The Wellcome Unit No. 1: twenty-one years of contributions in the field of tropical parasitology

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To give an account of the Unit's 21 years of research in these few pages is no mean task, embodying as it does some 200 publications on Leishmania, Trypanosoma and a variety of other parasites. Fortunately we have recently done this in the two-volumed book (ANONYMOUS, 1986) commemorating the 50th anniversary of the Instituto Evandro Chagas - also celebrated, by happy coincidence, in 1986. All the Unit's publications up to 1985 are listed in this fascinating account of the history and achievements of this famous Brazilian centre of biomedical research. Unless otherwise stated, reference to observations other than those by the Unit are also to be found in this book. Here, I propose to discuss only some of the Belem Unit's major contributions and a few of the difficulties we confronted in making them.

Leishmania and leishmaniasis

Leishmaniasis has always been our principal research project, which has been largely devoted to elucidating the epidemiology of the major forms of the disease in Amazonian Brazil (see ANONYMOUS, 1986, for reviews) - not only because of our privileged position in the centre of things, and the absurdity of dedicating our time to sophisticated bench-work more appropriately and easily done in London, but because such laboratory studies were in any case impossible until we knew with which parasite(s) we were dealing.

Cutaneous and mucocutaneous leishmaniasis

Although specific and subspecific names had been given to some causative agents of neotropical leishmaniasis - notably in Peru (Leishmania peruviana Velez), the Yucatan (L. mexicana Biagi, emend. Garnham) and French Guyana (L. braziliensis guyanensis Flocq) - a general view persisted that in Brazil the disease was due to the single parasite L. braziliensis braziliensis Viana. Some authors even regarded all American dermal leishmaniasis as due simply to "L. braziliensis".

Incrimination of silvatic rodents as reservoirs of L. mexicana in Belize led to the suspicion that a similar reservoir might exist for L. braziliensis in Brazil. I discussed this possibility with workers at the Instituto Evandro Chagas in 1963, and in particular with the virologist Otis Causey. He was struck by the similarity of lesions on the tails of Belize rodents and those he had noticed on the tails of rodents (Oryzomys spp.) in Belém. One can imagine his excitement when he found them to be teeming with Leishmania!

Causey's discovery (unpublished) was a key factor in our decision to locate the Wellcome Unit No. 1 in Belém - in spite of serious advice from the "House of Brazil", in London, that all cooking there had to be done on charcoal stoves, by the light of paraffin lamps! It was also fundamental to subsequent modifications of our whole conception of the American leishmaniasises, and the taxonomy of the parasites causing them. This, then, was the situation when the Unit was founded, in 1965, at the kind invitation of the Director of the Instituto Evandro Chagas, the late Dr Orlando Costa.

Naïve ideas - the formative years

The hardships confronting us in Belém were fortunately not of the magnitude envisaged by the House of Brazil (or the British Council, who also clearly regarded Belém as the back of beyond), but setting up the Unit certainly had its difficulties, some of which remain with us to this day. High on the list was the relaxed Latin American attitude regarding time and priorities: far from the old European adage of "never put off till tomorrow what you can do today", we found it to be more a question of "promise to do today what you cannot possibly do till tomorrow - or next week"! Another major problem, also still with us, has been the maze of bureaucratic red-tape - a national affliction which necessitated the creation of the special government post of "Ministro de Desburocratização". Language difficulties were, of course, a considerable obstacle in early days: less acute now, it nevertheless remains very time-consuming to write reports in two languages and execute all accountancy in two currencies (one of them highly unstable). Anyone facing the task of mounting and maintaining a long-term project under similar circumstances would be well advised to include an experienced, bilingual administrator among the staff, from the very beginning: a precautionary measure which we unfortunately did not take, largely due to our uncertainty regarding the Unit's permanency during its formative years.

When Jeffrey Shaw and I arrived in Belém, in 1965, it seemed that half the battle was over regarding the epidemiology of cutaneous leishmaniasis in the Amazon Region of Brazil. The reservoir hosts of L. braziliensis had seemingly been discovered, and it remained for us only to tidy up a few epidemiological details. The Wellcome Trust had been most generous, we felt, in granting a whole three-year period in which to do this.

The discovery of a parasite of the L. mexicana complex in South America

Our rosy illusion was soon shattered when we compared isolates of Leishmania from man with those from the rodents, for with few exceptions they proved to be totally different. Most parasites from man produced slow-growing, inconspicuous nodules in hamster skin which contained scanty, tiny amastigotes. In the sandfly they underwent a peculiar development attached to the wall of the hindgut, as
small rounded flagellates, in addition to the free, elongated promastigotes in the midgut and foregut. Most isolates from the rodents, however, produced huge, fast-growing tumours in the hamster, containing vast numbers of large amastigotes. They were ridiculously easy to grow in culture, and underwent no development in the sandfly hindgut.

By 1968 a number of facts had become clear. Not only was the parasite of rodents very different from that most commonly found in man, it was so strikingly similar to L. mexicana of Central America that we bestowed on it the new name of L. mexicana amazonensis. This close relationship was further emphasized when the vector was shown to be the sandfly Lutzomyia flaviscutellata - an insect closely related to Lu. olmeca olmeca, the proven vector of L. mexicana in Belize and the Yucatan.

Human infection with L. m. amazonensis is relatively rare, largely because the vector is not greatly attracted to man. The parasite remains of considerable importance, however, for two reasons: firstly because it is the cause of incurable “anergic” or diffuse cutaneous leishmaniasis in Brazil, and secondly because both the vector and the reservoir hosts can adapt to almost all types of forest - including plantations of such foreign trees as the Caribbean pine and gmelina, which are extensively used for paper-pulp in the Amazon Region.

Cutaneous and mucocutaneous leishmaniasis due to L. braziliensis braziliensis sensu lato

In 1970 we answered the request of a mining company in Central Pará to investigate the high incidence of cutaneous leishmaniasis among the men clearing forest in the range of hills known as the Serra dos Carajás. It was not difficult to pinpoint the vector - an aggressive sandfly which attacks man viciously at night, and during the day in overcast weather. It proved to be a new species, which we named Psychodopygus wellcomei in honour of Sir Henry Wellcome, and L. b. braziliensis has been isolated from infected flies on numerous occasions. Although extraordinarily abundant, we still remain ignorant of this insect’s resting-site(s) after taking blood meals, and the wild mammalian host(s) from which it becomes infected.

Cutaneous leishmaniasis due to L. braziliensis guyanensis

Our studies in the Carajas were suspended in 1974 following another urgent call from millionaire Daniel Ludwig’s forestry project on the river Jari, in north Pará, where some 300 cases of cutaneous leishmaniasis were being registered annually among men clearing primary forest for plantations of pine and gmelina trees, destined for his paper mill (this factory was actually rafted across from Japan, in full construction, to its present position on the bank of the river Jari - a truly remarkable feat).

The vector of L. b. guyanensis was shown to be a hitherto undescribed member of the Lu. anduzei group of sandflies, which we named Lutzomyia umbratilis. It is the predominant species of a number of treetrunk-dwelling sandflies, and on some occasions the infection rate was found to be as high as 7.0%. The major reservoir hosts were the two-toed sloth (Choloepus didactylus) and the lesser anteater (Tamandua tetradactyla).

As is so frequently the case in epidemiological studies of this sort, these results did not come easily. On one occasion over 200 traps set for terrestrial mammals were buried beneath fallen trees, after misdirected deforestation of our work area. Our early attempts to obtain sloths were frustrated by their culinary value in a community where protein was a relatively rare commodity - a problem resolved only by accompanying the deforestation on all fronts and offering a highly inflationary price for the unfortunate animals as they toppled to the ground with the falling trees.

Infection with L. b. guyanensis remains the most common form of cutaneous leishmaniasis in Brazil and the Guyanas, north of the Amazon river. Our detailed knowledge of the vector’s habits have, however, enabled some degree of protection for small work-parties by the spraying of the larger treetrunk bases with DDT, in the vicinity of their encampments. The efficacy of other insecticides is at present being investigated.

Finally, our observation that there is no cross-immunity between the above-mentioned leishmaniasis is of considerable importance in any consideration...
regarding the production of a non-living vaccine against these parasites.

Amazonian visceral leishmaniasis due to L. chagasi Cunha & Chagas.

The Instituto de Patologia Experimental do Norte, now the Instituto Evandro Chagas (Figure), was founded in Belém, in 1936, specifically for epidemiological studies on visceral leishmaniasis, which had been recorded for the first time in Brazil by Dr Henrique Penna in 1934. The investigation, headed by Evandro Chagas, was to be sadly short-lived, however, following Chagas’s tragically premature death in an air disaster in 1940. Although most of the work during the disease died with Chagas, important seeds had been sown: his dedicated team had indicated the dog as the major domestic reservoir of infection for man, and the sandfly Lu. longipalpis as the most likely vector due to its common presence in and around houses.

We felt that further studies in Amazonian Brazil might throw some light on the origin of this disease - the most important of the American leishmaniasis, and dismissed by many as simply due to L. infantum imported with the Iberian invaders in recent times. We were particularly excited, therefore, by our isolation of L. chagasi from the viscera of two seemingly healthy foxes (Cerdocyon thous), shot in a sparsely inhabited area on the outskirts of Belém in 1968. Our attempts to study the epidemiology of the disease remained thwarted, however, by a prolonged absence of human cases (the last one recorded was in 1962) and, although we succeeded in experimentally transmitting L. chagasi by the bite of Lu. longipalpis, we were unable to confirm the presence of this sandfly in Pará State, including Abaetetuba where Chagas and his collaborators had worked 30 years previously.

Our first break came in 1982, in a follow-up of two cases of visceral leishmaniasis in the little villages of Camará and Campinas, on the island of Marajó. We recorded the abundant presence of Lu. longipalpis (mostly in chicken-houses), and L. chagasi was isolated from a number of these sandflies, dogs, and another fox. Over the subsequent years the parasite has been obtained from 10 of 25 C. thous examined from Marajó (40%) and, as none of these animals has shown any visible sign of infection, we are led to the conclusion that this canid is the primitive, natural host of L. chagasi and that the parasite probably existed in the Americas, in this or other canids, long before the Iberian invasion (LAINSON et al., 1987).

In the same year we diagnosed the first case of what was to prove a serious outbreak of visceral leishmaniasis in Santarém, a small inland town some 700 km from Marajó and, during 1983-1986, recorded 150 human infections and 2066 infected dogs among 16 602 examined. Once again, Lu. longipalpis proved to be the vector, with infection-rates as high as 7% in some suburbs of the town.

Present and future field studies will be aimed at pin-pointing further potential foci of visceral leishmaniasis, largely by a search for Lu. longipalpis, and an examination of the fox/sandfly/parasite interfaces.

The multiplicity of leishmanial parasites: parasite identification and classification

During the studies described above a steadily increasing number of new leishmanial parasites has been found in man, sandflies and wild mammals. Of particular interest is L. deanei Lainson & Shaw, of porcupines (Coendou), and an as yet unnamed Leishmania of the armadillo (Dasypus): neither has yet been found in any other wild animal, or man, and the sandfly vectors are unknown. Among a number of other new parasites, one Leishmania from man shows a most unusual and characteristic morphology in both the amastigote and the promastigote stages.

When this multiplicity of neotropical leishmanias first became apparent, we prevailed on Dr B. E. C. Hopwood of the Wellcome Trust to convene a special meeting at the 2nd International Congress of Parasitology, in Washington D.C. in 1970, to discuss the possibility of using new biochemical methods for the identification of Leishmania. As a direct result there appeared, in 1972, the first of a series of publications from the Liverpool School of Tropical Medicine on the characterization of the parasite by enzyme electrophoresis and DNA buoyant densities and, since that time, a host of other biochemical techniques has added further criteria for separating taxa within the genus.

Stocks of the more important neotropical leishmanias, isolated and identified in Belem, now serve as WHO reference strains, and have formed the basis for the preparation of monoclonal antibodies by Drs John David and Diane McMahon-Pratt at Harvard Medical School. Their monoclonal antibodies have enabled us to detect “serodemes” within the L. braziliensis complex, and make parasite identification a much more rapid procedure (SHAW et al., 1987a, 1987b).

Increasing knowledge of the biology and biochemistry of the leishmanias has inevitably necessitated periodic revisions of our classification of these organisms. In the first of these we divided the parasites into two major “sections”, based on their development in the sandfly vector - namely, the Suprapylaria and the Peripylaria. More recently (LAINSON & SHAW, 1987) we have replaced these sections by the subgenera Leishmania Safjanova and Viannia Lainson & Shaw, respectively, and raised the subspecific names to specific level (Table).

Trypanosoma cruzi and Chagas disease, and T. rangeli

The first Amazonian cases of Chagas disease were recorded and investigated by this Unit in 1969, in Belém, and a follow-up serological survey of the suburban population revealed significant titres in only 14 of 5319 persons examined. Over subsequent years a small number of new autochthonous cases was registered, together with natural infections in silvatic triatomine bug species and a wide variety of wild animals.

Dr Michael Miles joined the Belém Unit in 1978 and worked with us until 1981 on T. cruzi and the characterization of our Leishmania isolates by enzyme electrophoresis. He demonstrated the presence of T. cruzi zymodemes 1 and 3 in Amazonian Brazil and indicated the absence of zymodeme 2 - the most common cause of Chagas disease in the more highly endemic regions elsewhere in Brazil. A major contribution was a study of reservoir/vector associations by tracking animals to their nests or retreats by an ingenious spool and line device attached to the liberated animal. Over 3000 isolates of T. cruzi have...
Table. Classification of the genus *Leishmania* to recognized species (abbreviated and updated from LAINSON & SHAW, 1987)

**Genus Leishmania** Ross, 1903.

   Definition: With the characters of the genus, life-cycle in the insect host limited to the midgut and foregut of the alimentary tract. Type species: *Leishmania (Leishmania) donovani* (Laveran & Mesnil, 1903) Ross, 1903. Old and New World.

   The *Leishmania (L.) donovani* complex:
   - *L. (L.) donovani* (Laveran & Mesnil, 1903) Ross, 1903.
   - *L. (L.) infantum* Nicolle, 1908.
   - *L. (L.) chagasi* Cunha & Chagas, 1937.

   Other species, outside the *L. donovani* complex:
   - *L. (L.) tropica* (Wright, 1903) Lühe, 1906.
   - *L. (L.) gerbilli* Wang et al., 1964.

   The *Leishmania (L.) mexicana* complex (New World):

   Possible additional members of the *L. mexicana* complex:
   - *L. (L.) garnhami* Scorza et al., 1979.

   The *Leishmania (L.) hertigi* complex (New World):

   Definition: With the characters of the genus: life-cycle in the insect host including a prolific and prolonged phase of development as rounded or stumpy paramastigotes and promastigotes, attached to the wall of the hindgut by flagellar hemidesmosomes, with later migration of flagellates to the midgut and foregut. Type species: *Leishmania (Viannia) braziliensis* Vianna, 1911, emend. Matta, 1916. New World.

   The *Leishmania (V.) braziliensis* complex:
   - *L. (V.) peruviana* Velez, 1913.

   Other parasites of the subgenus *Viannia*:

...
life-cycle and transmission of another new haemogregarine, Schellockia landauae, was described in the lizard Polychrus marmoratus and in experimentally infected Culex fatigans.

A new malarial parasite of bats, Polychromophilus deanei, was studied, in collaboration with Cyril Garnham who, in spite of a prolonged and mysterious fever, accompanied our mist-netting of bats during a hectic week's boat-trip in the local backwaters.

Among the trypanosomes, the important pathogen T. cruzi was recorded for the first time in Brazilian cattle, and T. evansi in the ocelot, Felis pardalis. New trypanosomes have been described in lizards and crocodiles, and T. boueroli of fish experimentally transmitted by the bite of the leech, H. lutei.

Twenty-three new species of coccidia, within the genera Eimeria, Isospora and Sarcocystis, have been described in a variety of lizards, snakes, turtles, bats and other mammals. Histoplasma, Pneumocystis and Toxoplasma infections have been found in a number of wild mammals.

Entomology

There are 93 recognized species of phlebotomine sandflies recorded from Pará, Brazil, and it goes without saying that a great deal of hard field-work has been needed to indicate the vectors of the four major leishmanial epidemiologies discussed above, and to study the ecology of these insects. Three of them (Lu. longipalpis, Lu. flaviscutellata and Lu. umbratilis) have been maintained as closed colonies for use in transmission experiments (together with other species), or to enable observations on the development of parasites in their natural vectors. The colonization of Ps. welcomrei so far eludes us.

In the taxonomic field, the Unit's succession of entomologists (Drs Habib Fraiha, Richard Ward, Paul Ready and Lee Ryan) have together collected and/or described 10 new species of Amazonian sandflies. Six of these are anthropophilic, and 3 are major vectors of cutaneous leishmaniasis. A recently published catalogue of all the known Pará species of sandflies (RYAN, 1986) has proved of great use in the field and for teaching purposes.

Rearing male sandflies from gravid females has enabled an accurate sex-association for 21 local species (often for the first time), and has been of prime importance in identifying species within the important Ps. squamiventris series (to which Ps. welcomrei belongs), among which some females are morphologically indistinguishable.

In recent years attention has been paid to "cryptic" species of phlebotomine sandflies. Richard Ward has investigated small variations in the pale tergal patches of allopatric and sympatric populations with dissimilar species of phlebotomine sandflies. Richard Ward has published a catalogue of all the known Para species of the "longipalpis" complex, which may be of significance in the epidemiology and distribution of American visceral leishmaniasis.

Separation of the morphologically indistinguishable females of Ps. welcomrei and Ps. complexus (a sympatric species) has been found possible by cutoxic analysis (RYAN et al., 1987).

Training and consultancies

Since its foundation, the Belém Unit has received 37 senior workers (from within and without Brazil) for training in laboratory or field aspects of leishmaniasis and Chagas disease. Two university students are attached to the Unit, each year, for a one-year training course and supervision of their thesis projects. Over 1000 strains of Leishmania, and many more T. cruzi zymoedemes, are available from this laboratory's cryobank, and we attend to many requests for the identification of isolates made in other institutions. Collaborative programmes with other research centres remain numerous.

Acknowledgements

This is the first opportunity I have had, in writing, of expressing my deep appreciation of Jeffrey Shaw's unwavering collaboration and close friendship during the 21 years in which we have shared both the successes and failures of our work in Belém. Joinly, we owe an immense debt of gratitude to the Wellcome Trust for the rare chance of studying the American leishmaniasis in such depth - a vital necessity if any sense is to be made of their complexities. Space does not permit me to list all those personalities involved, but special mention must be made of the following: Tony Duggan, who first listened so sympathetically to my dreams of such work when we met in Rio de Janeiro in 1963, and the late Brigadier Sir John Boyd whose enthusiasm was largely responsible for the chance of realizing them: Peter Williams, Tom Hopwood, Bridget Ogilvie, Len Goodwin, and all the Trustees involved, who have shown such staunch support and encouragement throughout. We owe much, too, to the Fundação SESP of Brazil for the generous provision of facilities in the Instituto Evandro Chagas, and to the various Directors of that Institute - in particular to the late Dr Miguel Cordiero de Azevedo (who welcomed us in 1965, and whose kindness and patience knew no bounds during the initial, difficult years), Dr Francisco de Paulo Pinheiro, and the present Director, Dr Alexandre da Costa Linhares. We are also indebted to our Brazilian colleagues, past and present - Drs Habib Fraiha, Marlene T. Póvoa, Adelson A. Souza, Fernando T. Silva, Rosili R. Braga, Isabel R. C. Rodrigues, Sebastião A. S. Valente and Edna A. Y. Ishikawa - and, last but by no means least, to our present and past technical staff, without whose help little of this work would have been possible: Antonio J. O. Monteiro, Antonio F. P. Martins, Augusto F. N. Filho, Cezarina N. Arcanjo, Constância M. Franco, Cristina A. Loureiro, Deocleciano G. Primo, Eurides L. Rocha, Francisco S. Gomes, Guilherme B. Nunes, Henrique W. S. Buna, Iorlando R. Barata, João B. P. Luz, José I. Almeida, José P. N. Cruz, José A. N. Lima, Julio C. J. Gama, Leonardo S. Carvalho, Leonidas S. Elizée, Manoel C. M. Souza, Maria A. Shaw, Maria G. S. Silva, Maria J. F. Santos, Marieleide D. Naff, Raimundo B. P. Luz, Roberto D. Naff, Sebastião F. Oliveira, Suéd N. P. Silva and Zulma J. C. Corrêa.

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