LEISHMANIASIS IN BRAZIL: XI. OBSERVATIONS ON THE MORPHOLOGY OF LEISHMANIA OF THE BRAZILIENSIS AND MEXICANA COMPLEXES

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Introduction

While studying strains of *Leishmania* isolated from man and animals in the Amazon we noted that they fell into two natural groups. These we called "fast" and "slow" strains (Lainson and Shaw, 1970) because of their behaviour in culture and hamsters. It must be emphasized that the terms "fast" and "slow" do not refer to their behaviour in man. When examining Giemsa-stained smears from infected hamsters we gradually became aware that we could distinguish the "fast" and "slow" strains. At first we thought that it was possibly due to differences in the cellular picture and number of parasites, but blind tests in fields with similar numbers of parasites and cells enabled us to distinguish parasites of the two groups, which we termed the braziliensis complex (slow) and the mexicana complex (fast) (Lainson and Shaw, 1972). Clearly we were recognizing basic morphological differences but we could not be certain what they were.

To determine the morphology of the amastigotes more precisely we decided to apply the classical procedures used to study the morphology of trypanosomes. It soon became apparent that camera lucida drawings of such small organisms were not very reliable. We therefore used photomicrographic techniques to determine the various mensural characters (Shaw and Lainson, 1972) of the amastigotes. This paper reports the basic morphological characters of some strains of the braziliensis and mexicana complexes.

Materials and Methods

The following *Leishmania* strains were used:

**PH7** *L. mexicana amazonensis*: isolated from a naturally infected *Lutzomyia flaviscutellata* captured in the Utinga forest, Belém. (Lainson and Shaw 1968).

**M22** *L. mexicana amazonensis*: isolated from a naturally infected *Oryzomys capito* captured in the Utinga forest, Belém (Lainson and Shaw, 1968).

**H6** *L. mexicana amazonensis*: isolated from a non-ulcerated lesion on the thigh of a man from the Paragominas region of Pará. (2°57' S - 47°13' W).

**H17** *L. mexicana amazonensis*: isolated from a lesion of a woman with extensive lesions on legs and back, many of which were ulcerated, from the River Maracá region of Amapá. (0°20' S - 51°35' W).

**H9** *L. braziliensis braziliensis*: isolated from a single ulcer infection below the knee of a man from the Itinga region of Pará. (4°36' S - 47°43' W).

**M1678** *L. braziliensis braziliensis*: isolated from a small single ulcer on the thigh of a man from the Serra Norte region of Pará (Lainson *et al*., 1973).

Amastigotes of the different strains were obtained from non-ulcerated cutaneous nasal lesions of golden hamsters (*Mesocricetus auratus*).

Previous morphological studies on *Sarcocystis* zooites (Lainson and Shaw, 1969) showed that the methods of fixation and collection significantly effect size. Preliminary observations on *Leishmania* amastigotes showed that direct smears from lesions were not a suitable source of parasites as there was considerable variation in shape depending on the presence or absence of blood. In general amastigotes tended to be torpedo shaped when associated with blood. Least variation in shape was noted in impression smears made from a piece of tissue from the lesion whose surface had been gently blotted on filter paper. Smears made in this way were fixed in Bouin’s fluid and stained with a modified Giemsa technique (Lainson, 1959; Wenyon, 1926). Photomicrographs of amastigotes were taken on Adox KB 17 or Agfa Isopan IF 35 mm film using a Zeiss WL research microscope with a Planapo 100/1·3 oil objective and the Zeiss II camera body mounted on a support arm. Each film included a photomicrograph of the scale of a slide micrometer which was
Results

A summary of the various mensural characters is given in Table I. Analysis by the F-test showed that there were highly significant differences between the lengths of *L. m. amazonensis* strains, ranging from 3.18 to 3.72 μm with a mean of 3.44 μm. The degree of overlap between the smaller strain M22 and the larger strain H17 was 23 per cent. The overlap between the two strains of *L. b. braziliensis* was 26 per cent, the mean major diameter being 2.4 μm. The overlap between the smaller *L. m. amazonensis* strain M22 and the larger *L. b. braziliensis* strain H9 was 12 per cent, while the overlap of the major diameter for the two groups was 13 per cent. The mean area of the amastigotes of strain M22 was 5.34 μm² and H9 was 3.78 μm² with an overlap of area of 24 per cent. The PK/PN ratio was greater than one for the strains of *L. m. amazonensis* and approximately one for the *L. b. braziliensis* strains indicating that the kinetoplast is more anterior to the nucleus in the former. The overlap of the distance P–K between strains M22 and H9 was 20 per cent and between M22 and M1678 15.9 per cent. The PK/KA ratio indicated that the kinetoplast is more posteriorly placed in the two *L. b. braziliensis* strains and the overlap of this character between strains M22 and H9 was 28.4 per cent. The PN/NA ratio showed that the nucleus is more centrally placed in the *L. b. braziliensis* strains.

The sizes of the nuclei of all the strains were similar ranging from 1.2 × 1.01 μm to 1.35 × 1.05 μm. The kinetoplasts of the *L. b. braziliensis* amastigotes were significantly larger than those of *L. m. amazonensis*. An F test of the major diameter of the kinetoplast was significant for the *L. m. amazonensis* strains but not...
TABLE I.
Mensural data in microns of four strains of Leishmania mexicana amazonensis and two strains of L. braziliensis braziliensis isolated from man and wild animals in the state of Pará, Brazil (Data expressed as means ± standard deviations and range).

<table>
<thead>
<tr>
<th></th>
<th>L. m. amazonensis</th>
<th>L. b. braziliensis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PH7</td>
<td>M22</td>
</tr>
<tr>
<td>Length</td>
<td>3.54±0.44</td>
<td>3.78±0.33</td>
</tr>
<tr>
<td></td>
<td>(2.6-4.7)</td>
<td>(2.4-3.8)</td>
</tr>
<tr>
<td>Width</td>
<td>2.41±0.38</td>
<td>2.10±0.34</td>
</tr>
<tr>
<td></td>
<td>(1.5-3.0)</td>
<td>(1.5-3.0)</td>
</tr>
<tr>
<td>P-K (posterior to</td>
<td>1.74±0.41</td>
<td>1.53±0.34</td>
</tr>
<tr>
<td>kinetoplast middle)</td>
<td>(1.0-2.9)</td>
<td>(1.0-2.3)</td>
</tr>
<tr>
<td>P-N (posterior to</td>
<td>1.09±0.36</td>
<td>1.16±0.34</td>
</tr>
<tr>
<td>middle of nucleus)</td>
<td>(0.5-1.8)</td>
<td>(0.5-1.7)</td>
</tr>
<tr>
<td>Kinetoplast length</td>
<td>0.66±0.08</td>
<td>0.60±0.07</td>
</tr>
<tr>
<td></td>
<td>(0.5-0.8)</td>
<td>(0.5-0.8)</td>
</tr>
<tr>
<td>Kinetoplast width</td>
<td>0.44</td>
<td>0.40</td>
</tr>
<tr>
<td>Nuclear length</td>
<td>1.32</td>
<td>1.2</td>
</tr>
<tr>
<td>Nuclear width</td>
<td>1.15</td>
<td>1.0</td>
</tr>
<tr>
<td>Number measured</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>PK/PN</td>
<td>1.60</td>
<td>1.40</td>
</tr>
<tr>
<td>PK/KA</td>
<td>0.97</td>
<td>0.98</td>
</tr>
<tr>
<td>PN/NA</td>
<td>0.45</td>
<td>0.57</td>
</tr>
</tbody>
</table>

for the two L. b. braziliensis strains. The overlap of the major diameter of the kinetoplast between strains PH7 and M1678 was nine per cent. Amastigotes of L. m. amazonensis in general had larger flagellar vacuoles. The mean mensural characters of strains H6 and H9 are shown in diagramatic form in Figure (i).

Discussion
Such statements as “the leishmania are a group of morphologically identical parasites” (Schnur et al., 1973) have often appeared in the literature. Muniz and Medina (1948), however, described a species of Leishmania from the guinea pig whose amastigotes measured 5.2 × 2.5 μm, which is considerably larger than species known to infect man. Adler (1963) states that Parrot and his colleagues showed that in dogs the amastigotes of L. tropica were considerably larger than those of L. infantum. The Russian workers have studied the sizes of the parasites causing urban and rural cutaneous leishmaniasis (see Kellina, 1962 for a review of these works). On the basis of size two subspecies of L. tropica were created L. tropica minor (L. tropica) and L. tropica major (L. major). Doubt as to the validity of this differentiation was expressed by some of the Russian workers but Kellina (1962), in a detailed study of the sizes of the parasites from 13 human cases of cutaneous leishmaniasis, concluded that there were significant differences in sizes of amastigotes associated with the urban and zoonotic forms of the disease. The mean sizes of parasites of the urban form (L. tropica) were 3.33 × 1.99 μm while those of the zoonotic form (L. major) measured 4.48 × 3.33 μm. She emphasized that great care was needed in the making of the smears from which amastigotes were measured.

Our results indicate that the amastigotes of L. m. amazonensis are larger than those of L. b. braziliensis, thus, as in the Old World, there appear to be two morphologically different groups of parasites. It was also noted that the major diameter of the kinetoplast of L. m. amazonensis was smaller than that of L. b. braziliensis. Apart from these two major differences there were also differences in the position of the kinetoplast and nucleus. In L. m. amazonensis amastigotes the kinetoplast was more central and the nucleus more pos-
terior. The closer approximation of the nucleus and kinetoplast in L b. braziliensis and the smaller size of this parasite often makes it difficult to determine the boundaries of the individual organelles in intracellular parasites and the amastigotes may appear as Histoplasma-like bodies.

The statistical differences between mensural characters is clearly significant if the technique is sufficiently standardized. Walker (1963) noted that batches of Giemsa stain of different ages caused notable differences in the total lengths of T. brucei trypomastigotes. We have measured the sizes of amastigotes of strain M22 from different hamsters stained with different batches of Giemsa but noted no significant variation in their major diameters. We are thus led to conclude that variations in size between the different L. m. amazonensis strains is most probably a strain specific variation rather than an artifact due to technique. What then is the taxonomic importance of statistically significant differences? Much has been written about this and it is generally taken that the degree of separation is the level for specific or subspecific ranking. Such levels are well beyond statistically significant differences and are clearly not equivalent in, for example, sexually and asexually reproducing organisms. Shaw (1969) suggested that a degree of overlap of 10 per cent or less approximated the morphological differences used by most workers for species of haemoflagellates. If the technique is at fault such distinctions may be accentuated but such factors as the quality of stain (Walker, 1963) or fixative (Lainson and Shaw, 1971) still only give rise to overlaps of 30 to 40 per cent in identical material.

Amongst the haemoflagellates morphology has for long been the standard character for the separation of species and at present much of the research on haemoflagellates is devoted to the determination of biochemical characters that may be of taxonomic use. The present studies, however, show that there are morphological differences between parasites classified as belonging to the mexicana and braziliensis complexes (Lainson and Shaw, 1972). Such differences are within the range of specific differentiation of the haemoflagellates and we therefore, see no reason why such old 'specific' names as L. mexicana and L. braziliensis should be soft pedalled as was implied by Lumsden (1974). Similarly the raising of major to specific status by Bray et al. (1973) seems to be quite reasonable especially in the light of Kellina's biometrical studies on L. tropica. It might be mentioned that it is rewarding to see that some biochemical characters, such as nuclear and kinetoplast DNA buoyant densities (Chance et al., 1973), conform remarkably well with identifications based on biological and epidemiological data.

It has been noted that larger strains of trypanosomes such as T. vivax (Fairbairn, 1953), T. evansi (Hoare, 1956) and T. congolense (Godfrey, 1961) all appear to be more pathogenic for domestic animals than are the smaller ones. In this respect it is interesting to note that the larger parasite, L. mexicana, is more virulent in hamsters than the smaller L. braziliensis, and that the larger Old World parasite L. major, is more pathogenic for mice that the smaller L. tropica (Moskovski and Southgate, 1971).

Summary
The present work shows that the amastigotes of L. m. amazonensis are morphologically distinguishable from those of L. b. braziliensis. The authors consider that such differences add further support to the use of the name braziliensis and mexicana at the specific level.

Acknowledgement
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We are most grateful to Roberto Naiff, Sebastião Oliveira, and Henrique Buna for their able technical assistance.

References


**CORRIGENDUM.**

Fig.(i). Amastigotes of *L. m. amazonensis* showing should read
Fig.(i). Amastigotes of *L. m. amazonensis* & *L. b. braziliensis* showing

The following two references included in the text did not appear in the References.
