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Scanning electron microscopy of the final phase of the life cycle of *Trypanosoma cruzi* in the insect vector

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Summary

Scanning electron micrographs showed that both epimastigotes and metacyclic trypomastigotes of *Trypanosoma cruzi* are attached by the flagellum to the epithelium of the rectal gland of *Triatoma dimidiata*. The flagellates tended to cover the surface of the gland and there was a marked predominance of epimastigotes with a round posterior end. Reproduction and metacyclogenesis seem to take place in situ, the latter apparently by twisting and elongation of the epimastigotes. Metatrypomastigotes remain attached for some time, probably by a weaker mechanism which easily allows them to loosen, facilitating expulsion with the urine or feces.

Key words: *Trypanosoma cruzi; Triatoma dimidiata;* metacyclogenesis; scanning electron microscopy; epimastigotes; life cycle.

Introduction

Some of the main ecological aspects of the development of *T. cruzi* in the insect vector, as well as the relevant anatomical and physiological features of the hindgut and excretory system, have been discussed previously (Zeledón et al., 1977). In the same paper it was shown by transmission electron microscopy that the flagellates attach themselves particularly to the epithelium of the rectal gland by flagellar hemidesmosomes, giving the appearance of a carpet.

We have now used scanning electron microscopy to study the tridimensional aspect of this phenomenon, which helps to clarify the location and metacyclogenesis of the agent of Chagas' disease in the insect.

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Materials and Methods

One adult, non-infected, *Dipetalogaster maximus* was used to assess the technique of exposure of the rectal gland, in view of its large size as compared with other species. The insect was paralyzed at 4° C and dissected under saline solution, separating the rectal sac together with a small piece of the midgut and the ampullae of the Malpighian tubes. Hematin was washed out with phosphate buffered saline (PBS) with the help of a fine capillary pipette introduced into the sac. The same procedure was followed with a naturally infected adult *Triatoma dimidiata*. The two rectal sacs were fixed in 2.5% glutaraldehyde for two hours at 4° C, washed three times with phosphate buffer, treated with 1% osmium tetroxide, dehydrated with ethanol and amyl acetate, opened longitudinally with small scissors, dried in a Hitachi HCP-1 critical point drier, mounted on double-sided adhesive tape and coated with gold in an ion coater (EIKO 1 B-3). Observations were made with a Hitachi HH 5-2R scanning electron microscope.

Results and Discussion

The rectal gland appears clearly delimited within the rectal sac, with the aspect of a flower (Fig. 1). When parasites adhere to it they tend to cover all its surface and folds giving it, under lower magnification, a granular appearance due to the predominance of epimastigotes with a rounded posterior free portion (Fig. 2). With higher magnification (Figs. 3 to 8) it becomes evident that both epi- and trypomastigotes, of the metacyclic type, are attached by the flagellum to the epithelium of the gland. Some epimastigotes present clefts of different sizes suggesting active division, and some were elongated and twisted, suggesting that these transform themselves in situ into trypomastigotes (Fig. 4). Nevertheless, the possibility of an asymmetrical division, producing an epi- and a trypomastigote at the same time, as observed by Camargo (1964) in cultures, cannot be ruled out, even though this was not evident in our pictures. Metatrypomastigotes probably can easily become loosened, particularly when the urine flow dilates the gland after a blood meal, and be flushed out to infect a new victim. We did not observe long and slender epimastigotes such as appear occasionally in feces; these are probably free in the lumen and not attached to the epithelium.

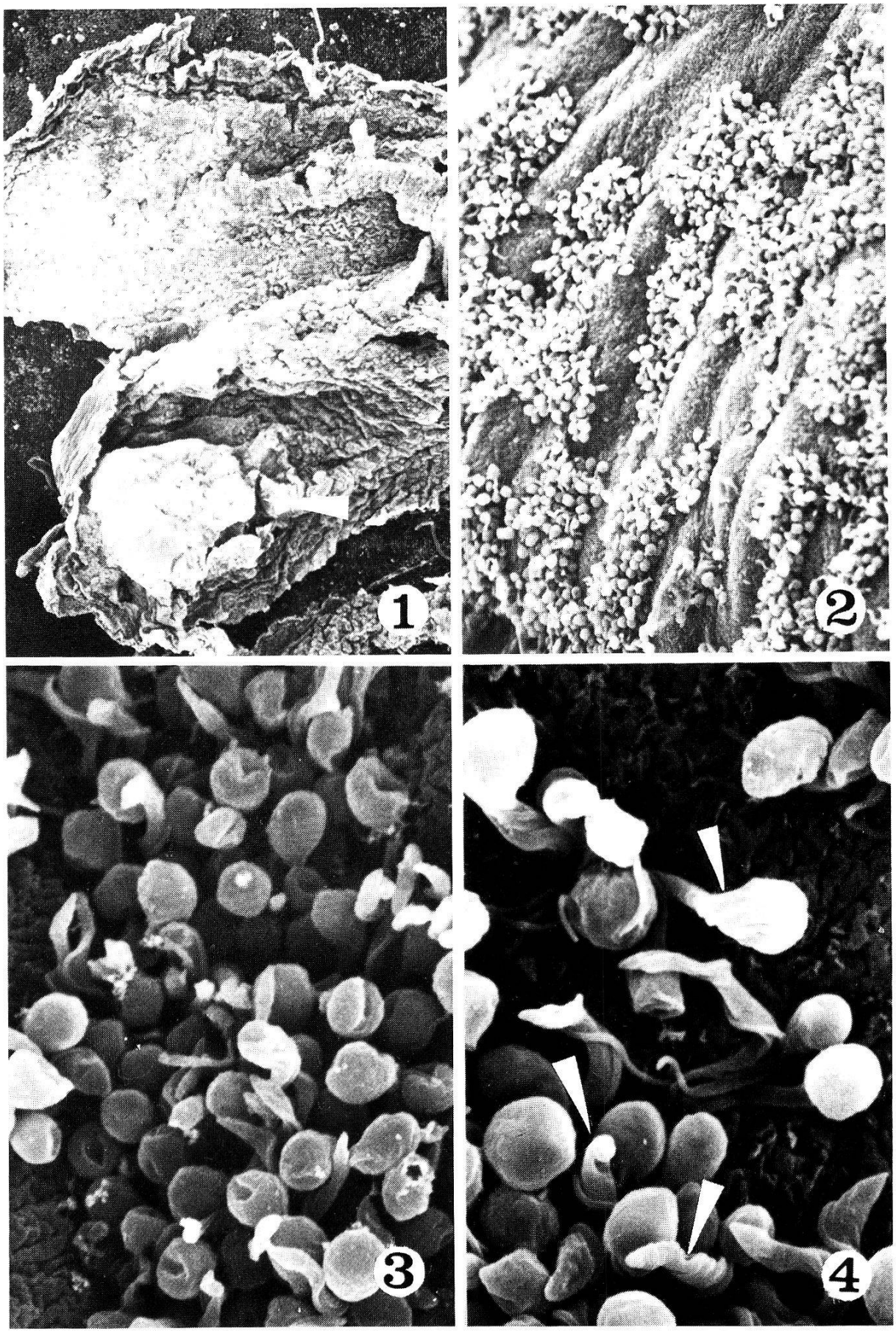
Recent observations (Zeledón et al., 1982) have shown that, in an experimental infection, flagellates become attached to the gland as early as eight days after the infective blood meal. Flagellates can be found, in both experimentally and naturally infected insects, within the folds of the rectal sac, particu-

Fig. 1. General view of the opened rectal sac of *Dipetalogaster maximus*. The rectal gland (arrowed) is clearly seen. ×20.

Fig. 2. General aspect of part of the epithelial surface of the rectal gland of *Triatoma dimidiata*, infected with *Trypanosoma cruzi*. Parasites tend to occupy all available space in the folds of the gland. ×490.

Fig. 3. Higher magnification of Fig. 2 to distinguish the palisades of flagellates and the predominance of epimastigotes with rounded posterior half of the body. ×2450.

Fig. 4. Higher magnification of Fig. 3 showing twisted epimastigotes (arrowed) apparently in the process of transformation to metacyclic trypomastigotes. ×3850.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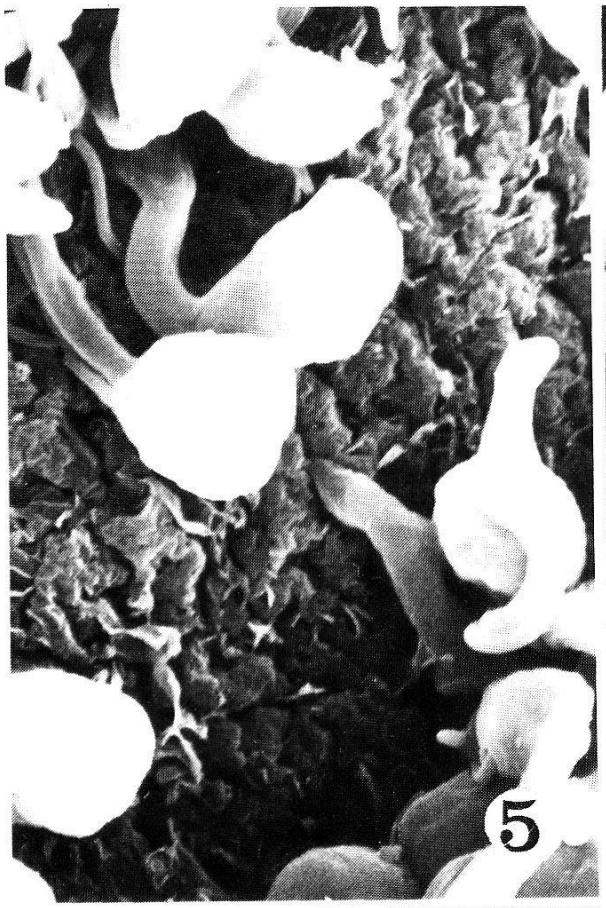
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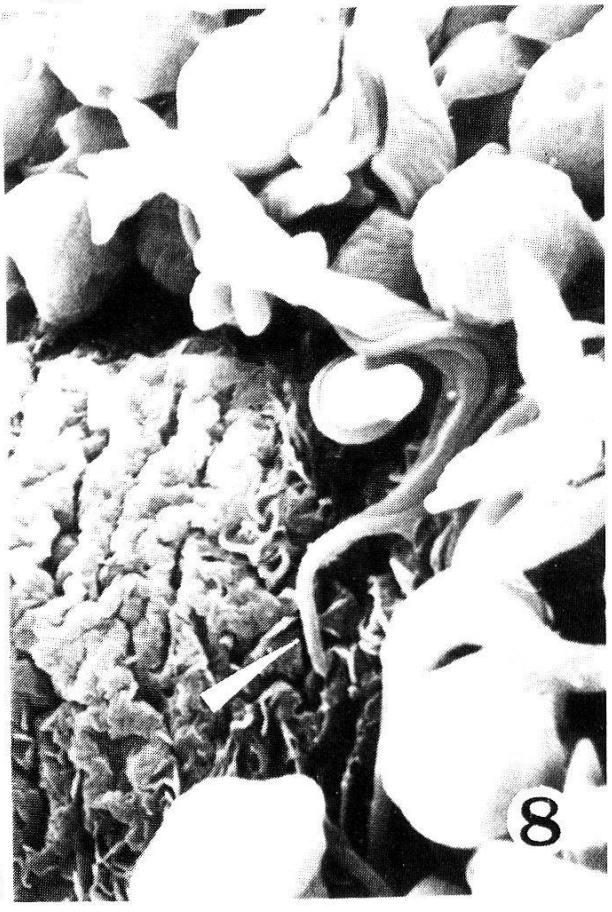
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larly in the vicinity of the gland, and occasionally in the ampullae or proximal parts of the Malpighian tubes. There was always a marked preference for the epithelium of the rectal gland.

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Figs. 5, 6, 7 and 8. Detail of epimastigotes and trypomastigotes anchored to the epithelium of the rectal gland. Different degrees of cleavage of epimastigotes are observed in Fig. 6, and the reservoir opening (arrowed) is evident in two forms in Figs. 6 and 7. A typical metacyclic trypomastigote appears in Fig. 8 with the flagellum inserted between folds of the epithelium of the gland (arrowed) and flagellar membrane surrounding the body. Figs. 5, 7 and 8, ×5600 and Fig. 6, ×7000.