The race to discover the insect vector of kala-azar: a great saga of tropical medicine 1903–1942

La course à la découverte de l’insecte vecteur du kala-azar : une importante saga de la médecine tropicale 1903–1942

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Abstract In the 19th century, a devastating epidemic of visceral leishmaniasis (kala-azar) swept through northeast India. After identification of the pathogenic agent, *Leishmania donovani*, in 1903, the question of its transmission remained to be resolved. In 1904, thanks to work by L. Rogers on cultures of this parasite it became probable that a haematophagous arthropod was responsible for transmission. J.A. Sinton suggested, in 1925, the distribution of the sand fly *Phlebotomus argentipes* was similar to that of the disease and, thereafter, two independent teams led by H.E. Shortt in Assam and R. Knowles and L. Napier in Calcutta concentrated on this potential vector. Parallel work was in progress in China, directed by E. Hindle and W. S. Patton for the Royal Society Kala-azar Commission, on another species of sand fly. In 1942 the Assam workers transmitted *L. donovani* to five human volunteers by the bites of colonised *P. argentipes* and the race was over.

Keywords *Leishmania donovani* · Kala-azar · Visceral leishmaniasis · Phlebotomine sand flies · *Phlebotomus argentipes*


Mots clés *Leishmania donovani* · Kala-azar · Leishmaniose viscérale · Phlébotomes · *Phlebotomus argentipes*

Introduction

Many people know who discovered the vector of malaria. But how many know who discovered the vector of visceral leishmaniasis, known as kala-azar on the Indian subcontinent? There were no Nobel Prizes awarded for this discovery and no fierce polemic about priority. The marathon to find out how kala-azar in India was transmitted is one of the great sagas of tropical medicine. The word saga is used here in the sense of a narrative of historic or legendary figures and events of an heroic age. The age was the first half of the 20th century. Six of the legendary figures involved in the discovery were eventually elected Fellows of the Royal Society of London. The principals were Henry Shortt (1887–1987), Robert Knowles (1883–1936), L. Everard Napier (1888–1967) and R.O.A. Smith. Others with supporting roles were Sir Rickard Christophers (1873–1978), Sir Leonard Rogers (1868–1962), Charles Wenyon (1878–1948), John Alexander Sinton (1884–1956), Edward Hindle (1886–1973) and W.S. Patton (1876–1960).

*Leishmania donovani* (Laveran & Mesnil) is the parasite that causes kala-azar on the Indian subcontinent [42]. Kala-azar is usually assumed to mean black sickness, an allusion to the clinical sign of darkening of the skin of a minority of
patients. However, Sir Ronald Ross investigated the etymology of the term and suggested kala-azar is probably best interpreted as deadly sickness. Kala (said to be Sanskrit by Ross) means either black or deadly. Azar, believed by Ross to be an Assamese word, means sickness but, in the Nowgong District, he noted that the people more frequently used the Urdu words jwar or bimari. Kala-dukh was another name for visceral leishmaniasis in Assam: in Sanskrit dukh means suffering or pain [42, p. 120]. This great scourge of West Bengal spread to Assam in the 19th century with an alarming mortality rate of 42 per thousand [19]. Assam was incorporated into British-administered Bengal in 1838 and the indigenous people associated the appearance of the first epidemic with colonial occupation: hence the name sakari bimari (literally government disease) for kala-azar [19]. Two reports show that the health authorities had no better idea of the cause than the people. In 1889, an expert, Surgeon-Captain G.M. Giles, was called in to report on kala-azar in Assam. He concluded that this disease was due to an infection with hookworms (ankylostomiasis) [42, pp. 255–270]. This idea was rejected by clinicians on the spot and, in 1896, a second expert, Surgeon-Captain (later Sir) Leonard Rogers, was asked to give an opinion: he maintained kala-azar was ‘nothing more or less than a very intense form of malaria’ [42, pp. 255–270], a view supported by Major (later Sir) Ronald Ross after an exhaustive clinical and post mortem study of cases in Assam that he conducted in 1896 [42, pp. 216–217].

The discovery of the cause of kala-azar

In 1903 Ross [33, 34] reported that kala-azar was caused by a protozoan parasite at first named Piroplasma donovani by Alphonse Laveran and Felix Mesnil earlier that year [23]. Sir William Leishman [24] and Charles Donovan [11] had independently seen amastigotes in spleen smears of Indian patients with prolonged fever, known as ‘dum-dum fever’, but neither they nor Laveran appear to have realised they had found the causative organism of kala-azar: primed by an extensive study of the disease in Assam in 1898, Ross did [42, pp. 101–234]. He had autopsied cases and noted the enlargement of the liver and spleen, but had never looked at smears of the tissues. He at once wrote to a colleague, Dr C.A. Bentley, in India and asked him to examine stained spleen smears from kala-azar patients. Bentley immediately found amastigotes, and cabled the result to Ross who quickly published two notes in the British Medical Journal announcing the discovery and transferring the species donovani to a new genus, Leishmania [33, 34]. Sir Leonard Rogers had had the same idea and had already seen the parasite in spleen smears of kala-azar patients sent to him in England by Dr J. Dodds-Price, one of his friends working in Assam: but Rogers was too slow in publishing and lost the priority [3]. The causative organism was now known, but how it was transmitted was not.

The discovery of the vector of kala-azar

Rogers may have missed priority in the discovery of the causative organism but he nevertheless made a crucial observation in 1904 indicating that the carrier of the disease was almost certainly an arthropod. He was the first to show that cultured amastigotes change into elongate, flagellated parasites, now known as promastigotes, with the same morphology as many monoxenous trypanosomatids parasitic in the guts of dozens of different species of insects [32]. The search began.

Before the First World War, workers followed completely false trails by experimenting with almost any blood-sucking creature including fleas, lice, reduviid bugs, bedbugs, mosquitoes, midges, stable flies, hippoboscids and ticks: suspicion was cast even on tsetse flies, house flies and hookworms [49]. Only leeches and vampire bats were not studied as possible vectors. The bedbug was the prime suspect. A student of Oscar Theodor in Palestine wrote that the ‘Kala-azar Commission was in fact established [in Jerusalem by the Royal Society] to incriminate the bedbug’ [16]. This never happened.

In the 1920s and 1930s, three teams were working on this problem, one in the Calcutta School of Tropical Medicine headed by Robert Knowles; a second in Assam directed at first by Sir Rickard Christophers and later by H.E. Shortt; and a third in Shantung Province, northern China—the Royal Society Kala-azar Commission staffed by Edward Hindle and W.S. Patton—that was abandoned after a few years because of civil unrest.

Several findings then led workers to focus their attention on sand flies. The first was a report by Charles Wenyon [48] of flagellates in the guts of unidentified sand flies caught at Aleppo in Syria. This is an ancient focus with a constantly high prevalence of anthroponotic cutaneous leishmaniasis caused by L. tropica—transmitted by Phlebotomus sergenti Parrot—and Wenyon was probably the first person to see a sand fly infected with Leishmania. At the time, this was not of immediate relevance to some of the people searching for the vector of leishmaniasis because infections of parasitic trypanosomatids of insects were well known, and this could have been one of numerous monoxenous species of no known importance in human disease [8, 31]. In 1914, in the highly active kala-azar focus in Assam, F.P. Mackie [28] found flagellates in the midguts of 7% (10/65) of specimens of a sand fly identified as Phlebotomus minutus [probably Sergentomyia punjabensis [43]]. Mackie knew the fly fed on geckos but he found no infection in ten geckos caught
in the same place as the flies. He concluded the parasite was ‘probably a natural parasite of the fly …’. This was a false trail: Shortt [37] later identified the parasite as a species of *Bodo*, a normally free-living genus of flagellates.

The second clue was the discovery by Edmond Sergent and colleagues [35,36] in Algeria that, when scarified on the skin, promastigotes from wild-caught *P. papatasi* (Scopoli) could give rise to cutaneous leishmaniasis caused by *L. major* (Yakimov & Schockov). Knowles and colleagues in Calcutta knew this in 1923 or before [21]. The third clue was founded on a fallacy. It was the belief that there appeared to be a close correlation between the distribution of kala-azar and that of one particular blood-sucking insect, a peridomestic phlebotomine sand fly (*P. argentipes* Annandale & Brunetti) that was abundant in houses in endemic areas of Indian kala-azar. This has been widely presented as a key observation in the steps to find the vector in India [50,51] but a comparison of the map of the distribution of kala-azar in India, as known in the early 1940s [29], with that of *P. argentipes* [26] shows there are many places where the vector has been recorded but kala-azar has never been reported. Before the Second World War, many parts of the subcontinent had not been surveyed for sand flies: now we can see there is no correlation [25].

Sinton [43] published his idea in 1925 but had told Knowles and colleagues at the Calcutta School of Tropical Medicine about it in 1922 [27]. It is perhaps no coincidence that, shortly afterwards, they were the first to show that, when wild-caught *P. argentipes* were fed on kala-azar patients, the parasite in the midgut of the fly changed from the amastigote to the promastigote form [22]. That was the fourth clue. According to Garnham [14], they showed the infections to Christophers, Shortt and Barraud who realised the importance of repeating this work with laboratory-bred sand flies that, unlike wild-caught flies, could not be harbouring natural infections. They therefore reared *P. argentipes* in the laboratory in Assam and, in 1925, quickly confirmed Knowles’s observation [6]. All that remained was to transmit the parasite experimentally to human volunteers by the bite of flies that had previously fed on kala-azar patients. That was easier said than done: it took Shortt a further seventeen years to prove beyond doubt that human kala-azar is transmitted by sand fly bites.

In the latter half of the 19th century, kala-azar had spread eastwards into Assam from Bengal with devastating effects. In the epidemic of 1900–1910, the mortality rate was 90%: in one District, a quarter of the population died and a third of the agricultural land lay uncultivated. It was not known how the infection was acquired and the only effective treatment at that time (with intravenous tartar emetic first used in India by Rogers) was not without danger. In 1924, a Kala-azar Commission was set up under the direction of Sir Richard Christophers in the middle of another epidemic with the main purpose of answering this question. Shortt was posted to the newly formed Commission from the time it was established. The Commission’s laboratory was situated on the banks of the Brahmaputra River at Golaghat in Assam where the scientific staff lived in houses with earthen floors, grass roofs and walls made of plastered bamboo. They had no electricity or running water: the laboratory was no better [40]. The team at the Calcutta School of Tropical Medicine headed by R. Knowles and L.E. Napier was an ancillary unit.

Shortt and Barraud, the Assam team’s first entomologist, began by colonising *P. argentipes*, now the obvious candidate as a vector. When Christophers was posted to another station after two years, Shortt became Director. By this time, the complete life cycle of the parasite in the fly had been revealed and work was in progress to see if the parasite could be transmitted to experimental animals by the bites of flies that had been infected by permitting them to feed on patients. It was not realised at that time that female sand flies fed not only on blood, but also on sugars. Despite this, they managed to keep some infected female flies alive long enough to feed a second time on laboratory animals or human volunteers, 11 of the latter were bitten a total of 11 537 times by infected sand flies: none became infected. Two hundred and forty-three mice were bitten by infected flies 9490 times: all remained negative. Thirty-five Chinese hamsters were bitten 3358 times: only one, bitten 1434 times, became infected [39]. This last animal was found in curious circumstances 511 days after the first bite. After the years of failure, Shortt became so depressed that he recommended the Commission be disbanded. The following day, while they were making a final examination of all the laboratory rodents that had been bitten by infected flies, the infected hamster was found [41].

Shortt’s competitors were not getting better results. At the Royal Society Kala-azar Commission in China, Hindle and Patton had obtained the development of *L. donovani* in another sand fly vector, *P. chinensis* Newstead, but had failed to transmit the parasite to Chinese hamsters (*Cricetus griseus* Milne-Edwards) by the bite of the fly [18,30]. Similarly, in Calcutta [now Kolkata], Knowles obtained only negative results after feeding 458 *P. argentipes* (previously fed on a kala-azar patient) on human volunteers. In the same year, in a review of the transmission of *Leishmania* problem, Adler [1] wrote: ‘It is impossible to abandon the sandfly [sic] theory of leishmaniasis, for the bulk of the evidence favours it, and all other biting insects so far suggested can be safely excluded.’ He concluded, however, that while he believed that Baghdad boil (a form of Old World cutaneous leishmaniasis) was transmitted by the bite of sand flies, the evidence available at that time suggested that ‘Visceral leishmaniasis in India is transmitted by the crushing of *P. argentipes*, and only occasionally by bite.’ The problem seemed insoluble.
The Commission in Assam survived partly, perhaps, because of the single infected hamster. But Shortt changed direction and, in spite of Adler’s opinion, he decided to concentrate on the question: did people become infected by the bites of sand flies? For this, he needed more human volunteers. The background to the struggle to find the volunteers for this investigation is given in an unpublished memoir by Shortt [40]. The Governor of Assam quickly squashed his tentative request that prisoners might play this role if offered some remission of sentence. Shortt decided he would have to use informed volunteers. He therefore prepared a budget and research proposal explaining what he wanted to do for submission to the Indian Research Fund Association of the Government of India. In spite of the fact that volunteers had already been used in experiments both in Shortt’s laboratory and in Calcutta, it was brusquely rejected as a preposterous idea with a clear instruction that he was categorically forbidden to carry out further experiments with human volunteers. He nevertheless sensed some nuances in the letter of refusal that gave him the impression his superiors wanted the work done providing they carried no responsibility. Accordingly, he resubmitted the same proposal with no mention of human volunteers and a different title: ‘Research with Insects’. It was approved. But he still had another hurdle to jump. His two colleagues at the time, Barraud and Craighead, considered the use of human volunteers unjustified and refused to take part unless ordered so to do. Shortt, who was senior to them, had no hesitation in giving the order. Exactly what part they played—if any—is unclear but it is noteworthy that they were not authors on the paper published later on the infection of volunteers by bite.

The Gologhal research station was not the ideal place to do rigorous experiments with human volunteers. There was no conveniently accessible place for the volunteers to be kept under observation without the risk of a natural infection: if a volunteer became infected while living there, it would be impossible to know if the infection were natural or experimental. Shortt decided to move. The place chosen was Gauhati at the foot of the Khasi Hills, within easy reach of Shillong, the capital of Assam that lies at an altitude of 2000 metres—well above the altitude at which P. argentipes is found. There were no cases of kala-azar in Shillong and it was an ideal place to recruit volunteers who had never been exposed to infection. On the other hand, cases with which to infect sand flies were easily found at the lower altitude around Gauhati. It was decided that (a) all volunteers would be individuals who had never left the capital, (b) they would be kept under observation in Shillong, safe from any risk of natural infection, (c) they would be generously compensated for their participation (400 rupees per month) and (d) if any became infected, they would be given treatment with urea stibamine until completely cured. Now good treatment was available, there was every reason to believe that none of the volunteers would be exposed to an unacceptable risk [38 [cited by 42, p. 84], 12].

Temporary houses were built of bamboo, mud and straw, and Shortt and the team settled in the new station in May 1930 with the sole objective of testing the idea that kala-azar was acquired by the bite of a sand fly that had previously fed on a person with the infection; it took more than ten years before the objective was reached. In spite of the living conditions, Shortt [40] described his spell at Gauhati as ‘one of the most enjoyable times we spent in India’.

The simple, foolproof plan of the experiments had to take into account that the natural biting activity of P. argentipes is between sunset and sunrise. Colonised sand flies were fed on patients in villages around Gauhati. Surviving flies were later fed on five volunteers recruited from Shillong who were brought down from the capital to the laboratory by car, arriving around noon. After infected flies had been fed on them, the volunteers spent the night in a sealed room with a single access—one window fitted with a large cloth sleeve through which the volunteers entered. When they were in the room, the sleeve was closed until after sunrise to ensure that wild flies did not bite them during the hours of the activity of this species. On the following day, they were taken back to Shillong by car and remained there under medical observation until the result of the experiment was known. All five volunteers became infected providing incontrovertible evidence that kala-azar could be acquired by the bites of sand flies that had previously fed on an infected human being [46].

**Discussion**

Robert Knowles, the “best man” at Shortt’s wedding—but nevertheless one of his competitors in Calcutta—aptly described the long search for the vector of kala-azar as follows: ‘The story of the discovery of how kala-azar is transmitted from man to man is one of the most amusing, also perhaps one of the sorriest in tropical medicine. It is a history of almost twenty years of wasted effort, of individual workers starting off with the highest hopes and ending in despair; of false starts and erroneous conclusions; of acute controversies and the flow of much ink; of wasted effort and the absence of co-ordinated enquiry’ [9]. One senses his feeling of disappointment and frustration that he and his colleague Napier failed to win the final prize. Moreover, it must have been particularly galling to know that their contribution helped Shortt to succeed.

Shortt’s outstanding success with his last series of volunteers was due to the technique of maintaining sand flies devised by R.O.A. Smith [44,45] whose contribution was not adequately acknowledged in the paper by Shortt and colleagues reporting successful transmission [46]. This omission was corrected in the following comments by the editor of the *Indian Journal of Medical Research* [13]:
‘The sandfly [sic] was again studied and new methods of breeding, maintaining and feeding *P. argentipes* were developed, and with their use a much greater success than formerly was obtained in the transmission of kala-azar to experimental animals, all hamsters used being infected.’ With reference to Shortt’s results, the editor then made it clear that ‘The new technique devised by Smith for obtaining sand flies in a highly infective state was employed’, almost as if he were anxious to put the record straight. In 1939, Smith was put in charge of an enquiry into an epidemic of kala-azar that erupted in Bihar State.

In his 1945 review, Shortt [39] mentions 11 human volunteers in the earlier disappointing studies who were bitten 11 537 times by infected sand flies but had remained apparently healthy. This negative series and Shortt’s numerous failures to infect laboratory rodents contrast sharply with the success with five out of five volunteers at Gauhati when the infected sand flies were maintained on raisins. It is probable that there is nothing special about raisins but, by giving the flies a source of sugar, it was possible to keep them alive long enough for the production of the metacyclic forms of the parasite that are infective to the vertebrate host. Certainly, Shortt (personal communication, telephone conversation 15 April 1987) did not acknowledge that raisins were the reason for his success. Later work suggests he was right: up to 1986, seven species of *Leishmania* were transmitted experimentally in the laboratory by the bite of sand flies on more than 30 occasions, all without raisins except for the work in India in the 1930s and early 1940s [20]. Sugar was the secret.

Suspicious that sand flies were vectors of kala-azar were reinforced by the results of an experiment by Sergent and colleagues [35], which is sometimes presented as the first proof that sand flies are vectors of cutaneous leishmaniasis [7,47]. Before the discovery of the genus *Leishmania* at the beginning of the 20th century, there was no reason to link the two diseases, one a cutaneous infection and the other, kala-azar, a visceral disease. But Knowles and Napier [21] were fully aware of the French work. There is, however, no record to show whether or not Shortt knew about it, although it is safe to assume he did. Shortt was in close contact with Saul Adler in Palestine who had repeated Sergent’s experiments and was eventually (with Ber) to prove sand flies are indeed the vectors of cutaneous leishmaniasis [2]. Shortt and Adler first met on the banks of the river Euphrates during the First World War when Lieutenant Adler introduced himself to Captain Shortt [4]; Shortt became a strong influence in Adler’s eventual choice of parasitology as a speciality. They kept in touch after the war. In 1931, after Adler had persuaded an eccentric Israeli zoologist, Israel Ahroni, to collect golden hamsters [5] for his work on leishmaniasis [C. Henwood (2008) gives a history of the hamster at http://www.hamsterbungalow.com/hamster-history.html]. Adler sent breeding stock to Shortt in India. Because of this contact, there is no doubt that Shortt must have known about Sergent’s work on cutaneous leishmaniasis and realised its relevance to his own work on the visceral form of the disease.

Shortt’s use of volunteers has been criticised as being unethical and it has been said that ‘many’ of the volunteers died as a result of the experimental infections [10]. By the time Shortt did his experiments, urea stibamine had been widely used for the treatment of Indian kala-azar since its discovery and introduction by Brahmachari in 1922 [17]. In 1932 Shortt wrote: ‘We found urea stibamine an eminently safe and reliable drug and, in seven years, in which we treated some thousands of cases of kala-azar, and saw thousands more treated in treatment centres, we personally came across no cases of death directly attributable to it’ [38; see 42, p. 84]. Shortt and his colleagues fulsomely acknowledged the courageous part played by the volunteers. They wrote: ‘We must acknowledge the self-sacrificing spirit of the human volunteers who submitted themselves for experimentation and helped in the final solution of a problem in tropical medicine of many years’ standing’ [46]. No mention was made of deaths. While it is probable that similar work would not be permitted now, Shortt’s decision to use human volunteers should be viewed against the background of the epidemics of a disease of unknown transmission that had ravaged northeast India for almost a century with appalling loss of life and devastation of the social fabric of the area. Critics should take into account that, while struggling to find the solution to the problem of the transmission of kala-azar, Shortt—often with his wife and occasionally with his two young children—lived for years in places where the disease was endemic and periodically epidemic.

It is curious that Shortt was not the senior author of the paper reporting the successful experiments [46]: it was C.S. Swaminath, an assistant entomologist and a junior member of the research staff [42, p. 90]. The contribution to the human experiments—if any—of the third author, Swaminath’s superior officer Lieutenant-Colonel L.A.P. Anderson, is uncertain. As a serving officer, Shortt could not publish without official permission and during the political turmoil in India at that time it was perhaps deemed inadvisable to reveal that a British doctor had used Indian volunteers as guinea pigs.

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1The latter accusation was a mistake: it does not appear in the subsequent 5th edition of this book, published in 2005; in fact, only five became infected and there is no record of any having died*.

*Additions to the original text by J.R. Baker

It is inconceivable that Shortt would have used volunteers with no certainty they could be treated if they became infected. [In his later review, Shortt [39, p. 20] recorded that ‘the failure of work with experimental animals had led us to the use of human volunteers, a step rendered justifiable, in spite of the dangerous nature of the disease, by the discovery of a very effective method of treatment.’]
pigs in a scientific medical study. Perhaps it was considered prudent that the senior author was an Indian member of the research team rather than the British instigator of the study.

Shortt was not elected to Fellowship of the Royal Society until 1950 after his second great contribution to parasitology: the first demonstration of the pre-erythrocytic stage of a malaria parasite in the liver of a human volunteer, discovered jointly with P.C.C. Garnham FRS and colleagues in 1949 [15].

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