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STUDIES ON *TRYPANOSOMA RANGELI* TEJERA, 1920. VI. DEVELOPMENTAL PATTERN IN THE HAEMOLYMPH OF *RHODNIUS PROLIXUS*

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The morphological sequence of Trypanosoma rangeli *development in the body cavity of* Rhodnius prolixus *is described. The metacyclic trypanosome is the product of successive division and transformation during the intra and extracellular development in the haemocoele.*

The significance of the early invasion of T. rangeli *into the haemolymph is discussed. The epidemiological importance of the developmental pattern of* T. rangeli *in the vector's haemolymph and the host-response to the parasite are considered.*

One of the most remarkable and unique features of *Trypanosoma rangeli* is its ability to invade the haemolymph of its Triatomine vectors on the way to the salivary glands where it completes the life cycle producing large numbers of forms infective to the vertebrate host.

Haemolymph infection occurs when parasites ingested during the infective meal penetrate the gut wall and reach the body cavity of the triatomine bugs. The time by which *T. rangeli* invades the haemolymph is unpredictable. Most authors agree that such invasion takes place several weeks after the infective meal, when the intestinal infection is usually well-advanced (Groot, 1954; Grewal, 1956; D'Alessandro, 1961). However, Añez (1980) observed the parasite in the haemolymph of *R. prolixus* as early as 24 hours after the infective meal.

In contrast to the constant occurrence of intestinal infection, *T. rangeli* does not invariably invade the haemocoele, and discrepancies are observed in the results reported by different authors using different isolates of the parasite or species of bugs (Tobie, 1961; Zeledon & Blanco, 1965; Sousa, 1972).

The present paper describes studies carried out on the haemolymph of *Rhodnius prolixus*, experimentally infected with two isolates of *T. rangeli.* An account is given of the morphology, development and behaviour of the parasite, following frequent observations from 1 to 55 days of the haemolymph of the infected bugs.

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MATERIALS AND METHODS

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

Infection of Triatomine bugs

A total of 50 *Rhodnius prolixus* were infected with blood forms of *T. rangeli* from infected mice. 25 fifth instar nymphs were fed on a mouse infected with Betijoque/78 isolate with a parasitaemia of 800 Tryps/mm^3 and the other 25 bugs were infected with Dog/78 isolate from a mouse which showed a parasitaemia of 600 Tryps/mm^3.

Fresh observation of the haemolymph

Sample of the haemolymph of each bug was examined daily from 1 to 7 days and then at 12, 15, 21, 25, 30, 35 and 55 days after infection. The bug to be examined was held abdomen upwards between the thumb and the index finger. The distal end of the middle or posterior leg was cut off with a small pair of scissors and a drop of haemolymph was collected on a clean glass slide and observed under a microscope at 200X. If parasites were detected, the drop of haemolymph was immediately smeared, allowed to dry, fixed with methanol for 2 minutes and stained with 10% Giemsa's Stain for 45-60 minutes.

The morphometric study was carried out as indicated in a previous paper of this series (Añez, 1983).

Quantification of infected haemocytes

At least 100 haemocytes were counted from samples taken at 7, 15, 21, 30, 35 and 55 days post-infection. The number of infected and uninfected haemocytes was recorded and the percentage of intracellular infections was estimated.

RESULTS

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

Although the extra and intracellular phases of the parasite in the haemolymph appear to occur simultaneously, they will be described separately here.

Extracellular Development

Once the haemolymph of infected bugs was invaded by flagellates from the gut (trypomastigotes or epimastigotes) (Fig. 1.1), they immediately started to divide and, during the first 3 days, most of the flagellates observed were dividing forms. On the first day, parasites dividing by binary fission were seen (Fig. 1.2). At the 2nd and 3rd days the number of dividing forms, in both binary and multiple division (Fig. 1.3), increased reaching a high proportion (93-98% of the flagellates) being together with a few short epimastigotes (Fig. 1.4), the only forms present in the haemolymph by this time. On the 4th day the number of dividing forms had fallen, while the proportion of short epimastigotes rapidly increased forming more than half of the population of parasites. At this time, the first intermediate epimastigotes (Fig. 1.5) and trypomastigotes (Fig. 1.7) appeared. Long epimastigotes (Fig. 1.6) first appeared on day 5th when intermediate epimastigotes and trypomastigotes predominated, and the numbers of dividing forms and short epimastigotes fell. During the 6th and 7th days after the invasion, the dividing forms disappeared from the haemolymph, the short and intermediate epimastigotes fell slightly, while the long epimastigotes and trypomastigotes increased significantly in number. By this time, a few metacyclic trypomastigotes (Fig. 1.8) were present. From the

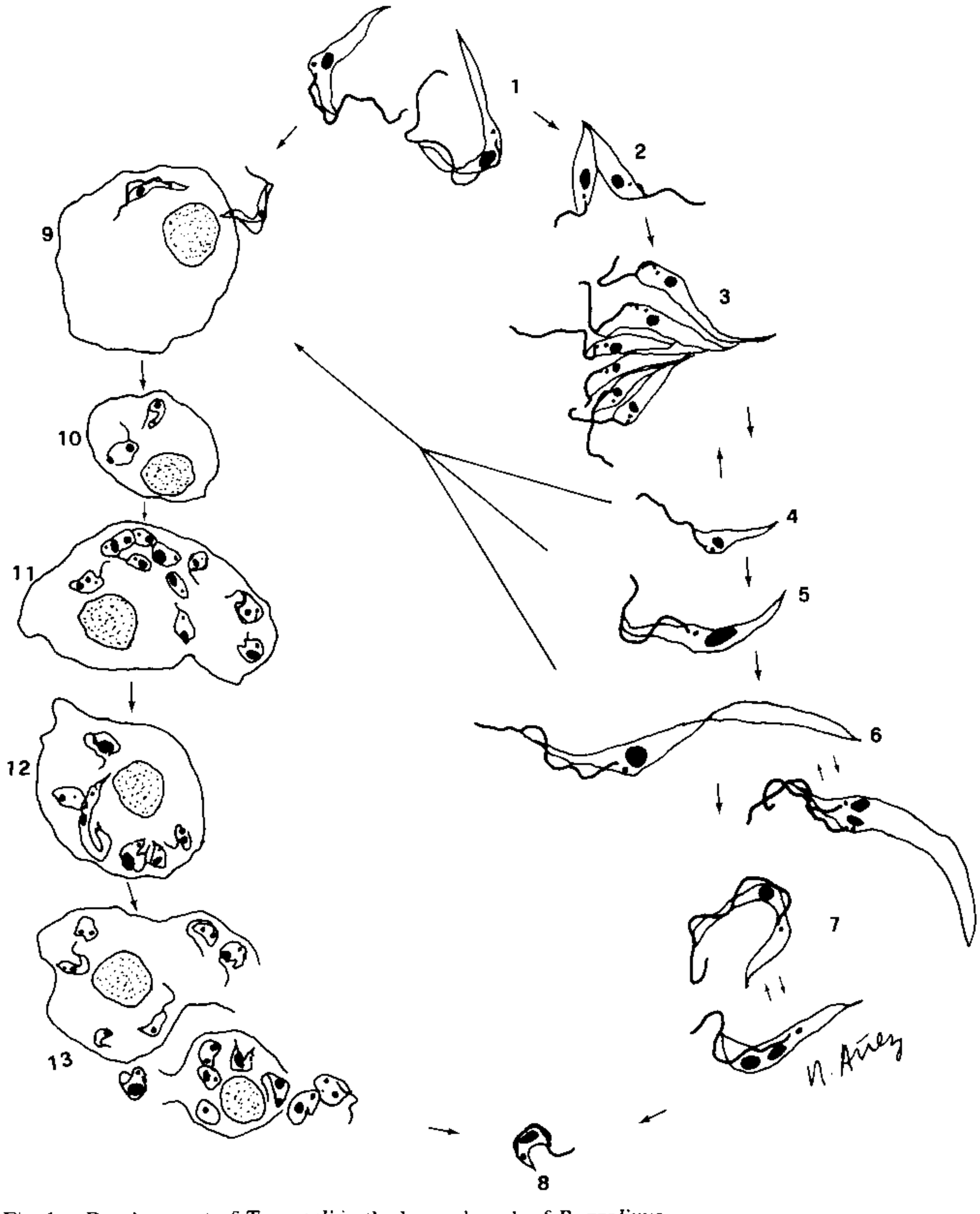

Fig. 1 – Development of *T. rangeli* in the haemolymph of *R. prolixus*.

12th day onwards all forms of the parasite described above were present in different proportions, with long epimastigotes as the predominant forms until the 30th day. From 35 to 52 days post-infection, most of the parasite in the haemolymph were trypomastigotes and at day 55 typical metacyclic trypanosomes predominated in the population.

Intracellular Development

Epimastigotes or trypomastigotes were seen within haemocytes from the 6th day onwards (Fig. 1.9). Once inside the haemocytes, the flagellates changed in shape, transforming into sphaeromastigotes or rounded forms (Fig. 1.10), followed by a progres-

sive division (Fig. 1.11). After division in the haemocyte, small parasites were seen inside which had a coiled body and an easily distinguishable nucleus and kinetoplast; the flagellum of these parasites was short (Fig. 1.12). The large number of parasites appeared to cause the rupture of the haemocyte and the subsequent release of parasites, most of which were metacyclic or rounded forms (Fig. 1.13).

Morphological distribution of *T. rangeli* in the haemolymph of *R. prolixus*

Table I shows a simplified model of the distribution of the developmental stages of *T. rangeli* in the haemolymph at different periods post-infection.

Proportion of haemocytes infected by *T. rangeli*

The proportion of haemocytes invaded by *T. rangeli* during the course of haemolymph infection in *R. prolixus* were estimated as 51, 52, 47, 47, 62 and 89% at 7, 15, 21, 30, 35 and 55 days post-infection, respectively.

DISCUSSION

This study revealed that the haemocoele was more frequently invaded by epimastigotes or trypomastigotes, penetrating the gut wall of infected bugs. Once in the haemolymph, the parasites underwent morphological transformations followed by continuous division, demonstrated by the high number of dividing forms observed during the first 3 days. Flagellates in binary or multiple division were frequently present by this time in infected bugs.

From 4 to 7 days after invasion of the haemolymph, most of the parasites were short (20.8 μm length) and intermediate epimastigotes (28.3 μm length) suggesting that the former were the product of the first division of the parasite and the latter a consequence of growth. During this time, parasites were found inside haemocytes. They were epimastigotes or trypomastigotes which, once inside the cell, coiled and transformed into amastigotes or sphaeromastigotes to initiate the intracellular division of *T. rangeli* in the haemolymph. From 12 to 30 days, long epimastigotes (58.2 μm length) predominated in the population of parasites in the haemolymph. Presumably they were the product of the growth of short and intermediate epimastigotes formed from the early divisions between 1 and 7 days after invasion by parasites from the gut. This agrees with the morphological sequence of development of *T. rangeli* in the alimentary canal of *R. prolixus* (Añez, 1983). Long trypomastigotes were the same size as long epimastigotes and had presumably arisen from the latter form simply by the migration of the kinetoplast from the anterior to the posterior position. Later, they became shorter (40 μm length) and the kinetoplast moved away from the nucleus, showing the typical trypomastigote shape. This form predominated from 35 to 52 days post-infection.

By the 55th day post-infection, most of the parasites seen were metacyclic trypanosomes (12.1 μm length) which were the final product of the successive transformation of the trypomastigote extracellularly and the result of the rupture of the infected haemocytes. It can be assumed from these results that the main dividing forms in the extracellular cycle is the epimastigote, which actively divides and then transforms into the trypomastigote which later becomes the infective metacyclic-form. In the intracellular phase, however, the principal dividing form is the amastigote which repeatedly divides within the haemocytes, leading to the rupture of the host cell and the release of metacyclic-forms. As can be seen, both the intra and extracellular phases of *T. rangeli* in the haemolymph of *R. prolixus* have the same product, the metacyclic trypanosomes, produced in different ways. These results differ slightly from those of Tobie (1970) using *R. prolixus* and a Venezuelan strain and Cuba (1975) working with *R. ecuadoriensis* infected with a Peruvian strain of *T. rangeli.* Tobie considered that the main dividing

TABLE I

Developmental pattern of *T. rangeli* in the haemolymph of *R. prolixus*.

days after infection	1 	2 	3 	4 	5 	6 
1	+++	-	-	-	-	-
2	+++	+	-	-	-	-
3	+++	+	-	-	-	-
4	+	++	++	-	+	-
5	+	+	++	+	+	-
6	-	+	+	+	++	-
7	-	+	++	+	+	+
12	+	+	+	++	++	+
15	+	+	+	+++	+	+
21	+	+	+	++	+	+

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

1.- Dividing forms
2.- Short epimastigote
3.- Intermediate epimastigote
4.- Long epimastigote
5.- Trypomastigote
6.- Metacyclic forms

form is the intracellular amastigote, producing short epimastigotes which, when free in the haemolymph, form the metatrypomastigotes. However, Cuba's opinion was that the main dividing form is the epimastigote seen in large masses in evident division and that metatrypomastigotes develop intracellularly from sphaeromastigotes.

The presence of metacyclic-forms in the haemolymph shows that *T. rangeli* is able to produce the final developmental stage without involving the salivary glands of the vectors. This assumption agrees with that of Tobie (1970) who indicated that the bug can be infective animals through contamination by the haemolymph if the insect is eaten or crushed.

The intracellular forms of *T. rangeli* in the haemolymph of triatomine bugs have been interpreted either as developing stages or as being phagocytosed to be destroyed by the haemocyte (Pifano & Mayer, 1949; Zeledon, 1954; Grewal, 1956). Although the two possibilities have been demonstrated in different circumstances (Tobie, 1970; Watkins, 1971; Cuba, 1975); it can be assumed from the present study that at least for *R. prolixus* and two Venezuelan isolates of *T. rangeli* the intracellular forms are actually developing forms, as demonstrated by the high proportion of haemocytes carrying numerous healthy parasites. The fact that about 50% of the haemocytes at 7th day and 89% at the 55th were invaded by parasites, supports this assumption. This appears to indicate that apparently *R. prolixus* has no-efficient mechanism to resist the capacity of *T. rangeli* to survive in its haemolymph.

The information obtained in the study of the morphological proportions of *T. rangeli* in the haemolymph at different times post-infection is summarized in a table showing the developmental pattern of this parasites in the haemocoele of *R. prolixus* kept at 25°C. This simplified guide is intended to provide field workers with an easy method for aging infections of *T. rangeli* in the haemolymph of naturally infected triatomine bugs. Together with another similar model of intestinal infections (Añez, 1983), this method may be of use in epidemiological surveys.

Haemolymph invasion in triatomine bugs infected with *T. rangeli* has been reported as occurring within the first 50 days after an infective meal, with a range of 15-183 days (Groot, 1953; Cuba, 1975; D'Alessandro, 1976). In the present study, however, invasion of the haemolymph was detected in *R. prolixus* 24 hours after a meal on mice infected with Dog/78 isolated of *T. rangeli.* The same species of bug infected with other Venezuelan isolate (Betijoque/78) showed parasites in the haemolymph from the 3rd day onwards. These isolates were recently isolated and have always been maintained by bug-mouse-bug passages. Presumably these isolates have been behaving as they do in nature, in the places where *T. rangeli* is endemic and where the vector is the same species used in this work. The behaviour of these freshly isolated parasites in its natural host also suggests that a primary characteristic of *T. rangeli* is its multiplication in the haemolymph rather than in the gut. This adaption to develop in the anterior station of its vector, gave Añez (1982) more arguments to re-consider the systematic position of *T. rangeli* and propose a new subgenus, to remove this parasites from the subgenus *Herpetosoma* where it had been maintained for a long period.

RESUMO

Descreve-se a seqüência morfológica de desenvolvimento do *Trypanosoma rangeli* na cavidade do corpo do *Rhodnius prolixus*. O tripanosoma metacíclico é o produto de sucessivas divisões e transformações durante o desenvolvimento intra e extracelular na hemocele. Discute-se o significado da invasão precoce da hemolinfa pelo *T. rangeli*. Fazem-se considerações sobre a importância epidemiológica do padrão de desenvolvimento do *T. rangeli* na hemolinfa do vetor e sobre a resposta do hospedeiro ao parasito.

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