pharyngeal sclerites,* which have each a slight anterior rectangular prolongation, joining it to the hypostomal sclerite of its side. The cephalo-pharyngeal sclerites are prolonged dorsally, into wing-like processes which are perforate near their outer edges, and ventrally, into a stout somewhat rectangular posterior process which has a curved incision at its extremity.

The cephalo-pharyngeal sclerites articulate ventrally with a broad chitinous plate, the floor of the pharynx, as do also the extremities of the posterior ventral processes.

The Puparium (fig. 14). The puparium is brown in colour, barrel-shaped and with clearly marked segments. Each segment bears a double series of hooks, one series pointing backward, the other forward. The rounded contour of the first segment is broken by the slightly projecting anterior spiracles, which are directed laterally outward. Posteriorly the puparium narrows distinctly into a short tubular portion which terminates in two bifurcated lobes, the post-abdominal spiracles. In the intersegmental space which separates the first and second abdominal segments are a pair of holes situated on the latero-dorsal side.

In a future paper we hope to deal with certain gaps in the foregoing account of the life history and to give a description of the structure and habits of the adult fly.

ACKNOWLEDGMENT.

We desire specially to express our thanks to Dr D. Keilin, of the Quick Laboratory, who has generously placed his extensive knowledge of the literature at our disposal, and also been most helpful with friendly criticism and suggestions. We have further found his recent work on the life-history and larval anatomy of *Melinda cognata* Meigen, parasitic in the snail *Helicella* (1919), of much assistance in determining the morphological details of the various larval stages.

REFERENCES.

Cholodkowsky, N. (1884). Über eine am Tracheensysteme von *Carabus* vorkommende *Tachina*-Art. *Zool. Anzeig.* vii. 316–318.

Dufour, L. (1827). Mémoire pour servir à l'histoire du genre *Ocyptera*. *Ann. d. Sci. Nat.* x. 248.

Keilin, D. (1919). Life history and larval Anatomy of *Melinda cognata* Meig. parasitic in *Helicella virgata*, Da Costa, etc. *Parasitology*, xi. 430.

Knuth, P. *Handbook of Flower Pollination.* English Translation, Oxford. Vol. iii. 1909.

Kunkel d'Herculais (1879). Observations sur les mœurs et métamorphoses du *Gymnosoma rotundatum*, Linn. *Ann. Soc. Ent. Fr.* v. sér., ix. 349.

Meade, R. (1891). Generic characters of *Siphona* genus. *Ent. Month. Mag.* xxvii. 126.

Nielsen, J. C. (1909). Jagttagelser over Entoparasitiske Muscidelarver los Arthropoder. *Entomol. Meddelelser*, 2 R. iv. pp. 28–42.

— (1918). Tachin-Studier. *Vidensk. Medd. fra Dansk naturhist. Foren.* lxix. 247–262.

Pantel, J. (1898). Le *Thrixion Halidayanum* Rond., essai monographique sur les caractères extérieurs, la biologie et l'anatomie d'une larve parasite du groupe de Tachinaires. *La Cellule*, xv. 290.

— (1909). Recherches sur les Diptères à larves entomobies. *La Cellule*, xxvi. 173.

Rennie, J. (1912). Note upon a Tachinid Parasite (*Bucentes geniculata* de Geer) of *Tipula* sp. *Proc. Roy. Phys. Soc. Edin.* xviii. 231.

Roubaud, E. (1906). Biologie larvaire et métamorphoses de *Siphona cristata* F.; adaptation d'une Tachinide à un hôte aquatique diptère; un nouveau cas d'ectoparasitisme interne. *C. R. Acad. Sci.* cxlii. 1348.

Schiner, J. R. (1862). Fauna Austriaca. *Die Fliegen*, Wien, pt i. 520–521.

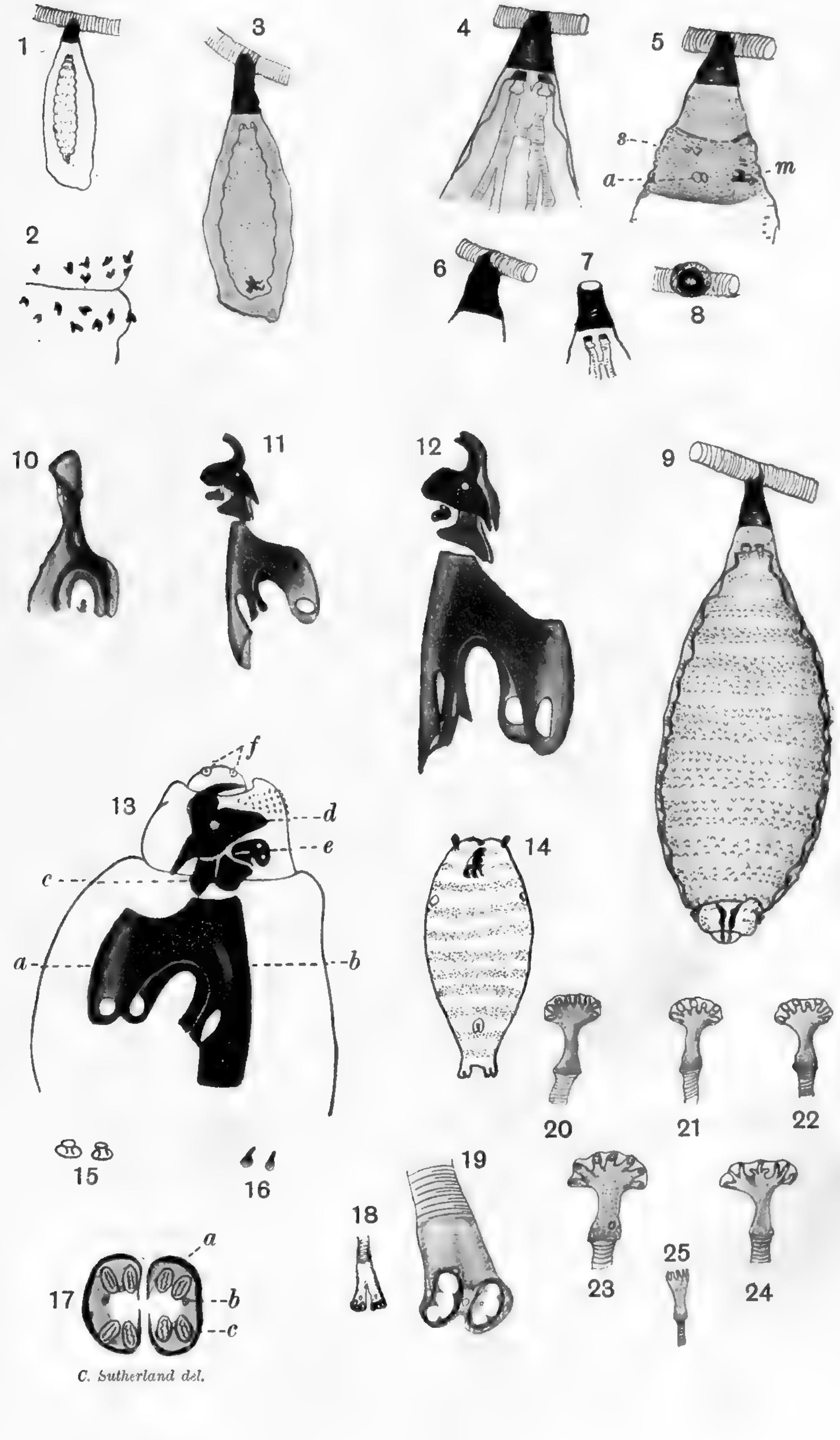
Walker, F. (1853). *Insecta Britannica* ii.

EXPLANATION OF PLATE XIV.

Fig. 1. First-stage larva within the sheath.
Fig. 2. Intersegmental area of first-stage larva showing spines.
Fig. 3. Second-stage larva within the sheath.
Figs. 4, 5, 6, 7 and 8. Different views of attachment of larva of *B. geniculata* to its host; Fig. 5 shows the terminal part of sheath containing moult of second larval stage. The post-abdominal spiracles (*s.*) and buccal apparatus (*m.*) of the second-stage larva can readily be made out; fig. 7 shows the sheath detached from the trachea, and fig. 8 shows the attachment to trachea (end view).
Fig. 9. Third-stage larva showing anterior end protruding from sheath.
Fig. 10. Bucco-pharyngeal apparatus of *B. geniculata*, first-stage larva.
Fig. 11. Bucco-pharyngeal apparatus of second-stage larva.
Fig. 12. Bucco-pharyngeal apparatus of third-stage larva.
Fig. 13. Head and bucco-pharyngeal apparatus of third-stage larva; *a.* dorsal process; *b.* cephalo-pharyngeal sclerite; *c.* hypostomal sclerite; *d.* mandibular sclerite; *e.* dentate sclerite; *f.* rudimentary antennae.
Fig. 14. Puparium.
Fig. 15. Rudimentary antennae.
Fig. 16. Chitinous segmental hooks of third-stage larva.
Fig. 17. Post-abdominal spiracles of third-stage larva; *a.* chitinous hoop; *b.* opening of peri-spiracular gland; *c.* peritreme.
Figs. 18 and 19. Post-abdominal spiracles of second-stage larva, showing three peritremes.
Figs. 20–24. Prothoracic spiracles of third-stage larva.
Fig. 25. Prothoracic spiracle of second-stage larva.

174

174



C. Sutherland del.

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.)

(Read November 1, 1920. MS. received December 4, 1920. Issued separately March 25, 1921.)

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. 190).

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 } Flying bees.
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.	Tarsonemus.	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.

LITERATURE CITED.

Anderson, John (1916), "The Connection of *Nosema apis* and Isle of Wight Disease in Hive Bees," *Proc. Roy. Phys. Soc. Edin.*, xx, p. 16.

Anderson and Rennie (1916), "Observation and Experiments bearing on Isle of Wight Disease in Hive Bees," *Proc. Roy. Phys. Soc. Edin.*, xx, p. 23.

Graham Smith, Fantham, and others (1912), "Report on the Isle of Wight Bee Disease," Supplement 8, *Journ. Board of Agric.*, xix.

—— (1913), "Further Report on the Isle of Wight Disease," Supplement 10, *Journ. Board of Agric.*, xx.

Imms, A. D. (1907), "Report on a Disease in Bees in the Isle of Wight," *Journ. Board of Agric.*, xiv, p. 129.

Malden, W. (1909), "Disease of Bees in the Isle of Wight," *Journ. Board of Agric.*, xv, p. 809.

Rennie and Harvey (1919), (1) "Isle of Wight Disease in Hive Bees," *Journ. Scott. Board of Agric.*, ii, p. 176.

—— (1919), (2) "*Nosema apis* in Hive Bees," *Journ. Scott. Board of Agric.*, ii, p. 511.

White, G. F. (1919), "Nosema Disease," *U.S. Dept. Agric. Bureau of Entomology Bull.* No. 780.

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.

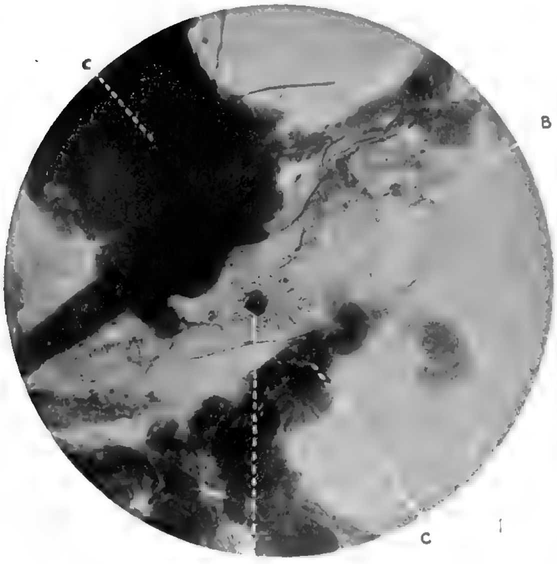
Fig. 5 shows blocking of wider tube with larval stages. $\frac{1}{6}$ in. obj., ocular No. 4.

Fig. 5, ocular No. 4.

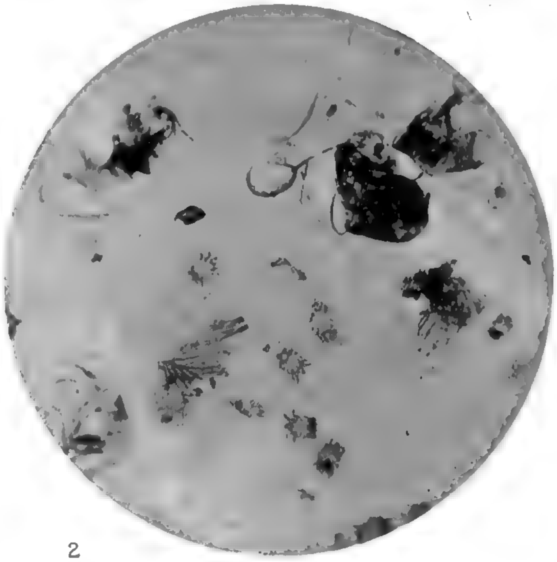
Fig. 6. Section of infested trachea wall. A. not badly affected. Mites seen in section. $\frac{1}{6}$ in. obj., ocular No. 4.

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

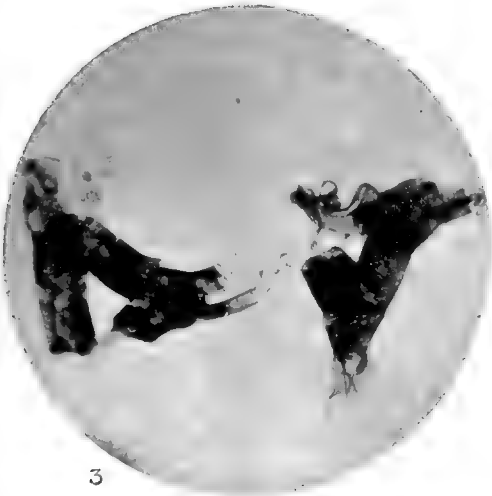
RENNIE, WHITE, AND HARVEY: ETIOLOGY OF ISLE OF WIGHT DISEASE.



1

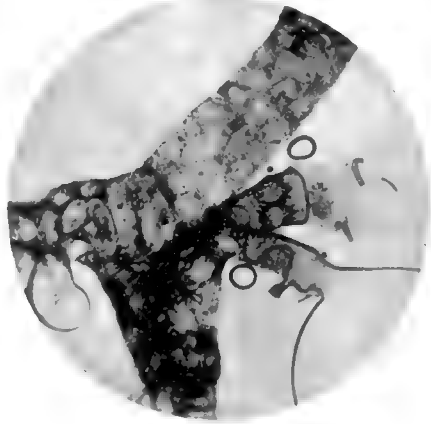


2

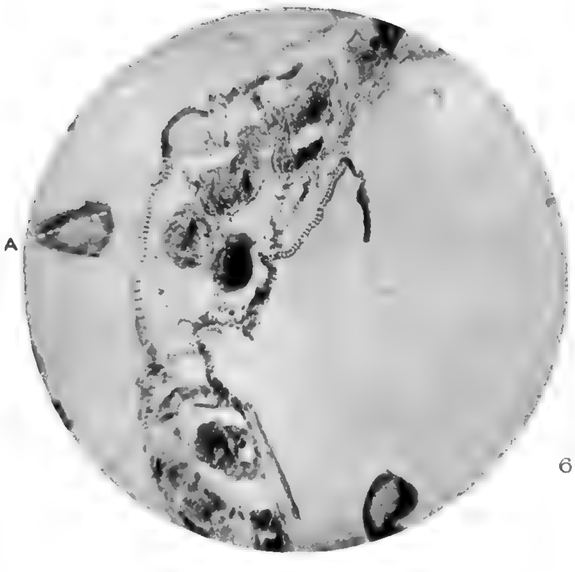


3

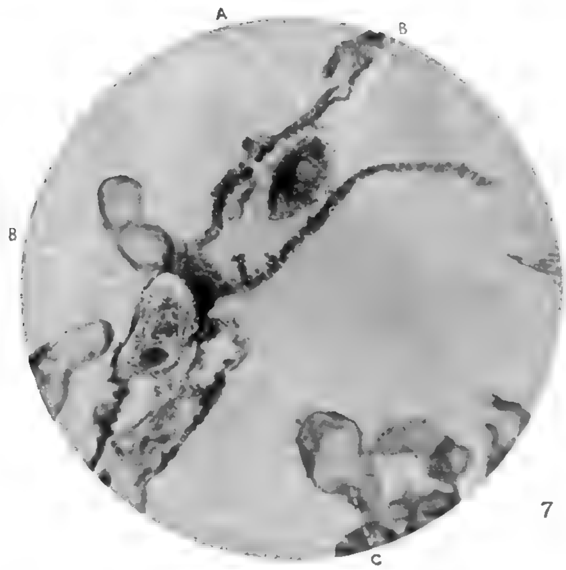
4



5



6



7

ZINCO COLLOTYPE CO., EDINBURGH

XXIX.

(2) The Pathology of Isle of Wight Disease in Hive Bees.

By P. Bruce White, B.Sc., Bacteriologist to the Bee Disease Investigation, University of Aberdeen and N. of Scotland College of Agriculture. *Communicated by* Dr John Rennie. (With One Plate.)

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

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

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

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

The parasitic invasion has two aspects.

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

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

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

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

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

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

It is usually only in the later stages of the attack that the mites attain the smaller tracheæ, the thoracic air-sacs, and the vessels of the head. In such cases, however, the young mites frequently advance as far as the calibre of the vessels permits.

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

Tracheal System.

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

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

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

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

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

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

Careful study of the tracheal wall for perforations reveals little. In two cases only has it been possible to observe the long piercing apparatus of the mite actually passing through punctures in the wall. The material damage done in this way is seemingly small.

Muscular System.

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

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

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

Average width of healthy fibres of muscles of flight = ·24 mm.
,, ,, atrophied ,, ,, ,, = ·12 mm.

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

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

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

A drawing of the low-power appearances of normal and atrophied fibres is shown in fig. 3, and a piece of atrophied fibre is drawn under the high power in fig. 4, while in fig. 5 the substance of a highly atrophied fibre (A) is contrasted with two normal fibrillæ and normal sarcosomes (B).

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

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

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

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

These muscle changes may be summarised as—

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

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

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

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

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

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

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

The Blood.

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

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

The Alimentary System.

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

The facts may be briefly outlined.

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

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

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

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

Malpighian Tubules.

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

Nervous System.

The examination of the nervous system for pathological changes is as yet very incomplete. The observations of the writer have been mainly restricted to the thoracic ganglia. No changes have been noted in the posterior thoracic ganglionic mass, and the few sections which have been examined of the anterior thoracic ganglia of sick and healthy bees show no alterations which cannot be accounted for as physiological variations due to senility.

Discussion.

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

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

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

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

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

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

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

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

The actual number of parasites distributed through the respiratory system is from this point of view of secondary importance, a fact which may explain the apparent vigour of many heavily infected bees.

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

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

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

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

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

From these experiments it may be stated that:—

Through closure of the first spiracle of one side, a condition of crawling is induced which bears a close resemblance in its symptoms to Isle of Wight disease, and that, further, the procedure may occasion atrophic muscle changes which are only known to occur in that disease. When the first pair of spiracles is closed, a state of complete incapacitation results, ending rapidly in death.

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

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

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

The 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.

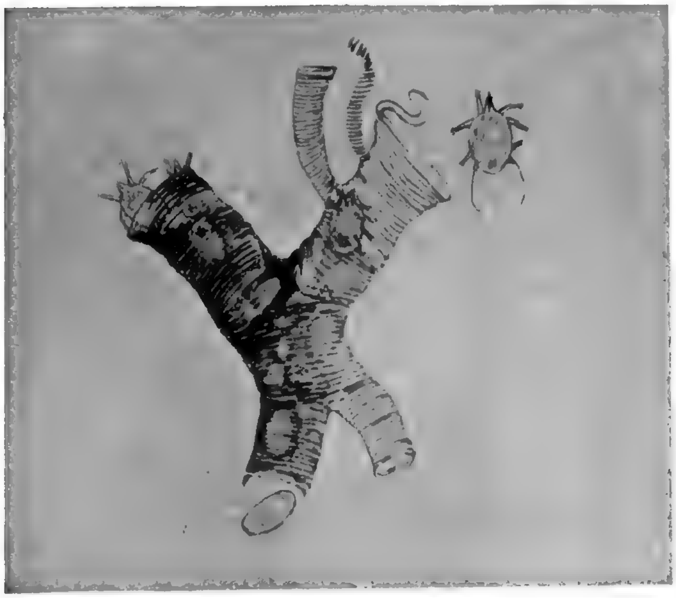
Fig. 3. Normal (*n*) and atrophied (*a*) fibres from the thoracic muscle of a crawling bee. Note the density, slenderness, and fractured ends of the atrophied fibres. (× 50.)

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.

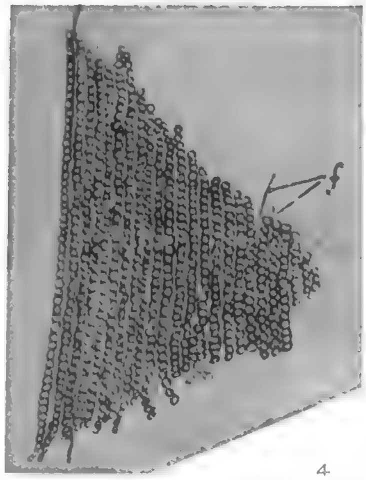
WHITE: PATHOLOGY OF ISLE OF WIGHT DISEASE.



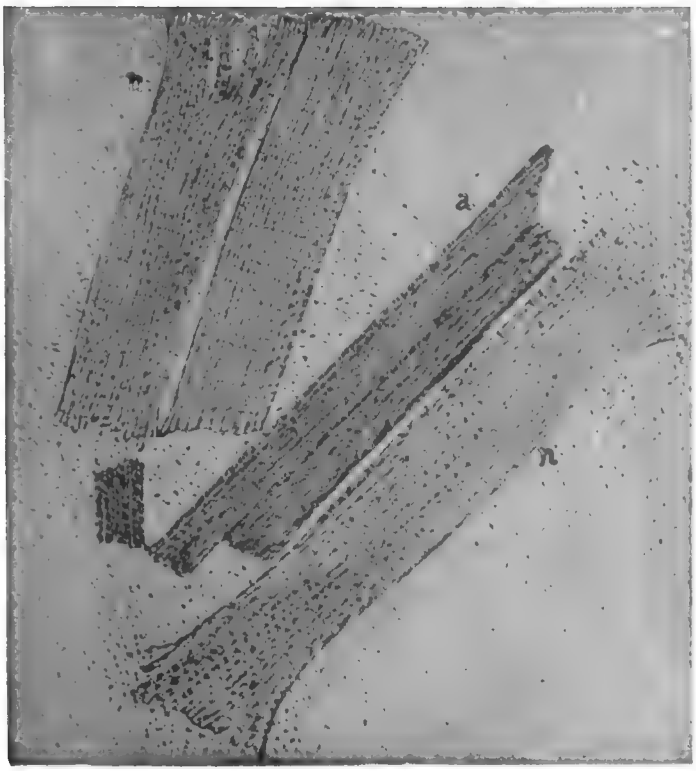
1

2

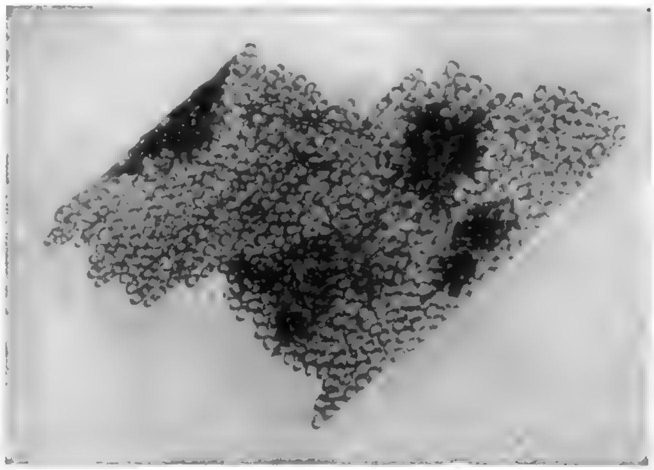
5



4



3



6

Zinco Collotype Co., Edinburgh

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.

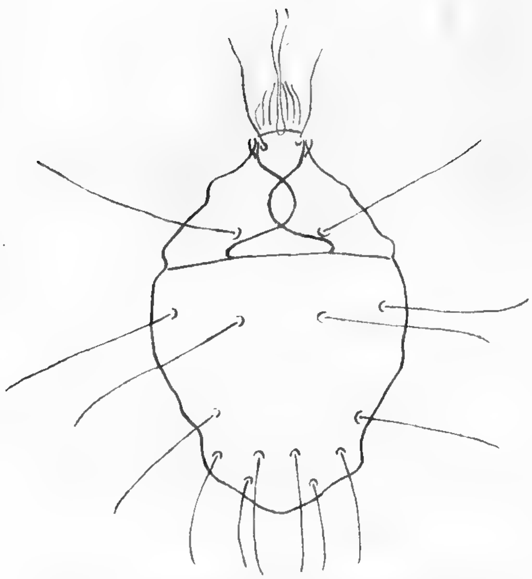
Ovigerous Female.—Seen from above, the body presents a somewhat oval form, broadest in the neighbourhood of the second pair of legs. The following are typical dimensions for a fully grown adult:—

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

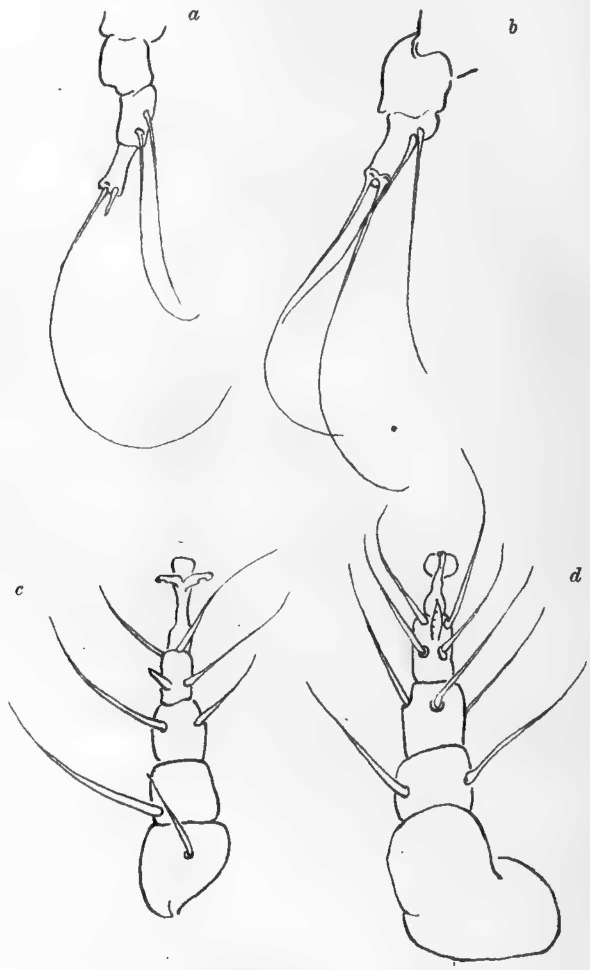
Total length from tip of gnathosoma to tip of longest hair of fourth pair of legs, ·25 mm.

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 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.



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.

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. 191).

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 cœca*, 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.

REFERENCES.

Banks, Nathan (1904), "A Treatise on the Acarina or Mites," *Proc. N. S. Nat. Mus.*, Washington, xxxiii, No. 1382.

Berlese, A. (1897), *Acari, Myriopoda, et Scorpiones huiusque in Italia reperta.*

Blanc et Rollet (1910), "De la présence chez l'Homme de *Tarsonemus hominis* Dahl," *Compt. Rend. de la Soc. de Biol.*, t. lxix, p. 233.

Canestrini, G. (1888), *Prospetto dele Acarofauna Italiana*, Padova.

Dahl, Fr. (1910), "Milben als Erzenger von Zellwucherungen," *Centralb. f. Bakt. u. Parasitenk*, Bd. liii, Orig. p. 524.

Myake and Scriba (1893), "Vorl. Mitth. über ein. neuen Paras. d. Mensch," *Berl. klin. Wochenschr.*, xvi, p. 374.

Oudemans (1906), *Entomologische Berichten*, v. 4, Nos. 91 and 92, p. 316 and p. 331.

Reuter, E. (1910), *Centralb. f. Bakt. u. Parasitenk*, Bd. lvi, Orig. p. 339.

Saul, E. (1913), *Centralb. f. Bakt. u. Parasitenk*, Bd. lxxi, Orig. p. 59.

Trouessart, E. (1892), "Considérations générales sur la classification des Acariens," *Revue Sci. Nat. de l'Ouest*, Paris, p. 20.

Zander, E. (1911), *Krankheiten und Schädlinge der erwachsenen Bienen*, Stuttgart.

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.

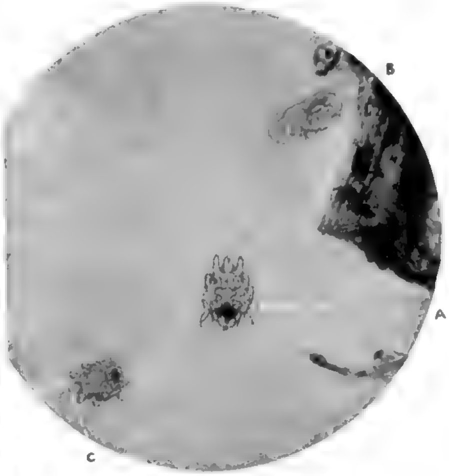
Fig. 7. Posterior end of male, showing last pair of legs and external genitalia. A, spine. B, genital lobes. Obj. $\frac{1}{6}$ in. Ocular No. 4.

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

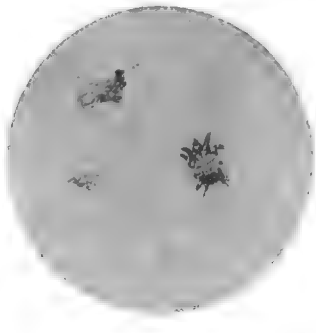
Fig. 9. Female, showing tracheæ. Obj. $\frac{1}{12}$ in. Ocular No. 4.

RENNIE: ORGANISM ASSOCIATED WITH ISLE OF WIGHT DISEASE.

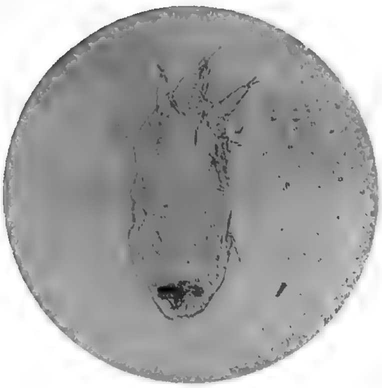
1



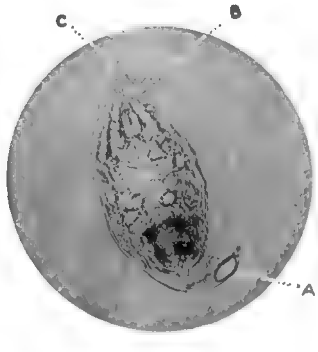
2



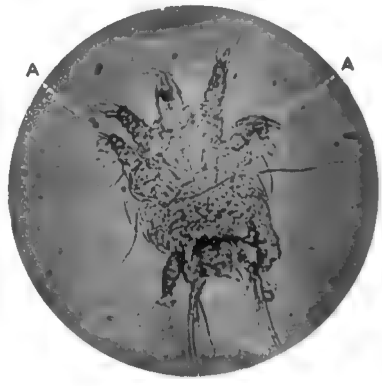
3



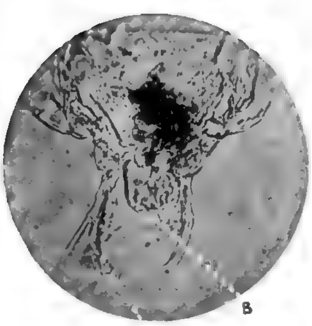
4



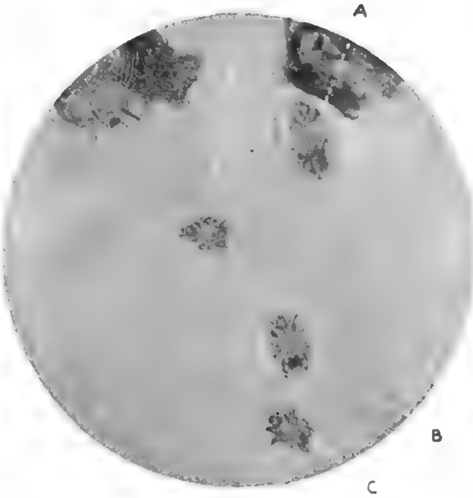
5



6



7



8

9

ZINCO COLLOTYPE CO., EDINBURGH.

ACARINE DISEASE EXPLAINED.

By JOHN RENNIE, *D.Sc.*

I.—INTRODUCTORY.

In Acarine Disease the thoracic breathing tubes of the Hive Bee are infested with mites, which pass through the whole of their growth stages from egg to adult in this situation. The mites are true parasites, members of the Family Tarsonemidae. All types of adult bees are liable to be affected—worker, drone and queen. The mites feed, breathe and breed in the region indicated, and the result of their secure establishment is the eventual disablement of the affected bees. This is primarily due to a continuous loss of blood, on which the mites feed. The degree of blocking of the air tubes, restricting the supply of oxygen and causing a deterioration of tissues directly connected with these, varies considerably. This is a secondary complication. Weakened bees may continue working for a long time, but eventually they become unable to fly and cease to share in the co-operative activity of the colony. They thus become not only useless members, but a burden upon their fellows in the hive. Such bees—popularly termed "crawlers"—while remaining associated with the stock are a continual source of danger to the healthy brood which is successively emerging from the cells.

As indicated above, this is a disease of the adult bee. Practically the only chance of brood becoming infested with *Tarsonemus* lies in the possibility of migrating females wandering upon the larvae within the cells and entering their thoracic spiracles. We have no clear evidence that this ever takes place. Such behaviour is probably not in accordance with the normal instinct of the mite. Experiments in which frames of brood from diseased stocks were transferred to healthy stocks failed to produce the disease. Such brood in healthy surroundings, easily traced, *e.g.*, when brood of an Italian queen is placed in a hive of ordinary black bees or *vice versa*, remains free from *Tarsonemus* infestation.

Classification of Insects and Mites.

The Natural Group, which embraces both the bee and its tracheal parasite, is known to science by the name *Arthropoda*. The term signifies " jointed footed." The group embraces more than half the known species of living animals of the present day. Representative examples are: lobsters, crayfish, crabs, woodlice, water fleas, all insects, centipedes, millipedes, spiders, scorpions, mites, ticks, king crabs. Diverse as these various creatures appear at first sight, they possess certain fundamental characters in common, and the group undoubtedly constitutes a natural branch of the Animal Kingdom. It will be useful to enumerate some of the most obvious features common to Arthropods before distinguishing more particularly the two types we are more immediately interested in, namely Insects and Mites. Besides possessing paired jointed limbs, Arthropods have a cuticular covering to the body, composed of the substance chitin. It is the tough, brownish, flexible substance we are familiar with, covering the exterior of bees, cockroaches, beetles, spiders, etc., or the transparent delicate "skin" upon "water fleas", or mites. A third character is shown in the jointed or segmented body, well marked in such forms as lobsters, crayfish, centipedes, caterpillars, bees; and less clearly in spiders and mites. Arthropods further have a brain which is connected, by a nerve loop enveloping the forepart of the alimentary canal, to a chain of nerve centres running down the under-side of the body beneath the skin in the middle line.

Two important classes within the Arthropod Phylum are the Insecta and the Arachnida, which without forgetting that they have in common the characters enumerated above, we may here conveniently contrast in tabular form.

Insecta.	*Arachnida.*
Possessing Antennae.	Without Antennae.
Compound eyes usual.	Never with compound eyes.
Three pairs of mouth appendages or jaws.	Two pairs of appendages at mouth — not exclusively jaws.

Insecta.	*Arachnida.*
Body clearly divided into three regions—head, thorax and abdomen.	Three divisions never clear in adult. Head and thorax often fused, *e.g.*, spiders.
Three pairs of walking legs.	Four pairs of walking legs.
Breathing by tracheae.	Modes of breathing various—Tracheae, lungbooks, skin.
Examples:—	Examples:—
Cockroaches.	Spiders.
Beetles.	Scorpions.
Bees.	Mites, *e.g.*, *Tarsonemus woodi.*
Flies.	Ticks.
Moths.	

I have endeavoured by the foregoing comparison and contrast to set out the relationship between the bee and its parasite, the mite *Tarsonemus woodi.* It is desirable that this should be understood when we are considering the features of the disease resulting from the invasion of the bee by the latter. It will be understood from the foregoing that as far as increase is concerned, there is no parallel with a bacterial or a protozoal invasion. Only one other point need be emphasised. The female *Tarsonemus* breathes by means of a set of tracheae, which derives its air supply from that within the trachea of the bee. The male mite and larva breathe through the delicate covering of the body.

Acarina or Mites.

In the simple scheme of Classification outlined above I have shown the general structural resemblances and differences between Insects and Mites. In these "Notes" I do not propose to digress from the main theme more than is immediately necessary, consequently it is not my intention to write at length or with any attempt at fulness on the subject of mites in general. They constitute a group of animals which have been studied seriously by only a limited number of workers in the past, and to-day there are surprisingly few acarologists of reliable repute in the whole world. Mites constitute a very numerous Order which includes both terrestial and aquatic forms. The popular impression which regards them all as parasitic is incorrect. A large number are so, but the majority are free living. Many which are found upon the bodies of various kinds of animals are not parasitic but are there temporarily for purposes of transportation only. These generally exhibit special adaptations such as adhesive discs or special claws for clinging purposes. These mites are usually immature stages, and much confusion has arisen in the past from their having been described as distinct species. The Order includes several important types, of which naturally the parasitic forms have called for serious attention from man, *e.g.*, the itch and mange mites of man, domestic animals and birds. The well-known *Dermanyssus* sucks the blood of poultry and cage birds; other forms attack the feathers only. Ticks are Acarina which are exclusively blood-sucking and are important not merely on this account but because they are the only natural agents for the spread of disease such as red water in cattle, malignant jaundice in dogs, etc., and tick fever in man in Central Africa. Some are destructive to vegetation, such as the familiar currant mite and the pear leaf blister mite, whilst gall forming mites are common on wild plants. Certain species, when introduced to houses and other buildings, sometimes multiply in such numbers as to constitute a veritable plague, and there are some forms *related to Tarsonemus* normally feeding upon plant juices which, should they get upon man, cause a highly troublesome itch accompanied by erythema. Within this large Order there are certain very definite differences in the manner of breathing, and the structural arrangements associated with this have been made the basis for the establishment of sub-orders. It is unnecessary to discuss these differences here, but it is desirable that they should be mentioned, since the respiration of *Tarsonemus* is a matter of practical importance and also as the division it belongs to presents a peculiar respiratory feature. Let me give one or two illustrations in classification. The familiar cheese mite and itch mites have no special respiratory tubes and appear to breathe through the soft delicate skin. Mites of this type are classed as *Astigmata*. The familiar *Gamasus* so common on beetles and humble bees, also found from time to time upon the hive bee, possesses breathing tubes whose external openings are placed between the third and fourth pair of legs. Mites of this type are classed as *Mesostigmata*. *Tarsonemus woodi*, the parasite of acarine disease in bees, belongs to a group whose males and young forms resemble the itch mites in not possessing breathing tubes, whilst the females have tracheae with a single pair of openings in the anterior region of the body. This group is known as the *Heterostigmata* on account of this remarkable difference. The term *stigmata* in these Group names refers to the openings of the respiratory tubes on the surface of the body.

Recognition Marks of Tarsonemus woodi.

Under certain circumstances mites of various species may be found in hives, on combs, or upon the bodies of bees themselves. In order to avoid the possible confusion in distinguishing *Tarsonemus woodi* from other mites I give here some simple particulars regarding this species. Taking the commonest of the cheese mites, *Tyroglyphus longior*, as a standard likely to be familiar to most readers, I quote first the actual maximum sizes recorded in comparison with those applicable to *T. woodi*. These are:—

	Cheese Mite, *Tyroglyphus longior*.	Bee Tracheal Parasite, *Tarsonemus woodi*.
	Millimetres.	Millimetres.
Adult Female ...	.75	.19
Adult Male	.65	.15

This shows that T. woodi is, as near as can be stated in simple figures, just one-fourth of the length of an adult cheese mite. These two types of mite resemble each other in possessing a delicately thin skin. Beyond this the comparison cannot be carried much further. In habit the one is free living, and relatively active in its movements, the other is an internal parasite. They differ also in many details of structure. Cheese mites have biting jaws (mandibles), bearing a general resemblance to the great claws of a lobster, with which they devour solid particles of cheese. These structures in the bee parasite are slender, hollow, needle-like parts which serve both to pierce the trachea of the bee and to imbibe its blood. The cheese mites have no special respiratory organs, and in this respect they resemble the male *Tarsonemus*, whilst, as we know, the female *Tarsonemus* possesses a tracheate breathing system.

Without wearying the reader with technical detail which only skilled observers can appreciate, it may be useful all the same to direct the attention of the amateur microscopist, who may be examining *Tarsonemus* for his own pleasure, to the various limbs. He will find that the first pair of legs is terminated in a single claw beneath which is a delicate pad or ambulacrum. The second and third limbs each possess two claws with ambulacrum. The claws are divergent. The last pair of legs in the female terminates in a pair of long sweeping hairs. The male, readily distinguished by its smaller size, will be found to have a short delicate spine and only one long hair at the termination of the last limb. In the adult cheese mites all the legs are terminated each in a single claw. With only one exception, all the colourless mites hitherto found in association with bee hives are of the same sub-order and general aspect as the cheese mites, and it is hoped that with this brief enumeration of distinguishing marks no difficulty will be experienced in recognising these less important and more or less casual associates of the bee.

The Respiratory System of the Bee.

The part of the body of the bee which is inhabited by the parasite of acarine disease is a very restricted region of the respiratory system. The breathing of a bee is effected by means of a series of tree-like branching tubes termed tracheae. These tubes arise originally as paired indimplings of the surface along the sides of the body. In the bee there are ten pairs of these. As development proceeds, the hollow inturned tubes extend further and further within the body, branching in all directions until eventually they form an extensive network in contact with the whole of the tissues. The inner terminal parts of the branches are extremely fine and it is from these that the supply of oxygen to the body is maintained.

These tracheae, in keeping with their origin from the outer layer of the body, are lined with a substance termed chitin. This is strengthened on the inner side by fine, more or less spiral, closely set ridges, which serve to maintain an open passage in these minute channels. In certain regions these tubes are dilated so as to form what are termed air sacs. In consequence of the very direct conveyance of air to all parts of the body by means of this system, the blood is relieved of the function of carrying oxygen and is nutritive merely, not respiratory. Contrary to what takes place in man, inspiration is a passive act, while expiration is active, and is effected by the muscles mainly of the thorax and abdomen.

The paired openings at the surface of the body are known as spiracles or stigmata. In many insects these are guarded by spines or bristles which serve to keep out dust, casual wandering organisms, and true parasites. Whilst in the hive bee protection of the abdominal spiracles is secured by their minute size, the anterior thoracic openings are larger and unfortunately are easily accessible to *Tarsonemus woodi*. Notwithstanding the provision for safeguarding the respiratory system of insects here referred to, there are many cases in this group both of the casual intrusion of stray organisms and of definitely established parasites. Well known examples of this are to be found in the flies of the Family Tachinidae. Rennie and Sutherland (*Parasitology* XII., 1920, pp. 199-211), have lately described such a case of parasitism in the common "leather jacket" (larva of Cranefly). Here, once the parasite insect is within the trachea, there is developed from the invaded tube as a result of the irritation set up, a small blind sac which envelopes the parasite. The latter, in course of time establishes an opening at the blind end, feeds through it upon the host's tissues, whilst utilizing at the other end of its sac or tube the air supply from the host's trachea. Eventually the host insect dies, the parasite pupates in the remains and in due course emerges as an adult fly. Other cases of a similar nature which have been recorded are briefly summarised in the paper referred to.

Whilst such examples of insects parasitic in the breathing system of insects are not uncommon, the case of an Acarine truly parasitic within the insect respiratory system, such as is found in *Tarsonemus woodi*, is the only instance on record. The reported casual occurrences of other mites in the respiratory system of the hive bee are not examples of parasitism and are of no direct importance in relation to acarine disease.

The Thorax of the Bee—the region of occupation by Tarsonemus woodi.

It can be readily seen that in the early stages of development the thorax of a bee is made up of three segments. In the adult body the limits of these divisions are very much obscured, although by careful dissecting, the several parts of the external covering pertaining to each can be distinguished. These three regions are appropriately known as the pro- meso- and meta-thorax. Internally there passes through the thorax on the under-side, the nerve chain upon which occur the ganglia of the thorax, while dorsally the tubular heart passes through the same region. The great mass of tissue within the thorax consists of the muscles for flight and for movements of the wing other than flying. There are three unpaired and two paired air sacs constituting part of the thoracic breathing system, and various tracheal tubes supply those muscles and nerves as well as the structures within the head and limbs.

Our immediate concern in acarine disease is with the thoracic segments and the organs they support. Externally the anterior segment consists ventrally in the adult bee of a number of movable pieces of hard chitin connected by a softer membrane. These ventral portions are readily detachable on removal of the first pair of legs, and if the division is effected by a sharp instrument, it is usual for them to be carried away along with the legs. The dorsal and side pieces are connected to form a slender hoop-like part, not very broad on the back, and tapering ventrally down each side to terminate in a somewhat slender point just behind the insertions of the first pair of legs. An important character of this piece of the bee's outer skeleton is the presence of a small rounded lobe which projects backward from each side and overlaps one of the plates belonging to the meso-thorax.

Concealed beneath this lobe, and the space between it and a side piece known as the episternal plate lies the fateful stigmatic opening, through which *Tarsonemus woodi* finds an entrance to the trachea of the bee. The trachea probably belongs to the prothorax, although its stigma lies in the soft chitin which joins the two regions, pro- and meso-thorax. It is probably useless to speculate as to whether the prothoracic tracheae were first found by the invading mite accidentally or whether the instinct to avoid light and enter crevices charateristic of many such like creatures, directively determined occupation of the bee. Anyhow, this particular stigma is unusual in not being exposed as are the others upon the body. These other stigmata all pertain to the abdomen, and lead almost directly into air sacs. The restricted capacity of the tracheae as compared with that of the air sacs is evidently more favourably to the mite, which although it has a free passage from the tracheae to certain of these in the thorax, yet never breeds in them. It is clear, at all events, that however originated, the occupation of the thoracic tracheae is not now a casual one, since these thoracic tracheae are the only breeding place of this species. *Tarsonemus woodi* is unquestionably a true endo-parasite, and I believe this is the only case of the kind known amongst acarines, although it is more than probable that future investigation will reveal other instances of a similar nature.

The stigmata leading to the tracheae are each provided with a blocking plate, by means of which the bee can at least partially close the tubes. Under what circumstances this function is exercised has not been determined, but as great difficulty has been experienced in endeavours to kill mites within the respiratory system by means of acaricides, it would appear as if where substances are offensive or irritant, the bees endeavour by closure to keep them out.

In contrast to the concealed stigma of the prothorax those of the abdomen are fully exposed on the exterior. The latter are smaller, being only about.07 mm. in their longer dimension and are too minute to permit of the entrance of an adult *Tarsonemus*. These further lead into a very short tube passing directly into large air sacs, and as has been mentioned above, it does not appear that *Tarsonemus woodi* is adapted to live in the larger spaces of these.

II.—THE PROGRESS OF INFESTATION.

(a) Within the Bee.

When the trachea of a bee is invaded by a female mite, egg laying commences very commonly quite close to the stigma. Cases of invasion may be observed in which the only occupants of the trachea are a female, along with an egg or eggs and unhatched larvae. This points to the fact that the immigrant female is already mated. I have observed many instances of infestations at the initial stage, but have never found males alone in such cases. When the conditions under which development is taking place are realised, the difficulty of obtaining exact information on such points as the length of life of a mite, the number of eggs produced by a single female, the total progeny of a single invasion, what determines migration of certain females and non-migration of others, and many other related problems, will be readily appreciated. To most of these questions I have no positive answer, as yet. In a series of examples in which the infestation may be described as definitely established, but in which migration had probably not commenced, I found the mites in all stages to number from 49 to 18. In these cases the adult females were never more than four, nor fewer than three. A crude deduction which may be accepted provisionally from such observations, gives us an average from 5 to 10 offspring from each adult. The following stages occur in the course of the complete life-history, viz: egg, six-legged larva, immature unmated adult, fertile adult of both sexes.

(b) Within the Hive.

From Bee to Bee.

As the mites bred within the bee increase in number and attain maturity, they pass to the outside of the bee, where by suitable methods of examination they may be found creeping amongst or clinging to the hairy covering. So far as we have been able to find out only the adult female migrates in this way. When bees carrying such mites upon their body move about in the cluster—rubbing shoulders, as it were with their healthy fellows—the mites are transferred from bee to bee. When this has happened, the adult females instinctively find their way to one or other of the first pair of thoracic air openings which they enter. Fertilisation has taken place previously in the bee, from which the mite migrated, and on the arrival in the new bee-host, egg-laying commences very usually, but not invariably, near the entrance to the tube. In due course, brood in all stages, from eggs to adults, are to be encountered right along the tracheal system of this part of the body as far as the diameter of the tube will permit the mites to pass. Fully developed mites pair within the bee and after fertilization migration takes place, although it seems to be the case that some females do not migrate, but lay eggs within the bee in which they themselves were bred.

The Queen in Relation to the Disease.

An important factor in the spread of *Tarsonemus* infestation within a hive-bee colony which calls for special mention is the queen.

I have found that nearly 50 per cent. of queens heading *Acarine* infested stocks, are themselves affected with the parasite.

The dangers from an infested queen are two-fold. The queen does not usually succumb to the disease during the life of the colony, and if infested early she may therefore remain a centre of dissemination of the parasite over a prolonged period. Secondly she is in a progressively weakened condition which directly affects both the rate of her production of bees and probably also the stamina of her brood.

The survival of the queen even when affected with the disease is a feature which has puzzled observers. It may be pointed out that the mortality which is associated with infestation results largely from the infested workers leaving the hive, and their being unable to return, owing to weakness and cold. If it were the habit of the queen to leave the hive (apart from her nuptial flight, at which stage she has had little opportunity to become infested) queenlessness would probably be a feature of acarine disease in a proportion of cases. It is probable also that the natural constitutional difference which enables queens, normally, to live very much longer than workers and drones enables them to survive also for a longer period while infested with acarine parasites.

Dead Infested Bees within the Hive.

A very large number of examinations of dead bees infested during life with this particular mite, *Tarsonemus woodi*, have been made, and all the evidence obtained has gone to show that the period of survival of the parasite under such circumstances is comparatively brief. It is usually possible, even upon very old bees which may also have become dry and brittle, to demonstrate an infection by the pathological condition of the remains of the tracheal system, and by the presence of dead mites, but I have not been able to find living examples in such bees. Consequently, I do not think that such bees are likely to be dangerous to healthy bees from the point of view of agents of infection with *Acarine disease*. There is a possibility that the eggs left in the tracheæ of dead mites are capable of prolonged survival, but little probability of their transference into the tracheæ of the living bee.

The whole of the earlier experimental investigations which were carried out with other workers, in which healthy bees were placed in hives previously occupied by infested bees, yielded negative results as regards infestation, and later, I have failed to find living *Tarsonemus woodi* on frames or other parts of hives apart from the living bee. The evidence on the whole points strongly to this disease being entirely a *contagious* one carried by the living bee.

(c) Outside the Hive.

Foragers.

It has been clearly established that bees infested with mites continue for a time to forage. They can fly, gather pollen and nectar, and carry out the usual duties of a mature bee. Whilst they are doing this, *e.g.*, visit-ing flowers, they may be carrying mites upon the exterior of their bodies, some of which are shed upon the blossoms they visit, perhaps to be picked up by other chance visitors to the same flowers.

Whether this means of dissemination is a frequent one is difficult to determine; so far as I know no direct evidence has yet been obtained, and this question remains to be investigated.

Danger from Crawling Bees.

Anxiety on behalf of healthy bees in an apiary is sometimes caused by the knowledge that a stock has crawled extensively, that bees have wandered all around, climbing grasses and flower stems, and getting lost amongst herbage. When the weather is warm, these survive over night, are revived by the heat of the following day, and this may continue for a period. Although it is quite true that Acarine infestation spreads usually in the cluster within the hive, no one can regard lightly the presence of hundreds and sometimes thousands of crawlers in the immediate vicinity of healthy stocks. The presence in the apiary of a few crawler traps which could be used in such an emergency even temporarily would go a long way to diminish this worry. The bees captured should be destroyed.

Robbing Bees.

When a stock weakened by disease or from any other cause is in possession of stores, robbing may be expected to take place.

As a means of infection, there is no question but that robbing plays a very important part. The weak stock may be a diseased one; it very often is so, and robbers attacking such a stock inevitably come into contact with infested bees and doubtless in so doing carry away to their own hives parasites on their bodies. Further, in robbing under such circumstances there is a mingling of bees from different stocks, amongst which there is always the possibility of the presence of a proportion of infested bees, so that even although the weak stock under attack is free from disease, the situation is one in which the danger of dispersal of the parasite amongst many stocks is quite real.

Drones.

An important agent in the spread of acarine disease is the drone. Drones readily become infested with *Tarsonemus woodi*, and as has already been pointed out with regard to workers, may be highly charged with migatory parasites whilst still capable of flight. In their habits of passing from hive to hive, therefore, there are significant possibilities of dissemination of *Tarsonemus* from even one stock over very large areas covering many apiaries. A consideration of this fact shows at once how in summer the disease may become established in a short space of time over a whole district. The problem of drone control, both in the matter of production, and of entrance to hives, assumes a new importance, and it will be evident that intelligent beekeeping generally is more and more essential for the proper control of disease in the adult bee. It is unquestionable that in those areas where the disease is present in summer the control of drones is fundamental for eradication, no matter what other preventive or remedial measures are adopted.

Drifting Bees.

As far as this disease is concerned, there is no question but that everything which tends to isolation of individual stocks favours freedom from disease. Consequently it seems worth while pointing out that the placing of hives of similar appearance in regular rows conduces to the mixing of bees of different stocks by drifting or otherwise. So long as disease is absent from the apiary this is a matter of small importance. But since the beekeeper unfortunately, in the early stages of disease, cannot tell, I would suggest that any arrangement of hives which would reduce the possibilities of the bees mingling is worthy of encouragement.

Sale of Swarms and Trading in Bees generally.

I consider that the chief agency in the dispersal of this parasite in the early days of its invasion has been the development of trading in bees within the British Isles, and particularly the practice of collecting driven bees in autumn from miscellaneous sources and disposing of these throughout the country. In this I do not implicate the more responsible traders of the present day who breed their own bees and exercise close scrutiny upon their apiaries. The sale of swarms by beekeepers, even within restricted areas, where it involves the dispersal of bees whose freedom from infestation has not been established by microscopical examination must also be recognised as dangerous. (See p. 42.)

III.—FACTORS AFFECTING THE COURSE OF THE DISEASE WITHIN A COLONY.

There exists a very general impression that a stock, once affected by Acarine disease is inevitably doomed to extinction. The impression doubtless originated in the fact that until recently it was not possible to recognise the presence of the disease until it had become securely established in the colony. When this stage has been reached, it is quite true, it has rarely been possible to save the stock. Whilst this is so, it is equally noteworthy that the course followed by the disease shows in different cases great variation in its rate of progress as well as definite fluctuations in its incidence upon the stock. It is now also clear that in a proportion of cases, where there is a combination of favourable circumstances, the infection may be shed and the stock become free of the disease.

In the belief that an understanding of the main contributing factors affecting the course of the disease is essential to its successful management, the following effort to make these clear is submitted. It is somewhat surprising that probably most beekeepers have failed to grasp the simple but really fundamentally significant fact that the problem is one which concerns the spread of an infestation in a continuously changing community rather than the progress of a disease in an individual. To make comparisons of this and other bee diseases with human infectious diseases leads only to confused thinking, unless the obvious distinction between the progress of these diseases within a community and their manifestations in suffering individuals is recognised.

There is no doubt that the progress of the disease in the individual bee is a very important feature in the main problem, and this matter is dealt with elsewhere (pp. 8 & 32). But it is not directly proportional to the spread of the disease in the colony, which is governed, as we shall see, by a number of independent factors. The beekeeper is primarily concerned with the increase and survival of the colony and this is controlled by various factors of which the development of broods of mites with the coincident pathology in certain individual bees may not be the dominant one. Whilst these factors are considered separately it will be understood that there is an interacting influence where they are present together.

Multiple Infestation from Outside Sources.

The commencement of infestation in a stock, may be due to the entrance to the colony of a single or of a limited number of infested bees. In such cases the spread of disease within the colony may be very slow or it may fail. On the other hand there may be within a limited time, repeated fresh infestations from outside sources.

Under certain circumstances, of which probably the most dangerous is *robbing*, infestation is conveyed by the robbing bees to their own colony. In this case it is started not from one or from a few centres within the hive, but more likely from hundreds. And this may take place in the course of a single day. Further, it may be repeated over a considerable period of time.

Under such circumstances a stock of bees, although otherwise most favourably situated, may be expected to show within a comparatively short period a well established infestation.

I have quoted robbing as a chief source of multiple infestations. Another will be found in the case where there are already affected stocks in the same apiary or in the immediate neighbourhood. Under such circumstances multiple infestations may be conveyed—apart from robbing—by the other means already enumerated, viz: drones, drifting bees, or casual visitors entering the wrong hive.

Effect of Numbers of Bees.

The larger the community, *i.e.*, the greater the number of worker bees, the longer time will elapse before the presence of the disease will become apparent to the observer or significant to the stock. We do not know how long a period elapses before a single bee which has become infested is in turn capable of communicating the infestation to its neighbours. But from the organisation of the parasite and the relatively small numbers of its progeny as compared with that of either a bacterial organism or Protozoon, we are certain that the process of increase is very much slower, and consequently so also is the rate of spread of the infestation. It is obvious, therefore, that in a crowded hive, some time must elapse before the proportion of infested bees is such that the prosperity of the stock is impaired beyond recovery. And when other favourable factors are present large numbers have an additional value in tending to save the stock.

Importance of a Good Queen.

In a bee population in the working season there is a continuous production of new individuals; young and disease free bees are daily crowding on to the frames and taking up their share of communal life. Normally this birth-rate greatly exceeds the rate of spread of disease with its associated mortality, and thus the incidence of disease in the stock will vary and must for the time being decline. Just to what extent this favourable condition develops depends primarily upon the age and constitution of the queen, whilst the degree to which her reproductive functions are exercised is again affected, amongst other factors by the size of the stock for the time being.

When *Acarine disease* is present in a stock during the working season, theoretically, there are three possibilities as regards increase, viz:—

1. Production may outstrip both the normal mortality and the losses from disease;
2. A balance may be maintained;
3. Production may fail to keep pace with the normal mortality and disease losses.

The results in each case should be obvious. In the first case the colony maintains itself in being. The proportion of incapacitated bees never in the course of the season rises so high as to threaten a breakdown of the social organization. The colony may yield surplus stores. But even in this case the productiveness is adversely affected—just to the extent to which bees have been rendered useless to the colony. At the same time the percentage of infestation may be brought to a low level or it may be extinguished. Such a colony may enter winter upon its own stores, but if infestation persist its incidence will tend to rise, and the colony, if alive in spring, is sure to be weakened in numbers.

In the second case the stock may survive till autumn. A yield of surplus stores is possible, but persistence until spring is improbable.

In the third instance before the summer is over, the colony becomes disorganised from the high proportion of bees in an advanced stage of the disease, and dies out.

These facts show the fundamentally important place of the queen in relation to this disease. Apart from the fact, already demonstrated, that she herself may contract infection and thus constitute a double danger, *viz.*, a source of infection to her bees, and a failure in production owing to progressive weakness, the absolute necessity for a high rate of increase ought to be evident. Consequently the value of timely re-queening in relation to the main honey flow has special significance in relation to disease, assuming that a first class selection both for vigour and fertility is made.

Seasonal Variations in Incidence.

Tarsonemus woodi breeds all the year round, and migrations from bee to bee are also continuous throughout the year.

Very far reaching results follow from this fact. We have already seen that of the circumstances which tend to counteract the spread of Acarine Disease in the working season, the most important is the rapidly changing character, both in individuals and numbers of the summer bee population. In winter, by contrast, we have a population in which normally such changes do not occur. *The winter population is static, not kinematic.*

In relation to this, winter is a distinctively critical period to a stock harbouring *Tarsonemus woodi*, and the circumstances favour a steady spread of the disease.

The result is inevitably unfavourable. Either the stock does not survive the winter, or it does so having a high proportion of infested and weakened bees. While this is so, my observations show that the actual rate of spread is slower in winter than in summer. I account for this by the fact that in winter movements amongst the bees of the cluster are limited and the opportunities of transference of mites from one host to another are correspondingly restricted. The fact that the mortality from Acarine disease is frequently less in winter than in summer is possibly also due to the constitution of the winter bee, whose normal longevity is greater than that of summer. Also, much of the summer mortality is accelerated by the bees leaving the hive as "crawlers."

Racial Vigour.

Since infection with *Tarsonemus woodi* in the majority of cases does not for some time incapacitate the bee, the practical measure of the harmfulness of infection lies in the extent to which it shortens the working period of the bee's life. It will thus be seen that the comparative vigour of different strains and also the standard of health maintained are important elements in enabling the members of a stock to bear the strain imposed upon them by the presence of mites breeding within their bodies and feeding upon their blood. The breeding of robust bees, well adapted in constitution to our climate and its foraging possibilities will pay in relation to this disease as in other aspects of beekeeping.

The term immunity is sometimes heard applied to bees in connection with Acarine Disease. Simply interpreted this term means capacity to withstand infection. It may be as well to point out to those who speak and write regarding races of bees which may or may not be immune to *Acarine disease* that we have no evidence of any kind that there exists a race or races of hive bees in which *Tarsonemus woodi* could not breed. It appears to me that until by controlled mating and artificial selection the scientifically trained bee expert can breed a bee whose thoracic spiracles are too small to admit *Tarsonemus* to the respiratory system—and this is a long way off—it is only confusing to talk about immunity in this connection. The bee experts should rather direct their energies to the production of vigorous and fertile races as the best natural antidote to *acarine* infestation.

It is also a matter to be regretted that the term "carrier" was ever introduced in relation to adult bee disease. No evidence has ever been brought forward to show that bees are capable of transmitting any disease whilst they themselves are free from its normal pathological accompaniments. What has been termed a "carrier" in Acarine disease is simply an infested bee which is nevertheless able to fly and work but which possesses no individual immunity from the disease. Every infested bee is a carrier in this sense in the earlier period of attack, for they all pass through a stage in which the presence of the disease cannot be recognised apart from microscopic examination. This has no reference to relative degrees of resistance which may be characteristic of different races of bees. When stocks having a percentage of such individuals are "carrying on" and even yielding surplus stores, the owner may possibly not appreciate the suggestion that the colony is diseased. He inclines rather to favour the counter proposition that his bees are "carriers" or "immune." While I have already endeavoured to make it clear that the subsequent history of such a colony will be determined by a combination of factors, the collapse of the stock eventually—more or less—may be fairly rapid or it may be long delayed. But experience shows us that the return either in bees or in surplus stores is never so good as it would have been had the stock been parasite free. (See further pp. 43-44.)

IV.—THE "SYMPTOMS" OF ACARINE DISEASE.

Prior to 1920, the presence of this disease in hive bees was recognised mainly by the incapacity of the affected bees for flight, manifested by their crawling upon the ground on leaving the hive. Other associated symptoms, usually present, were also commonly noted, such as retention of fæces and dislocation of wings, but for positive diagnosis, "crawling" in numbers of the adult members of the colony, associated with complete loss of flight power has been the feature relied on as diagnostic of this disease.

As we now know, "crawling" of the type referred to may be induced by more than one cause, and that under the original and now quite properly discarded designation, "Isle of Wight" disease, were included several maladies having analogous superficial symptoms.

In the course of now very extensive examinations of diseased bees from all parts of the country I have found that at least 99 per cent. of the stocks reported to me as failed or failing from what has been popularly termed "Isle of Wight" disease harbour the parasite *Tarsonemus woodi*, and the bees showing advanced stages of infestation are obviously in a diseased condition apart from their functional disability as regards flight. The disease of adult bees in this country which is of paramount importance at the present time is thus the one which is accociated with *Tarsonemus woodi*.

In Acarine Disease there are several features of "crawling" deserving special consideration.

Mass Crawling.

When the infestation has been multiple in origin, e.g., through robbing, one result that may be anticipated is that the disease will reach a critical stage about the same time in large numbers of bees. This will manifest itself eventually, under certain weather conditions, in a more or less sudden and extensive crawling affecting large numbers of bees. The beekeeper who is unfamiliar with the life history of *Tarsonemus woodi* looks upon the onset of "crawling" as the commencement of the disease, and regarding it in the same light as a bacterial infection, seeks the cause amongst the experiences of the stock during the previous week or two. We now know that in the majority of cases he must go back a much longer period of time for the origin of the disease.

In the case of sudden and extensive mass crawling the period is probably shorter than otherwise if we are correct in regarding this crawling as the first, resulting from the orignal infection from the outside. It may not, however, be so. In other cases where this symptom commences in limited numbers and increases gradually, it would appear reasonable to assume that the initial infestation has been slight and that the later increase in the numbers of infested bees is the result of the spread of the parasite within the four walls of the hive—all the offspring of the original intruders. The progress of the disease in this instance is therefore more gradual, and acute or sudden collapse of the stock is not an accompaniment.

Crawling in Association with Swarming.

A particular case of "mass crawling" and one of the commonest experiences of summer in relation to this disease is the appearance of sudden and extensive crawling in a swarm, whilst no such symptom has been recognised in the parent stock. In the course of examinations of affected bees of such swarms my experience has been to find that the degree of infestation in the majority of the crawling bees is an advanced one. Also not infrequently the proportion of crawling bees to the whole swarm is high.

If the stock yielding such a swarm be examined it will be found that it also has a proportion of infested bees although as yet mass crawling may be absent, and possibly even the solitary or stray crawler also.

Hitherto, under such circumstances, it has been usual for the beekeeper to speak of the swarm as contracting the disease before the stock of its origin, and it has sometimes been suggested to me that the source of infestation was under the circumstances almost certainly to be found in the hive in which the swarm had been placed, or on the old combs within it.

The actual facts are as follows: The origin of the swarm infestation and that of the primary stock is one. It may be due to any of the several causes already suggested. Where the proportion of affected bees is very high the infestation may date from the previous autumn or to early summer robbing of weak infested stocks. The older bees constituting the bulk of the swarm are in a more advanced stage of infestation than the younger, who largely make up the remaining colony. But whilst this may explain the earlier onset of crawling, it does not explain its association in mass with the event of swarming.

The mass crawling in this case is really a premature collapse brought about by the excitement of swarming in bees already weakened by disease. Crawling here cannot be associated with a critical position of the mites in the tracheal system both on account of its sudden and general distribution and of the varying degrees and position of the infestation actually present in the bees concerned.

Sometimes the crawling does not take place immediately after swarming, but may be delayed. On the other hand, I have witnessed its onset during the swarming, when the vast majority of the flying swarming bees came to the ground not far from the parent hive and never rose again, whilst others issuing, crawled around the doorway and upon the hive.

Periodicity of Crawling Symptoms in Acarine Disease.

It is a matter of common observation, more particularly in summer, that there are intermittent periods of crawling in *Acarine disease.* This feature is readily understood when we realise that an excessive amount of crawling, such as is seen to occur frequently on very warm days, rids the hive of the great bulk of individuals in the advanced stages of infestation, and in this way lowers the proportion of infested individuals in the bee population. Whilst this is going on new bees are being produced in large numbers, and even although a proportion of them may become infested quite early in their adult life, they continue to fly and work for a considerable period. There is no doubt that this is so. Thus a false impression of recovery from the disease is conveyed. During this period crawling may be virtually absent, and even when it commences again, the proportion may be a low one, because owing to the previous loss of infested bees, the opportunities meantime for infestation have been more limited. But all the time there may be a rising proportion of infested bees.

These facts explain those cases where a stock which has been reduced by crawling losses when strengthened by fresh bees, makes a good, though in most cases only temporary, recovery. But such a recovery may be effected at a critical time by fostering breeding by the queen and providing foraging bees whilst the nectar flow is at its height.

V.—PROFITABLE DISEASED STOCKS AND THEIR PROBLEMS.

A feature of this Disease which naturally perplexes those who have not seriously studied it, is the apparent non-susceptibility, or capacity for recovery of particular stocks. In illustration of this I give here a few examples.

1. In a letter from a correspondent, the following paragraphs occurred:—

"On January 9th I received your report that the bees from one of my stocks had *Tarsonemus woodi*.

"From this stock reported diseased on that date I had a swarm on the 23rd of May, and this swarm swarmed on the 28th of June. I have taken off 150 sections, and have three very strong stocks."

A sample of the stock originally reported on was sent me in the beginning of September and was found free from *Tarsonemus woodi*.

2. Another correspondent wrote:—"My best stock this year was from one found to be infected in April last. From this stock I made two good nuclei, gave combs of brood to other hives, and took off four crates of sections. It now shows no sign of sickness."

3. In another case I was furnished with particulars of an earlier history of acarine infection. According to the bee-keeper, the stock recovered. It yielded him a profitable return in both honey and bees, and with some pride he suggested that it was unlikely that I should find *Tarsonemus* in his bees. All the same *Tarsonemus* was found, and in most of his sample the disease was in an advanced stage.

It is important to remember that in all of these cases diagnosis of the presence of the disease was made in the first instance by the recognition of *Tarsonemus woodi* in the bees, before any external symptoms of disability had developed. Apart from this knowledge the colonies would undoubtedly have been classed as "healthy." These are not to be compared with those cases in which the disease has so permeated the colony that its presence is manifested in crawling symptoms obvious to the onlooker. But, prior to the discovery of *Tarsonemus woodi* as a pathogenic endoparasite of the tracheal system of the bee, this was the only indication. *Only such cases as reached this chronic condition were recognised.* This has given in the minds of the uninitiated an erroneous idea of the malignancy of the infestation.

I have no reason to minimise the gravity of this malady. But we have only to remember the complexity of factors and varying conditions under which the parasite seeks to maintain its existence, to recognise the absurdity of the claim that if this organism is present in a particular colony of bees, it ought to destroy such, and of the argument that if it does not, it cannot be the cause of failure of other colonies in which its presence and pathogenic character are manifest. Competent scientific observers will readily realise that in degree of original infestation, immediate environment, the breed or race of bee, age of queen, size of colony, season, climate, beekeeper, each colony presents to the invading parasite a complex of factors which, in the struggle for existence may be expected in a proportion of cases to prove effective either in its elimination or in the keeping of it at a relatively harmless level.

It must be recognised, therefore, that the presence of a proportion of acarine infected bees in a colony, *so far as that colony is concerned,* is not a sure indication of more or less immediate disaster. It is an unfavourable factor, certainly, but counter to it there may be a number of favourable ones.

VI.—DIAGNOSIS AND GRADING OF ACARINE INFESTATION.

Examination of Bees for Diagnosis.

The following advice is offered to the increasing number of expert beekeepers and microscopists who are now undertaking the examination of bees for Acarine Disease.

I. *The taking of the sample.* For a prognosis regarding any stock of bees suspected of disease, the sample should be as large as possible and consist of live bees taken at random off the frames. Samples which are made up of disabled bees (" crawlers ") alone are not satisfactory for this purpose, since if the disability is due to acarine disease, these may be expected to show a hundred per cent. infestation. Such samples are useful only in indicating the presence or absence of this disease.

II. In the sample taken as directed above, the proportion of clean and infested bees should be determined by examination of the bees individually. The larger the sample the more reliable is the result likely to be. A minimum limit of thirty-three bees is suggested. Examination of duplicate samples at an interval of 10 days will be found valuable.

Tarsonemus woodi, if present, will be found in the tracheae, occupying the anterior region of the thorax. In bees in which the infestation is well established, little difficulty will be experienced in detecting the presence of the mites. Should there be bronzing or blackening of portions of the tracheae this also will aid in diagnosis. The greatest source of error likely to arise in diagnosis will occur in connection with cases regarded as free from the parasite. To avoid error here it is imperative that in particular *the whole of the primary tracheal trunk on both sides of the body from the spiracular opening inward* should be examined. Failure to observe this rule will certainly result in cases of infestation being missed, and too low a percentage will be returned; also stocks which are not clear of the parasite may thereby be regarded as clean. Where mites are being imported into a stock, *e.g.*, by robbers, or in other cases of recent invasion, practically all of the infestations consist of single females and their young brood. They are almost invariably situated in the primary tracheae close to the spiracles. Unless the dissection is properly performed the initial portion of the respiratory tube is very liable to be lost. Such an error may prevent the detection of the disease at its very beginnings, when control measures can be most effective.

III. Not only is it necessary that every case of infestation in the sample should be noted, but it is further imperative that different degrees of this within the stock should be separately estimated. It has been found useful, for example, where a prognosis is aimed at, to recognise three degrees of infestation, viz.:—

(a) Recent. Shown by the healthy condition of the tracheae, the number of mites, which in this case is generally limited—total of all stages 10 or fewer,—and their situation—usually confined to the primary tracheal trunk below the first forking.

(b) Well-established. This is shown by the presence of larger numbers of mites of all stages, the more extensive occupation of the tracheae, which may or may not show bronzing or deterioration. Infestation of both sides of the body may be a feature in a proportion of these cases. Perhaps the best criterion of this stage is the clear evidence of breeding in active progress.

(c) Old infestation. The most obvious feature here is the greatly bronzed or actually blackened condition of the tracheae, with obliteration of their original structure. The number of living mites is noticeably fewer, or these may be absent. Dead mites are not uncommon in such cases, and bees may be found which, having well marked indications of a previous infestation, now show no mites at all. Dead mites, it should be borne in mind, may be found at any of the stages indicated by these three grades.

The relative proportions of each of these stages in a sample, or the preponderance of any one of them, are factors to be taken as a guide in prognosis regarding a stock.

Analytical Considerations,

Detailed analysis of different degrees of mite infestation has made clear to me that the rate of spread in a colony, apart from normal changes in the bee population, is itself uncertain. I have already emphasised this, pp. 20-24. This natural phenomenon directly related to the biology of the parasite and governed by external and internal factors, is not peculiar to Acarine disease but general to epidemics. But it emphasises the extreme importance of the early recognition of the presence of the parasite in the seemingly prosperous stocks. When this is done, success in the management of the colony is dependent on good beekeeping as much as anything. This means that beekeepers must be impressed with the necessity of obtaining satisfactory examination of their stocks and reports on the same.

The object of such an analysis is to provide a fair basis on which to make recommendations. In general it is not difficult to forecast the eventual fate of a stock suffering from acarine disease in those cases where the examination has been made because the stock is showing crawling and other symptoms. But it is quite another matter if the discovery has been made upon an apparently healthy colony previously unsuspected. In illustration, the time of the year may be early summer, the queen may be young, the season favourable, and the incidence of infestation low and the district fairly clear of disease. While it would not be fair to sell such a stock as healthy or clean, it would not be unreasonable on the part of the owner to endeavour to carry on with it, and I would recommend him, under certain conditions, to do so.

The *exact* course to be recommended must depend upon the degree and nature of the infestation, *i.e.*, the total percentage of infested bees, and the proportion of these in the different grades, and the condition of the mites, alive or dead. Affected stocks are frequently preponderantly of one type. For example, stocks showing no external signs of disease and yet infested are usually mainly of the type III*a* in the foregoing classification. Stocks showing a fairly high percentage, 50 or over, mainly in grade III*b*., are to be regarded as of doubtful value, and probably dangerous to other colonies. But here again, such factors as the time of year, the qualities of the queen, the amount of disease in the vicinity, and even the prevalence of drones, must weigh. I have known such stocks in a good year give a satisfactory yield in stores, and come through the subsequent winter, but it is impossible to tell to what extent they have been instrumental in disseminating the parasite amongst other colonies. If we judge stocks from the point of view of their danger to other colonies, such a type as is here referred to, is more to be feared than one with a much higher percentage infestation and an infestation of longer standing. The latter are approaching the stage of definite uselessness and are not usually worth saving, but the infestation appears to be spent, and migration of mites to be more or less absent.

In bees of grade IIIc. to which I am now referring, the tracheal tube may not only be blackened, but thickened or encrusted internally with granular debris. In such cases it is not unusual for the tube to be filled with masses of decomposing material in which bacteria are present. Amongst this, skins or moults of mites may be seen, and frequently no living mites are to be observed.

Such conditions are also sometimes to be noted in bees in which the tracheae are not markedly deteriorated, but in which there are many dead mites, but the group in which this is a more or less common feature is the one above referred to.

Dead Mites within living Bees.

An element of importance which must be recognised and appreciated in endeavours to estimate the characteristics of any infestation, is the presence of dead mites within the tracheae of the bees. There appears to be no doubt whatever that, the mites having performed their natural functions of growth and mating, certain of the females migrate to another bee host in which to exercise the function of reproduction. Others remain to multiply within the original host. Sooner or later after fulfilment of the reproductive faculty, death ensues in both sexes, and there is plenty of evidence to show that in large measure this takes place within the tracheae of the bees. There may be also from diverse causes a certain amount of mortality amongst immature forms as well. Since one of the tests of the gravity of an acarine infestation in the bee is the recognition of the number of living mites, it becomes important that the dead should be distinguished from these. This is a matter of some difficulty and trustworthy conclusions can only be arrived at with care and experience.

The first and most obvious sign of death in the mites is complete immobility. This feature alone is not to be depended on unless proper care is taken in manipulation of the bee, not to crush the mites while these are under examination, and further, that the observation of the mite is maintained for a sufficiently long period, during which no movement is detected. Living mites under a coverslip may remain practically immobile for hours, and unless these are closely watched, slight movements indicating that the mite is alive may be overlooked. A second feature, not universal but very common in mites which have died a natural death, is contraction of the body and limbs. The former becomes rounded and the latter are drawn inward and appear quite short. The body is also shrunken to smaller dimensions.

Mites not long dead usually remain colourless. The body eventually ceases to be granular and assumes a homogeneous, waxy-like appearance, and may have any tint from pale yellow to brown. The colouration is frequently deepest in mites found a long time dead in deeply bronzed parts of the tube. It rarely affects the appendages of the body. The association of browned tubes and bronzed mites is sometimes striking, where the mite has been dead for some time. It is not clear whether this deeper colouration of dead mites is caused by a staining from the contents of the tubes or not, but the association of yellowish or brown mites with a bronzed area of the tube may be frequently noted. In any case, the distinctly yellowish or brown mite is one which has been dead for a longer period than one not so discoloured but showing other signs of death. While this is so, many colourless dead mites may be encountered in these which have been dead for some time.

Vitality of Tarsonemus Woodi.

(a) *Away from the Bee.*—While discussing the question of the recognition of dead mites the following brief summary of experimental results so far obtained concerning the vitality of *Tarsonemus woodi* may be suitably given here. Mites which have been maintained in moist, deep-welled, glass slides at ordinary room temperature cannot usually be stimulated into activity after about twenty-four hours. Rarely, a mite has been found a short time beyond this period, which could move its limbs, but the majority of those maintained under the condition stated died before the end of the period. They have been maintained under observation and periodically moistened up till about seventy hours but no signs of life have ever been obtained during these later stages of experiment. Whilst recording these results I recognise fully the artificial conditions of such experiments. They should be accepted as giving only a general indication of the vital powers of the mite away from the bee. It is probable that mites which leave the tracheae of the bee naturally are capable of surviving longer outside its body. This is suggested in the results referred to in the following paragraph.

(b) *T. woodi in Dead Bees.*—Up to about 24-30 hours after the death of the host bee, living mites of both sexes may be readily encountered, although the majority appear to be dead by this time. While this is so, I have found living *Tarsonemus woodi* within bees which had been dead for five days. In accumulations of dead bees of much longer date I have never found them alive although mites of various other species may be very abundant.

Summary of Examinations.

By way of illustrating the results of examinations of bees made in accordance with the scheme already outlined, I give herewith a summarised statement of a few recent examinations which, while omitting all details noted with reference to the individual bees, sets forth the general results. I give these figures and facts in the hope that readers will apply themselves to the consideration of the question of what course, in their opinion, should be followed in each case with a view to the subsequent successful management of the stocks concerned.

Date.	Stock Number.	Bees taken from Combs.					Percentage of total Infection in sample.
1922		Total Number Examd.	Clean.	Infested.			
				III a	III b	III c	
Feb. 16	1	18	3	3	11	1	83
„ 20	2	33	1	2	30	0	97
„ 24	3	50	21	14	14	1	58
Mar. 1	4	33	27	4	1	0	15
„ 3	5	26	25	1	0	0	4
„ 6	6	33	27	5	1	0	18
„ 11	7	33	6	11	16	0	81

NOTES.—No. 1. Treatment Experiment, breeding noticeably marked.

No. 2. Mostly advanced stages of infestation, the proportion of dead mites not high, practically all the bees incapacitated, breeding in active progress.

No. 3. Stock under experimental treatment, bronzing of tubes marked in a few cases, many dead mites.

No. 4. Nearly all the mites dead, tracheae clean except in one case of slight bronzing.

No. 5. Infection here consisted of a single adult female mite and one ovum. A stock which has given negative results every month since previous September.

No. 6. A stock which has been untreated since November, bronzing of tube commenced, a few dead mites found.

VII.—DISSECTION ROUTINE FOR ACARINE INFESTATION.

Various enquirers have consulted me as to the best methods of examining bees for the accurate demonstration of Acarine infestation. These rightly desire not merely to break up the thorax of the bee and to trust to finding mites amongst the teased tissues, but to effect such a dissection as will demonstrate the extent of the infestation and the degree of deterioration of the tracheal tubes. In view of the desirability of having as many properly qualified experts throughout the country as possible, I have pleasure in offering the following assistance. The method described has been found perfectly satisfactory and easy of performance after a little practice. I need hardly add that students who have had some previous practice in minute dissection will have no difficulty in carrying out the operations and may modify the details to their own satisfaction as their experience increases.

(a) Requisites.

(1) Any ordinary type of compound microscope, having $\frac{2}{3}$-in. and $\frac{1}{6}$-in. objectives.

(2) A supply of glass microscope slides and No. 2 coverslips.

(3) A good pipette, with rubber bulb of good quality, similar in size to an ordinary fountain pen filler.

(4) One or two needles securely mounted on handles. These can be obtained both with smooth and with cutting edges. Both kinds will be found useful.

(5) One or more fine scalpels with narrow or arrow headed two edged blades. These must be kept sharp and clean.

(6) An ordinary forceps such as is used in dissecting.

(7) A hand lens, or better, for those whose eyesight is not very good, a set of magnifying binoculars.

(8) A small clean bottle containing water. One with neck just sufficiently wide to admit the pipette is best.

(9) A similar bottle containing weak glycerine will

be found useful, when it is desired to retain temporarily any preparation of particular interest.

(10) A clean duster, of some soft fabric.

(11) A few narrow strips of clean blotting paper.

(b) Preliminary Considerations.

The following directions are given with the view of providing preparations which will be suitable for furnishing information on the points named, *i.e.*, (a) The presence or absence of mites, both living and dead, and approximately their numerical proportions; (b) the type of infestation, *i.e.*, unilateral or bilateral; the degree, whether initial, actively increasing and showing all development stages, or in final stages; (c) the condition of the tracheae, to what extent pathologically affected.

Conclusions regarding these points can be safely drawn only if the preparation show all parts of the thoracic tracheae liable to be occupied by *Tarsonemus*.

For the purpose of differentiating between living and dead mites an anæsthetic cannot be used in killing the bees. This is best done by firmly compressing the head by means of a stout forceps. A few bees should be treated in this way before commencing operations, and a short interval of time allowed to elapse to permit the reflex movements of the limbs, etc., to cease.

(c) Dissection Routine.

After adjusting the microscope for examination with the low power (⅔-in.), by means of the pipette place a small drop of water in the middle of a clean slide and on this place the bee to be examined, ventral side uppermost. Move the bee to the edge of the drop of water so that the wings adhere to the slide. This helps to steady the bee during dissection.

Next, with a needle held in the left hand press firmly on the middle line of the thorax somewhere between the second and third pairs of legs. Avoid directing the needle too far forward. This operation should hold the bee steady.

Holding the scalpel in the right hand, pass its point through the soft chitin behind the first pair of legs, working right through, and gently drawing the fore part of the body away from the rest. It will be found that this operation removes the first pair of legs, the ventral plates of the prothorax, and the head. The honey sac may be drawn along with it, or it may be severed. These parts should be drawn to a corner of the slide and left there until the preparation is completed.

If the operation here described is properly performed it will be found that in the fore part of the thorax there are now exposed the muscles of flight, pale pink or whitish in colour, occupying the main part of the thoracic cavity. Towards the side of these lie the thoracic tracheae which may or may not be visible at this stage. In badly infested bees, whose tracheae are more or less bronzed, they can usually be readily observed.

Continuing the dissection, the operator still holds the bee in position by means of the needle held in the left hand. The scalpel is now inserted behind the narrow chitinous bands which form the ventral termination of the protergum. These form the anterior border of the exposed surface and lie in a little depression in front of the second pair of legs. They separate easily from the adjacent chitin. The scalpel is now worked along both sides towards the dorsal region, breaking through the chitin of the meso-thorax on each side where this lies adjacent to the backwardly directed lateral lobe of the pro-thorax which conceals the spiracle. The parts thus separated along with the soft tissues enclosed by them should now be drawn apart from the remainder of the bee. If the operation has been cleanly performed, they will contain the whole of the pro-thoracic tracheal system which is liable to be infested with the mites.

Until more familiar with the various tissues of the bee, beginners will find it best in their preliminary examinations to proceed as follows: Spread well with a pair of needles the soft parts thus separated out in a little water on the slide. Gently lower a coverslip on the top and wipe up any excess of water by means of a strip of blotting paper. The remaining parts of the bee, posterior to the pro-thorax, should be drawn to a corner of the slide until the examination is completed.

This is desirable since it sometimes happens that a part of the tracheal system has been left behind.

The freshly mounted preparation is now ready for examination. The parts of the tracheal system which are liable to be occupied by the mites will be recognised as transparent tubes with spirally lined interior. In an infested bee the tubes may be more or less bronzed or soiled, and mites will be present within. It must of course be remembered that in each bee there is a pair of such tubes in the thorax. It will be found that as regards details beyond the main trunks, there is a certain amount of individual variation in the branches, but for our purpose we are not concerned with these parts of the tracheal system which on account of their limited dimensions cannot be occupied by the parasite. Attention is also directed to the distinctive structure of the initial part of the system, which must always be observed if initial cases of infestation are to be recognised.

In making preparations by the foregoing method, it will be found, as manipulative skill increases, that it is best to separate out from each other by means of the needles the various tissues which are found in immediate proximity to the thoracic tracheae. Should the tracheae be ruptured in the course of dissection, it is well to examine these tissues also for any mites which, owing to this, may have been liberated from the respiratory tubes. The bulk of such tissues consists of the muscles of flight and of the thoracic glands.

VIII.—HOPES OF DELIVERANCE.

If we ask ourselves in the present state of our knowledge what are our hopes for deliverance from this plague, the answer is undoubtedly encouraging. While early and complete deliverance cannot come by any single empirical treatment, competent beekeepers have now at hand a number of agencies for control which already are of proved success. Some of these have been indicated already in the present paper, but may suitably be mentioned again for the sake of completeness in this survey.

Limiting the spread of the disease throughout the country.

The causal parasite is singularly helpless as regards capacity for its dispersal amongst bee colonies, and is dependent on external agencies. It cannot travel far. Bees themselves, particularly drones and "robbers" are mainly responsible for its transportation from colony to colony, and it is the beekeepers' business to avoid giving bees the opportunity for doing this.

The wider dispersal for which originally man himself must be held responsible continues to be effected by human agency. As long as beekeepers and bee dealers continue to transfer parasite infested colonies in the form of swarms or otherwise, from one area of the country to another, there will continue to be fresh outbreaks. This indicates that trafficking in bees must be limited to certified clean stocks.

In the absence of guarantees, transference ought to be prohibited.

Anti-Acarine Management.

Timely Intensive Breeding.

In previous writings, and again on earlier pages of this pamphlet, I have made reference to the extreme importance of high-grade breeding queens as a natural counteractive to the spread of disease within colonies.

The value of this procedure, coupled with scientific management in breeding bees before, and not after, the honey flow, has been proved such an effective check to the disease during the productive season, that Acarine disease has in the circumstances proved relatively unimportant as a check in honey-getting. It should never be forgotten that: *A good queen can produce bees faster than this disease can destroy them.*

Breeding Vigorous Long-lived Bees—Confusion Regarding Immunity.

But for complete success in reducing the causal parasite to relative impotence, beekeepers and breeders must go further and raise vigorous strains, long lived if possible, adapted to the British climate and conditions. It is quite true that many " experts " prefer to clamour for a " cure," or to talk of deliverance by immune strains or varieties of bees which they think will by some happy chance arise without efforts on their part. In this connection, I know nothing regarding the existence of any bees having any claim whatever to the quality of " immunity " from Acarine disease. No scientific evidence of their existence has ever been forthcoming. In most cases beekeepers themselves are merely displaying confusion of thought in their references to this subject. It may do some good and save misdirected confidence of beekeepers before accepting statements as to the existence of immune bees if with regard to such claims they will seek answers to the following questions:

Where claims are made that particular breeds are Acarine-immune,

(a) Does the term mean—
that the spiracles on the thorax of the bees for whom the claim is made are so small that the parasite mite—the cause of Acarine disease—is unable to gain an entrance to the body? Or,

(b) that although the mites are able to enter the bees' tracheae the bees' constitution is such that the mites cannot breed there and consequently perish? Or,

(*c*) that the bees (like ordinary bees) cannot hinder the entrance of mites nor prevent their breeding and obtaining sustenance within, but that no pathological results follow. In other words that, whilst not suffering from the effects of the presence of mites themselves these bees are potential nurseries of the disease for other and weaker bees, i.e., *they are true " carriers " of the disease.*

With regard to this last suggestion, it is well to mention that apart from experimental breeding a possible line of natural development which may take place is the evolution of a bee race of the type approaching the character referred to. Under present conditions, when almost all European bee races are being introduced to Great Britain, it is difficult to tell what the result in qualities will be of the heterogeneous hybrids that are being established in the apiaries of the general community. It may be good or bad; nobody knows. But until resistent strains tolerating the parasite preponderate, these can only be " nurseries of the disease." Hence we must continue our efforts at extermination of the parasite.

It may be that no proof exists regarding any of the forementioned (a), (b), (c) characteristics, but that the queens are exceptionally prolific, and possibly the bees are exceptionally robust, and that they thus *breed* faster than disease destroys. In other words, they are bees of the character emphasised above (paragraph 1 of this section), but which cannot be described as " immune " to the disease in any sense of the word.

Radical treatment of diseased Stocks.

Until the amount of disease present in the country has been materially reduced by such methods as are here outlined, a certain amount of radical treatment of diseased stocks is essential. There are two periods in the year when with comparatively small loss, if any, to the beekeeper, diseased bees should be destroyed. If at the end of the working season, stocks are found showing crawling symptoms and known to be heavily affected with Acarine disease, the owner is urgently recommended to destroy them. The bees should be sulphured and afterwards burned; any stores left should be securely placed beyond the reach of robbers. Money and time spent in feeding and otherwise preparing such bees for winter is wasted. To add driven bees or to unite with a healthy stock means at best only the perpetuation of the disease into the following season. Not only so, stocks suffering in autumn are reduced in numbers and they readily become the victims of robber bees. Robbing bees in such cases run very great risks of contracting the disease. Bees from strong stocks will travel considerable distances to rob weak ones, and this seems to me the most frequent means by which fresh infestations started in autumn are carried over the winter, not in the autumn diseased colony, but in the strong robbing one.

The colony with disease well established in autumn should be destroyed.

Again, in spring, while there may be hopeful circumstances inducing the beekeeper to nurse colonies which have survived the winter, but in which the presence of Acarine disease is recognised, he should do so only in the most favourable conditions. For example, it may be that he is desirous of saving a young queen who, favourably treated, e.g., under stimulating feeding to the colony, may be induced to quickly build up with young bees. The wintered bees may be expected to die soon, and there is a small chance that the young bees *may* escape the disease. Apart from this somewhat doubtful possibility, the chances of re-establishment of the colony are so slight as to render its rehabilitation into a strong and profitable stock highly improbable and not worth the trouble. While I am well aware that occasionally such stocks survive, destruction is probably in the wider interests of bee-keeping the better course.

Preventive Treatment for Diseased Stocks.

Wherever the introduction to bee hives of acaricides, deterrents, and the like has taken place, serious difficulties have been met with. Unfortunately, we cannot isolate from the healthy the sick members of a colony who are the centres of contagion, and all have to be treated together. Further, the bees actively set themselves to expel all odorous substances as quickly as possible. Again, owing to the nearness in organisation of host and parasite, substances intended to destroy the parasites have a harmful effect on the bee individual, and their use must always be limited as much as possible, to minimise such results. Excitement may arise causing disturbance of the normal routine, such as cessation of breeding, foraging, or any other hive duty. Brood which is passive cannot protect itself ; its health may be affected and loss of such brood in a number of cases results. Continued treatment, unless managed with extreme care and on proved experimental lines, may adversely affect the prosperity of the colony to a material degree, so that although the stock may survive it does not prove profitable as a honey-getting organisation. It may easily happen that a proportion of the adult bees may be killed or rendered useless, and in any case no treatment of this nature will restore the individual sick bees to health. By a curious irony,

> *that which the beekeeper has most ardently desired for the last three years, namely some specific which he may introduce amongst the bees to kill mites is the thing which on general principles of bee management ought not to be done.*

Such treatment must be an urgency measure only. And with the more potent of effective substances, the procedure to be safe must be exact as to quantities and periods of renewal; casual doses of such substances may prove useless or even disastrous.

Notwithstanding all this, on account of the undoubted value to the inexpert bee-keeper of having some simple procedure likely to yield even only partial success, in the hope of securing some effective substance with a minimum of harmful qualities and which may be used by a simple procedure, I have persisted with a very large number of experiments including a long list of likely substances.

Many things capable of destroying the mites when removed from the bees have been found, and no real difficulty in killing mites in bees isolated in cages has been encountered, but in illustration of the difficulties apart from those already enumerated, it may be mentioned that in most trials it has been the rule to experience considerable irregularity in results. The same substance in the same concentration, applied for the same length of time, may kill mites in one bee and not in another. The mortality amongst the bees in control cages varies in a similar way.

The same uncertainty has resulted when tests have been made with complete colonies. Substances which beyond question are lethal to the mites and which can be used without serious mortality amongst the bees, even when applied over prolonged periods, fail to absolutely clear the colony to the last mite. But notwithstanding this, by a combination of anti-acarine management such as I have already suggested, and treatment where this has been begun when the incidence of infestation was low, success in preventing the collapse of colonies and in maintaining such stocks profitably has been attained.

While it must be distinctly stated that I put forward no claim to have yet finally solved the problem of Acarine disease control, on which I am continuing my investigations, in response to many and continued appeals for the supply of provisional treatment, I have decided to commit myself to the following recommendations, selected from a list of tests all of which have had a measure of success. These are given in the midst of much highly promising but uncompleted work, and are put forward not as final advice, but more particularly for seasonal reasons, in order that some help may be available in the present autumn. On general grounds, as regards treatment, autumn undoubtedly offers the safest and most effective opportunities for the introduction of noxious substances to bee hives, and it is hoped the advice will be followed now by those who approve this method of control. Spring, to a lesser degree, affords similar advantages, and the suggested treatment is applicable there also; summer, on the other hand, is the season in which effective management rather than treatment should be pursued. In this way all risk to brood or of contamination to honey is avoided, and the normal working of the colony is not disturbed.

Recommendations for Autumn.

Apart from those cases in which the presence of disease has become evident in autumn, and as regards which I have already recommended destruction, there are those others where the parasite is present to a lesser extent, and external symptoms are not evident. A proportion of such stocks will survive the winter, and thus be the means of continuing the disease into the following spring unless before that time the parasites have been destroyed. Since however the general body of beekeepers cannot distinguish such stocks from clean ones, it is therefore recommended that after foraging and breeding has largely ceased, but before the bees have settled to form their winter cluster, the following routine should be carried out with *all* stocks.

1. *Fumigation.* (a) With sulphur in smoker. The material may be prepared simply by sprinkling flowers of sulphur on the usual smoking material. Corrugated paper is very satisfactory for the purpose. There should be a daily routine of this nature for at least a week, and again at intervals of a few days until the bees settle for winter. At dusk, a few puffs of the sulphur smoke should be blown in strongly from the top of the frames, and the hive quickly closed down while the door is contracted. The fumigation should be carried out by a beekeeper sufficiently experienced as to be able to avoid overcoming the bees and yet give the maximum safe dose. A limited amount of loss of older or weaker bees—possibly infested members of the colony—should not be regretted.

(b) With Sulphur and Nitre. A variation of the foregoing procedure which, though a slightly more expensive method of preparation is one which gives a smoke of more uniform composition and on the whole better results. It is to use corrugated paper, made up in a roll to fit the smoker, and treated as follows:

Add excess of flowers of sulphur and nitre to about ¾ pint of carbon bisulphide. Shake well from time to time to dissolve to saturation. Steep the prepared paper in this, shake up to coat with excess of sulphur, remove and allow to dry. The paper is then ready for use. Carbon bisulphide is highly inflammable and must not be handled near a light. If fumigation by this means be carried out efficiently and extensively throughout the country, *as late in the season as possible*, when intermingling of bees of different stocks has ceased, I anticipate a significant reduction in the amount of disease next year. Material made up as described can be obtained along with that referred to in par. 3 below.

2. "*Flouring*" *of Bees.* It is a useful procedure in autumn to dust the bees with ordinary flour, frame by frame, while they are still working. If this is done in a fine spray from a suitable syringe, better results will be obtained. The process is specially helpful when the bees are suspected of robbing other stocks, and acts in the way of obstructing the migrating mites while still outside the bodies of the bees. The cleaning and fanning which takes place assists further in preventing these gaining the spiracles. It is hoped to publish at an early date an account of an extension of this method of treatment together with a description of a specially made syringe suited to the work.

3. The following recommendation is not intended to be adopted, in the case of stocks, known by microscopical examination to be free from disease. It is applicable only to such colonies, as have been shown to be diseased and which are regarded on general grounds as worth saving. In addition to the foregoing, just before the bees settle for winter, a few drops of the preparation to be issued by this Department (made up in appropriate strength and supplied with all necessary directions), should be allowed to soak into some absorbent material and placed within the hive. It may be simply dropped on to the tops of the frames. This treatment should be repeated five or six times at intervals of 2 days and renewed on specially mild days throughout the winter when the bees fly. It is a proved "mite killer" to the extent already indicated. In view of the care required

in its use, and the fact that its ingredients may be modified in the course of experience, it is to be used only in the doses stated and according to the directions supplied. Information as to the conditions on which it will be available, and where, will be made in the *Bee Press* and elsewhere without delay.

Agricultural Zoology Department,
North of Scotland College of Agriculture,
Marischal College, Aberdeen,
August, 1923.