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ABSTRACT

RESEARCHES ON MALARIA

BEING

THE NOBEL MEDICAL PRIZE LECTURE FOR 1902

BY

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Contents.

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RONALD ROSS.

(Being the Nobel Medical Prize Lecture for the year 1902, delivered at Stockholm on the 12th December.)*

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:

* It should be understood that the lecture as given on this occasion was only an abstract of the present publication.

Average Strength of Troops 60,653.

Disease	Admissions	Deaths	Constantly sick
Malarial Fever	19,445	50	710
Enteric Fever	970	290	141
Other Fevers	1,479	2	67
Dysentery	1,561	52	108
Hepatic Congestion . .	1,010	5	68
Hepatic Abscess	156	95	15
Heat Stroke	174	52	8
Cholera	107	89	2
Contagious Diseases . .	18,049	14	1,650
Total	42,951	649	2,769

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 whlch it has always given to the march of civilization 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 civilization, 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 civilization 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 civilization, which have flooded and fertilised Asia, Europe, and America, have broken themselves in vain upon its deadly shores.

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: —

(1). That Laveran's parasite is the cause of malarial fever.

(2). That it is a sporozoon belonging to a group probably allied to the Coccidiidæ, of which other members are found in birds; and that somewhat similar but more distantly related hæmocytozoa are found in other vertebrates.

(3). That the organisms propagate in the blood by spore formation.

(4). That there are probably at least three varieties of the human parasites, which cause respectively the quartan, the tertian, and the irregular (pernicious or æstivo-autumnal) fevers.

(5). That the paroxysm of fever commences with the release of the spores.

(6). 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 effect 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 aërial 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 [8, 7, 4], 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 amoeba (*Amœba guttula*) which they thought might be the parasites in their free condition [10]. 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 localized prevalence than could be explained on any theory of aërial 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

* 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.

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 fell into the mistaken conception, parallel with that of Broussais, that the disease may be due to intestinal autointoxication; 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.

* Vandyke Carter had accurately followed Laveran in Bombay in 1887; but I did no see his work until 1894.

** As I found subsequently, one reason for this was that I had been working principally with old aestivo-autumnal infections in which the larger and more obvious parasites (crescents) were scarce.

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 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 *Amœba 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 Nott 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

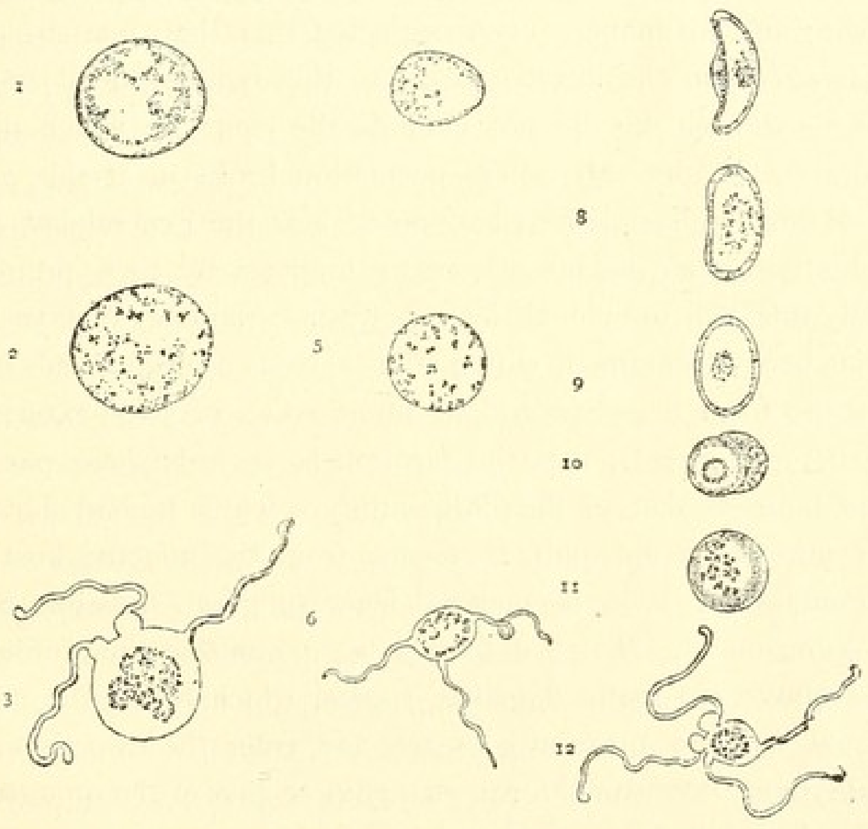

Figure I. Gametocytes producing motile filaments; the tertian parasite (1—3); the quartian parasite (4—6); the æstivo-autumnal parasite (7—12). From page 644 of Manson's paper [26].

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 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 conjected 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 filariæ, or by the old machinery of the aërial miasma. Thus Manson's hypothesis suggested a clue only to the departure of the parasite from the human host; it did not attempt 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 filariæ. 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'

* Or possibly »by piercing the integuments».

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 exceptional 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 rôles 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

* 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.

** 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.

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 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.*

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 encyclopedias; and I did not even clearly recognise the identity of mosquitoes and gnats, but thought that

* 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 æstivo-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.

the former constituted a special division of the Culicidæ.* 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 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 æstivo-autumnal fever (cresents) 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 10 to 30 minutes after the blood is drawn from the patient; but it was now necessary to follow it in the mosquito. The in-

* In spite of repeated attempts to obtain such literature I remained in the same predicament until I returned to Engtand in 1899.

sects 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 the 13th May. 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 five per cent of the crescents give issue to the motile filaments; but I now found that in the mosquito's stomach something like sixty 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

* It was easy to distinguish those which had produced the filaments by their collapsed condition afterwards.

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 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

* It might now be possible, though still difficult, to follow them by staining them either in dehæmoglobinised blood or in section; but the Romanowsky reaction was not known to me at that time.

into almost any form — amoeboid, 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 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 larvæ 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 blind 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,

* Under a magnification of a thousand diameters a mosquito appears as large as a horse.

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; 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-navicellæ 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 1895 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;

* The experiment was justifiable owing to the slight degree of illness usually produced by malarial fever in natives when properly treated.

but the failure of many subsequent attempts of the same kind forced me later to reject any definite conclusion on the point (section 11).*

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 the 9th September 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 æstivo-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

* 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.

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 pseudo-navicellæ 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 equation». 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

* 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.

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 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 larvæ 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 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 the 12th October, 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 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, Sacharoff 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].

* 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 24 hours, the crescents no longer reacted, and indeed showed clear evidence of death by their vacuolisation and other structural changes.

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 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 æstivo-autumnal. But though each insect was exa-

* Not once as yet had I come across the dappled-winged mosquitoes; though, be it noted, many of my larvæ were collected from ditches and the edges of ponds.

mined 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 explanation. 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 were 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.

12. *The Sigur Ghat; 1897.* — I arrived at Ootacamund, the great hill station of the Nilgherry Hills, at the beginning of April, 1896. This station which is about 8,000 feet above sea-level, is surrounded by numerous tea and coffee plantations, scattered here and there in the rich valleys of the hills, and even for some distance out on the plains which encompass the hills like a sea. After enquiry it was determined to begin the investigation in the Sigur Ghat, a long natural trench which cuts at one stroke from the Ootacamund plateau right down to the plain, and which had the worst reputation for malaria. A *dâk bungalow* (rest house) and a small plantation existed near the top of the trench, at a place called Kahutti about 5,500 feet above sea-level; and owing to the fact that a single night spent lower down the valley was thought enough to ensure

a bad and perhaps fatal attack, I determined to lodge here and visit the lower valley only during the day time. Nevertheless even at Kalhutti I found almost everyone suffering from fever — which was ascribed to miasmata floating up the ravine from the plains below; and I had been there only a few days and had paid only one diurnal visit to the plain when I myself suffered a bad attack of æstivo-autumnal infection, the diagnosis being confirmed by the microscope.*

After two weeks' energetic treatment with quinine I was well enough to resume operations; and this time went direct to the plantations at the foot of the Sigur Ghat. The owner of one of them, Mr. Kindersley, wise enough to reside in the hills during the intensely malarious season of the year, very kindly placed his house in the plantation at my disposal; so that I was able to make a thorough survey of the locality. Both plantations are situated in the midst of luxuriant forest and undergrowth close under the declivities of the mountains, and are copiously watered by irrigation channels. Almost all the native *employés*, as well as some families of aborigines, were suffering from severe malaria — anæmia, emaciation, and enlarged spleen; and the parasites were easily found in the blood of some of them. But I was not a little astonished when I discovered that mosquitoes appeared to be almost absent in all the houses. In spite of considerable rewards which were offered for their capture, and in spite of the efforts of my trained servants and myself, scarcely any were secured. I was informed indeed by some of the *employés* that they were often bitten at night by insects which escaped in the morning; but these nocturnal visitors were not procurable.** Later however, we were told of some insects which haunted the jungle and bit in the daytime under the trees. I found these to be a small kind of brindled mosquito, and strongly suspected that they might be the culpable species; and accordingly examined them closely and called them *Culex silvestris*.

A part of my mission here was to enquire whether the mosquitoes in this highly malarious spot did not contain parasites which were not con-

* This case was remarkable for the brevity of its incubation period. I had never suffered before from malaria, and was not likely to have acquired the infection either at Bangalore or Ootacamund. I had arrived at Kalhutti at 6 p. m. on the 22nd April, and my attack commenced at 10 p. m. on the 25th April. I ascribed it at the time to my visit to the plain made on the 23rd April; but there is now little doubt that the infection was acquired at Kalhutti itself, which was swarming with mosquitoes, and where the servant of the dâk bungalow and all his family were ill. At the same time I do not remebember to have been bitten by mosquitoes, and said so in my published account.

** Judging by our present knowledge, these must have been the offenders.

tained in the mosquitoes of the less malarious Bangalore. If they did so these parasites might reasonably be suspected of being the mosquito stage of the malaria parasite; and the question could subsequently be tested by experiment. These mosquitoes were at once found to contain two new kinds of parasites, namely crowds of active swarm-spores in the intestine, and, secondly, clusters of spores (each cluster containing eight bright oval spores) in the ventral nervous system. A close study was made of these organisms; but they did not appear in some of the jungle mosquitoes which had been fed on patients. Strangely enough however, a person who volunteered to swallow a number of the swarm-spores in water was attacked subsequently with fever, the malaria parasites, however, not being found in his blood; but I heard afterwards that, contrary to his statements, he had had fever just previously.

It will be remembered that Manson's secondary hypothesis suggested that the motile filaments, after living for some time in the mosquito, pass from it into the water, and thence by ingestion or inhalation into man. My experience, however, tended to convince me that if such infection of water takes place at all it must be very limited — in other words, that after their escape from the dead mosquito, the organisms can neither travel far in the water nor live long there. For if they could do this, almost all water in India would be infected, and the disease would be universal, instead of being confined, as it is, to certain spots. For the same reason the miasmatic theory never appealed strongly to me. I thought it most likely that men became infected from small stores of drinking water such as wells, cisterns, and even pots and ewers, into which infected mosquitoes often fall and die while laying their eggs — a theory which would easily account for the isolation of the malady, because, as I had observed over and over again, mosquitoes seldom wander far from their haunts. As, according to hypothesis, the organism escape from the gnat into the water in which she lays her eggs, it followed that water which contained most larvæ should contain most malaria parasites, and, conversely, that drinking water free from larvæ would probably be free from parasites. Now in attempting to apply these considerations to the case of the Sigur plantations, I found them at once opposed by many facts. Not only were there few adult mosquitoes there, but the larvæ could be found only in a few stagnant puddles in the depth of the jungle, while the drinking water was obtained from rapid streams just issued from pure mountain springs, in which larvæ neither existed nor were likely to exist.

These facts again forced me to reconsider the whole of Manson's secondary hypotheses, and to search for more plausible theories. Three such theories occurred to me. I had long observed that while they are sucking blood, gnats deposit minute drops of excretæ on the skin every ten seconds or so; and I had actually shown that these drops may contain the pseudo-navicellæ of gregarines. It was therefore possible that they might contain the spores of the parasites of malaria, which might then be able to work their way through the skin and into the blood of the victim. Another hypothesis of mine was that the malarial spores might be voided by the insects, not upon the skin, but upon rotting vegetation or damp earth (e. g. the floor of the houses and huts of natives), and might there possibly develop into some extracorporeal form capable of infecting man by air-borne spores.* The third theory was that infected mosquitoes could in some mysterious manner introduce the parasites directly into the blood during the acts of puncture and haustellation. This view was similar to that of King and Bignami, with this difference that while these observers thought that the mosquitoes derived the parasites from marshes, I held, in consequence of Manson's induction, that they derived them from patients. In the account of my work in the Sigur Ghat which was published a few months later [40] it was stated that this was the hypothesis which I now held to be the probable one.**

It was during these researches that I first noticed the »dappled-winged mosquitoes«. While looking for mosquitoes in a vacant rest house at the

* This was by no means an idle conjecture, and was indeed strictly based upon the analogy of Cunningham's life history of the *Amoeba coli*, which that observer stated was voided from the intestines of cattle and afterwards formed pseudo-plasmodia in the exposed dung — men and cattle being infected by the air-borne spores of these pseudo-plasmodia. He thought that the organisms were related to the Mycetozoa and called them *Protomyxomyces coprinarius*. His important statements have been ignored but not disproved by subsequent writers. Similarly I thought that the parasites of malaria might possibly be extracted from the circulation by mosquitoes, be deposited by them upon the damp floors of dwelling houses and there develop in a like manner. This hypothesis was at that time as cogent as any other.

** I said, »On the whole from a consideration of the epidemiological facts I should be inclined to favour the idea of contact being the mode of infection; and may add that one of my servants who was employed in catching the adult *silvestris* by allowing them to settle on his legs and arms was attacked five days afterwards by the quartan parasite.« By contact I meant contact of the mosquito with the skin as explained further on by the following words: »Since the presence of a human being in the jungle at once causes a number of *silvestris* mosquitoes to attack him on all sides, it is very clear that he may readily be infected by their agency, either by injection of the parasite through the puncture, or by its deposition on the skin in the shape of spores contained in the insect's fæces, which, observation shows, are always discharged in quantity during the act of haustellation.« My theories regarding infection are also referred to in my previous paper [30].

foot of the *ghat* I captured an insect resting in a peculiar attitude with the body-axis at an angle to the wall (as I noticed at the moment). On examination, its wings were found to have a series of black marks along the anterior nervure; but as I saw no more individuals of the species, I did not think the observation to be of sufficient importance to be included in my paper. Yet, had I only known it at the time, this was the very species I was in search of!

Indeed the whole of this investigation afforded a clear example of the well-known ambiguity of epidemiological work. Of the kind of insect which was really causing the disease at the time, I saw but a single individual! The reason is now quite apparent. Unlike the grey and brindled mosquitoes which rest in the dark corners of dwellings by day in large numbers, many species of dappled-winged mosquitoes fly out at daybreak. It is true that other species of this genus have more domestic habits and can therefore be more easily found; and if fortune had been my friend in those days she would have brought me to a place where these species abound—such as places afterwards visited by me in Assam and the Darjeeling Terai. Nor does it follow in any case that the predominant species of mosquito in a locality must be the malaria-bearing species there; there is no reason why the innocent species should not outnumber the dangerous species even in the most malarious spots: while lastly, it is now known that the dangerous species may abound where there is no malaria at all. Hence, though I did not know it at the time, it is impossible to indicate, much less to certify, the malaria-bearing species by its numerical relations with other species in malarious localities.

One of the principal results of my work in the Sigur Ghat was that it led me to doubt the probability of infection by drinking water. I should have liked to remain there much longer; but on the expiry of my leave was forced to return to my regiment at Secunderabad, five hundred miles away, and was never able to visit the place again.*

13. *Secunderabad; 1897. The Fundamental Discovery.* — On my return to Secunderabad (July 1897), the first thing I noticed was that the malaria had continued unabated during almost two years since I had left; if anything it was worse, and many recruits who had recently joined the regiment had been attacked—as they averred, for the first time. This clearly

* I had been offered an appointment in Berar, but had declined it in order to carry on these researches in the Sigur Ghat. I suffered severely for this later on.

showed that these cases were not merely relapses, and that some cause of infection was actually at work among the troops. It was for me to discover the cause; and I determined to return to my old method, and to test experimentally all the kinds of mosquitoes prevalent anywhere near the barracks. I had now been studying the subject almost constantly for over two years, and had become so very familiar with the microscopical appearance of the various structures of the mosquito* that I felt the mosquito stage of the parasite could no longer escape me if it existed at all. Numerous »cases of crescents» suitable for the experiments were in my hospital, and it was obvious from the number of fresh cases occurring that the proper kind of mosquito must be somewhere about. If I failed it could only be because there was some flaw in Manson's induction.

At the same time a possible fallacy was detected in the logic of that part of the theory which suggested that the motile filaments after their escape from the parent cells in the mosquito's stomach must take up their abode *in the tissues* of the insect. The vital and inevitable part of the induction consisted only of the reasoning which inferred that the stomach of the mosquito is the natural *locus* for the escape of the motile filaments. It was only conjecture to say that they must enter the tissues; because for all we knew it was possible that they might *remain in the intestine* for some time and then be voided, probably in some altered form, either upon the ground or upon the human skin (see my hypotheses in the previous section). It was therefore now necessary to examine the evacuations as well as the tissues of my subjects.

I commenced work by making a careful survey of the various kinds of mosquitoes which were to be found in the officers' quarters, in the regimental hospital, and in the numerous little houses of the native soldiers, which constituted the barracks or »lines», as they were called. I found first, the insects with which I was familiar during my previous studies here in 1895, namely (a) several species of brindled mosquitoes, and (b) two species of grey mosquitoes. But at the same time I was astonished at observing that the whole place was overrun by swarms of (c) a small and delicate variety of mosquitoes, which were at once observed to rest with the body-axis at an angle to the wall, and which had spotted wings. In fact they were evidently of the same genus (though not of the same species) as the mosquito which had been previously found

* This does not mean that I was equally familiar with the *macroscopical* anatomy of the mosquito—a subject which has only recently been dealt with fully.

in the Sigur Ghat—a genus, or perhaps family, quite distinct from those of the grey and brindled mosquitoes with which I had hitherto been working.

It is now time to speak more particularly of all these mosquitoes. I had written repeatedly to Manson, to various booksellers in England, and to several persons in India who I thought might help me, for some literature on the subject; but could obtain nothing except a few notes by popular authors, such as Thomas, who wrote on piscatorial subjects in India. I could not even obtain any adequate works on the anatomy of insects in general. Of Ficalbi's work on European gnats — which would have helped me immensely—I was ignorant, and received no copy. Manson had found the name of one species of mosquito which I sent to him; but this did not help me, for what I required was a scientific work on the structure and classification of the mosquitoes as a group. I was therefore obliged, as mentioned in section 10, to trust to my own rough methods of classification; and these were based, not on the criteria of entomologists, such as the structures of the mouth parts or the nervures of the wings, but on the general appearance and markings, the eggs, the habits, etc., of the insects. It was only the working classification of an amateur without literature to guide him, and made for his own convenience; but, as events have proved it was roughly correct. Up to July 1897 I recognised the two following groups:—

(a). *Brindled Mosquitoes* (now recognised as belonging to the genus *Stegomyia*, Theobald). Body and legs boldly marked black and white, or brown and white. Wings plain. Biting voraciously, mostly in the daytime. Resting with abdomen hanging towards the surface of attachment, and the last pair of legs tilted on the back. Breeding mostly in pots of water. Larvæ floating head downwards and possessing short stumpy breathing tubes. Eggs black, oval, and laid separately.

(b). *Grey Mosquitoes* (now recognised as belonging to the genus *Culex*, Linn. as defined by Theobald). Back barred with transverse brown and white stripes. Legs and wings plain. Biting somewhat timidly, mostly at night. Resting with abdomen hanging towards surface of attachment. Breeding mostly in wooden tubs, ditches, garden cisterns, and drains. Larvæ floating head downwards and possessing long breathing tubes. Eggs elongated and somewhat lanceolate and laid simultaneously in »rafts.»

I had found mosquitoes of the same genera, though possibly of different species, at Bangalore and at several spots in the Nilgherry Hills; and also at Bombay, Poona, and Madras during short visits made to these cities in connection with my sanitary duties at Bangalore. I remembered also to have seen similar insects in Burma and the Andamans; so that it was reasonable to suppose that they constituted the common or ordinary kinds of mosquitoes in India. The new mosquitoes which I now and subsequently met with, and named dappled-winged mosquitoes, were evidently of quite another genus to the foregoing, and were distinguished by me by the following characteristics:—

(c). *Dappled-Winged* or *Spotted-Winged Mosquitoes* (now recognised as belonging to the genus *Anopheles* Meig.). Body, legs, and proboscis marked brown and white, or dark and light brown. Wings with several dark blotches on or near the anterior nervure. Resting with abdomen pointed outward from the surface of attachment. Body more elegant, and shaped like that of a humming-bird moth. Breeding mostly in natural pools of water on the ground. Larvæ floating flat on the surface of the water like sticks, and possessing no breathing tube at all. Eggs laid singly; cohering in triangular patterns, and shaped like an ancient boat with raised prow and stern, and surrounded with a membrane which — when the egg is seen in profile — gives the appearance of a bank of oars to the boat.

In the spotted-winged mosquitoes which I now found at Secunderabad I noticed at once the general difference of shape, the peculiar attitude of the insects when at rest, the marks on the wings, and the appearance of the eggs (as seen within the body of the female when dissected); but the larvæ could not be studied until later.* The adults were very delicate, pale brown creatures, which by common consent seemed scarcely to bite man, though they were numerous enough to have caused much irritation had they done so. They swarmed in my own quarters, but seldom bit me. They abounded also in the houses of the other officers of the regiment, who, with their families had remained quite free from malarial fever. Consequently I was not disposed to think that they had anything to do with the disease. On the other hand the grey mosquitoes swarmed in the barracks, but were much less numerous in the officer's quarters (situated some hundreds of yards to leeward of the barracks). Suspicion therefore first attached to the latter variety.

I determined, however, not to be swayed by such considerations, but to make a most complete and exhaustive test of all the varieties which I could procure—even at the cost of repeating much of my old negative

* It was principally my assistant, Mahomed Bux, who ascertained, as a general rule, the attitude of the larvæ.

work, during which, laborious as it was, I may have overlooked the object I was in search of. A number of natives were employed to collect larvæ from far and wide round the barracks. These larvæ were kept in separate bottles, and when the adult insects appeared they were released within mosquito nets in which the patients were placed. The insects were applied sometimes during the day in a darkened room; and were sometimes fed all night. After feeding, the gorged insects were collected in small bottles containing a little water and were kept for several days before being dissected. The procedure was therefore the same as before; but now, in order to ensure at least definite negative results, redoubled care was taken; almost every cell was examined; even the integument and legs were not neglected; the evacuations of the insects found in the bottles, and the contents of the intestine were scrupulously searched; at the end of the first examination staining reagents were often run through the preparation, and it was searched again with care. The work, which was continued from 8 a.m. to 3 or 4 p.m. with a short interval for breakfast, was most exhausting, and so blinding that I could scarcely see afterwards; and the difficulty was increased by the fact that my microscope was almost worn out, the screws being rusted with sweat from my hands and forehead, and my only remaining eye-piece being cracked, while swarms of flies persecuted me at their pleasure as I sat with both hands engaged at the instrument. As the year had almost been rainless (it was the first year of plague and famine) the heat was almost intolerable, and a punkah could not be used for fear of injuring the delicate dissections. Fortunately my invaluable oil-immersion object-glass remained good.

Towards the middle of August I had exhaustively searched numerous grey mosquitoes and a few brindled mosquitoes. The results were absolutely negative; the insects contained nothing whatever. Then, I think for the first time, I began to feel that the long quest had been in vain and that a flaw existed somewhere in the induction. The disease was there, the mosquitoes were there—how was it that I found nothing? I may perhaps be pardoned for dwelling on my personal feelings during that time, and the astonishing time which followed. Science too has its drama; and the actor on that real scene cannot help being moved when he remembers it — although it may appear trivial enough to others.

I had remembered the small dappled-winged mosquitoes, but as I could not succeed either in finding their larvæ or in inducing the adult insects to bite patients, I could make no experiments with them. On the

15th August, however, one of my assistants brought me a bottle of larvæ, many of which hatched out next day. Among them I found several dappled-winged mosquitoes, evidently of the same genus as those found about the barracks, but much larger and stronger. Delighted with this capture I fed them (and they proved to be very voracious) on a case with crescents in the blood. Expecting to find more in the breeding bottle and wishing to watch the escape of the motile filaments in this new variety, I dissected four of them for this purpose immediately after feeding. This proved to be most unfortunate, as there were no more of these insects in the bottle, and the results as regards the motile filaments were negative. I had, however, four of the gorged dappled-winged mosquitoes left; but by bad luck two of the dissections were very imperfect and I found nothing. On the 20th August I had two remaining insects both living. Both had been fed on the 16th instant. I had much work to do with other mosquitoes, and was not able to attend to these until late in the afternoon when my sight had become very fatigued. The seventh dappled-winged mosquito was then successfully dissected. Every cell was searched, and to my intense disappointment nothing whatever was found, until I came to the insect's stomach. Here, however, just as I was about to abandon the examination, I saw a very delicate circular cell apparently lying amongst the ordinary cells of the organ, and scarcely distinguishable from them. Almost instinctively I felt that here was something new. On looking further, another and another similar object presented itself. I now focussed the lens carefully on one of these, and found that it contained a few minute granules of some black substance exactly like the pigment of the parasite of malaria. I counted altogether twelve of these cells in the insect, but was so tired with work and had been so often disappointed before that I did not at the moment recognise the value of the observation. After mounting the preparation I went home and slept for nearly an hour. On waking, my first thought was that the problem was solved; and so it was.

Next morning I returned to the hospital with much apprehension lest the eighth and last dappled-winged mosquito should have died and become decomposed during the night. It was alive; and was killed and dissected with much anxiety. *Similar bodies were present in it, only they were distinctly larger.* The seventh mosquito had been dissected four days after finding; the eighth five days after feeding; the parasites in the latter had lived a day longer than those in the former and were consequently larger. Both insects had been bred from larvæ in captivity; both had been fed for the first time on the same person—a case of malaria; no such objects as these pigmented cells—as I then called them—had ever before been seen in the hundreds of mosquitoes examined by me; the objects lay, not in the stomach cavity of the insects, but in the thickness of the stomach wall; all contained a number of black granules precisely similar in appearance to those contained by the parasites of malaria, and quite unlike anything which I had ever seen in any mosquito previously. Lastly, these two mosquitoes were the first of the kind which I had ever tested.*

The mind long engaged with a single problem often acquires a kind of prophetic insight, apparently stronger than reason, which tells the truth, though the actual arguments may look feeble enough when put upon paper. Such an insight is mainly based, I suppose, on a concentration of small probabilities each of which may have little weight of itself; but in this case at all events the insight was there and spoke the truth.

These two observations solved the malaria problem. They did not complete the story, certainly; but they furnished the clue. At a stroke they gave both of the two unknown quantities—the kind of mosquito implicated and the position and appearance of the parasites within it. The great difficulty was really overcome; and all the multitude of important results which have since been obtained were obtained solely by the easy task of following this clue—a work for children. We may rest assured that if these observations had not been made we should still have remained ignorant of the mode in which this important disease, with its annual death roll of millions, is propagated—aye, and would have remained ignorant of it until some one else had taken up the same investigation by the same method.

And no other method would have solved the problem. It was necessary to find not one but two unknown quantities, and neither could be found by itself. There are no phenomena which would serve to indicate the kind of mosquito. In nearly all malarious places there are many kinds of mosquitoes, and, as in the Sigur Ghat and other places, the malaria-bearing species are in no way predominant among them either in

* On the assumption that these cells had developed from the motile filaments it was difficult at the moment to explain the pigment within them — as the motile filaments have no pigment. I thought it possible, however, that after fixing themselves in the stomach wall they might be able to derive hæmoglobin from the contents of the organ, and afterwards convert this into the pigment.

numbers or in any other way. Indeed the malaria-bearing species occur in places where malaria has not been known in the memory of man, as around Liverpool. By what process of reasoning then could we isolate the species? It might possibly have been practicable to detect it by a very long series of experiments aimed at infecting men by the bites of successive species of mosquitoes; but no one would have undertaken such a work without the guide of a very strong theory in favour of inoculation by the bite; and the theory of King and Bignami to this effect was little more than a conjecture. It was not likely that the first species tried would have given successful results, as my own experiments of 1896 showed. Even if, after a multitude of costly and dangerous experiments, a positive result had been attained by this method, it would always be open to doubt (seeing that the experiments would have to be done in a malarious country) whether the case was not merely one of relapse; and another long series of experiments would be required to eliminate this doubt. And then, even when the proper species of mosquito was detected, there would still be no guide to the form and position of the parasites within it, or even to the way in which they enter the insect (Bignami thought that they enter the larvæ from marsh water). No, the thing was not practical. Bignami himself abandoned his experiments on his own theory after the first failure [29] and did not resume them until after my work had clearly indicated both the kind of mosquito implicated and the route of infection. The only practicable method was to attempt to find both unknown quantities simultaneously by the »trial and failure system»—such as I adopted.*

The discovery of the pigmented cells, therefore, ended for me at least the old research, the period of doubt, the groping in the dark. The secret spring had been touched, the door flew open, the path led onward full in the light, and it was obvious that science and humanity had found a new dominion. But it was necessary to follow the clue forthwith; to watch the development of the pigmented cells in mosquito after mosquito; to ascertain what became of them; to fathom the mystery of the route of infection; and then — to save human life in the gross, perhaps to open continents to civilization.

The first thing was to obtain more — hundreds — of these large dappled-winged mosquitoes. Alas, the man who had found them had, contrary

* I mention these facts because many writers on the subject seem to think that the original discovery was made merely by catching the first mosquito and finding the pigmented cells within it.

to my orders, put the larvæ from many sources in the same bottle! All the larvæ from all these sources were collected — but no more dappled-winged mosquitoes! I turned then to the small but similar variety which swarmed about the barracks. Being evidently of the same genus, they too would probably harbour the parasites; but though my men and myself searched high and low for their larvæ, we could not find them. I could scarcely even persuade the adults to lay their eggs in captivity.

Thinking that in spite of all my care I may have overlooked the pigmented cells in the grey and brindled mosquitoes, I now searched for them in the stomachs of a number of these, but without result. A number of the small dappled-winged mosquitoes caught about the hospital were also examined for them in vain. These observations served however for a »control» on the two positive cases.

Owing to the great heat at Secunderabad I had been obliged to leave my family at Ootacamund, and was now compelled to go to Bangalore for a few days in order to settle them there for the remainder of the summer. This gave me leisure for writing a report to the government of India on the discovery of the pigmented cells, and also a short paper on the same subject for publication. The latter was of course intended only as a preliminary to a detailed report which I hoped to be able to publish in a few months and which I thought would contain the full explication of the whole problem. I described my method in a few opening lines, being careful to note that the mosquitoes used by me had been »bred in bottles from the larva». The mosquitoes were then described as well as possible — the spots on the wings and the peculiar shape of the eggs being noted, but reference to the peculiar attitude being inadvertently omitted. Next I gave in detail the circumstances under which the pigmented cells were found, together with a description of them; and finally discussed, very guardedly, their probable relation to the parasite of malaria. I had brought the original preparations with me, and now showed them to my friend Surgeon Major John Smyth, who at my request

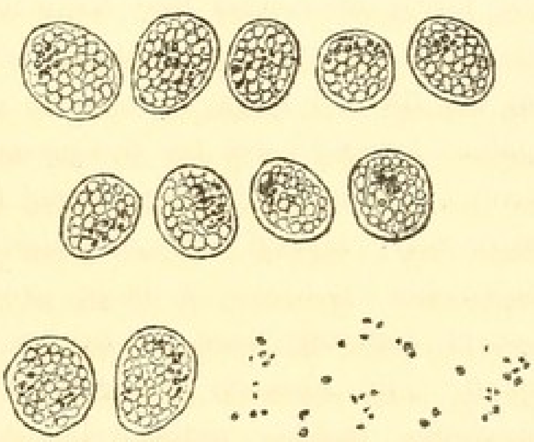

Figure 2. Pigmented Cells (zygotes) of æstivo-autumnal parasite in dappled-winged mosquitoes (*Anopheles*). From Ross's paper, British Medical Journal, 18th December 1897, page 1787.

kindly added a note to my paper, corroborating my description. They were then despatched by post to Manson. My paper, however, did not appear until December [38]; but when it did so it was accompanied by an excellent drawing of the pigmented cells furnished at the instance of Manson, and also by remarks of Manson, Bland Sutton, and Thin, who discussed the new objects — the last holding that the cells were ordinary cells of the stomach wall into which malarial pigment had entered in some manner from the stomach cavity. This preliminary article was published by me for the express purpose of guiding the researches ot others; and in fact anyone who had read my description of the pigmented cells and of the dappled-winged mosquitoes would now have had little difficulty in repeating my work.*

On my return to Secunderabad I was much disappointed to find that the larvæ of neither the large nor the small species of dappled-winged mosquito had yet been collected. Consequently in the intervals of searching for them, I spent my time in examining the stomachs of all the mosquitoes I could catch for the pigmented cells. I hoped especially to find them in the small dappled-winged insects caught about the hospital, where there were several cases of malaria, but was disappointed. On the 18th September, however, a large grey mosquito was observed feeding on a patient suffering from the benign tertian parasites and was promptly secured. The stomach was full of black blood, so that it must have fed previously (freshly imbibed blood showing red in the insects) as well as on this occasion. It was kept until the 21st and was then dissected. To my delight the pigmented cells were again found, in considerable numbers; but they were larger even than those of the mosquito of the 21st August. As this particular insect had not been bred from the larva in captivity I could not say for certain where it had become infected, but I thought it likely that it had been feeding on the case of tertian all the time (that is from about a week before it was killed) as the patient was in a bed by himself in a corner of a large nearly empty ward. Hence I naturally inferred as a probability that the pigmented cells in this insect were derived from that case; and I thought that their large size suggested that they must have been so derived about a week before the insect was killed. But of course I could not speak with absolute assurance on these points.**

* This is exactly what was done by the Italian observers fifteen months later (see section 23).

** This mosquito also contained a number of the swarm-spores which I had observed in the Sigur Ghat.

Meanwhile swarms of small grey larvæ had been found in an isolated pool of rain-water, which I had overlooked because it was on the top of a hillock where pools were not likely to exist. On hatching out, these were found to be the long-sought larvæ of the small dappled-winged mosquitoes. I observed at once that they had no breathing tubes and that their attitude was peculiar as compared with the larvæ of other mosquitoes; and noticed also that the pool in which they were found seemed too shallow and evanescent for the latter — facts shown by me and my colleagues in 1899 to be of the greatest importance in connection with the prevention of malaria. Directly enough of the adults appeared from the larvæ in the breeding-bottle, they were released in large numbers within the mosquito-net of a patient with crescents in his blood. Next morning only two of them were found to have fed themselves. One was killed next day, but nothing was found in it. The second was killed the day after, and was found to contain a large number of very small pigmented cells! This really almost clinched the matter; for three out of four dappled-winged mosquitoes bred from the larvæ in captivity and fed on cases of crescents had been found to contain pigmented cells; while these cells could not be seen in insects of the same kind which had not been so fed. Just at this time I wrote to Manson, in a state of unbounded delight, that he might expect to know the full life-history of the parasites of malaria in the mosquito within a few weeks.

Next day however, I received telegraphic instruction from Government ordering me to proceed forthwith to Kherwara in Rajputana — a place a thousand miles distant!

14. *Interruption; September 1897—February 1898.*— It would be difficult for others to understand the effect of this cruel blow. Here in Secunderabad I had numerous cases of malaria in my own hospital, and, moreover, the men had been trained to submit to mosquito bites—a matter often of some difficulty with the superstitious natives of India. I had also experienced assistants hired by myself for the work; and, above all, the proper kind of mosquitoes, including their larvæ, just found in abundance. There is no doubt whatever that, had I been left at Secunderabad, I could easily have traced the whole life history of the human parasites in dappled-winged mosquitoes within a few weeks. But at Kherwara I did not know what would happen. It was in the north; winter was approaching; and I knew that mosquitoes would refuse to bite in

the cold. I failed even to guess the reason for this sudden transfer. The astonishing discovery of the pigmented cells had been officially and fully reported to the Government through the chiefs of my own department; malaria is the most important disease of India; and I thought that my superiors were taking the greatest possible interest in researches which touched so vital a subject — I thought that they would make every effort to leave me undisturbed, if not to give me active help.

But the orders were peremptory and not to be discussed. Within two days (26th September) I was on the week's journey to Kherwara. I saw only one gleam of comfort. It was impossible that my chiefs, medical men, would consent to interrupt my work at such a moment. There must undoubtedly be a bad outbreak of malarial fever at Kherwara which would throw great light on my subject.

When I arrived at the place however—a petty station with three or four Europeans (whom I shall always remember for their kindness), and part of a native regiment of Bhils, isolated in the midst of miles of wild country far removed from civilization—I was told that there was no malaria there; there had not been a case for months!

This then was my Elba — almost my Île du Diable; and I saw no prospect of escaping from it for a year at least. After excusing myself from accepting the appointment in Berar, I had indeed later asked to be remembered for a permanent appointment to which I thought my long service (more than sixteen years) and my work at Bangalore had at least given me some claim. But this was only a temporary and insignificant one, generally held by juniors; and I do not know why the transfer was made, unless possibly (though not certainly) for reasons connected with the Afridi war. At all events it was made without reference to my researches. I wrote officially to my superiors, begging to be allowed to return to Secunderabad to continue my work; but received only a reprimand in consequence. There was no escape; but my pension was due to me the following April, and I made up my mind to apply for it as soon as the war was over, and to continue my researches as a private person.

The cold weather came on apace, and at first it appeared to be utterly impossible to work. There were no cases of malaria and scarcely any mosquitoes. Much to my pleasure, however, I found a few dappled-winged gnats, and observed again that their larvæ lived in water *on the ground* — namely in a pit and an old well — apparently almost as dormant as the adults were. I kept a single one alive in a bottle for two months without its developing.

Shortly after arrival at Kherwara I wrote down a brief account of the finding of the pigmented cells in the third and fourth mosquitoes. At the end of January the British Medical Journal containing my previous paper on the cells [38], together with remarks by Manson, Bland Sutton, and Thin, reached me. I therefore rewrote the beginning of my second paper; and added a reference to some work which I had been able to do with pigeons, and also a long discussion of Thin's remarks, in which I showed that his position with regard to the pigmented cells was untenable. The paper was published in February. I did not explicitly say that the third dappled-winged mosquito had been bred from the larva in captivity, because it was evident that this fact would be inferred from the opening of the first paper of which the second was obviously a continuation. But I said that the grey mosquito in which pigmented cells had been found was »*observed* feeding on a patient», and that »I judged for many reasons that it had been feeding occasionally on the same man for several days» — showing clearly enough that this insect had not been bred from the larva in captivity. The facts might have been put more explicitly at the time; but they are apparent enough to any candid reader.* In the paper the order of the third and fourth mosquitoes is changed for purposes of description — the case of the grey mosquito being put last because it was doubtful.

The work with pigeons just referred to was as follows. Being unable to obtain cases of human malaria I turned to the malaria of birds which had long been known to harbour parasites closely similar in appearance and life-history to the malaria parasites of man. Both Manson and I had long recognised the technical advantages of working with these organisms. I immediately found the parasites of Labbé's genus *Halteridium* in the pigeons of Kherwara; but could not induce mosquitoes to bite the birds. Observing, however, that they were infested by a species of blood-sucking fly, I examined thirty of these, and some lice, fed on infected pigeons. No pigmented cells were, however, found in them.

* When I wrote these papers I did not suspect that every line of mine, even in some of my private letters, would be subjected to a minute and unscrupulous analysis in the hope of finding discrepancies which would serve to discredit my observations. Every possible artifice has been used for this purpose by the very men who learnt all they knew from these very publications.

At last when the weather became warmer in February several cases of quartan fever occurred among the troops, probably relapses. The dappled-winged mosquitoes still refused to bite; but I succeeded in feeding a number of brindled mosquitoes of a peculiar brown species on the cases. The results were again negative in thirty-four of these insects.

I was just about to apply for my pension when welcome news arrived. I had of course given full details of my sudden transfer to Manson; and he had exerted himself to influence the government of India and the Director General of the Indian Medical Service (then Surgeon General Cleghorn) to put me on special duty to continue my researches. I had urged the same thing upon the Director General; but, unfortunately as it happened, suggested that one good place for the work would be Assam, where an epidemic of *kala-azar* — a disease which Rogers had recently reported to be malaria — had long been raging. However, I now received a telegram stating that I had been placed on special duty to investigate malaria and kala-azar in Calcutta and Assam for six months.* My five months' imprisonment was at an end. I arrived in Calcutta on the 17th February 1898; and was joined there by my family, with all my books and notes which had been with them at Bangalore all this time.

15. *Calcutta; February—April, 1898. The Theory Proved.*— Now in recompense for the tribulations of Kherwara, opened a glorious time, during which the amazing story of malaria was unrolled little by little. The great induction had given the clue; now, following the clue step by step, we were to be led into regions where nature revealed herself wonderful beyond the imagination of any of us. In the background was something greater still — the possibility of saving human life on the large scale.

I am happy to be able to begin this part of the narrative with a brief account of the brilliant and important discovery of MacCallum. It will be remembered that Manson had thought the motile filaments to be flagellated spores; that I had studied them much without being able to learn anything new about them except that they are certainly living organisms; and that when I finally found the pigmented cells I thought that these were derived from the motile filaments, and had absorbed their melanin from the hæmoglobin in the stomach cavity of the insects. In his letter of the 11th August however, Manson sent me a paper by Si-

* Afterwards extended to one year.

mond, suggesting that the similar motile filaments of certain *Coccidia* are not of the nature of flagellated spores at all, but of the nature of sperms [35]. How were these facts to be reconciled?

In a letter dated the 17th November 1897 Manson informed me that a discovery had been made by W. G. MacCallum in America regarding the motile filaments, showing independently that they are of the nature suggested by Simond's work. He did not send me the literature; and as his letter reached me at Kherwara I could not then obtain it. Shortly after my arrival at Calcutta however, I procured a copy of the Lancet [36] which gave an abstract of MacCallum's work. The discovery was as follows.

In 1897 Mac Callum undertook a study of the motile filaments. Working with the *Halteridium* of birds he noticed first that the gametocytes seemed to be of two kinds, namely one kind which produced the motile filaments, and another kind which did not do so. On watching two of these cells, one of each kind in the same field of the microscope, he observed (July 1897) that the filaments escaped from one as usual; that it moved about actively for a time; and then approaching the other gametocyte actually entered it. Other observations of MacCallum and Opie, made both on Halteridium and on the crescentic gametocytes of the æstivo-autumnal parasite of man, confirmed this beautiful discovery. The fact, as previously shown by Sacharoff, that the filaments contain chromatin was now explained; and also the facts that they escape and move about in the blood. They are, indeed, sperms which are emitted from the one kind of gametocytes, the males, and which fertilise the other kind, the females. Thus these minute parasites, among the lowest of creatures, have their sexes, and a form of sexual reproduction precisely like that of the highest animals.

More than this, MacCallum observed in the case of Halteridium of the crow that the female cell, motionless before fertilisation, afterwards becomes elongated and vigorous, and moves across the field *in vitro*. This motile form had apparently long been seen by Danilewski and had been called by him a *vermicule*.*

So much for the motile filaments, but now what were the pigmented cells? Everyone seems to have thought that as soon as the flagellate

* I should certainly have observed these facts when I was making a special study of the motile filaments in 1895 and 1896. I repeatedly saw them apparently attacking leucocytes [42, page 14]. The reason why I found that only a percentage of crescents emit the filaments in the mosquito's stomach is now explained — the remainder were females (section 11).

spores disappeared, so did Manson's theory. But it was not so. The induction remained as strong as before; the locus of the phenomenon was still in all probability the stomach-cavity of the mosquito. MacCallum's work seems to have reached Manson shortly after my discovery of the pigmented cells came to him. He connected the two groups of facts in a moment. *My pigmented cells were the vermicules, or fertilised female cells, which had burrowed into the insect's tissues for the purpose of undergoing further development there.* This, and not my hypothesis made before MacCallum's paper was known to me, explained the presence of pigment in the cells. He communicated his views to me in his letter of the 7th February, and published them later [41].

Meanwhile, after another struggle, I was again in sight of the pigmented cells. On my arrival at Calcutta I found myself installed in the convenient little laboratory which had been formerly used by Professor D. D. Cunningham. There was a native assistant there; but I hired at my own expense several others, especially a most intelligent Mahommedan named Mahommed Bux, who after he had been trained showed great enthusiam and gave me much assistance. To my delight I at once noted several varieties of dappled-winged mosquitoes, besides many kinds of grey and brindled mosquitoes, actually within the laboratory, and found the breeding-places of the latter just outside. Those of the dappled-winged mosquitoes were detected a little later; and were again seen to be pools of water on the ground. The next thing was to obtain cases of malaria; but here I was met by an unexpected and most unforeseen misfortune. The plague had been raging all this time in India; and on the Government's trying to introduce Haffkine's prophylactic inoculation in Calcutta just before my arrival, serious riots, during which many of the Europeans had felt themselves obliged to go about armed with revolvers, had occurred. The ignorant populace, thinking that the British were trying to inoculate them with and not against plague, flew into paroxysms of terror at the very sight of a European *hakim* (physician), while anything remotely resembling inoculation made them frantic. The physicians of the Calcutta hospitals were evidently very unwilling that I should use their cases for my experiments under these circumstances; and, as I had no hospital of my own as in Secunderabad and Bangalore, I was forced to send my assistants into the bazaar (native parts of the city) in order to try to induce patients to come to me on payment. Calcutta is not very malarious, especially at that time of the year, and it was only on large payment that several beggars with fever were induced to come to me; but when I proposed to prick their fingers in order to examine their blood they generally left their money, took up their crutches, and fled without a word! This placed me in complete perplexity as to what to do, until I remembered the malaria of birds. A number of crows, pigeons, weaver-birds, sparrows, and larks were then immediately procured, and experiments commenced on them without delay.

The malarious parasites of birds are exceedingly closely related to those of men, and together with these and the malaria parasites of bats and monkeys form a group which is quite distinct from the intracorpuscular protozoa of some mammalia, such as the *Pyrosoma bigeminum* of cattle, and of reptiles, such as *Drepanidium*. The true malaria parasites (namely the intracorpuscular protozoa of man, birds, bats, and monkeys) are distinguished by their generally amœboid character, by their possession of the characteristic black or brown pigment (melanin), and by an identical life-history as regards the production and appearance of the spores within the corpuscles, and of the motile filaments shortly after the blood containing them is drawn from the host. The parasites of birds differ from those of man only in some very small morphological details; and are so similar that in the earliest sub-classification of the group by Grassi, one of the parasites of birds commonly called *Proteosoma* is placed with two of the human species, the quartan and tertian, in one genus; while the other parasite of birds, commonly called *Halteridium*, is placed in another genus together with the remaining parasite of man, that of the pernicious, remittent, or æstivo-autumnal fevers. The latter part of Grassi's classification was wrong; and we now recognise that both the parasites of birds must be placed in one group with the quartan and tertian parasites of man; while the third human species must be placed in a group by itself, owing to the distinct shape of its gametocytes (crescents). Thus zoologically, the avian species are actually more nearly related to two of the human species than these are to the third human species. Anyone who had actually studied all these parasites, moreover, would have little doubt that they would be found to possess practically identical life-histories outside the vertebrate hosts, or at least life-histories which, if not identical, would be closely similar. It did not of course follow with certainty that the carrying agents of the avian parasites would be the same as those of the human species; but we could safely assume that they would be some kind of blood-sucking arthropod. At all events it was certain that the discovery of the life-history of the avian parasites would immediately open up that of the human organisms; while the practical difficulties of working with birds and infecting them would be less than with men. In fact I should have been wise to have begun my researches with birds in 1895. I therefore determined to employ birds at once pending the subsidence of the plague-scare, when I purposed of course to return to the human parasites; and there is no doubt that this was the right course.

It was first advisable to see whether mosquitoes would not carry one or both of the avian parasites. A number of crows and pigeons had been found to contain *Halteridium*; but without waiting to examine the other birds, I placed one crow, two pigeons, four larks and six sparrows in several cages all within the same mosquito netting, and then in the evening released within the net a number of grey and brindled mosquitoes bred from the larva in captivity. Next morning many of the grey mosquitoes were found gorged and were collected and kept for several days according to my rules. On the 13th and 14th March I dissected them one by one. When thirteen had been examined with negative results I began to fear that I had committed myself to another tedious search for the proper kind of host of the avian parasites. But fortune was kinder on this occasion; the fourteenth mosquito had pigmented cells precisely similar to those which I had found in the dappled-winged mosquitoes fed on patients with crescents.

Next I examined the larks and sparrows used in this experiment together with the crows and pigeons, and found that they contained not *Halteridium* but *Proteosoma;* so that it was doubtful from which kind of parasite the pigmented cell had been developed. Consequently I now put the birds with *Halteridium* in one net and those with *Proteosoma* in another, and released within both nets numbers of grey mosquitoes bred in the same bottle. Of thirty-four of these fed on the birds with *Halteridium* all were negative; but out of nine fed on the birds with *Proteosoma*, no less than five contained pigmented cells.

This result was obtained on the 20th March and practically proved the mosquito theory of malaria. Out of hundreds of grey mosquitoes previously examined none had contained pigmented cells except one which had been caught feeding on a case of tertian (section 13), and one which may have bitten one of the birds with *Proteosoma* in the experiment of the 14th March. Now, however, no less than five out of nine fed on birds with *Proteosoma* contained them. Mathematically therefore the probabilities were enormous (amounting almost to certainty) in favour of the view that the pigmented cells in this experiment had been derived from the *Proteosoma*. The cells were in the tissues of the insect; the parasite must therefore be able to make its way into and live in mosquitoes; precisely similar cells had been found in mosquitoes fed on men with malaria — and the chain of proof was complete.

But the fact that the pigmented cells in the mosquitoes are indeed derived from the parasites in the birds was of such fundamental importance that it required the most formal and rigid proof — especially as no life-history of a protozoal organism able to transfer itself from one host to another was then known to science.*

I therefore now commenced a long series of differential experiments in order to establish the fact thoroughly. Grey mosquitoes bred from the larva in captivity were fed (a) on birds with *Proteosoma* and (b) on birds without *Proteosoma*, and the results compared. The details will be found in my Report [42]. Out of 245 grey mosquitoes fed on birds with *Proteosoma*, 178, or 72 per cent, contained pigmented cells, while out of 249 of them fed on blood containing other parasites or no parasites, not a single one contained them.

Another experiment was the following. Three sparrows were selected, one with no parasites, one with a few *Proteosoma*, and one with many *Proteosoma*. They were placed in separate nets, and numbers of grey mosquitoes from the same breeding bottle were fed simultaneously but separately on them. Ten mosquitoes fed on each bird were then examined, and the total number of pigmented cells in all of them were counted. The results from a hasty enumeration made by myself were as follows. No pigmented cells were found in the ten mosquitoes fed on the sparrow without parasites; 292 in the ten mosquitoes fed on the sparrow with a few *Proteosoma*; and 1009 in the ten fed on the one with many Proteosoma [42]. The preparations were sent to Manson, who made a more careful enumeration and found 0, 571, and 1084, pigmented cells in the three sets of mosquitoes separately [41].

The fact then was proved, and the theory that the parasites of malaria develop in mosquitoes was practically established. Meanwhile I had been proceeding in the fascinating task of watching the progress of that

* The life-history of *Pyrosoma* in ticks is not even yet known; and the transference of trypanosomes by flies appears to be merely mechanical.

development. A number of grey mosquitoes would be fed on an infected bird and would be dissected two, three, four days, and so on, afterward. It was thus found that the pigmented cells grew rapidly in size until about the eighth day, when they became so large as to be almost visible to the naked eye. At this point they seemed to become mature; and it could be seen that many of them burst within the insect; because mosquitoes which had been infected more than eight or nine days before dissection were found to contain not the mature pigmented cells, but only their empty capsules. For the moment I could not ascertain what became of their contents.

This part of the work led to an interesting observation which influenced all subsequent researches om mosquito-borne disease. It will be remembered that Manson had always thought that a few days after her meal of blood the female mosquito laid her eggs and died; at this moment he considered both filariæ and malaria parasites escape into the water from the insect [26]. I had accepted this view, but had frequently observed that the insects do not die immediately after laying their eggs; and now, as I watched the pigmented cells growing larger and larger without apparently ripening, even five days after the insect was fed, it occurred to me that we had been allowing our mosquitoes to die so early owing to a very simple reason—we had omitted to feed them again! I therefore fed my infected mosquitoes a second and a third time, and more; and found that I could easily keep them alive for a month.* This enabled me to work out the development of the malaria parasites completely; and also helped others subsequently to find a further stage in the development of filariæ, and to ascertain the mode of infection in yellow fever.

I did not succeed, and, indeed, scarcely attempted to find the host of *Halteridium*. Nor was there time to work out the formation and behaviour of the »vermicules» in the stomach cavity of the mosquito — although this could have been done very easily; but on one occasion I saw the motile vermicule of crow's *Halteridium* in a brindled mosquito.

Of course all this time anxious efforts had been made to obtain cases of human malaria for experiment. Early in March I succeeded after much difficulty in finding an old beggar with a few crescents willing to submit to the dreaded operations; and I examined 41 grey mosquitoes and 15

* I refed them on healthy birds; but Bancroft subsequently found that they could be kept alive for some time on bananas.

dark greenish dappled-winged mosquitoes which had been fed on him. The first kind were tried merely as controls, and were of course negative; but, much to my surprise and disappointment, so were the latter. I attributed the failure to the facts that the crescents were very scarce in the patient, that the mosquitoes fed very sparingly, and that there was a spell of very cold weather (for Calcutta) at the time. A few unsatisfactory experiments with grey mosquitoes fed on a child with mild tertian parasites also failed. In spite of all efforts no other cases could be procured.

A full list of all these experiments, beginning with my earliest work in 1895, will be found in my Report written a few weeks later [42].

Recognising, of course, the inadequacy of my nomenclature for mosquitoes and the urgent necessity for employing the correct entomological names for the various species used by me, and having failed to obtain any literature on the subject, I now applied for assistance at the Indian Museum in Calcutta; but I received a brief reply to the effect that the *savants* there could give me no information on the subject. Once more I had to depend on myself; and I therefore took special note of the dappled-winged mosquitoes found near my laboratory. No less than four species were detected — a large brown species, a large greenish one (with which the experiments just described were made), a small black one, and a small brown one. The first was named later by Giles from specimens brought to England by me, and was called by him *Anopheles rossi;* and from the studies of Stephens and Christophers made in Calcutta some years subsequently it is almost certain that the second species was *A. fuliginosus.*

Numerous specimens of *Proteosoma* in grey mosquitoes were sent to Manson on the 30th March.

By the middle of April I had overworked myself, and was obliged to ask for ten days' leave to the Himalayan hill-station Darjeeling, where I hoped for time to write my report in a cool climate. I had heard also of several intensely malarious spots at the foot of the Darjeeling mountains, and hoped to be able to carry on there the studies on human malaria which were debarred in Calcutta, and at the same time to continue my work on avian malaria. I therefore left Calcutta on the 17th April.

16. *The Darjeeling Terai; April—June, 1898. Efforts to Obtain Assistance.*—The results with *Proteosoma* were obviously so important

that it was necessary to give them to the world at once, in the hope that many observers would now be easily able to follow the work, and also that I might obtain assistance in consequence of my success. Consequently I devoted my time at Darjeeling to writing a report to my chief, the Director General of the Indian Medical Service, on my latest work. The report begins with a brief statement of my first discovery of the pigmented cells,* followed by a list of the experiments, both positive and negative, which I had made with a view to infecting mosquitoes with human malaria. Then comes a detailed account of experiments and positive results with *Proteosoma*, followed by a minute description of the necessary *technique*, and of the appearance, position, and development of the pigmented cells. Next I discuss several points, including the bearing of MacCallum's work on mine. As I had brought my microscope and some of my specimens with me, I was able to add to the report large plates giving drawings of the pigmented cells up to the stage to which they had as yet been traced.† The work was, however, hurriedly executed, as I had only a few days in which to write it. The pigmented cells are called in it »proteosoma-coccidia», a term which has been criticised. I thought at that time that the parasites of malaria really belong to the Coccidiidæ, the early stages of their life being passed in man and birds, and the later stages (to which the name Coccidia might more appropriately be attached) in the mosquito; just as the early and later stages of the sexual forms of *C. oviforme* occur respectively in the bile ducts and the intestine of the rabbit. At the end of the report a description of the grey and brindled mosquitoes with drawings is furnished by Mr. G. C. Dudgeon, a gentleman who was acquainted with entomology; and the report concludes with the words, »These observations prove the mosquito theory of malaria as expounded by Dr. Patrick Manson. . . .»

The report after some delay, was dated 21st May, and was despatched at once, with an urgent request that it might be published as soon as possible. To my surprise I was informed that publication was not allowed without the permission of the Secretary of State for India. This meant writing to England and several months delay; but the report was printed very soon and numerous copies were sent at the end of June to Manson for private circulation among persons interested in malaria. In the meantime my success had been described in detail both to Laveran and Man-

* In the twelfth line the word »ordinary» is a slip of the pen for »other».

† These plates are reproduced at the end of this publication.

son in letters dated 22nd April — the letters being accompanied by a series of seventeen more preparations; and, as my results could not be published by myself, I now asked Manson to publish them for me.

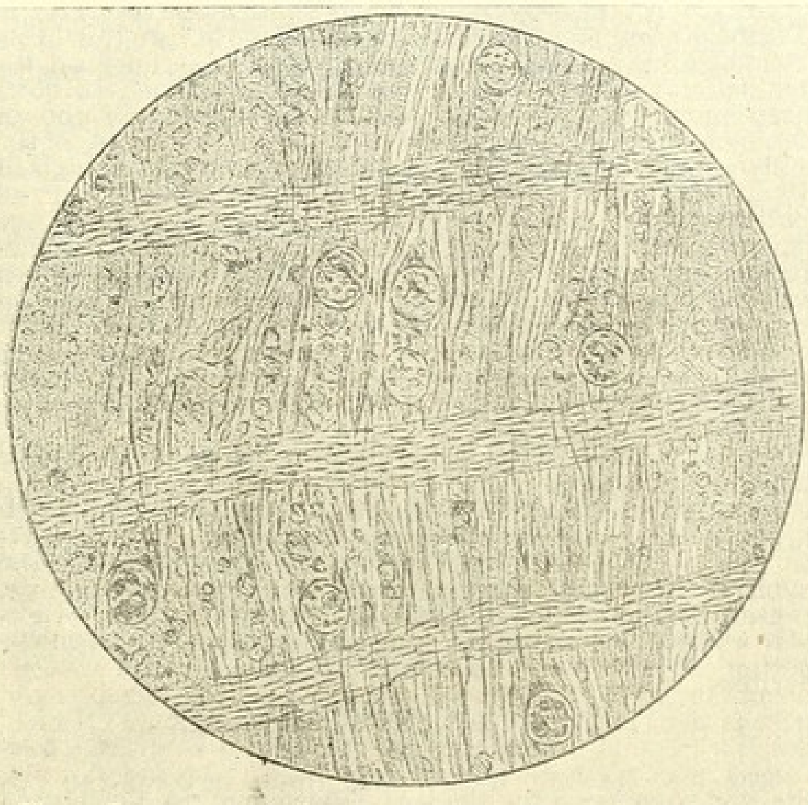

Fig. 1.—From a preparation of mosquito's stomach dissected thirty hours after the insect had fed on bird's blood containing proteosoma. The pigmented cells evidently lie between the longitudinal musc lar fibres which they have to some extent disassociated.

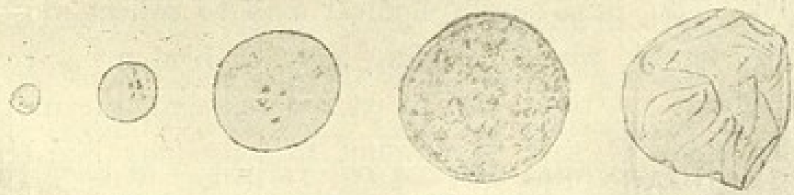

Fig. 2.—Development of the pigmented cell. The figure marked "6th day" is intended to represent what is evidently a capsule from which its contents have escaped.

Figure 3. From paper by Manson, British Medical Journal, 18th June 1898, page 1577. After Ross's drawings.

On the 18th June Manson published an able paper on the subject. The article commences with a *resumé* of my original discovery of pigmented cells in dappled-winged mosquitoes fed on a human patient with malaria, and gives the references to my papers describing the observation [41]. It goes on to describe the new results with *Proteosoma;* giving drawings of the pigmented cells up to the sixth day of development, and a diagram showing the connection between MacCallum's observation and my own; and it concludes with letters from Nuttall and Laveran accepting my results. Laveran said, »It appears to me to be undoubted that the elements discovered by Dr. R. Ross in the stomach of mosquitoes fed on the blood of birds, the subjects of hæmosporidiosis, are really parasites, and that these parasites represent one of the phases of the evolution of the hæmatozoa .... I have shown the preparations to M. Metchnikoff, who shares my opinion.»*

This paper drew general attention to my work, to which previously little credence had been attached; and, as many of my preparations had been sent to England and France, not only were those competent to form an opinion enabled to judge of the truth of my statements, but those who wished to follow my steps were now easily able to do so. In fact a most amusing comedy now commenced, in which we witnessed the hasty efforts of those who had been sceptics, not only to follow my steps but to persuade the world that their labours were original. During several years since that date every observation of mine has been independently discovered by various writers.

Recognising the vast significance of these preliminary results with *Proteosoma*, and also feeling that it was quite beyond the power of one man to complete as quickly as the interests of humanity demanded the work which remained to be done, I now made strong efforts to obtain assistance. The help of a single medical man to collect mosquitoes and cases of malaria for me would certainly have enabled me to reach the last proofs in a month or two; and be it remembered, the mortality from fever in India alone is said to amount to something like ten thousand persons every day. When, however, I asked the Director General for the services of one or more junior medical officers, I was told that none could be spared at the time. As a matter of fact there are always many medical officers in military employment in India, who can be spared if they are urgently called for; and the truth is that the necessary trouble was not taken. I then wrote to Manson begging him by all means in his power to obtain assistance for me from England; and thought that the Royal Society, which is subsided to a small amount by Government, might afford to give it. The matter was considered; and it was finally agreed

* Owing to a misapprehension, this paper erroneously states that *Halteridium* also had been cultivated.

to appoint, with the help of the Colonial Office, a commission of three gentlemen to investigate malaria. Two of these were sent in the autumn to study the subject — in Italy; and, after much difficulty, the third was allowed to come to me. He arrived at Christmas with orders to stay for two months — not to help me but to verify my statements!

That was all the help I received. The excuse is that my work had not been confirmed. But it had been accepted by Laveran, Manson, Metchnikoff, and Nuttall, who at least knew the subject. Was not this enough to justify the expenditure of a few hundred pounds in so greàt a cause? I mention these facts because it was largely this failure to obtain assistance which drove me from India some months later; which delayed the completion of my work for more than a year, and which postponed the adoption of an energetic prophylaxis in India until the present. Not mine the fault: the truth is that for some inexplicable reason men will never recognise the transcendent importance of investigation into the causes of those great diseases which destroy them.

The rest of my time in this district was spent in making attempts to find a suitable place in the intensely malarious areas at the foot of the mountains for researches on human malaria. This alone was a matter of no little difficulty, as the locality was new to me and I could obtain no accurate information regarding the disease. I worked especially at a place called Punkabari, situated a few hundred feet above the plain. A hospital and plantation existed here, and there was a large village some miles away on the plain. But the results were not gratifying; few dappled-winged mosquitoes could be found, as the rainy season had not yet commenced; while to my grief I discovered that the plague-scare was, if anything, stronger here than in Calcutta. So terrified were the natives, that on one occasion, when one of my men shot a sparrow for me in the village, all the coolies in the neighbourhood ran away for miles into the jungles, costing the planters much money and trouble before they could be induced to return. In fact I was given to understand that scientific investigations were not required there at the moment! Indeed it soon became apparent that I was only wasting much valuable time; and I consequently determined to complete my researches on *Proteosoma* at Calcutta without further delay.

17. *Calcutta; June—August, 1898. The Route of Infection.* On my return to Calcutta (4th June) I found it still quite impossible to obtain

cases of human malaria for my work, and therefore proceeded at once with the life history of *Proteosoma*. The most wonderful of all the phases of this history was now to be revealed. I had traced the development of the pigmented cells up to their maturity and subsequent rupture and discharge of their contents into the body-cavity of the grey mosquitoes. I could not see at the moment what happened to these contents; yet upon this point depended the vastly important question of the route of infecsion in malaria. But, when I had broken off my work a few weeks previously, the contents had appeared to consist of little more than a pure fluid.

Hitherto my mosquitoes had been dissected in water or a weak solution of salt, and I had had no time for methodical staining. A strong salt solution was now used and the secret was revealed. The contents of the mature pigmented cells did not consist of clear fluid, but of a multitude of delicate thread-like bodies, which, on the rupture of the parent cell, were poured into the body-cavity of the insect, and which were evidently spores.

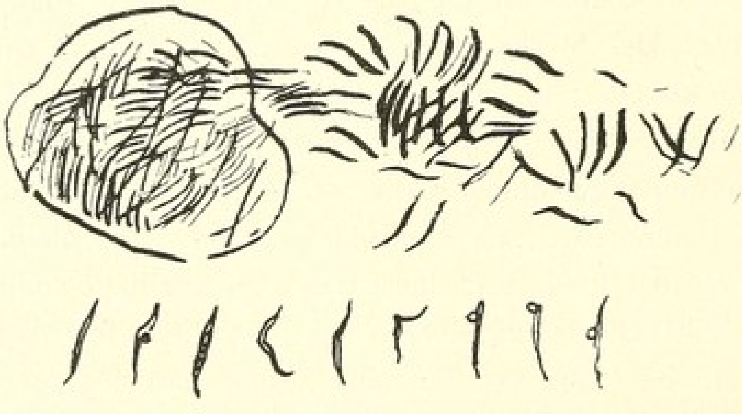

Figure 4. Sketch of thread-like bodies (sporozoids or blasts) escaping from mature ruptured pigmented cell (zygote). From letter of Ross to Laveran, dated 18th July 1898.

What happened now to these spores in view of the theories mentioned in section 12? Did they escape into the water according to Manson's ideas; or were they voided by the intestine according to mine; or did they in some mysterious manner work their way into healthy persons during puncture, according to the theories of King and Bignami and later of myself? But the staff of theory was no longer necessary; plain research would suffice.

Here there was another sharp but short struggle. I saw that the thread-like bodies, although apparently without motion themselves, were soon scattered by the insect's circulation all through its body; but beyond this I could not follow them for some time, in spite of the most assiduous endeavours. They seemed to have been created without object.

On the 2nd July however, I found in the thorax of a mosquito a large cell which, surprising to state, contained within it several of the thread-like bodies. They were able then to work their way into cells; but what was the cell? On the 4th July, while working upon another mosquito, I found that the thread-like bodies seemed to become more and more numerous towards a point in the thorax — as if they were converging toward some destination. At that point there were numerous cells such as I had seen on the 2nd July. They were attached to a duct and were

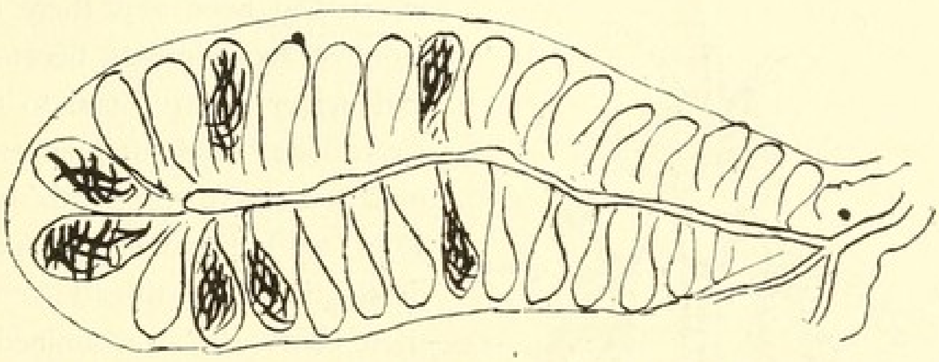

Figure 5. Thread-like bodies (sporozoids) in cells of salivary gland of mosquito. From letter of Ross to Laveran, dated 18th July 1898.

all contained within the same capsule — they constituted in fact some kind of gland. In all these cells there were hundreds of the thread-like bodies, floating loosely at all angles to each other like fish in globes of glass. Close by was another lobe of the gland similarly full of the spores. I was at the summit but not on it. I did not know what the gland was. I knew the appearance of the cells it is true, but in spite of my thousand and more dissections I had by no means acquired a full knowledge of the macroscopical anatomy. I found it by no means easy to meet with the gland again. On the 8th July the mystery was solved. The gland lay in the neck and upper thorax — the throat— of the mosquito. It consisted of three lobes on each side. The ducts of each lobe unite together like the midrifs of a trefoil. The duct so formed runs forward and meets the similar duct of the other side under the chin — so to speak — of the mosquito. The common duct advances still further and enters through the round base of the central stylet or stabbing weapon of the mosquito's proboscis. It was easy now to recognise the nature of the gland;

it was the *salivary gland*, which secretes the irritating fluid which the mosquito injects in the wound made by her in the skin, perhaps to dilate the vessels, perhaps to prevent speedy coagulation of the blood.*

The exact route of infection of this great disease, which annually slays its millions of human beings and keeps whole continents in darkness, was revealed. These minute spores enter the salivary gland of the mosquito and pass with its poisonous saliva directly into the blood of men. Never in our dreams had we imagined so wonderful a tale as this.

But still all this was inference only; the last proof was demanded. If the infection can be given in this way, give it. I had long possessed in the laboratory five old birds — four sparrows and one weaver bird — which had been kept there for my »control» experiments, because they had never been found to contain *Protesoma*, even after several examinations, On the 25th June, as soon as I began to suspect the destination of the thread-ilke bodies, these birds were all examined again, and were found to be still quite healthy. On that and the following nights, a large number of grey mosquitoes which had been long previously fed upon infected birds and many of which had been found to contain the thread-like bodies in their salivary glands, were released within a mosquito net in which the five healthy birds were placed. On the following mornings I satisfied myself that the infected mosquitoes had gorged themselves freely on the birds; and then, fascinated by the study of the parasites in the salivary glands of mosquitoes, I forgot all about even this important experiment.

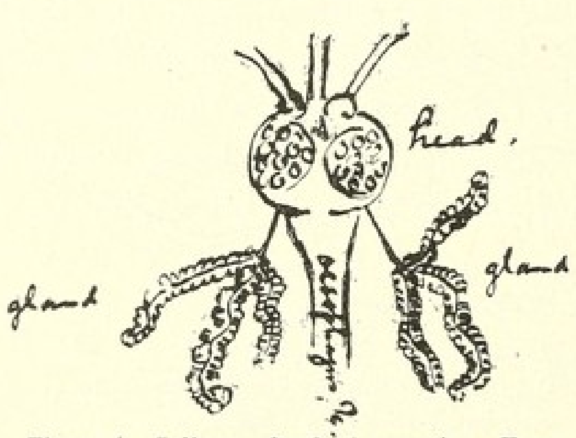

Figure 6. Salivary gland of mosquito. From letter of Ross to Manson, dated 6th July 1898.

Now only a small percentage of birds in Calcutta are infected with *Proteosoma*. Out of 111 wild sparrows examined by me I found the parasites only in 15, or 13.5 per cent. Moreover, even in infected birds, the parasites were scarce, seldom more than one being found in each field of the microscope. On the 9th July I suddenly remembered my experi-

* This gland had been discovered in 1888 by Macloskie [5], but I did not know it at the time and still had received no literature on the subject.

ment and examined the previously healthy birds. All of them without exception were now found swarming with *Proteosoma*, as many as twenty or even more being found in each field.

But not content even with this I repeated the experiment over and over again; and within the next few weeks I succeeded in infecting 22 out of 28 healthy sparrows (79 per cent), and also a crow and four weaver birds, and, moreover, gave a more copious infection to four sparrows which previously contained only a few parasites. At the same time I kept as controls a number of healthy birds in mosquito-nets, safe from the bites of mosquitoes, and found that none of them became infected (with one exception probably due to an error).

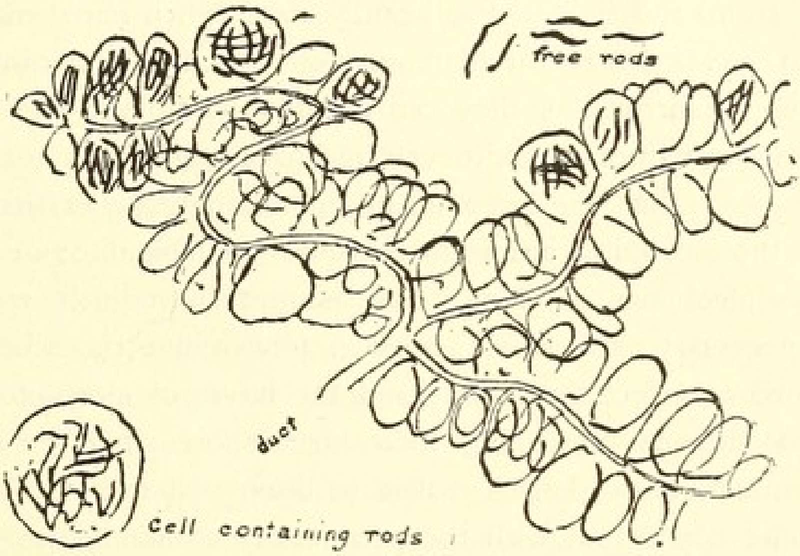

Figure 7. Thread-like bodies (rods, sporozoids) in salivatory glands of mosquito. Published from Ross's drawing by Manson, British Medical Journal, 24th September 1898, page 852.

Manson, to whom I had sent full details, told me that he would expound all my results, with demonstrations of my specimens, at the meeting of the British Medical Association to be held at Edinburgh at the end of July. I now announced the successful infection of birds to him by a telegram, which reached him just as he was setting out (though ill at the time) for the meeting; and he was therefore able to communicate the complete life-history of the parasites in his address.

His exposition as Dr. Charles said, »created quite a furore», and was quickly made known everywhere. His papers was published on the 24th September [43] and gave a full account of the subject up to the infection of healthy birds, together with several drawings of the thread-like bodies, both free and in the salivary glands, taken from my letters.

It was interesting during these researches to watch the gradual invasion of the birds by the parasites. From five to eight days after they were bitten by infected mosquitoes no parasites could be found in their blood; then a few appeared; then many; and at the last large numbers. The first five birds all died, and so did some of the others; and their liver was found to be full of the characteristic pigment of malaria. But many recovered, the parasites gradually decreasing in number.

At the same time I was temporally not a little delayed by finding inside the mature pigmented cells certain large brown or black bodies which I provisionally thought might be connected with their life-history. As proved by the researches just described, malaria could be carried by mosquitoes from the sick to the healthy, but as we know malaria clings intensely to location. It therefore seemed not at all unlikely that these black bodies, occurring as they did actually within the pigmented cells, might be of the nature of sporocysts meant in some way to infect other mosquitoes — so that the infection might not only be carried from man to man by the mosquito, but from mosquito to mosquito; or they might be meant to infect man, as Manson had thought, through the water. It was of course necessary, for sound science, to examine these bodies; and I therefore tried to infect both birds and the larvæ of mosquitoes by feeding them on insects containing these black spores; but the results were negative. Subsequently I saw reason to doubt whether the black spores really had any connection with the parasites (section 20).

But there was little time for such researches, necessary as they seemed at the moment. Although there could be no doubt that the human parasites have the same history as *Proteosoma*, still it was a necessary formality to complete the partial demonstration of this fact which had been already attained, if only to persuade Government to take active measures against the disease; and I was at last free to undertake the work. But now precisely occurred my last and most annoying interruption.

Before coming to this, however, let us consider the results which had already been attained and which have been the basis of nearly all that has been subsequently done.

(1). The general life-history of *Proteosoma* in the grey mosquito and the mode of infection being now ascertained, we could foretell to a practical certainty that the life-history and mode of infection of all the other parasites of the same group, including the human ones, would be closely similar in all their stages; that is, that if they differed at all, they would differ only in small details. The result of this was that if anyone wished to trace the life-history of any of these organisms in a second host he would now find the task an extremely easy one, because (a) he would know exactly the appearance of the parasite he was in search of, and (b) he would know exactly in what part of the anatomy of the second host to look for it. And if he wished to ascertain whether a given animal was or was not the second host of the parasite, he could easily make sure of the fact by ascertaining whether or not it harboured the described parasites, after feeding and dissection by the methods laid down by me.

It is in fact solely by this means that we have been able to demonstrate the proper hosts of the human parasites in many parts of the world.

(2). More than this, the pigmented cells of the æstivo-autumnal parasite of man had been demonstrated to be exactly similar to those of *Proteosoma* on the second, fourth, and fifth days after infection of the mosquito; and the hosts of this important organism were shown to be at least two species of a special genus which could be recognised by its possessing spotted wings and boat-shaped eggs (section 23); and were clearly shown not to be my grey and brindled mosquitoes, the former of which had been described sufficiently for recognition [39 and 42].

(3). The important law that not all species of mosquitoes can harbour a given parasite of this group had been established, both with regard to the æstivo-autumnal parasite and *Proteosoma* and *Halteridium;* and several important facts regarding mosquitoes had slowly become evident to me — but were not published until later.

(4). Lastly full directions of *technique* had been given in my report [42]. These consisted of numerous essential details, acquired during several years' experience, regarding dissection and feeding, etc. — without a knowledge of which the observer would be very likely to go wrong (as for instance by attempting to section his mosquitoes for searching for the parasites, and omitting to feed them regularly and change their soiled habitations for clean ones).

On the other hand my researches had given little or no information about the quartan and tertian parasites — except of course the all important analogy with *Proteosoma*. The observation of the grey mosquito caught feeding on the case of tertian was doubtful (section 14). Moreover they had not directly and absolutely demonstrated the final stages even of the æstivo-autumnal parasites in the dappled-winged mosquitoes, nor the mode of infection. But nevertheless they had reduced the demonstrasions still required to an easy formality which was within the capacity of any tyro with sufficient material and a microscope.

I am sorry to have to write such a summary of my work as this one; but it is rendered necessary by those who, during the long interruption of my labours which now followed, were able to work out some details of the subject before me, and who have wished to conceal the assured fact that their efforts were simply a repetition and imitation of mine. It should be pointed out that, by a generally recognised zoological rule, the discovery of the life-history of *Proteosoma* in mosquitoes covers that of other members of the same group of organisms, which have precisely the same development. By that rule, the right of priority in discovery belongs to him who first works out the life-history of one species of a group of animals; not to those who merely perform the easy task of extending the known facts to other species. Discovery is discovery; the determination of parallel facts, the filling in of details, the publication of pretty illustrations, and the furnishing of formal proofs of matters which are already certain, are useful — but do not constitute discovery.

My infection experiments on birds were completed early in August, and, as will be related presently, I was now no longer able to defer my work on *kala-azar*. Consequently I was obliged to leave Calcutta on the 13th August for my new duties — much exhausted by work and heat in the plains. Before doing so, however, I released my host of little feathered prisoners, which had unwillingly been of such assistance in the investigation.

It should be mentioned that from the first discovery of the thread-like bodies I had wondered whether they have any other destination besides the salivary gland. The eggs were especially suspected, but the results of investigation were negative. I therefore now concluded that malaria is communicated *only* by the bites of insects.

18. *Darjeeling District; August—September, 1898. Kala-dukh.* — It was mentioned at the end of section 14 that I myself had proposed to Government that *kala-azar* should be included in the programme of my year's special duty; because I then hoped that this disease might shed light upon the mosquito theory; but now when the theory was established and it was necessary to press on with the study of the human malaria, I wished to escape this additional duty, as I dreaded lest it should involve me in much pathological work which would interfere with the principal

line of research. I hinted as much to the Director General, but was told that he expected me to adhere to the programme. The disease was exciting much comment because it was new and was taking some thousand lives annually in Assam; but it was forgotten that malaria, though it is not new, takes some millions of lives annually in India alone!

Harold Brown had recently studied a disease which existed at the foot of the Darjeeling mountains, and which was called *kala-dukh* (black sickness) and was evidently closely allied to *kala-azar* (black-fever). Consequently I obtained permission to investigate this disorder first, partly because an opportunity might be afforded me of making some further studies at the same time on malaria in my old haunts at Punkabari. Fixing my head quarters at Kurseong in the hills on the road to Darjeeling, I made numerous visits to this locality, but was dogged by ill-luck. The plague-scare, though waning, was still present; and difficulties of transport impeded the work. On the 25th August I arrived at Naxalbari, an intensely malarious plantation and village on the plain beyond the foot of the hills, and found swarms of small and large dappled-winged mosquitoes (probably *Anopheles listoni* and *A. rossi*). There was no time to make formal experiments, and the people would not have allowed them; but I examined some dozens of these mosquitoes caught in the houses of infected persons, both for the pigmented cells and the thread-like bodies; but without success.* Nearly all my time was however taken up in patholgical enquiries on *kala-dukh* — as I feared would be the case. But now it was no longer possible to postpone the evil hour without dereliction of duty, and I was obliged to set out on the long journey to Assam.

19. *Assam; September—November, 1898. Kala-Azar.* — I arrived at Nowgong, the centre of the epidemic of *kala-azar*, on the 13th September. It was at once obvious that my worst fears were well-founded, and that I would be plunged for months into a difficult pathological problem and a long pathological report. But the work was not without interest, and I may be pardoned for touching upon it briefly. The disease had been first noticed by McNaught in 1882. A few years later Government sent Giles to investigate it; and Giles, who probably did not come much in contact with the real disease, seemed to have been considerably

* How unfortunate I was in this respect may be gathered from the papers of Stephens and Christophers [71] who later found a large percentage of these mosquitoes infected in this very district.

misled, and in a report (which was nevertheless a very able one) pronounced the malady to be ankylostomiasis [77]. Many of the practitioners in the locality were not satisfied however, and in 1896 Government sent Rogers to make a further report. Rogers certainly saw the real disease and concluded that it was a virulent form of malaria [78]. As it was evidently communicable, this implied that he held malarial fever to be communicable — a thing which no one would believe at that time; but he maintained his opinions with great courage and success. I was now sent in order, if possible, to decide the question; and as my researches had shown that contrary to accepted views malaria must be communicable from the sick to the healthy, Rogers position was justified. But the exact nature of *kala-azar* still required definition; and, as I was called upon to judge between opposite opinions, I was forced into a tedious enquiry — though it was my immediate personal impression that the disease is malaria.

Mixed with the cases of *kala-azar* there were numerous cases of ordinary malaria; and I found that the local practitioners could not distinguish which was which until the cases became exceedingly severe, when they were declared to be *kala-azar*. This generally happened only in the later stages of the cases — so that in fact *kala-azar* seemed to be simply another name for a very severe and frequently fatal form of malarial cochexia. As, moreover, many of the patients had ankylostomes, those who are familiar with the subject will understand that my task was indeed a complex one. The plague-scare not having penetrated here, I attacked the problem by examining the blood of all the cases, both of malaria and of *kala-azar*. My results showed that while the parasites were easily found in the early cases, they became more and more scarce as the disease advanced; until, in the old typical cases of malarial cachexia and *kala-azar* neither parasites nor pigment were to be found, even in blood taken from the spleen. I inferred then that *kala-azar* is probably only malaria, though it was possible that some secondary infection might account for the gravity of the cases. I also inferred — what no one would accept before then — that the spontaneous disappearance of the parasites must be due to the gradual establishment of immunity; and that the low fever present in these old cases was due, not to the parasites, but to some secondary intoxication from the greatly enlarged liver and spleen. And the same theories seemed to me to apply to *kala-dukh*.*

* It has just become highly probable that these diseases are due to a new parasite recently discovered by Leishman and Donovan.

This investigation required repeated examination of the blood of all the cases which I could procure in the town; and, being made at high pressure, involved another extreme strain on the eyesight. Nevertheless I examined several batches of dappled-winged mosquitoes fed on cases with parasites, but the insects selected for the work were like some of those abounding at Calcutta, namely *Anopheles rossi*. All proved negative. My disappointment was considerable, but I was not satisfied that the feedings, which were left to assistants, were properly done. Many of the same insects caught in the houses of patients were also negative. By the aid of my assistants, however, many fresh examples of the law that the dappled-winged mosquitoes breed in pools of water on the ground were obtained.

During my stay at Nowgong I wrote a short report, dated the 11th October, on the infection of birds by the bites of mosquitoes [46]. This was not published until some months later; but of course the principal facts had long previously been published by Manson [43].

At the conclusion of my work on *Kala-azar* I returned, now utterly exhausted, to Calcutta.

20. *Calcutta: November 1898—February 1899. The Work Confirmed.* — Arriving at Calcutta on the 19th November I set to work to pick up the threads of my work on *Proteosoma;* to obtain cases of human malaria (the plague-scare having abated); and to write my report on *kala-azar* — this being a tedious business requiring a full discussion of many intricate details. But my health had now suffered greatly from the continuous exertion made under very trying circumstances; and I felt scarcely able to complete even my report. The labour, the disappointments, even the successes, of the long and anxious investigations of a single subject had been too much for me.

The cold and dry weather had now commenced in Calcutta; and the result was that the malaria parasites had become much more difficult to find, either in men or in birds. Added to this, as I had no room in my laboratory which could be warmed by a wood fire (a gas-stove injured the insects), the mosquitoes could scarcely be persuaded to bite. And when they did so, it was observed that the parasites developed in them much more slowly than in hot weather.

Moreover, all this time I had failed to obtain any assistance in India, and saw no prospect of obtaining any. I had been told indeed by Manson that Dr. Daniels was to arrive shortly; but he was being sent, not really to assist me, but to enquire into the correctness of my statements; and was to remain with me only for a month or two. The only persons who had hitherto taken sufficient interest in my proceedings even to look at my preparations (I mean from the beginning of my work in 1895) had been Drs. Smyth, Maynard, Dyson, and Cooke; and it was clear that no one really credited my results. Even the Director General, who was then in Calcutta, would not visit my laboratory. It was the case of Galileo and the satellites of Jupiter over again!

I was, however, much cheered by the arrival of Dr. Rivenburg of the American Mission, who, hearing of my work, came all the way from a distant part of Assam with his wife and children at his own expense to assist me. He had been previously quite unknown to me; and I shall never forget his disinterested action and the help he gave me.

I had also been delighted to hear from Manson that the work was now being taken up by Koch and the Italians. My papers had been published as described; copies of my *Proteosoma* Report [42] had been sent to many persons interested in malaria. On the 8th November Manson wrote again informing me that he had just despatched some of my preparations to Rome, namely to Bignami and Charles.

I did not become acquainted with the admirable work of Koch until later (section 23); but the efforts of Bignami and Grassi were now communicated to me in a series of interesting and well instructed letters by Dr. Edmonston Charles — a gentleman then staying in Rome, but whom I had never met, nor corresponded with before. From these and their own papers it was clear that the Italian writers had been inspired by my work and had been desperately endeavouring to follow it; that they had detected the genus (*Anopheles*) of my dappled-winged mosquitoes, and, after having seen my preparations, had succeeded at the end of November in finding my pigmented cells in an Italian species of this genus caught in infected houses. Bignami also claimed to have infected healthy persons by mosquitoes obtained in this manner; and, by a lucky stroke, one of the persons bitten by some *A. claviger* was found to have acquired the mild tertian parasite.

I shall return to this subject when describing the confirmations of my work. The efforts of Bignami and Grassi were, however, obviously hasty and unreliable; while their writings were historically most inaccurate. They therefore did not impress me, and exercised no influence whatever in the completion of my own labours. For several years afterwards, however, nearly all my work was credited to these writers.

One interesting fact, however, I learnt from the Italians through Charles, namely that my grey mosquitoes belonged to the genus *Culex* and my dappled-winged mosquitoes to the genus *Anopheles*. Manson appears to have sent some of the former to Grassi, and, according to Charles's letters of the 19th November, he thought they were *Culex pipiens* — as a matter of fact they were *C. fatigans*. Also from Charles's letter of the 25th November and from their own publications [48, 51], it was clear that the Italians thought that my dappled-winged mosquitoes were *Anopheles claviger*; and in his letter of the 6th January it was stated that Grassi considered some dappled-winged mosquitoes which I sent him were *A. pictus* — they were really *A. rossi*, Giles. I satisfied myself more fully next year at the British Museum regarding the zoological names of the mosquitoes studied by me. The Italians had no difficulties in these respects as they had Ficalbi's works on gnats to guide them. They received some more of my preparations, through Dr. Charles, early in January 1899.

On the 22nd December Dr. Daniels, of the Medical Service of British Guiana, arrived. Though somewhat sceptical at first, he was soon convinced after seeing my preparations and repeating the experiments with care; and he fully confirmed my work in a paper, which however was not published by the Royal Society until much later [71]. I am much indebted to him for his assistance and advice in connection with my report on *kala-azar* — no one has a profounder knowledge or a larger experience of malaria. In the time remaining to us we attempted several series of experiments on human malaria (cases now being more obtainable), mostly with the large brown dappled-winged mosquito of Calcutta with which I had made most of my few but negative experiments recently in Calcutta and Assam. These too proved to be negative. We ascribed our failure to some mistake; but the cause was ill-fortune. The mosquitoes were chiefly *Anopheles rossi* which in Bengal certainly do not easily take malaria.*

* It was, of course, the cold weather in Calcutta, but Daniels and I used incubators. Stephens and Christophers later obtained positive results in Nagpur with *A. rossi*, but only by very careful artificial regulation of the temperature [71].

It should be clearly understood that all my experiments on human malaria since 1897 were made under very unfavourable circumstances, and that I never considered my negative results to be at all final.

Several distinguished visitors came to us at this time — F. Plehn, A. E. Wright, and A. Ruffer. They all accepted our work, except the »black spores» mentioned in section 17. Nevertheless Daniels and I did not think it right to abandon them without clear evidence; but when, later, I found closely similar bodies in large numbers in mosquitoes which had not been infected at all, my faith was shaken; and it was disturbed still more when I failed to find them in the infected mosquitoes of Sierra Leone. My doubts were mentioned in the concluding sentences of my report on *kala-azar*.

That report was finished on the 30th January [79], and contains eighty-one closely printed folio pages. My years' special duty was now almost finished; but I could obtain no definite assurance from my chief that I was to be retained on the same duty for an extended period. Yet the matter was vital to me. Nearly all the money at my disposal had been spent in consequence of these researches, chiefly because of the expenses connected with the constant changes of station to which my family and

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At this time I informed Daniels of some other details; and, in view of a controversy which arose later, he has kindly testified to this fact in the following letter: —

October 8th 1900.

Dear Ross,

I shall have great pleasure in testifying to the following facts: —

(1). Shortly after my arrival in Calcutta in December, 1898, you showed me living specimens of your »grey», »brindled», and »dappled-winged» mosquitoes.

You pointed out to me the attitude assumed by the last, the position of its larvæ in water, and the peculiarities of its eggs.

Since then I have learnt that these are characteristics of the genus *Anopheles*.

You contrasted these with the eggs and larvæ of other mosquitoes, which I now know to belong to the genus *Culex*.

(2). You showed me two species of »dappled-winged» mosquitoes, and I sent specimens of them to the British Museum where they now are. They have been described by Major Giles, I. M. S., as *Anopheles*.

You also showed me a specimen of a stomach of a mosquito with what are now known as »zygotes». This you stated was the stomach of a »dappled-winged» mosquito, similar to those you had shown me, which had been fed on a patient with crescents in 1897.

(3). I may add that the development of Proteosoma, as demonstrated to me by you in the »grey» mosquito, is essentially the same as the development of »crescents» in *Anopheles* as observed by me in British Central Africa.

I am,
Yours very truly,
C. W. Daniels.

P. S. As regards breeding-places, you informed me that the »dappled-winged» mosquitoes breed in puddles, the »brindled» mosquitoes generally in flower-pots, and the »grey» mosquitoes in tanks, ditches, etc.

C. W. Daniels.

Major R. Ross.

myself had been subject; and if I were now compelled to return to Secunderabad, I should not be able later to pay for my passage to England. Moreover, both Daniels and Rivenburg were now leaving me, and it was evidently foolish to expect any further assistance in India — much more that of a trained entomologist, which I especially required for the completion on my work on human malaria. I therefore determined to leave India forthwith and to return to England, trusting to fortune to give me an opportunity for finishing the investigation in a manner which I thought suitable. I mention these personal details as I have been blamed for leaving India at that moment.

Before doing so, I urged upon Government the importance of taking active measures for the prevention of malaria in accordance with my observations. Besides advising the strict use of mosquito-nets for a personal prophylaxis, I urged especially a campaign against mosquitoes as the best measure for towns and cantonments — particularly against the dappled-winged mosquitoes, which I said breed principally in water on the ground. My letter was published later [55], and I hope that the advice will soon begin to be taken.

I had also written a brief abstract of my work dated the 31st December 1898. This was presented by Laveran to the Académie de Médecine on the 24th January 1899, and was published soon afterwards [54]. In this paper my obligations to Manson and Laveran were acknowledged, I hope, in the full manner which honourable science demands. I wrote: —

> «Pour éviter tout commentaire erroné, qu'il me soit permis de déclarer ici que mes travaux ont été entièrement dirigés par Manson, et que j'ai eu l'assistance de ses conseils et de son influence à toute occasion; je dois aussi remercier le Dr. Laveran de m'avoir envoyé ses avis si autorisés. Quand, en mai dernier, je lui envoyai des spécimens de mes corps pigmentés du moustique, il reconnut immédiatement la vraie nature de ces éléments.»

And I added in conclusion,

> «Je considère comme probable que la malaria est communiquée à l'homme uniquement par les morsures des moustiques et peut-être d'autres insectes.»

21. *England; March—July 1899. Foundation of the Liverpool School of Tropical Medicine.* — On the voyage to England (February 1899) I

had full time to consider the present condition of our knowledge about malaria, especially in relation to the all important subject of prevention. It was almost certain that infection is caused solely by the bites of insects — but of what insects only? My long negative work had almost proved that the commonest Indian mosquitoes, the grey and brindled genera, do not carry æstivo-autumnal infection, at least. On the other hand, it was certain that two species of dappled-winged mosquitoes in Secunderabad, and one species in Rome, do carry it; while, if Bignami's observation was to be trusted, the last species carries also the mild tertian infection. But Secunderabad and Rome are not the whole world; even in Bengal, Daniels and I had not succeeded in infecting dappled-winged mosquitoes. The question as to which species do or do not carry malaria might prove to be a very complex one, not to be solved only by a few local experiences; there are probably hundreds of species of mosquitoes in the world, each of which would have to be tested unless we could find some good reason for limiting the enquiry. I therefore sought for some such reason, and found one. For centuries it had been known that malaria is connected with stagnant water on the ground — not with water in the pots, tubs, and tanks which abound close to all habitations, but with marshes and pools on the surface of the earth. Again malaria was known to increase every year at the rainy season, and subsoil-drainage was known to mitigate if not remove the disease. Hence *it was extremely probable that the insects which carry malaria breed only, or chiefly, in terrestial water.* For years we had assumed that the disease is caused by organisms which spring from marshes. We had been partially right, but not wholly right; it is not the infective but the infecting organism which springs from the marsh — not the germ but the carrier of the germ. Now, referring to mosquitoes alone, which varieties of these insects breed only or chiefly in terrestial waters? I remembered my frequent observations on this point (sections 14, 15, and 19). The grey and brindled mosquitoes breed chiefly in tubs and pots in India; but *the dappled-winged mosquitoes breed in pools on the ground.* Now it was only these last which, hitherto, had certainly been connected experimentally with malaria.*

What a weapon for good was now placed in our hands! Hitherto when we wished to remove malaria we were obliged to drain a whole area, recognising only that all terrestial waters seemed to be dangerous.

* This reasoning was by no means obvious or even known until after our work at Sierra Leone. In temperate climates grey mosquitoes (*Culex*) also breed often in terrestial water.

Now we should be able to go to a place and to point out the actual pools which cause the disease, by showing that they contain the larvæ of the culpable insects. The expense of dealing only with these would be much less.

Shortly after my arrival in England in March I learnt something about the zoological classification of mosquitoes from Mr. E. E. Austen of the British Museum. I found that, as I had already partially learnt through Charles, my dappled-winged mosquitoes were those of Meigen's genus *Anopheles* and that both my grey and brindled mosquitoes belonged to the same genus, namely *Culex*. I was dissatisfied with this because it seemed to me certain that they were of different genera. Recently Theobald in his fine work on mosquitoes [75] has separated them, placing the brindled mosquitoes in the genus *Stegomyia*, and reserving the name *Culex* for the grey mosquitoes. Later, Giles determined that the grey mosquitoes which carry *Proteosoma* are *Culex fatigans*, and called the large negative dappled-winged mosquitoes of Calcutta *Anopheles rossi.* *

Meanwhile Manson had been urging his great scheme of creating special schools for the teaching of tropical medicine, and had now received the support of Mr. Chamberlain. In Liverpool, Sir Alfred Jones, supported by Professer Boyce of University College, Mr. Adamson, Chairman of the Royal Southern Hospital, and many other gentlemen, had warmly taken up the scheme, and now appointed me the first lecturer of the Liverpool School of Tropical Medicine. I therefore found myself no longer an isolated worker, but a member of a company determined to advance the interests of life and health in the tropics. And it was an auspicious moment; for the great weapon which had just been forged for the prevention of the most important of tropical diseases needed strong hands to lift and wield it.

Almost my first care on returning to England was to consult eminent zoologists regarding the proper nomenclature for use in connection with the developmental stages of the parasites in mosquitoes. With the aid of Professor Herdman I published a paper on the subject [59], in which, abandoning the hasty provisional nomenclature hitherto used by me, I called the motile filaments, *microgametes;* the pigmented cells, *zygotes;* and the thread-like bodies, *blasts*. I also suggested a classification for the

* Owing to an error he thought that it was this kind in which I had first found the pigmented cells in 1897.

parasites of men and of birds. But there is still great divergence of opinion on these subjects.

Evidently West Africa, a rich and enormous country hitherto paralysed by malaria, was destined to be the first objective. I lost no time in urging the advisability of sending me there in order to complete my studies of the disease and determine its agents on the spot. In July I delivered my inagural lecture and demanded attention for my scheme for extirpating malaria by attacking the pool-breeding mosquitoes [58]. At the end of July 1899, accompanied by Mr. E. E. Austen of the British Museum and Dr. H. E. Annet, Demonstrator of the Liverpool School of Tropical Medicine, I left England for Freetown, Sierra Leone.

22. *Sierra Leone; August—September, 1899. The Investigation Completed.* — We have now reached the last chapter of this history — which I fear has become tedious. If a literary simile may be allowed in a scientific narrative, I had at least come to my Ithaca, after many mischances sent by many opposing deities. Two years had elapsed since I had seen the pigmented cells of the human parasites — two years of fruitless efforts, interruptions and bad fortune; and seven years had elapsed since I had commenced the special study of malaria; but now assisted by my able colleagues and myself, I needed but a week or two to demonstrate all the stages of the human parasites in dappled-winged mosquitoes, and also to ascertain the fundamental principles upon which State sanitation against tropical malaria should be based. I will be brief; the details are given in the publications [60, 67].

On the day after landing (10th August) we found two species of dappled-winged mosquitoes (*Anopheles costalis*, Loew, and *Anopheles funestus*, Giles) in abundance. On the 13th August, we detected a pigmented cell, evidently of the mild tertian parasite, in one of them. A few days later, in some barracks where there was much malaria, we ascertained that a quarter of the mosquitoes (almost exclusively *A. costalis*) were infected; and found in them pigmented cells evidently derived from all three varieties of parasites — quartan, tertian and æstivo-autumnal. We also made a few formal feeding experiments, and could have made as many more as we pleased. The material was unlimited; but our time was short, and the proof was already sufficient.

We then investigated the conditions under which the *Anopheles* breed and propagate malaria. It was the rainy season and the place was full of stagnant pools. Everywhere the larvæ of the dappled-winged mosquitoes were in these pools, while those of the grey and brindled mosquitoes occurred in tubs and pots. The great law of malaria — its connection with stagnant water on the ground — was explained. Moreover, simply by noting the presence of the larvæ, we could tell at a glance which pools were dangerous to health and should be dealt with in the public interests.

The habits of the insects were noted and found to be precisely similar to those of the Indian species. We studied particularly the characteristic attitude of the larvæ and adults of the dappled-winged mosquitoes, as formerly observed in India — invaluable tests for the immediate and easy recognition of the agents of the disease; we noted the evidence demonstrating the short flight of the insects, and their connection with rank tropical vegetation; we disposed of the ideas that tidal swamps cause malaria, but showed how earth-works produce outbreaks by the formation of pools of rainwater. In fact we were able to give a thorough explanation of the manner in which the old paludic and telluric theories of malaria originated.

We were also able to establish for the first time the fundamental principles which the State must adopt in order to extirpate malaria in tropical cities. These are (1) scrupulous drainage of the soil; (2) pending this, the persistent treatment of *Anopheles*' breeding-pools by culicicides; (3) the segregation of Europeans. We also recommended the protection of public buildings, such as barracks, gaols, hospitals, and rest-houses by wire gauze screens; the isolation of the sick; and the habitual employment of mosquito-nets and punkahs by individuals.

Our results and recommendations were immediately communicated to Government and also published in the medical press [60].

After our return to England in October we published a full report of our experiences [67]. In this book, written by myself and endorsed by my colleagues, I collected the principal results of all my researches on malaria made during seven years; and illustrated the life-history both of the human and avian parasites in mosquitoes by numerous photomicrographs made by myself. This work, therefore, which records the completion of these labours by the successful demonstration of the whole evolution of the human parasites in *Anopheles*, constitutes the summary and conclusion of all my previous papers. It has been said that the book was based on the writings of those who, as a matter of fact, learnt everything from me; but I can say with exact truth that if no one except MacCallum and Koch had touched the subject since 1895, scarcely a word in the Report would have been different.

It should be added that in March 1900 I gave an abstract of the history of my work in a lecture at the Royal Institution [68]; and particularly, that toward the end of the same year the president of the Royal Society, Lord Lister, formally accepted my results in his address to the Society.

From this time my own effortts have been devoted almost entirely to the practical campaign against malaria. Few people are aware of the fact that even the most solid discoveries of science may be allowed by the public to remain quite disused and inoperative unless strenuous efforts are made to urge them upon the popular attention. Even yet, in spite of the constant endeavours of many persons, very little has really been done towards the extirpation of malaria. This has been principally due to the fact that, for some inexplicable reason which wholly escapes me, the chief prophylactic measure recommended by me, namely a campaign against mosquitoes by drainage and petrolage, has been generally held to be impossible; yet it is the only general prophylactic measure possible in tropical towns. The struggle over this matter has been almost as severe as that over the original problem; but it is now drawing to a close. It is impossible to discuss the matter here. Suffice it to say that in the two principal towns, Havana and Ismailia, in which the measure has been adequately employed, the reduction of malaria has already been as much as eighty per cent.

This then is the conclusion of the history. I fear that some of the personal details may have appeared out of place in the narrative; but they have been introduced — though unwillingly — for a special reason. No form of enterprise is of such transcendent importance to humanity in general as the investigation of disease — the principal enemy of every man. The interests of all nations, not only in the present but in the future, demand that every possible encouragement should be given to such investigations — particularly that medical men, who are in an excellent position to undertake them, shall receive the warmest assistance in their self-imposed task. The story, however, which I have felt it a duty to record in this lecture adds but one to the many instances of medical history which show that little attention is given to this point. My labours will be abundantly repaid if earnest students in this field of science receive, in the future, in consequence of this narrative, a little more assistance thau was given to me.

23. *Confirmation and Extensions.* It is impossible for me to describe here, even in detail, the vast amount of work which has been done in many parts of the world on the mosquito theory of malaria since 1899; but it is necessary just to touch upon some of the more immediate verifications of my observations.

(1). Undoubtedly the first verification was due to Koch and his assistants [63, 64]. Professor Koch was kind enough to communicate to me at my request, in a letter dated the 15th May 1901, the origin and progress of his researches on the mosquito theory of malaria. He says: —

»The idea that mosquitoes may be the cause of malarial infection occurred to me on my first visit to the tropics in British India in the winter of 1883—84, and since then I have always spoken in this sense in my lectures and to my assistants. I have not indeed myself published anything about these views; but you will find a notice in R. Pfeiffer's work *Beiträge zur Protozoen-Forschung*, Berlin, 1892 (near the end).

»The fact that malaria, when it occurs epidemically, is often confined almost entirely to the children, the adults remaining free and therefore having become immune, I discovered first in villages in Java which lie in the valley of Ambarawa. That was at the beginning of November 1899. I reported on it on the 9th of December 1899, and my letter was published in the Deut. Med. Woch. No. 5. 1900, beginning of February. I obtained my first, successful cultivation of *Proteosoma* in mosquitoes in company of Prof. R. Pfeiffer in Rome in September 1898. We continued the investigation in Berlin; and in the middle of November we followed the developmental stages of the parasite up to the sickle-shaped bodies in the poison glands of the mosquito — that is up to the end. We were able to determine the form of *würmchen* (vermicule) in Proteosoma so easily because I, with Professor Kossel, had already in June of the same year (1898), without knowledge of MacCallum's investigation, detected the origin of the spermatozoa, the process of fertilization, and the formation of the *würmchen* in Halteridium.

»The publication of this investigation was very much delayed in consequence of the long time taken for the reproduction of the photographs in a way which satisfied me.

»At all events I have not thought it necessary to attempt to assert my priority on this occasion as the matter concerned only the confirmation of already known things.»

Professor Koch has the honour of having been one of the first, not only independently to conceive the mosquito theory of malaria, but also to attack it by experiment. He and Kossel independently observed the function of the motile filaments by the employment of correct methods of staining: this is practically admitted by Bignami (Lancet 1898, Vol II, page 1898), who was later able, probably through his instruction, to demonstrate the chromatin in the motile filaments [50] — a thing which he had refused to credit before. Koch also was the first to fill a gap in my own researches on *Proteosoma* by demonstrating the passage of the vermicule through the wall of the mosquito's stomach — a subject in which Grassi merely followed him later. That he succeeded in cultivating Proteosoma in Rome and Berlin, in September to November 1898, shows that he was the first to confirm my own observations. About that time Dr. Annett of the Liverpool School of Tropical Medicine saw some of his preparations of pigmented cells in Berlin. Koch's discovery of the frequent infection in native children in the tropics was one which I had entirely failed to make — although I should have made it; and is an addition to our knowledge of the very highest importance, being of far greater intrinsic value than much of the trifling matter which has been put forward in other quarters with much *réclame*. It enables us to explain with ease the source of most malarial infections in the tropics, and, besides, gives a complete revelation regarding the possibility of immunity in malaria, a thing in which no one would previously believe. Added to this Professor Koch has pressed still further onwards, and pushed with authority and ability the great subject of the practical prevention of the disease in the tropics — a matter the importance of which few writers on the subject have seemed able to comprehend. The methods recommended by me consist principally of the use of mosquito nets and the extirpation of mosquitoes; but Koch at once inaugurated a new conception which had not occurred to me and which consisted in the cinchonisation of the people in malarious localities. Although this measure is not always possible in its full extent, still experience shows that in a modified form it is most useful; and I have come to the conclusion that it should always be enforced as much as practicable in addition to the measures which I advocate. It was also Koch who was the first to call general attention to the important fact that a sudden and ill-advised dose of quinine is apt to precipitate attacks of blackwater fever in certain persons and localities.*

(2). Many erroneous ideas have been formed about the Italian work (referred to in section 20) by those who have no practical knowledge of malaria or full acquaintance with the literature. The facts are exactly as follows.

The South of Italy affords unparalleled advantages for the study of malaria, because abundance of material is there combined with great facilities in connection with laboratories, literature, and scientific communion; hence, though the principal discoveries have been made elsewhere, the writers of Southern Italy have been able to add to them much detail, which has proved more or less correct. It was hoped after Laveran's discovery that they would be able to find the extracorporeal phase of the parasite; but, unfortunately, misled by fondness for hypotheses, they fell into fundamental errors. Most of them hastily concluded that the motile filaments are »agony-forms»; and, as described in sections 6 and 11, A. Bignami rejected Manson's induction on this account; and another writer, G. B. Grassi, abandoned the whole mosquito-theory (1) because mosquitoes do not bite birds, (2) because they abound in places where there is no malaria, and (3) because the malaria parasites die in the stomachs of mosquitoes. At the same time he maintained that the extracorporeal stage of the parasite is a free-living amœba [10]. Bignami however, while rejecting Manson's version of the theory, adopted King's, and stated that he had even made some experiments on the subject in 1894; but these were only referred to as a past event [29], and seemed to have been quickly abandoned.

It is probable that these writers would have remained indefinitely in this position but for the researches of others. In 1895—97, Sacharoff [23], Simond [35], MacCallum [36], and myself [32] destroyed the Italian theory regarding the motile filaments; and then the publications of the 18th December, 1897 [38], the 26th February 1898 [39], the 21st May [42], the 18th June [41], the 24th September [43], and the 11th October [46], completely demonstrated the life-history of this group of parasites in mosquitoes; clearly indicated the genus concerned in the propagation of æstivo-autumnal fever; and gave other details mentioned in section 17.

* I should like to add, in contradiction of many inaccurate statements which have been made, that his acknowledgment of my own observations has been the most complete possible.

As all these papers, except those of the 21st May and the 11th October (which were in fact covered by Manson's papers of the 18th June and the 24th September), were published in such a prominent organ as the British Medical Journal, it is to be assumed that they were from the first known to the Italian writers, who have always shown a prompt knowledge of the labours of others. In his first publication [44], Grassi refers to my work without mentioning my name or giving references — as if it were then perfectly well-known in Italy. The later publications of Bignami and Grassi [48, 47] show that they were quite intimate with it before they themselves attained any definite results.

Such being the case, in order to follow my work in Italy and elsewhere, all that was now needed was to determine the genera of my grey and dappled-winged mosquitoes from such indications as I had been able to give. The former had been described in two papers, and was most evidently closely allied to *Culex pipiens*; and the latter, in which the æstivo-autumnal parasites had been shown to develop, were described in 1897 [38] as follows: —

»The latter are a large brown species biting well in the day-time, and incidentally found to be capable of harbouring the filaria sanguinis hominis. The back of the thorax and abdomen is a light fawn colour; the lower surface of the same, and the terminal segments of the body a dark chocolate brown. The wings are light brown to white, and have four dark spots on the anterior nervure. The haustellum and tarsi are brindled dark and light brown. The eggs — at least when not properly developed — are shaped curiously like ancient boats with raised stern and prow, and have lines radiating from the concave border like banks of oars — so far as I have seen, a unique shape for mosquito's eggs. The species appears to belong to a family distinct from the ordinary brindled and grey insects; but there is an allied species here, only more slender, whiter, and much less voracious.» In the next paper [39] these small insects also are called »dappled-winged».

At that time in Italy the Culicidæ had been carefully studied by Ficalbi in several works [31], and it was an easy task for anyone possessing these works, and also having fresh mosquitoes for dissection, to determine the genus of my dappled-winged mosquitoes from my description alone. Although I did not give the entomological criterion of the genus *Anopheles* (the long palpi of the female), I gave three other details which sufficed for the identification. First, the dappled-winged mosquitoes belonged to a group distinct from the grey mosquitoes (*Culex pipiens* type). Secondly, both species of this group had spotted wings; and still more particularly, one of them (certainly) had exactly »four dark spots on the anterior nervure». Now it is well-known that very few *Culices* and *Stegomyiæ* have spotted wings, while *Anopheles* almost always have them. The *Anopheles*, however, not only generally possess spotted wings, but the spots are *generally four in number and arranged along or close to the anterior nervure*. Lastly, if any doubt remained the observer would only have to catch the first spotted-winged female gnat and to examine the eggs within her, when they would be immediately seen to possess the characteristic *boat-like shape*, with the well-known clasping membrane simulating *oars on either side*.

It is curious that some of those who have written on the subject have overlooked the fact that the very first Italian mosquito which from its name alone would be suggested by my description was *Anopheles claviger*. Two of the synonyms of this insect are *Anopheles quadrimaculatus*, Say, and *Anopheles maculipennis*, Meigen!

There is, however, no doubt whatever that the Italians detected the genus of my dappled-winged mosquitoes, because they themselves say so in two of their articles of November, [48] and [51]. Nuttall admits the fact [74]. But there is reason to suppose that they recognised the insects long before November.

It was evidently Manson's paper of the 18th June 1898 which stimulated the Italians to renewed activity, because they set to work shortly afterwards. But their success was delayed by efforts towards originality. Grassi endeavoured to find the guilty species of mosquito by its prevalence in malarious localities. His efforts were a close repetition of mine in the Sigur Ghat — even his servant was attacked by malaria as mine had been. He discovered three species of guilty mosquito, namely *Culex penicillaris*, *C. malariæ* (so named by him — really *C. vexans*), and *Anopheles claviger* — principally (per lo meno) the first [44]. I have already shown in sections 11 and 12 how useless it is to attempt to identify the malaria-bearing species by its preponderance in malarious places; and it has now been demonstrated that even in Italy there is no such relation between the disease and its agent. *Anopheles* abound where there is no malaria — even round Liverpool. Needless to say then, two out of the three species isolated by Grassi have nothing to do with the disease. He was right regarding the third, *A. claviger;* but it is quite reasonable to suppose that he detached this simply from my description of the dappled-winged mosquitoes. As a matter of fact all these epidemiological efforts of Grassi, though interesting in a small way, were nothing but a series of vague speculations.*

Meantime Bignami, after four year's inaction, had returned to his old method of attempting to infect men by the bites of mosquitoes brought from malarious places. His results are minutely recorded in his paper [48]. He set to work in August — that is, after Manson had proclaimed at the British Medical Association that I had succeeded in infecting birds by the bites of mosquitoes [43]. Bignami's task was now vastly simplified; with the guidance of my work he collected his mosquitoes from infected houses; whereas if he had continued to act in accordance with his own theory he would have collected them from marshes — which would have led to constant failure (section 13). He claimed his first success early in November, but still could not say which of the various kinds of mosquitoes employed by him had produced the result.**

Up to November therefore the Italians had failed either to find the guilty species of mosquito or to demonstrate the life-cycle of the parasite in the insects. At this point Charles's series of eight letters addressed from Rome to me (dated from the 4th November to the 14th January) commence. They have been printed by me with his consent; and show clearly (what however can be also demonstrated from their own writings) that the Italians were then intimately acquainted with my work; that they had received my report [42] giving full details of *technique:* and that they had detected the genus of my grey mosquitoes (from specimens sent by Manson) and of my dappled-winged mosquitoes (from my description). In his letter of the 8th November 1898, Manson records having sent some of my preparations to Charles and Bignami (on or before that date); and Charles in his letter of the 25th November records showing one of these to Grassi (on or before that date). It is possible, however, that the Italians had seen

* The writers of some zoological text books, who have evidently had little personal experience of the disease, seem to have actually believed that Grassi determined the »*Anopheles* malariferi» by these efforts. That is not the case. In an early work [54] I said that they were made independently of Manson and myself; but this was written before I studied the Italian work with close attention; and since then I have withdrawn the statement [72].

** That human malaria is conveyed by the bites of mosquitoes had of course been proved — practically to a certainty — by my infection of numerous birds three months previously. Bignami's experiment was merely a formality of which the success could already be foretold with confidence. The statement, frequently made, that he was the first to give experimental demonstration of this fact may be set aside without comment.

my preparations long before this, as numbers of them had been sent to Manson and Laveran in the spring and summer; and they may also have seen those of Koch, who had cultivated *Proteosoma* in Rome in September.

Bignami, Bastianelli, and Grassi had now evidently determined to resort to the correct method for determining the guilty species of mosquito, and imitated exactly the experiment by which I had ascertained the second host of *Proteosoma* in the previous March. The experiment was recorded by them on the 28th November [51]. They fed six *Culex pipiens*, one *Anopheles nigripes* and four *Anopheles claviger* on some cases of crescents, and at last found my pigmented cells in two of the last species. They do not record the exact date on which this observation was made, but from Charles's letters it would appear to have been on the 25th November or later.

This, if correct, was the first definite demonstration of the guilty species of mosquito in Italy. It was made fifteen months after my original demonstration of the same parasite in the same genus of mosquitoes in Secunderabad on the 20th August 1897 [38], and nearly four months after Manson had announced the whole life-cycle of *Proteosoma* at the British Medical Association [43]. The Italian experiment was, however, of doubtful correctness, because the authors do not state that the mosquitoes used by them had been bred from the larvæ [51]. At the same time they actually impute to me the very fault which they themselves were committing, and do so contrary to the printed evidence of my own words.*

In their next paper [53] they claim to have found the various developmental stages of the æstivo-autumnal parasites in *A. claviger* caught in houses and stables, or fed on patients in hospital. Here again, examination of the publication shows that none of the insects employed seem to have been bred from the larvæ; and, what is still more important, the number of insects on which the observations were made is not exactly given. For all we know, the whole paper may have been written on the strength of only a very few positive results; and this is the more possible because it describes a life-cycle which is an exact repetition of that of *Proteosoma*. The authors give no precise differential experiments in order to prove the connection between the pigmented cells seen by them and

* They say that my experiment was doubtful because my mosquitoes may have previously bitten other animals [51, p. 314]. Now it is clearly stated in my publication [38, p. 1786] that the insects used by me had been bred in »bottles from the larvæ«; and from the whole tenor of my researches it was evident that such was the case.

the hæmatozoa. For this proof they rely upon my *Proteosoma* report [42], to which, however, they scarcely refer. Although their paper gives to the ignorant the impression of being original, it is in reality merely a re-script of mine.

Meanwhile Bignami and Bastianelli had been continuing their attempts to infect men by *A. claviger* taken from houses; and claimed a second positive result early in December. In this case, however, by good fortune, the infection proved to be a mild tertian one. Some months later, these two authors published a paper [56] recording the development of this parasite also in *Anopheles*. This was the first, and, indeed, only important Italian result which had not been previously indicated by me; I had made no observation connecting the tertian parasite also with the dappled-winged mosquitoes.

Subsequently the same authors and Grassi claimed to have demonstrated the development of the parasites in *Anopheles nigripes*, *A. bifurcatus*, and *A. superpictus*.

They also claimed to have shown that the members of the old genus *Culex* do not carry malaria; but this had long previously been abundantly proved by me in India, at least with regard to the æstivo-autumnal parasite; and the fact is that Grassi had only identified my grey mosquitoes, which I had shown to be negative to this parasite. Their first drawings of the parasites in mosquitoes were not published until the spring.*

In my first reference to the Italian work I accepted it with some reserve; but after a careful examination of their writings made in 1900 I felt much more scepticism. Their work during the winter of 1898—99 is evidently hasty and deficient in exact details regarding the various observations; and the general tenor of their historical passages is so inaccurate as to inspire grave doubts regarding the whole literature. I think that at that time they found my pigmented cells in a few, possibly a very few, *A. claviger* and that Bignami and Bastianelli also showed that the tertian parasite develops in the same insects; but beyond this it is impossible to speak with confidence. Many of their details also are derived from me.

My work was completed in the autumn of 1899 at Sierra Leone (section 22) and was published immediately [60]. Many of the details are incorporated in Grassi's book published in June next year [69]. This

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* For an independent account of all these researches the detailed history of Nuttall [65] and especially his *critique* on the priority question [74], should be consulted.

See also [72, 73, 76].

work, which is dedicated to Manson, is principally a compilation of the researches of others — the historical passages being quite inaccurate. At the bottom of page 31 of the first edition, the author says, »Giova infine far risaltare che io arrivai agli *Anopheles* malariferi indipendentemente da Ross, le cui ricerche sui parassiti malarici degli uccelli furono pubblicate quasi contemporaneamente alla mia prima Nota preliminare.» He and his colleagues found the »*Anopheles* malariferi» in Italy by detecting the genus of my dappled-winged mosquitoes; but they did not incriminate it with certainty until the end of November, five months after Manson published my work on the malaria of birds [41]. Grassi's »first preliminary note» [44] was published more than three months after this paper of Manson's, and, moreover, refers to my work as a well-known matter even then. I found the »*Anopheles* malariferi» in two species of mosquito in India *fifteen months* before Bastianelli, Bignami, and Grassi found it in Italy. Speaking quite strictly and accurately it is the principal merit of Grassi to have discovered, not the »*Anopheles* malariferi», but its correct entomological name.*

Excepting the discovery of the host of the tertian and perhaps the quartan parasites, the Italian work was simply a local affair, done, like the work of my colleagues and myself in Sierra Leone and of other observers in many parts of the world, on the basis of my Indian researches culminating in July 1898 (section 17).

Of the sixteen and more species of *Anopheles* which have now been definitely connected with malaria, only three or four were incriminated by the Italians; it is therefore quite incorrect to attribute the determination of this relation to them — much more to attribute it, as some have done, to Grassi alone. The connection between *Anopheles* and malaria has been determined by the united efforts of many observers in many parts of the world.

* Early in 1903 this writer published a pamphlet purporting to be a translation of important papers on the subject (*Documenti riguardanti la storia della scoperta del modo di transmissione della malaria umana;* Milano). It contains no bibliography nor accurate history of the events; and omits most of the principal publications of my work. It purports to give in full my paper recording the original discovery of the pigmented cells [38]; but on examining this copy I find that the drawings of the cells given by Manson, and the remarks of Manson, Bland Sutton, and Thin (all of which, of course, absolutely establish the genuineness of the discovery) are omitted without the smallest explanation. The author then proceeds to claim the discovery for himself. This work also is dedicated to Manson — a fact which may lead many to believe in its accuracy: but Manson has publicly stated that it was dedicated to him without his permission (Lancet and British Medical Journal, 28th March, 1903).

(3). Certainly not less important than the Italian work has been that of the Malaria Commission of the Royal Society, consisting of Drs. Daniels, Stephens, and Christophers. After confirming my results in Calcutta as mentioned in section 20, Dr. Daniels proceeded to British Central Africa, where he met Drs. Christophers and Stephens, who had proceeded there after a month's stay in Italy in the autumn. These observers had great trouble at first in obtaining suitable cases for experiment, but finally succeeded in doing so. Daniels confirmed our results in Sierra Leone, and added many useful and interesting details. Stephens and Christophers afterwards followed us in Sierra Leone and elsewhere in West Africa, and then proceeded to India.

The researches of all these gentlemen are given in the admirable reports to the malaria committee of the Royal Society [71]. These researches have had the effect of completely consolidating previous work on the subject. The authors have shown no less than eight species of *Anopheles* to be amenable to the malaria infection, and that *Culices* and *Stegomyiæ* are always refractory; they have demonstrated many of the habits of these insects in various parts of the world; and, besides, have given us much invaluable information regarding the pathology of the disease, especially of blackwater fever. Stephens and Christophers also found independently the great law of Koch regarding the prevalence of malaria amongst native children in the tropics.

The perusal of the writings of these gentlemen and of many other observers will convince any one that it is impossible to do justice to them in the form of a brief review; it is scarcely even fair to attempt to describe such labourious work in a few words; and I shall therefore now draw this lecture to a close with the remark, that I hope soon to deal with all these investigations in a manner which is due to them. But I should like to conclude with the names of a few of those who have more recently added valuable information to our store of knowledge regarding the mosquito theory of malaria — particularly Ziemann, Manson, T. Manson, the members of the numerous expeditions of the Liverpool School of Tropical Medicine (Drs. Fielding-Ould, Annett, Dutton, Elliott, Logan Taylor), Fernside, James, Low, Sambon, Van der Scheer, Van Berlekom, Celli, Nuttall, Shipley, Ruge, Howard, Theobald, Schaudinn, and Sir William MacGregor. I omit to refer to their works, and those of many others, only because it is impossible to do so properly within the limits of this work.

It would not be right, however, to conclude without referring to those most conspicuous examples of the success of anti-malaria measures for the improvement of public health — the cases of Havana and Ismailia. A campaign against yellow fever and malaria was commenced at Havana early in 1901; and Colonel and Assistant Surgeon General Gorgas of the United States army, who was in charge of the work, has recently reported as follows on the success of it, in a lecture delivered on May 22nd at the New York Post Graduate Clinical Society.

»The results of these combined measures were very marked. Mosquitoes entirely disappeared from many parts of the city, and were decreased everywhere. On the first inspection made in January, 1901, 26,000 collections of fresh water were found in the city, containing mosquito larvæ, this exclusive of the cess-pools. In January 1902, the consolidated inspection reports covering the same area, showed less than 300; but the most striking evidence was its results on yellow fever. It must be borne in mind that yellow fever had been constantly in Havana since 1760, that it was not, as it had been in our North American cities, some years present and some years absent, but steadily every year and every month and every day, in all that time. The deaths from yellow fever had been since 1889, about as follows: — 1890, 303; 1891, 364; 1892, 352; 1893, 482; 1894, 388; 1895, 549; 1896, 1355; 1897, 743; 1898, 127; 1899, 118; 1900, 301; 1901, the first year of our mosquito work 5, and since September 1901, not a single case.

The work with regard to malaria is not quite so striking, and this necessarily follows from the nature of the disease. But I think the results, as shown by the sanitary reports, are very hopeful, with regard to malaria, and indicate that in the course of time, malaria can be also eradicated. In 1900, the year before mosquito work, the deaths from malaria were 344; in 1901, the first year of mosquito work, they had fallen to 151; in 1902, the second year of mosquito work, they had dropped to 90, and, for the first four months of 1903, 16.»

At the end of 1902, the Suez Canal Company asked me to go to Ismailia on the Suez Canal, in order to advise regarding the best measures to take against the malaria which had long been prevalent in that town. I advised active operations against the mosquitoes; and this advice was followed with great energy and success. The following table of statistics, kindly supplied by Prince D'Arenberg, the President of the Company, speaks for itself.

Statistique du paludisme à Ismailia.

(From the statistics of the Suez Canal Company).

Mois	1897	1898	1899	1900	1901	1902	1903	1904
Janvier	83	94	201	156	128	162	13	
Février	103	83	165	139	83	105	20	
Mars	129	126	129	266	99	101	16	
Avril	135	127	109	175	100	64	14	
Mai	173	77	126	169	82	133	9	
Juin	180	43	126	114	68	154	15	
Juillet	188	81	104	145	74	120	23	
Août	242	86	107	166	123	130	19	
Septembre	336	128	128	258	244	176	25	
Octobre	254	178	172	228	372	139	39	
Novembre	178	271	209	244	352	* 174	12	
Décembre	88	251	208	182	265	73	4	
	2,089	1,545	1,784	2,284	1,990	1,551	209	

* Operations commenced.

Not only, however, from this place do we hear of reduction of sickness and mortality. Undoubtedly the whole West Coast of Africa is much improved, and good accounts continue to flow in from Lagos, the Gold Coast, British Central Africa, Hongkong, and further India; and it is to be hoped that within a few years malaria will, as Sir William MacGregor says, have lost its terrors, at least for Europeans who are called upon to serve in the tropics.

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(This list of works, chronologically arranged, includes chiefly my own writings, many of which are omitted in bibliographies, and such others as are referred to in the text. R. Ross.)

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DESCRIPTION OF PLATES.

(These Plates are copied from those given in my *Report on the Cultivation of Proteosoma, Labbé, in Grey Mosquitoes*, dated the 21st May 1898; but in the description I have substituted for the appellation *proteosoma-coccidia*, temporarily used by me for the *pigmented cells*, the word now generally employed, namely *zygotes*. R. Ross).

Note. All the figures and plates were drawn by me accurately according to scale from actual preparations, most of which were preserved in formalin. Plates II to IX are faithful representations of entire fields.

Plate I. **Drawings of zygotes from the second to the twelfth day.**

Figures 1—5 zygotes of the second day. Figure 6, stained.
Figures 7—11 zygotes of the third day. Figure 12, stained.
Figures 13, 14, 15, zygotes of the fourth day. Figure 16, stained.
Figure 17, zygote of the fifth day.
Figures 18—22, zygotes of the sixth day and later.
Figures 23, drawing in outline of the stomach of a mosquito studded with zygotes of the sixth day, seen by a low power.

Plates II—VIII. **Fields of Leitz oel. imm. 170 minim.** $^1/_{12}$th (inch).

Plate II. External coat of stomach studded with young zygotes of about 30 hours. Air vessel, crossing muscular fibres, and some oil globules, are seen.
Plate III. External coat of stomach studded with young zygotes of about 40 hours,
Plate IV. Zygotes of third day. Vacuolated forms.
Plate V. Zygotes of third day. Hyaline forms.
Plate VI. Zygotes of fourth day. One vacuolated and three hyaline forms. One zygote of a second generation derived from a second feeding.
Plate VII. Zygotes of the fifth day.
Plate VIII. Zygotes of the sixth to seventh day. One zygote of a younger generation.
Plate IX. Pyloric end of stomach studded with zygotes of the seventh day, seen by a power of medium strength.

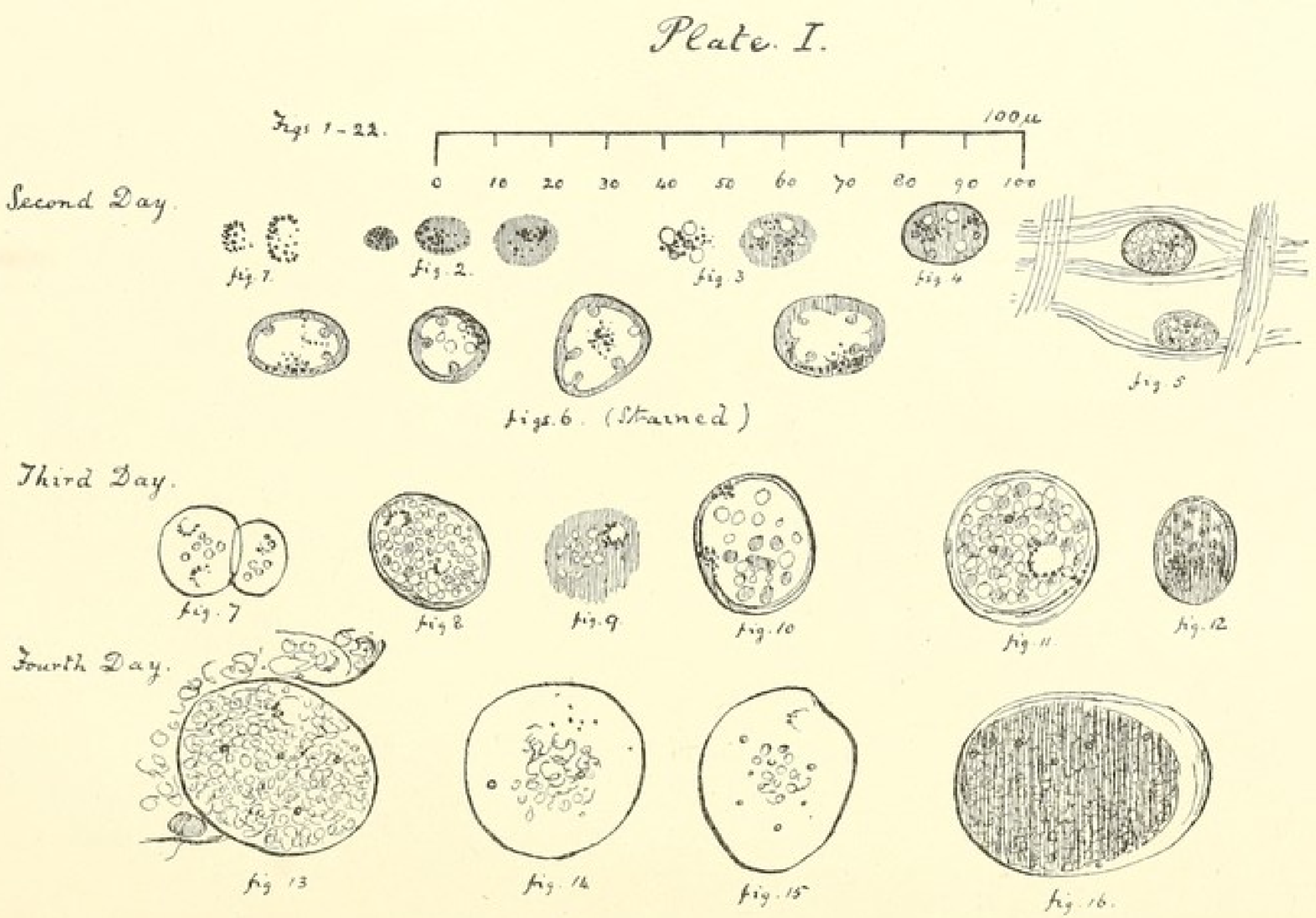
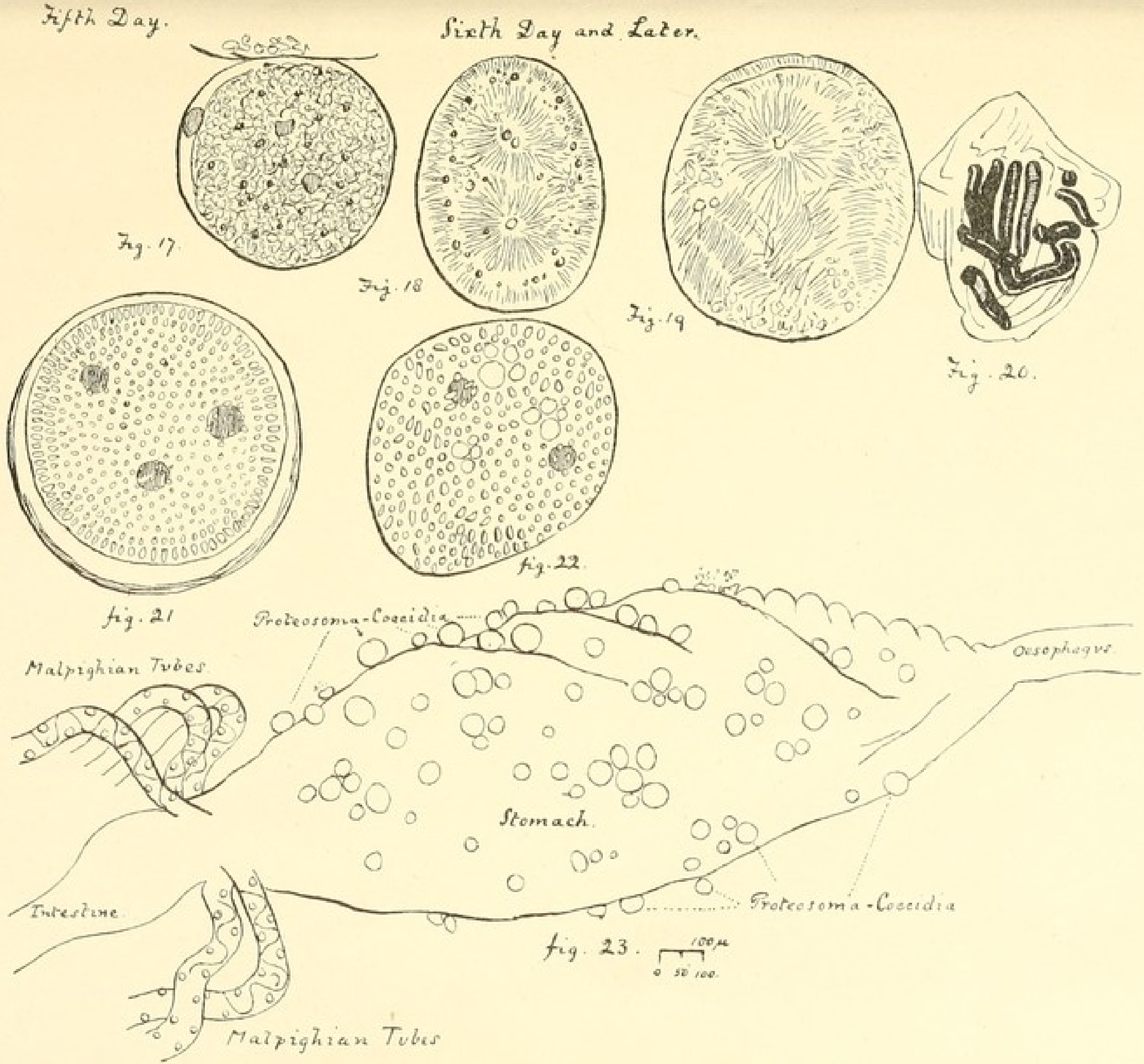
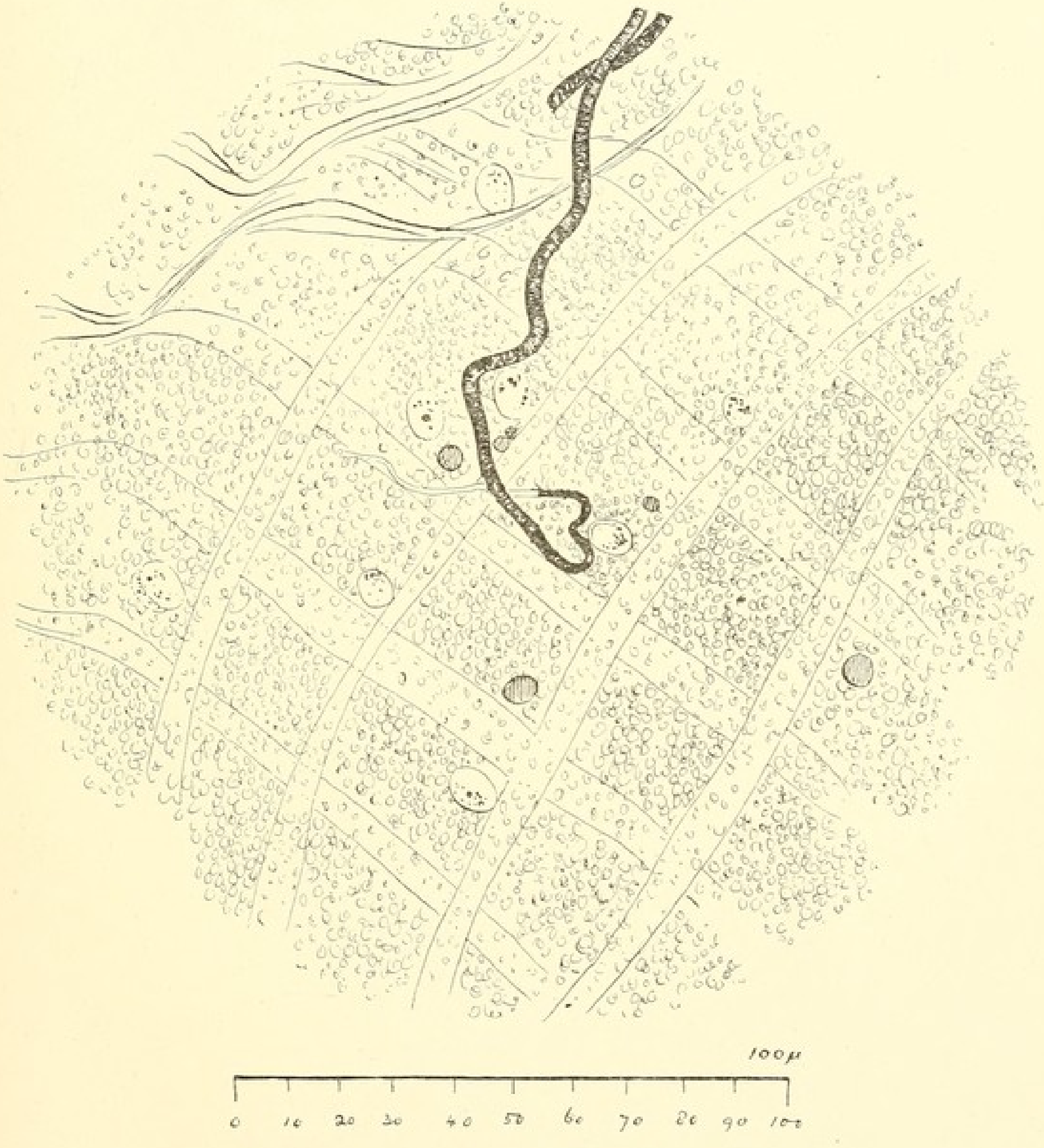


Plate. I.
Figs 1-22.
100μ
0 10 20 30 40 50 60 70 80 90 100
Second Day.
fig. 1.
fig. 2.
fig. 3
fig. 4
fig. 5
figs. 6. (Stained)
Third Day.
fig. 7
fig. 8
fig. 9
fig. 10
fig. 11
fig. 12
Fourth Day.
fig. 13
fig. 14
fig. 15
fig. 16.



R. ROSS: Researches on Malaria.

Plate II.



R. ROSS: Researches on Malaria.

Plate III.

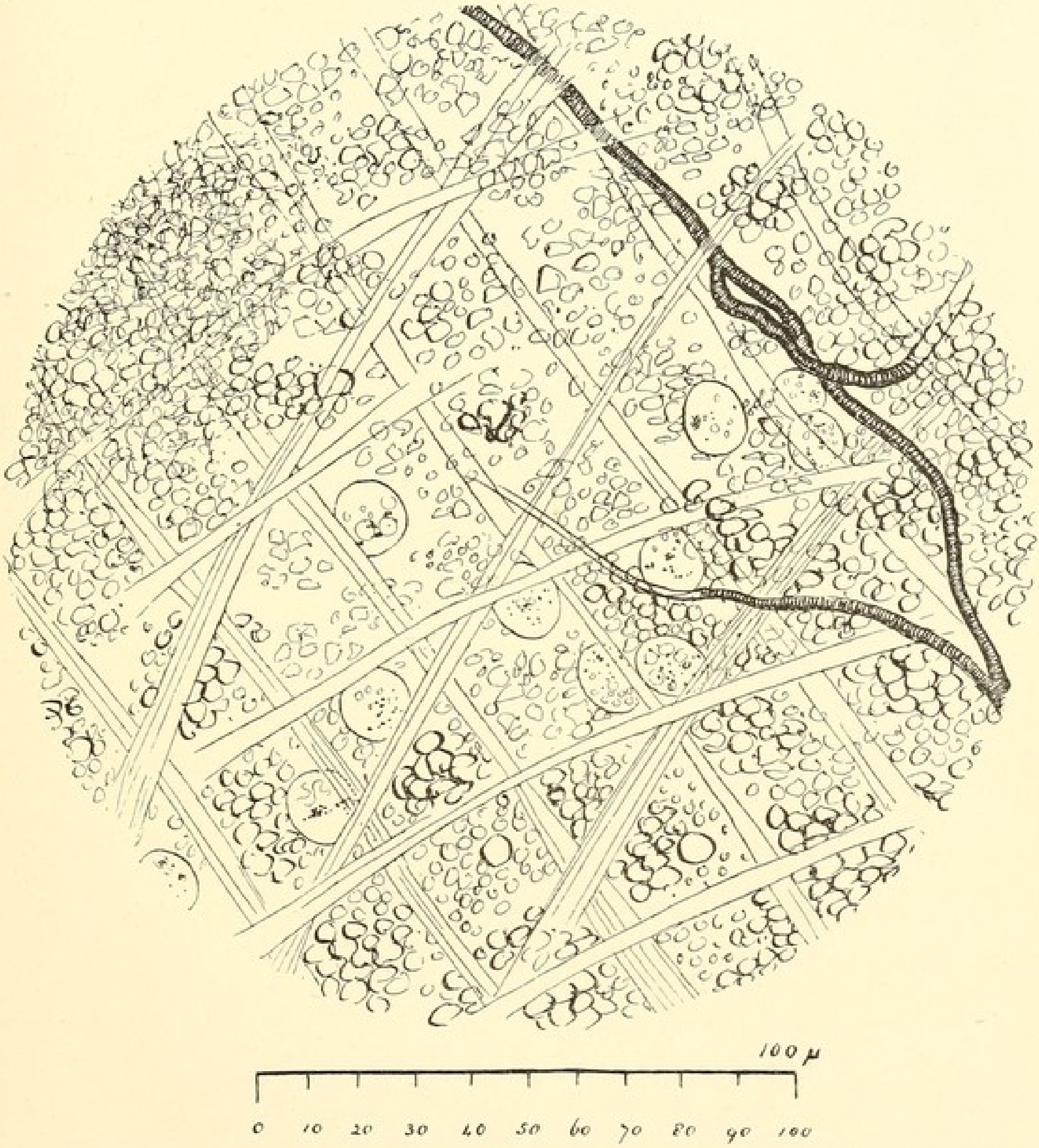
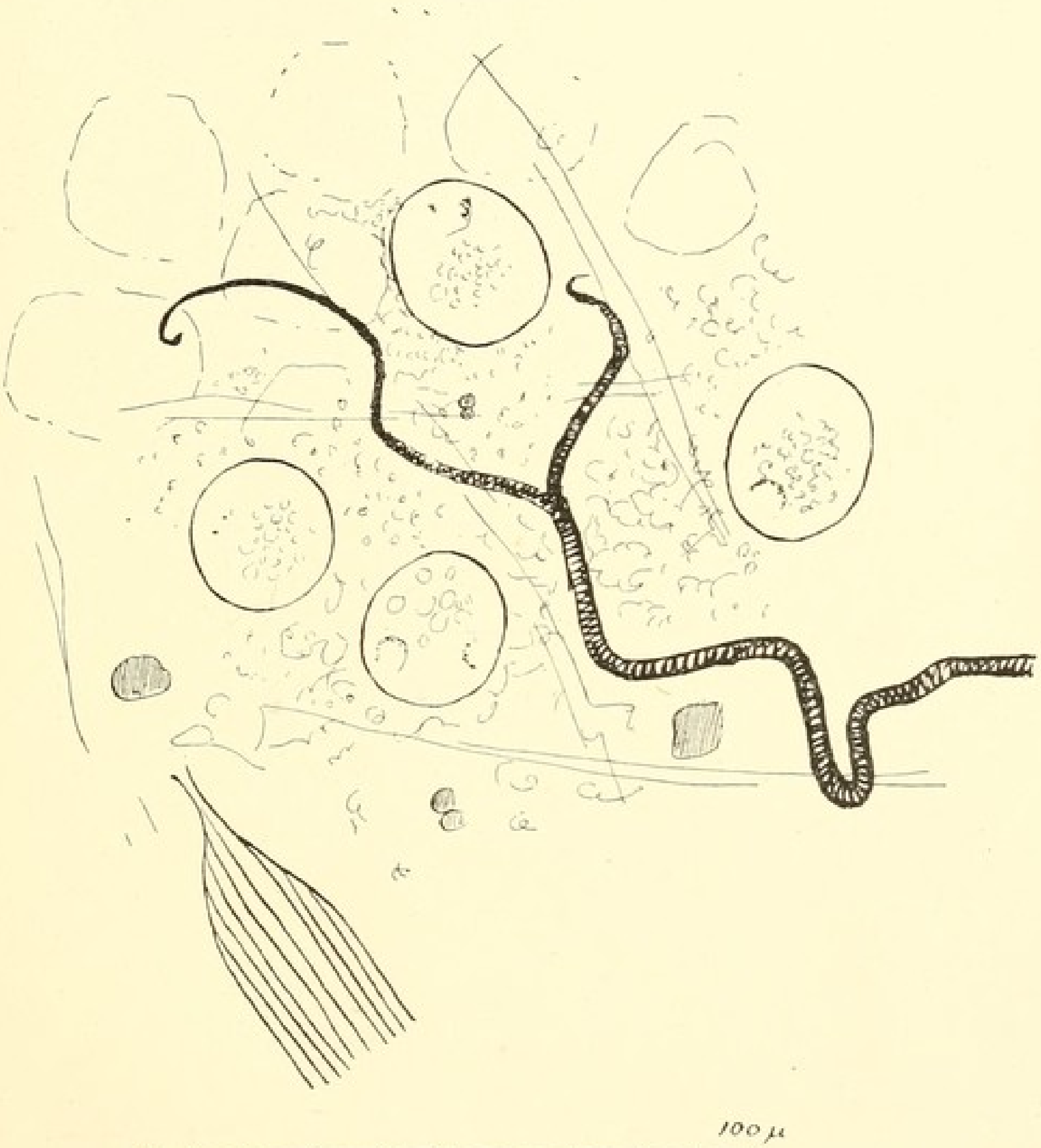


Plate IV.

Plate V.



R. ROSS: Researches on Malaria.

Plate VI.

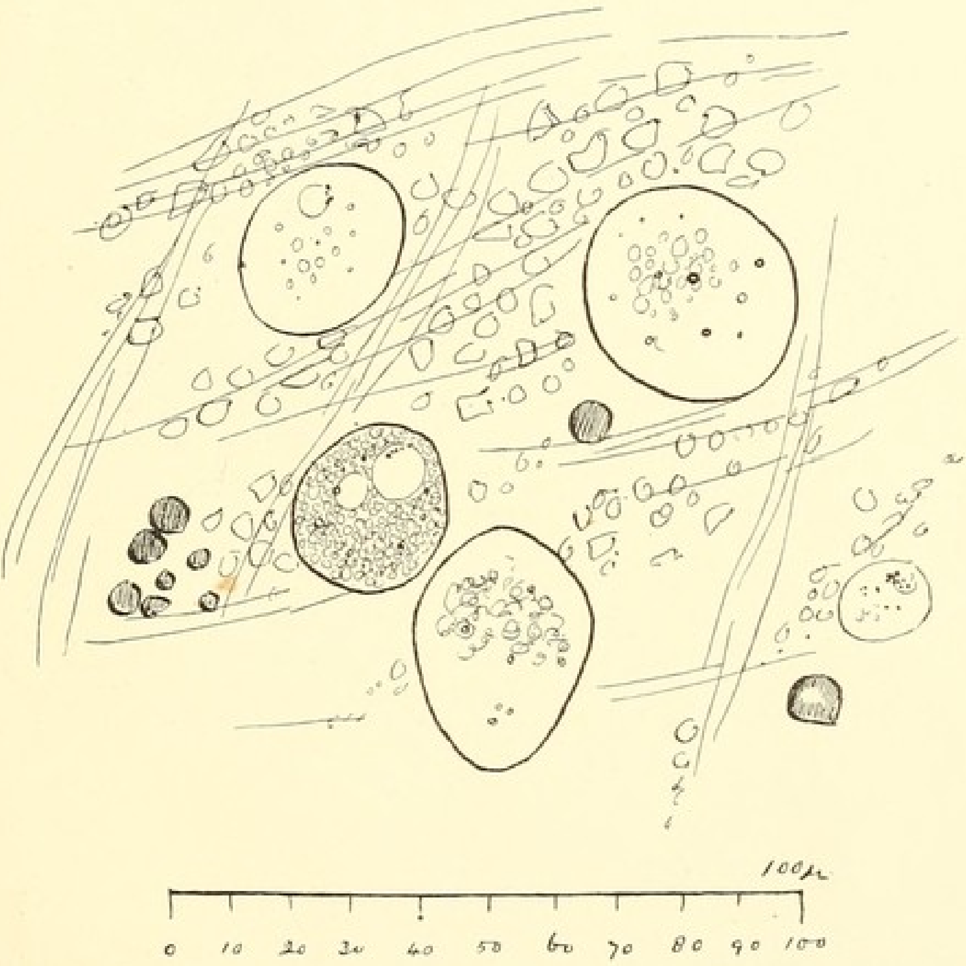


Plate VII

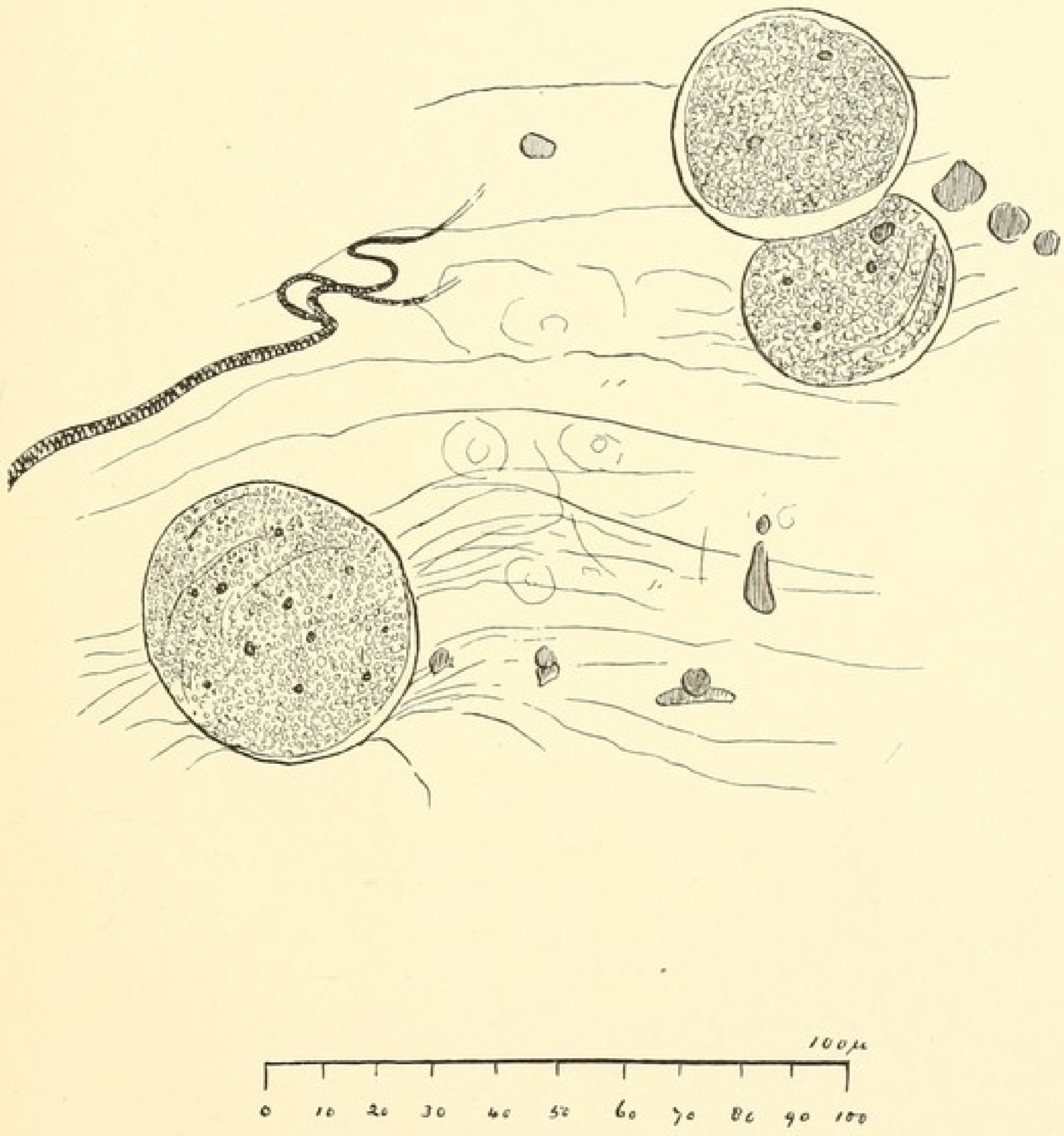


Plate VIII.

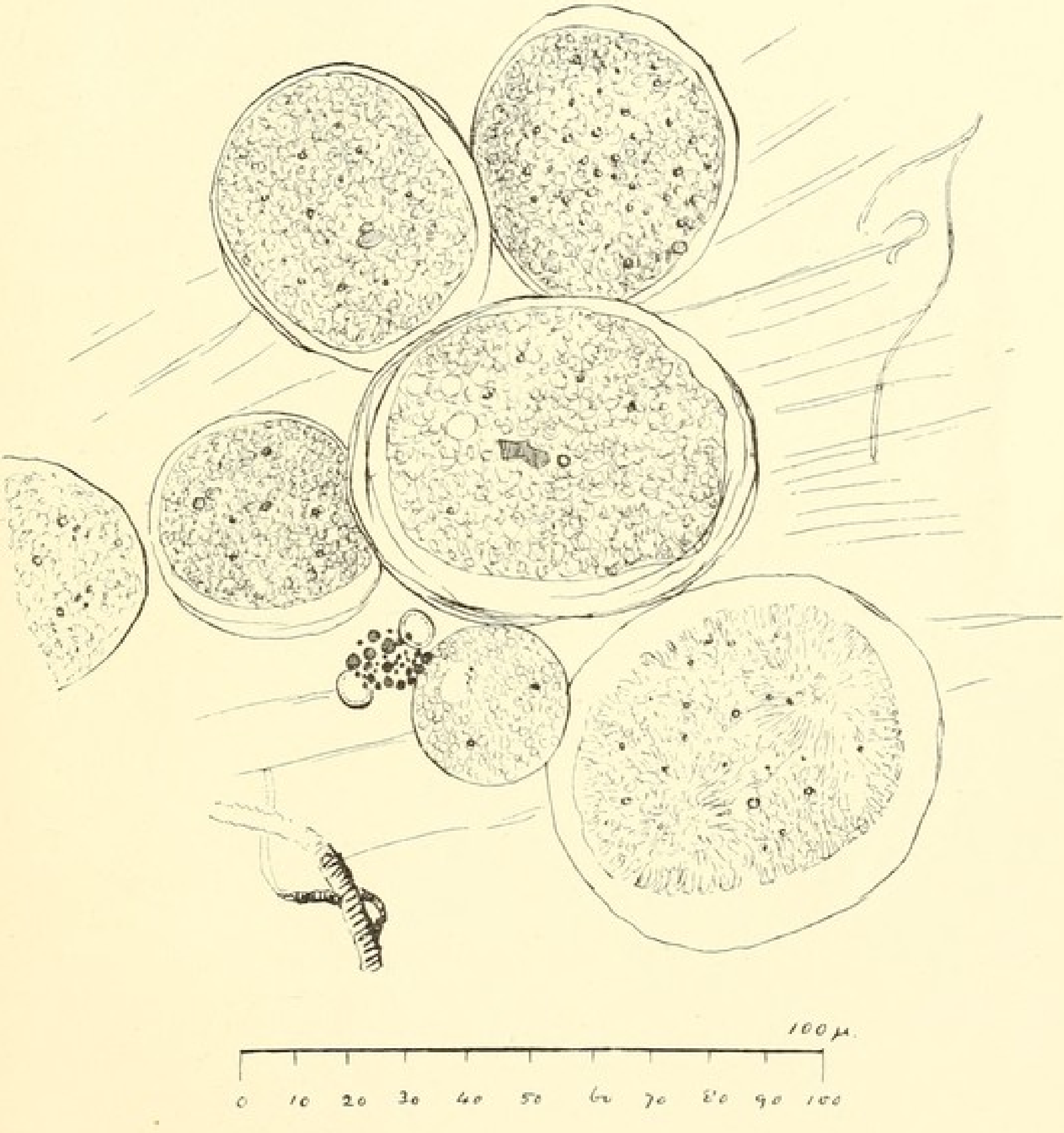


Plate IX.

R. ROSS: Researches on Malaria.

IV

The Health Cycle Table is a remarkable one, made out of the published annual mortality returns for 30 years. Here is a phenomenon to be explained, but I have no full explanation to offer. There are, however, four points in connection with it.

1) acclimatisation. The high death rates to a certain extent appear to be affected by the numbers recent to the Penal Settlement in given series of years, which have varied very greatly for several general administrative reasons — from as many as 1936 in 1876 to as few as 143 in 1871.

2) survival of the fittest. In unhealthy years the weakly are thinned out, & the result in an isolated island community is likely to be an improved death rate in the years immediately following.

3) medical administration. This may to some extent affect the figures for given years, though I should not put much stress on it. A series of high death rates has a tendency to produce strict sanitary efforts, & a series of low death rates a tendency towards relaxation of irksome sanitary regulations.

4) mosquitoes. All insect pests, generally from unascertained causes, breed much more rapidly in certain series of years than in others. If then malarial fevers cases are the result of the bites of mosquitoes, it is arguable that they are more numerous & injurious in some years than in others. But nothing has been ascertained as to the basis for such an argument & it cannot amount therefore to more than a conjecture.

5) rainfall. It might be possible to show that rainfall cycles affected health cycles. But I have failed in this.