ON THE METAMORPHOSIS OF THE YOUNG FORM OF
FILARIA BANCOFTI, COBB, [Filaria sanguinis hominis, Lewis; Filaria nocturna, Manson] IN THE BODY OF CULEX CILIARIS, LINN., THE "HOUSE MOSQUITO" OF AUSTRALIA.

By Thos. L. Bancroft, M.B. Edin.

[Read before the Royal Society of N. S. Wales, June 7, 1899.]

Dr. Patrick Manson in a paper read before the Linnean Society of London, March 6th, 1884, remarked:—"Six years ago I described the metamorphosis undergone by the embryo Filaria sanguinis hominis, in the body of the mosquito. I hoped that (considering the practical importance of a correct knowledge of the life-history of this parasite) the statements I then made would, long ere this time, have been thoroughly confuted or confirmed. . . . With the exception of Lewis in India, Myers in Formosa, and Sonsino in Egypt, I do not know that anyone has worked seriously at the subject. And although both Lewis and Sonsino have confirmed my statements as to the entrance of the Filaria into the mosquito, and followed up part of the metamorphosis, neither of them has advanced his observations so far as to be able to confirm my statements as to the later stages of this, or positively to prove that the mosquito is or is not, the intermediary host. Some eminent helminthologists in England accept my statements and endorse the inferences I have drawn—Cobbold for example. But in other quarters, so far from securing acceptance of my theory, the work of Lewis, on account of the hesitation and scientific caution with which he expresses himself, has had the effect of inducing a certain amount of scepticism. Leuckart is sceptical;

and of course the scepticism of so eminent an authority is of great weight in influencing opinion, especially in Germany."

In answer to an inquiry from me as to whether there was any recent work on the subject of "filarial metamorphosis," Dr. Manson wrote, November 15th, 1898:—"So far as I know, nothing has been done in "filarial metamorphosis" since my Linnean Society's paper. Lewis did not go very far with the work. There is an excellent opportunity for work on this subject, and were I in your place, I should certainly go on with it."

In writing to Dr. Manson, I had mentioned the circumstance of my being able to verify his "filarial metamorphosis," but that I had never seen the "actively moving filaria," which he stated left the mosquito's body and lived a free life in water until transferred to the human host.

To this he replied in these words:—"I have seen the "actively moving filaria" in the seven days' old mosquito a good many times; I used to be able to pick out the mosquitoes containing it. Their thoraces looked plump and juicy to the eye. Of course you must have hundreds of mosquitoes from which to select such."

Now in my former investigation there was no difficulty in finding the early stages of the metamorphosis in every mosquito (Culex ciliaris) without exception, which had imbibed filariated blood; those mosquitoes which lived seven days—and none ever lived longer—never contained any actively moving filariae; they contained forms more or less resembling Figure 4.

It were useless to make further search for this "actively moving filaria"; either Manson must be in error I thought, or the Culex ciliaris was not an efficient host.

The recent work in India on the metamorphosis of the Malarial parasite in mosquitoes induced me to study the habits of these insects in this district. I found that I could keep certain kinds of mosquito, particularly Culex ciliaris and a large black species hitherto undescribed, alive in confinement for about two months; one individual actually lived seventy days. Banana was found
to be a good food for them. It was ascertained that unimpregnated mosquitoes lived the longest; those that had been impregnated lived two or three weeks, whilst the males rarely lived a fortnight. In my former investigation into filarial metamorphosis, it never occurred to me to feed my filariated mosquitoes whilst in confinement, accepting the common belief of their only feeding once and dying within a week. Manson, Lewis and Myers, who had worked at the subject, never fed the mosquitoes, and it never dawned upon me that my mosquitoes were dying from starvation.

Having discovered that mosquitoes could be kept alive long periods in confinement if fed on banana, I was anxious to ascertain what would become of the filaria, which were to be seen in mosquitoes that lived seven days; would they go on developing if the mosquitoes lived longer?

Unfortunately E.S., the filariated subject, a girl of sixteen, from whom I had obtained filariae had left the district, having secured a situation as domestic servant; she was the only person affected with filariasis I knew of.

Dr. Manson's encouragement and a grant of £7 from the Queensland Branch of the British Medical Association to defray the cost of E.S. returning and living with her parents for three months and submitting to be bitten by mosquitoes, induced me to enter upon a fresh investigation on February 1st, 1899. It was found that the actively moving filariae were to be seen, but not before the sixteenth or seventeenth day, sometimes in cold weather not until twenty days, and that no further development occurred in them even after a sojourn of sixty days in the mosquito's thorax.

The final stage of the metamorphosis, i.e., the preliminary alternation of generations, is attained in sixteen or seventeen days; (Fig. 6) the young filariae are then \( \frac{1}{15} \)" in length by \( \frac{1}{10} \)" in breadth, some only \( \frac{1}{15} \)" \( \times \) \( \frac{1}{10} \)"; there is no apparent difference except in size; there is a well marked intestine with oesophageal bulb, also some differentiation of the body protoplasm into reproductive organs (ovary and testicle) but I have not been able to make out any sexual difference.
The young filariae are generally only to be found in the thorax, yet a few occur in some instances in the abdominal cavity. There are usually three or four filariae present, sometimes as many as twenty-five. In twenty filariated mosquitoes that were killed and examined between sixteen and sixty days, every one of them contained actively moving filariae.

Mosquitoes bearing filariae do not appear to be injured seriously; one that was killed fifty days after its meal of blood contained eleven filariae in the thorax and two in the abdominal cavity.

In mosquitoes fed on non-filariated blood no filariae could be detected.

When the mosquito's thorax is torn across several times with dissecting needles in a watch-glass containing water on the stage of a dissecting microscope, the filariae are liberated and sink to the bottom; they can be seen fairly easily with the naked eye and by aid of a needle picked out; they cannot swim nor move away from the spot where they happen to sink, yet they twist and wriggle about in a violent manner; by means of what appear to be caudal suckers some of them stick to the glass, also to the dissecting needle when touched by the same.

Water is injurious to them for after three or four hours therein they die. Water therefore cannot be the medium, as was generally supposed, by which they ultimately reach the human subject.

Directly after having seen the first "actively moving filariae" wriggling about in water for a couple of hours, I concluded that water was the medium, and wrote a letter to the Editor of the Australasian Medical Gazette\(^1\) to that effect, being anxious to correct a former statement\(^2\) of mine to the effect that the young filariae died in water; [as subsequent observation has shewn that statement did not require correction]. Shortly after having written the letter I found the young filariae were dead but concluded that they must have been injured by the cyanide of potassium by which the mosquito was killed. Many experiments

\(1\) Australasian Medical Gazette, March 20, 1899. \(2\) Ibid., June 20, 1898.
were afterwards made with filariz from mosquitoes that had been dissected whilst alive to insure no injury to the filarie they contained; it made no difference however, for the young filarie always died after three or four hours' immersion in water. In mosquitoes that had died a natural death, when examined twenty-four hours afterwards, the filarie were dead; this occurred whether the mosquito died on water or not. The filarie never escape naturally from the mosquito's body.

In order therefore, for the young filarie to reach the human subject, it would appear that the mosquito must be swallowed. It is not uncommon to meet people, who have accidentally swallowed one of these insects, and it seems possible enough that such might occur, especially in those who sleep with the mouth open. In the act of killing mosquitoes with the hand their bodies are ruptured and any young filarie, that might be present, would be extruded on to the fingers and afterwards transferred to the mouth. Mosquitoes when aged frequently get bogged in jam and honey, and by such food it is possible, although somewhat improbable, they could gain entrance into the human stomach. To be infected, some may imagine that there must be an easier way than by swallowing mosquitoes; they must remember, however, that Nature has not ordained that the life-cycle of entozoal parasites shall be easily attained; obviously for the reason that were it easily accomplished, gross infection would occur causing the death of the host and with him the parasites.

Leuckart in his work¹ makes the following reference to Manson's discovery, p. 64, footnote:—"From the observations of Manson² there can no longer be any doubt that the few embryos which can pass without danger to themselves through the intestine of the mosquito undergo further development in the body-cavity, in consequence of which they now differ in size and in the structure of the mouth parts from the embryo at an earlier stage. Manson is of opinion that embryos, having thus reached a certain stage in

¹ Parasites of Man by Rudolph Leuckart—Young J. Pentland, 1886.
the body-cavity, get into water only on the death of the host, and that they are taken into the human body with the water. This statement still requires demonstration, but even were this proof forthcoming there would yet remain a possibility that the embryos evacuated with the urine (which probably no more represent a useless production than the eggs of intestinal worms which pass out with the faeces) may be transported to certain small hosts, and by these means human beings may perhaps be infected more commonly than in the way pointed out by Manson."

From these remarks it would appear that Leuckart imagined that it was a normal occurrence for embryo filariae to pass out of the body with the urine; such is not so, however, and is by no means common in those affected with filariasis; it occurs in cases only when there is rupture of a lymphatic or blood vessel in the kidney or bladder; the filariae when mixed with urine are rapidly altered by endosmosis or exosmosis and live but a short time. The same applies to dogs affected by the Filaria immitis in which however it is even of rarer occurrence.

How did it come about that Manson saw the final stage of the metamorphosis in mosquitoes seven days old?

This I believe to be the explanation:—The filariated mosquitoes upon which he made his observations were not bred out and thus in confinement from the moment of their emergence from the pupa state; they were free mosquitoes obtained from a room where filariated persons slept. A few of the mosquitoes that were captured doubtless had imbibed blood weeks before and already contained advanced stages of the metamorphosis. They were imprisoned and never fed, consequently they died about the sixth or seventh day, when they were microscopically examined. Manson evidently believed that their last meal of blood was their first.

Manson has remarked 1 "that various stages of the metamorphosis were occasionally to be seen in the same mosquito." Such

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a thing never occurred to me, and is inexplicable except on the
supposition that his mosquitoes had imbibed filariated blood on
several different occasions.

In the following details my observations differ from those of
others, who have worked at this subject.

1. Pressure of the cover-glass more particularly and endosmosis
are the cause of rupture and escape of material at the anus in the
young filariae; such is not a natural phenomenon; it will not
happen if the thorax be teased out in Müller's fluid and examined
without a cover-glass or with a small piece of cover-glass.

2. After the meal of blood is digested, the mosquito's stomach
and intestine contain no filariae.

3. The filariae after imbedding themselves in the thoracic muscles
lie quiescent until about the fourteenth or fifteenth day, when
very slight movements can sometimes be detected.

4. I have been unable to satisfy myself that the embryo filariae
cast their sheaths before leaving the mosquito's stomach; when
seen in the thorax they appear to have lost the long collapsed
sheath following tail; the sheath may however have only shrunk
or it may be filled out by the worm, which has already grown
longer and thicker; the tail is peculiar in the early stages, which
may be due possibly to retention of the sheath.

5. The filariae, which emigrate to the thorax, do so directly they
are withdrawn from the human host; those that are to be seen
in the mosquito's stomach several hours later are they, that for
some reason, whether being too young, or from injury or from
having been already acted upon by the digestive juices, are not
destined to enter upon a metamorphosis. Loss of sheath, striation
of body, changes in the body protoplasm in them are due to
endosmosis and digestion.

6. No apparent sheath can be seen in the embryo filariae in
freshly drawn blood, but a flagellum-like body generally following
the tail (Fig. 1); sometimes the flagellum-like body is momently
protruded from the head, and pari passu disappears from the tail;
this only occurs when the worm is swimming tail first; such appearance cannot be seen in every embryo, and I am inclined to think that it is not normal (Fig. 2). The flagellum-like body is the collapsed sheath, which can only be diagnosed as a sheath when endosmosis has taken place. Those who have figured the embryo, have represented a worm inside a distended sack; such appearance is unnatural. The purpose of the sheath is possibly to anchor the worm to the side of a blood vessel when the latter is resting.

Manson in his recent work¹ p. 460, has remarked:—"It is also manifest that the purpose of the "sheath" with which it is provided while circulating in the human host, is to muzzle the embryo filaria and prevent its breaking through the blood-vessels, and so missing its chance of gaining access to the mosquito."

If any should care to decide the question for himself, let him prepare a slide of filariated blood and paint a little oil round the edge of the cover-glass to prevent evaporation and examine under the microscope twenty-four hours afterwards, when a certain amount of coagulation and crystallisation has taken place; this forms some resistance to the filariae and they may be seen crossing from one edge of the cover-glass to the other in a tortuous but definite course with the collapsed sheath following tail.

I cannot agree with Manson that the sheath muzzles or impedes the filaria in any way; normally I believe the sheath is never separate from the body. The embryo in freshly drawn blood wriggles about but never seems to leave the same spot; this peculiarity was considered due to some impediment caused by the sheath, but the embryo of *Filaria immitis*, which is not possessed of a sheath, wriggles precisely in the same manner.

7. Some writers would lead you to imagine that there is but a single pair of adult filariae in each filariated subject; judging from analogy of what occurs in other animals harbouring filariae, I believe that there are generally a good many present, a dozen or

¹ Tropical Diseases—Cassell and Coy. Ltd., 1898.
so, or possibly in some cases fifty. The number of embryos that
are to be found in a drop of blood is some criterion of the number
of adults in the subject; if the embryos are scarce, it is likely
that there are few adult females, but if plentiful it is probable
there are many females.

It is not known how long the embryo filaria lives in the blood,
probably it is several months, and probably the adult worms live
several years.

Provided a filariated subject could prevent reinfecting himself,
it is very likely that in course of five years, he would be entirely
free from the parasites. To accomplish this, it might be wise to
emigrate to a country where there are no mosquitoes, and failing
that, to sleep under perfect mosquito-net bed curtains.

Fortunately it is easy to rid the house of the *Culex ciliaris*; it
appears that this insect was introduced into Australia;\(^1\) it will
not go wild but always frequents habitations, breeding in recep-
tacles holding water in or about the house; such receptacles
should be covered with gauze, net, perforated zinc or other material
to exclude mosquitoes; cattle and poultry water troughs should
be emptied out at least every ten days, as by so doing, the mos-
quitos larve could never mature; it takes fourteen to twenty days
from the mosquito egg to the perfect insect.

In this investigation the following methods were found the best.
In breeding "house mosquitoes" it is necessary to obtain their
eggs or larve. Galvanised iron washing tubs are convenient
vessels wherein to rear the larve; these are filled with fresh water
and placed in a shady spot; into them is put a handful of rotting
leaves and a small piece of flesh, preferably flesh that has passed
the putrefactive state in water, having been converted partly into
adipocere. When animal matter forms part of the diet, the larve
grow faster and to a larger size. The larve soon die should the
water become foul. In a fortnight or so the larve will have
changed into pupæ; by means of a miniature scoop-net (the size of

a tablespoon) made of wire and mosquito net the pupae are transferred to a glass vessel of water such as a fish bowl (about six inches in diameter at the mouth). The mouth of bowl is covered with muslin the material known as "white leno" was found very serviceable; mosquito net is not suitable as mosquitoes can, when they try, creep through the meshes, especially when the net is stretched tightly. The pupae do not require food, and in a day or two the perfect insects will have emerged from them. The male mosquitoes are easily distinguished by their large feathery antennae; they do not suck blood. Transference of mosquitoes to a glass cell is performed by means of a "collecting tube"; this is a hollow glass cylinder conveniently four inches long and one and a half inches in diameter, one end is covered with mosquito net, whilst a cork is loosely fitted to the other (Fig. 8); pieces of Argand gas-lamp chimney make good collecting tubes.

Glass cells, about ten inches high and six inches in diameter, are convenient wherein to store living mosquitoes; they are fitted
up as follows:—At the bottom is placed a little dry sand, also a vessel holding three or four ounces of water; the sand serves to weight the cell and steady the water vessel; into the vessel of water is put two or three bits of straw or cork, this is to assist the mosquitoes rising from the water; as the mosquitoes age they get infirm and frequently get drowned unless they reach some floating object. Over the mouth of the cell is stretched a piece of wet leno and tied tightly with twine; when the leno is dry a circular hole an inch in diameter is cut out of the centre, and this hole is covered with a watch-glass, concave side upwards (Fig. 7).

The transference of mosquitoes to a glass cell is done in the following way:—The mosquitoes are allowed to escape under the mosquito-net curtains; the cork being removed, the mouth of a "collecting tube" is placed over a mosquito, which then flies up the tube; the cork being now replaced the tube is brought close to the glass cell, the cork being directly over the watch-glass; the cork is removed and the tube put right on to the watch-glass, and at the same time the watch-glass is slid aside, the open mouths of the tube and cell are now together; a puff of air blown down the tube causes the mosquito to fly down into the cell; the watch-glass is again placed in position. By such means a dozen mosquitoes might be put into a cell in a minute without any danger of injuring them.

Female mosquitoes bred out by me were put into an empty cell of the capacity of forty ounces of water and sent to the home of the filariated subject, who liberated them under the bed curtains upon retiring; next morning any with distended abdomen she captured by means of a collecting tube transferred back to the cell and returned the same to me; they were again liberated under curtains and transferred to larger vessels. In the cell storing mosquitoes a section of ripe banana is suspended; it was found best to cut the banana at right angles to its length in pieces one and a half inches in length with the skin left on. Moulds very soon grow on the cut ends when the mosquitoes prefer to pierce the rind and thus get at the sound tissue. It is advisable to
remove the piece of banana and replace by fresh every three or four days. Should the air in the cell become foul from the decomposition of banana, or from the odour of mould fungi or the water at times contaminated by banana juice, it is advisable to liberate the mosquitoes under a mosquito net curtain and transfer them to a clean cell. It is also well to place a plug of cotton wool in the hole in leno and over this the watch-glass, concave side down. The cells are placed in a room in the house where the light is subdued, or shaded by brown paper from too strong a light. Half a dozen mosquitoes is a sufficient number to put into one glass cell of the capacity of one hundred ounces of water.

When mosquitoes are required for examination, they are liberated under the curtains, captured and killed in the entomologist's cyanide bottle, or by means of chloroform etc. Two pairs of ciliary forceps are useful with which to pull off the wings, legs and head; afterwards the body is divided by dissecting needles into thorax and abdomen, and each portion examined separately, teased out in water, or better in Müller's fluid with or without a cover-glass under a magnification of fifty diameters.

The following is a short account of the life-cycle of *Filaria Bancrofti*:—Commencing with the mature parasites in the human subject; these are three or four inches in length by \( \frac{3}{8} \) in breadth, they live in the lymphatic vessels; they produce the embryo *filariae*, which are \( \frac{3}{8}^{\prime\prime} \times \frac{3}{8}^{\prime\prime} \); these latter live in the blood vessels, swimming about when the host is sleeping and resting themselves when he is awake.

Mosquitoes when biting a filariated subject during the night withdraw together with blood some of the embryo *filariae*. Soon after the embryos reach the mosquito's stomach they pierce the stomach wall and find their way to some muscular mass, particularly the thoracic muscles, in which they imbed themselves (Fig. 3); there nourished by the mosquito's plasma they grow at a prodigious rate, becoming longer and thicker and assume by the fifth day the appearance represented in Fig. 4, in which a distinct line,
Metamorphosis of *Filaria nocturna* in Mosquito's thorax. × 200 diameters.

Fig. 1 Normal appearance of filaria in the blood.

Figs. 3, 4, 5, 6, Stages of the metamorphosis, 1—5—10—16 days respectively. A = Head; B = Tail.
the rudimentary intestine, can be seen from the mouth to the anus; the body protoplasm, at first homogeneous, has been changed into large cells with numerous vacuoles; in ten days the intestine presents a double line, the large cells have given place to very small cells, Fig. 5; from this time on to the seventeenth day most remarkable changes occur too intricate and difficult to describe; in seventeen days thereabout the young filaria has attained its maximum development as far as its life in the mosquito is concerned; it now awaits the chance of gaining entrance to the human host; in the event of which, we presume that it will start upon a second metamorphosis, the final alternation of generations, in which it grows to the length of three or four inches and becomes sexually mature.

It remains to be proved that these young filariae will become sexually mature in the human host; I have elsewhere suggested how this might be accomplished, viz., by inducing a life-sentenced prisoner to swallow some mosquitoes bearing filariae on condition that he be given a free pardon.

Besides proving that the Culex ciliaris, Linn. is an efficient host for Filaria nocturna, I have shewn that two other species of mosquito are not hospitable, viz., Culex notoscriptus, Skuse, and C. annulirostris, Skuse. Both these mosquitoes will live in confinement at least twenty days. Culex notoscriptus sucks out plenty embryos, but as far as I have seen none of these ever migrate to the thorax; they appear to have been killed by the salivary juice. Only rarely do some embryos migrate in the case of Culex annulirostris; after two days however, any that did reach the thorax have died and been absorbed. Other mosquitoes have been experimented upon, but as I have been unable to keep these alive sufficiently long for the final stage of the metamorphosis, it is impossible to say definitely that they are not hospitable, yet every thing tends to that conclusion.

In the case of Culex hispidus, Skuse and C. vigilax, Skuse, these two species live about seven days in confinement, and a

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1 Australasian Medical Gazette, March 20, 1899.
number examined about that time contained no filariae. In the
case of Culex nigrithorax, Macquart, C. procax, Skuse, and
Anopheles musivus, Skuse, I have been unable to keep them alive
more than three days; a good many experiments were made with
Anopheles musivus; this mosquito sucks out a very large number
of embryos, and the most of these migrate to the thorax.

For the scientific names of the mosquitoes I am indebted to
Henry Tryon, Esq., Entomologist to the Queensland Government.
Thanks are due also to E.S., the filariated subject, without whose
assistance this investigation could not have been carried out, and
Manson's important discovery might for some time to come have
remained unbelieved.

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EXPLANATION OF FIGURES.

Figs. 1 to 6—Stages of Filarial Metamorphosis. \( \times \) about 200
diameters.

Drawings to scale were made of the filaria \( \times \) 1,000 diameters,
afterwards reduced by photography.

Figs. 7 and 8—Apparatus for collecting and storing mosquitoes.

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Added June 1st.

A number of mosquitoes imbibed filariated blood on April 26th
and the final stage of the metamorphosis did not occur in them
until May 31st, \( i.e. \), thirty-five days. The weather was cold.

It has occurred to me that the young filariae may gain entrance
to the human host whilst mosquitoes bearing them are in the act
of biting. The entrance of warm blood into the mosquito may
excite the young filariae in consequence of which they pierce the
cæophagus and pass down the proboscis into the human skin.
In this way injury from the human digestive agents would be
avoided; it is not unreasonable to suppose that like water the
digestive fluids would soon kill the young filariae, but it is probable
that those that may have been set free by rupture of the mosquito's
body would immediately pierce the mucous membrane and enter
a lymphatic or other vessel.

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