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Trypanosoma cruzi: MODIFICATION OF ALKALINE PHOSPHATASE ACTIVITY INDUCED BY TRYPOMASTIGOTES IN CULTURED HUMAN PLACENTAL VILLI.

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SUMMARY

Human term placental villi cultured "in vitro" were maintained with bloodstream forms of **Trypanosoma cruzi** during various periods of time. Two different concentrations of the parasite were employed. Controls contained no **T. cruzi**. The alkaline phosphatase activity was determined in placental villi by electron microscopy and its specific activity in the culture medium by biochemical methods. Results showed that the hemoflagellate produces a significant decrease in enzyme activity as shown by both ultracytochemical and specific activity studies and this activity was lower in cultures with high doses of parasites. The above results indicate that the reduction in enzyme activity coincides with the time of penetration and proliferation of **T. cruzi** in mammalian cells. These changes may represent an interaction between human trophoblast and **T. cruzi.**

KEY WORDS: Trypanosoma cruzi; Placental villi, human; Tissue coculture; Alkaline phosphatase; Chagas' disease, congenital.

INTRODUCTION

Trypanosoma cruzi, the causative agent of Chagas' disease, may be congenitally transmitted from mother to fetus and the role of placenta in congenital transmission has been demonstrated[2, 4, 21].

T. cruzi infects the host cell by a phagocytic mechanism[3, 19]. During the process of penetration, the parasite interacts with the surface of the host cell, and the plasma membrane of the two cells may be modified by this interaction[6, 7, 23]. Thus, it is possible that some components of the apical membrane of the syncytiotrophoblast, like alkaline phosphatase, are modified by interaction with **T. cruzi,** indicating an early step of the paghocytic process[13].

The alkaline phosphatase (EC: 3.1.3.1) is an integral enzyme of the apical plasma membrane of the trophoblast, and its activity can be distinguished from other isoenzymes by its thermostability[1].

To analyze the changes induced by **T. cruzi** on the membrane of the host cell, human placental villi were cocultured with the parasite. The activity of alkaline phosphatase was determined by electron microscopic studies of placental villi and by detection of specific activity in the culture medium.

MATERIAL AND METHODS

Placentae. Human term placentae from cli-

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nically and serologically healthy women were used. Samples of 2 mm thickness were obtained from the subchorionic areas of the central part of cotyledons, with an average weight of 40 to 70 mg, and employed for tissue cultures.

Trypanosoma cruzi. The bloodstream form of the Tulahuen strain was used; it was obtained from male mice, C_3H strain, about 20 to 30 g weight, infected and bled at the 7th day of inoculation. Isolation was performed according to ANDREWS & COLLI[3] and MEIRELLES et al[19]. Blood was centrifuged at 100 g for 10 min, and after being kept at 37°C for one hour, the supernatant was centrifuged at 1000 g for 15 min. The pellet containing the parasites was washed three times in Eagle's minimal essential medium and resuspended in fresh medium.

Cultures. Organs culture technique was used[15], employing 1.5 ml of Eagle's minimal essential medium (Gibco, New York, USA), at pH 7.35. Cocultures were maintained at 37°C and harvested after 15, 30, 60 and 150 min and 16 and 48 h respectively. Two concentrations of parasites were employed for each infected group: 3 x 10^3 and 1 x 10^5 parasites per ml of medium. Controls contained no **T. cruzi.** No changes of medium were performed to the end of each period of culture either in experiments or controls. The cultures were harvested at each time and the villous tissues were prepared for cytochemical and structural studies and the respective media were used for alkaline phosphatase activity assay.

Ultrastructural and ultracytochemical techniques. Placental tissues were washed and fixed in Karnovsky's solution[16] for 2 h, at room temperature, and postfixed in 1% osmium tetroxide. Samples were dehydrated in acetone and embedded in Araldite. The ultrathin sections were contrasted with uranyl acetate and lead citrate. For determination of alkaline phosphatase, samples were fixed in Karnovsky's solution for 30 min at 4°C. The rest of the technique was performed according to JONES & FOX[15], using sodium 3-glycerol phosphate (Laboratory BDH Ltd, England) as substrate. Controls were incubated in substrate-free medium. Other controls were processed for electron microscopy without lead contrast. Observations were made in a Siemens Elmiskop 101 electron microscope, at magnifications ranging from 2000 to 20.000 x.

Specific activity. The thermostable alkaline phosphatase activity was determined in the culture medium after centrifuging at 1000 g for 15 min. The supernatant was heated for 30 min at 65°C. The enzyme activity was analyzed according to the technique of MESSER et al[20], and expressed as umol of p-nitrophenol released per mg of proteins per h. Protein was measured by the method of LOWRY et al[17].

Statistical analysis were performed using bifactorial variance procedures.

RESULTS

Ultracytochemical data in control cultures showed intense activity of alkaline phosphatase on the surface of the microvilli as well as in vesicles of the syncytiotrophoblast (Fig. 1a).

The activity of alkaline phosphatase in villi exposed to **Trypanosoma cruzi** appeared on the apical surface of the syncytiotrophoblast as small and electron microscopic dense particles and also in areas where parasites were not seen (Fig. 1b). These deposits were scarce, isolated and located on the outer limiting plasma membrane, as observed in cross sections of microvilli. Vesicular elements could be appreciated in the syncytial cytoplasm, with scarce lead salts deposits attached to the inner surface of the vesicular membrane. In other cases, enzyme activity was found in cellular membrane invaginations. At 48 h of infected culture, a marked reduction in enzyme activity was observed in placental villi as well as a striking diminution of microvilli (Fig. 2). Amastigotes were evident within the trophoblast in some sections of the placental villi (Fig. 2). In other sections where parasites were not evident, a diminished enzyme activity was also seen.

Enzyme activity in control cultures media increased noticeably during the first 150 min. At 16 and 48 h, the values of activity were reduced. The cultures infected with 1 x 10^5 parasites showed an increment of specific enzyme activity until 150 min, and markedly decreased values in the later periods. However, this activity was

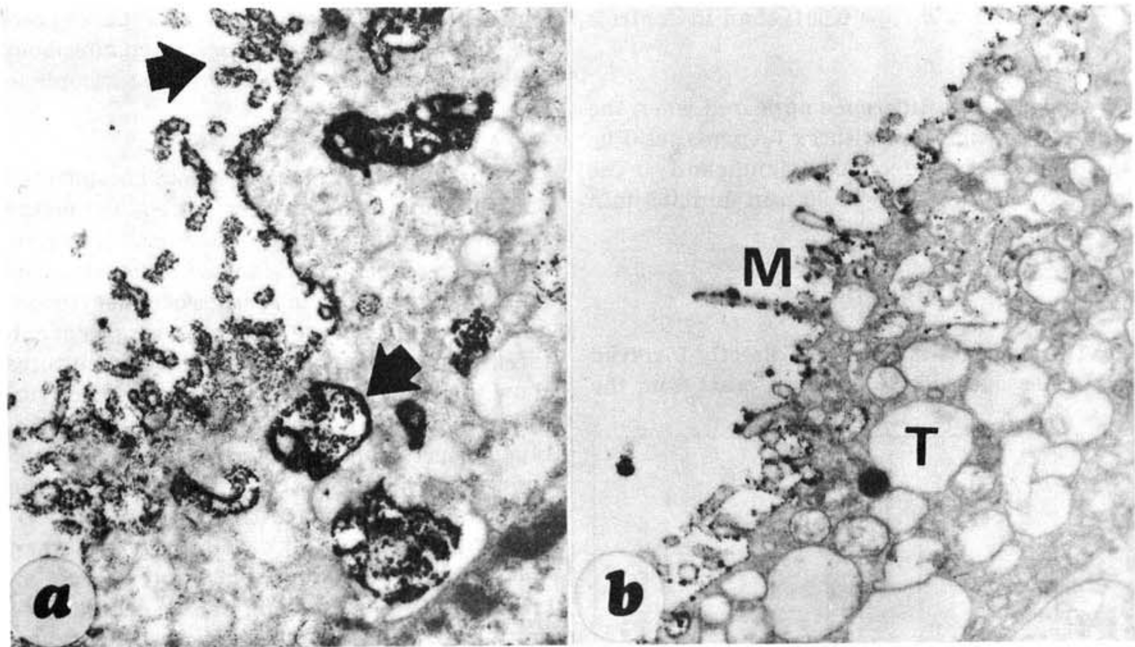

Fig. 1 — Human term placenta from a 60 min tissue culture stained for alkaline phosphatase. (A) Control. Enzyme activity in microvilli and endocytic vesicles of trophoblast (arrows). 12.600 X. (B) Infected culture with 1 x 10^5 trypomastigotes of **Trypanosoma cruzi.** Enzyme activity is decreased even in areas where parasites are not seen. T: trophoblast; M: microvilli. 16.200 X.

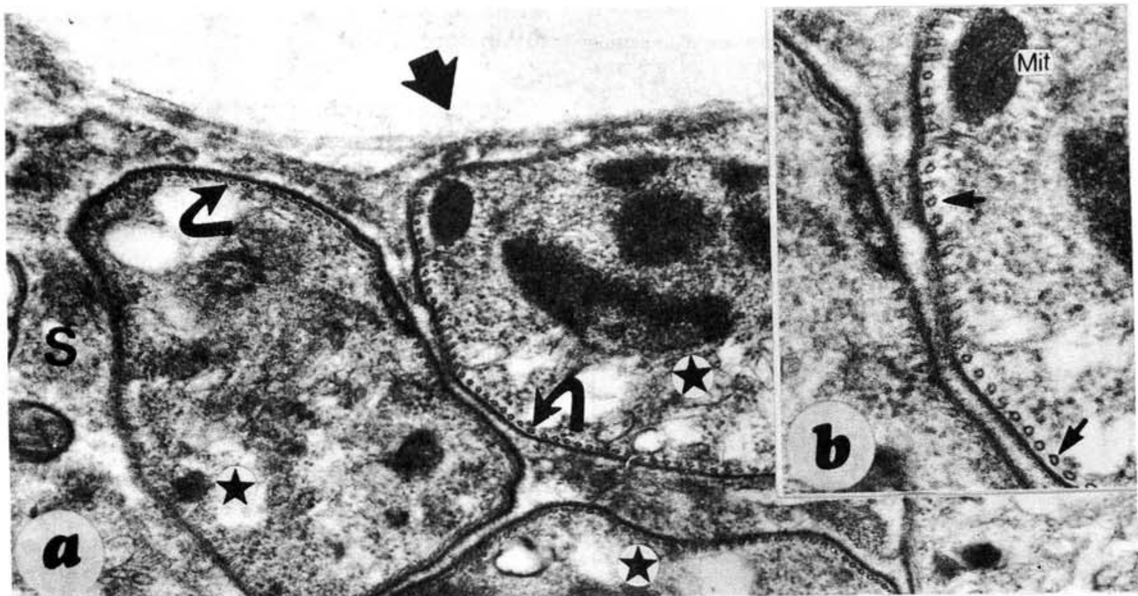

Fig. 2 — Human term placenta from a 48 h tissue culture infected with 1 x 10^5 trypomastigotes of **Trypanosoma cruzi** and stained for alkaline phosphatase. (A) Virtually neither microvilli nor enzyme activity is demonstrable (arrow). Amastigotes (★) in the trophoblast and subpelicullar microtubules (curved arrows) are shown. 28.800 X. (B) Detail of (A) showing two parasites within the trophoblast. Subpelicullar microtubules (arrows) and mitochondrion are evident. 57.600 X. Mit: mitochondrion; S: syncytiotrophoblast.

significantly lower ($p < 0.001$) than in controls (Fig. 3).

A significant difference appeared when the cultures were infected with 3 x 10^3 parasites (Fig. 4). Specific activity decreased significantly in the infected cultures only at 60 min and 150 min ($p < 0.001$).

DISCUSSION

The placenta is an organ directly involved in the passage of **Trypanosoma cruzi** from the mother to the fetus, and in this case it undergoes alterations that have been described morphologically at the light and electron-microscopic level[4, 24].

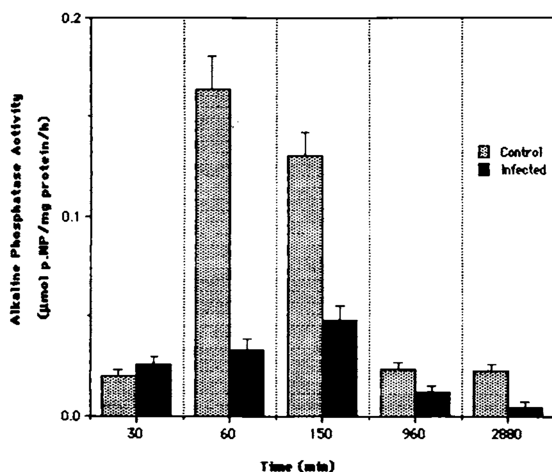

Fig. 3 — Specific activity of alkaline phosphatase in the culture media of human term placentae cocultured with 1 x 10^5 **Trypanosoma cruzi** (trypomastigote forms) and non infected (Control). Bars show standard errors of four experiments.

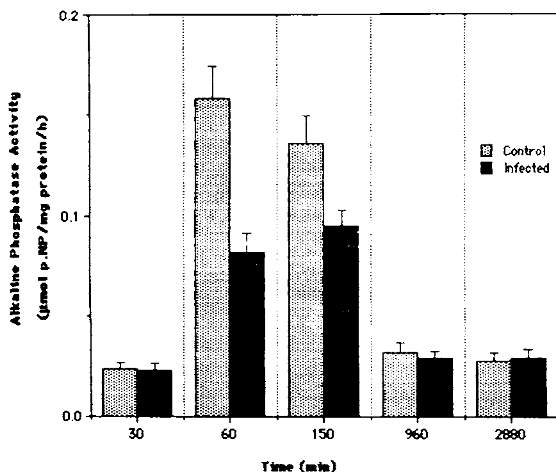

Fig. 4 — Specific activity of alkaline phosphatase in the culture media of human term placentae cocultured with 3 x 10^3 **Trypanosoma cruzi** (trypomastigote forms), and non infected (Control). Bars show standard errors of four experiments.

Our studies on placentae from non-infected cultures show an intense enzyme activity on the microvillous surface of the trophoblast, in agreement with earlier observations[15, 18]. Besides, this activity may be seen in vesicles of endocytic origin and becomes more evident in long term cultures. Placental samples from infected cultures show a significant decrease of enzyme activity in the microvilli and endocytic vesicles. Probably this decrement, detected by electron microscopy, could be due to the parasite action on the placental membrane. CALDERÓN & FABRO[7] and CALDERÓN et al[8] have proved that when membranes of the trophoblast and **T. cruzi** interact, changes in lipid and protein contents are produced. In addition, the parasite might induce fusion with the cellular membrane of the host cell. It is also possible that the parasite uses lytic factors and/or proteases to facilitate its penetration[5, 9, 22].

Our results on the specific activity of alkaline phosphatase in control cultures media agree with the delta values obtained by GALSKY et al[14] in normal villi.

In infected cultures, the reduction of specific enzyme activity is greater with increasing concentrations of infecting parasites, and the comparison between specific enzyme activity in infected and non-infected culture media, shows a significative decrease of activity in cultures with **T cruzi.** These results agree with the time of penetration and multiplication of parasites into the cells[10, 11]. Probably, the presence of **T. cruzi** in the culture media would not exert a direct action on the alkaline phosphatase activity, insofar as this activity was not reduced at 30 min in the infected cultures.

T. cruzi produces a decrease in alkaline phosphatase activity, as shown in the ultracytochemical studies of the placental samples as well as in the specific activity detected in the culture medium. Therefore, we consider that the reduction in enzyme activity is due to an interaction between placental villi and **T. cruzi.**

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RESUMO

Trypanosoma cruzi: **alteração da atividade de fosfatase alcalina induzida por tripomastigotas em culturas de vilos placentários humanos.**

Vilos placentários humanos a termo foram mantidos "in vitro" em interação com formas tripomastigotas sangüíneas de **Trypanosoma cruzi**, durante diversos períodos de tempo. Foram utilizadas concentrações diferentes de parasitas. Os controles não continham **T. cruzi**. Determinou-se a atividade de fosfatase alcalina em vilos placentários mediante microscopia eletrônica e sua atividade específica no meio de cultura, mediante métodos bioquímicos. Os resultados mostraram que o hemoflagelado produz uma diminuição significante da atividade enzimática tanto pelos estudos ultracitoquímicos como de atividade específica e esta atividade de fosfatase alcalina foi menor em culturas com altas doses de parasitas. Estes resultados são indicadores de que a redução de atividade enzimática coincide com o tempo de penetração e proliferação do **T. cruzi** nas células. Estas mudanças podem representar uma interação entre o trofoblasto humano e o **T. cruzi**.

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