Sir Ronald Ross and the Malarial Parasite

Discovery of its Route – From Man to Mosquito and Back

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Malaria: A Scourge

Although the weather was sultry and warm, the boy shivered with high fever. Even a thick blanket was not adequate for his shivers. He was having these fevers for the last few days periodically. What he did not know was that within the next day he would go into a coma, and unless he was given quinine immediately, he might die. In the late nineteenth century, such malaria attacks were very common all over the tropical countries including several temperate ones, extending all the way up to Sweden and Canada. Around 1880 Alphonse Laveran had demonstrated convincingly that malaria was caused by parasites present in the blood, but the important question was 'How do the germs get there?' The disease could not be prevented unless the route of infection was established!

Young Ross Enters the Scene

In 1857, a General in the Indian Army, Sir C C G Ross and his wife Matilda gave birth to Ronald Ross in the small town of Almora, at the edge of the Himalayas. At the age of eight Ronald was sent to England to be educated and early in his life he developed interests in poetry, literature, music, and mathematics. Young Ronald had no great affinity for medicine. However, following his father's wishes, he began his medical studies at St Bartholomew's Hospital Medical College, London, in 1874. In 1881, young Ross joined the Indian Medical Service. He held temporary appointments in Madras, Burma, and the Andaman Islands, and by 1892 developed an interest in malaria. He married Miss Rosa Bloxam who accompanied him to Bangalore where Ross reported for duty as a staff surgeon.

Keywords
Ronald Ross, malarial parasite, *Anopheles* mosquito.

Malaria Germs: Air or Water Borne?

Around those years, there was great confusion regarding the malarial parasites and the route of its transmission. Speculations were strife. The name malaria comes from the Italian words '*male*' which means bad and '*aria*' which means air. This name was coined since the disease was associated with odorous marshy land. Like most diseases known at that time, it was believed that the germs bred in marshy water and that the infection spread through drinking such water, or through breathing the marshy air. Several scientists tried to prove this, but the results were largely negative. For some time it was believed that an amoeba, *Amoeba guttula* as reported by Grassi and Feletti [1], was the free living stage that caused malaria. However, these results could not be substantiated. Ross also tried the oral routes to infect people and indeed, published some early papers regarding intestinal route of infection [2, 3]. Later he realized that the forms he saw under the microscopes then were not malarial parasites at all, but some artifacts [4]. Ross recalls in his Nobel lecture "Unfortunately at that time it was extremely difficult to obtain in India any of the more recent literature on the subject; and even the discovery of Laveran (1880) had scarcely penetrated there as yet – much less the work of Golgi, Danilewsky, Marchiafava and Celli. I was therefore forced to rely almost solely on my own observations and thoughts" [5]. However, these trials built up Ross's expertise in the examination of blood samples, which came to his aid in his subsequent research.

Commemorative postage stamp issued by the Govt. of India on August 20, 1997, on the occasion of 100th anniversary of the discovery of Plasmodium oocytes in mosquitoes.

Enter Manson and his Theory of Mosquitoes as the Carrier of Malaria

In 1894, Ross obtained furlough to return to England, and used the opportunity to discuss extensively with Dr. Patrick Manson, considered by many to be the father of tropical medicine. Manson showed Laveran's results to Ross, and the identity of malaria infected blood became clearer in Ross's mind. By 1878 Manson had already demonstrated that the filarial parasite, which causes the disease elephantiasis, could transmit from man to

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mosquitoes. Manson believed the same for malaria. The idea appealed to Ross immensely. After all, the parasites are found in the blood, and mosquitoes feed on blood. Later in his memoirs, Ross writes that there were several scientists who had hypothesized mosquitoes to be the carrier of malaria, based on several epidemiological observations [5,6]. However, proof was needed, and there was no place like India to do such experiments.

In 1895 Ross was appointed as a medical officer of a regiment of native soldiers at Secunderabad. These men suffered much from malarial fever. There were also plenty of mosquitoes waiting to be examined. Ross observed that the crescent shaped infected blood cells (now known as gametocytes) would change into filamentous flagellated shapes which moved about rapidly. This was, in itself, not a new observation. The Italian scientists Grassi and Bignami had described the movements as the death dance of the parasites. Neither Ross nor Manson believed in the death theory and Manson urged Ross to follow these motile filaments in the mosquito gut, as he was convinced that there lied the secret of malaria transmission. Ross set up his experiments with great care. He got his men to bring him mosquito larvae and hatched them in captivity. Thus these mosquitoes were free from any 'earlier' infective forms. The mosquitoes would then be fed on malaria patients inside a bed-net for several hours, subsequently kept alive in jars with sugared water for several days and dissected from time to time. Ross describes his problems in detail [5]. Apart from infected blood of patients (which he could determine unequivocally by then), all other parameters were absolutely unknown. What will he look for in the mosquitoes, and how long after the feeding? The flagellated forms, visible in the mosquito gut soon after feeding, vanished within minutes of exflagellation. Ross was aware of the possibility that the parasites could develop in tissues other than the mosquito stomach and in peculiar shapes too. Therefore he could not limit his examinations to merely the gut and had to observe each part of these mosquitoes. He was however convinced that whatever forms they acquire in the mosquitoes, they must grow larger by the day.

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For two years Ross searched in mosquitoes without avail. He could not find any reproducible change in these mosquitoes. He noticed, however, that the release of the flagellar forms was favored in the mosquito gut environment, as opposed to that on microscope slides. Ross was convinced that the release and the motility of the flagella in mosquitoes were towards a natural development of the parasite – and not a death dance.

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There was also the question of infection back to man. The favorite hypothesis (proposed and supported by many, including Manson) was that mosquitoes deposited the germs into the water on which they laid eggs. The drinking of this contaminated water could be the route of infection. Ross carried out such experiments as well. He describes an initial experiment where he fed three persons, who swore that they had not had fever for several years, with extracts of several infected mosquitoes, and one of the persons developed fever with malaria parasites in his blood. Ross was naturally thrilled with this result, but several attempts to repeat such results showed merely three out of twenty-two persons to be infected [7]. Ross decided that the positive reactions, though interesting, were too few and too slight to warrant any definite conclusion.

Ross described later "I am now inclined to think that these (positive cases) may have been due to the following circumstances. The persons on whom the experiments were made were generally low-caste Indians who required a fee before drinking the water and also an assurance that they would receive more if taken ill. Now it is well recognized that many natives are constantly infected with malaria and get relapses on any extraordinary demand being made upon their systems, as by fatigue, chill, or dissipation. I have even heard it stated by medical men possessing large experience of natives that they can often produce fever in themselves by exposure when they wish to do so. In this case at all events it was possible that some of the subjects spent their preliminary fees in dissipation, thus producing the supposed reaction after the experiments" [5].

Search for the Correct Species of Mosquitoes

All in all Ross had drawn blanks in his investigations. This is when he began to question the mosquito species which might be the true carrier of the disease. He was working largely with the abundant brindled or grey mosquitoes (now known to belong to genus *Stegomyia* and *Culex*), and he started searching for rarer species. During his short posting at Ooty from April 1897 for a few months, he spotted the dappled wing mosquitoes (*Anopheles*) in the Sigur Ghat, but could not get many to investigate.

In July 1897 he was back at Secunderabad, and ready for his experiments once again. The monsoon was late and the heat was intense. In his 'Nobel lecture' Ross recalls "The work, which was continued from 8 am to 3 or 4 pm 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".

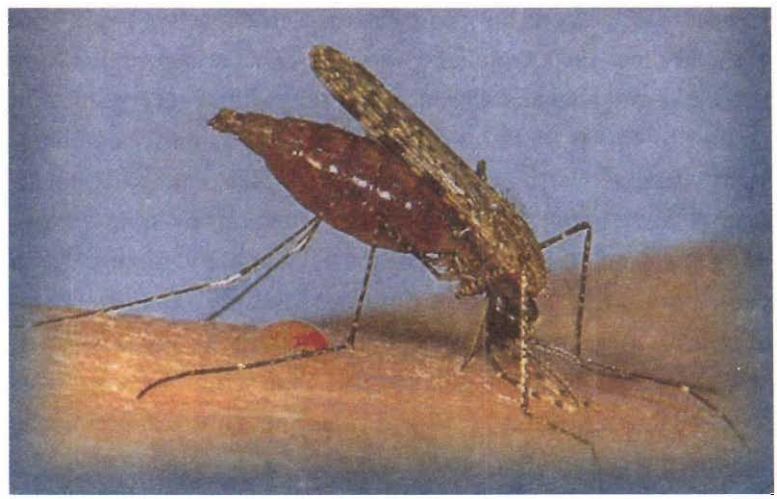

Figure 1. Female Anopheles after a blood-meal.
(Taken from 'Pictorial Identification Key for Indian Anophelines', National Institute of Malaria Research (NIMR), India; with permission).

Sighting of Gametocytes in *Anopheles* Mosquitoes; Success at last!

In mid-August 1897 one of his assistants brought him some larvae that hatched into dappled wing mosquitoes. Along with his work with other mosquitoes, Ross fed these on a malaria patient on August 16. Somehow he lost several of these fed dappled wing mosquitoes and by August 20, Ross was down to last two of such blood-fed dappled wing mosquitoes. He was exhausted with his work on other mosquitoes, and by the end of the day when he dissected one of these last two dappled wing mosquitoes he saw nothing unusual. He was examining the last tissue – the gut – and was about to abandon the mosquito when he observed a very delicate circular cell, different from others. He looked further and spotted another and another. Altogether he found twelve such cells. On the next day, August 21, 1897, Ross killed the last blood-fed dappled wing mosquito and found similar forms, but larger, and quite distinct from the gut cells. Although these were just two mosquito samples, Ronald Ross was quite convinced that those cells in the gut were the parasites. At a stroke Ross was now aware of two unknown quantities – the kind of mosquito implicated and the position and appearance of the parasites within it. Ross took ten days' leave to write a paper, 'On some peculiar pigmented cells found in two mosquitoes fed on malarial blood', which was published in the 18th December, 1897, issue of the *British Medical Journal*. Before sending his work for publication, Ronald Ross was cautious enough to have his work verified by a colleague, Surgeon-Major John Smyth.

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Within some weeks Ross was able to collect enough larvae of the dappled wing mosquitoes, and was set up to pursue his experiments. With great delight he wrote to Manson that he expected to unveil the life-cycle of the malaria parasite in the mosquito within a few weeks. Next day however, he received telegraphic instruction from the Government, ordering him to proceed forthwith to Kherwara in Rajputana – a place thousand miles away in Western India, and with very little malaria!

In his writings, Ross has elaborated on his disappointment in the attitudes of his superiors, and their indifference towards his experiments and results.

Setback and Disappointment

It was a cruel blow for Ross. In Secunderabad he had numerous cases of malaria in his own hospital. He had trained men and had the right kind of mosquitoes hatching out from larvae. He had reported all his research work to his superiors, and thought that they ought to have taken greatest possible interest in such research which touched so vital a subject. But the orders were peremptory and not to be discussed. Within two days (26th September, 1897) Ross set out for his journey to Kherwara. Upset at this disruption, Ross had almost decided to opt for voluntary retirement. However, possibly due to Manson's appeal to the then Director General (Cleghorn) of the Indian Medical Service, Ross was subsequently posted on special duty to investigate malaria and kala-azar in Calcutta.

In his writings, Ross has elaborated on his disappointment in the attitudes of his superiors, and their indifference towards his experiments and results. He was quite sure of his discoveries, and wanted to complete his research on so important a subject. He was also aware that the Italians were close on his heels and were actively searching for the mosquito stages. He just could not understand as to how the authorities could be so insensitive and interfere with his experiments that he had set up so painstakingly.

Finally – Elucidation of Life Cycle of Parasites in Mosquitoes

In Calcutta in February 1898, Ross used a laboratory which had been formerly used by Professor D D Cunningham, and found an able and intelligent assistant named Mahommed Bux. However, he could not find adequate numbers of human volunteers. So he turned to bird malaria. He was quite convinced that the life-cycle of bird malaria was very similar to that of the human malaria. Mosquitoes were fed on birds with and without *Proteosoma* (the bird malaria parasites) and the results compared. Out of 245 grey mosquitoes fed on birds with *Proteosoma*, 178, or

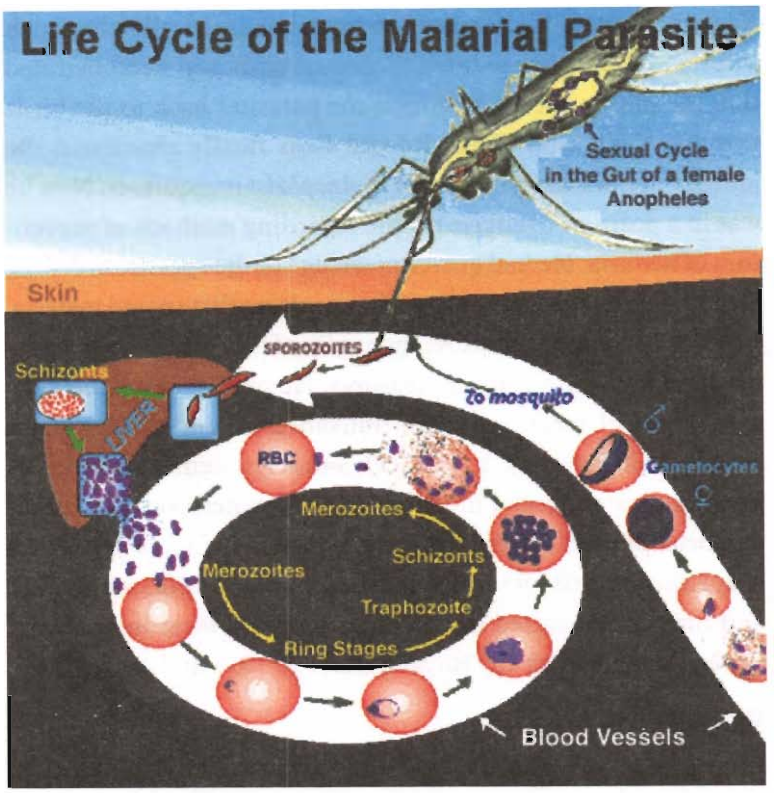

Figure 2. Life cycle of the malarial parasite.
Compiled by TIFR, India (http://www.malaria.org/lifecycle.html).

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. Ross also carried out quantitative experiments. 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 0, 571, and 1084, pigmented cells in the three sets of mosquitoes were found [5]. Where did the parasites go from the gut? Ross meticulously followed the zygotes in the mosquitoes and discovered that the thread like sporozoites released from burst zygotes, were found to concentrate in certain glands in the thorax of the mosquitoes. He traced the glands and concluded that these were salivary glands of the mosquitoes, and immediately realized that the parasites were introduced through the saliva when the mosquitoes had their blood meal.

Ross then made his final set of experiments and demonstrated that the mosquitoes transmitted the parasites back to the birds through their bites.

Ross then made his final set of experiments of allowing such infected mosquitoes to bite uninfected birds and demonstrated that the mosquitoes transmitted the parasites back to the birds through their bites. Thus Ronald Ross finally elucidated the journey of the malaria parasites in *Anopheles* mosquitoes. Now he was in a position to advise people regarding methods of prevention of malaria. He had great knowledge of the specific breeding grounds of the specific species of mosquitoes that spread malaria. In the subsequent years Ross spent a lot of time in the implementation of mosquito control measures. He published mathematical models of malaria disease transmission, and such malaria models still hold true in tropical countries, demonstrating his great understanding of the disease. The greatest were his mathematical models on epidemiology, initiated in his report on Mauritius in 1908, described in his *Prevention of Malaria* in 1911 and further elaborated in a more generalized form in scientific papers published by the Royal Society in 1915 and 1916 [8].

Recognition

In 1899 Ross resigned from the Indian Medical Service and returned to England, and joined the Liverpool School of tropical medicine. In 1901 Ross was elected a Fellow of the Royal College of Surgeons of England and also a Fellow of the Royal Society, of which he became Vice President from 1911 to 1913. In 1902 he was awarded the Nobel Prize for Medicine "for his work on malaria, by which he has shown how it enters the organism and thereby has laid the foundation for successful research on this disease and methods of combating it". He was honored with several other prestigious awards including knighthood in 1911. In 1926, the Ross Institute and Hospital for Tropical Diseases was opened on Putney Heath, London, by the Prince of Wales as a memorial to and in recognition of Ross' work.

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Last Years of Ross

The last few years of Ross were perhaps not very comfortable financially. In 1928, Ross advertised his papers for sale, making it known that he needed the money to support his wife and

family. Ross had preserved all of his letters, prose, poems and experimental records meticulously. He even collected back many of the letters written by him to others. Many of these papers are now held by the London School of Hygiene & Tropical Medicine and are preserved and catalogued [9]. The Ross collection includes a) correspondence with those whose influence had been great on him; Sir Patrick Manson, Charles Alphonse Laveran, William Crawford Gorgas, and Joseph Lister; b) notebooks containing details of his scientific research; c) manuscripts and published articles on malaria and other diseases; d) correspondence about his dispute with Italian scientists over the mosquito-malaria theory; and e) records of Ross' expeditions overseas to develop and implement mosquito control measures.

While Ross is remembered most for his malaria work, we must note that he was also a mathematician, epidemiologist, sanitarian, editor, novelist, dramatist, poet, amateur musician, composer, and artist. He died, after a long illness, on 16 September 1932.

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Suggested Reading

[1] Grassi and Feletti, Contribuzione allo studio dei parassiti malarici, *Atti Accad. Gioenia Sci. Nat. Catania, 5,* Ser. 4a, 1892.

[2] Ross, Entero-septic fevers, *Indian Med. Gaz.,* p.230, 1892.

[3] Ross, Solution of corpuscles mistaken for parasites, *Indian Med. Record,* p.310, 1893.

[4] Ross, A list of natural appearances in the blood which have been mistaken for forms of the malaria parasite., *Indian Med. Gaz.,* Dec.p. 441, 1894.

[5] R Ross, Researches on malaria, Nobel Lecture, December, 12, 1902. (From *Nobel Lectures, Physiology or Medicine 1901-1921,* Elsevier Publishing Company, Amsterdam, 1967). Available at http://nobelprize.org/medicine/laureates/1902/ross-lecture.html

[6] King, Insects and disease, mosquitoes and malaria, *Popular Sci. Monthly,* Sep. 1883.

[7] Ross, Some experiments in the production of malarial fever by means of the mosquito, *Trans. South Indian Branch, Brit. Med. Assoc.,* Dec. 1896. (Read Oct. 30, 1896). Also *Indian Med. Gaz.*

[8] http://www.malariasite.com/malaria/ross.htm

[9] http://www.lshtm.ac.uk/library/archives/rossproject.html

[10] http://www.cdc.gov/malaria/history/ross.htm

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