Trypanosomiasis and Leishmaniasis with special reference to Chagas' disease

Ciba Foundation Symposium 20 (new series)

Held jointly with the Venezuelan Academy of Sciences and 'La Trinidad' Medical Center, Caracas

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Trypanosomiasis and Leishmaniasis

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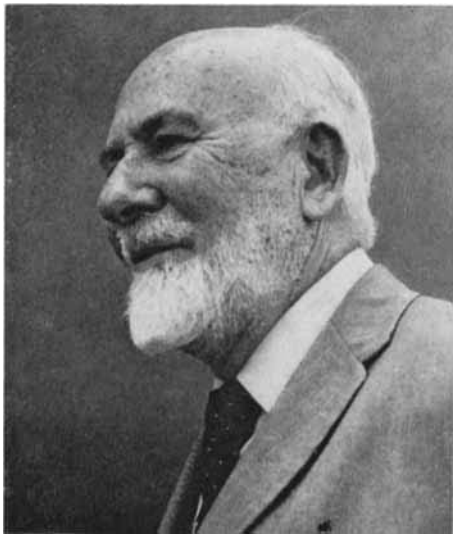

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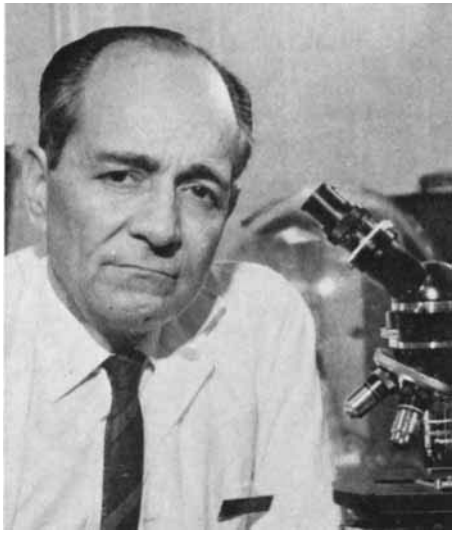

Professor F. Pifano

Guests of honour at the symposium

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Introduction

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The South American and African forms of trypanosomiasis, together with the cutaneous and visceral forms of leishmaniasis, affect in diverse ways many millions of people in tropical and subtropical areas of the world. Over the years since the causative organisms were identified, we have learned a great deal about these diseases and about the parasites, but in spite of this progress methods for prevention and control are still far from adequate.

The parasites differ in many ways but as we learn more about them we are coming to appreciate that they also have some important features in common. I believe there is much to be gained by workers concerned with these three diseases coming together now to exchange information and to discuss common problems in an attempt to define the most important lines for future research.

This is the aim of our symposium—and to that end the programme is necessarily a broad one but it has a logical structure. We start with a general comparison of the parasites from a taxonomic point of view and go on to discuss epidemiology, pathogenicity and problems of immunity. Then we take a closer look at the parasites, their ultrastructure, nutrition and metabolism, and finally consider problems of chemotherapy and drug resistance.

However, the very breadth of this programme and the fact that the people here represent many different disciplines creates a hazard we must try to avoid if the symposium is to be a success, namely the danger of specialists using the jargon of their subject. I therefore appeal to the speakers to present their material in a form which can be readily understood by us all and not just by a specialist group.

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Leishmaniasis and trypanosomiasis: the causative organisms compared and contrasted

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Abstract The order Kinetoplastida comprises those protozoa which exhibit extranuclear DNA in the form of a kinetoplast—a self-replicating organelle associated with the mitochondrion; practically all its members are parasitic. Of the 18 or so genera into which the order is divided, most are of minor importance, being mainly parasites of insects; the main mass of the huge literature on the order relates to *Leishmania* and *Trypanosoma* because these genera include organisms seriously pathogenic to man and to his domestic animals.

Despite the close taxonomic affinity between these two genera, studies of the diseases which they respectively cause have remained rather separate. Nevertheless, there are clearly many aspects of these diseases where comparison between the two would be likely to be rewarding. Common to both is the manifest ability of organisms of identical morphology to be associated with different pathological outcomes or with different patterns of transmission in nature. How far such differences are determined by differences in the potential of the infecting organisms and how far by differences in the reactions of the hosts is at present largely unknown. Before the host component of the association can be properly appreciated, much more precise characterization of organisms is essential. Morphological criteria are clearly inadequate and other criteria for the recognition of populations of organisms of particular biological potential need to be found, such as those based on immunological or biochemical experiment. Studies for this purpose should be concentrated on populations of organisms of known homogeneity (clones), stabilated as close as possible to their natural origins.

A peculiarity of tropical medicine has been a tendency for its various sections to develop rather in isolation from one another, even when they might have been expected to be related scientifically. Many factors have contributed to this situation—the discontinuous geographical distribution of many tropical diseases, the geographical separation of the areas where the diseases occurred from integrative centres of medical science, the partition of the tropics into spheres of influence of extra-tropical states, the complexity of the epidemiology

of many of the diseases which led necessarily to specialization by workers on individual diseases, and so on. However, in recent years many influences have been at work to dissolve these divisions and to promote the exchange of ideas and methods between sections. In this context it is impossible not to mention the influence of Dr N. Ansari, Chief, Parasitic Diseases, World Health Organization, who has greatly contributed to the integration of work on African trypanosomiasis in eastern and western Africa, and more recently to the integration of this field with that of South American trypanosomiasis.

The present symposium is another welcome influence in this respect and leads to a situation which would have been impossible a few years ago—that of a worker primarily experienced in arbovirus epidemiology, and then in trypanosomiasis in East Africa, attempting to contribute to the comparison of two fields in which he has had no direct practical involvement, leishmaniasis and Chagas' disease. I hope that from this somewhat detached position I shall be able to make some useful contribution.

MORPHOLOGICAL TAXONOMY

The essentials of the taxonomic position of the organisms under discussion, extracted from the classification of Honigberg *et al.* (1964) and relying mainly on Kudo (1966) for the placing of genera, are as follows:

Phylum Protozoa

Subphylum I Sarcomastigophora (of four Subphyla). Flagella, pseudopodia, or both types of locomotory organelles; single type of nucleus except ... Foraminiferida; typically no spore formation; sexuality, when present, essentially syngamy.

Superclass I Mastigophora (of three Superclasses). One or more flagella typically present in trophozoites; solitary or colonial; asexual reproduction basically by symmetrogenic binary fission; sexual reproduction unknown in many groups, nutrition phototrophic, heterotrophic, or both.

Class 2 Zoomastigophorea (of two Classes). Chromatophores absent; one to many flagella; additional organelles may be present in mastigonts; amoeboid forms with or without flagella, in some groups; sexuality known in a few groups; species predominantly parasitic.

Order 4 Kinetoplastida (of nine Orders). One to four flagella; kinetoplast

argentophilic and Feulgen-positive, present as self-replicating organelle with mitochondrial affinities; most species parasitic.

Suborder 1 Bodonina (of two Suborders). Typically two unequal flagella, one directed anteriorly, one posteriorly; no undulating membrane; kinetoplast absent secondarily in some species; free-living or parasitic.

Genera: Bodo, Pleuromonas, Rhynchomonas, Proteromonas, Phyllomitus, Colponema, Cercomonas, Cryptobia.

Suborder 2 Trypanosomatina. One flagellum, either free, or attached to the body by means of an undulating membrane; all species parasitic.

Genera: Proleptomonas, Leptomonas, Phytomonas, Crithidia, Blastocrithidia, Herpetomonas, Rhynchoidomonas, Leishmania, Trypanosoma, Endotrypanum.

We may, at this stage, neglect the Bodonina and concentrate on the Trypanosomatina. These latter flagellates may exist in a variety of morphological forms distinguished primarily by the site of origin of the single flagellum; a convenient nomenclature for these morphological forms is that proposed by Hoare & Wallace (1966) (Fig. 1). This nomenclature is simple and straightforward and has rapidly come into general use. There are, however, some difficulties with regard to forms whose cell body is round or nearly so.

The round form in the Hoare & Wallace (1966) system is without a flagellum—an amastigote. For certain flagellated round forms of *Trypanosoma* (*Schizotrypanum*) *cruzi* seen in the gut of *Rhodnius prolixus*, Brack (1968) introduced the term 'sphaeromastigote'. This term seems to me to be unfortunate in that it departs from what Garnham (1971) described as the 'uniform Greek etymology' of the terms proposed by Hoare & Wallace (1966), which relate specifically to the condition of the flagellum, whether or not it exists and, where it does, to its position of origin. The sphaeromastigote term refers not to this, but to the shape of the body. It is further unfortunate that the term is being applied without distinction to rounded forms with a short uncomplicated flagellum (Fig. 1g), to rounded forms with a long flagellum and a circumferential undulating membrane (Fig. 1i), and to organisms whose flagellar development is intermediate between these extremes (Fig. 1h). These different forms, all at present designated as 'sphaeromastigotes', may well be stages in significantly different lines of development, as indeed was suggested by Brack herself. It would seem preferable, so as not to distort the excellent Hoare and Wallace system, and yet give a full description of the round forms occurring, to separate the descriptions of body form and those of flagellar position. By and large, these flagellated round (so-called 'sphaeromastigote') forms appear to run through the same gamut of flagellar modification as do the more elongate forms and they could be classified, respectively, as promastigote,

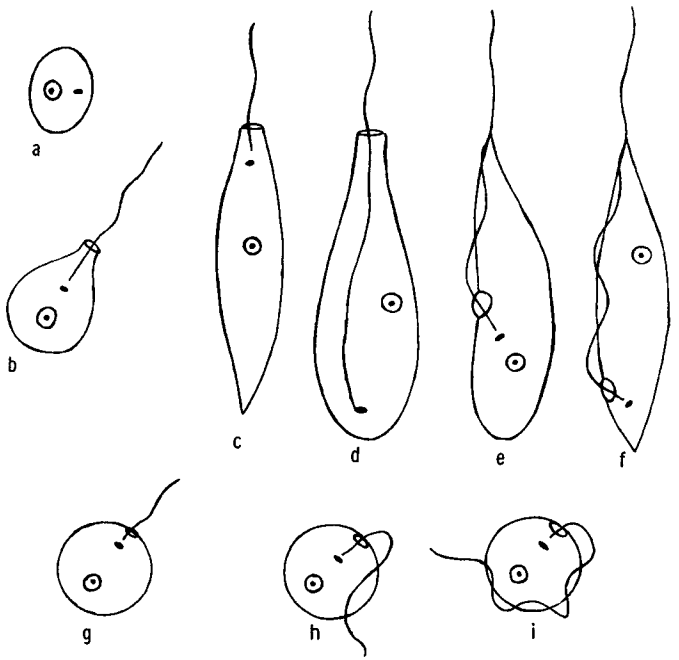

FIG. 1. The forms adopted by the Trypanosomatina (after Hoare & Wallace 1966). (*a*) Amastigote, (*b*) choanomastigote, (*c*) promastigote, (*d*) opisthomastigote, (*e*) epimastigote, (*f*) trypomastigote (Hoare & Wallace, 1966); (*g*)–(*i*) sphaeromastigotes (Brack 1968).

epimastigote or trypomastigote, depending on whether the flagellum arises immediately from the kinetoplast, or runs round the organism about 90°, or about 180°, before becoming free. Thus, Fig. 1g would be a round promastigote, Fig. 1h a round epimastigote, and Fig. 1i a round trypomastigote.

These round forms which occur in the cycles of development of vertebrate-infecting organisms are not apparently associated with any attribute of resistance to adverse circumstance. They are located in the interior of the cell, in vessels or in the insect gut. There are, however, other types of amastigote to which are accredited faculties of resistance and which occur in the midgut of insects infected with *Leptomonas* (McGhee 1968). Two kinds of true cysts are described for insect trypanosomatids (Wallace 1966): 'strap-hanger' cysts which arise from unequal division of the parent organism, the small daughter cell adhering to the flagellum of the larger, moving distally and encysting; and the secretion of a jelly-like coat around the flexed organism within which it continues to be motile. Wallace (1966) quotes, also, a number of cases in which flagellates survived 'drying' for long periods, suggesting that such cysts may be

important stages in the life history of the organism. In fact he states later that transmission of insect flagellates is generally through contamination, in which such stages would seem likely to be of great importance. Little attention has been paid to the possibility of resistant stages occurring in the trypanosomatids parasitic in vertebrates, and so the relevance of these observations on insect trypanosomatids is at present uncertain.

Individually, all these named forms are of little use taxonomically, as any given form may occur, and be morphologically indistinguishable, in the course of the developmental cycle of two or more organisms which are clearly differentiable on other grounds. McGhee (1968) provocatively states the problem by pointing out that: '...one may not state with certainty, for example, whether the trypanosomatid [? promastigote form] seen in the rectum of a phytophagous hemipteron, *Oncopeltus fasciatus*, is of the genus *Phytomonas*, *Leptomonas*, *Crithidia*, *Blastocrithidia*, *Rhynchoidomonas*, *Trypanosoma*, *Leishmania*, *Herpetomonas*, or even possibly *Proleptomonas*, much less to allocate it to a 'species'.'

Within the Trypanosomatina, present classification depends on the summation of a number of characteristics ranging from morphological to clinical and epidemiological. Levine (1972) provides a useful table of these characteristics: positions of kinetoplast and reservoir, state of development of the undulating membrane, the number of forms occurring in the life cycle, host relationships and number and provenance of the species described. This is a very useful summary of the group but it must be remembered that the numbers of species described in groups are largely products of the amount of attention devoted to the groups and of the propensities of the workers concerned, whether they were 'lumpers' or 'splitters'. Thus the validity of a 'species' inevitably varies widely from genus to genus.

TAXONOMY OF LEISHMANIA

Leishmania is primarily characterized by existing in two forms—amastigote in the cells of its vertebrate host and promastigote in the gut of its insect vector. Because of the virtual morphological identity of the organisms throughout the genus, they are here classified mainly according to the clinical conditions which they produce in man. Lainson & Shaw (1972) say, in discussing the classification of New World *Leishmania* on the basis of vernacular terms applied to the clinical condition:

'...while a given *Leishmania* may produce an over-all similar clinical picture in man, individuals may react differently to the same parasite and the appearance of the disease may vary greatly at different stages of the infection'.

Reacting, thus, from the confusion likely to be engendered by too great reliance on clinical appearance alone as a basis of classification, Lainson & Shaw (1972) proposed a more widely based classification based on a variety of characters—rate of growth in culture or in experimental animals, geographical distribution, epidemiological patterns, as well as clinical characters. Briefly, this classification, extended on the same principles to include the other *Leishmania* spp. summarized by Garnham (1971) and Belding (1965), is as follows:

Leishmania donovani complex

Organisms tending to 'visceralize' in man (i.e. with a predilection for infecting cells in the viscera, particularly in the spleen); main vectors *Phlebotomus* of the 'major' group.

L. d. donovani: Asia, mainly India and China; infects adults; no known extra-human vertebrate host.

L. d. infantum: Mediterranean countries; infects mainly children; extra-human vertebrate hosts, dogs.

L. d. chagasi: South America; infects adults and children; extra-human hosts, wild dogs.

Leishmania tropica complex

Organisms confined to cutaneous locations, not tending to visceralize; main vectors *Phlebotomus papatasi* and *P. sergenti.*

L. t. tropica (= minor): Asia; aetological agent of 'dry' sore; no known extra-human hosts.

L. t. major: Asia; aetiological agent of 'moist' sore; extra-human vertebrate hosts, *Rhombomys* (gerbil), *Meriones* (jird), etc.

Leishmania mexicana complex

'Fast-growing' organisms in culture and in hamsters. In hamsters cell response is poor and lesions are packed with amastigotes; there is metastatic spread. Lesions in man are mild, cutaneous, with no nasopharyngeal involvement. Lesions in wild animal hosts (rodents, *Didelphis*) inconspicuous,

mainly on tail. Vectors *Lutzomyia intermedia* group, mainly *L. olmeca* and *L. flaviscutellata*; promastigotes not developing in the hindgut triangle.

L. m. mexicana: Mexico, Guatemala, British Honduras. Causes mild infection, a single cutaneous lesion which is self-healing, or persistent chronic ear lesions; but no nasopharyngeal involvement. Chiclero's ear, Bay sore; one recorded case of 'anergid' diffuse cutaneous leishmaniasis (DCL).

L. m. amazonensis: Amazon basin to Mato Grosso State, Brazil; Trinidad; perhaps elsewhere. Rarely infects man, causing mild, single or limited, cutaneous lesions; no predilection for ear tissue or for nasopharynx. Occasional DCL cases.

L. m. pifanoi: Venezuela. Only known from DCL cases.

Leishmania enriettii

Brazil. An anomalous species discovered in laboratory *Cavia porcellus* (guinea pig); not infective for *C. aperea* (Brazilian wild guinea pig).

Leishmania braziliensis complex

'Slow-growing' organisms in culture and in hamsters. In hamsters cell response is marked and lesions have moderate or scanty amastigotes; there is no metastatic spread. Lesions in man, single or multiple, often extensive and disfiguring; nasopharyngeal involvement in one species. Lesions in wild animal hosts (rodents, procyonids, marmosets), limited discrete inconspicuous skin lesions, or inevident. Vectors *Lutzomyia intermedia* and *Psychodopygus* groups; parasites developing in the hindgut triangle.

L. b. braziliensis: Brazil, and forest areas east of Andes in other states. Causes destructive cutaneous leishmaniasis, lesions frequently large, persistent and disfiguring, with frequent nasopharyngeal metastases. Espundia.

L. b. guyanensis: Guyanas, Surinam, Brazil, Venezuela. Causes single lesion or spreads to many crateriform ulcers over body, metastasizes along lymphatics; probably not metastasizing to nasopharynx. Pian bois.

L. b. panamensis: Panama, possibly extending to north and south. Causes single, or few, shallow crateriform ulcers, metastasizing as nodules along lymphatics; probably not metastasizing to nasopharynx.

L. peruviana

Peru, western slopes of Andes to 3000 m; the only form not associated with forest areas. Causes single or limited number of self-healing lesions; no naso-pharyngeal involvement. Uta.

TAXONOMY OF TRYPANOSOMA

Trypanosoma is primarily characterized by manifesting, in at least some stage of the life history, trypomastigote forms; amastigote and epimastigote forms may also occur. There may be, also, a variety of flagellate round forms of as yet uncertain significance (Ormerod & Venkatesan 1971*a*, *b*).

A classification of the genus has been proposed by Hoare (1966, 1972) but this deals only with the species infecting mammals. It is, in summary, as follows:

(A) *Stercoraria*

Free flagellum present; kinetoplast large, not terminal; posterior end of body pointed; multiplication in mammal discontinuous, typically in epimastigote or amastigote forms; typically non-pathogenic; development in vector in posterior station, transmission contaminative.

Subgenus *Megatrypanum:* Large species; kinetoplast typically near nucleus, far from posterior end of body; includes *T. (M.) theileri, tragelaphi, ingens, melophagium* and others.

Subgenus *Herpetosoma:* Medium-sized species; kinetoplast subterminal; includes *T. (H.) lewisi, duttoni, nabiasi,* and others.

Subgenus *Schizotrypanum:* Small species, trypomastigotes typically curved; kinetoplast voluminous, close to posterior end of body; includes *T. (S.) cruzi, vespertilionis, pipistrelli* and others.

(B) *Salivaria*

Free flagellum present or absent; kinetoplast terminal or subterminal; posterior end of body usually blunt; multiplication in mammal continuous in trypomastigote stage; typically pathogenic; development in vector (*Glossina*

—tsetse-fly) in anterior station and transmission inoculative; includes also some atypical species transmitted non-cyclically by arthropod vectors, or by coitus.

Subgenus *Duttonella:* Monomorphic species; posterior end of body rounded; kinetoplast large, terminal; free flagellum present; development in *Glossina* in proboscis only; includes *T. (D.) vivax, uniforme.*

Subgenus *Nannomonas:* Small species; monomorphic or polymorphic; kinetoplast of medium size, typically marginal; free flagellum usually absent; development in *Glossina* in midgut and proboscis; includes *T. (N.) congolense, dimorphon, simiae.*

Subgenus *Pycnomonas:* Short, stout species; monomorphic; kinetoplast small, subterminal; free flagellum short; development in *Glossina* in midgut and salivary glands; includes *T. (P.) suis.*

Subgenus *Trypanozoon:* Typically pleomorphic species with small subterminal kinetoplast; development in *Glossina* in midgut and salivary glands; includes some aberrant species transmitted non-cyclically or by coitus; includes *T. (T.) brucei, gambiense, rhodesiense, evansi, equinum, equiperdum.*

Levine (1972), discussing Hoare's (1966, 1970) classification of the *Trypanosoma* spp. of mammals, observes: (*a*) that, as the classification of the genus does not include its type species—*Trypanosoma rotatorium* (Mayer, 1843)—it will be necessary to erect one subgenus additional to the seven proposed by Hoare (see also Hoare 1972), and (*b*) that all members of the 'group' (= genus) are so similar morphologically that splitting them into several subgenera is not justified.

The first proposal is unexceptionable, but not the second. In fact a main criticism of Hoare's classification could be based on the morphological heterogeneity of the trypanosomes of mammals, in particular the occurrence of amastigote intracellular forms in some of the subgenera but not in others, e.g. in *Herpetosoma* in the insect host and in *Schizotrypanum* in the mammal host. Although amastigote forms may not be confined to these subgenera—Ormerod & Venkatesan (1971*a*, *b*) advance the occurrence of a similar phase in *(T. T.) brucei*, though not as an intracellular organism—this seems to be an important distinction, as recognized in Baker's (1965) phylogenetic chart by the grouping of *T. (S.) cruzi* with *Leishmania* rather than with the other species of *Trypanosoma* (see below).

TAXONOMY OF ENDOTRYPANUM

Brief mention should be made here of this genus, included by Hoare (1966) as a subgenus in the Stercoraria but deserving generic status. Epimastigote and trypomastigote forms occur within the erythrocytes of the host (*Choloepus* and *Bradypus*; sloths). The genus includes only two species, *E. schaudinni* and *E. monterogeii* (Shaw 1969).

Insufficient is known of the genus to make it very profitable to discuss it. However, it has a similarity to *Leishmania* and to *T. (S.) cruzi* in that it shows an intracellular form in its vertebrate hosts. However, this is only a slender connection in that the invaded cells are erythrocytes and the cell-invading form is not especially adapted to intracellular existence—it is an epimastigote or trypomastigote, not an amastigote.

PHYLOGENY OF THE TRYPANOSOMATINA

Conjectures as to the phylogeny of the Trypanosomatina are based mainly on the variety and upon the order of the morphological forms through which the organisms pass in the course of their development. Wallace (1966) distinguishes between a 'life cycle' (in which a more or less constant and obligate sequence of stages is followed by an individual organism) and a 'population cycle' (in which a sequence of changes is superimposed on an indeterminate number of individual generations). By these definitions the developmental processes of the Trypanosomatina are probably population cycles. Considerable confusion exists in described life histories because the infections studied were frequently mixed; Wallace (1966) emphasizes the need to study clone populations.

Wallace (1966) suggests that the kinetoplast originated as an adaptation in organisms subject to sudden changes in the oxygen (or nutrient) content of their surroundings. He points out that the *Bodo*-like flagellates are subject to such changes and selects them, therefore, as the group in which the kinetoplast may well have originated. Such flagellates, later established in the intestine of a vertebrate host, found themselves ready equipped to invade the tissues and become transmitted by haematophagous arthropods. From this point (Fig. 2) Wallace derives the two 'parasitic' lines *Leishmania* and *Trypanosoma*, differentiated by the position of the reservoir (equivalent to kinetoplast), these leading, respectively, to arthropod parasites of similar morphology. He does not explain the loss of one of the two flagella.

Baker (1965) proposes a line of evolution almost diametrically the opposite

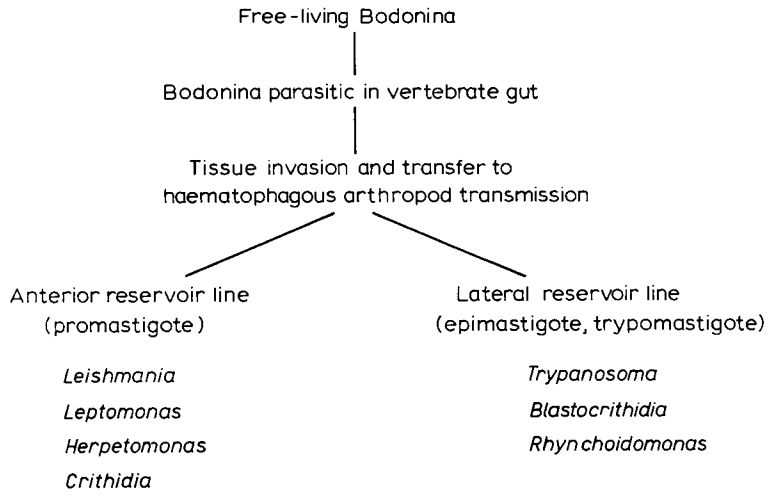

FIG. 2. Phylogenetic history of the genera of the Trypanosomatina, according to Wallace (1966).

of this (Fig. 3), starting from primitive (free-living) *Leptomonas* by a progressive elaboration through forms parasitic in insects to those parasitic in vertebrates, though again, as with Wallace's theory, in two lines, one promastigote, one epimastigote.

One may not place too much reliance on such conjectural evolutionary plans but it is interesting to note that: (*a*) both theories regard *Leishmania* as a homogeneous genus; (*b*) one theory (Wallace's) does the same for *Trypanosoma*, but the other (Baker's) splits *Trypanosoma* into three widely separated groups: the generality of the insect-transmitted *Trypanosoma* grouped with the flagellates of insects; the annelid-transmitted and *Glossina*-transmitted trypanosomes; *Trypanosoma cruzi* grouped with *Endotrypanum*.

One wonders why such weight is given to the promastigote–epimastigote difference, with so much less to other of the form differences and to even more fundamental differences such as habitat in the vertebrate body. One of the most striking of the last is whether or not the organisms show an essential stage of their life cycle within cells of the vertebrate host.

This happens in three groups—*Leishmania* spp., *Trypanosoma (Schizotrypanum) cruzi* and *Endotrypanum* spp. We may disregard *Endotrypanum* as a probably highly aberrant genus and consider the other two vertebrate-infecting genera.

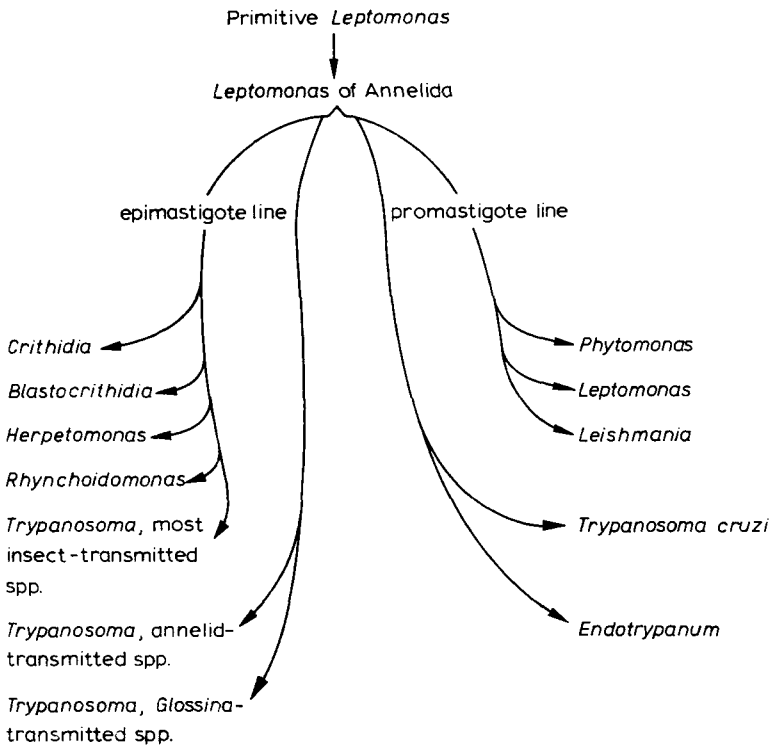

FIG. 3. Phylogenetic history of the genera of the Trypanosomatina; modified from Baker (1965).

COMPARISON OF LEISHMANIA AND TRYPANOSOMA

Morphology and transmission

Leishmania. These occur, as far as the vertebrate host is concerned, in reptiles and in mammals.

The *Leishmania* spp. occurring in reptiles may be rather separate. They exist in the vertebrate host mainly in the promastigote form (Garnham 1971). According to Garnham (1971), there is a consistent difference between reptile-infecting and mammal-infecting *Leishmania* in the interval between the sub-pellicular microtubules, which is 58–67 nm in the reptile-infecting and 35–42 nm in the mammal-infecting species. Considering the antiquity of the reptiles phylogenetically, it is remarkable that the reptile-infecting *Leishmania* are (? completely) confined to the Old World; this might indicate that the leish-

manial colonization of the reptile was a rather recent event. The absence of reports of leishmanial infection in birds, also, is arresting.

The *Leishmania* spp. infecting mammals seem to be a very homogeneous group. Only two morphological forms are exhibited, amastigotes related to intracellular existence in the vertebrate host and promastigotes related to extracellular life in the gut of the insect host. All species are transmitted by phlebotomine (Diptera) flies. The range of mammalian hosts which they infect is comparatively restricted—only rodents, canids and primates.

Trypanosoma. This seems to be a much less homogeneous collection of organisms than is *Leishmania.* Organisms may exhibit themselves as amastigotes, epimastigotes and trypomastigotes. The variety of form exhibited within the trypomastigotes is extremely wide, varying from nearly spherical forms to extremely long, slender, ribbon-like forms. *Trypanosoma* spp. are transmitted by a wealth of vectors, mainly annelids (leeches) and a wide range of insects, including Siphonaptera, Hemiptera, Diptera. The range of vertebrate hosts infected is also very wide, including fish, reptiles, amphibia, birds and mammals.

Considering this heterogeneity in relation to Hoare's (1966, 1972) classification of the *Trypanosoma* spp. infecting mammals, it may be seen that the heterogeneity lies mainly in the Stercoraria. The Salivaria are a homogeneous group comprising species transmitted by the highly aberrant muscid fly genus —*Glossina*—in cycles which involve only artiodactyls, perissodactyls and primates, together with species likely to be derived directly from that transmission pattern.

The Stercoraria, on the other hand, include organisms transmitted by Hemiptera, Siphonaptera and Diptera (Tabanidae) and cover an extremely wide range of mammal host species—rodents, marsupials, canids, felids, artiodactyls, primates, cheiroptera, etc. Most of this diversity of mammal host species is, however, contributed by the one subgenus, *Schizotrypanum*, which includes *T. (S.) cruzi.* This subgenus is also aberrant among the Stercoraria in showing intracellular amastigote forms in the vertebrate host.

In summary, then, it appears that *Leishmania* is a very homogeneous genus while *Trypanosoma* is not. Further, the subgenus *Schizotrypanum* is the most aberrant one in the genus *Trypanosoma* and shares with *Leishmania* the ability to invade the cells of the host vertebrate and multiply within them. There seems, therefore, possibly to be some phylogenetic affinity between *Leishmania* and *T. (S.) cruzi* to justify further consideration of their relationship. Baker (1969) regarded *Trypanosoma (S.) cruzi* as at the end of the *Trypanosoma* spectrum most closely related to *Leishmania.*

Distribution in the vertebrate host

The distribution of these two groups of organisms in the body of the vertebrate host is as follows:

Leishmania appear to invade exclusively cells of the mononuclear phagocyte system (van Furth *et al.* 1972).

Trypanosoma (S.) cruzi. Andrade & Andrade (1971) accept that the earliest cells invaded are cells of the mononuclear phagocyte system but very soon a wide variety of other cells are involved. In fact *T. (S.) cruzi* is vastly catholic in the cells which it invades—cells of the mononuclear phagocyte system, glial cells, muscle cells, vascular endothelial cells, neurons, fat cells, etc. (Köberle 1968; Weinman 1968). However, muscle cells of all types—cardiac, intestinal, skeletal—are those especially affected.

The contrast between the types of cells selected by these two different groups of organisms is paralleled by the amount of attention which seems to have been devoted to consideration of the mechanisms of transfer of infection from cell to cell within the host in the two groups. No discussion of this matter with relation to *Leishmania* has been noticed; it does not seem to be known whether this is accomplished by phagocytosis of organisms in whole or disrupted infected cells, or by conversion of the amastigotes to promastigotes and reinvasion, or whether the dissemination of infection simply accompanies multiplication of the cells. In this latter respect it is interesting to recall the studies of Hulliger *et al.* (1964, 1966), who found that *Theileria* multiplied at about the same rate as their host cells. The organisms were associated with the spindle fibres of the host cells and were pulled apart and distributed to the daughter cells. Van den Ende & Edlinger (1971), from studies of the multiplication of *Theileria annulata* in mixed male and female lymphoid cell cultures (separable on the basis of their chromosome patterns), concluded that cell-to-cell infection *in vitro* did not take place. Cells containing parasites were induced to multiply more actively than unparasitized cells. One may conjecture that something of the same sort occurs with *Leishmania*-infected macrophages.

On the other hand, this matter of cell-to-cell infection seems to have been the focus of considerable interest in *T. (S.) cruzi*, as discussed by Köberle (1968). Transformation of amastigotes to trypomastigotes takes place within the pseudocyst and on rupture the transformed organisms distribute themselves and infect other cells. Amastigotes not transformed at the time of pseudocyst rupture are believed to be non-viable and it is to their degeneration in the vicinity of the pseudocyst that lesions of nearby ganglion cells are ascribed (Köberle 1968).