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# ON THE MIGRATION OF THE LARVAE OF *ONCHOCERCA GIBSONI* THROUGH THE CAPSULE OF THE WORM NODULE

BY

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The life-history and the mode of transmission of the parasitic Nematode which causes worm nodules in cattle still await elucidation. Many observations have been made and a considerable amount of experimental work has been carried out, almost entirely in Australia, but so far the results obtained have hardly been commensurate with the amount of labour expended. The morphology of the worm itself, and the structure of the fibrous nodule in which it is invested, may be regarded as fairly exhaustively known. How and where the parasite enters its host, how it reaches its destination, and by what means the larvae are transmitted to another host, are questions on which we have but scattered items of information. The great majority of transmission experiments have had negative results, and there are very few positive observations at our disposal on which to base any manner of feasible hypothesis.

An excellent summary of the results obtained up till 1911 has been compiled by Harvey Johnston (1911). In that paper an account is given of the various lines along which experimental researches have been conducted, and of the few hypotheses which have been formulated. The observations and suggestions of previous workers are carefully examined and criticised, and the

conclusion is arrived at that, even in spite of serious objections, the most likely transmitting agents are to be found amongst the mosquitoes, the true lice or a cattle fly (*Musca* sp.).

Since Johnston's review four further important contributions have been made on the subject. The first of these was by Cleland (1912) in Sydney; the second by Gilruth and Sweet (1912); the third by Breinl (1913) in North Queensland, and the most recent by Cleland (1914). Cleland made the somewhat unexpected discovery that three calves, born and bred on Milson Island, Hawkesbury River, New South Wales, had become infected with *Onchocerca* nodules. As these calves had not at any time been removed from the island, it is evident that infection must have occurred there, and that therefore all the factors concerned in the transmission of the parasite are present on the island. These important discoveries, though not immediately furthering our knowledge of the mode of transmission, cannot fail to help in materially lessening the number of possibilities. Thus, for instance, Cleland notes that the mothers of these calves were not infected, a fact which excludes the possibility of direct infection from mother to foetus or calf. The absence of leeches and ticks is also commented upon, so that they may apparently be definitely excluded from any participation in the life-history of the parasite. Another point of some significance is the absence of areas of bog and marsh on the island. This appears to preclude the possibility of the infection being water-borne, either directly or by the intervention of aquatic animals. The presence of a single deep water-hole on the island does not, in Cleland's opinion, invalidate this conclusion, as he does not consider it a likely or suitable place for infection to take place. This, however, cannot be admitted without some definite proof, and in view of Breinl's observations, which will be mentioned presently, the possibility of water-borne infection cannot be absolutely excluded.

Cleland's summary of the remaining possibilities differs somewhat from Johnston's. While including lice and mosquitoes, he does not mention the cattle-fly, but adds *Stomoxys calcitrans* as the most likely intermediary, with Mallophaga, Tabanidae and the sand-fly *Culicoides molestus*. He evidently attaches most importance to *Stomoxys* and mosquitoes.

Gilruth and Sweet's paper consists almost entirely of negative evidence, but they have made several observations which may prove of value in later work. They assert, for instance, that fresh infection rarely, if ever, occurs in cattle more than two years old. They also note the occurrence of a very large percentage of degenerate nodules. This may possibly explain the negative results obtained in many experiments to determine the mode of transmission of the larvae. Gilruth and Sweet dismiss the possibility of direct infection or of infection from the soil, and they also appear to abandon the idea which they formerly entertained of lice being the intermediate hosts. Like Cleland, they come to the conclusion that a biting fly is the most probable intermediary.

Breinl's observations are of the first importance, as they are amongst the few definitely positive results which have been obtained in any direction. They confirm a suspicion which had been hinted at by previous observers, namely, that the larvae could make their escape from the worm nodule, pierce the intact skin of their host, and so find their way to the exterior. Breinl's discovery is of interest from the fact that it is the first record of such an occurrence in the life of any parasite of this class. Although it is true that the event was only observed in a few cases in the course of a large number of experiments, still it cannot be denied that even these few positive observations are sufficient to establish the fact that the *Onchocerca* larvae can make their escape through the unbroken skin of their host either naturally or under experimental conditions. The only objection which can be urged against this statement is that some slight abrasion of the skin may have been caused in the act of shaving or scraping. So far as appears, however, stringent precautions were taken that this should not occur.

Cleland's latest paper (1914) is a comprehensive account of an extended series of experiments directed on the one hand towards ascertaining the path taken by the worm in the infected animal, and on the other towards incriminating biting flies or mosquitoes as the intermediate host. He has elucidated several interesting points, and has made the case in favour of *Stomoxys calcitrans* a very strong one. His feeding experiments with *Stomoxys* are not by any means conclusive, but they show that the larvae can live

in it for at least as long as they live in water and much longer than in non-biting flies. Of more importance is his discovery of the worms in their passage up the hind leg of the infected animals. His suggestion that the formation of nodules is an accident in the life history of the worm and that larvae are chiefly produced while the worm is free is not a new one, and appears not altogether necessary in view of the experiments to be detailed later. It shows, however, that it is not necessarily in the vicinity of the nodule that larvae are to be found, but that they may be met with in the subcutaneous tissues over a considerable extent of the body.

It was with the view of controlling and confirming these observations that the present investigations were undertaken. In the first instance a series of over thirty experiments, similar to those of Breinl, were carried out. Two moderately infected, middle-aged, cows were used. Each had about half-a-dozen palpable nodules varying in size from 15 mm. to 30 mm. in diameter. Each of the nodules were selected in turn for experiment. In most cases the skin was previously depilated with a mixture of barium sulphide, zinc oxide and starch, but in some instances the hair was not removed.

The experiments were started in March, 1913, and continued till the end of July. The wet season was just ending, though there were several rainy days during the course of the investigations. The experiments were started at different hours of the day, from 8 a.m. till 6 p.m., on the chance of there being some particularly favourable time for the escape of the larvae. Various other modifications were also adopted. In every case the water used was tap-water previously boiled, filtered, and allowed to cool to body-temperature. In some of the early experiments the skin over the nodule was washed with absolute alcohol and then with sterile water, but in the later experiments the alcohol was omitted.

In the first nine experiments a calico bandage folded to form a square pad was soaked in sterilized water, covered with oiled silk, and strapped over the selected area. This was left on for one-and-a-half to three hours, after which the pad was carefully washed in sterile water and wrung out and the skin scraped with a blunt scalpel. In the later experiments a small circular vessel of three

inches diameter was substituted for the calico pad. This was filled with sterile water and applied continuously to the selected part.

On the few occasions which offered, the cow was exposed to the rain for two or three hours before the experiment was started, in the expectation that rain might have some influence in stimulating the egress of the larvae.

Notwithstanding the various modifications adopted, not one of the experiments gave a positive result. In no case were any larvae observed, either in the water or in the scrapings from the skin, although a very exhaustive examination was made on every occasion. These negative observations do not vitiate the validity of Breinl's results, but they show that the exodus of the larvae does not follow simply on the application of water to the skin over every or any nodule. Evidently some other factor or factors are essential. These may be associated with climatic or environmental conditions, or may be inherent in the condition of the nodules themselves. It may be, as will be gathered from what follows, that none of the nodules in either of the two cows used were in a suitable state, i.e., for the emigration of the larvae.

Further experiments were conducted with nodules excised from the carcasses of cattle slaughtered at the meat works, the object being to ascertain whether the larvae are in the habit of penetrating the capsule of the nodule in large numbers or if the occurrence is only occasional.

In the first experiment two freshly excised nodules were used. One measured about one inch in diameter, the other half an inch. The first was surrounded by a mass of muscle, the second by a small amount of connective tissue. From the first nodule the muscle was carefully cut away and divided into two portions. The two nodules and the two pieces of muscle were placed in separate, covered, glass capsules, nearly filled with filtered rain-water. The capsule containing the larger nodule and that containing one piece of muscle were placed in the incubator at 37° C. The other two were allowed to remain at room temperature (21° to 26° C.). At the end of an hour the water was poured off from each of the capsules, which were cleaned out and filled with fresh water. This was poured off at the end of another hour and a half, the capsule again carefully cleaned, and fresh water added. The same process

was repeated three times during the ensuing seven hours and once on each of the following three days. The fluid poured off from each capsule was centrifugalised and the centrifugate examined for larvae.

The larger nodule gave a negative result throughout, as did the piece of muscle which had been left at room temperature. The other piece of muscle showed one larva on the second examination (i.e., at the end of two and a half hours). The smaller nodule showed two larvae at the end of six hours, and an additional one was found at the expiry of another three hours. No larvae were found later than this.

In the second experiment eight nodules (nodules 5-12) were used. It was similar to the first, but two nodules were placed in acidulated water (1% HCl.) and two were placed in alkaline water (1%  $\text{NH}_3$ ). In the case of the other four, ordinary filtered rain-water was used. Two cross-shaped incisions about 1/16th of an inch deep were made in the capsule of nodule No. 12. Half of the capsules were placed in the incubator at 37° C., the others left at room temperature. The same procedure was adopted as in the first experiment. The results are tabulated below.

Experiment started at 1 p.m., 30th July.

	30th July			31st July	1st August
	2.15 p.m.	5.15 p.m.	9 p.m.	9 a.m.	12 noon
5. Acidulated water, 37° C. ...	4	0	2	0	0
6. Acidulated water ... ..	0	3	0	0	0
7. Ammoniated water, 37° C. ...	2	2	0	6	1
8. Ammoniated water ... ..	2	12	0	0	0
9. 37° C. ... ..	42	8	0	0	3
10. Room temperature ... ..	439	2,000	Very numerous	30	7
11. 37° C. ... ..	47	18	0	0	0
12. Room temperature ... ..	42	230	20	14	0

At the end of the experiment the nodules were cut open and their contents examined. In numbers 5 and 6 the worm appeared

as a caseous mass. In numbers 7 and 8 the worm was in good condition but the larvae were all dead. A similar condition was found in 10 and 12, but in 9 and 11 the interior of the nodule was in a state of putrefaction.

From this experiment it would appear that increase of temperature does not notably, if at all, favour the escape of the larvae from the capsule. It would also appear that acid and ammonia, at least in the strength used, have a decidedly retarding effect.

It may be mentioned that all the nodules were carefully examined before the experiment to ensure that no cut or injury was present in the capsule. The result in the case of No. 10 was so unexpected that the nodule was subjected to an especially careful examination after the experiment, but no sign of any cut or other perforation could be detected. The capsule was perfectly intact, and was surrounded by a considerable amount of connective tissue.

At the same time as the above experiment was being conducted a number of smaller nodules surrounded by fibrous tissue were placed in two glass vessels with water and kept at room temperature. These were examined after seventeen hours, when it was found that 101 larvae had made their escape in one case and twelve in the other. After another twelve hours a second examination showed eight in one and twenty-two in the other. A third examination fifteen hours later proved entirely negative.

This experiment appears to indicate that in many *Onchocerca* nodules, if not in all, larvae are constantly present in the capsule, and are continually migrating through it in greater or less numbers into the surrounding tissues.

A third series of experiments was started on the 12th August, at 11.30 a.m. Examinations were made at intervals throughout this and the following two days. The procedure was identical with that in the preceding series, but all the nodules were kept at room temperature. At each period of examination one nodule was transferred to Zenker's fluid, fixed and prepared for sectioning. At 3 o'clock on the same day a fourth series of experiments was started, and these were examined at the same intervals as the others.

The results of these experiments are set forth in the following table:



	12th August		13th August			14th August	
	2 p.m.	5 p.m.	9 a.m.	12 noon	4 p.m.	9 a.m.	2 p.m.
15	2	3	0	0	0	1	—
16	2	2	0	1	0	0	0
17	0	0	0	0	0 (fixed)		
18	3	2	1	0	1	0	0
19	0	0	5	0	0	0	0
20	1	0	2	0	0	0	0
21	0 (fixed)						
22	0	5 (fixed at 3 p.m.)					
23	0	2 (fixed at 6 p.m.)					
24	2	0	0 (fixed)				
25	0	2	3	1	2 (fixed at 3 p.m.)		
26	Started at 3 p.m.	0	1	0	0	0	0
27		1	0	0	1	0	0
28		0	0	1	0	0	0
29		1	34	42	45	6 (fixed)	
30		0	2	0	1	1	0

From the above table it will be seen that fewer larvae were liberated from the nodules in these experiments than in the previous series. Only in No. 29 was there anything approaching a free production. It will also be noticed that in the case of these nodules the larvae only began to make their escape in any numbers several hours after the experiments had been started. This might be taken to indicate that the water in which the nodule was immersed had a distinct, though slow, effect in stimulating the migration of the larvae through the capsule.

The result in No. 15 is of interest, for in this case a period of at least twenty-three hours elapsed during which no larvae made their escape. In No. 27 a similar period of at least nineteen hours occurred. In both cases a single larva made its appearance some time later. No. 17 was entirely negative, and when sections from it were examined, absolutely no larvae were found in the capsule,

though in the centre of the nodule there was a small mature female worm.

In the sections from No. 21 there were very numerous larvae throughout the capsule. They were arranged roughly in three concentric strata. These corresponded practically to the three densest layers of the capsule. There were extremely few in the looser tissue, and none at the periphery of the capsule. In the sections from Nos. 22 and 23 there was a considerable number of larvae scattered irregularly throughout. No larvae were found in No. 24, but there were several in No. 25. In No. 29 a concentric arrangement of the larvae could be made out, but not so distinctly as in No. 21. Some of the adherent loose connective tissue was removed from this nodule and cut into sections, but no larvae could be detected in it.

A series of experiments similar to the foregoing was started on October 13th. At the beginning, six nodules were fixed and prepared for sectioning. Others were fixed at varying intervals.

The results of the experiments are as follows:

Started 1 p.m.	13th October			14th October			15th October	
	3.30 p.m.	5.15 p.m.	9 p.m.	9 a.m.	12.30 p.m.	5.30 p.m.	11 a.m.	3.30 p.m.
32	40 (fixed)							
33	—	5 (fixed)						
34	30	0	16 (fixed)					
35	—	36	40	12	—	2	0	0
36	6	—	10	4 (fixed)				
	—	4	12	0	—	1	0	0
38	1	—	2	4	0 (fixed)			
40	4	—	12	0	—	3 (fixed)		
41	—	0	0	0	—	0	0	0

These results are rather similar to those obtained in the previous series. The longest time during which larvae continued to escape from any of the nodules was twenty-eight hours in the case of Nos. 35, 37 and 40. These three nodules were cut open at the end

of the experiment and the larvae inside the parent worm were found to be all dead. No. 41 was entirely negative throughout.

Examination of the sections made from nodule 31, which had not been immersed in water, showed that the capsule was entirely devoid of larvae. Three series of sections from different parts of the capsule were made and a large number of sections carefully gone through, but no trace of larvae could be detected. A considerable amount of effused blood was present in the capsule, so that the nodule had apparently been recently bruised.

In the sections from nodule 32 only a very few larvae were present. In No. 33 they were more numerous, but still comparatively scanty. The sections from No. 34 were rather interesting. The inner layers of the capsule showed an extremely large number of larvae, but in the outer layers there were absolutely none at all. One might infer from this that the influence of the water had caused all the larvae already in the capsule to escape, and had induced a fresh migration from the interior of the nodule. In the case of Nos. 32 and 33 it might be supposed that the action of the water had not been allowed to continue for a sufficiently long period.

The foregoing inference receives support from the fact that in nodule 36, which had been subjected to the action of the water for twelve hours longer than No. 34, an almost exactly similar condition was found, with the difference that a number of larvae were met with in the median layers and a few in the outer layers. In nodule 38, however, the result was discordant, as only a few larvae were seen, and there almost entirely in the outer layers of the capsule. This might be explained by the death of the adult worm. In No. 40 the result was somewhat similar to that in Nos. 34 and 36, and here again the larvae were much more numerous in the walls of the capsule even than in No. 36.

Of the other nodules which had not been immersed in water, two showed numerous larvae in the walls of the capsule, one was entirely negative, while the others had a few.

On November 13th a series of experiments somewhat different from the foregoing was started. The following are the details. Nodules were immersed in water continuously for varying lengths of time. The nodules were then fixed and the water examined for larvae.

44 fixed in formalin immediately							Result
45	"	"	after 1 hour in water	...	...	...	No Larvae
46	"	"	" 2 "	"	"	...	40 "
47	"	"	" 3 "	"	"	...	12 "
50	"	"	" 6 "	"	"	...	12 "
48	"	"	" 18 "	"	"	...	No "
49	"	"	" 24 "	"	"	...	No "
51	"	"	" 24 "	"	"	...	No "

It must be noted that nodules 48, 49 and 51 were kept in the ice chest for a few hours previous to the experiment, and this had probably been sufficient to kill all the larvae.

On examination of the serial sections no larvae were found in the capsule of No. 44, and only a few in No. 45. In No. 46, however, the larvae were very numerous. In 47, 48 and 50 they were extremely scanty, while in 49 and 51 they were entirely absent.

The result of this experiment is not at all conclusive, yet it shows that in the case of a suitable nodule, such as No. 46, a considerable number of larvae may make their escape from the capsule in quite a short space of time.

In all the foregoing experiments attempts were made to keep the larvae which had emerged from the nodules alive in water for as long as possible. In the most successful case they remained alive for only seventeen hours. Gilruth and Sweet (1911) and Breinl have made similar observations, but with larvae removed mechanically from the interior of the nodule. They found that the life of these larvae in water could not be prolonged beyond two days.

#### SUMMARY AND CONCLUSIONS

The results of these experiments, while not confirming Breinl's observations, go to show that *Onchocerca* larvae can and do make their escape through the capsule of the worm nodule. They usually do so in small numbers, but may at times, or in some cases, migrate in comparatively large numbers.

It is evident that the results of Breinl's work form a strong

argument in favour of water-borne infection. Against this, however, must be placed two very weighty facts, namely, that the larvae are not adapted for independent existence in water, and that their life in that medium, so far as observed, does not extend beyond two days. Notwithstanding these objections, water-borne infection, direct or by intermediary host, must be considered as a possibility.

Taking everything into consideration, insect-borne infection appears to present the greatest measure of probability. Leiper (1911) supported this view on purely morphological grounds. Anatomically the adult worm is so closely allied to the *Filariae* that there is some justification in expecting an analogous life-history. On the other hand the habitat of the worm is different and approaches more closely that of the Guinea-worm (*Dracunculus*). This fact has not failed to lend some bias in favour of water-borne infection.

Practically all observations go to show that this difference in habitat from that of the *Filariae* is correlated with a difference in the life-history. De Does (1904) is reported to have found *Onchocerca* larvae in the blood, but no other observer has succeeded in doing so. It has, again, been suggested that the larvae may circulate in the blood like *Microfilariae* before the fibrous capsule is formed around the adult, or that they may only occur in the blood of young cattle. Both of these seem quite unnecessary hypotheses in view of the fact that the larvae are in the habit of migrating through the capsule of the nodule into the surrounding tissues, and eventually finding their way into, or even through, the cutaneous surface.

As has been shown in the foregoing notes, the number of larvae so liberated may, in certain cases, be very considerable, and quite sufficient to ensure a moderate chance of a few being taken up by a blood-sucking or biting insect. If it be the case that the larvae are in the habit of emerging through the skin of their own accord there is the possibility of some non-biting insect becoming infected, but in such a case it would be difficult to understand how re-inoculation could be effected.

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