RESEARCHES ON MALARIA.

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Preliminary.—Malarial fever, or as it is often called, paludism, or intermittent fever, is perhaps the most important of all diseases which afflict humanity. Broadly speaking, it is spread over almost the whole of the Tropics, and also extends into many countries which possess temperate climates—being found as far north as Sweden and Canada. Although, happily, it is not a very fatal disease; yet it is generally so prevalent in the countries in which it exists that the sum of the illness which it causes is immense. To take, for instance, the great country of India, with its enormous population of nearly three hundred millions, we find from the sanitary returns of the Government that the deaths from fever alone are given at 4,919,591 for the single year 1900; and average roughly about five million deaths yearly—a population nearly as large as that of Sweden and Norway. Although it is not possible to state that all this fever is malarial fever, there are reasons for thinking that most of it must be such. From the more exact returns of the Army and of the jail prisoners in India—returns attested by medical men—we find that in 1900, out of the total of 305,927 persons, no less than 102,640 were admitted into hospital for malarial fever during the year; and even this large figure is below the truth, because in India many slight cases of fever are not admitted into hospital at all. The following table, taken from the returns of the British troops in India for 1900, will enable us to compare the sickness due to malaria and to other diseases respectively:

1. Average Strength of Troops 60,653.

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Admissions</th>
<th>Deaths</th>
<th>Constantly Sick</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malarial fever</td>
<td>19,445</td>
<td>50</td>
<td>710</td>
</tr>
<tr>
<td>Enteric fever</td>
<td>970</td>
<td>290</td>
<td>141</td>
</tr>
<tr>
<td>Other fevers</td>
<td>1,479</td>
<td>2</td>
<td>67</td>
</tr>
<tr>
<td>Dysentery</td>
<td>1,561</td>
<td>52</td>
<td>108</td>
</tr>
<tr>
<td>Hepatic congestion</td>
<td>1,010</td>
<td>5</td>
<td>68</td>
</tr>
<tr>
<td>Hepatic abscess</td>
<td>156</td>
<td>95</td>
<td>15</td>
</tr>
</tbody>
</table>

1 It should be understood that the lecture as given on this occasion was only an abstract of the present publication.
It should be noted that the death rate for malaria is here far below the truth; because, the disease being often very chronic, many of the worst cases are invalided to Europe, while in others death is often recorded as being due to intercurrent affections, such as pneumonia or dysentery, even though malaria may have been the original or principal cause of the fatal result.

Similar statistics will be found in most of the tropical countries of the world where statistics are kept at all. Even in such a temperate climate as Italy, the annual number of cases' amounts, according to Celli, to something like two millions, while the number of deaths may be fifteen thousand a year. For the great continent of Africa we have, of course, no figures; but we know from the important discovery of Koch, confirmed by many German and British workers, that between fifty and a hundred per cent. of the negro children always remain infected—from which also we may assume that the terrible infantile mortality among negroes is largely due to this disease.

But malarial fever is important, not only because of the misery which it inflicts on mankind, but because of the serious opposition which it has always given to the march of civilisation in the Tropics. Unlike many diseases, it is essentially an endemic, a local, malady, and one which unfortunately haunts more especially the fertile, well-watered and luxuriant tracts—precisely those which are of the greatest value to man. There it strikes down, not only the indigenous barbaric population, but, with still greater certainty, the pioneers of civilisation, the planter, the trader, the missionary and the soldier. It is therefore the principal and gigantic ally of barbarism. No wild deserts, no savage races, no geographical difficulties have proved so inimical to civilisation as this disease. We may almost say that it has withheld an entire continent from humanity—the immense and fertile tracts of Africa; what we call the dark continent should be called the malarious continent; and for centuries the successive waves of civilisation, which have flooded and fertilised Asia, Europe and America, have broken themselves in vain upon its deadly shores.
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(2) The Discovery of the Parasite of Malaria.—From the first, then, the study of this potent foe of mankind has given a great occupation to science. It is not within my province at this moment to detail the early steps by which science gradually penetrated the mystery—steps, however, which are not the less interesting to follow. Though it was well known to the ancients, the disease was not clearly differentiated from other fevers until much later. Towards the middle of the seventeenth century, however, physicians recognised that in cinchona bark we possess a drug which is a specific for a certain class of fevers, namely the intermittent fevers. As Kelsch and Kiener remark, this discovery was not only an immense therapeutical benefit, but also led to a notable pathological advance, because it enabled Morton and Torti to prescribe the exact limits of the disease curable by the medicine, namely, malarial fever; and the works of these writers, especially Torti, who without possessing thermometer or microscope accurately described the intricate course of the disease, are among the most admirable works of medical science. At the end of the seventeenth and the beginning of the eighteenth century Morton and Lancisi elaborated another important conception, that the disease is produced by some poison which enters the body from without; and the latter especially clearly understood what may be called the great law of malarial fever—that it is connected with stagnant water on the ground. The next great advance was made in the middle of last century by Meckel, Virchow, Planer, Arnstein, Frerichs, and others, who discovered that the disease is characterised by the presence in the blood and some tissues of a peculiar black granular substance, the malarial pigment or melanin; and this observation led directly to the great discovery of Laveran in 1880, that the melanin is produced within multitudes of minute amoeboid parasites which live within the blood corpuscles of the patient—a discovery which not only illuminated the whole subject of malaria, but by opening a new department of parasitic pathology, has put the name of Laveran in the place of honour beside those of Pasteur, Lister and Koch.

The work of Laveran and of those who followed him affords one of the most beautiful and useful chapters in the whole book of science; and I wish that it were possible to deal with it here at length. We owe to Danilewsky, Theobald Smith and others, the discovery of similar parasites in the blood of many vertebrates, and to Laveran and Golgi the determination of several important laws concerning the whole group of these organisms. Marchiafava, Celli, Mannaberg, Metchnikoff, Canalis, Antolisei and many others added
important details; Kelsch and Bignami made minute clinical and pathological studies; Romanowsky discovered the best method of staining the parasites; Gerhardt and others produced infection by inoculating the blood of patients into healthy persons; and Richard, Councilman, Vandyke Carter, Osler, Plehn and numerous other skilled observers confirmed those results in many parts of the world. The principal conclusions reached by this mass of investigations are as follows:—

(a) That Laveran's parasite is the cause of malarial fever.
(b) That it is a sporozoon belonging to a group probably allied to the Coccidiidae, of which other members are found in birds; and that somewhat similar but more distantly related haemocytozoa are found in other vertebrates.
(c) That the organisms propagate in the blood by spore formation.
(d) That there are probably at least three varieties of the human parasites, which cause respectively the quartan, the tertian and the irregular (pernicious or aestivo-autumnal) fevers.
(e) That the paroxysm of fever commences with the release of the spores.
(f) That with all varieties of the parasites, there are certain forms which do not produce spores, but which, shortly after blood containing them is drawn from the host, emit certain singular motile filaments; and that the nature and functions of these forms still required further investigation.

(3) The Problem of the Mode of Infection.—But even after all these fine discoveries, there still remained for solution a problem of the greatest difficulty and of the greatest importance. We had discovered the pathogenetic organisms of malarial fever, and had studied them and their effects with the greatest care. This was much, but not all; it sufficed for the treatment of individual cases; but not for the prevention or extirpation of the disease on a large scale. For this we were obliged to seek a wider knowledge; the parasites occur in the human blood—but how do they arrive there? On this scientific question turned the whole prophylaxis of malaria—a subject the importance of which in connection with the future development of many of the richest portions of the world's surface I need not enlarge upon. Ignorant of the route of entry, we could rest our prophylaxis only upon an unsatisfactory empirical basis; cognisant of it, we might hope to stamp out the plague even in its most redoubtable haunts. It is my privilege in this lecture to describe particularly the steps by which this great problem has at length received its full solution.
In what manner precisely does the malarial infection reach the human blood? From early times certain cardinal facts regarding the disease have been known to us and have limited the area of investigation regarding this question. It has been recognised, first that malarial fever is essentially an endemic disease—that is, that it does not easily spread from man to man independently of locality, as do, for instance, small-pox or plague; secondly, that it adheres especially to warm localities where there is much stagnant water, such as marshes. Upon these facts, themselves perfectly true, numerous hypotheses have been constructed; notably the one dating from the times of Lancisi and Morton, that the disease is due to miasmata exhaled from the stagnant water—whence indeed the word malaria has originated; and later the allied theory of the telluric miasm, according to which the soil possesses a poisonous effluent so powerful at certain spots that it can there produce fever in man. It was even thought that when the surface of the ground is disturbed, this effluent escapes like a gas, infecting all those who live in the vicinity. These speculations afford an interesting example of the manner in which the human mind is apt to embroider fact with hypothesis. It is a fact that malarial fever is connected with stagnant water; but that the connection is due to an aerial emanation from the stagnant water was only an hypothesis which has never received experimental verification. Nevertheless it was almost universally accepted until the true explanation of the connection referred to was given in the manner which I shall presently describe.

The discovery of the pathogenetic organism by Laveran produced but little change in our ideas on this point. It was simply thought that the parasites must be capable of saprophytic life in stagnant water, and may enter the body by the inhalation of watery vapour or by infected drinking water; and, indeed, efforts to obtain experimental proof of these conceptions were quickly made, especially by Calandruccio, Marino, Agenore, and Celli, who endeavoured to infect healthy persons by means of water brought from notoriously unhealthy sources. The experiments proved, however, entirely negative—somewhat to the surprise of those who were acquainted with them. At the same time parallel enquiries were commenced to ascertain the saprophytic stage of the parasites; and Grassi and Feletti found an amœba (Amœba guttula) which they thought might be the parasites in their free condition. Their work recalls that of Crudeli and Klebs, who, before Laveran's discovery, claimed to have found the
cause of malaria in the form of a bacillus—which they asserted abounds in the water and soil of malarious localities, especially in the lowest stratum of the air, and gives typical intermittent fever to rabbits and other animals. All these observations are now proved to have been unsound.

(4) First Researches in India, 1889-94.—It is, I understand, the principal duty of those who are called to the high honour of presenting the lecture of the Nobel Medical Prize to give in it an account of their own researches; and I shall therefore begin my personal narrative at this point. I had entered the medical service of the Government of India in the year 1881; but although many opportunities for studying the malarial problem had been given me, I was not specially attracted to it until the year 1889, when I first began to observe many facts at variance with the telluric hypothesis which had been instilled into me during my curriculum. I noted especially that the disease had a much more limited and localised prevalence than could be explained on any theory of aerial convection; I found that outbreaks often appeared to occur among troops merely as the result of chill or fatigue; and that in many instances the symptoms accorded ill with the classical descriptions. These observations provoked in me much dissatisfaction with accepted theories; and gradually led me to the task of reviewing the whole subject by close analysis. Unfortunately at that time it was extremely difficult to obtain in India any of the more recent literature on the subject; and even the discovery of Laveran (1880) had scarcely penetrated there as yet—much less the work of Golgi, Danilewsky, Marchiafava and Celli. I was therefore forced to rely almost solely on my own observations and thoughts; and at first

1 More or less brief abstracts of these investigations have already been published [54, 68], but their brevity has only had the result of permitting the genesis of many errors regarding the real nature of the task. I have therefore thought it best to give in this publication a fuller, and indeed almost autobiographical, narrative of the successive events. It is scarcely possible, except by this means, to present a true picture of the difficulties in the way of resolving this intricate problem. As so often happens in science, the most important part of the investigations really consisted of the initial failures; and I have therefore described these negative results in as much detail as is given to the discovery of the pigmented cells and of the life history of Proteosoma which afterwards gave the fundamental solution of the problem. It should be added that my work was minutely recorded, not so much in publications, as in a long series of letters to Manson, Laveran, and Nuttall, and that extracts from these letters are now about to be published, together with reprints of some of my papers.
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fell into the mistaken conception, parallel with that of Broussais, that the disease may be due to intestinal auto-intoxication; and I published some papers supporting this view [12-16]. In 1892, however, several writers began to ventilate Laveran's work, but most unfortunately described, not the parasites of Laveran, but a number of artifacts. The error was speedily detected and exposed [17, 18, 20, 21], but naturally led me (and many others in India) to doubt the whole discovery. As happened with many others, although in pursuance of these studies I had made a laborious examination of malarial blood for some years, I had failed entirely to find the true parasite at all. Up to the year 1894, therefore, my work, though it gave me an invaluable training for what was to come, remained in itself quite ineffective.

(5) Return to England, 1894.—In 1894 I obtained furlough to England, and immediately on arrival sought the advice of Professor Kanthack. He assured me that I was mistaken in doubting the truth of Laveran's discovery, and referred me to Dr. Manson (now Sir Patrick Manson). Manson, to whom the parasite had been previously demonstrated in England, now in his turn showed it to me, and also made me acquainted with the invaluable and illuminating monographs of Mannaberg, and of Marchiafava and Bignami. I now collected my studies in the form of an essay (unpublished) in which I discussed the position of the malarial problem at the time, and which was accorded the Parkes' Memorial Prize for 1895. In November, 1894, Manson communicated to me his hypothesis, just formed by him, that the mosquito is the intermediary host of the malaria parasite, as he had proved it to be of *Filaria nocturna*. I was immediately and powerfully struck with this hypothesis, and at once determined to give it close experimental examination on my return to India. At the same time I remembered that the same hypothesis had been mentioned by Laveran, and I told Manson of the fact. It was not until 1899, after the solution of the problem, that Nuttall informed me of the earlier theories of King and Koch enunciating the same view. Consequently I have always thought it proper to state that my own work on that part of the malarial problem which flowed from the

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1 Vandyke Carter had accurately followed Laveran in Bombay in 1887; but I did not see his work until 1894.

2 As I found subsequently, one reason for this was that I had been working principally with old estivo-autumnal infections in which the larger and more obvious parasites (crescents) were scarce.
mosquito theory was based on the hypothesis of Manson and Laveran. But I do not wish by this admission to underrate those of King and Koch; and I shall now enter upon a short digression in order to examine all these very interesting hypotheses together.

(6) The Theories of King, Laveran, Koch and Manson.—As already mentioned, when the malaria parasite was discovered everyone who remembered the old telluric and miasmatic hypothesis thought that it must live a saprophytic existence in marshes, and up to 1894 Grassi's *Amoeba guttula* was looked upon as being possibly the free form of the organism in water. Another interpretation of the connection between malarial fever and stagnant water had, however, been noted as early as 1883 in a remarkable paper by King [2]. King advanced the view that the malarial poison is carried from the marsh to the human being by the bites of mosquitoes which breed in marshes; and he gave with great dexterity no less than nineteen reasons in favour of this position—reasons based entirely on epidemiological considerations, such as the frequency of infection in warm, moist climates in the evening, in the lower storeys of houses, and so on. He refers to a previous enunciation of this conjecture in papers by Crawford in 1807, and Notts in 1847, now apparently lost; and he quotes Manson's filaria-mosquito work as a strong reinforcement of his views; but he is evidently ignorant of Laveran's discovery, which was then slowly fighting its way into recognition. A fuller account of his excellent paper will be found in Nuttall's history [65, 66].

Laveran's conjecture was first given briefly in 1884 [3, p. 457,] evidently independently of King. Seven years later he mooted the same idea still very briefly and without giving many reasons [11, p. 147.] The similar conjecture of Koch was not published at all; but in a letter to me he says that the mosquito theory occurred to him during his first visit to India in the winter of 1883-4, and that R. Pfeiffer mentioned the matter publicly in 1892 (see Koch's letter in section 23).

As already stated, Manson did not arrive at his hypothesis until near the end of 1894, when he drew attention to it (after mentioning it to me) in a short article [22]. He based it, not upon the epidemiological considerations of King, but upon a very powerful parasitological argument of his own—which was as follows. The work of Laveran, Golgi, Marchiafava, Celli and others had clearly established that the general life-cycle of the parasites within the vertebrate host consists of a process of schizogony or asexual spore formation by means of which the organisms proliferate indefinitely
in the blood. But in addition to the sporocytes existing for this purpose, all observers from the time of Laveran had observed certain large cells, which, while they were evidently mature as regards size, did not produce spores and appeared to have no function within the body. Laveran showed, however, that a few minutes after blood containing these cells was withdrawn from the circulation they underwent a singular change—that is, they gave issue to

![Diagram of gametocytes producing motile filaments](image.png)

**Fig. 1.**—Gametocytes producing motile filaments: the tertian parasite (1-3); the quartan parasite (4-6); the estivo-autumnal parasite (7-12). From page 644 of Manson's paper [26].

a number of long, actively motile filaments, capable of separating themselves entirely from the parent cell, and progressing independently among the blood-corpuscles. There had already been a long discussion about these forms. Grassi, followed by Bignami and many other Italians, considered them to be forms of degeneration, and held that the motile filaments were products of a kind of death agony *in vitro*. The reason given for this view was that the motile filaments contain no chromatin (which is not true); but in my opinion these observers had not considered them with sufficient
attention, or they could not have thought them to be dying bodies. On the other hand, Laveran, Danilewsky and Mannaberg, who had studied them closely, came to the opposite conclusion, that they constitute in some way the highest stage of the parasite: and Mannaberg even conjectured that they may be concerned in the passage from the intracorporeal to the extracorporeal stage of the organisms—though he did not indicate the route by which he thought the passage was made. Manson's speculation broke in at this point. He accepted Mannaberg's position, and noted also the general law that parasites must attain some means of passing (at least by their progeny) from the already-infected individual into a fresh individual; that the parasites of malaria being contained within the closed cavity of the circulation cannot escape from it except by the intervention of some external agency (e.g., a suctorial insect); that the position as regards these parasites was indeed the same as that of the filaria embryos, which he had shown require the intervention of a mosquito to escape from the infected host; and that the epidemiological laws of malarial fever suggest a possible connection with the same insect. Hence it flashed upon him that the motile filaments mentioned above are really flagellate spores, which, when the parent cells are ingested by the mosquito, escape and enter the insect's tissues, developing in them into some form analogous to that of the organisms in the human blood.

Manson continued the speculation to a further point, especially in a later publication [26]. It will be remembered by those who have studied his works that in his original investigations on the development of filaria embryos in mosquitoes he had failed to ascertain that the insects live for more than a few days; he had thought that after a single meal of blood the mosquito lays her eggs on the surface of water and dies in the act of doing so. Consequently when he had traced the development of the embryos to the stage which they reach in the insect's thorax he inferred that that was the whole development, and that after the death of the host on the surface of the water the embryos escaped into the latter and finally infected man by the digestive tract. And he now applied the analogy to the malaria parasites, and thought that, similarly, after the insect's death, they enter the water and infect man either by drinking-water, as he assumed for the filarie, or by the old machinery of the aerial miasma. Thus Manson's hypothesis suggested a clue only to the departure of the parasite from the human host; it did not attempt

1 Or possibly by piercing the integuments.
to define the route of entry, the exact mode of infection. In these points he admitted that his speculation was looser, and research has shown that he was wrong—certainly with regard to the malarial parasite and probably even with regard to filaria. In another point, also, he was wrong—the motile filaments are not flagellate spores, as he thought they were. I remember mentioning to him at the time that they might possibly be of the nature of sperms—an idea which was suggested to me by Lewis, who conjectured that his trypanosoma might be that of nature [6, p. 638]. We shall see later what they really are. But these errors were immaterial. The fundamental part of his hypothesis was the close and powerful argument to the effect that the motile filaments and the parent cells from which they spring must be meant to infect the mosquito in some manner. This was more than a hypothesis; it was a great and illuminating induction. It gave the required clue to further research; and without it I am convinced the malaria problem would not have been solved at all, and we should still be engaged in a laborious and hopeless search for the parasites in water and air.

Cogent as Manson's arguments appeared to me, they were far from convincing to most other students of malaria; and in fact no one else took the trouble to investigate the matter in spite of its immense importance to humanity. In 1896, indeed, Bignami wrote a long and dexterous article attacking the whole induction [29]. He still refused to believe that the motile filaments were anything but the result of death in vitro, and added that if the induction were true, malarial fever could be propagated by patients living in the presence of mosquitoes—which he refused to consider possible. At the same time he evolved a theory of his own to the effect that mosquitoes become infected with the parasites when in the larval stage in water, and then inoculate them into man during puncture. Thus while Manson thought mosquitoes carry the parasite from man to the marsh, Bignami thought that they carry it from the marsh to the man. The latter view does not appear to me a very philosophical one, since it presupposes the possibility of organisms living normally partly a saprophytic and partly a parasitic existence in the mosquito, and then being suddenly transferable on exceptionable occasions to man. Bignami's theory, however, was exactly that of King, given much more forcibly thirteen years earlier. He mentioned that he had previously attempted to infect men by gnats brought from malarious localities, but that the attempts had failed. He also referred to experiments made by Calandruccio, who had failed to observe any development of the parasites in the stomach.
of mosquitoes fed on malarial blood. Lastly, he cites another most valuable analogy in favour of the mosquito theory of malaria, namely, that of the *Pyrosoma bigeminum*, a parasite of cattle allied to the parasites of malaria, and known by the brilliant researches of Theobald Smith and Kilborne to be carried by ticks [19]. Koch also had used this analogy, but I think that both Manson and myself had overlooked it somewhat unduly. Curiously enough all this time it seems to have occurred to no one that the mosquito may act in both roles imagined by King and Manson severally—that it may both take the parasite from the patient and also inoculate it into healthy persons. I traversed Bignami’s criticisms in an article which will be referred to later (section 11).

In considering the merits of these various hypotheses we must always remember that all of them have found no little support from Manson’s original discovery of the development of *Filaria bancrofti* in mosquitoes.

(7) **Nature of Proposed Investigation.**—We must now return to my own labours. As already mentioned, directly I became acquainted with Manson’s induction, I determined to continue my investigation of the malaria problem entirely on this new basis.

Before my departure for India I discussed with Manson the best method of procedure. We agreed that the proper course would be to select patients whose blood was rich in gametocytes (the name now given to those forms of the parasite of which some produce motile filaments), to allow mosquitoes to bite these patients, and to attempt to trace in the tissues of these insects the development of the said motile filaments (which we thought were flagellate spores). In fact, it was proposed that I should adopt exactly the procedure employed by Manson in regard to *Filaria*

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1 The development of the *Pyrosoma* in ticks is still unknown, though the second host has long been recognised. It should be understood that the history of this organism, and of the filaria in mosquitoes, while adding great force to the mosquito theory of malaria, gave us no information regarding the form and position of the malaria parasites in mosquitoes, nor of the species of insect concerned. The *Pyrosoma* is not very nearly related to the malaria parasites, and the filaria not at all. I was not aware of the work of Smith and Kilborne until much later.

2 We thought that Manson himself could not undertake the work in England, but this perhaps would not have been as difficult as we supposed. The parasites have now been cultivated in the local *Anopheles* at Hamburg and have been found in them in Holland. Work might easily have been done in this direction at home, while I was labouring in India. At all events I should have been greatly assisted by a study of British gnats.
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 bancrofti. It seemed necessary only to follow the motile filaments, after their escape from the gametocytes contained in the ingested blood in the mosquito's stomach, to their supposed destination within some kind of cells of the insect's tissues (e.g., the stomach or blood-cells)—apparently an easy task. It was true we anticipated, on the analogy of the filaria, that not all species of mosquitoes would be amenable to the malarial infection, and we recognised that this doubt would increase the difficulties; but we hoped readily to distinguish the proper species by its particular prevalence in very malarious localities. The motile filaments being traced to their habitat in particular cells of the insect, we thought that it would be easy to observe their further development, and to watch their escape into water after the host's death. This done we should be able to identify the extra corporeal form of the parasites in water, air or dew, and to ascertain exactly the route of infection of man.

(8) Preliminary Observations at Secunderabad, 1895.—I reached India in 1895 and found myself appointed medical officer of a regiment of native soldiers stationed at Secunderabad and suffering much from malarial fever. A survey was immediately made of the malarial parasites existing among these men, and I found myself able to confirm for India, in almost every detail, the specialised work of the Italians and of Mannaberg.1

At the same time I examined the mosquitoes which abounded in the barracks and hospital. Before leaving England I had made many attempts to obtain literature on mosquitoes, especially the Indian ones, but without success, except for some brief notes in encyclopædias; and I did not even clearly recognise the identity of mosquitoes and gnats, but thought that the former constituted a special division of the Culicidae.2 Consequently I was forced to rely entirely on my own observations; and I noted that the various species of mosquitoes of the locality belonged apparently to two

1 My regiment was stationed near a small marsh and suffered badly, while another regiment situated only a mile to leeward of the same marsh escaped. My regiment suffered from the estivo-autumnal and tertian varieties of parasite, quartan being quite absent; but in the neighbouring regiments of this great garrison quartan abounded—a fact which confirmed me in favour of the view that these varieties are distinct and not interchangeable forms. The work of Crombie and myself was the first done in India on this basis, that of Vandyke Carter (1887) being done on the basis of Laveran's works.

2 In spite of repeated attempts to obtain such literature I remained in the same predicament until I returned to England in 1899.
different groups, separated by many traits, and called these groups for my own convenience, *brindled mosquitoes and grey mosquitoes*. It was not until 1897 that I clearly recognised a third group, which I called *spotted-winged mosquitoes* (see sections 12 and 13).

As the grey and brindled mosquitoes abounded round the infected barracks, it was naturally thought likely that they were concerned in the propagation of the disease. After some initial difficulties I caused numbers of them (especially the brindled mosquitoes) to be fed on persons with the gametocytes of *estivo-autumnal fever* (crescents) in their blood. It should be noted that from the first I employed for this purpose only mosquitoes bred in captivity from the larvæ, and not insects caught at random in the houses. There were two excellent reasons for this; first, that the insects caught at random might have already fed themselves previously and have thus, for all I knew, acquired various parasites which might confuse my results; and, secondly, because it is easier to obtain the insects in numbers by collecting their larvæ and keeping them in vessels until they hatch out from the pupæ, rather than by catching each separately by hand. The mosquitoes were fed by being released from the breeding-jar into a mosquito-net, within which the patient was placed, the gorged insects being subsequently caught in bottles and dissected as required.

From the first I kept careful notes of my observations, and also recorded them in letters to Manson sent by almost every weekly mail, except when later, being very busy at Bangalore, I was obliged to reduce both notes and letters. The note-books and letters are still in my hands.

(9) *Secunderabad, 1895. The Motile Filaments in Mosquitoes.*

—The first point requiring study was the process by which the motile filaments escape from the parent cells (gametocytes) within the stomach cavity of the mosquitoes. The process had been frequently watched *in vitro* (in slides of liquid blood prepared for the microscope), and was known to occur in from about ten to thirty minutes after the blood is drawn from the patient; but it was now necessary to follow it in the mosquito. The insects were killed from one minute to several hours after feeding, the stomach being then extracted and its contents examined in the fresh state. I was obliged to invent the technique for myself; and my first successful dissection was made on May 13th. Within a few weeks I made a fairly complete study of the subject and ascertained an encouraging fact. *In vitro*, doubtless owing to the unnatural conditions, only about 5 per cent. of the crescents give issue to the
motile filaments; but I now found that in the mosquito's stomach something like 60 per cent. of them do so. It was also noted that the preliminary stages of the process, namely, the swelling up and rounding of the crescents, were much more constantly seen in the blood ingested by the insect than in vitro. This was of importance, because it showed at least that the insect's stomach is a more favourable locus for the process than an ordinary specimen of blood is. I observed also that a considerable percentage of the crescents (about one-third) never produced motile filaments at all, even after the lapse of several hours and within the insects; and I noticed that those which refused to emit them had a slightly different appearance to the others. At the time I thought that they were parasites which had been killed in some manner during ingestion; but when I repeated the experiments next year I saw cause to doubt this, and felt some difficulty in explaining why all the crescents did not emit filaments, as they should have done, according to Manson's hypothesis regarding their nature.

A description of these first results was written in June, but was not published until the end of the year [24].

(10) Difficulty of the Task. New Methods Devised.—The fact, then, was established that the gametocytes are not immediately killed in the mosquito's stomach (as might well have happened), but indeed emit their motile filaments more readily there than in vitro. It was necessary now to seek the destination of the latter in the insect's tissues; and here the true magnitude of the task to which I had set myself became manifest. Manson had been able to follow the migrations of the filaria embryos with comparative ease because they are large organisms readily distinguishable from the fluids or tissues which surround them; but the motile filaments are exceedingly delicate bodies, the movements of which are very difficult to follow even with the highest powers of a good microscope and in the clear spaces of an ordinary preparation of blood; but the blood in a mosquito's stomach shortly becomes a thick grumous mass in which it is impossible even to see the filaments unless they are in active movement. Moreover, even this assistance was denied me; for I speedily ascertained that within a few minutes of their escape they seemed to lose their movements. At least they constantly disappeared as if by magic; and in spite of all artifices I

1 It was easy to distinguish those which had produced the filaments by their collapsed condition afterwards.
failed to ascertain what had become of them. In fact, to trace them further, to follow the migrations of these well-nigh invisible bodies through the masses of cells of which so large an animal as a mosquito is composed, was indeed an impossible task with the means which I possessed.

Hence, though Manson then and later constantly advised me in his letters to adhere to the plan of following the motile filaments, I determined to abandon the quest and to employ other methods; and he himself failed to obtain any results with the insects which I sent to him from time to time. It was most fortunate that I came to this decision so early, because events have proved that the motile filaments migrate nowhere, and do not enter the mosquito's tissues at all.

The first method, which I now adopted and which ultimately led to success, was the following: By hypothesis, the motile filaments, after reaching the particular cells of the mosquito for which we thought they were destined, should grow in them into some unknown but larger form. It was impossible to predict what this form would be. Manson conjectured that it would very likely be some intracellular form similar to the intracorpuscular stages of the organism in the human blood—fixed perhaps in the stomach cells or blood-cells of the insect. Personally, however, while I thought this view possible, I had no full faith in it. It seemed to me that the mosquito stage of the parasite might be anything, so long as it was of a protozoal character.

The protean changes of many of the parasitic worms warned us that nature was capable of ordering any extraordinary transformations in the interest of parasites; and as no case was yet known of a protozoon capable of wandering from one species of host to another, I had no guide as to what might happen with the organisms which I was studying, and conjectured that, for all we could say, the motile filaments might develop into almost any form—amœboid, coccidiform, gregarinoid, or even infusorial, small or large. What was nearly certain, however, was that they were likely to grow in size after a few days' residence in the mosquito—to become more visible, and, if they were to pass into the water as we thought they would do, to take a definite form of resistance which ought to be easily recognisable. My new method then was to give up the

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1 It might now be possible, though still difficult, to follow them by staining them either in dehaemoglobinised blood or in section; but the Romanowsky reaction was not known to me at that time.
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attempt to follow the newly escaped and almost invisible motile filaments, and to dissect the mosquitoes, not at once, but after the lapse of some days, during which time the motile filaments should by hypothesis develop into something more tangible. I proposed then to feed the mosquitoes as before on cases with crescents in the blood, to keep them alive for some days; and then search their tissues for any parasites which might occur in them. The parasites found, it would be easy to determine whether or not they are derived from the motile filaments, simply by ascertaining whether or not they also occur in mosquitoes of the same kind fed on healthy blood. Throughout the investigation it was of course necessary to employ only what I called in bacteriological parlance “sterile mosquitoes,” that is, mosquitoes freshly hatched from the larvae in captivity, and therefore not contaminated by previous feedings.

Such was the procedure now adopted, but the difficulties involved even in it were very great. As the situation of the sought-for parasites could not be indicated with any certainty, it became necessary to search for them through all the tissues of each insect examined, to scrutinise by a powerful microscope, one by one, all the minute cells composing the huge aggregate of which the insect consists. To investigate a single insect thoroughly in this manner required at least two hours’ exhausting and blinding work. Added to this difficulty, I had no clue as to the form and appearance of the object which I was seeking for; nor was I even sure that the kind of insect under examination was amenable to the infection at all. I was looking for a thing of which I did not know the appearance, in a medium which I did not know contained it. In short, it was a mere blinding groping for some clue which I trusted fortune would give in the end. As an instance of the difficulty of such work, I may mention that neither the organisms of yellow fever, which is now known to exist in a particular kind of mosquito, nor the Pyrosoma of Texas cattle-fever, which is known to exist in a tick, have yet been found in these animals, though long searched for by competent observers. Nevertheless, I am confident that, hopeless as the method may appear, it was the only one capable of solving this difficult problem.

At the outset of the investigation it was necessary for me to become thoroughly acquainted with the normal histology of the mosquito—for which I had again to trust to my own observation.

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1 Under a magnification of a thousand diameters a mosquito appears as large as a horse.—“Researches on Malaria.”
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in spite of all efforts no literature on the subject could be obtained by me. It was also necessary to note and study the ordinary parasites of these insects, of which I found a number during the ensuing years. Indeed, at the commencement of the work, I found one which required careful working out. It was a pseudo-navicella occurring in the malpighian tubes of the brindled mosquitoes (Stegomyia). After a little study it was ascertained that pseudo-navicellas have no connection with the parasites of malaria, being the sporocysts of a species of gregarine. Next year Manson published an account of these interesting organisms taken from my letters to him [26]. I refer to them also in my publication at the end of the year [24].

The second method alluded to above was based on the following considerations. According to Manson's more advanced hypothesis, the motile filaments, after living some days in the mosquito, would probably pass from it into the water, on the surface of which we then supposed it usually died after laying its eggs. Such water, then, ought to be infective to human beings, either when ingested, or perhaps when inhaled as a vapour. It would be easy to test this speculation by experiment. I caused a number of mosquitoes, both of the brindled and grey varieties, to feed on a selected patient, and then kept them in large jars containing water at the bottom, until they died one by one. The water was then exposed to sunlight, and otherwise allowed to remain in the condition of marsh water. Different batches of fed mosquitoes were introduced into the jars from time to time so as to make sure that the water should indeed contain the parasites which by hypothesis should escape from the insects. In May, 1891, I gave draughts of this water to three natives, who volunteered themselves for the experiment. All of them declared that they had not suffered from fever for years. Strangely enough, one of the men developed a mild but marked attack of fever in eleven days, the parasite being found in his blood. I was naturally much pleased with the success of the experiment, and began to hope that the mode of infection had been found; but the failure of many subsequent attempts of the same kind forced me later to reject any definite conclusion on the point.

1 The experiment was justifiable owing to the slight degree of illness usually produced by malarial fever in natives when properly treated.
2 Owing to the interest of Surgeon-Major Owen, the Maharajah of Patiala had at this time offered the Government of the Punjab to employ me at his own expense to study malaria in his dominions. The Government of the Punjab, however, refused the offer.
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(11) Bangalore, 1895-97. Progress of Work.—Possessing abundance of material, together with plenty of leisure, I was now progressing excellently with my researches (though without definite results) when I received my first interruption. On September 9th, 1895, I was placed on special duty by the Government of India to combat a serious outbreak of cholera in the large town of Bangalore, and also to report on the general condition of sanitation there. This duty was of great interest and value to me because it afforded me an unrivalled opportunity for examining in the closest detail the general sanitation of a tropical city—an experience which has stood me in good stead during later years. For four months, however, I was so busy with my new labours that I had little time for research. Bangalore I already knew well, having, indeed, made some of my first studies of malaria there when Staff-Surgeon of the town from 1890-93. I now easily ascertained, by the light of Laveran's great discovery, that the cases of fever which I had attributed to intestinal auto-intoxication were nothing but examples of aestivo-autumnal infection among partially immunised natives. I found also that, as at Secunderabad, my brindled and grey mosquitoes abounded all over the place. I dissected a few mosquitoes as time allowed, and when my more arduous sanitary duties began to diminish in March, 1896, I found that I could give an hour or two a day to the work. My results, however, remained constantly negative, in spite of the closest scrutiny of many mosquitoes. At the same time I continued my attempts to produce infection by water.

In March, 1896, Manson delivered the three Goulstonian Lectures at the Royal College of Physicians in London, and again put the case of his hypothesis in an admirable manner, supporting his arguments largely upon my observations of the previous year [26]. He wrote to me frequently for fed mosquitoes, which I sent to him whenever I could. He also urged me to keep on the track of the flagellated spores; advised me to try infection experiments with the insufflation of dried and powdered mosquitoes, and with the vapour of an artificial marsh in which fed mosquitoes had died. These devices did not appear as promising to me as they evidently did to him. It was scarcely likely that dead mosquitoes could do much in regard to the dissemination of malaria in nature, at least in the form of dust, owing principally to the fact that dead insects seldom escape the ants in the Tropics. All dust, moreover, is generally subject to the intense heat of the sun, which, except in the presence of water, must be very inimical to most unprotected organisms. The small amount of time at my disposal was therefore devoted to the methods already attempted.
Towards the middle of the year I had made nineteen experiments with a view to carrying infection by drinking water; and together with three more cases, I described these in a publication at the end of the year [30]. Water, in which mosquitoes fed on cases of malaria had died, or which contained large numbers of the pseudonavicelle of the gregarines of mosquitoes, was given to various persons by the mouth. The majority of the attempts were entirely negative; but nevertheless a slight but noticeable reaction did occur in three of the whole number of twenty-two cases. This still remains a very curious circumstance; but the facts were published exactly as they were found, without the influence of the personal equations. At the end of the paper I summarised my results and decided that the positive reactions, though interesting, were too few and too slight to warrant any definite conclusion. I am now inclined to think that they may have been due to the following circumstances. The persons on whom the experiments were made were generally low-caste Indians who required a fee before drinking the water and also an assurance that they would receive more if taken ill. Now it is well recognised that many natives are constantly infected with malaria and get relapses on any extraordinary demand being made upon their systems, as by fatigue, chill, or dissipation. I have even heard it stated by medical men possessing large experience of natives, that they can often produce fever in themselves by exposure when they wish to do so. In this case, at all events, it was possible that some of the subjects spent their preliminary fees in dissipation, thus producing the supposed reaction after the experiments.

These results not being as decisive as I had expected from the first experiment of the kind made in the previous year, I began to consider whether some other route of infection was not possible or probable; and it soon grew upon me that Manson’s induction was exigent only as regards the entry of the parasites into mosquitoes, and that his secondary hypothesis regarding their escape from the insects and their infection of man through drinking water was not so strong. I quickly thought of several other routes for infection—which will be examined presently; and first I considered it possible

1 My actual words were, “While we cannot dream of stating definitely on the strength of these experiments that there is something connected with the mosquito which is capable of imparting fever, the three positive results are still curious and tend to be in favour of the truth of Manson’s theory.” Yet one of my Italian critics has attempted to prove, by ignoring this passage, that I pretended to have established infection by drinking water.
that the insects, previously infected from diseased persons, or possibly from other mosquitoes, might then inoculate the parasites into healthy persons during puncture, or might deposit them on the skin during haustellation. It was easy to test this view immediately by experiment, and early in August I made a small series of observations which were published in the same paper [30].

A number of mosquitoes all bred from larvae in captivity, and of all the kinds which I could collect (many specimens of brindled and grey mosquitoes) were fed upon several patients with numerous parasites in their blood. One of these patients had all three kinds of parasites in him; and I specially employed this case, as well as many varieties of mosquitoes, in order to increase the chances of one at least of the species of mosquitoes present being appropriate for one at least of the species of parasites. After feeding, the insects were kept alive for one or two days and were then applied in considerable numbers on two occasions to Mr. Appia, Assistant-Surgeon of the Bowring Civil Hospital at Bangalore, who courageously volunteered for the experiment. Mr. Appia had suffered from malarial fever some years previously, but not since then; so that if he should be attacked by fever shortly after the experiment, it would be strong evidence, if not proof, in favour of the inoculation theory. He remained, however, absolutely free from fever. He was then bitten by five mosquitoes which had been partially fed immediately before on a case of crescents—on the supposition that the insects may carry the infection mechanically, as the tsetse-fly carries nagana; but the result was again negative. Lastly, two other individuals were bitten by mosquitoes fed from three to five days previously; still without effect. I judged, then, either that infection is not produced in this way, or that the proper species of mosquitoes had not been employed, or that they had not been kept for the proper period after feeding; and I proposed to return to the subject again. It should be noted that these experiments of mine were made quite independently, and before I had heard of the theories of King and Bignami—as indeed was stated in another publication of mine at the end of the year [32, p. 251].

In July, 1896, Bignami’s criticism of Manson’s hypothesis, referred to in section 6, appeared in Italy [29]. I heard nothing about it whatever, until I received Manson’s letter of October 12th, which was accompanied by a translation of the critique. Bignami’s paper was not a profound one, and consisted only of a copious and dexterous rendering of ideas which were new only to those who had not already fully considered the subject. His objection to Manson’s
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theory was based principally on Grassi’s loose speculation that the motile filaments are the result of the death of the parasites in vitro. As this was a vital point in the chain of reasoning I now set to work to examine the subject experimentally, and was soon able to show that the escape of the filaments depends on certain proper conditions, and not at all on the death of the parasites. Thus they escape more readily when the specific gravity of the blood is altered, either by the abstraction of water by partial evaporation or, as Marshall proved, by the addition of a little water. On the other hand, they do not escape at all, even when the parent cells perish, so long as the blood is kept scrupulously unchanged. In order to prove this, I drew the blood from the finger into a small mass of vaseline placed upon the skin, and then mounted the whole for the microscope in such a manner as to prevent the blood coming even into momentary contact with the air. The result was that not a single crescent emitted motile filaments or even underwent the preliminary change of spherulisation, although it was evident they all died after a time. This experiment completely disposed of the death-agony theory of the Italians. Previously to this, however, Sarcharoff had shown that, contrary to Grassi’s statements, the filaments do contain chromatin; but I could not procure a copy of his work [23]. I should add that after long observation of the filaments I could never bring myself to believe that they are merely the result of the spasmodic movements of dying protoplasm; and this tale was in fact never anything but a gratuitous assumption.

These researches were published later [32, 33], and were confirmed by Manson and Rees in London [37]. The conditions required by the crescents for emitting filaments were now clearly seen to be those obtaining in the mosquito’s stomach, where the blood is rapidly altered by abstraction of water; and I therefore continued my work without further reference to Bignami’s objection.

His view that infection may be caused by inoculation had already been considered and experimented on by me, as just mentioned. But it should be understood that Bignami’s hypothesis (which was the same as that given long previously and much more

1 If the preparation was opened and the blood momentarily exposed to the air within some hours after abstraction from the patient, the crescents could be seen at once to resume their functions. But if this experiment was delayed about twenty-four hours, the crescents no longer reacted, and indeed showed clear evidence of death by their vacuolisation and other structural changes.
strongly by King) was very different from mine. King and Bignami thought that mosquitoes bring the poison from marshes to man; this speculation had not occurred to me until I read Bignami's paper in October, and then it did not appeal to me at all, because it was self-evident that the connection between malaria and marshes could be sufficiently explained by the fact that mosquitoes breed in stagnant water. My speculation was that mosquitoes become infected from men (according to Manson's induction) and possibly also from other mosquitoes, and then communicate the parasites to healthy persons—perhaps by inoculation. It will be seen which view is right; but in consequence of my negative experiments, the inoculation theory was not much favoured by me until I made my researches in the Sigur Ghat (Section 12).

My duties at Bangalore continued for a year and a half. At first placed upon special duty to report on the sanitation of the town (80,000 inhabitants), I was afterwards appointed officiating Residency Surgeon there and was required to reorganise the whole of the sanitary arrangements, to create a health department, to participate in a committee designated to reconstruct the municipal regulations, and to contend against several outbreaks of cholera. Consequently I did not possess as much time as at Secunderabad for my researches on malaria, but nevertheless, in addition to the experiments last referred to, I was able to dissect many hundreds of mosquitoes in pursuance of my principal plan of campaign. Several agents were employed to collect the larvæ of as many kinds of mosquitoes as possible, especially from several spots whence most of the cases of fever came; and these insects, belonging to many species of the brindled and grey groups of mosquitoes, were all tested by direct feeding on cases of malaria, especially autumnal. But though each insect was examined with the utmost care, almost every cell being scrupulously searched for parasites, the results still remained entirely negative.

Towards the end of my stay in Bangalore, as failure followed failure, I was naturally forced to reconsider the whole basis of my work. But no; the most critical examination of Manson's induction failed to exhibit any flaw in the fundamental reasoning. The gametocytes, and the process by which the motile filaments escape from them after the blood is drawn from the patient, could only be meant for infection of the mosquito. There was no other explana-

1 Not once as yet had I come across the dappled-winged mosquitoes; though, be it noted, many of my larvae were collected from ditches and the edges of ponds.
tion. Nature does not create these complex phenomena for nothing; and the theory must be—was—sound. What, then, was the cause of my repeated failures? Was it possible that the kinds of mosquitoes which I had tested hitherto—very many kinds—were all of the wrong species?

The reasons for and against this view were as follows: In all the districts and towns of India in which I had served or stayed during fifteen years—Madras, Bangalore, Moulmein, the Andamans, Secunderabad, Upper Burma, Bombay, Poona, Calcutta, Karachi, Quetta, the Nilgherry Hills, malaria was undoubtedly present, especially among the natives; and in all of them without exception I remember to have noticed mosquitoes belonging to both the grey and the brindled classes. This naturally suggested a connection between the disease and the insects; but, on the other hand, were not the latter perhaps too common? So far as I could ascertain, the disease was generally limited to certain spots and localities (by no means always near marshes); whereas the insects were everywhere, and were indeed often commonest at points where malaria was rare, as in the houses of Europeans. After all, may not the true malaria-bearing variety, or varieties, have been overlooked by me? Possibly they were comparatively rare species, or species occurring only at a certain season—a hypothesis favoured by the well-known fact of the seasonal variation of malaria. Now, as I was fully aware at the time, malarial fever is a relapsing disease, in which attacks continue to occur for years after infection; so that it does not follow by any means that the infective variety of mosquito must always be present in a locality, even though numerous cases of malarial fever are present. And it was to be specially noted that most of the cases occurring in Bangalore were probably only cases of relapse.

These arguments were not strong enough to be conclusive on either side of the question. I had done quite right in spending so much time over the grey and brindled mosquitoes; there was enough *prima facie* evidence against them to demand a full enquiry. But before spending more time over them it was now advisable to see whether further light could be obtained by epidemiological investigation. The towns in which I had worked hitherto could scarcely be considered more than moderately malarious; I now proposed to visit an intensely malarious spot, at the height, too, of the malarious season, in order to ascertain what kind of mosquitoes prevailed there at the time, and reasonably hoped that this kind would prove to be the guilty species.

Being a servant of Government I could not of course go where I
pleased without leave, and I therefore first attempted to interest Government in my work. Owing to my representations the United Planters' Association of Southern India took up the matter; and the Honourable Mr. Bliss, Member of Council of the Madras Government, and also Surgeon-General Sibthorpe, head of the Madras Medical Service, were kind enough to give their warm assistance—for which I shall always be much indebted. The result was that the Government of Madras made a proposal to the Government of India that I should now be placed on special duty to investigate malaria. Most unfortunately, however, in addition to the plague, the Afridi war broke out just about that time, and owing to the paucity of medical officers the Government of India was obliged to reject the proposal—May, 1896. But in the meantime I had determined to begin the enquiry at once at my own expense during two months' leave which was due to me; and accordingly, on the completion of my duty in Bangalore, I went to the Nilgherry Hills for the purpose of studying the point referred to in some of the intensely malarious plantations at the foot of these mountains.

(To be continued.)