the
WELLCOME
TRUST
ILLUSTRATED HISTORY
OF
TROPICAL DISEASES

EDITED BY PROFESSOR F E G COX
RIGHT: Adolfo Lindenberg (1872–1944), the first person to detect leishmanial parasites in patients suffering from úlcera de Bauru.
Professor C. S. Lucaz
Leishmaniasis occurs in both the Old World and the New World. In both regions the parasites belong to the genus *Leishmania* and are transmitted by sandflies. The species of *Leishmania* in the New World are different from those in the Old World and the genera of sandfly vectors are also different. In the New World, there are essentially three forms of the disease: cutaneous, mucocutaneous and visceral. These are caused by several species of *Leishmania*, most of which have a range of wild mammal reservoir hosts.

Mucocutaneous leishmaniasis occurs in the New World, where it is known by a variety of names and causes permanent, disfiguring lesions. Control measures depend largely on the use of insecticides to destroy the sandflies and on the destruction of reservoir hosts.

The history of New World leishmaniasis is tied up with that of the Old World disease. This concerns the recognition of the disease, the discovery of the parasites that cause the disease and their vectors, the recognition of the numerous forms of leishmaniasis in the New World and the association of these with particular species of parasites, vectors and reservoir hosts.

**Fifth to tenth centuries – the first clues**

Ancient Peruvian and Ecuadorian ceramics, estimated to date from AD 400 to AD 900, are frequently in the form of human figures (*huacos*) and sometimes depict individuals with faces disfigured by ugly lesions, particularly of the nose and lips. These quaint vessels and figurines long remained mere museum curiosities but, in 1895, Samuel Mathewson Scott took a collection of them to the USA, where they became the subject of much speculation as to the nature of the mutilations. In 1897 Ashmead presented a paper at the Congress on Leprosy, in Berlin, and suggested that the most likely diseases shown by the *huacos* were syphilis and leprosy. This hypothesis was largely abandoned, however, when medical historians indicated that both these afflictions were
probably imported into the New World in post-Columbian times. Tamayo, in 1909, appears to be the first to link the huaco lesions with a disease referred to by the Peruvian indians as 'uta', contracted in the valleys of the Andean highlands. This form of cutaneous leishmaniasis rarely, if ever, produces destructive nasal or buccal lesions and is characterized by relatively simple skin lesions. These are often on the face, and some of the huacos with such facial disfigurations might well be depicting uta.

The huacos most popular among authors discussing the antiquity of leishmaniasis, however, are those showing extensive destruction of the nose and mouth – a common sequel to infection with a different leishmanial parasite, the aetiological agent of 'espundia' acquired in the lowland rainforests on the eastern side of the Andes. Most of the huacos are the work of lowland, coastal civilizations (notably Chimú and Mochico indians) who were, therefore, more likely to make contact with uta cases than espundia sufferers, a much longer and more hazardous journey away on the far side of the mountains. Another view is that the huacos depict artificial lesions of criminals who had been mutilated as a form of punishment, or following rituals in worship of the potato, when noses were cut off to imitate the potato 'eyes'.

ABOVE: Peruvian huaco showing facial disfigurements thought to be caused by leishmaniasis.

Sixteenth to eighteenth centuries – the written record

Spanish historians at the time of the conquistadores wrote of skin lesions seen among the Peruvian indians which resulted in mutilations similar to those of some huacos. In 1571 Pedro Pizarro described a disease of coca-growers on the lower, eastern slopes of the Andes, which often destroyed the nose and lips. As this is, today, a known endemic area for espundia, it is reasonable to suppose he was describing this disease. Father Rodrigo de Loayza, in 1586, noted that skin lesions were very common among the Peruvian Indians, and later reference to them was also made by Diego de Morales, in 1602, who commented on the 'plague of mosquitoes' affecting all in that region and the terrible skin ulcers or 'llagas' among the local inhabitants. Such reports were common throughout the ensuing centuries; of particular interest is that of Cosme Bueno, in 1764, who implicated sandflies as the probable vectors of both uta and Carrion's disease (bartonellosis), and thus anticipated by almost one and a half centuries any other suggestion that these insects are the transmitters of pathogens to man.

Slowly it became apparent that diseases resembling uta and espundia were widespread throughout most of the Latin American continent, where they became known by a variety of local names. For the less destructive skin lesions: uta seco, úlcera de Velez, úlcera de los chicleros, buba, úlcera de Bauru, ferida brava, botão do oriente, forest-yaws, Bay-sore, chicleros ear, pian-bois, and bosch-yaws. For espundia: laga corrosiva, cancro espúndico, nariz de tapir, tiacarana, gangosa, ferida esponjosa and cancro fagendênico.

Nineteenth and early twentieth centuries – is uta ‘oriental sore’?

In 1852 Professor Julian Bravo suggested that Peruvian uta was identical with 'oriental sore' – an infection producing similar skin ulcers among the inhabitants of many Mediterranean and Asian countries – the aetiology of which was also still in doubt. Although the causative agent of Old World oriental sore had been described in 1903, and named Leishmania tropica in 1906, association of a leishmanial parasite with the various skin diseases in Latin America was not achieved until 1909, when Lindenberg detected 'Leishman–Donovan bodies' in the lesions of patients with úlcera de Bauru in São Paulo State, Brazil. Oddly, he first published his finding in a newspaper,
O Estado de S Paulo, on 30 March 1909, and the discovery was confirmed by Carini and Paranhos who recorded their observations in the same newspaper on the next day. Finally, Miranda in 1910 and Splendore in 1911 both demonstrated the parasite in classical cases of espundia.

Until 1911 the causative agent of New World cutaneous leishmaniasis was thought to be *L. tropica*. In that year, however, Gaspar Vianna, a young clinician, working in the Instituto Oswaldo Cruz in Rio de Janeiro, claimed to have detected morphological differences between that parasite and the one responsible for American cutaneous leishmaniasis. He named the latter *L. braziliensis* (later corrected to *L. braziliensis*) and although his observation was subsequently disproved, there was a general acceptance of the name.

For the next few years the general opinion was that all American cutaneous and mucocutaneous leishmaniasis was due to a single parasite, *L. braziliensis*. In 1913, however, Velez decided that the parasite responsible for Peruvian uta was neither *L. tropica* nor *L. braziliensis*, and patriotically named it *L. peruviana*.

The incrimination of vectors – early beginnings

In 1921, in north Africa, Sergent and his colleagues produced oriental sore in a man inoculated with flagellates from a naturally infected sandfly, *Phlebotomus papatasi*. It was natural, therefore, that phlebotomine sandflies should be suspected as the vectors of leishmaniasis in the Americas, where the phlebotomine fauna is extraordinarily rich and varied. In 1922, Aragão, impressed by the abundance of the sandfly *Lutzomyia intermedia* in and around houses in rural areas of Rio de Janeiro State, Brazil, fed some on the lesions of patients with cutaneous leishmaniasis, found flagellates in a saline triturate of the flies and produced a lesion.
containing *Leishmania* after intradermal inoculation of the material into the nose of a dog.\(^{17}\) Pessôa and Pestana examined large numbers of sandflies in São Paulo State, Brazil, in 1940, and found ‘leptomonad’ infections in *Lutzomyia migonei*, *Lu. whitmani*, and *Lu. pessoai*.\(^{18}\) Although the true nature of the flagellates was not determined, these sandflies remained high on the suspect list as vectors of *L. braziliensis*.

**A new form of cutaneous leishmaniasis**

A bizarre form of cutaneous leishmaniasis of man in Venezuela was described by Convit and Lapenta in 1946.\(^{19}\) It was characterized by nodular lesions scattered over the body and containing enormous numbers of unusually large leishmanial parasites. The disease would not respond to treatment, and patients showed a negative reaction to the Montenegro skin-test. The condition became referred to as ‘diffuse cutaneous leishmaniasis’ (DCL).

**The second half of the twentieth century – the growing complexity of American leishmaniasis**

By the middle of the twentieth century it had become clear that the different forms of leishmaniasis were caused by different parasites. In 1953, Biagi gave the name of *L. tropica mexicana* to the causative agent of ‘chiclero’s ulcer’ in Yucatán, Guatemala and Belize,\(^{20}\) and Floch adopted this trinomial nomenclature in 1954 when he used the name *L. t. guyanensis* for the parasite of ‘pian-bois’ in French Guyana.\(^{21}\) Cutaneous leishmaniasis in other parts of South America he considered to be due to *L. t. braziliensis*. However, in common with a great many others, Medina and Romero did not favour use of the name *tropica* and gave the name of *L. braziliensis pifanoi* to the parasite causing Venezuelan DCL,\(^{22}\) described by Convit and Lapenta. The Brazilian parasitologist, Pessôa, thought along the same lines, and in 1961 listed the recognized American leishmanias as *L. braziliensis braziliensis, L. b. guyanensis, L. b. peruviana, L. b. pifanoi* and *L. b. mexicana*.\(^{23}\) Garnham raised the parasite of chiclero’s ulcer to specific rank, in 1962, as *L. mexicana*;\(^{24}\) to be followed by Medina and Romero, who referred to the causative agent of Venezuelan DCL simply as *L. pifanoi*.\(^{25}\)

**Animal reservoirs of cutaneous leishmaniasis**

Incrimination of both dogs and wild animals as reservoirs of *Leishmania* in the Old World was greatly to influence studies on leishmaniasis in the Americas. In 1951 Herrer found *Leishmania* parasites in skin lesions of dogs from foci of *uta* in the Peruvian Andes.\(^{26}\) Whether or not the dog
was an effective reservoir or merely a ‘victim host’, like man, remained debatable. Elsewhere cutaneous leishmaniasis was clearly an occupational hazard of forest workers, and it was logical to suspect forest animals as the source of infection. In 1957–59, workers in the Gorgas Memorial Laboratories, Panama, isolated a *Leishmania* from forest rodents and showed it to be infective to man, and in 1960 Forattini also demonstrated infections in rodents from São Paulo State, Brazil. In neither case, however, was it conclusively shown that the organism was identical with that commonly responsible for human cutaneous leishmaniasis. This link was firmly established by Lainson and Strangways-Dixon, working on the epidemiology of chiclero’s ulcer, in the forests of Belize, during 1959–62. There they found three different rodent species harbouring *L. mexicana* in their skin and showed that the parasite was identical with that causing the disease in man, following the inoculation of volunteers and by comparative biological study of the parasites. Sandflies were infected by feeding them on hamsters with skin lesions caused by *L. mexicana*, and the parasite was transmitted to a volunteer by the bite of one of them – the first conclusive proof of the transmission of American leishmaniasis by the bite of phlebotomine sandflies.

During a trip to Amazonian Brazil, in 1963, Lainson met Otis Causey and showed him photographs of rodents infected with *L. mexicana* from Belize. Causey had seen similar lesions on the tails of the forest rodent *Oryzomys* sp. in Belém. Between 1965 and 1972 Lainson and Shaw studied the parasite found by Otis Causey in *Oryzomys* and isolated it from other species of rodents and opossums. They named this parasite, that produced both curable, simple skin lesions and incurable DCL in patients with no specific cell-mediated immunity against the parasite, *L. mexicana amazonensis*.

**The vectors of Leishmania mexicana discovered**

Following Strangways-Dixon and Lainson’s transmission of *L. mexicana* to man by the bites of experimentally infected sandflies, Johnson and her colleagues reported flagellates in a number of Panamanian sandfly species. Those from a specimen of *Lutzomyia trapioid* produced cutaneous leishmaniasis when inoculated into the skin of a volunteer.

While Paul Williams and Henry Disney were continuing the epidemiological study of chiclero’s ulcer in Belize, Disney perfected a trap with which to collect sandflies attracted to the rodent reservoir hosts. He noted the abundance of the sandfly *Lu. olmeca olmeca* on them and, in October 1965, found his first infected specimen. Armed with this knowledge, Biagi and his colleagues confirmed the role of *Lu. o. olmeca* as the vector of *L. mexicana* in neighbouring...
Yucatán, in December of the same year. Three years later Lainson and Shaw, using 'Disney-traps', found the vector of *L. m. amazonensis* to be a rodent-loving sandfly, *Lu. flaviscutellata*, relatively unattracted to man and essentially nocturnal – features which were responsible for the relative infrequency of infection in man.

‘Bosch yaws’

During a study of the epidemiology of cutaneous leishmaniasis in Surinam, in 1966, Wijers and Linger recorded flagellate infections in a tree-trunk-dwelling sandfly which they referred to as *Lutzomyia anduzei*. It was thought to be the most likely vector of 'bosch yaws', but attempts to infect hamsters with the insect flagellates failed and the parasite remained unidentified.

One parasite or many?

During the 1970s and 1980s it became clear that there were numerous species or subspecies of *Leishmania* responsible for cutaneous and mucocutaneous leishmaniasis in the New World. In an attempt to bring some order into an area of possible chaos, Lainson and Shaw divided the neotropical leishmanias into the 'mexicana complex' (*L. mexicana mexicana*, *L. m. amazonensis*, *L. m. pifanoi* and *L. m. enriettii*) and the 'braziliensis complex' (*L. braziliensis braziliensis*, *L. b. guyanensis*, *L. b. panamensis* and *L. b. peruviana*) based on their morphology, development in hamster skin, *in vitro* culture, biochemistry and behaviour in their vectors. Intensifying ecological and epidemiological investigations continued to indicate a diversity of leishmanial parasites and biochemical techniques, employing enzyme electrophoresis mobility patterns, DNA buoyant densities and DNA fingerprinting, soon confirmed the validity of the existing neotropical species and subspecies.

During the 1980s several new species were described and Lainson and Shaw revised their earlier classification, introduced two subgenera, *Viannia* and *Leishmania*, and elevated all pre-existing subspecies to specific level. This classification easily accommodated the more recently described species, *Leishmania* (*Viannia*) *laimoni*, *L. (V.) naiffi*, *L. (V.) shawi*, *L. (L.) venezuelensis*, *L. (V) colombiensis* and *L. (V) equatorensis*.

At the time of preparation of this volume, the named species of *Leishmania* from the New World are listed in Table 1. Descriptions of some others are in preparation and, without doubt, there must be many yet to be discovered, particularly in the extraordinarily rich fauna of Amazonia.
Table 1. Named species of *Leishmania* from the New World

<table>
<thead>
<tr>
<th>Subgenus</th>
<th>Vianna, 1911</th>
<th>Lainson &amp; Shaw, 1987</th>
<th>Subgenus</th>
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<tr>
<td><em>L. (V.) braziliensis</em></td>
<td>Vianna, 1911</td>
<td>Lainson &amp; Shaw, 1987</td>
<td><em>L. (L.) chagasi</em></td>
<td>Cunha &amp; Chagas, 1937</td>
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<tr>
<td><em>L. (V.) peruviana</em></td>
<td>Velez, 1913</td>
<td>Muniz &amp; Medina, 1948</td>
<td><em>L. (L.) enriettii</em></td>
<td>Biagi, 1953</td>
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<tr>
<td><em>L. (V.) shawi</em></td>
<td>Lainson &amp; Shaw, 1989</td>
<td><em>L. (L.) venezuelensis</em></td>
<td>Yoshida et al, 1993</td>
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</tr>
<tr>
<td><em>L. (V.) equatorensis</em></td>
<td>Grimaldi et al, 1992</td>
<td><em>L. (L.) venezuelensis</em></td>
<td>Yoshida et al, 1993</td>
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Known to infect humans

The beginnings of chemotherapy – tartar emetic the wonder drug

Vianna's 1911 contribution to medicine was to prove far greater than merely providing a scientific name. Impressed by the hopelessness of those suffering from the horrific nasopharyngeal lesions frequently produced by *L. braziliensis*, he turned his attention to treatment, which till then had been singularly unsuccessful. Tartar emetic (antimony potassium tartrate) had been effectively used in the treatment of African trypanosomiasis by French and British workers in 1906–7, and Vianna was quick to realize its potential against the related organism, *Leishmania*. In 1912 he published the results of his highly successful trials and tartar emetic soon became widely used in other countries, particularly in Asia, where its use against visceral leishmaniasis cut the mortality rate by about 95%. Vianna's discovery was to pave the way for future development of less toxic antimony derivatives, which remain our principal armament against leishmaniasis to this day.

Diagnosis and identification

In 1926 Montenegro published a technique for the preparation of an antigen from the flagellate stage of *Leishmania*, cultured in blood-agar medium. This has been widely used as a simple skin test analogous to the tuberculin and lepromin tests. Further advances that have proved invaluable in an understanding of the epidemiology of leishmaniasis include the introduction of the DNA restriction endonuclease ('fingerprinting') technique to demonstrate 'schizodemes' of different *Leishmania* species by Lopes and his colleagues in 1981, and the use of DNA hybridization probes to characterize *Leishmania* species by Barker and his colleagues.

American visceral leishmaniasis – first recorded cases

The history of visceral leishmaniasis in South America is relatively short and dominated by arguments about whether or not it was imported from the Old World in relatively recent times. The first documented case was from Paraguay in 1913. Migone noted the presence of 'corpuscles', which he was convinced were leishmanial parasites, in the blood of a sick man who had been...
American visceral leishmaniasis is characterized by a swollen abdomen caused by enlargement of the spleen and liver. Professor R Lainson

FAR RIGHT: Henrique Penna (1901–1985) (second from left) revealed the extent of American visceral leishmaniasis when he began to use a viscerotome to examine liver samples. Professor R Lainson

BELOW: Gladstone Deane, a member of the Chagas team, boarding a two-seater aeroplane for Abaetetuba, the only means of access. Evandro Chagas died in an aeroplane crash in 1940, three years after this photograph was taken. Professor L M Deane

working on the construction of the São Paulo–Corumbá railway in Brazil before arriving in Paraguay. The man showed symptoms consistent with visceral leishmaniasis, failed to respond to anti-malarial treatment, and eventually died. Although conclusive autopsy diagnosis was not obtained, it is likely that this represents the first record of American visceral leishmaniasis. The man had emigrated to Brazil 14 years previously, and the possibility that he brought the disease with him from Italy seems remote. Undoubted autochthonous cases were not registered until 13 years later when, in 1926, Mazza and Cornejo recorded visceral leishmaniasis in two Argentinian children who had never left that country, and from that time onward cases were rapidly recognized in almost all Latin American countries, from Mexico in the north to Argentina in the south.

The public health importance of American visceral leishmaniasis was not to become really apparent, however, until 1934 when Dr Henrique Penna used the viscerotome to examine liver samples from cases suspected to have died from yellow fever in various rural areas of Brazil. He uncovered 41 deaths due to visceral leishmaniasis, principally in children, and indicated the major foci of the disease to be in the northeastern States, particularly Ceará. Carlos Chagas,
famed discoverer of *Trypanosoma cruzi* and human American trypanosomiasis (Chagas disease) caused by that parasite, was at this time Director of the Instituto Oswaldo Cruz, Rio de Janeiro. He sent his son, Evandro Chagas, to investigate the principal foci of visceral leishmaniasis in the northeast of Brazil where, in Sergipe, he described the first living case of the disease in Brazil and indicated its similarity to Mediterranean infantile visceral leishmaniasis. He also made the important observation that the most common haematophagous insect found in and around the patient’s house was the sandfly *Lu. longipalpis*.

The Visceral Leishmaniasis Commission – field studies 1936–1940

Evandro Chagas headed a Commission to study the epidemiology of American visceral leishmaniasis and, for bureaucratic and financial reasons, was offered support only in the Amazonian State of Pará, where the number of cases was low. In Belém, a huge old colonial-style mansion was made available to him for laboratories and received the imposing name of the Institute of Experimental Pathology for the North (IPEN). In the absence of roads, Chagas and his colleagues were flown (one by one!) to their study area near the small township of Abaetetuba in antiquated, single-motored aeroplanes, normally used for carrying mail to the distant villages. In spite of these difficulties, the Commission uncovered more cases of visceral leishmaniasis, in humans and dogs, indicated the essentially rural nature of the disease, confirmed the abundant presence of *Lu. longipalpis* and suggested the likelihood of a wild-animal reservoir of the disease.

In 1937 Cunha and Chagas gave the name *Leishmania chagasi* to the parasite of American visceral leishmaniasis, in honour of Carlos Chagas who had died the year before. In 1938, however, Cunha decided that it could not be separated from *L. infantum* of the Old World. Other authors referred to the organism as *L. donovani*. On 8 November 1940, Evandro Chagas died in a mid-air aeroplane collision; he was only 35. The IPEN was re-named the Instituto Evandro Chagas in his honour but, dismayed and disoriented, his little band of dedicated workers never recovered from the loss of their brilliant and colourful leader, and interest in visceral leishmaniasis in Brazil went into steady decline.

The latter half of the twentieth century – stricken Sobral: a rude awakening

In 1953 an outbreak of visceral leishmaniasis in the vicinity of the small town of Sobral, in the interior of Ceará, northeast Brazil, estimated to have resulted in the deaths of over 100 inhabitants, startled the health authorities into setting up another inquiry into the epidemiology of the disease, in the hope of initiating some form of control. Three prominent figures in Brazilian tropical medicine were involved, E Alencar, L M Deane and M P Deane; the Deanes had both been members of the Evandro Chagas team, in Pará. Between 1953 and 1955, Leonidas and Maria Deane found foxes, *Lycalopex vetulus*, infected with *L. chagasi*, and recorded heavy flagellate infections in wild-caught *Lu. longipalpis*. They considered these to be promastigotes of *L. chagasi*, and infected further *Lu. longipalpis* by feeding them on a sick fox. By 1955, Alencar and the Deanes had registered nearly 1000 new cases of human visceral leishmaniasis in Ceará and other states of northeastern Brazil. They found the major areas of transmission to be in the boqueirões (humid, wooded foothill valleys); cases were only sporadic in the arid, lowland plains (seridões) and the hills (serras), where dryness and strong winds were unfavourable for sandflies.
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Reservoir hosts of American visceral leishmaniasis

As a result of these studies, dogs were considered the major reservoir for human infection, and up to 100% of *Lu. longipalpis* fed on sick dogs became infected. Human visceral leishmaniasis cases, however, proved to be poor sources of parasites for sandflies. Specific control measures took the form of a three-pronged attack – the slaughter of infected dogs, treatment of human cases, and the regular spraying of houses with DDT, all of which greatly reduced human infection.

By 1960, workers in Venezuela had recorded 51 cases of visceral leishmaniasis and, once again, the dog was considered the major source of infection for man.52,53 Most other Latin American countries recorded only sporadic cases, although this might have been more a reflection of inadequate interest in the disease and poor diagnosis.

In the search for a wild reservoir host, Torrealba and Torrealba in Venezuela in 1964 inoculated a fox, *Cerdocyon thous*, with *L. chagasi* and found the parasite in the bone marrow of the animal seven months later.54 The fox showed no signs of disease. The significance of this host was confirmed by Lainson and his colleagues who isolated *L. chagasi* from *C. thous*, shot on agricultural land close to forest near Belém, Pará, Brazil, where neither human nor canine visceral leishmaniasis had been recorded.55 The infected animals appeared in excellent condition. Subsequently, *L. chagasi* was isolated from 11 out of 26 *C. thous* in Marajó and, in the same area, over half the foxes were serologically positive. *Leishmania chagasi* was transmitted experimentally to a clean fox by the bite of infected *Lu. longipalpis* and the fox later used to infect clean sandflies.56 These findings suggest a silvatic enzootic of *L. chagasi*, maintained in foxes by *Lu. longipalpis*, that might be of importance in the maintenance of the parasite in uninhabited or sparsely populated regions. Infected foxes might serve as a 'silent' reservoir from which peridomestic foci of the canine and human disease arise.

In 1984 Sherlock and colleagues isolated *L. chagasi* from an opossum, *Didelphis albiventris*, caught in a focus of visceral leishmaniasis in Jacobina, Bahia, northeast Brazil,77 the first isolation of the parasite from a non-canid. These workers, however, considered it unlikely that the opossum *Didelphis* was an important reservoir of *L. chagasi* because of the low infection rate in this animal (two out of 84). On the other hand, Corredor and colleagues identified a *Leishmania* from *D. marsupialis* as *L. chagasi* in El Callejon district, Colombia, and felt that this animal had all the hallmarks of a good reservoir, including lack of disease caused by the parasite, its peridomestic habits and extreme abundance.58
American visceral leishmaniasis – indigenous or imported?

While various scientists accepted the use of the name *L. chagasi* for the New World parasite, they did not rule out the possibility that imported *L. infantum* (the organism responsible for Mediterranean infantile kala azar) was present in South America, and in 1981 Lainson, Miles and Shaw found no differences between three isolates of *L. chagasi* (two from Brazil and one from Honduras) and *L. infantum* from France, on isoenzyme profiles. However, a year later, Decker-Jackson and his colleagues detected distinct differences between *L. donovani*, *L. infantum* and *L. chagasi* on radiorespirometry profiles, and Jackson and his colleagues, using the restriction endonuclease digestion technique, found these parasites to have distinctly different DNA fragment patterns. Subsequently, a number of investigators recognized *L. chagasi* as being distinct from *L. infantum*, although Moreno and his colleagues failed to differentiate *L. chagasi* from *L. infantum* on enzyme profiles using 15 different enzymes and concluded that there has been ‘...a recent or contemporary importation of *L. chagasi* from natural *L. infantum* foci of the Palearctic Region.’

The chain of evidence against *Lutzomyia longipalpis* completed – transmission by the bites of naturally infected sandflies

In 1977, Lainson and his colleagues obtained the first experimental transmission of *L. (L.) chagasi* among hamsters by the bites of infected laboratory-bred *Lu. longipalpis* and, in 1984 and 1985, they found this sandfly to be the only species consistently present in an outbreak of visceral leishmaniasis in Santarém, Pará, Amazonian Brazil. The parasite was transmitted to hamsters which had been subjected to the bites of a large number of *Lu. longipalpis* caught in the backyard of a house in which active human and canine cases of American visceral leishmaniasis were living. Dissection of these sandflies revealed an infection rate of 7%, and 16 isolates of the parasite were shown to be *L. chagasi* using enzyme profiles and monoclonal antibodies.

Epilogue

The recognition that several species of *Leishmania* cause different forms of disease in the New World has provided a basis for a thorough understanding of the epidemiology of the diverse forms of New World leishmaniasis which can be used for rational control programmes. At present, control is concentrated on the vectors and reservoir hosts and the treatment of infected individuals. The drugs in use are basically antimonials and amidines based on those developed early in this century. Both types of drug have adverse side effects and are difficult to administer. Current research is concerned with developing new drugs and combination therapy with existing drugs and new treatment regimens. The possibility of vaccines is being considered, particularly against the cutaneous forms of the disease, and biochemical, immunological and molecular techniques are being developed for the early diagnosis of the various forms of the disease and for evaluating the possible outcomes. Studies are also continuing on the identification of the parasites and vectors involved, using biochemical, serological and molecular techniques, and determining patterns of parasite–vector–reservoir interactions.