is formed in the gelatinous masses in the bone and excreted in the urine, but no proof of this view has been offered, and against it we have the fact that similar new growths occur in other parts of the body without the appearance of anything unusual in the urine. Whatever their true connection may be, it cannot be denied that the formation of albumose in the body and its excretion in the urine is no less an essential part of the disease than is the existence of multiple myelomata. I think, therefore, that the term "myelopathic albumosuria" is a convenient one to apply to it, as it expresses the leading features of the condition without binding us to any theories as to the way in which they are associated.

Tabulated List of Published Cases in which "Bence Jones Albumose" has been recognised in the Urine—"Myelopathic Albumosuria."

Number.	Date of Publication.	Observer.	Diagnosis during Life.	Condition found at Necropsy.
1	1847	Macintyre and Bence Jones (London)	?	Mollities ossium rubra; description corresponds to multiple myeloma. ³
2	1883	Kühne	Osteomalacia	No necropsy made. ⁴
3	1889	Kahler (Prague)	Osteomalacia	Multiple myeloma. ⁵
4	1891	Stokvis (Amsterdam)	?	Bones brittle; marrow replaced by a red gelatinous mass. ⁶
5	1895	Stintzing (Jena)	Osteomalacia or multiple myeloma	Multiple myeloma. ⁷
6	1897	Senator (Berlin)	Nephritis with albumosuria	"Myelogenous round-celled sarcoma, so-called myeloma." ⁸
7	1897	Bozzolo (Turin)	"Kahler's disease"	No record. ⁹
8	1898	Naunyn (Strassburg)	Multiple myeloma	No record. ¹⁰
9	1898	Bradshaw (Liverpool)	Multiple myeloma	Multiple myeloma. ¹¹
10	1899	Ellinger (Königsberg)	None made	Multiple myeloma. ¹²
11	1900	Fitz (Boston, U.S.A.)	Multiple myeloma	Multiple myeloma. ¹³

NOTE.—Since this paper has been set up in type my attention has been drawn to a case published in 1899 by Buchstab and Schaposchnikow, of Odessa, in which the discovery of albumosuria led to a correct diagnosis of the bone affection being made during life.¹⁴

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CONTRIBUTION TO THE TECHNIQUE OF BACTERIOLOGY.

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In the course of some investigations we found it necessary to invent a method by which the diffusible products of the life of micro-organisms could be obtained without subjecting the micro-organisms themselves, or the culture media, to the action of heat, filtration, or chemical reagents.

It is known that if certain microbes be introduced in a bag of filter paper or a sack of collodion and this bag placed under the skin of an animal, the toxins produced by the microbe pass through the walls into the surrounding tissues and give rise to toxic symptoms in that animal. We endeavoured to discover a technique by which these diffusible poisons or other diffusible products could be obtained in an ordinary culture tube.

The diagram shows how the problem was solved. The apparatus consists of an ordinary test tube A filled to any required level with bouillon or some other fluid cultivating medium, and plugged with cotton wool at b. Through this plug (b) another smaller glass tube (a) is introduced to the lower extremity of which is attached a collodion sack d. This inner tube is filled to any required level with some cultivating fluid and is plugged at the upper extremity (e) with cotton wool.

We may now give some hints as to the preparation of these collodion bags, and their fixation, as we have not found in the literature at our disposal a good account as to the best method of making them. A small test tube is rapidly dipped bottom downwards into a small tumbler filled with collodion, until 2 or 3 inches of the tube are covered with collodion. It is then withdrawn and allowed to dry bottom upwards. After drying, the same process is repeated two or three times. The collodion is then found to stick tightly to the glass tube.

In order to separate the collodion from the glass tube, the whole is dipped alternately for a few seconds in strong spirit and then into water. The water and spirit diffusing through the

collodion gradually loosen it from the glass. After a few minutes the collodion bag can easily be peeled off from the glass tube. A small glass tube of such a size that it can just enter the bag is then introduced into the open extremity of the collodion bag, and the whole is then placed in the incubator at 37° C. The collodion bag now shrinks and tightly fits on the glass tube. All other methods of fastening the collodion bag have proved faulty in our hands, as the fastenings invariably allowed microbes to get through.

In order to sterilise the collodion glass tube (now called inner tube), it is fixed in an empty test tube in the manner shown in the diagram, and the whole is sterilised at 150° C. by dry heat on one or several occasions.

When this has been done the inner tube (a) together with the plug b are transferred aseptically to a tube of sterilised bouillon or gelatine of equal size, the plug b now serving to close the tube of bouillon into which the inner tube is dipped. The bouillon in tube a is now inoculated with the micro-organism to be studied, and the whole placed in the incubator. The inner tube may also be filled to the required extent, introduced into the bouillon tube, and the whole sterilised in the autoclave. Experience, however, has shown us that the first method is preferable. After a time the inner tube (a) is withdrawn, and the outer tube then contains the diffusible products which have been elaborated in the inner tube, and which have diffused through.

It is clear that this method can be used for a variety of purposes, as, for instance, the study of those products of the life of micro-organisms, which dialyse through a collodion membrane. By filling the outer tube with therapeutic serum, for example, antidiphtherial serum, and the inner tube with a diphtheria culture, the action of serum on the dialysable products of the diphtheria bacillus may be studied, and inversely the action of dialysable constituents of therapeutic serum on the bacillus diphtheriae and on its toxins.

By inoculating the liquid contained in the outer tube with one kind of micro-organism, and that of the inner tube with another, the antagonism between two microbes may also be studied.

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.

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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 palpi during the puncture remain raised. When the mosquito proceeds to penetrate the skin the labium, as Reamur admirably described, buckles up at first towards the base, forming an obtuse angle. As the stylet