ON FILTRATION OF MICROFILARIAE BY LYMPH NODES.

BY

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In 1933, one of us (C. K. D.) took part in series of experiments on the filtering efficiency of the popliteal and iliac lymph nodes in the dog. It was shown that when erythrocytes or streptococci, suspended in an artificial lymph, were perfused through these nodes at rates of flow and pressure consistent with normal conditions in the dog, a very complete filtration occurred. In the paper which reported these results* it was suggested that similar experiments utilizing microfilariae would be of decided interest.

Through the kindness of Dr. M. C. HALL, Chief, Zoological Division of the Bureau of Animal Industry, Washington, a dog was secured which was infected with Dirofilaria immitis. During the morning the blood of this animal

was heavily charged with microfilariae, and it proved easy to centrifuge the organisms out of the heparinized and haemolyzed blood. They were then resuspended in a solution of dog serum and physiological saline such that the protein content fell between 1.2 and 2.3 per cent., concentrations of the blood proteins normal for leg lymph in the dog. The microfilariae lived for many hours in such artificial lymph and were extremely active.

Just as in former experiments on filtration by lymph nodes, two types of experiment were performed.

In the first, an afferent lymphatic to the popliteal node was isolated and cannulated, the cannula pointing centrally. This cannula provided inflow of the lymph containing microfilariae. It is a fortunate fact that the afferent vessels to such a node all empty into the cortical sinus, and fluid introduced through a single vessel thus takes a path identical with that provided if all the afferent vessels are cannulated. Even more fortunate is the fact that the effluent from such nodes leaves ordinarily by a single short vessel which may be picked up at the hilus of the gland and in which it is easy to insert a relatively large cannula so as to avoid back pressure and distention. For convenience in perfusion, the inflow cannula was connected with a graduated reservoir in which necessary degrees of pressure could be maintained steadily and which permitted stirring of the perfusate so as to assure an even distribution of microfilariae. As soon as perfusion was started the total effluent lymph was collected in separate samples during short periods and all the microfilariae counted in each of these.

A typical experiment of the first type was as follows.

14th November, 1934. Weight of dog, 22.0 kg. Anaesthetized with 18 c.c. of 5 per cent. nembutal intraperitoneally. The afferent and efferent vessels of the left popliteal node were cannulated. Artificial lymph used for perfusion contained 1.37 per cent. dog serum proteins and 1,530 microfilariae per c.c. Table I presents the results.

Table I.
Filtration of Microfilariae. Inflow through an Afferent Vessel of the Popliteal Node. Effluent from the Efferent Vessel of the Node.

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<tbody>
<tr>
<td></td>
<td>C.c.</td>
<td>C.c./Min.</td>
<td>C.c.</td>
<td>C.c./Min.</td>
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<tr>
<td>0–10</td>
<td>1-45</td>
<td>0.145</td>
<td>1.6</td>
<td>0.16</td>
<td>0</td>
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<td>10–25</td>
<td>1.70</td>
<td>0.11</td>
<td>1.2</td>
<td>0.08</td>
<td>1</td>
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<tr>
<td>25–40</td>
<td>1.85</td>
<td>0.09</td>
<td>1.32</td>
<td>0.32</td>
<td>12</td>
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<tr>
<td>40–55</td>
<td>2.95</td>
<td>0.10</td>
<td>2.70</td>
<td>0.18</td>
<td>53</td>
</tr>
<tr>
<td>55–70</td>
<td>1.55</td>
<td>0.10</td>
<td>1.60</td>
<td>0.10</td>
<td>33</td>
</tr>
<tr>
<td>70–85</td>
<td>1.65</td>
<td>0.10</td>
<td>1.60</td>
<td>0.10</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>10.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>10.02</td>
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As soon as all the perfusate had passed into the lymph node the animal was killed by bleeding to death and the perfused node removed promptly. It was cut in two parts, one
of which was fixed for sectioning. From the other, scrapings were made which showed active microfilariae with no tendency whatsoever to adhere to any of the cells scraped from the node.

The filtration accomplished in this and in duplicate experiments is in sharp contrast to what occurs when streptococci were perfused in a similar manner. These organisms rarely passed through the node, and on section were found adherent to reticular strands and in and upon phagocytic cells. The microfilariae meet no such biological restraint, and their imprisonment in a node is a simple expression of the mechanical complexity of the nodal filter.

In the second type of experiment, an afferent vessel to the popliteal node was cannulated to provide perfusion inflow. The effluent was secured by cannulating the thoracic duct at the entrance into the left subclavian vein. In these circumstances the artificial lymph containing microfilariae passed through two nodes, the popliteal and iliac, and entered the thoracic duct to be delivered with the normal lymph flow from the effluent cannula.

A typical experiment of this second variety was as follows.

27th November, 1934. Weight of dog, 16·3 kg. Anaesthetized with 12 c.c. of 5 per cent. nembutal intraperitoneally. An afferent vessel leading to the left popliteal node was cannulated and connected to the perfusion apparatus. The thoracic duct was isolated and cannulated. The artificial lymph for perfusion contained 1·8 per cent. dog serum proteins and 6,410 microfilariae per c.c. In order to indicate the time of arrival of the perfusate in the thoracic duct cannula, a small amount of 2 per cent. trypan blue in physiological saline was added to the perfusate. This dye has no observable effect on the activity of the microfilariae.

Table II.
Filtration of Microfilariae. Inflow through an Afferent Vessel of the Popliteal Node. Effluent Collected from the Thoracic Duct.

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<td>C.c.</td>
<td>C.c./Min.</td>
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<td>0-14</td>
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<td>0-10</td>
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<tr>
<td>25-41</td>
<td>1·4</td>
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<td>0-84</td>
<td>8</td>
</tr>
<tr>
<td>41-56</td>
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<td>13-3</td>
<td>0-88</td>
<td>110</td>
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<td>0-14</td>
<td>13-9</td>
<td>0-92</td>
<td>102</td>
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<td>0-16</td>
<td>13-2</td>
<td>0-88</td>
<td>197</td>
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<tr>
<td>86-99</td>
<td>0-95</td>
<td>0-073</td>
<td>13-3</td>
<td>1-02</td>
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<td>99-114</td>
<td>1-00</td>
<td>0-066</td>
<td>12-3</td>
<td>0-80</td>
<td>511</td>
</tr>
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Table II presents the results. In this case, the first specimen, which contained no microfilariae, showed no trypan blue. The dye appeared during collection of the second specimen. A small amount of heparin was added to each collecting tube to prevent coagulation of the lymph and the tubes were at once centrifuged at high speed. The counts of microfilariae represent all
the organisms obtained from the sediment thrown down in each tube. At the close of the perfusion the animal was bled to death and the popliteal and iliac nodes removed for scrapings and sectioning. Organisms recovered from nodes were normally active. Lymph taken by syringe from a large vessel just central to the iliac node contained 35 microfilariae per c.c., indicating the rapidity with which the organisms were passing through the nodes. The flow was somewhat higher than in preceding experiments but both grossly and microscopically the nodes were normal. The popliteal and iliac glands were thoroughly coloured with trypan blue, indicating passage of the perfusate through both structures.

HISTOLOGICAL EXAMINATION OF PERFUSED LYMPH NODES.

Sections 8μ thick showed small numbers of organisms when the entire cross-section of a perfused node was examined. The organisms were most frequently in intermediary sinuses in the depths of the gland, occasionally in cortical vessels, and rarely in the afferent vessels of the capsule. In no case was there the slightest evidence of reaction. The microfilariae lay free and apparently so far as the cells of the node were concerned were entirely without significance as foreign bodies.

In three experiments microfilariae were injected into nodes through the afferent vessel, and while perfusion was in progress the efferent vessel was tied so that the organisms were imprisoned in the glands. The microfilariae survived this treatment with great success. In the second experiment, the left popliteal node was injected with normally active organisms at 11.30 a.m., and the right node with organisms which were thought to have been killed by heat and which were non-motile when injected into the right popliteal node at 11.50 a.m. Both nodes were removed 22 hours later and scrapings from both showed normally active microfilariae. In the case of the right node, the contained organisms started their period of habitation in a somewhat precarious condition and their recovery is a good expression of the innocuousness of the lymph node environment. In the third experiment, living microfilariae were injected into a lymph node and imprisoned by ligation of the efferent vessels. Numerous microfilariae were found alive and normal at the end of 5 days. We cannot at present furnish any estimate as to the number that may have escaped from the node by migrating through the capsule or even possibly through the walls of large lymphatics. In none of these instances of imprisonment in nodes was there the slightest evidence of phagocytosis.

DISCUSSION.

These experiments show quite clearly that the microfilariae of *Dirofilaria immitis* pass through normal lymph nodes with great ease. They show further that the nodal environment for very considerable periods of time is in no way destructive.

The question at once arises as to how far these results with *Dirofilaria* can
be applied to human infection with *Wuchereria bancrofti*. *Dirofilaria* lacks a sheath and there is a rather general belief that unsheathed microfilariae are more motile in the sense of being able to travel from one point to another than are sheathed forms. In order to test this question of comparative motility, blood containing microfilariae was taken from our infected dog in heparin. The movements of single organisms were then charted, using a camera lucida and employing preparations both with and without cover glasses. These observations were made at room temperatures between 70 and 75°F. The results of such observations are shown in Figs. 1 and 2. In Fig. 1, pathways A and B have been

![Diagram 1](https://academic.oup.com/trstmh/article-abstract/29/1/51/1942504)

**Fig. 1.**—Courses of two microfilariae (*Dirofilaria immitis*) in undiluted, heparinized blood, plotted at 5-minute intervals. Observed in uncovered droplet.

![Diagram 2](https://academic.oup.com/trstmh/article-abstract/29/1/51/1942504)

**Fig. 2.**—Courses of two microfilariae (*Dirofilaria immitis*) in undiluted, heparinized blood, plotted at 5-minute intervals. Observed under cover glass rimmed with vaseline.
travelled by microfilariae in blood without a cover glass. In Fig. 2, pathways C and D were made by organisms in blood and sealed under a cover glass by vaseline. When the four paths are measured and related to time the results are as follows: A travelled at an average of 0.18 mm. per minute; B, 0.16 mm.; C, 0.19 mm.; and D, 0.14 mm. Unfortunately no infection with Wuchereria was available to us, but through the kindness of Dr. F. W. O'CONNOR we were able to obtain blood from a patient infected with Loa loa and thus could obtain comparative data upon a sheathed organism.

Fig. 3 is a chart of the movements of a single microfilaria of Loa loa. The examination was carried out in heparinized whole blood under a cover slip just as in the case of C and D, Fig. 2. The rate of travel was 0.37 mm. per minute, much faster than that observed for Dirofilaria. Notes made by the observer, D. L. A., are pertinent:

Laboratory of Dr. F. W. O'CONNOR, Presbyterian Hospital, New York.

12th March, 1935. 11.25 a.m. Blood to the amount of 5 c.c. is drawn from a vein in the arm of the patient and heparinized. Tracings of courses of microfilariae would seem.
to indicate much crossing and recrossing of path. This is not always true. Straight paths are often seen extending across the microscopic field after the preparation is several hours old. No unsheathed larvae are seen.

1.5 p.m. All larvae are as active as when the blood was first drawn. No unsheathed organisms are found in blood diluted with saline, in whole blood or in stained specimens. The tube specimen was transported to Boston without refrigeration.

Laboratory of Comparative Pathology, Boston, Mass.

13th March, 1935. 10.15 a.m. The microfilariae are as active as on 12th March. No unsheathed larvae are found.

14th March, 1935. The blood is contaminated and all microfilariae in the tube specimen are dead. Larvae on a slide under a vaseline-rimmed cover glass prepared yesterday are active. One larva has penetrated the vaseline with the anterior third of its body directed into the vaseline. The posterior two-thirds is lashing vigorously about and creating a fan-shaped area clear of red cells.

These observations show beyond possible question that sheathed microfilariae are capable not only of movement but of actual travel, and that their travel is forcible as indicated by the organism which penetrated the vaseline. So far as migration is concerned the microfilaria of *Loa loa* is even more effective than that of *Dirofilaria*, and it is reasonable to believe that the ease with which the latter organism traverses lymph nodes would be equalled by *Loa*. If the behaviour of *Loa* is a fair index of what one may expect from *Wuchereria*, it may be expected that microfilariae in lymphatics will pass to the blood stream at least as rapidly as does the lymph current in which they find themselves, and that healthy organisms will experience no serious check in passing through lymph nodes.

A further point, which will be treated in a second paper but which is pertinent here, is that if one cannulates lymphatics in various regions in a dog infected with *Dirofilaria*, microfilariae are found plentifully in the lymph. This means that these organisms readily get out of unbroken blood capillaries, traverse a certain distance in the tissues, and enter lymphatics. Experiments are planned to determine this point. At this time the presence of microfilariae (*Dirofilaria*) in lymphatics is merely further evidence of forcible movement on the part of these larvae. At autopsy of this particular animal adult *Dirofilaria* were found only in the right ventricle.

**Summary.**

1. Experiments have been accomplished in which microfilariae (*Dirofilaria immitis*) have been perfused through the normal popliteal lymph nodes of dogs. It has been shown that there is no phagocytic filtration of the organisms and that they pass through the nodes with comparatively slight hindrance, differing from bacteria and even from the dog’s own red cells which are phagocytosed in
the nodes, leaving the perfusate practically free of them when collected from the efferent vessel.

2. It has been shown that microfilariae of *Loa loa* although sheathed are more motile, *i.e.*, they travel farther per minute, than do those of *Dirofilaria* which possess no sheath.

3. By analogy it is suggested that microfilariae of *Wuchereria bancrofti* deposited in the lymph stream will not be measurably impeded by lymph nodes in their journey to the blood stream, and that if mechanically checked in nodes normal organisms will not suffer by such residence.