Advances in
PARASITOLOGY

VOLUME 16

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PREFACE

It is a tribute to the excellence of the editorship of *Advances in Parasitology* by Professor Ben Dawes that I felt a certain pride at being invited by Academic Press to carry on the series when he died. I had frequently reviewed volumes of the series and recorded my high opinion of the quality of the contributions which Professor Dawes presented. The invitation was therefore a challenge which could hardly be declined. It is a further tribute to Professor Dawes that, in accepting the invitation of Academic Press, I felt that I could not undertake the task single-handed and made the proviso that I should need some support. In seeking this I have been most fortunate in attracting the aid of Drs J. R. Baker and R. L. Muller, both with extensive editorial experience added to established basic scientific reputations. I trust that, as a team, we may continue the high standard set by Professor Dawes single-handed.

Such is the time-scale of solicitation, editing and publication that the content of the present volume bears fundamentally the mark of Professor Dawes. It is a mix of Protozoology and Helminthology, with the latter predominating. Doubtless this same mix will continue to contribute largely to future volumes, but there may be an advantage—so as to provide freer flow of thought and concept—in interpreting "Parasitology" more widely than simply as Protozoology and Helminthology (together with Entomology, a discipline introduced mainly because arthropods provide most of the vector mechanisms), and bringing into consideration some of the manifold other kinds of organisms which, beside protozoa and helminths, follow the parasitic way of life.

W. H. R. LUMSDEN
August 1978

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Taxonomy and Transmission of *Leishmania*

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I. Introduction

The review by Adler (1964), by directing attention to "problems most likely to engage research workers in the near future", undoubtedly influenced many research programmes in the late 1960s and early 1970s and, for several years after his death, the genius of Saul Adler continued to act as a stimulus for many studies on *Leishmania*.

Because leishmaniases continue to be major public health problems in many parts of Asia, Africa, Europe and America, and because they are undoubtedly spreading (Anonymous, 1971), several important reviews on the subject have appeared in recent years. Reference should be made, in particular, to works by Bray (1972, 1974), Garnham (1971a,b), Heyneman (1971), Lainson and Shaw (1971, 1972, 1973, 1974), Lysenko (1971), Manson-Bahr (1971), Marsden and Nonata (1975), Moškovskij and Dunhamina (1971), Moškovskij and Southgate (1971), Neronov and Gunin (1971), Petriščeva (1971), Saf'janova (1971), Zuckerman (1975) and Zuckerman and Lainson (1977).

Lainson and Shaw (1971) succinctly reviewed the evidence incriminating sandflies (Diptera: Psychodidae—Phlebotominae) as the normal insect hosts

for *Leishmania* spp. Lewis (1971, 1974) dealt with the biology of Phlebotominae, with special reference to their rôle as vectors of leishmaniases. A book by Forattini (1973) contains a wealth of information about leishmaniases in the New World and the biology of Phlebotominae, especially that of Neotropical species. However the mass of facts was not so critically assessed as it was by Lainson and Shaw (1971, 1973, 1974), whose work now needs some revision in the light of later discoveries. The species of sandflies listed as vectors of leishmaniases by Bray (1974) are, in general, those which have been found naturally infected with promastigotes causing leishmanial infections when inoculated into susceptible laboratory animals; slight amendments in nomenclature of sandflies are needed and a few species can now be added.

Several reports, some with extensive bibliographies, on long term epidemiological investigations have also been published. Reference should be made to works summarizing studies in USSR (Perfil'ev, 1966; English translation, 1968), Belize (Lainson and Strangways-Dixon, 1963, 1964; Strangways-Dixon and Lainson, 1966; Disney, 1968; Williams, 1970), France (Rioux and Golvan, 1969), Sudan (Hoogstraal and Heyneman, 1969) and Ethiopia (Ashford *et al.*, 1973a). Studies on leishmaniasis in Panama, spanning about 30 years, have not yet been brought together in a single monograph; it is to be hoped that the important work carried out by the Wellcome Parasitology Unit in Belém will, eventually, be summarized in book form.

Whereas the review by Adler (1964) provided guidelines for future research, the present review attempts to summarize achievements in the last decade or so. Little reference is made to subjects authoritatively examined by the authors already cited. Only two topics are dealt with: taxonomy and nomenclature of *Leishmania*; and Phlebotominae as insect hosts for trypanosomatid parasites. Greater emphasis is given to the results of investigations in the New World. This, perhaps, reflects personal interests but it has been in the Americas that field studies have made great advances since the publication of Adler (1964).

II. Taxonomy and Nomenclature of *Leishmania*

The species of *Leishmania* infective to man are morphologically identical even though they give rise to a number of distinctly different clinical syndromes. The traditional methods for recognizing species were based on the disease states in man and, when urging the need for new standards for separation of species, Kirk (1950) commented that clinical differences were the only characteristics which represented "hereditarily stable differences in the parasites concerned". No modern student of *Leishmania* would give wholehearted support to such a statement; most would support Moškovskij and Southgate (1971) in considering clinical criteria, alone, as unsatisfactory taxonomic tools. In presenting a provisional classification of human leishmaniases on clinico-epidemiological evidence, Moškovskij and Southgate (1971) completely refrained from referring to the parasites by either generic or specific names.

It has become increasingly clear that a classification system based on human disease patterns has been a hindrance to understanding the variety of clinical forms of leishmaniases and to appreciating the specific and infraspecific relationships that exist between the parasites themselves. However the traditional framework for classifying *Leishmania* does not, in fact, conflict with modern methods for defining species and subspecies.

A. CLINICAL MANIFESTATIONS

Adler (1964) discussed the difficulties inherent in accepting a clinically-based taxonomy. He pointed out that, in all parts of the world where it occurs, clinically active visceral leishmaniasis presents the same symptoms; but, in different foci of infection, visceral manifestations may be preceded, accompanied or succeeded by cutaneous symptoms. He referred to cases from eastern Africa (Sudan and Kenya) in which patients may develop skin lesions, similar to oriental sore, several months before the onset of visceral signs of infection. Manson-Bahr (1955) referred to a report of the same phenomenon in southern USSR. Adler (1964) also discussed the difficulties in attempting to use clinical criteria to define the organisms responsible for the various forms of cutaneous and mucocutaneous leishmaniases in the Americas.

Cahill (1964) described cases of leishmaniasis acquired in an area of kala-azar in Upper Nile Province of Sudan by six North Americans. Four of the patients, described as healthy, well-nourished individuals, developed cutaneous lesions only. The three who had been taking antimalarial drugs presented a single lesion. The fourth, who had not been taking antimalarials, developed 18 separate lesions. The other two patients, who were older, in poorer physical condition and had not been taking antimalarial drugs, developed classical features of visceral leishmaniasis (fever, weight loss, anaemia, hepatosplenomegaly) without prior cutaneous manifestations. Subsequent serological and immunological studies on the parasites isolated from these patients revealed that the men were infected with the same strain (Adler *et al.*, 1966). The clinical manifestations in the patients were not due to distinctive properties of the parasites but depended on host characteristics—age, physical condition, dietary and drug (use of antimalarials) habits.

The use of serological and immunological techniques (Bray and Lainson, 1966, 1967; Bray and Rahim, 1969; Bray and Bryceson, 1969; Bray *et al.*, 1973a) established that diffuse cutaneous leishmaniasis, leishmaniasis recidiva and post-kala-azar dermal leishmaniasis are not produced by distinctive characteristics of the parasites concerned but represent different host reactions to them. Bray and Lainson (1966, 1967) could not differentiate between parasites isolated from a case of diffuse cutaneous leishmaniasis from the State of Pará, Brazil and *Leishmania* isolated from a case of espundia in the State of Ceará, Brazil. Bray and Rahim (1969) were unable to detect differences between the organisms isolated from cases of oriental sore and leishmaniasis recidiva in Iraq. (They showed, however, that organisms causing leishmaniasis recidiva in Iraq and Iran were distinctive and that the parasites causing oriental sore in Iraq and Ethiopia can be separated by serological tests.) Bray

and Bryceson (1969) proved that the same strain* of *Leishmania* in Ethiopia can cause diffuse cutaneous leishmaniasis, tuberculoid leishmaniasis, oriental sore and visceral leishmaniasis. Bray *et al.* (1973a) showed that the organisms causing kala-azar in India are serologically identical with parasites of post-kala-azar dermal leishmaniasis isolated from human cases in the same country.

Garnham (1971a,b) and Zuckerman (1975) reviewed the evidence that diffuse cutaneous leishmaniasis arises from a failure of cell-mediated immune processes in the human host. Zuckerman (1975) examined the evidence that leishmaniasis recidiva is the result of the development of hypersensitivity by the human host.

Apart from the advances in understanding the immunopathology and serology of *Leishmania* in man, a clinically-based taxonomical system of separating species would be realistic only if man were the prime mammalian host. In most cases, man is an incidental and, more often, an accidental, host of the parasites. With the exception of visceral leishmaniasis in the Indian subcontinent (and, perhaps, kala-azar in parts of eastern Africa), man is not the prime host of the parasites and, in fact, plays an insignificant rôle in their propagation. Although the genus *Leishmania* is in a state of active diversification, man is but one of many mammalian hosts for the parasites and has, probably, played little part in evolutionary sequences. As Bray and Lainson (1967) stated: "Speciation has occurred in rodents, canines and sandflies."

B. SEROLOGICAL AND IMMUNOLOGICAL STUDIES

Zuckerman (1975) reviewed recent studies on the immunology of leishmaniases, giving particular attention to the fundamental changes in ideas about immunopathological processes that have occurred since the subject was considered by Adler (1964). Here, reference is made only to some of the studies which have a bearing on understanding the taxonomic relationships of *Leishmania* spp.

1. *The Adler test*

Adler (1964) reviewed the earlier serological methods which had been used, often with inconsistent or contradictory results, to define species of *Leishmania;* and he described a technique which he had found useful in differentiating between the organisms causing espundia, chiclero's ulcer, oriental sore and kala-azar. The test also revealed slight but consistent differences between the parasites causing the moist and dry forms of oriental sore.

The Adler test entails growing a known and unknown *Leishmania* on media

* Following the recommendations of a meeting on the characterization, nomenclature and maintenance of salivarian trypanosomes held in London, 27–30 September 1976, under the chairmanship of W. H. R. Lumsden, the word "strain" as used throughout this paper should be replaced by "stock" ("population derived by serial passage *in vivo* and/or *in vitro* from a primary isolation, without any implication of homogeneity or characterization"). [Eds]

containing immune rabbit serum. Adler *et al.* (1966) and Garnham (1971a) described the sequence of events when parasites are seeded in cultures incorporating homologous or heterologous serum. Wertheim *et al.* (1970) studied the early extracellular and intracellular changes leading to the formations described by Adler (1964).

By use of this test, Adler *et al.* (1966) demonstrated that parasites isolated in Sudan from clinically different forms of leishmaniasis and from non-human sources were serologically similar. Strains from two human cases of kala-azar and three human cases of cutaneous leishmaniasis (isolated from five of the six cases described by Cahill, 1964), isolates from two naturally infected sandflies and from five naturally infected mammals (*Rattus rattus, Arvicanthis niloticus, Acomys albigena, Genetta genetta, Felis serval*) were shown to belong to the same species of *Leishmania*. The three cases of cutaneous leishmaniasis were considered to be examples of primary leishmanioma or abortive kala-azar.

Saf'janova (1966) used a modified form of the Adler test to distinguish between strains of parasites in sandflies, of human and lizard origins. He found that one species of fly had been infected with promastigotes which were antigenically close to human strains but that another species of fly had been infected with parasites of reptilian origin.

In Israel, Gunders *et al.* (1968) used the Adler test to identify strains of *Leishmania* isolated from *Meriones* (? species) and *Psammomys obesus*. The parasites from these two sources were indistinguishable from *Leishmania* isolated from a typical case of human oriental sore in Israel.

The Adler test provided a means of recognizing the origins of promastigote infections in sandflies, of identifying non-human hosts in a focus of human leishmaniasis and of the demonstrating that the same strain of parasite can give rise to distinct clinical conditions in man. In general the results tended to support the traditional clinically based taxonomic system for identifying the *Leishmania* infective to man.

2. *Fluorescent antibody staining technique*

Bray and Lainson (1965) demonstrated that this technique is of no value in identifying strains and species of *Leishmania*.

3. *Ouchterlony double diffusion tests*

Using this technique, Bray and Lainson (1966) found antigenic differences between strains but obtained insufficient evidence to identify parasites with certainty. They showed that a strain of parasite from Belize was distinct from strains isolated in Panama and Costa Rica; the Belize strain, isolated from a forest rat in an area of chiclero's ulcer, shared only one antigen with an Israeli strain of oriental sore even though the two strains had previously been found (Adler and Gunders, 1964) to produce cross-immunity; strains isolated from cases of visceral leishmaniasis in Brazil, Sudan, Kenya and India had three or more antigens in common with all other strains tested, including those

from cases (or from the areas) of cutaneous and mucocutaneous leishmaniases.

Schneider and Hertig (1966) found that antigenically distinct groups of *Leishmania* exist in Panama. Each of two groups included isolates from man and sandflies; the various strains of the two groups had no distinctive geographical patterns; both Panamanian groups were distinguishable from Guatemalan and Belize strains from cases of chiclero's ulcer, and from an old strain isolated from a case of uta in Peru. Schneider and Hertig (1966) were unable to group three isolates from Panamanian sandflies. The identity of one of these strains was later established by electron microscopy (Wallace and Hertig, 1968). By further double diffusion tests, Schneider (1968) showed that the other two ungrouped strains were closely related to a *Leishmania* which Herrer *et al.* (1966) isolated from a porcupine, *Coendou rothschildi*. Schneider found that the parasites from the porcupine were not closely related to those causing human cutaneous leishmaniasis in Panama; Herrer (1971) subsequently defined the porcupine parasites as *L. hertigi*.

4. *Cross absorption/passive haemagglutination test*

This test, using sensitized tanned sheep erythrocytes, was described by Bray and Lainson (1967) and Bray (1969).

Bray and Lainson (1967) found that the test consistently revealed antigenic differences between several of the strains examined but they refrained from naming the different serotypes demonstrated. The test confirmed the complexity of the situation existing in Central America: a strain from Panama was distinct from parasites isolated from a Brazilian case of espundia, from two strains from Costa Rica, and from a strain from Belize; the two Costa Rican strains were distinctive one from the other. Parasites from Indian kala-azar were differentiated from those of visceral leishmaniasis from Kenya. No antigenic difference was found between post-kala-azar dermal leishmanoid of India and oriental sore from Israel, or between typical espundia from the State of Ceará, Brazil and a case of diffuse cutaneous leishmaniasis from the State of Pará, Brazil.

Reference has already been made to results obtained by Bray and Rahim (1969), Bray and Bryceson (1969) and Bray *et al.* (1973b).

5. *Serological tests and the taxonomy of* Leishmania

The serological/immunological techniques used by Bray and his colleagues were aimed at the detection of humoral antibodies. Later studies showed that the immunopathology of leishmaniases can be better understood in terms of cell-mediated rather than humoral immunological processes (Zuckerman, 1975). The search for humoral antibodies, however, revealed consistent differences between certain strains, showed strain similarities even when the clinical syndromes differed considerably, and provided a more rational basis for understanding the relationships between strains of *Leishmania*.

C. GROWTH CHARACTERISTICS

Schneider and Hertig (1966) mentioned that some Panamanian strains of *Leishmania* did not thrive well in culture and could best be described as "slow growers". Bray and Mumford (1967) reported that a strain from Guyana grew slowly when inoculated into the nose of golden hamsters, the resultant lesion containing few amastigotes. The strain would not grow well in NNN medium containing rabbit blood and could be maintained only in medium prepared with hamster or rat blood.

Further evidence of differences in growth characteristics of *Leishmania* were obtained from studies on strains isolated in forested areas of Brazil and in Panama. Lainson and Shaw (1969a) briefly reported that *Leishmania* isolated from man and small forest rodents in the State of Mato Grosso, Brazil, could be separated into slow-growing and fast-growing strains. Further details were given by Lainson and Shaw (1970) and were later discussed by Lainson and Shaw (1971).

In the Mato Grosso study area, Lainson and Shaw (1969a; 1970) encountered both cutaneous and mucocutaneous lesions in man. Smears prepared from some single sore cutaneous lesions were found to contain many amastigotes whereas other, similar, lesions contained scanty amastigotes. Lesions found on small forest mammals were, with one exception, on the tail; in the exceptional case, the lesion was on the ear. All tail lesions were similar in appearance. Leishmanial lesions were found in 21 of the 107 small mammals examined (94 rodents, 13 marsupials); 20 of the infected animals were rodents and only one of the marsupials was infected. The behaviour of seven strains (one from a marsupial and six from rodents) in culture and after inoculation into golden hamsters was similar to that of strains isolated earlier in the State of Pará, Brazil (Lainson and Shaw, 1968, 1969b). The parasites grew profusely in cultures and no difficulty was experienced when transferring the organisms to new cultures; in hamsters, the parasites rapidly produced large histiocytomata containing many amastigotes. Three strains (one from a rodent, two from humans with single leishmanial lesions) behaved differently. Growth in culture was poor, transfer to new cultures proved difficult and the parasites often died after one or two transfers. When inoculated into hamsters, parasites developed slowly, producing small lesions containing few amastigotes; the lesions were surrounded by inflamed reactionary tissue. In comparing strains isolated from man and small forest mammals in the States of Mato Grosso, Pará and Maranhão, Lainson and Shaw (1970) found that both fast- and slow-growing organisms were isolated from single sore cutaneous lesions but only slow-growing parasites were recovered from cases of mucocutaneous leishmaniasis. In both man and hamsters, the slow-growing parasites provoked strong tissue reactions which were quite different from the host responses to fast-growing parasites. Lainson and Shaw (1970, 1971) discussed these findings in relation to the taxonomy of *Leishmania* in forested areas of Brazil but refrained from making new nomenclatorial proposals.

In Panama, Johnson and Hertig (1970) studied the growth characteristics