The Development of Extra-Intestinal Cycle of *Trypanosoma cruzi* in *Triatoma infestans* and *Panstrongylus megistus* *

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(With 11 text-figures)

Chagas (1909) mentions the possibility of *Trypanosoma cruzi* having better conditions for development in the haemolymph of the insect than in the digestive tract, where the intestinal flora is abundant.

In 1930, Dias reported the presence of *T. cruzi* in the Malpighian tubes and later in the body-cavity of *Panstrongylus megistus* (1932), but in 1934 he stated that the cycle of the parasite occurs only in the digestive apparatus of the vector. Naquira (1962-1963) injected blood culture forms of *T. cruzi* into the coelomic cavity of *Triatoma infestans* and suggested the possibility of the multiplication and development of the parasite in this situation. Zeledon *et al.* (1977) found that *T. cruzi* clustered or adhered to the cells of the rectal gland. Ribeiro, Belda Neto *et al.* (1977a, b) demonstrated the cyclic transformation of the parasite in the haemolymph of the vector. Lacombe (1980) likewise described the extra-intestinal development of *T. cruzi* in *Triatoma infestans* and later in *Panstrongylus megistus*, *Triatoma vitticeps*, *Triatoma pseudomaculata* and *Rhodnius prolixus* (1980a, b). In the latter work, reproduction of *T. cruzi* was seen in some haemocytes of the haemolymph and later in the epithelial cells of the rectal ampullae (Lacombe, 1981). It was necessary to compare the two routes followed by the parasites up to the trypomastigote phase which occurs in the large rectal ampullae.

Through histological techniques, it can be observed that no valve separates the postmesenteron from the ampullae of Malpighian tubes (Fig. 1) and that the parasite can go directly from the postmesenteron to the pylorus in the intracellular cycle (see diagrammatic representation in Fig. 1). The amastigote phase occurs in haemocytes and the transformation into infective forms takes place in the ampullae of the Malpighian tubes; later, when fully adults, the parasites move to the rectal glands. The parasite pass through the piloric valve, with their flagella adhering to the edge of the rectal glands cells.

TECHNIQUES AND METHODS

The histological techniques applied in this work are based on those of Romeis, 1928; Pantin, 1948; Barth, 1958; Thompson, 1966; Gabe, 1968 and others.

The first stage consisted in feeding hungry specimes of *Triatoma infestans* and *Panstrongylus megistus* on mice heavily infected with *T. cruzi* (strain "Y"). Two hours later the insects were anaesthetised and after removal of tergites etc. they were fixed in one of the following fixatives: Carnoy, Bouin, Helly, Susa, Zenker of Fleming. Dehydration was done by the alcohol-benzol method: Immersion for four hours in each of the following (1) 75% alcohol and 25% benzol,

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(2) 50% alcohol and 50% benzol, (3) 25% alcohol and 75% benzol, (4) 7 ml alcohol and 100 ml benzol, (5) and (6) benzol only. The tissues were then embedded in histosec paraffin latex. Sections were cut at 3μ, 5μ and 7μ. The sections were stained with iron

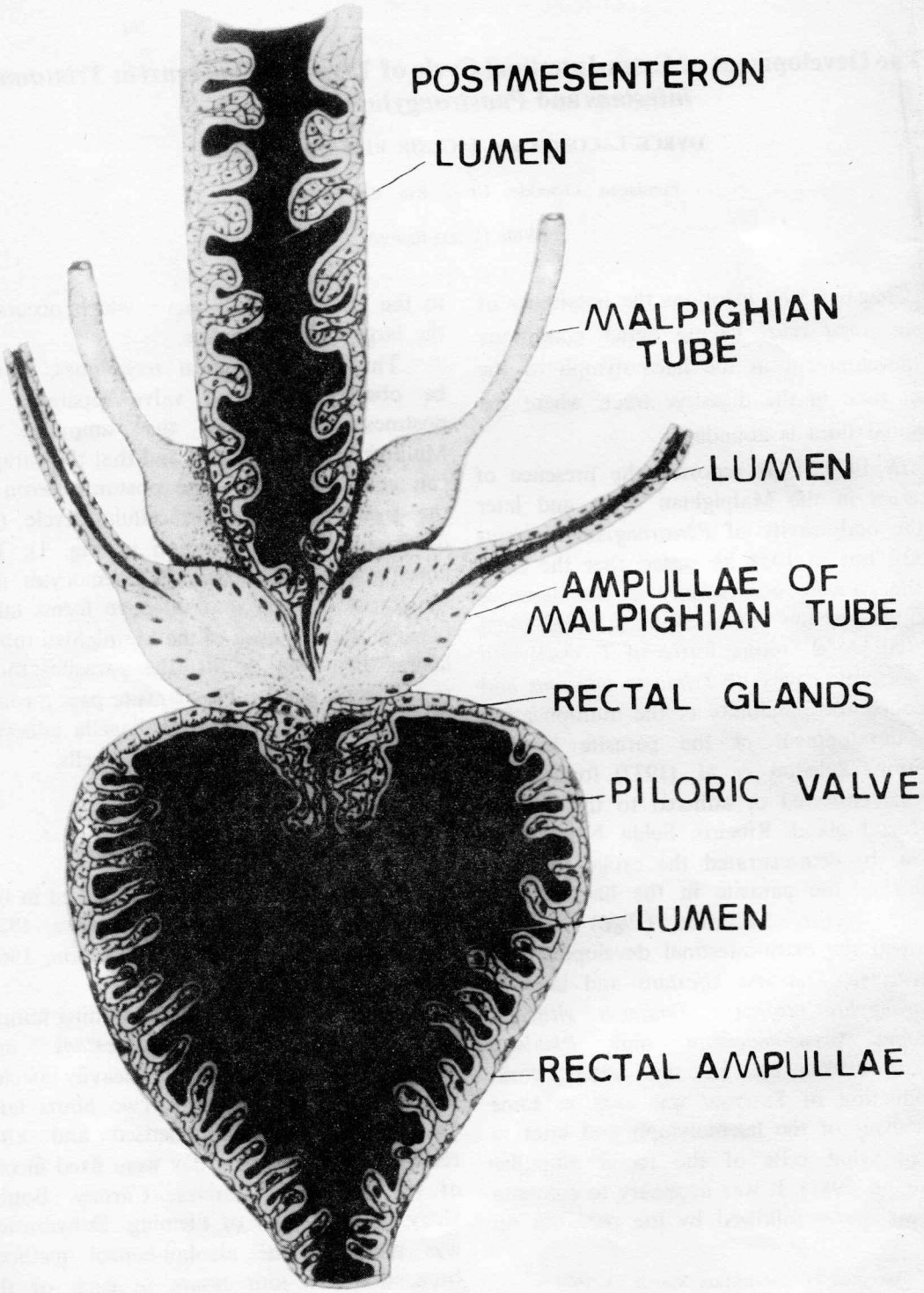


Fig. 1 – Schematic drawing of the excretory and digestive apparatus.

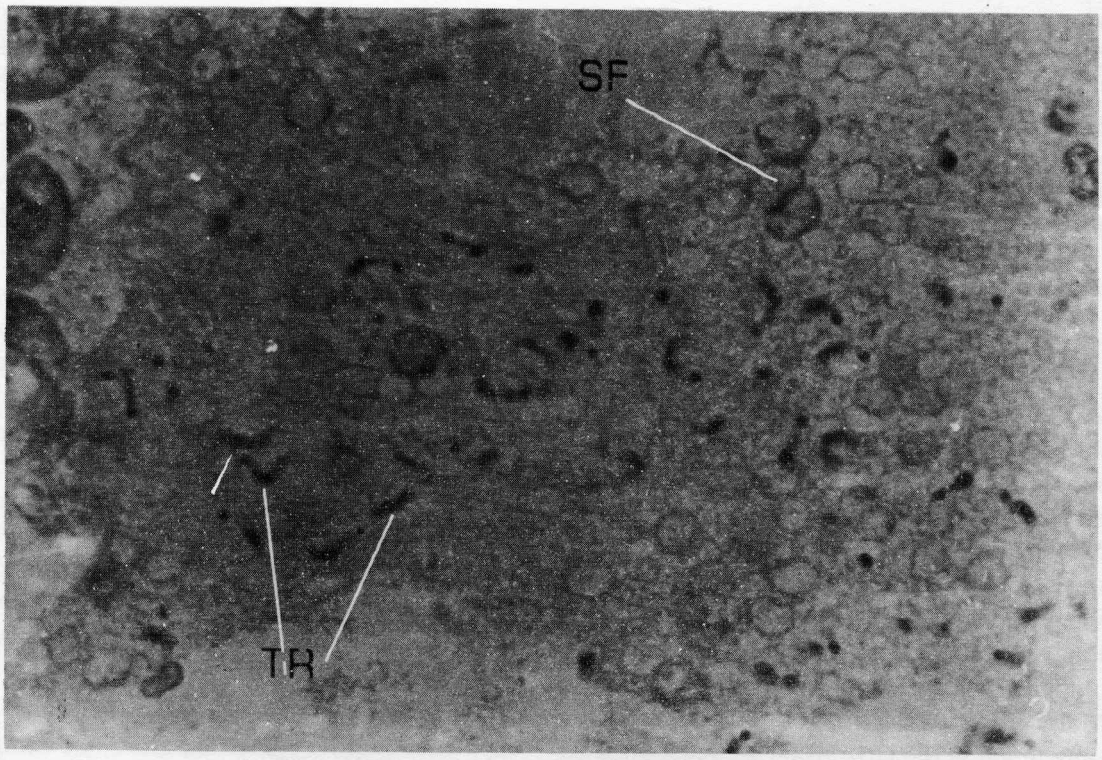
hematoxylin (Heidenhain's), Chromotrop 2R, Giemsa, Gallocyanin, Masson's Trichrome, Chrome-hematoxylin (Gomori) and Periodic Acid-Schiff (P.A.S.). This routine work was repeated at intervals of 4 and 6 hours, and 1, 2, 5, 7, 10, 15, 20 and 30 days, always after feeding the insect.

RESULTS

The extra-intestinal pathway of *T. cruzi* was observed and phases recorded by microphotographs of the sections. Figure 1 shows the hind part of the postmesenteron and its connection with four ampullae of the Malpighian tubes, which reach the rectum through the pyloric valve. At the connection there is a strong muscular sphincter around the long instestinal cells and among the four ampullae formed at the base of the Malpighian tubes. The piloric valve is therefore only found in the large rectal ampullae situated at the beginning of the glandular epithelium with the Malpighian tubes ampullae. Four series of sections were made, 4, 6 or more hours after feeding the insect on heavily infected mice (Fig. 2). Some trypomastigotes and sphaeromastigotes (SF) were found in the promesenteron. The trypomastigotes penetrate the cells that form the walls of the promesenteron and continue in the direction of the haemolymph. The penetration of trypomastigotes into cells (and their transformation into amastigotes) was observed by Pereira da Silva (1959) in tissue culture. The sphaeromastigotes proceed in the lumen of the promesenteron to the postmesenteron. Brack (1968), Brenner (1971, 1972, 1973), Ferrioli *et al.* (1978) and others mention a great number of amastigotes in the stomach of *Triatoma infestans* 72 and 96 hours after and infected meal. In the present work and earlier a great number of elements with a rounded form showing a similarity to sphaeromastigotes were seen. The parasites which reach the haemolymph enter the haemocytes of the leucocyte type and the latter are abundant near the promesenteron, postmesenteron, Malpighian tubes, gonad and dorsal vessel. These haemocytes contain numerous parasites (Fig. 3, PA). It is well known that cells of the haemolymph contain many inclusions but these can be distinguished from parasites by histochemical techniques, as described by Wigglesworth in *Rhodnius prolixus* (1934, 1959, 1973), Menezes *et al.* (1981), etc.

The haemocytes of *Rhodnius prolixus* infected with *Trypanosoma rangeli* show the parasites clearly in the form of epimastigotes leaving their interior (Cuba-Cuba, 1975). This cannot be seen in *Trypanosoma cruzi* under light microscope, but Camargo *et al.* (1980a, b) show evidence of the phenomenon by indirect specific immunofluorescence (Ferrioli *et al.*, 1978). After smearing the haemolymph of *Panstrongylus megistus* and *Triatoma infestans*, the parasites were observed inside the vacuoles from three or more days after infection.

The cell membranes of haemocytes 3 days after the insect has fed allow the escape of small elongated parasites into the haemolymph (Fig. 4, HM) where they are transformed into epimastigote and trypomastigote forms (Ribeiro *et al.* 1977a, b). The free parasites invade the Malpighian tubes. Our previous work (Lacombe & Rangel, 1979) indicated that the Malpighian tubes serve adequately for the return of the parasites into the digestive tract. The parasite inside the Malpighian tubes are excreted (with toxins etc.) into the lumen (Fig. 5). Figure 6 shows the young parasites set free in the lumen of the Malpighian tubes (Fig. 7). They move right towards the base and start to grow (Fig. 8). The parasites lie free in the cytoplasm of the ampullae cells which function in their nutrition (Lacombe, 1981). It is believed that the parasites (epimastigotes) feed on protein and eventually proceed to the base where they develop into trypomastigotes. In infected animals, the cells of the ampullae become degenerate. *Panstrongylus megistus* has a rapid metabolism compared to *Triatoma infestans* and collection of haemolymph becomes extremely difficult after feeding the former on infected mice, whereas it is relatively easy



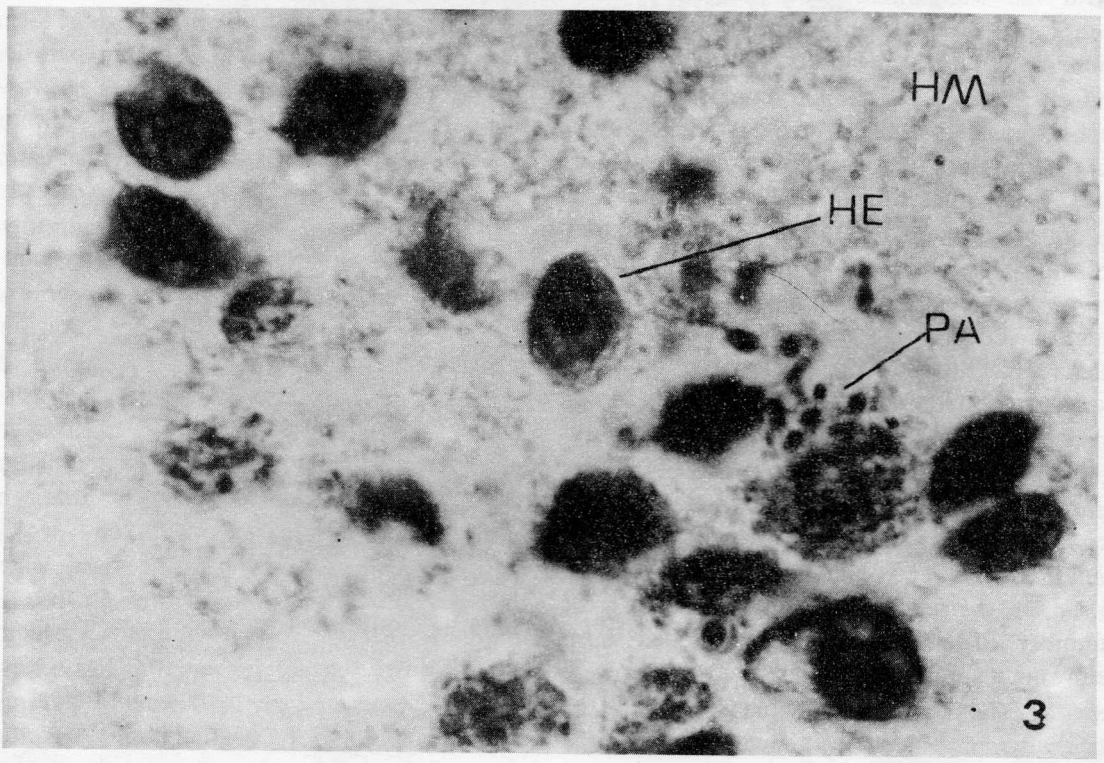
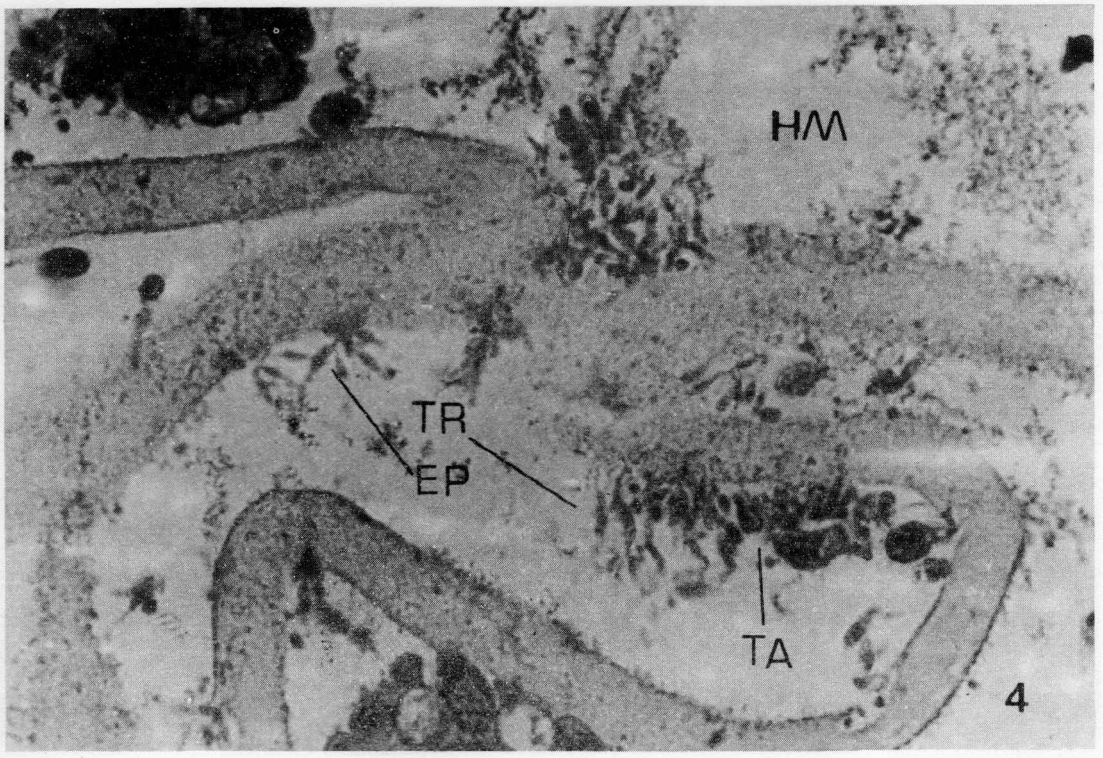


Fig. 2 – Trypomastigotes in the stomach of *Triatoma infestans* 1000 ×; SP Sphaeromastigote; TR Trypomastigote.
Fig. 3 – Haemocytes in haemolymph with parasites 1000×; HE Haemocyte; HM Haemolymph; PA Parasites.



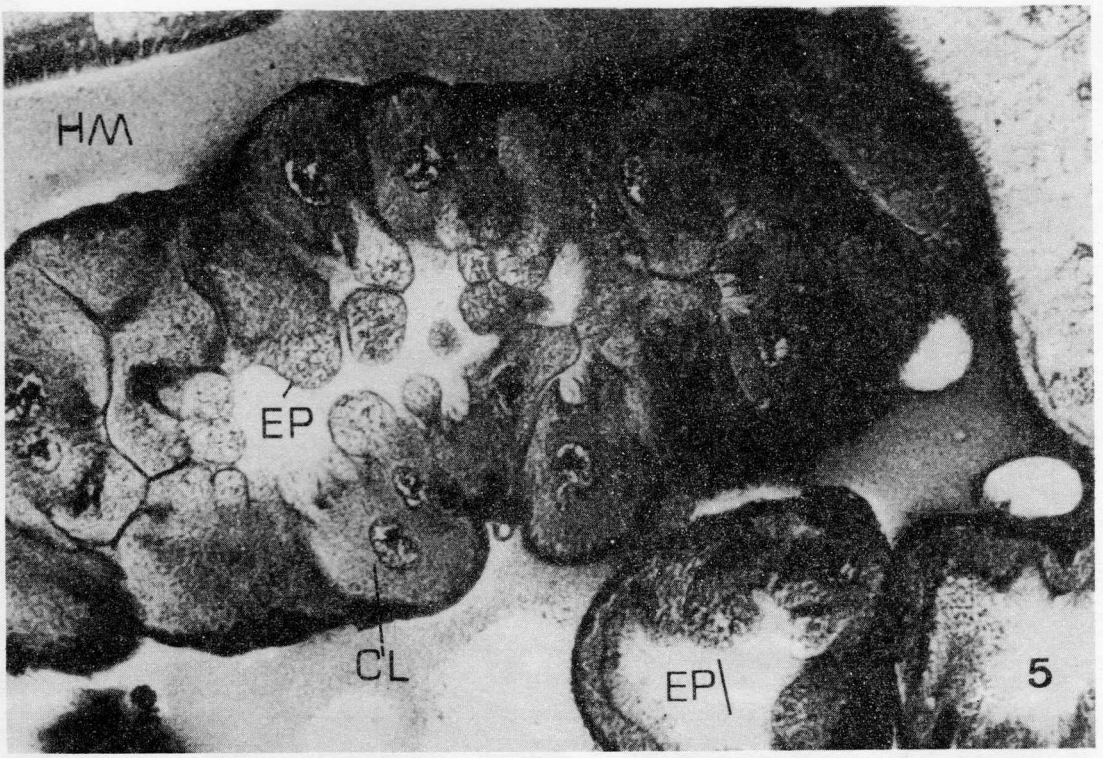
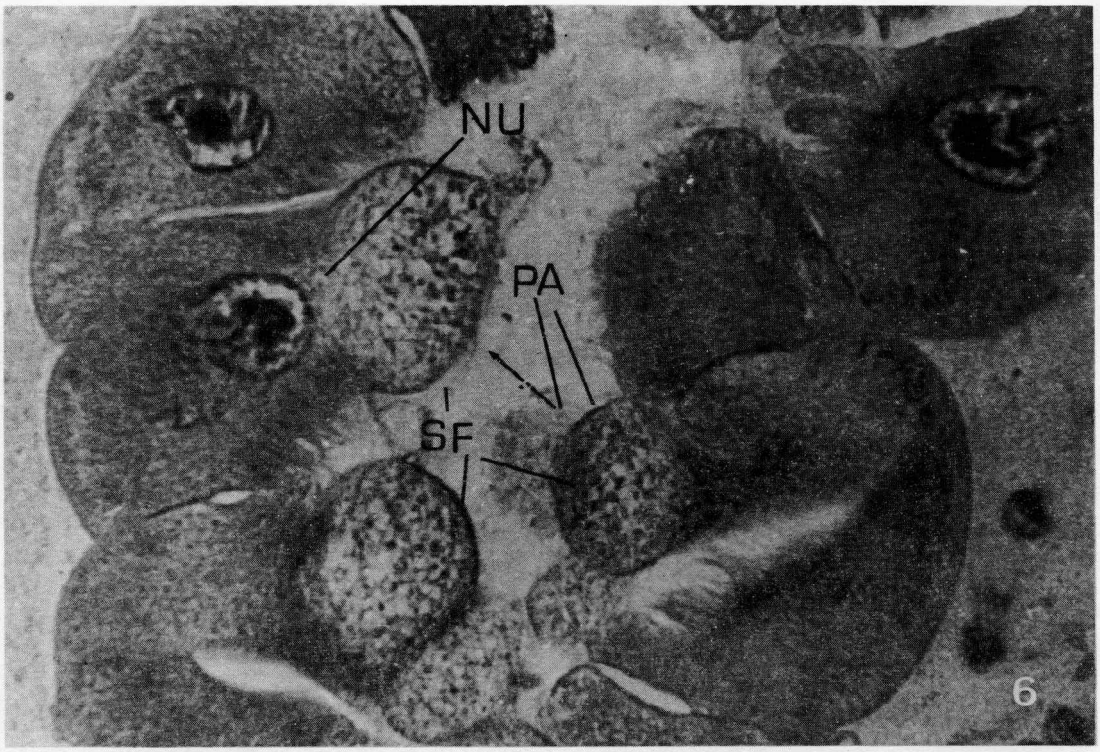


Fig. 4 – Epimastigote and trypomastigote forms in haemolymph 1000×; EP Epimastigote; HM Haemolymph; TR Trypomastigote. Fig. 5 – Longitudinal section by Malpighian tubes 250×; CL Malpighian tubes cells; EP Young epimastigotes; HM Haemolymph.



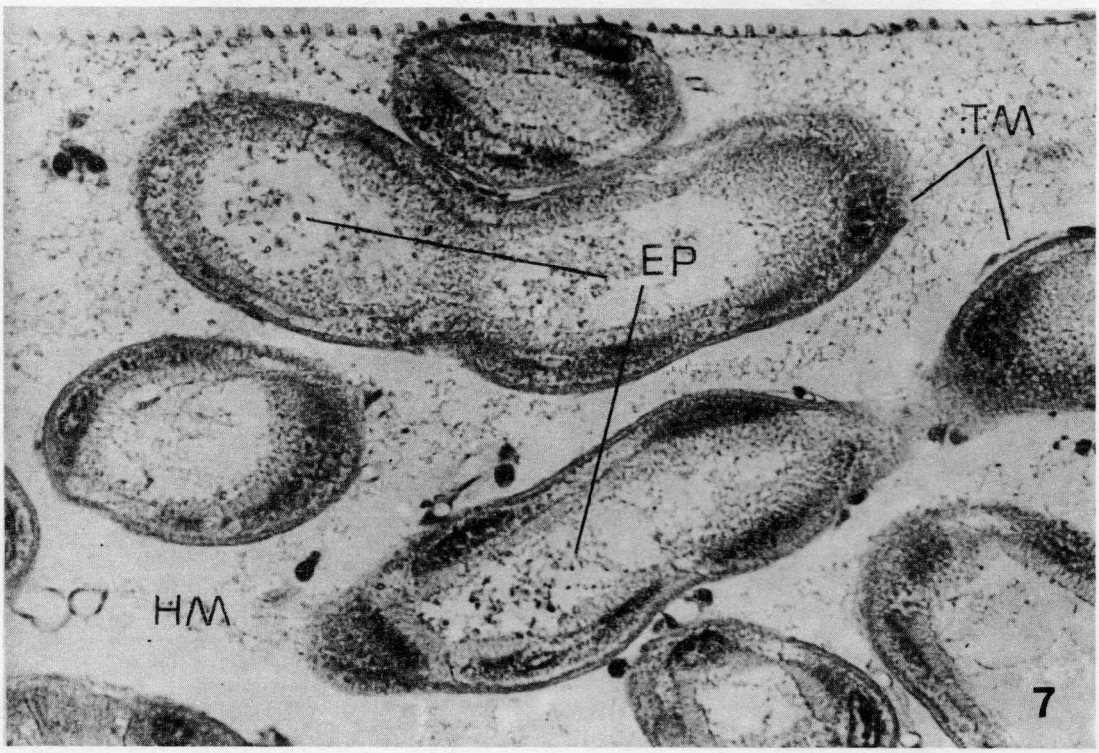
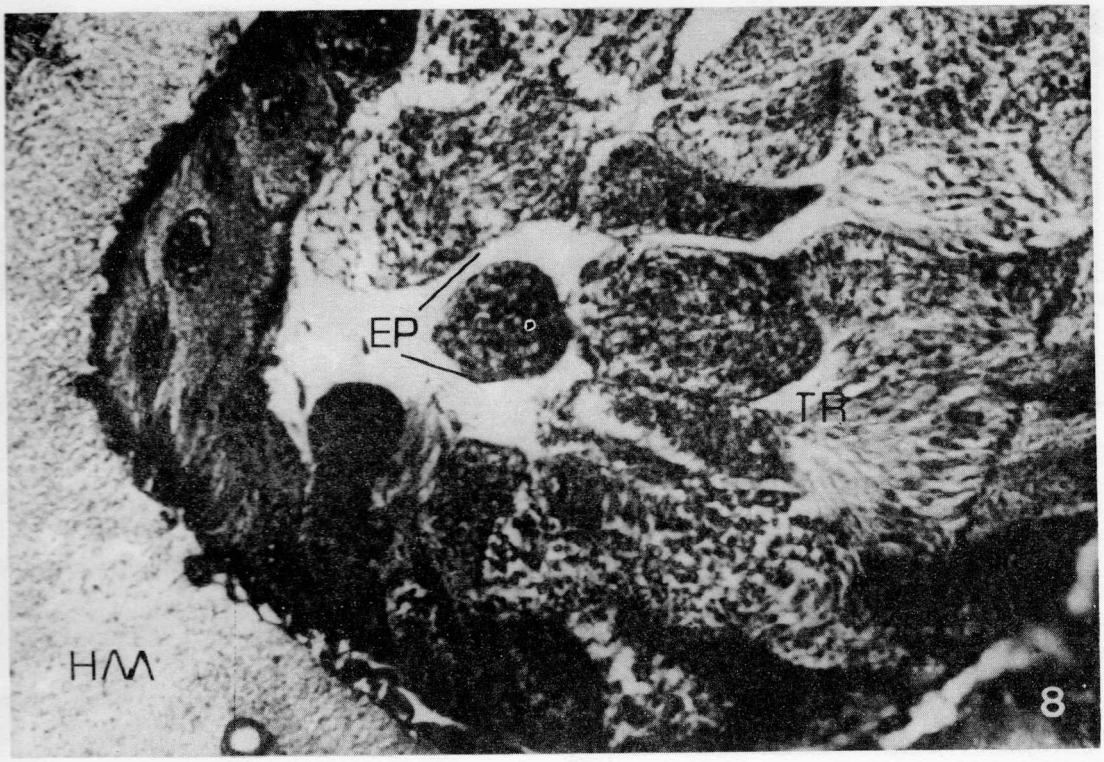


Fig. 6 – Spheres with parasites 450×; NU Nucleus of cells of Malpighian tubes; PA Parasites; SF Spheres. Fig. 7 – Section of Malpighian tubes 250×; EP Young epimastigotes; HM Haemolymph; TM Malpighian tubes.



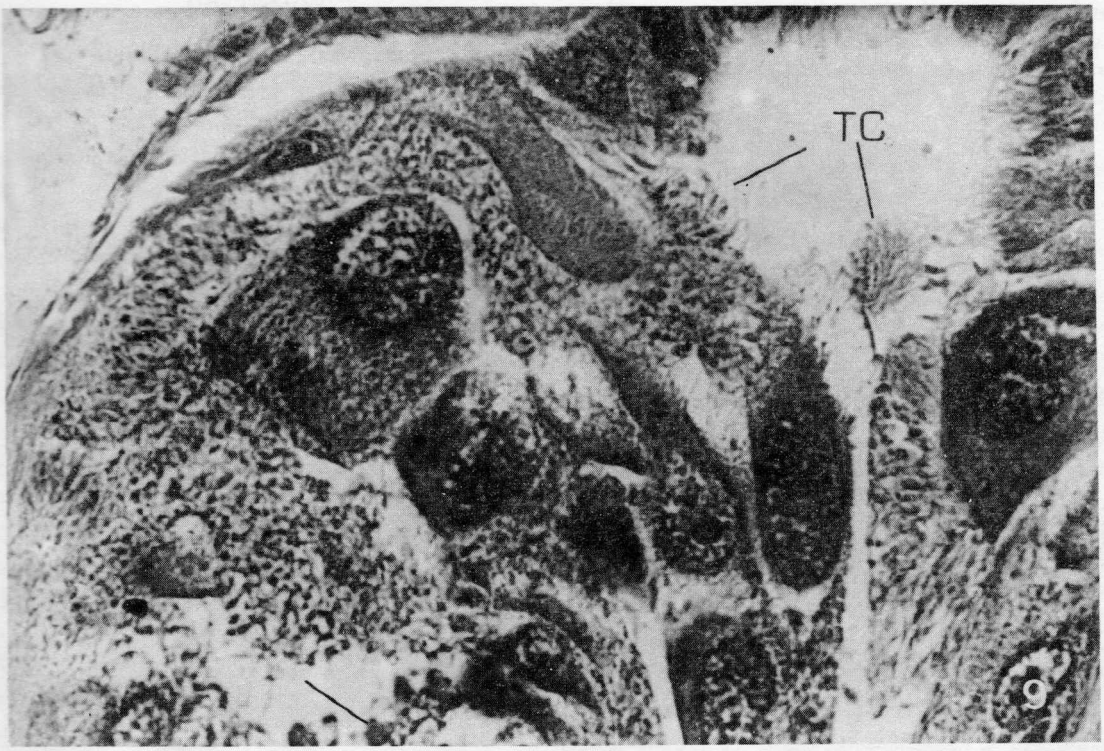


Fig. 8 – Section of ampullae of Malpighian tubes 450×; EP Epimastigotes; HM Haemolymph; TR Trypomastigotes.
Fig. 9 – Many *Trypanosoma cruzi* forms in ampullae of Malpighian tubes 450×; TC *Trypanosoma cruzi*.

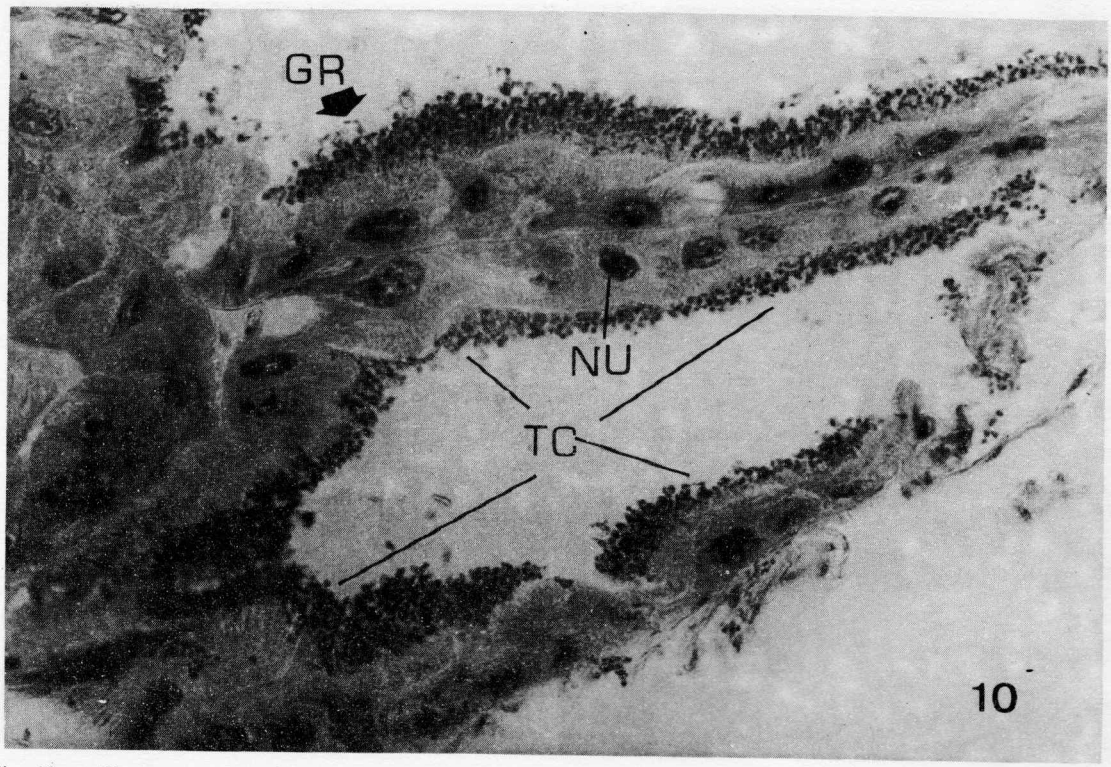


Fig. 10 – *Trypanosoma cruzi* in rectal gland 450×; NU Nucleus of rectal gland; TC *Trypanosoma cruzi;* GR Rectal gland.

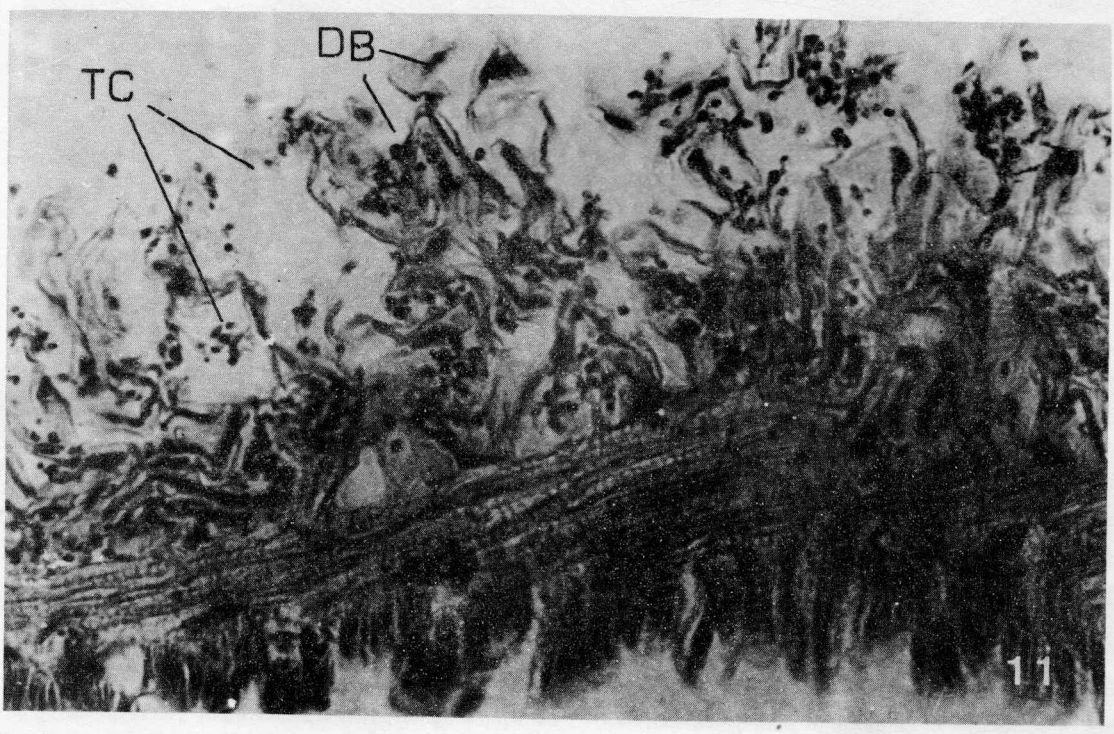


Fig. 11 – Epithelium of rectal ampullae 450×; DB Folds of rectal wall; TC *Trypanosoma cruzi* in rectum wall.

in *Triatoma infestans*. This is probably due to the recent appearance of parasites in Malpighian tubes and ampullae of *Panstrongylus megistus*. Figure 9 shows abundance of *T. cruzi* (TC) in Malpighian tube ampullae of *Panstrongylus megistus*, 7 days after feeding. From the 7th day, we also observed the route of the parasites in the direction of the rectum and the development of epimastigotes and trypomastigotes. The parasites pass through the pyloric valve and then adhere by the flagellum to the cells of the rectal gland (Fig. 10).

The abundance of these parasites (TC) on the edge of this gland (GR) increases daily. Among the folds of the rectum (Fig. 11) accumulate a great quantity of epimastigotes and trypomastigotes. Many trypomastigotes were observed in long and thin forms in smears of the rectum; they may be a result either of the extra-intestinal cycle of development or of the classical intestinal cycle of the *T. cruzi*.

DISCUSSION

The details of the extra-intestinal cycle were well demonstrable in sections. Invasion of the haemolymph was noted by Dias (1934) and more recently by Camargo *et al.* (1980a, b). The return of the parasite to the digestive tract was described by Lacombe (1979) who noted the presence of parasites in the haemolymph and in the cells of the four long Malpighian tubes as well as their immediate excretion. The parasites are located in the cells of the Malpighian tubes in the form of spheres. After being liberated by rupture of the membrane of the spheres, the parasites move towards the rectal ampullae being, however, retained in the epithelium of the four ampullae of the Malpighian tubes. The parasites in this region can be seen clearly as epimastigotes and trypomastigotes. The time for the development of the parasites varies in different vectors (Lacombe, 1980). A considerable difference in time was observed in the classical cycle of *T. cruzi* confined to the digestive tract as compared with the route of the parasite through the haemolymph and return via the Malpighian tubes. The latter route is much quicker.

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SUMMARY

By applying various histological techniques the results obtained were similar to those in Lacombe's previous publications referring to the development of *Trypanosoma cruzi* in haemolymph, the later penetration in the Malpighian tubes and their return to the digestive tract.

Trypomastigotes and sphaeromastigotes are found in the promesenteron after two hours of feeding the insect with mice blood containing numerous *Trypanosoma cruzi*

Some parasites move to the promesenteron from postmesenteron and others from the haemolymph of the insect.

The amastigotes form inside the haemocytes, multiply, occupying nearly all the cells of the haemolymph.

The parasites liberated inside the Malpighian tubes move to the ampullae where they begin their growth.

The parasites in epimastigote and trypomastigote forms cross the pyloric valve and adhere to the folds of the rectum.

RESUMO

Utilizando técnicas histológicas diversas, os resultados obtidos são similares àqueles encontrados em publicações anteriores do autor, referentes ao desenvolvimento do *Trypanosoma cruzi* na hemolinfa, a posterior penetração nos tubos de Malpighi e seu retorno ao trato digestivo.

Trypomastigotas e esferomastigotas foram encontrados no promesentero duas horas após a alimentação do inseto em sangue de camundongos contendo numerosos *Trypanosoma cruzi*.

Alguns parasitos seguem do promesentero para o postmesentero, e outros seguem em direção à hemolinfa do inseto.

A forma amastigota penetra nos hemócitos, multiplica-se, ocupando quase todas as células da hemolinfa.

Os parasitos liberados no interior dos tubos de Malpighi dirigem-se à ampola retal onde iniciam seu crescimento.

Os parasitos nas formas epimastigota e trypomastigota passam pela valvula pilorica e ficam aderidos às dobras do reto.

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