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Localization of Acid Phosphatase in *Trypanosoma cruzi*

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Acid phosphatase activity has previously been reported in several species of trypanosomes (Lehman, 1963; Brooker and Vickerman, 1964; Seed *et al.*, 1967). However, the intracellular localization of the enzyme and its relation to trypanosomal organelles and inclusions are still unclear. The objectives of the present ultramicroscopical study were to localize acid phosphatase activity in the trypanosome cell, and to clarify its relationship to the organelles of the organism.

Materials and Methods

The strain of *Trypanosoma crusi* used was maintained by weekly passage on liver infusion tryptose medium. The cultured organisms, crithidial form of the trypanosome, were collected and washed by centrifugation in 0.2 M tris-maleate (TM) buffer, pH 5.0. They were prefixed in 2.5 % glutaraldehyde for 10 to 20 minutes at 4°C, then were washed again in TM buffer using cold-centrifuge.

The incubation medium for the acid phosphatase reaction (Barka & Anderson, 1962) was composed of: 10 ml of 1.25 % sodium β-glycerophosphate, 10 ml of TM buffer, 20 ml of 0.2 % lead nitrate, and 10 ml of distilled water. As a control, trypanosomes were incubated in a medium devoid of β-glycerophosphate, or in a fluoride solution, a known inhibitor of acid phosphatase.

Following 15 to 30 minutes of incubation at 37°C, the organisms were washed in TM buffer, and were then fixed in 1.0 % osmic acid solution for 60 minutes at 4°C. The material was then dehydrated in absolute alcohol, and embedded in epoxy resin. Ultrathinsections were cut with a Porter-Blum microtome. To avoid artefacts, lead acetate was not used for staining. Photographs were taken with a Hitachi HU-11B ultramicroscope at 5,000 to 20,000 magnifications, and were further enlarged 2 to 3 times for publication.

Results

The electron-dense deposition of lead phosphate indicating acid phosphatase activity was found on the cell wall, and especially on the sub-microfibrils (Figs. 1-a, 2, 3). The enzyme activities in the sub-microfibrils were demonstrated in their tubules (Fig. 1-b). The most distinct areas of enzyme activity were in the region of the reservoir at the flagellum, and in neighboring vesicles (Fig. 1-a). In addition, the wall/or inclusions of vacuoles, and the flagellar sheath were also positive for acid phosphatase activity (Figs. 2, 3). Furthermore, the reaction products were weakly in Golgi area (Figs. 2, 3). Control specimens did not demonstrate positive reactions (Fig. 4).

Discussion

Lehman (1963) reported acid and alkaline phosphatase in the cytoplasm of culture

forms of *Trypanosoma ranarum* by light microscopic methods. The enzymes were indiscriminately located in the cytoplasm, and no areas of specific concentration were seen. Soon after, Brooker and Vickerman (1964) demonstrated acidphosphatase activity in discrete bodies in the region of the reservoir surrounding the base of the flagellum. They suggested that this enzymatic activity, marking the localization of lysosomes, might be associated with the digestion of material taken into the flagellate by pinocytosis.

These preliminary reports were followed by a more comprehensive study by Seed *et al.* (1967) on the biochemical characteritics and localization of acid phosphatase activity in *T. gambiense*, using biochemical techniques and electron microscopy. They found that acid phosphatase activity was associated with subcellular vesicles distributed throughout the cytoplasm, with the greatest concentration at the base of the flagellum. Seed and his colleagues identified these phosphatase-positive subcellular vesicles as lysosomes. In the present study as well, acid phosphatase activity was detected in and around the reservoir of the flagellum, and in nearby vesicles. These vesicles may originate in the area of the reservoir, and might be involved in the formation of phagolysosomes, the fusion of phagosomes with lysosomes.

Steinert and Novikoff (1960) studied pinocytosis in *T. mega*, using a ferritin solution as a marker. They found that pinocytic vacuoles coalesced with inclusion bodies at the posterior end of the flagellate, leading them to postulate that the inclusion bodies were lysosmes. Brooker and Vickerman (1964) supported this reasoning by demonstrating acid phosphatase activity at the sites mentioned by Steinert and Novikoff (1960).

Additional evidence correlating phagosomes and lysosomes was supplied by the studies of Müller and Törö (1962), and Müller (1963), who investigated the appearance of lysosomal enzymes in *Paramecium* food vacuoles by cytochemical methods. Although the actual transfer of enzymes into the vacuoles was not described in detail, it did appear to be very similar to the fusion of phagosomes and lysosomes in mammalian cells.

Similar findings to the present experiments were reported more in detail by Ohashi (1972) using *Trichomonas vaginalis* and *T. tenax*; the enzyme activities were recognized markedly in Golgi apparatus, hetero- and auto-phagosome but weakly on periplast. The present authors also demonstrated the enzyme in the microtubules of periplast. This observation suggests that the hydrolysis of acid phosophatase might be operative at the site of microtubules without the participation of other organelles like lysosomes and the reservoir.

Summary

Localization of acid phosphatase activity in crithidial form of *Trypanosoma cruzi* was demonstrated by means of electron microscopical techniques. The enzyme activities were localized especially in the region of the reservoir at the base of flagellum and in neighboring vesicles. These vesicles may originate in the area of the reservoir, and might be involved in the formation of phagolysosomes. Furthermore, the reaction products were recognized in Golgi areas, phagosomes in cytoplasm and microtubules in periplast. Detection of the marked reaction product in the microtubules suggests that the hydrolysis of acid phosphatase might be operative at the site of microtubules without the participation of other organelles like lysosomes and reservoir.

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REFERENCES

1) Barka, T. and Anderson, P. J. (1962) : Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. J. Histochem. Cytochem., Vol. 10, 741–753.
2) Brooker, B. E., and Vickerman, K. (1964) :

Acid phosphatase in trypanosomes. Trans. Roy. Soc. Trop. Med. & Hyg., 58, 293-294.
3) Lehman, D. L. (1963) : The biology *Trypanosoma ranarum* (Lankester, 1971). Localization of acid and alkaline phosphatase in flagellates grown at different temparatures. J. Proto., Vol. 10, 399-400.
4) Müller, M. and Törö, I. (1962) : Studies on feeding and digestion in Protozoa, III. Acid phosphatase activity in food vacuoles of *Parameciun micronucleatum*. J. Proto., Vol. 9, 98-102.
5) Müller, M. (1963) : Lysosome, Ciba foundation symposium.
6) Oohashi, O. (1972) : Studies on acid phosphatase in Trichomonads. II. Electronmicroscopical demonstration of the activity. Jap. J. Parasit., Vol. 21, No. 1, 16-27.
7) Seed, J. R., Byram III, J., and Gam, A. A. (1967) : Characterization and localization of acid phosphatase activity of *Trypanosoma gambiense*. J. Proto., Vol. 14, 117-125.
8) Steinert, M. and Novikoff, A. B. (1960) : The existence of a cytostome and the occurence of pinocytosis in the trypanosome, *Trypanosome Mega*. J. Biochem. Biophys. Cytol., Vol. 8, 563-569.

トリパノソーマ・クルジーにおける酸性フォスファターゼの局在

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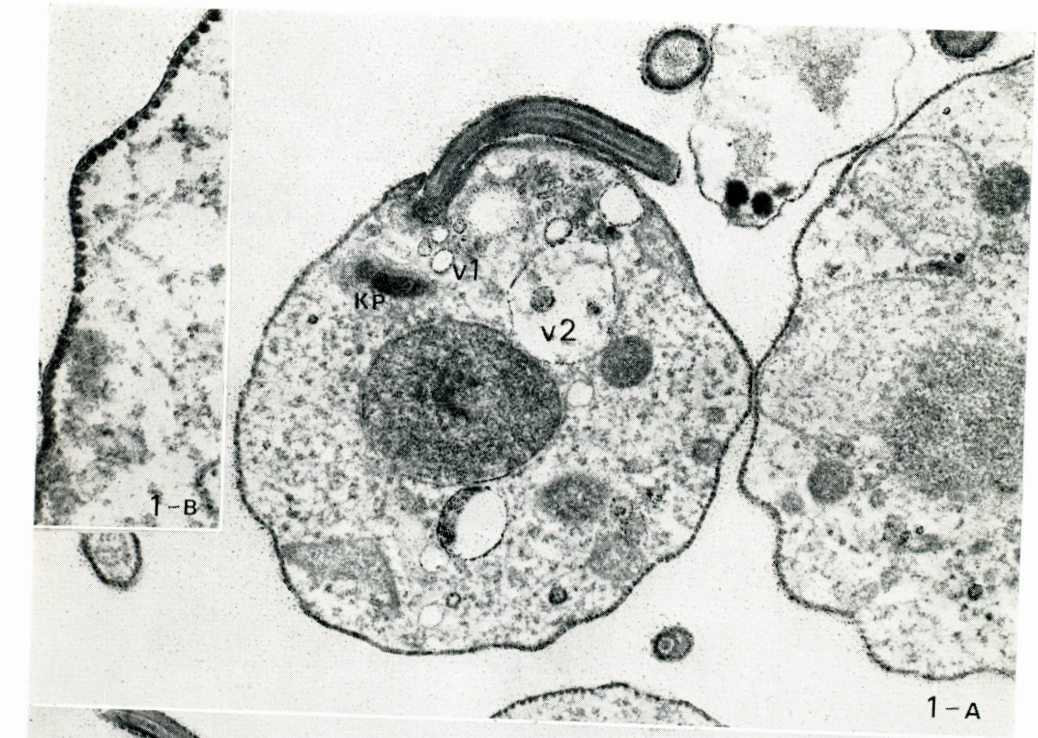
トリパノソーマ・クルジーの培養した株でクリシジア型における酸性フォスファターゼの局在を電顕細胞化学的手法で証明し, organella との関連及びその生理的意義を明らかにすることを目的として本実験を行つた. その結果, 酵素活性が認められたのは, ゴルジ体, 空胞 reservoir, inclusion body, 虫体細胞膜 (特に sub-microtuble) 等である. 最も強い反応の認められた場所は reservoir で, そこから形成される pinocytotic vesicle にも活性が認められた. 更に phagolysosome と phagosome を形成している場合にも両者に活性が認められた. 一方 sub-microtubule に活性が認められたことは, 虫体細胞膜の代謝を考える上で興味深く, 他の organella と同様に代謝機能を有していることを示唆している.

Explanations of Figures

Fig. 1-a : The reaction products of acid phosphatase are deposited on the cell, sub-microtubules, vacuoles, reservoir and in the neighboring vesicles. KP : Kineto plast, N : Nucleus, V1 : Vesicles, V2 : Vacuole. (× 25,000)
Fig. 1-b : The high magnificated picture of the periplast. The enzyme activities were observed in sub-microtubules. (× 50,000)
Fig. 2 : The acid phosphatase activities were found in the Golgi apparatus (G), reservoir (R), vacuoles (V) and inclusion bodies (I). (× 25,000)
Fig. 3 : The acid phosphatase activities were found in the Golgi apparatus (G), inclusion bodies (I) and microtubules in periplast. (× 25,000)
Fig. 4 : The reaction products were not seen completely in the control specimens. N : Nucleus, R : Reservoir, V : Vacuole.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v2
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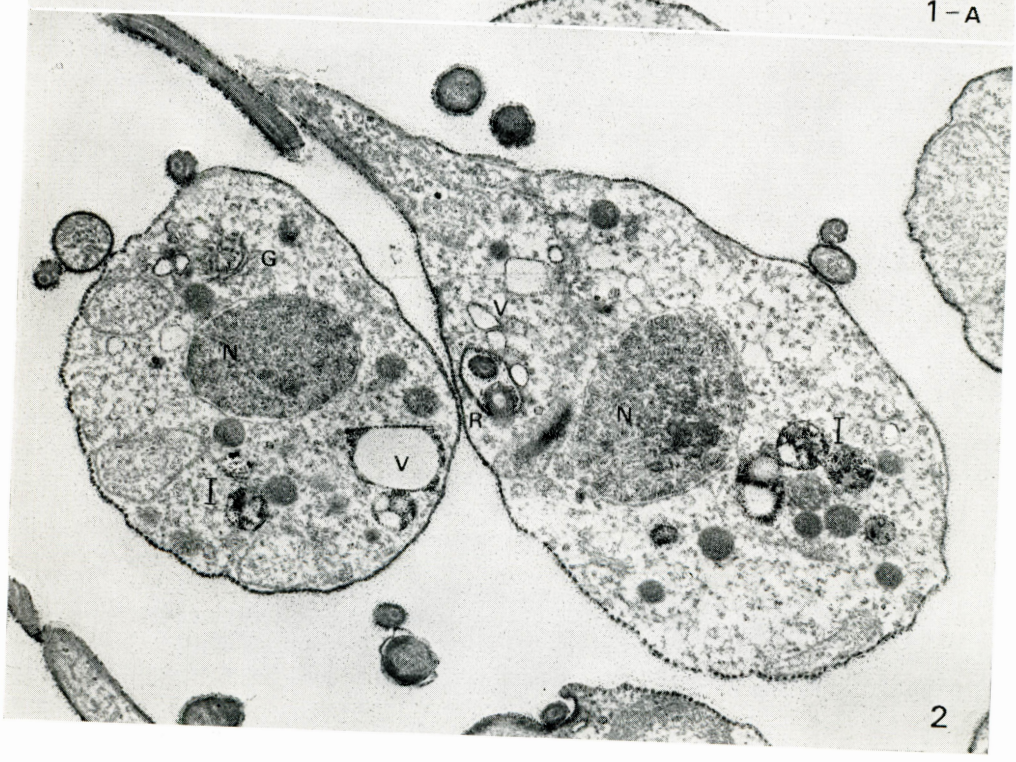
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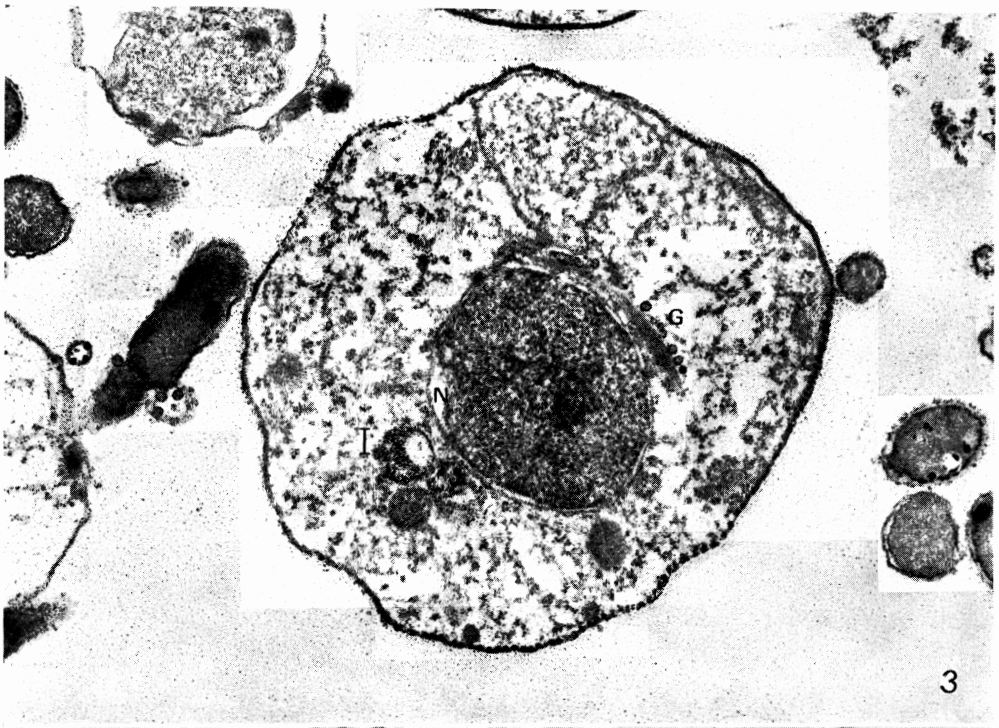
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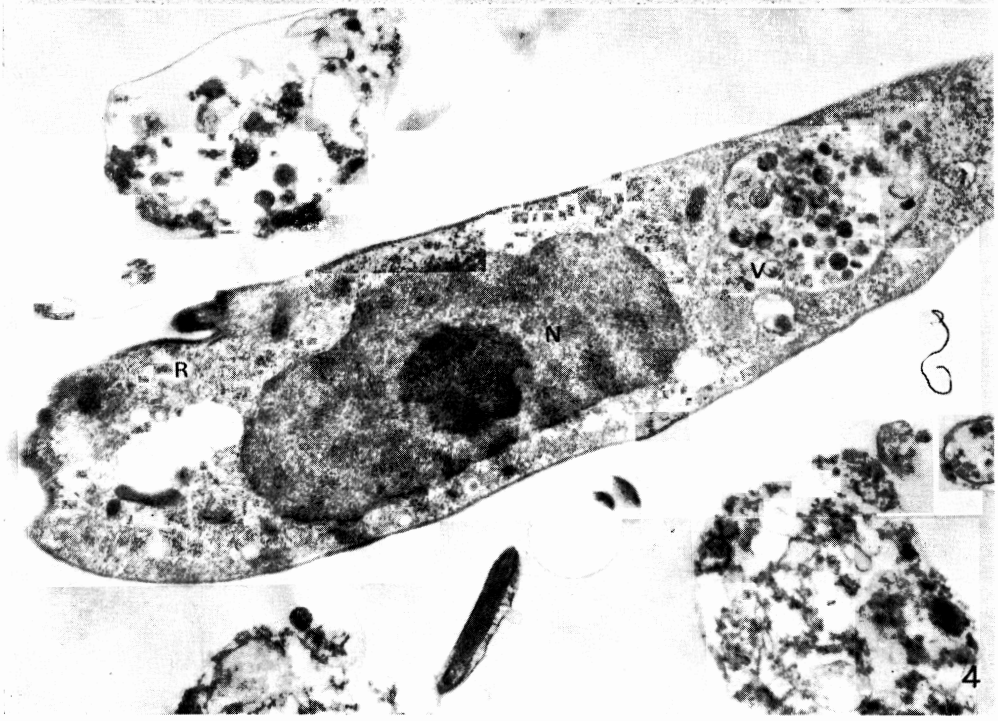
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