numerical analyses of isoenzyme data provide information of systematic value as opposed to parameters which provide information useful only for identification purposes.

Isoenzyme studies have made several major contributions to the study of leishmaniasis (Chance, 1979). The divisions of the genus can now be delimited with considerable confidence. Within the Old World the separation of the parasites causing cutaneous disease into *L. major* and *L. tropica* is now well established, with *L. aethiopica* not closely related to either. All the parasites causing visceral leishmaniasis, including those in the New World, have proved to be closely related. Within the New World the complexes of *L. mexicana* and *L. braziliensis* have clearly been shown to be separate entities confirming their separation on the biological characters of size and patterns of development in mammalian and invertebrate hosts. Many of these findings are confirmations of previously accepted divisions within the genus; of much greater significance are the contributions made by the ability to identify individual isolates, thus allowing a fuller understanding of epidemiological cycles with the identification of parasites in potential reservoirs and vectors. An important example is the realisation that visceral infections in rodents in Africa are usually *L. major* and are not therefore reservoirs of *L. donovani* as had been previously suspected. We also now have a clear understanding of what type of disease can be caused by each parasite, thus for example *L. donovani* s.l. can cause both visceral and cutaneous disease, and *L. tropica* may on occasions give rise to visceral infections. Thus we see a re-emergence of the spectral theory of leishmaniasis in which the same parasite may cause a variety of clinical syndromes according to the reactivity of the host.

In addition to the isoenzyme electrophoresis many other biochemical and immunological techniques have been used to characterise *Leishmania* (Chance, 1985). The two techniques of most current interest are the use of monoclonal antibodies and nucleic acid hybridisation. A major feature of both these approaches are attempts to develop rapid methods of identification based on the use of radioactive, fluorescent, or chromogenic probes for the detection of parasites in biopsy material. Unlike current methods in use, the aim of these methods is to be of immediate use in patient management. Some progress has been made in this area with the use of radioactive kinetoplast DNA probes to identify flagellates in sandfly guts.

Clinical Detection of Leishmania

Indirect methods of detecting the presence of leishmanial parasites, i.e. detecting antibodies to leishmanial antigen have the obvious advantage of avoiding invasive diagnostic methods. Although the sensitivity of present immunological methods is considerable, they are handicapped by problems of

specificity. In an attempt to develop and improve antibody detection tests, we have examined the surface of *L. donovani* for an antigen which elicits a strong response in humans and which does not cross react with antibodies to other organisms, and which preferably, for technical reasons, is present in promastigotes.¹²⁵ I labelled promastigotes, surface labelled by the iodogen method were immunoprecipitated with kala-azar serum and run on SDS-PAGE. One major protein band of 63 kilodaltons was detected which was found to be common to all geographic variants of *L. donovani* and also to other species of *Leishmania*. This molecule was not immunoprecipitated by human sera against other infectious organisms known to cross react with leishmanial antigen, and would appear to be an ideal candidate for investigation as an immunodiagnostic reagent. Studies in this area are continuing.

There has clearly been a great deal of progress in our understanding of the divisions within the genus *Leishmania* and the hope for the future is that clinically useful techniques will become available for the rapid detection and identification of *Leishmania*.

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Part I.II Current Methods in Studies on the Ecology and Epidemiology of the Leishmaniasis with Particular Reference to the Americas

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Introduction

Most forms of human leishmaniasis are zoonoses, with a primary origin in wild animals, and occasionally involving domestic animals—in particular the dog. Elucidation of the “epidemiological triangle” is the

logical first step leading to prevention and control of these diseases: namely, indication of the mammalian reservoir(s), the sandfly vector species, and conditions under which the parasites are transmitted to man. This will only be possible if we have reliable methods to demonstrate and identify the organisms concerned, for we now know that there are many more species of *Leishmania* circulating among wild animals than we previously supposed.

Qualitative Studies

A. The Human Population

(a) The Cutaneous Leishmaniasis

(1) Microscopic Diagnosis

Clinical aspects can be deceiving, and differential diagnosis is required from dermal lesions due to a variety of other aetiological agents, particularly in neotropical regions. There is no more sure diagnosis than seeing and isolating the parasite, but in inexperienced hands or in poor laboratory conditions, detecting amastigotes in stained smears may be sadly inefficient. Badly prepared smears, containing a host of contaminative bacteria, fungal spores, dust particles and staining deposit, may result in the failure to find *Leishmania*, or registration of false positives. Points to remember are the use of ultra-clean and dry slides, avoidance of the necrosed central part of ulcers, adequate cleansing of the skin surface before making a biopsy or scraping from the margin of the lesion, avoidance of excessive blood in the smear, and rapid drying of **thin** smears for **immediate** fixation in absolute methanol which has not been allowed to absorb atmospheric moisture. Geimsa stain is that most widely used, but the quality of staining may vary with the brand: buffered distilled water should be at 7.0–7.6 pH, and free of algal contaminants. Placing the slides face down in staining dishes, rather than Coplin jars, will avoid staining deposit if the surface scum is pushed to one side.

(2) *In Vitro* Culture

Parasites may be so scanty in old, chronic lesions that they defy detection in stained smears. Diagnosis will now depend on the culture of tissue juice or biopsied skin in a suitable blood-agar medium, and the inoculation of such material into laboratory animals. Isolation of the parasite for subsequent identification is of utmost importance in epidemiological studies: the use of monoclonal antibodies and DNA analysis for immediate identification of *Leishmania* species on glass slides or other substrates may hold promise for the future: but these techniques are still not fully adapted to most field conditions, and are of little use when lesions contain extremely scanty numbers of amastigotes. Growth of different *Leishmania* species may vary greatly in different culture media and laboratories must ascertain those which are most suitable for the parasites likely to be encountered in their study areas.

(3) Isolation of Laboratory Animals

The Syrian hamster remains the animal of choice: the mouse is much less susceptible to some leishmaniasis, particular those of the *braziliensis* complex. Aspirated tissue juice or a saline triturate of biopsied tissue is usually inoculated intradermally into the skin of the nose or feet, and the animals examined periodically for the appearance of skin lesions. Some *Leishmania* species will produce inapparent skin infections, and for this reason it is advisable to make cultures from the hamster skin, at the point of inoculation, even if the animals are seemingly negative.

(4) The Intradermal ("Leishmanin") Test

This widely used skin-test is highly specific and of great use in quantitative epidemiology. In **diagnosis**, however, the following points must be borne in mind: (a) a positive reaction may be due to a past infection and nothing to do with the present lesion of the patient; (b) positive reactions may be registered in persons with no present or past evidence of infection; (c) a small percentage of parasitologically positive patients may be negative, and the test is regularly negative in cases of diffuse anergic leishmaniasis.

(b) Human Visceral Leishmaniasis

It is best to first check suspect cases for leishmania antibody (IFAT, ELISA or other serological tests). If positive, material for microscopic examination, culture and animal inoculation may be obtained from spleen, bone-marrow or lymph-node biopsy. Patients with Indian or Kenyan kala-azar regularly show amastigotes in white cells of the peripheral blood, and the "tail" thin blood films may be profitably examined for parasites. The Leishmanin test is negative in **active** visceral leishmaniasis, but becomes positive after cure in a high proportion of cases.

B. The Sandfly Population

With the parasite(s) isolated and **characterised** from an adequate number of patients, it remains to determine the sandfly vector(s). This may be relatively simple in regions where there are few sandfly species, and is clearly more difficult in areas where there are large numbers of anthropophilic species - as in neotropical rain-forest. Logical steps are as follows: (a) Accurate pin-pointing of the **exact** area of transmission. (b) a variety of trapping methods must be employed, throughout the year, to indicate **all** the anthropophilic sandfly species. Initially this will necessitate human bait (with all possible efforts to diminish risks of infection), but once man-biters are recognised, alternative methods should be initiated using animal bait (e.g. the horse and other equines) and such well-tried devices as the "Shannon" and C.D.C. light-traps. When the habits of sandflies are known, they may be collected from their resting-sites, such as tree-trunks and animal burrows etc. Peridomestic species may be taken from the inside

walls of houses and animal shelters. (c) incrimination of the vector species: this will depend on isolating and identifying the parasite; finding a significant number of that species infected; demonstration of promastigotes in the anterior station of the gut; indicating an intimate reservoir-host/sandfly/man contact; and experimentally transmitting the parasite by the bite of the suspected vector species.

C. The Mammalian Population: Reservoir-hosts

(a) Anthroponoses

In India, parts of China and possibly Kenya, visceral leishmaniasis is thought to be an anthroponosis, with transmission directly from man to man.

(b) Zoonoses

Among domestic animals, the only proven and effective reservoir is the dog, which has become the "liaison" host of visceral leishmaniasis due to *L. infantum* and *L. chagasi*. The canine disease is usually fatal, but infection in wild canids (foxes, wolves and jackals) is mostly benign and inapparent. These animals offer, therefore, a constant threat of new outbreaks of visceral leishmaniasis when control measures are diminished. Entomological data on biting habits, preferential hosts and distribution will give clues as to wild mammalian hosts: analysis of fresh blood from sandflies is potentially useful, but the results must be interpreted with care because many sandflies have very catholic feeding habits. Finally, the screening of wild animals should always include the examination of both skin and viscera: the procedure (Lainson, 1982) is similar to that used for the isolation of *Leishmania* from man.

D. Identification, Description and Preservation of *Leishmania* Isolates

Correct identification of the parasite by recognised biological and biochemical criteria (Lainson & Shaw, 1986) is imperative in epidemiological studies – especially where a number of different leishmanias are circulating in nature. The original code of the laboratory in which an isolate is made should always be incorporated into the descriptive labelling of that parasite, using the coding method recommended by the World Health Organisation (Anon., 1984). Strains should be preserved in liquid N₂ as soon as possible after isolation, and deposited in one or more International Reference Centres.

Quantitative Studies

These should accompany the qualitative studies, to provide information on the prevalence and incidence of the human disease in a given area, ecology and population fluctuations of both sandfly vectors and mammalian reservoir-hosts, and the prevalence and incidence of canine visceral leishmaniasis – all of which forms a basic necessity in initiating control measures and measuring their efficacy.

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Part I.III Mate Recognition in a Sandfly (Diptera : Psychodidae)

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Introduction

The present paper summarizes preliminary studies on the sexual behaviour of the sandfly *Lutzomyia longipalpis* Lutz & Neiva. This phlebotomine is the vector of *Leishmania donovani chagasi* in South America, though it is now believed that *Lu. longipalpis* consists of at least two sibling species, which are as yet not clearly defined.

There is little information on the reproductive biology of psychodids including phlebotomine sandflies. Early observations by Fuerborn (1922) of psychodids described the presence of "scent glands" which it was thought emitted chemical secretions used in sexual signalling. An early description of sandfly sexual behaviour was from Malta, where Whittingham and Rook (1923) described the courtship of *Phlebotomus papatasi* Scopoli. To my knowledge there are no further descriptions of sexual behaviour in sandflies until Chaniotis (1967) described courtship in *Lu. vexator occidentis*. An earlier report from Brazil described the existence of an "odiferous gland" in male *Lu. longipalpis*, which it was thought was involved in stimulating the female before copulation (Barth, 1961). Other recent references to sexual behaviour in sandflies are those of Gemetchu (1976) on *P. longipipes* and Beach *et al.* (1983), who described the relationship between age in female *P. martini* and rates of insemination.

Morphological Variation and Crossing Relationships

Morphological variation in *Lu. longipalpis* was first noted by Mangabeira (1969). He observed that males captured in Pará state, north Brazil bore a single pair of pale tergal patches on segment four, in contrast to specimens he captured from the north-eastern state of Ceará which showed an additional pair of patches on segment 3. The different distributions of the two forms of the vector are of interest in view of the apparently uneven distribution of human kala-azar cases. For example, in the New World 97.5% of cases occur in Brazil and of these, 68% are recorded from Ceará. The suspicion that the distributions of the two-spot form in