RESEARCHES ON MALARIA.

By Major Ronald Ross, C.B., F.R.S., D.Sc.

Indian Medical Service (R).

(Continued from p. 474.)

(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 Kalhutti, 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 estivo-autumnal infection, the diagnosis being confirmed by the microscope.¹

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 luxurious 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—anaemia, 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.² Later, however, we were told of some insects which haunted the jungle and bit in the day time 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 contained 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

¹ 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 April 22nd, and my attack commenced at 10 p.m. on April 25th. I ascribed it at the time to my visit to the plain made on April 23rd; 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 dak bungalow and all his family were ill. At the same time I do not remember to have been bitten by mosquitoes, and said so in my published account.

² Judging by our present knowledge, these must have been the offenders.
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 escapes from the gnat into the water in which she lays her eggs, it followed that water which contained most larvae should contain most malaria parasites, and, conversely, that drinking water free from larvae 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 larvae 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 larvae 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 excreta on the skin every ten seconds or so; and I had actually shown that these drops may contain the pseudo-naviculae 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.

1 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.

2 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 *silenstris* 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 *silenstris* 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 feces, 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].
It was during these researches that I first noticed the "dappled-winged" mosquitoes. While looking for mosquitoes in a vacant rest house at the 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 expiration 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.¹

¹ 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.
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 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,¹ 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

¹ 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.
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 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, &c., 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. Larvae 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. Larvae floating head downwards and possessing long breathing tubes. Eggs elongated and somewhat lanceolate, and laid simultaneously in rafts.
Researches on Malaria

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 hummingbird moth. Breeding mostly in natural pools of water on the ground. Larvae 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 larvae 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 officers' quarters (situated some hundreds of yards

1 It was principally my assistant, Mahommed Bux, who ascertained, as a general rule, the attitude of the larvae.
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 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 larvae from far and wide round the barracks. These larvae 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 August 15th, 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 August 20th, 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 larvae 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 insect, 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

1 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 hemoglobin from the contents of the organ, and afterwards convert this into the pigment.
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 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 larvae 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 pig-

---

1 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.
mented 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
civilisation.

The first thing was to obtain more—hundreds—of these large
dappled-winged mosquitoes. Alas, the man who had found them
had, contrary to my orders, put the larvae from many sources in the
same bottle! All the larvae 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 larvae, we could not find them. I could scarcely even
persuade the adults to lay their eggs in captivity.

![Fig. 2.—Pigmented cells (zygotes) of estivo-autumnal parasite in dappled-winged
mosquitoes (Anopheles). From Ross's paper, British Medical Journal, December 18th,
1897, p. 1,787.](image)

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 observa-
tions 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 larvae." 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 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 of 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 larve 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 September 18th, 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.

---

1 This is exactly what was done by the Italian observers fifteen months later (see section 28).
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 August 21st. 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.  

Meanwhile swarms of small grey larvae 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 larvae 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 larvae 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 larvae 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 larvae 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 1,000 miles distant!

1 This mosquito also contained a number of the swarm-spores which I had observed in the Sigur Ghat.
Researches on Malaria

(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 larvae, 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 (September 26th) 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 civilisation—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 larvae 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 re-wrote 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.1 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.

1 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.
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.

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 February 17th, 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 wonderfully beyond the imagination of any of us. In the background was something greater still—the possibility of saving human life on the large scale.

1 Afterwards extended to one year.
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 haemoglobin in the stomach cavity of the insects. In his letter of August 11th, however, Manson sent me a paper by Simond, 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 November 17th, 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 MacCallum 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 aestivo-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*.¹

So much for the motile filaments: but now what were the pigmented cells? Everyone seems to have thought that as soon as the flagellate 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 February 7th, 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 enthusiasm 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

¹ 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).
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 ameboid 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 estivo-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
Researches on Malaria

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 Halteridiun; 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 larve 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 March 13th and 14th, 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 Halteridiun 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 Halteridiun 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 Halteridiun 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 March 20th, 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 March 14th. 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.¹

I therefore now commenced a long series of differential experiments in order to establish the fact thoroughly. Grey mosquitoes bred from the larvae 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 1,009 in the ten fed on the one with many

¹ The life-history of Pyrosoma in ticks is not even yet known; and the transference of trypanosomes by flies appears to be merely mechanical.
Proteosoma [42]. The preparations were sent to Manson, who made a more careful enumeration, and found 0, 571, and 1,084, 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 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 on 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 filariae 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.1 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 filariae, 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

---

1 I re-fed them on healthy birds, but Bancroft subsequently found that they could be kept alive for some time on bananas.
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 forty-one grey mosquitoes and fifteen 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 March 30th.

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 April 17th.

(16) The Darjeeling Terai: April—June, 1898. Efforts to Obtain Assistance.—The results with Protosoma 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 Protosoma, followed by a minute description of the necessary technique, and of the appearance, position, and development of the pigmented cells. Next I discussed 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 "protosoma-coccidia," a term which has been criticised. I thought at that time that the parasites of malaria really belonged to the Coccidiidae, 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 May 21st, 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

1 In the twelfth line the word "ordinary" is a slip of the pen for "other."
2 These plates are reproduced at the end of this publication.
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 Manson in letters dated April 22nd—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.

On June 18th Manson published an able paper on the subject. The article commences with a résumé 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 hemosporidiosis, are really parasites, and that these parasites represent one of the phases of the evolution of the hematozoa. . . . I have shown the preparations to M. Metchnikoff, who shares my opinion." ¹

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

¹ Owing to a misapprehension, this paper erroneously states that Halteridium also had been cultivated.
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 subsidised to a small amount by Government, might afford to give it. The matter was considered; and it was finally agreed 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 great 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 (June 4th) 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 infection 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.

![Image]

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

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.

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...
Ronald Ross

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.

![Thread-like bodies (sporozoids) in cells of salivary gland of mosquito. From letter of Ross to Laveran, dated July 18th, 1898.](http://militaryhealth.bmj.com/)

On July 2nd, 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 July 4th, 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 July 2nd. They were attached to a duct and were 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 July 8th the mystery was
Researches on Malaria

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.  

![Salivary gland of mosquito. From letter of Ross to Manson, dated July 6th, 1898.](http://militaryhealth.bmj.com/)

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 June 25th, as soon as I began to suspect the destination of the thread-like bodies,

---

1 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.
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.

![Fig. 7.—Thread-like bodies (rods, sporozooids) in salivary glands of mosquito. Published from Ross's drawing by Manson, British Medical Journal, September 24th, 1898, p. 852.](http://militaryhealth.bmj.com/)

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 fifteen, 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 July 9th I suddenly remembered my experiment 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 twenty-two out of twenty-eight healthy sparrows (79 per
Researehes on Malaria

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).

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 were published on September 24th [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 temporarily 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.
Ronald Ross

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.

(a) 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 (i.) he would know exactly the appearance of the parasite he was in search of, and (ii.) 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.

(b) More than this, the pigmented cells of the aestivo-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].
Researches on Malaria

(c) 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 estivo-autumnal parasite and Proteosoma and Halteridium, and several important facts regarding mosquitoes had slowly become evident to me—but were not published until later.

(d) 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, &c., 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 parasities—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 estivo-autumnal parasites in the dappled-winged mosquitoes, nor the mode of infection. But, nevertheless, they had reduced the demonstrations 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 August 13th, 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 headquarters 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 August 25th 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 pathological enquiries on *kala-dukher*-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 September 13th. 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 the 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 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

1 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.
Ronald Ross

frequently fatal form of malarial cachexia. 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-duk.1

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 October 11th, 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.

(To be continued.)

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