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STUDIES ON *TRYPANOSOMA RANGELI* TEJERA, 1920. V – DEVELOPMENTAL PATTERN IN THE ALIMENTARY CANAL OF *RHODNIUS PROLIXUS*

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The morphological sequence of Trypanosoma rangeli *development in the alimentary canal of* Rhodnius prolixus, *is described, with observations made in dissected guts from 6 hours to 45 days post-infection. No metacyclic-forms are produced in the digestive tract at any time, and transmission by the contaminative route must be considered atypical. Amastigotes appear to be an essential stage in the development of* T. rangeli *in the gut of* R. prolixus.

The epidemiological importance of the developmental pattern of T. rangeli *in the vector's gut is discussed, and its usefulness for aging infections is considered.*

Tobie (1961,1965) stated that the morphological transformation and multiplication of *Trypanosoma rangeli* in the alimentary canal of triatomine bugs are constant features in the life cycle of this parasite. In spite of the typically low parasitaemia in the mammalian host the parasite always succeeds in establishing an infection in the gut of the vector.

The gut infection may persist for months after the infective meal, and the flagellates can be found in the faeces of 31 to 70% of infected bugs (Pifano et al, 1948; D'Alessandro, 1961; Cuba, 1975). However, higher rates of infection are detected by dissection of the bug's mid and hindgut, which provides more reliable information than that obtained by examination of faeces alone (D'Alessandro & Mandel, 1969).

There is conflicting information about the role played by the intestinal phase in the transmission of *T. rangeli.* Some authors have failed in their attempts to infect rodents by inoculation of faeces from infected bugs (Renjifo Groot & Uribe, 1950; D'Alessandro, 1961; Tobie, 1964), whereas others have reported success in infecting these and others mammals by this method (De Leon, 1950; Coutinho & Nussenzweig, 1952; Pifano, 1954; Grewal, 1956).

The possible failures to produce infections in experimental animals could be the unnatural route of inoculation (e.g. I.P.) or the lack of infective metatrypanosomes in the inoculum (Hoare, 1972).

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D'Alessandro (1976) pointed out that although long and short trypomastigotes from the intestine of reduviids have been indiscriminately called metatrypomastigotes, their infectivity has not been demonstrated. Indeed, Tobie (1964) compared short trypomastigotes from the faeces, and metatrypomastigotes from the salivary glands of infected *Rhodnius prolixus*, and found morphological differences. She suggested that the trypanosomes in the faecal material were not metacyclic in form and not infective. She also concluded that although the first stage of development of *T. rangeli* takes place regularly in the alimentary canal of *R. prolixus*, the transmission by the contaminative route does not occur.

The present paper describes the morphological sequence of development and distribution of *T. rangeli* in the gut of *R. prolixus*. Observations with both the light and electron microscope, of the different gut regions from 6 hours to 45 days after infection are described.

MATERIALS AND METHODS

Details of the experimental animals including triatomine bugs, *T. rangeli* isolates and infected mice, have been given in a previous paper (Añez, 1981a).

Infection of R. prolixus *with blood forms of* T. rangeli

A total of 32 fifth instar nymphs of *R. prolixus* were infected with blood forms of *T. rangeli* from infected mice. One group, of 16 bugs was fed on a mouse infected with Betijoque/78 isolate, which showed a parasitaemia of 570 Tryps/mm^3. Each bug ingested an average of 0.055 ml of blood (about 31,350 flagellates). The other group, also of 16 bugs, fed on a mouse infected with Dog/78 isolate with a parasitaemia of 450 Tryps/mm^3. The bugs ingested an average of 0.059 ml of blood (26,550 flagellates). After infection the bugs were returned to a constant temperature room at 25°C and 75% relative humidity.

Dissection of infected bugs

A pair of infected bugs were chosen at random and killed with ether at 6, 24, 48 and 72 hours and at 7, 15, 30 and 45 days after infection. The alimentary canal was removed from each bug after cutting the lateral edges of the abdomen and lifting the dorsal cuticle. The oesophagus, anterior midgut (stomach),* posterior midgut and hindgut were separated and placed on a slide. They were then teased apart, smeared, allowed to dry, fixed with methanol for 2 minutes and stained with 10% Giemsa's stain for 1 hour.

Biometric record

Observations were made using a Wild microscope at 1000X. Parasites were drawn with a camera lucida at 1250X. The measurements were made with a pair of dividers calibrated to give a separation equivalent to 1 μm. The size categories for epimastigote were established as follows: short epimastigotes from 9 to 22 μm, intermediate epimastigotes from 23 to 34 μm and long epimastigotes from 35 to 81 μm.

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* Anterior midgut corresponds to the dilated section of the midgut, the pro-mesenteron, while posterior midgut is the narrow cylindrical section, the post-mesenteron.

Ultrastructural study

Two pairs of fifth instar nymphs of *R. prolixus* were infected with *T. rangeli* from a mouse infected with Dog/78 isolate. When a well established infection was detected, the bugs were killed and the anterior midgut and hindgut dissected out. These organs were fixed in 3% Glutaraldehyde in 0.1 M Cacodylate buffer for 1 hour at 4°C, washed 3 times in the buffer solution and post-fixed in 1% Osmium tetroxide in the same buffer for 1 hour at 4°C. The guts were then dehydrated in an acetone series (30, 50, 70, 80, 90% and 3 washes in 100% acetone, 15 minutes each) and embedded in Epon 812 resin. Sections were cut on a L.K.B. ultramicrotome, stained in lead citrate and uranyl acetate and examined on a Phillips EM 300 electron microscope at 60 K.V.

RESULTS

Morphology

All the stages of development including blood trypomastigotes, amastigotes, short, intermediate and long epimastigotes, dividing forms, sphaeromastigotes and trypomastigotes, were detected during the course of the infection (Fig. 1). No metacyclic forms were seen.

Development of T. rangeli *in the gut of* R. prolixus

All the intestinal development of the parasite occurred in the gut lumen. Early development was confined to the anterior and posterior midgut. After blood trypomastigotes (Fig. 1A) were ingested by the bug, they rapidly began to round up. They became shorter, the kinetoplast and nucleus became closer to each other, but the free flagellum remained long. These short-stumpy flagellates (Fig. 1B) seen 6-24 hours after infection, then lost the free flagellum and formed the amastigotes (Fig. 1C) seen from 24 h onwards. Later a free flagellum was again produced, the resultant sphaeromastigotes (Fig. 1D) multiplying by binary fission (Fig. 1E) to form short epimastigote (Fig. 1F) which moved into the hindgut. From 72 hours onwards, division in the anterior midgut continously produced short epimastigotes. These always moved to the posterior midgut and hindgut where they increased in size, producing intermediate (Fig. 1H) and long epimastigotes (Fig. 1I). The short epimastigotes themselves began to divide (Fig. 1G) and division forms and sphaeromastigotes were found in the posterior midgut and hindgut. The long epimastigotes eventually transformed into trypomastigotes (Fig. 1J) which were found throughout the mid and hindgut from the 7th day onwards. The morphological sequence of the parasite in the gut of *R. prolixus* is summarised in Fig. 1.

Distribution of T. rangeli *in the gut of* R. prolixus

Samples taken at frequent intervals (6h to 45 days) from anterior midgut, posterior midgut and hindgut of bugs infected with blood forms of *T. rangeli*, revealed parasite transformation in all of these sites. Ingested blood trypomastigotes soon began to change shape and could not be found later than 24 hours post-infection. Table I shows a simplified distribution of the various developmental stages in the 3 gut regions and shows the changes that occur in the morphological composition with time. No parasites were observed in the oesophagus at any time.

Ultrastructure

Ultra thin section of the gut of *R. prolixus* infected with *T. rangeli*, revealed the presence of a group of parasites surrounded by a membrane in the gut lumen. Because of

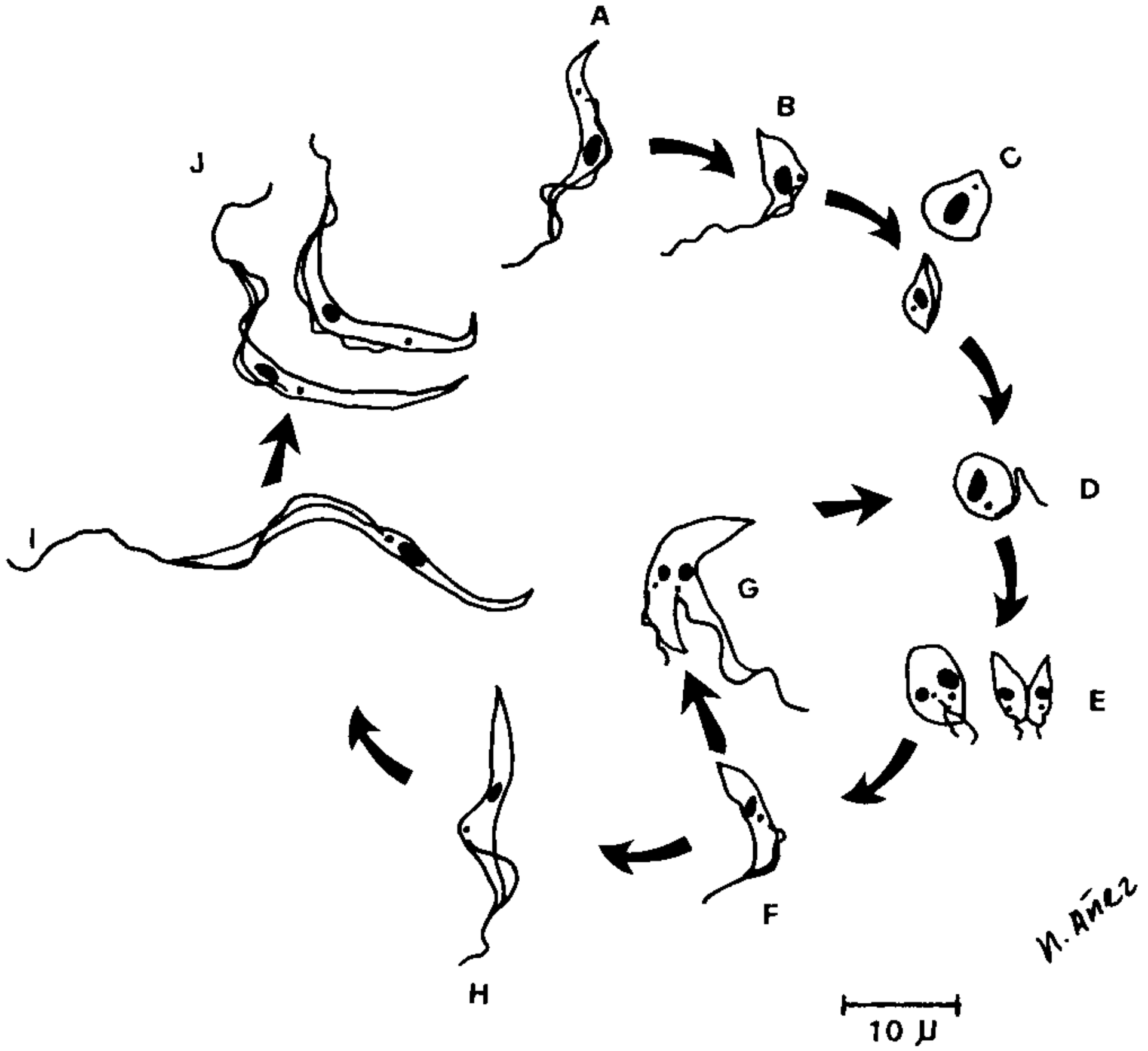

Fig. 1 – Morphological sequence of the development of *T. rangeli* in the gut of *R. prolixus*.

the absence of a real cystic wall, it is convenient to call these structures "Cyst-like bodies" (Fig. 2). Moreover the free parasites and the "Cyst-like bodies" observed in the lumen, parasites were frequently seen between the cuticular intima and the epithelial layer of the gut of infected bugs (Fig. 3).

DISCUSSION

When the blood of an infected mammal is ingested the blood-trypomastigotes of *T. rangeli* undergo certain changes in the lumen of the intestine of triatomine bugs. The forms normally found in the gut are a few small flagellate or a flagellate, round or oval forms, many epimastigotes (short and long) and trypomastigotes (Hoare, 1972; D'Alessandro, 1976). According to Hoare (1972) the amastigote are not considered an essential stage in the life cycle of *T. rangeli*.

In the present study, the sequence of structural changes displayed by *T. rangeli* in the alimentary canal of *R. prolixus* has been systematically observed in specimens kept at 25°C.

During the first 24 hours after the ingestion, the typical blood trypomastigotes (26.6 µm lenght) were transformed into shorter flagellates. These forms had a stumpy

TABLE I

Developmental pattern of *T. rangeli* in the alimentary canal of *R. prolixus*.

days after infection	1	2	3	4	5	6	7	8	gut regions
1/4	+++	-	-	-	-	-	-	-	
1	++	+	+	-	+	-	-	-	
2	-	+++	-	+	++	-	-	-	anterior
3	-	+++	-	+	+	-	-	-	
7	-	+++	+	+	+	-	-	-	midgut
15	-	++	-	+	+	-	+	+	
30	-	+	+	-	+++	+	+	+	
45	-	+	+	+	++	+	+	+	
1/4	+++	-	-	-	++	-	-	-	
1	-	-	+	-	+++	-	-	-	
2	-	+	+	-	++	+	-	+	posterior
3	-	+	+	-	++	+	+	-	
7	-	-	+	-	++	+	+	-	midgut
15	-	+	+	+	+	+	++	-	
30	-	-	+	+	++	+	+++	+	
45	-	-	+	-	+	+	+++	+	
1/4	-	-	-	-	-	-	-	-	
1	-	-	-	-	+++	+	-	-	
2	-	+	+	+	++	+	+	-	
3	-	-	+	+	++	+	+	+	hindgut
7	-	-	+	-	++	++	+	-	
15	-	+	+	+	+	+	+++	+	
30	-	-	+	-	++	+	+++	+	
45	-	-	+	+	++	+	+++	+	

+: 1 - 30%
++: 31- 60%
+++: 61-100%

1.- Blood trypomastigote.
2.- Amastigote.
3.- Sphaeromastigote.
4.- Diving forms.
5.- Short epimastigote.
6.- Intermediate epimastigote.
7.- Long epimastigote.
8.- Trypomastigote.

body (14 μm lenght) with the same long free flagellum (10 μm) as normal blood trypomastigotes. This transformation was seen only in the anterior and posterior midguts. No blood trypomastigotes were detected in the hindgut. From 24 hours onwards, the

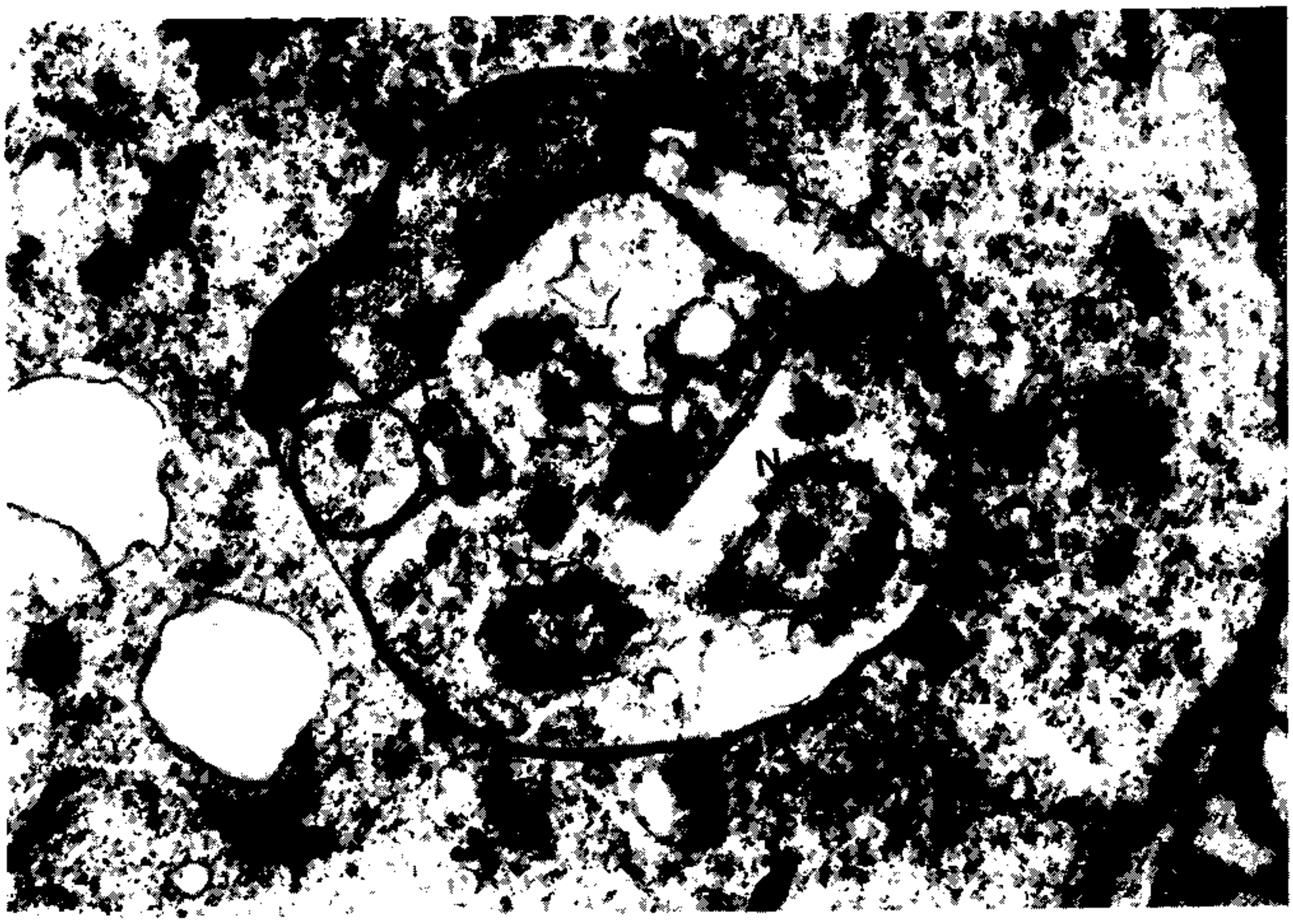

Fig. 2 – Electron micrograph showing "Cyst-like bodies" of *T. rangeli* in the gut of *R. prolixus*. Note membrane (arrowed). Nucleus (N). Flagellum (F). (Mag. x 15,900).

Fig. 3 – Transversal section showing a parasite between the cuticular intima (C.L.) and the ephitelial layer (E.L.) of the gut. Flagellum (F). (Mag. x 26.291).

stumpy forms started to disappear, to be replaced by amastigote forms (5.7 x 4.7 μm in diameter) most of which had an axoneme visible with the light microscope.

The amastigotes were detected at 24 hours in the anterior midgut and at 48 hours in the posterior midgut and hindgut. The major proportion of amastigotes was noticeably greater in the anterior midgut (80%) than in the rest of the gut (20%). Apparently the presence of blood in the anterior midgut provides the nutritional requirements which allow constant and repeated multiplication of the parasites as amastigotes, before their transformation into the next stage of development. Contrary to Hoare's (1972) opinion, amastigotes may therefore be an essential stage in the development of *T. rangeli* in the gut of *R. prolixus*. The next developmental step is the transformation of amastigote into sphaeromastigotes. These forms were short-lived and rapidly divided by binary fission before transforming into epimastigotes. This stage soon became the most common and stable morphological form during the development of *T. rangeli* in the gut, comparable in proportion only with that of the amastigotes in the anterior midgut. The proportion of the population formed by the 3 epimastigote categories varied with time. Short epimastigotes were detected earlier than intermediate and long epimastigotes, which were seen from the 7th day onwards. It can be assumed from their time of appearance that short epimastigotes grow into intermediate and then long epimastigotes. The same sequence was observed in faeces of infected bugs (Añez, 1981b). Trypomastigotes started to appear in the 3 gut regions from 7 days onwards, although they were always in lower proportion than the epimastigotes. No metacyclic-forms were observed at any time in any of the dissected parts of the gut. Although the size range of trypomastigotes varied from 22 to 55 μm, the smaller ones cannot be considered as metacyclic because of their punctforme kinetoplasts and very high kinetoplastic indices (K.I: 3.4). These observations support Tobie's (1964) opinion that the trypanosomes in faecal material are not metacyclic and are therefore unable to infect by the contaminative route. The same observation gave Añez (1982) elements to re-consider the systematic position of *T. rangeli* and propose a new subgenus to locate this and similar parasites.

Although the bugs examined had heavy gut infections, no intracellular stages were observed during the ultrastructural study of *T. rangeli* in the gut of *R. prolixus*. It seems likely that the development of this parasite in the gut of its vector is entirely extracellular. The electron microscope revealed the presence of groups of parasites surrounded by a membrane, which were not detected with the light microscope. The term "Cyst-like bodies" was adapted for these structures because: i) although they were observed within a membrane, this cannot be considered as a real cystic wall, and it is more convenient at the moment to follow the nomenclature used by Deane & Milder (1972). They described similar structures for *T. conorhini* maintained in culture medium. ii) the nature of such a membrane, which has not been reported before for *T. rangeli* is unknown. Although the disposition of the parasites within the membrane suggests a process of reproduction, it is perhaps wise not to speculate until further studies are made.

As regards the parasites observed in the gap between the cuticular intima and the epithelial layer of the gut of infected bugs, it can be assumed from this observation that this is the normal way used by *T. rangeli* to invade the haemocoele of triatomine bugs in its migration to the salivary glands. The fact that no desmosomes junctions were observed between the parasites and the gut layers could suggest an active penetration of the parasite to the haemolymph.

Since neither infective metacyclic forms of *T. rangeli* nor intracellular stages were seen in the gut of *R. prolixus*, the alimentary canal of triatomine bugs can be considered as an important reservoir of parasites, giving the parasites a long period in which they can invade the haemocoele. Once in the haemolymph the parasite can reach the salivary glands and produce the forms infective to the vertebrate host. The intestinal infection can then be lost without the vector losing its ability to transmit the parasite.

Information gathered in the present study from dissected bugs was used to make a table indicating the developmental pattern of *T. rangeli*. This combines the morphological diversity of the parasite in the 3 gut regions with the estimated proportion of every form at different times post-infection. The table is intended to be a simplified guide for aging infections in the gut of *R. prolixus* by *T. rangeli*, in places where the average temperature is about 25°C. This and similar models could be used in epidemiological surveys to detect recent transmission i.e. young infections, and gives clues to possible vertebrate reservoirs (e.g. the younger the infection the nearer the vertebrate). This model needs to be tested in the field to check its sensitivity and usefulness for workers who are always in need of simple and accurate methods for the collection of data.

RESUMO

A seqüência do desenvolvimento morfológico do *Trypanosoma rangeli* no canal alimentar do *Rhodnius prolixus*, é descrita, segundo observações, em intestinos dissecados desde 6 horas até 45 dias pós-infecção. Não se produzem formas metacíclicas no trato digestivo em tempo algum, e a transmissão por via contaminativa deve-se considerar atípica. Os amastigotos aparentam ser um estágio essencial no desenvolvimento do *T. rangeli* no intestino do *R. prolixus*.

A importância epidemiológica do padrão de desenvolvimento do *T. rangeli* é discutida e a sua utilidade na determinação da idade da infecção é considerada.

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