Some of these problems are now being studied by us, with interesting results.

We had already been using this method for some time in the laboratory, when our attention was drawn to a paper by Paul Carnot and Louis Fournier,¹ who, independently and unknown to ourselves, have described a similar method for obtaining the toxin produced by the pneumococcus in cultivating media.

We are greatly indebted to Dr. Pinching Bey and Dr. Schiess Bey for allowing us to begin this work in the bacteriological laboratory of the Government hospital, Alexandria. To the latter gentleman we are especially indebted for much valuable assistance.

REFERENCE.

¹ Recherches sur le Pneumococque et ses Toxines, *Arch. de Méd. Expér.*, May, 1900.

THE PROPAGATION OF THE FILARIÆ OF THE BLOOD¹ EXCLUSIVELY BY MEANS OF THE PUNCTURE OF PECULIAR MOSQUITOS.

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PRELIMINARY NOTE.

LAST winter an English Commission, including Dr. Patrick Manson, came to Rome, as is well known, to examine the results obtained by the Italians in their study of malaria. The illustrious English pathologist, when visiting on this occasion the Laboratory of Comparative Anatomy, showed one of us (Grassi) some of his specimens bearing on the development of *filaria nocturna* in *Culex ciliaris*, syn. *Culex pipiens*. One of those specimens was of special interest, as it contained a larva of *filaria*, at its maximum stage of development, in the head of the insect, thus suggesting a probability of its being inoculated into man by means of puncture, as are the parasites of malaria. Grassi pointed to the analogy which he had observed in certain larval *filariæ* harboured by *Anopheles claviger* in the neighbourhood of Rome. Manson mentioned that Bancroft not long before had likewise advanced the same idea. The question was very attractive, and promised brilliant results. Manson and Grassi, on parting, understood all its importance.

Grassi put off to a favourable season the experiments which he proposed to carry out with the other writer of this paper (Noë) experiments which might resolve the problem in an exhaustive manner; meanwhile in his lectures on comparative anatomy he explained to his pupils the probability that the *filariæ* of the blood were propagated by means of insect punctures. However, regarding as superfluous a special publication on this point which would not positively and indisputably advance the question, and so as not to cast on the scientific field seeds of uncertain destiny, he abstained from so doing. On May 14th he received a letter from Dr. Manson which announced that having had sectioned some material sent to him by Bancroft, he had obtained the demonstration of the above-mentioned hypothesis. Grassi was pleased to hear of the confirmation of an idea which he held very probable, but at the same time it seemed to him that sufficient light had not been shed on this point, which had always seemed obscure to him. We had also seen the larvæ of *filariæ* in the general body cavity of *Anopheles* and also in the head, but we could not understand how the larvæ, devoid of any physical or chemical means for perforation, could pass through the walls of the body and especially through the chitinous carapace. It is exactly on account of this difficulty that we regarded as only probable the above-mentioned hypothesis, and that in the memoir, *Studies of a Zoologist on Malaria*, published on June 4th Grassi wrote:

The *filaria* which develops in *Anopheles* in Rome belongs to a domestic mammal into which it is inoculated to all appearances by the same *Anopheles* by means of its punctures. The experiments necessary to confirm this hypothesis, based on the position taken by the *filaria* arrived at its maximum development in the body of *Anopheles*, are now being conducted [p. 30].¹

Manson had informed us that it had been found in sections free in the proboscis, but we doubted that this might be due to the imperfect preservation of the material obtained from

Australia. It was also possible that the nematodes seen by Manson in the proboscis were not *filariæ nocturnæ*, especially as larvæ of nematodes somewhat similar to those are found in the proboscis of ordinary flies which do not puncture. This demonstrates that the above-mentioned localisation of *filariæ* is not sufficient to furnish the proof of the transmission by the puncture made by the intermediary host. Lastly, it was possible that the larvæ might have been found casually in the prolongation of the body cavity within the labium, just as sometimes are found within the labium of *Anopheles claviger* encysted larvæ of nematodes, which may be also found encysted in other parts of the body. Meanwhile, on June 16th, appeared a note by Low, a pupil of Manson's, in which² the author declares probable the transmission of *filaria nocturna* by means of punctures, because, as he says:

The larvæ of *filariæ* arrived at their maximum development, open for themselves an independent passage through the base of the labium, proceeding forward along the proboscis between the labium and the hypopharynx amidst the stylets.

But consulting the illustrations of his work, and making a due concession to the imperfections of lithography, it results, if we are not in error, that the *filariæ* are not already free, as Low describes, but are instead still enclosed in the prolongation of the general body cavity within the labium, exactly as we said before.

Fully recognising the importance of Low's work, we still consider the question of inoculation as only probable. It remains yet unexplained how the larvæ of *filaria*, which have no organ capable of perforating, came out through the carapace, and the obscurity on this point was, as may be easily understood, a serious omission, because it might have bred many other hypotheses. Only direct experiment might have thrown light on the question, and in this the English school failed; nor could it have been otherwise, because it was not possible to make any experiment on man without risking giving him a terrible disease which, so far, is incurable.

Contemporaneously our experiments progressed on the larvæ of *filaria* harboured by *Anopheles claviger*. We had already previously determined, by subjecting dogs to punctures, that the larva is derived from *filaria immitis* (Leidy).⁴

Thus was admirably explained the fact that one of us (Grassi) had noticed several years previously that *F. immitis* has a geographical distribution corresponding to that of malaria, and naturally, as we know to-day, to that of *Anopheles*.⁵

The embryos of *filaria immitis*, sucked up with the blood by *Anopheles*, migrate into the Malpighian tubes, where they continue their development, behaving more or less like the other blood *filariæ* already known. The larvæ, arrived at the maximum development possible in the body of *Anopheles*, abandon the Malpighian tube to enter the general cavity of the body, leaving behind the old cuticle,⁶ then they progress towards the head, and collect themselves there rapidly (as our transverse sections show very clearly) in the prolongation of the general cavity of the body within the labium, called also inferior labium,⁷ (exceptionally also in the palpi).

By proper experiments⁸ we have demonstrated that when *Anopheles* bites these larvæ come out of the labium, and are thus inoculated in the bitten animal. The mechanism of exit represents one of the most singular and admirable phenomena that one can imagine for the diffusion of parasites.

As is known, the *Culicidæ* introduce only six of the nine appendages which constitute their mouth apparatus. Of these two form two canals, a wide canal constituted by the superposition of the labium to the hypopharynx, the other narrow, grooved along the hypopharynx itself.⁹ The wider canal serves very likely at the beginning of the puncture to emit some gases enclosed in the three sacs annexed to the anterior intestine (which, as we know now, have certainly a sucking function); later it serves for the passage of the blood: the narrow canal serves for the emission of saliva; the four stylets (mandibles and maxillæ) cut with a saw movement. Of the remaining three pieces which constitute the mouth apparatus, the two palps during the puncture remain raised. When the mosquito proceeds to penetrate the skin the labium, as Reamnur admirably described, buckles up at first towards the base, forming an obtuse angle. As the stylet

gradually penetrate this angle is advanced towards the middle of the labium, becoming extremely acute, so much so that when complete penetration is effected the labium appears doubled up on itself, forming a narrow kind of loop, and thus forms, through the conformation of the parts which close together, a new canal. The two halves of the olive and the little tongue, resting against the skin of the animal which is punctured, embrace the six pieces penetrating the skin. It is certainly through the bending of the labium stuffed with filariæ that is brought about the rupture of the integuments of the labium; along the dorsal groove and through the rupture thus produced come out the filariæ to penetrate the body of their definitive host. It is difficult, as everyone will understand, to enter into further particulars. In some cases we believe that we positively found the rupture in the middle of the length of the labium in correspondence with the loop. It seemed to us also that the two halves of the olive and the little tongue, being in the above-mentioned position, had an importance in directing the movements of the filariæ towards the wound made by the stylets. Perhaps also the gases emitted in the first moment of the bite help the entry of the filariæ into the body of the definitive host. However, it is certain that up to now all the *Anopheles* that have punctured seemed free of the filariæ they had previously carried in their probosces.¹⁰

If the punctured animal is a dog, the filariæ continue their development, as is seen by the following experiment:

On July 19th we injected under the skin of a non-infected dog some larvæ of filariæ which we had collected in a drop of normal saline solution by pulling apart the labium of two infected *Anopheles claviger*. On August 4th, on making a *post mortem* examination of the dog, we found in the subcutaneous connective tissue near the genitals a very small female filaria, which must be judged as *F. immitis*, still immature. We were able to preserve only its anterior half—sufficient, however, for the diagnosis. Anyone who knows the difficulty of finding filariæ, especially when young, will not be surprised that we found only one.

Another dog which contemporaneously had swallowed larvæ of filariæ did not present any of these parasites in process of development, which induces us to exclude infection through the alimentary canal, as one could foresee after the facts above mentioned.

Naturally we want to obtain all the intermediate stages; therefore we have extended our experiments on many more dogs, experiments of which we shall report in due time. The facts here referred to show without doubt that the blood filariæ, as the parasite of malaria, are inoculated by the puncture of special mosquitos, although in a different way. The exit of the larvæ does not take place, as Bancroft had supposed, through the œsophagus and pharynx, nor in the way that Manson and Low had believed they had seen; the mechanism discovered by us is far more perfect and rational.

NOTES AND REFERENCES.

¹ I mean by "blood filariæ" those filariæ whose embryos circulate in the blood. ² The details of the observations on which this note is based will be published shortly by Noël. ³ BRITISH MEDICAL JOURNAL, i, 1900, p. 1456. ⁴ What Grassi and Calandruccio had long ago sustained against Sorsino is thus indirectly confirmed, that filaria immitis has nothing in common with the larvæ of filariæ which are found in the dog flea. Nuttall in his exact critical historical study has reproduced a letter of Sorsino which contains several errors referring to one of us (Grassi). I limit myself to pointing out that in the above mentioned letter Sorsino confesses to having confused *Hematoptenus* with *Trichodectes*. "Mais je reconnus mon erreur bien avant la publication de mes études"; whilst in the paper of 1888 Sorsino has written: "I must point out that the species of louse I examined, and in which I found the larvæ phases of the hæmatozoon of the dog as in the flea is not *Trichodectes latus*, as I believed at first and as I said in a preliminary note published in the BRITISH MEDICAL JOURNAL of July 15th, 1888, but is instead *Hematoptenus pilifer*, which here seems more common in dogs than the parasite above mentioned." We must notice also what Calandruccio in 1892 found in a mosquito, the larvæ of a filaria that he supposed might be in relation with filaria immitis. However, he abandoned this subject, and his observations remained doubtful because, as far as we know, *F. immitis* has not been found in Sicily. ⁵ It should be noted that for the development of filaria temperata has the same great importance as for the development of malarial parasites. ⁶ Of the many embryos which reach the Malpighian tubes only few (3, 4, 2) reach that stage of development which in *Anopheles* can be called complete. ⁷ The complete development within Malpighian tubes in summer requires about ten days, the passage into the labium takes place very rapidly (one or two days). We keep *Anopheles* for a long time alive nourishing them with fruit, especially with melons. They not only continue to live, but also deposit their eggs. We may add that the nourishment is likewise sufficient for the regular development of the malarial parasites. ⁸ Of a large quantity of *A. claviger* which had punctured a filarated dog about August 16th and 17th, 23 were introduced into a small room with a healthy dog on August 23rd. On the morning of August 24th all were full of blood; on examination, none were found to contain filariæ; many of them, however, show the sure traces of their recent presence. On the same morning of August 24th we examined 15 *A. claviger* taken from the same vessel from which had been taken the preceding, and which had not yet bitten; 5 of these showed the labium invaded by filaria; we took 20 more out of the same vessel and intro-

duced them on the evening of August 24th into the small room with above-mentioned dog. On the morning of August 25th all except one were full of blood, this one not having bitten. The first 20 were all without filaria, showing, however, evident traces of their recent presence. The last which had not punctured had the labium full of filariæ. The experiment was repeated on August 26th with 25 more *Anopheles*; of 9 sectioned without having bitten, 3 were infected; 16 introduced into the usual room after puncture were found non-infected. ⁹ In many copies of the memoir, *Studies of a Zoologist on Malaria*, the picture 12 in Plate IV representing the section of the proboscis shows this canal opened through a lithographic deficiency. We have included the possibility of the filariæ escaping through these blind ends that in the above-mentioned work we supposed were receptacles of saliva, a supposition which we now believe to be unfounded. ¹⁰ We have reason to believe that the exit of the filariæ from the labium, and therefore from the proboscis takes place also when the *Anopheles* puncture fruit. This we deduce from the fact that a great quantity of *Anopheles* certainly infected within the labium on August 5th submitted to a fruit diet presented the labium on August 10th completely empty.

SUPRARENAL GLAND EXTRACT AS A HÆMOSTATIC.

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SOMEWHAT more than eighteen months ago I suggested¹ the administration of suprarenal gland in cases of hæmorrhage from the walls of the alimentary canal, a condition which makes itself evident as hæmatemesis or melæna.

PHYSIOLOGICAL ACTION.

The rationale of the treatment was based on Schäfer's observation that suprarenal extract caused contraction of the plain muscle fibres of blood vessels when applied locally and acted as an efficient hæmostatic in epistaxis, together with the result of a series of experiments which led me to the conclusion that administration of the gland by the mouth did not cause a rise of blood pressure in normal individuals.

MODE OF ADMINISTRATION.

At that time I did not enter into detail as to the method of administration, nor the pathological conditions in which the exhibition of the preparation would probably be followed by satisfactory results.

Since many of the liquid extracts are robbed of their efficiency by time, one or two crushed 5-grain tablets given by the mouth is the most satisfactory method of administration in hæmatemesis.

THERAPEUTIC USES.

The advantage of suprarenal extract over the more popular hæmostatics lies in the fact that it acts in very dilute solution, and does not tend to combine with albumen and become inert, in a similar way to ferric chloride; it must, however, be borne in mind that it does not cause coagulation of the blood, and therefore does not seal the bleeding points with clot.

In hæmatemesis the most rational method of using iron is by administering one large dose, since some of it will combine with the blood already shed into the stomach, and an excess is required to cause coagulation of the blood oozing from the vessels and thus seal them. The correct administration of suprarenal extract in the same pathological condition offers considerable contrast; for small doses may be given, since the drug acts in very dilute solution, but it must be repeated at short intervals, for only while the vessel wall is under the action of the extract will it remain contracted; coagulation of blood is not accelerated.

Practice substantiates theory, for in cases where suprarenal extract has been administered temporary cessation of bleeding has occurred, but often recurrence of hæmorrhage has ensued after several hours when no repetition of the dose has been ordered.

Hæmorrhage from a small artery is more likely to be efficiently controlled than capillary oozing from a hyperæmic mucous membrane. It is no easy matter to foretell whether its exhibition in typhoid ulceration and hæmorrhage would be followed by satisfactory results, since it is questionable whether the extract would reach the site of the ulcer in a potent form. I have not had opportunity of making practical observations.

Two or three tablets crushed and mixed with a few ounces of water and injected into the rectum is the most satisfactory