

Malaria and mosquitoes / Major Ronald Ross.

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Royal Institution of Great Britain.

WEEKLY EVENING MEETING,

Friday, March 2, 1900.

HIS GRACE THE DUKE OF NORTHUMBERLAND, K.G. F.S.A.,
President, in the Chair.

MAJOR RONALD ROSS, D.P.H. M.R.C.S.

Malaria and Mosquitoes.

OUR knowledge of the disease called malarial fever first emerges from chaos in the seventeenth century, when, owing to the recent discovery of quinine, the great Italian physician, Torti, was able to differentiate this malady from other fevers, and to describe its symptoms with accuracy. Next century, Morton, Lancisi, Pringle and others observed the connection of the disease with stagnant water and low-lying ground, and first emitted the theory—which in one form or another has found general acceptance up to the present date—that the fever is due to a miasm which rises from the soil or water of malarious localities. The next great advance was made in the middle of the nineteenth century by Meckel, Virchow and Frerichs, who ascertained that the distinguishing pathological product of the disease is a black substance, which is distributed in collections of minute coal-black or brown granules in the blood and organs of patients, and which is called the *malarial pigment* or *melanin*. This line of research culminated in the great discovery of Laveran in 1880—to the effect that the melanin is produced within the bodies of vast numbers of minute parasites which live in the red blood-corpuscles of the patient.

Ray Lankester had already opened the science of the parasitology of the blood-corpuscle by his discovery of *Drepanidium ranarum* in frogs; and it was at once apparent that the parasites found by these two observers are somewhat nearly allied—that is, that Laveran's parasite is a *Protozoal* organism, and not a vegetable one like the pathogenetic organisms recently discovered by Pasteur, Lister, Koch and many others. And our knowledge of the subject was quickly increased by the discovery of similar hæmatozoa in certain species of reptiles, birds, monkeys and bats, and in cattle, by Danilewsky, Kruse, Labbé, Koch, Dionisi, Smith and Kilborne. In 1885 a further advance was made by Golgi, who ascertained that the human parasites propagate within the body of the host by means of ordinary asexual spore-formation; that the exacerbations of fever in a patient are coincident with the disruption of the clusters of spores produced by the organisms; and that there are at least three varieties of the parasites in man in Italy

These observations were confirmed and extended by a large number of persons working in various parts of the world—most prominent among whom are Marchiafava, Celli, Vandyke Carter, Grassi, Osler, Bignami, Antolisei, Councilman, Mannaberg, Romanowsky, Labbé, Koch, Manson, Thayer and MacCallum. In short, the work of all these observers, and of many others scarcely less meritorious, has not only absolutely established the fact that the parasites are the cause of malarial fever, but has given us a very thorough knowledge both of the parasites themselves and of their pathological effects, direct and indirect; until the science of malaria—for it may almost be described as a science in itself—has become a brilliant exemplar of the modern methods of research as regards the science of disease in general.

But I am not here concerned with questions of pathology in malarial fever. At the conclusion of the labours to which I have just referred, we had, it is true, grasped the nature of the disease itself; but a question of the greatest moment still required an answer. We had studied side by side the morbid process and the parasites which cause it; but we had still to find out how infection is caused, how these parasites effect an entry. We had ascertained the life-history of the parasites within man, and of the kindred parasites within other animals; but, even after all these investigations, the life-history of the parasites *outside* man and *outside* other vertebrate hosts remained to be discovered. Until this was done our knowledge was not complete. It is now my privilege to describe the interesting theories and investigations which led to the solution of this great and difficult problem.

The *importance* of the problem need not be enlarged upon. In the British army in India during the year 1897, out of a total strength of 178,197 men, no less than 75,821 were admitted into hospital for malarial fever! Fortunately the death-rate of the disease is low in most places; but on the other hand the cases are so numerous that in the aggregate the mortality from malarial fever is very large indeed. For instance, in India alone, among the civil population (who do not take adequate treatment), the mortality from "fevers" during the single year 1897 amounted to the enormous total of 5,026,725—over five million deaths—being nearly ten times that due to any other disease. Although undoubtedly thousands of deaths are wrongly attributed to fever in these statistics, such figures can point only to a very great mortality due to malaria. Yet India on the whole is not nearly so malarious as many localities—such, for instance, as places on the coasts of Africa. In short, next perhaps to tuberculosis, malarial fever is admittedly the most important of human diseases.

But if the problem to which I refer was an important one, its solution presented difficulties which I, for one, formerly thought to be insuperable. It has been mentioned that Lancisi and Pringle connected the disease with stagnant water; and their views have

been generally endorsed by innumerable observations made since their time—by the general experience of mankind, by statistics, and by the fact that malaria can often be actually banished by means of drainage of the soil. But Laveran had now shown the disease to be due to a parasite of the blood. How reconcile these facts? There appeared to be but one way of doing so—namely, by supposing that the organism lives a free life in the water or soil of malarious places, from which it enters man by the respiratory or digestive tracts. To prove this it was necessary to discover it in the water or soil of malarious places. But how make this discovery? The organism is not a bacterium, but an animal parasite. It cannot be taken from the living blood and sown on the surface of a gelatine film. Experiments have proved that it can be inoculated from man to man by the intravenous injection of fresh infected blood; but this is a very different thing to cultivating it in an artificial medium. At all events, experiments in this line have always failed and are not in the least likely to succeed. The parasites simply perish when taken from their natural habitation, the blood. It was therefore extremely unlikely that we should ever be able to follow up their life-history by this means—which has proved so successful as regards the bacteria. It remained only to find them in the soil or water by direct search. But how identify them among the host of Protozoa which live in these elements? Certainly not by their form or appearance. As known to us at that time, they were simply minute amœbæ ensconced in the red corpuscles and accurately adapted for such a life. Now red corpuscles do not exist in soil and water; if the parasites live in the latter, they must possess some other form to that which they possess in the blood, and the clue afforded by identity of appearance fails us. The only remaining method open to us would have been to attempt to produce infection by each one in turn of the numerous species of Protozoa found in the water and soil of malarious places—a task of great magnitude, and one which we now know would have failed. Indeed, it was actually attempted by several observers, and actually did fail.

Such was the state of things up to the end of the year 1894. Speaking for myself, I can well remember the hopeless feelings with which I then regarded the problem. Fortune, however, was to be kinder to us than I had dared believe. At this very moment the key to the solution of the problem had already been indicated by Dr. Patrick Manson.

I have said that since the original discovery of Ray Lankester numerous hæmatozoa—or rather hæmocytozoa—have been found in man and various animals. All these are generally classed by zoologists in Leuckart's order of the Sporozoa, and are usually divided into three groups—groups which are not very closely related, except for the fact that all the organisms concerned are parasites of the red corpuscles of the blood. One group—found in reptiles—consists of parasites closely allied to the Gregarinidæ, another is found in oxen,

and is the cause of Texas cattle-fever; the third, for which I adopt the name of *Hæmamœbidæ* Wassielewski—is found in man, monkeys, bats and birds. It is to this third group—the *Hæmamœbidæ*—to which we must now direct our attention, because it includes the parasites of malarial fever. There are, at least, two known species found in birds, two in bats, one in monkeys, and three in man. The human parasites are those which respectively cause the three varieties of malarial fever—quartan, tertian, and remittent or pernicious fever. For these three species I adopt the names *Hæmamœba malarix* (quartan), *Hæmamœba vivax* (tertian), and *Hæmomenas præcox* (remittent fever).* According to Metchnikoff the group belongs, or is allied, to the *Coccidiidæ*. All the species have a close resemblance to each other, and all contain the typical melanin of malarial fever. The youngest parasites are found as minute *amœbulæ* living within the red corpuscle and generally containing granules of this melanin (which, indeed, is derived by the parasite from the hæmoglobin of the corpuscle within which it makes its abode). The *amœbulæ* grow rapidly in size, until, after one or more days (according to the species) they reach maturity. At this point many of them become *sporocytes*—that is, give rise to ordinary spores by vegetative reproduction. These spores presently attach themselves to fresh corpuscles, become fresh *amœbulæ*, and so continue the life of the parasites indefinitely within the vertebrate host. Others of the *amœbulæ*, however, instead of becoming *sporocytes* like the rest, become *gametocytes*.

Now it is to these *gametocytes* that an extreme interest attaches, because it is to them, and to Manson's study of them, that we owe the solution of the malarial problem. Numerous observers had examined them before Manson's time, but all had failed in arriving at a correct idea as to their function. It had been often observed that they circulate in the blood of the vertebrate hosts without, apparently, performing any function at all. As soon, however, as they are drawn from the circulation—as when the blood containing them is made into a fresh specimen for microscopic examination—they undergo the most remarkable changes. They swell up and liberate themselves from the enclosing corpuscle; and then some of them are suddenly seen to emit a number of long *motile filaments*. These filaments can easily be watched struggling violently, and may sometimes be seen to break from the parent cell and to dart away among the corpuscles, leaving the residue of the *gametocyte*, with its melanin, an inert and apparently dead mass.

Now it is not to be supposed that such an extraordinary phenomenon as this—which was observed by Laveran during his first investigations—could be witnessed without exciting the liveliest curiosity. As a matter of fact a hot controversy rose regarding it. Laveran, Danilewsky and Mannaberg maintained that the phenomenon is a vital one—that the *motile filaments* are living organisms, and

* Nature, August 3, 1899.

constitute a stage in the history of the parasite. Antolisei, Grassi, Bignami and others of the Italian school fell back upon the old theory—which we always like to employ when we cannot explain a phenomenon—that it is a regressive phenomenon, a disintegration of the parasite due to its death *in vitro*. Here, however, the controversy practically stayed. While the Italians, in conformity with their views, attached no signification to the motile filaments, Laveran, Danilewsky and Mannaberg, who held an opposite opinion, did not expressly or exactly state what their signification is. Mannaberg, indeed, held that they are meant to lead a saprophytic existence, but did not explain how they could escape from the body in order to do so.

It was reserved for Manson to detect the ultimate (though not the immediate) function of these bodies. He asked why the escape of the motile filaments occurs only after the blood is abstracted from the host (a fact agreed upon by many observers). From his study of these filaments, of their form and their characteristic movements, he rejected the Italian view that they are regressive forms; he was convinced that they are living elements. Hence he felt that the fact of their appearance only *after* abstraction of the blood (about fifteen minutes afterwards) must have some definite purpose in the life-scheme of the parasites. What is that purpose? It is evident that these parasites like all others must pass from host to host; all known parasites are capable not only of entering the host, but, either in themselves or their progeny, of leaving him. Manson himself had already pushed such methods of inductive reasoning to a brilliantly successful issue in discovering by their means the development of *Filaria nocturna* in the gnat. He now applied the same methods to the study of the parasites of malaria. Why should the motile filaments appear only after abstraction of the blood? There could be only one explanation. The phenomenon, though it is usually observed in a preparation for the microscope, is really meant to occur *within the stomach cavity of some suctorial insect, and constitutes the first step in the life-history of the parasite outside the vertebrate host.*

It is perhaps impossible for any one, except one who has spent years in revolving this subject, to understand the full value and force of this remarkable induction. To my mind the reasoning is complete and exigent. It was from the first impossible to consider the subject in the light in which Manson placed it without feeling convinced that the parasite requires a suctorial insect for its further development. And subsequent events have proved Manson to have been right.

The most evident reasoning—the connection between malarial fever and low-lying water-logged areas in warm countries—suggested at once that the suctorial insect must be the *gnat* (called *mosquito* in the tropics); and this view was fortified by numerous analogies which must occur at once to any one who considers the subject at all and which it is not necessary to discuss in this place.

Needless to say, since Manson's theory was proved to be right, it

has been shown to be not entirely original. Nuttall, in his admirable history of the mosquito theory, demonstrates its antiquity. Eleven years before Manson wrote, King had already accumulated much evidence, based on epidemiological data, in favour of the theory. A year later (1884), Laveran himself briefly enunciated the same views, on the analogy with *Filaria nocturna*. Koch, and later, Bignami and Mendini, were also advocates of the theory—partly on epidemiological grounds and partly because of a possible analogy with the protozoal parasites of Texas cattle-fever which Smith and Kilborne had shown to be carried by a *tick*. Hence many observers had independently arrived at the same theory by different routes. But I feel it most necessary to point out here that there is a difference between a fortunate guess and a true scientific theory. Interesting and suggestive as were many of the hypotheses to which I have just referred, they were to my mind far from convincing. *Filaria nocturna*, and even *Apiosoma bigeminum*, are not in close enough relationship with the *Hæmamoebidæ* to admit of very forcible analogies in regard to the respective life-histories. The epidemiological arguments of King and Bignami (some of which were also used by Manson) were scarcely solid enough to support by themselves a theory of any weight. All these were hypotheses—little more: I can scarcely conceive a practical man sitting down to laborious researches on the strength of arguments like these. On the other hand, Manson's theory was what I have called it—an *induction*—a chain of reasoning from which it was impossible to escape.

I have wished to defend this work of Manson's because it has been much misunderstood and much misrepresented, and even (in a somewhat amusing manner) completely ignored by some who, though they once strongly opposed his theory, now, as soon as it has done its work, wished to forget it. It is true that he endeavoured to predict the history of the parasites a little too far, and that he was in error (as will presently appear) regarding the immediate nature of the motile filaments; but the core of his theory was invaluable. I have no hesitation in saying that it was Manson's theory, and no other, which actually solved the problem; and to be frank, I am equally certain that but for Manson's theory, the problem would have remained unsolved at the present day.

Dr. Laveran's theory was unfortunately enunciated with great brevity; but it appears to me to have been really founded on many if not all the arguments independently advanced by King and Manson. To him we owe not only the discovery which made all these researches possible; but also an early and correct prediction as to the future life-history of the organisms with which his name will be inseparably connected.

To leave these interesting theories and to return to actual observations—I should begin by remarking that Manson thought the motile filaments to be of the nature of zoospores—that is, motile spores which escape from the gametocytes in the stomach cavity of the gnat,

and then occupy and infect the tissues of the insect. In this he was proved, two years later, to have been wrong. The motile filaments are not spores, but *microgametes*—that is, bodies of the nature of spermatozoa. I have said that some of the amœbulæ in the blood-corpuseles of the host become sporocytes, which produce asexual spores (nomospores); while other amœbulæ become gametocytes, which have no function within the vertebrate host. As soon, however, as these gametocytes are ingested by a suctorial insect they commence their proper functions. As their name indicates, they are sexual cells—male and female. About fifteen minutes after ingestion (in some species), the male gametocyte emits a variable number of microgametes—the motile filaments—which presently escape and wander in search of the female gametocytes. These contain a single *macrogamete* or ovum, which is now fertilised by one of the microgametes, and becomes a *zygote*. We owe this beautiful discovery to the direct observation of MacCallum (1897), confirmed by Koch and Marchoux, and indirectly by Bignami. Metchnikoff, Simond, Schaudinn and Siedlecki have also demonstrated what are practically sexual elements in some of the Coccidiidæ. Directly MacCallum's discovery was announced Manson saw the important bearing of it on the mosquito theory. Admitting that the motile filaments themselves do not infect the gnat, he at once observed that it was probably the function of the zygote to do so—and this time he was perfectly right.

I must now turn to my own researches. Dr. Manson told me of his theory at the end of 1884, and I then undertook to investigate the subject as far as possible. I began work in Secunderabad, India, in April 1895; and should take the present opportunity for acknowledging the continuous assistance which I received both from Dr. Manson and from Dr. Laveran, and later from the Government of India. Even with the aid of the induction, the task so lightly commenced was, as a matter of fact, one of so arduous a nature that we must attribute its accomplishment largely to good fortune. The method adopted—the only method which could be adopted—was to feed gnats of various species on persons whose blood contained the gametocytes, and then to examine the insects carefully for the parasites which by hypothesis the gametocytes were expected to develop into. This required not only familiarity with the histology of gnats, but a laborious search for a minute organism throughout the whole tissues of each individual insect examined—a work of at least two or three hours for each gnat. But the actual labour involved was the smallest part of the difficulty. Both the form and appearance of the object which I was in search of, and the species of the gnat in which I might expect to find it, were absolutely unknown quantities. We could make no attempt to predict the appearance which the parasite would assume in the gnat; while owing to the general distribution of malarial fever in India, the species of insect concerned in the propagation of the disease could scarcely be

determined by a comparison of the prevalence of different kinds of gnat at different spots with the prevalence of fever at those spots. In short, I was forced to rely simply on the careful examination of hundreds of gnats, first of one species and then of another, all fed on patients suffering from malarial fever—in the hope of one day finding the clue I was in search of. Needless to say, nothing but the most convincing theory, such as Manson's theory was, would have supported or justified so difficult an enterprise.

As a matter of fact, for nearly two and a half years, my results were almost entirely negative. I could not obtain the correct scientific names of the various species of gnats employed by me in these researches, and consequently used names of my own. Gnats of the genus *Culex* (which abound almost everywhere in India) I called "grey" and "brindled" mosquitoes; and it was these insects which I studied during the period I refer to. At last, the persistently nugatory results which had been obtained with gnats of this genus determined me to try other methods. I went to a very malarious locality, called the Sigur Ghat, near Ootacamund, and examined the mosquitoes there in the hope of finding within them parasites like those of malaria in man. The results were practically worthless (except that I observed a new kind of mosquito with spotted wings); and I saw that I must return to the exact method laid down by Manson. The experiments with the two commonest kinds of *Culex* were once more repeated—only to prove once more negative. The insects, fed mostly on cases containing the crescentic gametocytes of *Hæmomenas præcox*, were examined cell by cell—not even their excrement being neglected. Although they were known to have swallowed living Hæmamœbidæ, no living parasites like these could be detected in their tissues—the ingested Hæmamœbidæ had in fact perished in the stomach cavity of the insects. I began to ask whether after all there was not some flaw in Manson's induction; but no—I still felt his conclusion to be an inevitable one. And it was at this very moment that good fortune gave me what I was in search of.

In a collecting bottle full of larvæ brought by a native from an unknown source, I found a number of newly-hatched mosquitoes like those first observed by me in the Sigur Ghat—namely, mosquitoes with *spotted wings* and *boat-shaped eggs*. Eight of these were fed on a patient whose blood contained crescentic gametocytes. Unfortunately I dissected six of them either prematurely or otherwise unsatisfactorily. The seventh was examined, on August 20, 1897, cell by cell; the tissues of the stomach (which was now empty owing to the meal of malarial blood taken by the insect four days previously being digested) were reserved to the last. On turning to this organ I was struck by observing, scattered on its outer surface, certain oval or round cells of about two to three times the diameter of a red blood-corpuscule—cells which I had never before seen in any of the hundreds of mosquitoes examined by me. My surprise was complete when I

next detected within each of these cells a few granules of the characteristic coal-black melanin of malarial fever—a substance quite unlike anything usually found in mosquitoes. Next day the last of the remaining spotted-winged mosquitoes was dissected. It contained precisely similar cells, each of which possessed the same melanin; only the cells in the second mosquito were somewhat larger than those in the first.

These fortunate observations practically solved the malaria problem. As a matter of fact, the cells were the zygotes of the parasite of remittent fever growing in the tissues of the gnat; and the gnat with spotted wings and boat-shaped eggs in which I had found them belonged (as I subsequently ascertained) to the genus *Anopheles*. Of course it was impossible absolutely to prove at the time, on the strength of these two observations alone, that the cells found by me in the gnats were indeed derived from *Hæmamoebidæ* sucked up by the insects in the blood of the patients on whom they had been fed—this proof was obtained by subsequent investigations of mine; but, guided by the presence of the typical and almost unique melanin in the cells, and by numerous other circumstances, I myself had no doubt of the fact. The clue was obtained; it was necessary only to follow it up—an easy matter.

The preparations of the stomachs of the two *Anopheles* were sealed, and were afterwards examined by Drs. Smyth, Manson, Thin and Bland-Sutton; and an account of the work, and of the observations of these gentlemen, was published a little later. Unfortunately, my labours now met with a serious interruption; but not before I had succeeded again in finding the zygotes in two other mosquitoes—one, another species of *Anopheles*, also bred from the larva, and also fed on a case containing crescentic gametocytes; the other, a "grey mosquito" (*Culex pipiens* type), which had been caught feeding on a case of tertian fever, and which I now think had become previously infected from a bird with *Hæmamoeba relictæ*.

Early in 1898, mainly through the influence of Dr. Manson, Sir H. W. Bliss and the United Planters' Association of Southern India, I was placed by the Government of India on special duty in Calcutta to continue my investigations. Unable to work with human malaria—chiefly on account of the plague scare in Calcutta—I turned my attention to the *Hæmamoebidæ* of birds. Birds have at least two species of *Hæmamoebidæ*. I subjected a number of birds containing one or the other of these parasites to the bites of various species of mosquitoes. The result was a repetition of that previously obtained with the human parasites. Pigmented cells precisely similar to those seen in the *Anopheles* were found to appear in gnats of the species called *Culex fatigans*, Wiedemann, when these had been fed on sparrows and larks containing *Hæmamoeba relictæ*. On the other hand, these cells were never found in insects of the same species when fed on healthy birds or on birds containing the other parasite, called *Hæmamoeba danilewskii*.

It will be evident that this fact was the crucial test both as regards the parasitic nature of these cells and as regards their development from the hæmocytozoa of the birds; and it was not accepted by me without very close and laborious experiment. The actual results obtained were as follows:—

Out of 245 *Culex fatigans* fed on birds containing *H. relictæ*, 178, or 72 per cent., contained "pigmented cells." But, out of 41 *Culex fatigans* fed on a man containing crescentic gametocytes, 5 on a man containing immature tertian parasites, 154 on birds containing *H. danilewskii*, 25 on healthy sparrows, and 24 on birds with immature *H. relictæ*—or a total of 249 insects, all carefully examined—not one contained a single "pigmented cell."

Another experiment was as follows:—Three sparrows, one containing no parasites, another containing a moderate number of *H. relictæ*, and the third containing numerous *H. relictæ*, were placed in separate cages within three separate mosquito curtains. A number of *Culex fatigans*, all bred simultaneously from larvæ in the same breeding bottle, were now liberated on the same evening partly within the first mosquito netting, partly within the second, and partly within the third. Next morning many of these gnats were found to have fed themselves on the birds during the night. Ten of each lot of gnats were dissected after a few days, with the following result:—

The ten gnats fed on the healthy sparrow contained no "pigmented cells." The ten gnats fed on the sparrow with a moderate number of parasites were found to contain altogether 292 "pigmented cells"; or an average of twenty-nine in each gnat. The ten gnats fed on the sparrow with numerous parasites, contained 1009 "pigmented cells"; or an average of 100 cells in each gnat. These thirty specimens were sent to Manson in England, who made a similar count of the cells.

I may mention one more out of several experiments of the same kind. A stock of *Culex fatigans*, all bred from the larva, were fed on the same night partly on two sparrows containing *H. relictæ*, and partly on a crow containing *H. danilewskii* (placed, of course, under separate mosquito-nettings). Out of twenty-three of the former lot, twenty-two were found to have pigmented cells; while out of sixteen of the latter, none had them.

Hence no doubt remained that the "pigmented cells," really constitute a developmental stage in the mosquito of these parasites; and this view was accepted both by Laveran and Manson, to whom specimens had been sent. In June 1898, Manson published an illustrated paper concerning my researches, and showed that the pigmented cells must in fact be the zygotes resulting from the process of fertilisation discovered by MacCallum.

It remained to follow out the life-history of the zygotes. For this purpose it was immaterial whether I worked with the avian or the human parasites, since these are so extremely like each other. I elected to work with the avian species, chiefly because the plague-

scarcely in Bengal still rendered observations with the human species almost impossible. By feeding *Culex fatigans* on birds with *H. relictæ* and then examining the insects one, two, three and more days afterwards, it was easy to trace the gradual growth of the zygotes. Their development briefly is as follows: After the fertilisation of the macrogamete has taken place in the stomach-cavity of the gnat, the fertilised parasite or zygote has the power of working its way through the mass of blood contained in the stomach, of penetrating the wall of the organ, and of affixing itself on, or just under, its *outer coat*. Here it first appears about thirty-six hours after the insect was fed, and is found as a "pigmented cell"—that is, a little oval body, about the size of a large red corpuscle or larger, and containing the granules of melanin possessed by the parent gametocyte from which the macrogamete originally proceeded. In this position it shows no sign of movement, but begins to grow rapidly, to acquire a thickened capsule, and to project from the outer wall of the stomach, to which it is attached, into the *body cavity* of the insect host. At the end of six days, if the temperature of the air be sufficiently high (about 80° F.), the diameter of the zygote has increased to about eight times what it was at first; that is, to about 60 μ . If the stomach of an infected insect be extracted at this stage, it can be seen, by a lower power of the microscope, to be studded with a number of attached spheres, which have something of the appearance of warts on a finger. These are the large zygotes, which have now reached maturity and which project prominently into the mosquito's body-cavity.

All this could be ascertained with facility by the method I have mentioned; and it should be understood that gnats can be kept alive for weeks or even months by feeding them every few days on blood—or, as Bancroft does, on bananas. But a most important point still required study. What happens after the zygotes reach maturity? I found that each zygote as it increases in size divides into *meres*, each of which next becomes a *blastophore*, carrying a number of *blasts* attached to its surface. Finally the blastophore vanishes, leaving the thick capsule of the zygote packed with thousands of the blasts. The capsule now *ruptures*, and allows the blasts to escape into the body fluids of the insect.

These blasts, when mature, are seen to be minute filamentous bodies, about 12–16 μ in length, of extreme delicacy, and somewhat spindle-shaped—that is, tapering at each extremity. Just as the zygotes recall the shape of the Coccidiidæ, so do these blasts recall the "falciform bodies." Prof. Herdman and I have adopted this word "blast" for these bodies after careful consideration—but others prefer other names. They are, of course, *spores*, but spores which have been produced by a previous sexual process—and are, in fact the result of a kind of *polyembryony*. Just as a fertilised ovum gives rise to blasts which produce the cluster of cells constituting a multicellular animal, so, in this case the fertilised ovum or zygote gives rise to blasts, each of which, however, becomes a separate animal.

Prof. Ray Lankester suggests for the blasts of the Hæmamœbidæ the simple term "filiform young."

At this point the investigations took a turn of extreme interest and importance, scarcely second even to what attached to the first study of the zygotes. Since the blasts are evidently the progeny of the zygotes, they must carry on the life-history of the parasites to a further stage. How do they do so? What is their function? Do they escape from the mosquito, and in some manner, direct or indirect, set up infection in healthy men and birds? Or, if not, what other purpose do they subserve? It was evident that our knowledge of the mode of infection in malarial fever—and perhaps even the prevention of the disease—depended on a reply to these questions.

As I have said, the zygotes become ripe and rupture about a week after the insect was first infected—scattering the blasts into the body-cavity of the host. What happens next? It was next seen that by some process, apparently owing to the circulation of the insect's body-fluids (for the blasts themselves appear to be almost without movement), these little bodies find their way into every part of the mosquito—into the juices of its head, thorax, and even legs. Beyond this it was difficult to go. All theory—at least all theory which I felt I could depend upon—had been long left behind, and I could rely only on direct observation. Gnat after gnat was sacrificed in the attempt to follow these bodies. At last, while examining the head and thorax of one insect, I found a large gland consisting of a central duct surrounded by large grape-like cells. My astonishment was great when I found that many of these cells were closely packed with the blasts—(which I may add are not in the least like any normal structures in the mosquito). Now I did not know at that time what this gland is. It was speedily found, however, to be a large racemose gland consisting of six lobes, three lying in each side of the insect's neck. The ducts of the lobes finally unite in a common channel which runs along the under surface of the head and *enters the middle stylet, or lancet, of the insect's proboscis.*

It was impossible to avoid the obvious conclusion. Observation after observation always showed that the blasts collect within the cells of this gland. It is the *salivary* or *poison* gland of the insect, similar to the salivary gland found in many insects, the function of which, in the gnat, had already been discovered—although I was not aware of the fact. That function is to *secrete the fluid which is injected by the insect when it punctures the skin*—the fluid which causes the well-known irritation of the puncture, and which is probably meant to prevent either the contraction of the torn capillaries or the coagulation of the ingested blood. The position of the blasts in the cells of this gland could have only one interpretation—wonderful as that interpretation is. The blasts must evidently pass down the ducts of the salivary gland into the wound made by the proboscis of the insect, and *thus causes infection in a fresh vertebrate host.*

That this actually happens could, fortunately, be proved without any difficulty. As I had now been studying the parasites of birds for some months, I possessed a number of birds of different species, the blood of which I had examined from time to time (by pricking the toes with a fine needle). Some of them were infected, and some, of course, were not. Out of 111 wild sparrows examined by me in Calcutta, I found *H. relictæ*—the parasite which I had just cultivated in *Culex fatigans*—in 15, or 13·5 per cent. As a rule, non-infected birds were released; but I generally kept a few to use for the control experiments mentioned above, and the blood of these birds had consequently been examined on several occasions, and had always been found free from parasites. At the end of June I possessed five of these healthy control birds—four sparrows and one weaver-bird. All of them were now carefully examined again and found to be healthy. They were placed in their cages within mosquito-nets, and at the same time a large stock of old infected mosquitoes were released within the same nets. By “old infected mosquitoes” I mean mosquitoes which had been previously fed repeatedly on infected birds, and many of which on dissection had been shown to have very large numbers of blasts in their salivary glands. Next morning, numbers of these infected gnats were found gorged with blood, proving that they had indeed bitten the healthy birds during the night. The operation was repeated on several succeeding nights, until each bird had probably been bitten by at least a dozen of the mosquitoes. On July 9, the blood of the birds was examined again. I scarcely expected any result so complete and decisive. Every one of the five birds was now found to contain parasites—and not merely to contain them, but to possess such immense numbers of them as I had never before seen in any bird (with *H. relictæ*) in India. While wild sparrows in Calcutta seldom contain more than one parasite in every field of the microscope, those which I had just succeeded in infecting contained, ten, fifteen, twenty and even more in each field—a fact due probably to the infecting gnats having been previously fed over and over again on infected birds, a thing which can rarely happen in nature.

The experiment was repeated many times—generally on two or three healthy birds put together. But I now improved on the original experiment by also employing controls in the following manner. A stock of wild sparrows would be examined, and the infected birds eliminated. The remainder would then be kept apart, and at night would be carefully secluded from the bites of gnats by being placed within mosquito nets. These constituted my stock of healthy birds. From time to time two or three of these would be separated, examined again to ensure their being absolutely free from parasites, and then subjected to the bites of “old infected mosquitoes,” and, of course, kept apart afterwards for daily study. Thus my stock of healthy birds was also my stock of control birds. Until they were bitten by gnats, I found that they never became infected (except in a

single case in which I think I had overlooked the parasites on the first occasion), although large numbers of healthy birds were kept in this manner. The result in the case of the sparrows which were subjected to the bites of the infected gnats was different indeed. Out of 28 of these, dealt with from time to time, no less than 22, or 79 per cent., became infected in from five to eight days. And, as in the first experiment, all the infected birds finally contained very numerous parasites.

It was most interesting to watch the gradual development of the parasitic invasion in these birds; and this development presented such constant characters that, apart from other reasons, it was quite impossible to doubt that the infection was really caused by the mosquitoes. The course of events was always as follows. The blood would remain entirely free from parasites for four, five, six or even seven days. Next day one or perhaps two parasites would be found in a whole specimen. The following day it was invariably observed that the number of the organisms had largely increased; and this increase continued until in a few days immense numbers were present—so that, finally, I often observed as many as seven distinct parasites contained within a single corpuscle! Later on, many of the birds died; and their organs were then found to be loaded with the characteristic melanin of malarial fever.

I also succeeded in infecting on a second trial one of the six sparrows which had escaped the first experiment; and also a crow and four weaver-birds; and lastly, gave a new and more copious infection to four sparrows which had previously contained only a few parasites.

These experiments completed the original and fundamental observations on the life-history of the *Hæmamoebidæ* in mosquitoes. The parasites had been carried from the vertebrate host into the gnat; had been followed in their development in the gnat; and had finally been carried back from the gnat to the vertebrate host. The theories of King, Laveran, Koch and Bignami, and the great induction of Manson, were justified by the event; and I have given a detailed historical and critical account of these theories, and of my own difficulties and experiences, in the hope of bringing conviction to those who might, perhaps, otherwise think the story to be too wonderful for credence.

But work of great importance remained to be done. I had intended, immediately after making this study of one of the parasites of birds, to extend the investigation more fully to those of man—a work which now presented no difficulty, since both the kind of mosquito hospitable to them (*Anopheles*) and the form of the parasites in the mosquito were well known to me. Unfortunately I was obliged to attend to other and less important duties, which kept me fully occupied for several months—an interruption which practically put an end to my own study of the mosquito-theory at a very interesting point. No time, however, was really lost. In December

1898, Dr. Daniels, of the Malaria Commission of the Royal Society and the Colonial Office, arrived in Calcutta to examine and report upon my results. After carefully repeating the various experiments he fully confirmed the statements made by me.* At the same moment, the work was taken up with great brilliance and success by Dr. Koch and by Prof. Grassi and Drs. Bignami and Bastianelli, in Italy. I must now describe the investigations of these observers—though I have scarcely space to do so at the length they deserve.

Ever since the discoveries of Laveran and Golgi, the Italian observers of the Roman school have done much important work on malaria, facilitated by the well-known prevalence of the disease near Rome; work, if not of much originality, yet full of careful detail. More recently, however, this work had been practically arrested by their theory—wholly gratuitous, but which they accepted as a dogma—that the motile filaments are forms of disintegration *in vitro*. When Manson propounded his theory, Bignami, for instance, rejected it on this ground. But at the same time he evolved a gnat-theory of his own—a theory that malarial fever is inoculated by gnats which carry the parasite from marshy areas. The arguments he used were the epidemiological ones already advanced by King, and which can scarcely be said to amount to more than a plausible hypothesis: the only solid basis for the theory—that of Manson—was opposed by him. Later, however, the work of Simond, Schaudinn, Siedlecki, MacCallum and myself, explained by Manson, rendered the Italian position concerning the motile filaments quite untenable; and Bastianelli, Bignami and Grassi now undertook a study of the mosquito-theory on sound principles. My own results, with descriptions of the technique employed and with illustrations of the zygotes, had been published from time to time; a summary of them had been given by Manson in June 1898, and another, including the infection of healthy birds, before the British Medical Association, early in August; and there could therefore be no difficulty in following up the observations therein recorded. In September, Grassi published a paper in which he described certain investigations made in Italy with a view to ascertaining the species of gnats which are associated with the prevalence of malaria in that country. Such investigations are not, I think, trustworthy; and as a matter of fact two out of the three species of gnat then selected by Grassi as being malaria-bearing ones, have now been rejected by him. The third species was an *Anopheles*, namely *A. claviger*, Fabr.

At the same time Bignami resumed his study of the subject. Some years previously, following his theory, he had endeavoured to infect healthy persons by the bites of gnats brought from malarious places. He had failed and abandoned his efforts—and I believe that his method would of itself never had led to a solution of the problem. In the autumn of 1898, however, he renewed his efforts; but was

* Nature, August 3, 1899.

again unsuccessful until he used a number of *Anopheles claviger*, brought from a house containing infected persons. The result was successful, the subject of the experiment becoming infected after some time. This important experiment gave the first confirmation with human malaria of my previous inoculation experiments with the malaria of birds; but since other species of gnats as well as *A. claviger* had been employed, it failed to fix suspicion entirely on the latter. In order to obtain this result, these observers were finally obliged to resort to the correct method of Manson and myself—namely that of direct cultivation of the parasites in the gnat. Success was now immediate. The zygotes and blasts of the parasites were found, exactly as previously described by me, in the tissues of *A. claviger*; and lastly, healthy persons were infected by the bites of these insects. Pushing forward with admirable rapidity, the Italian observers next found that all three species of the human Hæmamebidæ are cultivable in *A. claviger*; and not only in this, but in other Italian species of *Anopheles*, while, like me, they failed in cultivating the parasites in *Culex*.

Almost simultaneously Koch repeated and confirmed with the weight of his authority most of the results which had been obtained as regards both the human and avian parasites. In August 1899, the malarial expedition sent to Sierra Leone by the Liverpool School of Tropical Medicine (of which expedition I was a member), found the human parasites in two species of *Anopheles* in that colony, namely *A. costalis*, Loew, and *A. funestus*, Giles. I hear also that the same result has been obtained with *Anopheles* in two other parts of the world, so that it would appear that something like nine species of *Anopheles* have now been inculcated—while as yet every species of *Culex* which has been tried has failed to give positive results.

From this point it becomes impossible to follow in detail the researches carried out in connection with the mosquito theory in various parts of the world. The facts already collected would fill a small volume; and every month witnesses additional publications on the subject. I shall therefore, in conclusion, content myself with a brief reference to three points of leading importance.

I shall first try to indicate how completely the recent discoveries explain the well-known laws regarding the diffusion of malaria. As mentioned at the beginning of this lecture, malarial fever has long been known to be connected with the presence of stagnant water. That is to say, we generally, though not invariably, find that the disease is associated with low-lying flat areas, where water tends to collect to a considerable extent. It was indeed the general appreciation of this law which led to the old miasma-theory of the disease—the theory on which the word “malaria” was based. We assumed that the poison is one which rises from marshy areas in the form of a mist, and which thence infects all living within a given distance. Later, when the pathogenetic parasite was discovered in the blood of febricitants, many observers, still clinging to this conception, thought

that the parasite is an organism which in its free state dwells in such places, and diffuses itself in such mists. It is interesting to note how near to the truth this almost instinctive conception took us. It is right in idea, wrong in fact. It is not the parasite itself which springs from the marshy ground, *but the carrier of the parasite.*

This was one of the many interesting points made by King in his mosquito-theory of seventeen years ago. But King fell into an error which could have been used as a powerful argument against his hypothesis. He seemed to have assumed that all mosquitoes rise from marshes. Hence, he said, malaria exists in the presence of marshes; hence it is a disease of the country, rather than of towns, and so on. As a matter of fact, mosquitoes as a rule do not rise from marshes at all; they do not all even rise from pools of water on the ground; the commonest species, at least of those which habitually annoy human beings, spring from tubs and pots of water in the vicinity of houses, and are indeed more common in cities than in country places, at any rate in the tropics. Now it is not the least interesting feature of recent researches that they have shown where the error lay. As soon as I have succeeded in cultivating the human parasites in my "dappled-winged mosquitoes," which were really gnats of the genus *Anopheles*, I began to study the habits of these insects, and soon ascertained the remarkable fact that while gnats of genus *Culex* generally breed, in India, in vessels of water round houses, gnats of genus *Anopheles*, which I had just connected with malaria, breed in *small pools of water on the ground.* This point was made the subject of a special investigation by the recent expedition to Sierra Leone; and we found that the law holds good there as in India. While *Culex* larvæ were to be seen in almost every vessel of water, or empty gourd or flower-pot in which a little rain-water collected, in only one case did we find *Anopheles* larvæ in such. On the other hand, *Anopheles* larvæ occurred in about a hundred small puddles scattered through the city of Freetown—puddles mostly of a fairly permanent description, kept filled by the rain, and not liable to scouring out during heavy showers. What was almost equally significant, the larvæ seemed to live chiefly on green water-weed. Hence it follows that while *Culex*, the apparently innocuous genus of gnats, are essentially, or at least often, domestic insects, *Anopheles*, the malaria-bearing genus, are essentially gnats which spring from stagnant water on the ground. And numerous other facts in the history of malaria can be explained by the same discovery. It is supposed, for instance, that malaria originates from freshly-turned earth; now we actually noted examples where railway embankments and the like had produced *Anopheles* pools; and it is easy to see that disturbance of the soil may often produce depressions in the ground capable of holding a little rain-water suitable for the larvæ of these insects. Again malarial fever often appears on board vessels which have touched at malarious ports; as an explanation of this we ascertained that *Anopheles* visit ships from the shore. In short, on study-

ing the matter from every point of view, I must confess to being ignorant of any well-established fact about malarial fever which is not explained by the mosquito-theory.

This brings me to the subject of *objections* to the mosquito-theory. In view of the exact and copious microscopical and experimental evidence which has now been collected in proof of the theory, it is no longer permissible to doubt the main facts; and the objections which one still finds, both in the lay and the medical press, are generally based on a complete ignorance of these facts, and need not be discussed here. But there is one objection—frequently made, in spite of corrections as frequent, by persons who reside in malarious places—which deserves comment. This is, that malaria exists where there are no mosquitoes, and that so-and-so has had fever without being bitten by gnats at all. Generally speaking, we must always remember that malarial fever is a disease in which relapses occur perhaps for years after the first infection, and that it is this first infection and not the relapses which are due to the bite of *Anopheles*. It is thus possible to suffer from any number of attacks of fever without being bitten by *Anopheles* (except on one occasion), and without invalidating the theory—a fact of which those who argue in this manner are generally ignorant. Again, it is well known that one may be bitten without perceiving it; that some persons are singularly callous to the punctures of these insects; and, lastly, that many others have very limited powers of observation. I may say at once that, personally, I cannot accept any statement to the effect that gnats are absent in any locality in the tropics, until such a statement is made by a competent observer after direct search; because I have never been in any place in the tropics—and I have been in a large number—where there were no gnats. On the other hand, I have often found numerous gnats in localities where I was previously told there were none. I was once actually informed that there are no mosquitoes in Sierra Leone! The fact is that those who will trust the statements of the general public on such matters must be very credulous.

I turn lastly to the all-important subject of *prevention*, but can do no more than touch upon it here. Two methods suggested themselves at once. I need not refer to that of guarding against the bites of these insects by the use of mosquito-nets and so on—an obvious and, I believe, an exceedingly useful measure, which may reduce the chances of infection to a small fraction. Unfortunately such methods will never be employed on a large scale in the majority of malarious localities; and we must resort to the *destruction of malaria-bearing species of gnats*. Early in 1892 I reported to the Government of India that it may be possible to exterminate *Anopheles* in *some* localities—especially some towns, cantonments and plantations—owing to the habit the insects have (in some places) of breeding only in selected pools. Since then, a considerable literature has already grown up round the subject. Reviewing this literature, it seems probable that we may be able to exterminate *Anopheles* or at least

largely reduce their numbers, in towns where, owing to the conformation of the ground, the low level of the subsoil water or the small rainfall, surface pools suitable for the insects are comparatively few. The methods which can be adopted against the larvæ are numerous—such as brushing out the pools with a broom, draining them away, filling them up, or treating them with various *culicides*, such as paraffin and numerous other substances (recently investigated by Celli and Casagrandi). On the whole the most promising method which suggests itself is the employment of some cheap solid material or powder which dissolves slowly, which kills the larvæ without injuring higher animals, and which renders small pools uninhabitable for the larvæ for some months. If, for instance, a cartload of such a material would suffice to extirpate the larvæ for a square mile of a malarious town, the result would be a large gain to its healthiness. Dr. Fielding-Ould has lately reported favourably on *tar*. Grillet recently reports a case in France where a large district was rendered free of malaria by the extensive use of *lime* for agricultural purposes. *Gas-lime*, or even common *salt*, may be suggested. In short, though the question of the possibility of attacking these insects with success is still entirely in the experimental stage, we may reasonably hope that the mosquito-theory of malaria may some day prove to be as useful to humanity as it certainly has proved interesting to the student of science.

In conclusion, however, I should add that this result is not likely to be attained unless we, as a nation, determine to pay more attention to scientific discoveries in the field of tropical medicine than hitherto we have done. During the last fifty years discovery after discovery in this field has been made without finding any adequate reflex in medical and sanitary practice in our tropical possessions. The discoveries, for instance, of Lösch, Davaine, Dubini, Bilharz, Bancroft, Koch, Laveran, Manson, Carter and Giles, though nearly concerned with the lives of thousands of human beings, have been generally treated either with scepticism or neglect—have been neither sufficiently followed in the laboratory nor sufficiently acted upon in the region of practical sanitation.

