Research Problems in Connection with Leishmaniasis—Submitted 12 October 1961 by **Dr. Marshall Hertig**, Medical Entomologist, Gorgas Memorial Laboratory, Panama, Republic of Panama.

The many aspects of leishmaniasis which are in need of investigation fall into two main categories: (1) the several diseases comprising the leishmaniases themselves (epidemiology, method of transmission, animal reservoirs, therapy), and (2) control. In this memorandum it is at times convenient to consider the individual diseases separately or in terms of different geographic situations, and at other times to consider phenomena or problems common to all.

The leishmaniases include: (i) kala azar, or visceral leishmaniasis, of man and dogs, caused by *Leishmania donovani*, distributed from the Mediterranean littoral to North China and extending southward into the Sudan and East Africa, and also found in certain areas in Brazil and several other countries in the New World; (ii) oriental sore, or cutaneous leishmaniasis (*L. tropica*), extending from the Mediterranean through the Middle East into India; (iii) American muco-cutaneous leishmaniasis (*L. brasiliensis*) which occurs from Yucatan to northern Argentina; (iv) the form of cutaneous leishmaniasis known as "lepromatous" which has been described from Venezuela.

All the leishmaniases have in common the fact that the two most fundamental parts of the whole problem, the reservoirs and method of transmission, remain partly or wholly unsolved. With regard to transmission it is quite true that the ability of *Phlebotomus* sandflies to transmit by biting has been demonstrated for Indian and Chinese kala azar and for oriental sore in Palestine. Even in the absence of comparable demonstrations

for other species and regions, the now considerable body of information about sandflies and leishmaniasis,—distribution, abundance, association with leishmaniasis, the striking and characteristic growth of *Leishmania* in sandflies in the laboratory, the occurrence of natural infections in wild-caught sandflies,—fully justify the hypothesis that all the leishmaniases are transmitted by *Phlebotomus*.

1. *The reservoir problem:* Is there a reservoir other than man in all cases or only for certain species in certain places?

A. Situations where reservoirs have been demonstrated or are probable

1. Oriental sore

a. Turkmenistan. Semi-desert region; high endemic human rate; infected desert rodents found, especially gerbils; *Phlebotomus* found resting and breeding in burrows of these rodents; sandflies also infected with *Leishmania*. While no experimental transmissions were carried out, this is one of the few situations where the observed epidemiology seems to be satisfactorily explained.

b. Soviet Middle Asia. Agricultural development forced rodents into open country, leaving oasis free of infection except around periphery.

c. Iran. Similar to Turkmenistan, with infected gerbils found.

d. Dead Sea region. Superficially the situation resembles that of Turkmenistan, although details have never been worked out. The desert far from human habitations has an abundance of sandflies and there are rodent burrows under nearly every bush.

2. American cutaneous leishmaniasis. The disease is closely linked with forests, often sparsely inhabited. The epidemiology "calls for" a reservoir other than man and there is general agreement that there must be such reservoir. Naturally infected rodents have been found in Panama and Brazil, but the reservoir problem is still far from solution.

3. Kala azar

a. Sudan and East Africa. In the Sudan kala azar has been contracted by military patrols in otherwise uninhabited areas. In East Africa Heisch has recently recovered *Leishmania* from rodents in a kala azar area.

b. Soviet Middle Asia. Scene of reclamation of previously uninhabited land in Tadjikistan. Naturally infected jackals found. Elsewhere, in established towns with endemic kala azar, domestic dogs are found infected.

c. Brazil. Deane has found wild foxes naturally infected, as well as domestic dogs in association with human cases.

d. Mediterranean region; China. Infected domestic dogs are frequently found in association with human cases. Various investigators believe that dogs are the reservoir in these areas.

B. No reservoir demonstrated

1. Kala azar in India. It seems that domestic dogs are not infected (for reasons not understood) and this has led many investigators to believe that man himself is the sole reservoir. This point of view is supported by the fact that sandflies can be quite easily infected by feeding on Indian kala azar patients, whereas in other countries it is only rarely that sandflies can be infected by patients.

However, these considerations should be borne in mind: In India kala azar is largely a rural disease and it seems to me that a reservoir is eminently possible. There is also a curious feature of the early history of kala azar in India. In the 1860's and 1870's the disease began to spread in epidemic form in the lower end of the Brahmaputra valley. The epidemic produced panic and was in effect depopulating, not only on account of the high mortality but also the flight of people from infected villages. At that time the Brahmaputra throughout its 300-mile course in Assam had steamboats, commercial traffic, pilgrimages, etc. If the factors necessary for transmission were all present and lacked only the introduction of the parasite via the human host, there should soon have been foci established throughout the valley. However, the disease spread at a steady rate of about 10 miles a year and it took 30 years to reach the upper part of the valley. The slow spread would correspond better with the advance of an efficient vector or reservoir or a combination of them. This, of course, does not exclude the possibility that man himself was and still is the sole or chief reservoir. Nevertheless I believe that in the rural setting of this disease in India, a reservoir other than man should still be looked for.

2. Oriental sore in some Near East and Mediterranean cities. Wild rodent reservoirs have been demonstrated or are probable in the desert regions mentioned above. On the other hand, various cities, closely built up and densely inhabited, have been classic foci of oriental sore. In these cases no reservoir has been demonstrated and various investigators believe that transmission takes place from person to person via the bite of sandflies. In this case it is necessary to assume that sandflies become infected by feeding on the narrow area of unulcerated skin surrounding the ulcer. I know of no observational or experimental evidence that enough sandflies feed accurately on such restricted areas, with resulting infections, to account for the observed epidemiology.

II. Identification and nomenclature of species and strains of *Leishmania*. The three names, *Leishmania donovani*, *L. tropica* and *L. brasiliensis*, are commonly accepted,—for convenience at any rate,—for the respective parasites of human and canine kala azar, oriental sore and American cutaneous leishmaniasis. In the case of kala azar, the disease differs in a number of respects from country to country, e.g., as to age incidence, relative response to treatment (Sudanese kala azar is more resistant to treatment than the others), frequency of dermal involvement after treatment. With oriental sore, two general clinical types, "dry" and "moist", are recognized in some places, as in Soviet Middle Asia, where they are associated respectively with urban or rural environment. In American cutaneous leishmaniasis there are several geographic strains recognizable clinically by the proportion of cases in which invasion of the mucosa occurs (virtually none in Yucatan and northern Guatemala; few, probably not over 3 or 4 percent in Costa Rica and Panama; around 80 percent in Brazil and Paraguay). Furthermore, Guatemalan and Panamanian strains behave differently in experimentally infected hamsters and sandflies and these two strains can be separated by a precipitin reaction.

It will eventually be necessary to revise the nomenclature to take account of these differences of varying types and degrees. A few investigators have already given subspecific names to local strains. At the other extreme there are some who urge the concept of "leishmanial unity," i.e., that all the leishmaniases are caused by one species, with differences in behavior, clinical syndromes, etc., the result of local conditions, and depending on the species of host or vector.

I believe that this phase of the problem can be clarified only by an extensive comparative study of as many species and strains of *Leishmania* as possible. Such study should be done by one group of investigators, with initial objectives the determination for all strains (1) their behavior in a broad series of experimental animals, including hamsters, mice, gerbils, cotton rats, monkeys, etc., as well as any available wild animals which lend themselves to laboratory procedures. (2) Behavior in *Phlebotomus*. (3) Cultural characteristics. (4) Serology, specific or group reactions, including precipitin reactions. (5) Immunology: cross immunity; the Montenegro reaction, its validity other than for American cutaneous leishmaniasis in man. (A workable test which would reveal prior subclinical infections in man, as well a test which could be used in a survey of wild-caught animals, would be enormously useful.) In addition to the mammalian infections, this comparative study should also include those leishmaniae which have been recovered from lizards or other vertebrates.

III. *Method of transmission.* Valid experimental transmission of leishmaniasis has been achieved on only three occasions

(kala azar in India and China, oriental sore in Palestine). It is highly desirable that transmission experiments be carried through in other regions with other species of *Leishmania* and *Phlebotomus*, to determine not only which species can and do transmit the disease in a given locality, but also, and of probably greater importance, to determine the factors underlying transmission.

It is well known that mere infection of the sandflies is not enough to insure transmission. There is the record of the many years of failure to transmit either kala azar or oriental sore in the laboratory before hitting upon the very simple devices of giving the Indian sandflies access to boiled raisins and of adding salt to the mixture of culture and blood fed the Palestinian sandflies. In the Chinese transmission, wild sandflies taken from the kennel of an infected dog transmitted kala azar to hamsters. In this case the physiological equivalents of raisins or salt were conceivably available to the sandflies in nature before they entered the experiment. This phenomenon,—the effect of these "extraneous" substances in making possible experimental transmission and the determination of their equivalents in nature, has never been investigated. It may be noted that our group in Panama has been carrying on transmission experiments using both animals and man, so far with negative results, in spite of having infected sandflies and using raisins or salt or both.

The whole kala azar problem is at present being studied in both the Sudan and East Africa, and we may hope for important results. With regard to other kala azar areas it has seemed to me that canine kala azar in the Mediterranean, which is so important in the epidemiology, offers a thoroughly neglected opportunity to work out the transmission problem. Much of the ground-work has already been done. The Mediterranean species of *Phlebotomus* have been worked on for many years and a great deal is known about their distribution, habits, laboratory rearing, and their probable, but not demonstrated, role as vectors. Infected dogs, with large areas of the skin loaded with leishmaniae, are known to be excellent sources of experimental sandfly infections. Differential infection rates as to species of artificially fed sandflies have been determined. Curiously, however, no transmission experiments with sandflies have ever been done with Mediterranean dogs. Work of this sort should throw important light on the whole Mediterranean kala azar situation.

Ecology of Phlebotomus. Any work with *Phlebotomus* in connection with any of the leishmaniases obviously involves not only experimental procedures in the laboratory but a study of the field ecology. Satisfactory techniques of rearing sandflies and their experimental manipulation have been pretty well worked out, but there is still a great deal to be done in the field. Very little is known about the breeding places of various of the supposed vectors of leishmaniasis. For example, *P. sergenti* and members of that group, generally assumed to be the vectors of oriental sore in certain areas of the Mediterranean and Near East, often occur in densely built-up urban communities, such as parts of Baghdad and the old Turkish part of Canea in Crete (at least this was true before DDT). I can't recall that anything is known about the breeding places of this group or its habits outside of buildings. *P. perfiliewi*, a Mediterranean species belonging to the *P. major* group (which group incidentally is ordinarily implicated in the transmission of kala azar) is apparently the transmitter of oriental sore in the Abruzzi, an Italian province on the Adriatic. This is a well cultivated area and these sandflies can be enormously abundant in villages, but again nothing is known about their basic ecology.

Our own group in Panama, starting with almost nothing known about the breeding places of New World species, has been able to recover larvae in large numbers and thus demonstrate the larval habitats (in soil and leaf litter) of 15 species, as well as the diurnal shelters of some of the common man-biting species which were previously unknown. There remains, however, the determination of the natural hosts other than man, flight habits, food other than blood,—in short, the whole natural history of *Phlebotomus* in relation to reservoirs of leishmaniasis.

Much of the work on kala azar and oriental sore in the Mediterranean and Near East has been done in urban and village communities where sandflies freely enter houses and stables and remain during the day. Most of the work has concerned these abundant and easily studied sandflies, with certain species thought (probably correctly) to be the vectors of the local disease. However, the concentration of effort on these house-haunting species has resulted in the neglect of the surrounding countryside. By and large the natural history of sandflies outside Mediterranean villages is a blank. Surveys should be made in the open country, gallery woodland along streams, groves, hedge rows, etc., searching caves, hollow trees, rock crevices and the like, making night time catches with animal bait (e.g., man, horses, cattle) and employing animal-baited and light traps. It could well be that catches of sandflies in areas away from villages would be scanty compared with house catches, but any information secured could be of great significance in connection with sandflies and potential reservoirs, and hence with the basic epidemiology. Any work on the ecology of sandflies in any leishmaniasis area would be a contribution to the whole problem.

IV. Clinical phases; therapy; pathology

1. It would be desirable to develop an oral method of treatment for the sake of rural and forest populations for whom visits to clinics or health centers are difficult or out of the question. In the case of American cutaneous leishmaniasis pyrimethamine has shown much promise.

2. American cutaneous leishmaniasis; some questions:

a. Lymphatic involvement. To what extent does this occur outside of Panama? and in oriental sore?

b. Nature of satellite lesions without parasites?

c. Method of transport within the body? Where does the parasite hide out to reappear years later in the nasal mucosa?

d. Multiple lesions; How caused? Separate bites? Autoinoculation? Transport by blood or lymph? Contrast with typically single lesion of oriental sore.

V. *Control. Phlebotomus* is extremely vulnerable to residual DDT. Experimental projects in the Dead Sea region of Palestine, in Peru and in the Abruzzi in Italy, have shown that sandflies can be promptly and effectively controlled by DDT applied in dwellings and stables as in anti-malaria campaigns. As a by-product of DDT spraying undertaken primarily for malaria control it has been shown (e.g., in Greece) that a single annual application virtually wipes out *Phlebotomus*, indoors and out, within the entire area of a village where the dwellings are close together. The explanation is undoubtedly that sandflies, with their typically short flight range, are bound to encounter lethal surfaces quite promptly. Effective control of oriental sore following DDT has been demonstrated in Crete and Italy. The results in terms of kala azar control seem not to have got into the literature,—at least I know of no clearcut large-scale project in which the results of DDT, whether primarily for malaria control or otherwise, have been measured in terms of kala azar. Anti-malaria work in places such as India must surely have included kala azar areas. The effects on sandflies and kala azar should be evaluated.

So far no effective resistance to DDT has been demonstrated for *Phlebotomus*.

In the early history of DDT the urgency of malaria overshadowed the concurrent leishmaniasis problems and little attention was paid to the incidental effects on the latter. Non-malarious pockets of leishmaniasis were left untreated and may still remain untreated. Within the past year or so I have learned that in areas in Italy, in at least one case the site of a successful oriental sore project, DDT treatment has been discontinued. It seems to me that with the demonstrated effectiveness of DDT in compact communities, urban or village, it would be worth while to undertake systematic control of leishmaniasis in various Old World endemic areas, notably the Mediterranean region and India. This work would, of course, be properly combined with anti-malaria programs, but leishmaniasis should have its place in its own right.

The control of American cutaneous leishmaniasis, with the population at risk scattered in forests, is another matter. Separation of dwellings from the forest by a cleared space of several hundred meters apparently gives a certain amount of protection in Panama. On the basis of work in Peru it is probable that some protection would be given by the DDT treatment of dwellings and stables and also large surfaces nearby, such as stone walls, rock outcrop, large tree trunks. DDT has not yet been tried in a forested environment with or without clearings around houses. At the invitation of the Costa Rican Government and the International Cooperation Administration, Dr. C. B. Fairchild and I visited Costa Rica in connection with leishmaniasis. Among other things we suggested an experimental test of DDT along these lines, which, we understand, may be carried out in the near future.