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# GROWTH AND DIFFERENTIATION OF *TRYPANOSOMA CRUZI* CULTURE FORMS KEPT IN LABORATORY FOR DIFFERENT PERIODS OF TIME

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## SUMMARY

Cultures of two *T. cruzi* strains (Y and MR), kept in LIT medium for different periods of time after isolation from vertebrate host, presented similar growth curves. Differentiation (epimastigotes → metacyclic trypomastigotes) rates, however, were consistently different in the cultures of Y strain, ranging from 5% (PF culture) to 30% ($Y_{77}$ cultures). Similar variation was observed with cultures of MR strain, which steadily displayed higher differentiation rates than those of Y strain. The factors responsible for the reported differences in the rate of metacyclic morphogenesis could not be identified so far.

## INTRODUCTION

*Trypanosoma cruzi* cultivated in LIT ("liver infusion-tryptose") liquid medium presents a 4-day period of exponential growth followed by a stationary phase CAMARGO [3]. The differentiation of epimastigotes into metacyclic trypomastigotes begins at the end of the exponential growth and proceeds all along the stationary phase. The usual proportion of metacyclic forms in the cultures (10% to 15%) CAMARGO [3]; FERNANDES et al. [5] may significantly increase by using dog-heart infusion instead of liver infusion and lowering the medium pH; in such medium, ~70% of metacyclic forms could be obtained at the end of a weeks growth (CASTELLANI et al. [4]). All those experiments have been performed only with cultures of Y strain SILVA & NUSSENZWEIG [7]. The present paper reports the experiments on growth and differentiation, in LIT medium, of several cultures of two different *T. cruzi* strains (*Y* and *MR*), kept in artificial medium for variable periods of time after their isolation from the vertebrate host.

## MATERIAL AND METHODS

*The following T. cruzi strains were used*:

*Y* strain, isolated from an acute case of Chagas' disease SILVA & NUSSENZWEIG [8] and *MR* strain, isolated from a naturally infected *Triatoma infestans* BRENER & CHIARI [2]. Both strains were kept in mice by repeated intraperitoneal blood passages performed weekly for the *Y* strain and every 10 days for the MR strain. In different periods, the blood of heavily infected mice was inoculated into LIT medium, the flagellates being then serially transferred to fresh LIT medium about every 15 days.

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*Culture media* — LIT ("liver infusion-tryptose"), described by CAMARGO [3] and HIL ("dog-heart infusion lactoalbumin"), described by CASTELLANI et al. [4] have been used.

*T. cruzi cultures used in the growth and differentiation experiments*:

*Y strain*: culture "$Y_{77}$" having undergone after isolation from experimental infected mice, 77 passages in LIT within 3 years; culture "PF" cultivated for about 17 years in blood-agar and then, in LIT medium for 1.5 years.

*MR strain*: Culture "$MR_{44}$", with 44 passages in LIT within 2 years and culture "$MR_{147}$", with 147 passages in LIT within 6.5 years.

For some experiments, hemocultures in LIT medium, were carried out with the blood of animals previously inoculated with the aforementioned cultures. Before the experiments, the flagellates were gradually adapted to growth in LIT medium by weekly passage performed for a period of two months. Those culture were identified a "$Y^{II}_{77}$", "$MR^{II}_{44}$" and "$PF^{II}$".

All cultures were gradually adapted to exponential growth and only flagellates presenting growth of at least 100% in 24 hours were used as inocula for the experiments.

*Growth and differentiation experiments* — *T. cruzi* culture forms at exponential growth were inoculated into 50 ml Erlenmeyer flasks containing 8 ml of medium so that an initial density of about 15 x $10^6$/ml flagellates was obtained. Cultures transferred to a different medium, were previously washed twice in the new medium by centrifugation at 400 g. All cultures were kept at 28°C. In the experiments with HIL medium, the cultures were previously in cubated at 21°C for 48 hours and, then, kept at 28°C.

After being homogenized in a mechanical spinning shaker the cultures were accordingly diluted in Paul's solution PAUL [7] and the number of flagellates daily determined in a Fisher-Autocytometer with the threshold adjusted to 72.5. The percentage of metacyclic trypomastigotes was determined by microscopical examination of 500 to 1000 non-selected flagellates in Giemsa-stained smears.

## RESULTS

No significant differences regarding growth in LIT medium was detected with all studied cultures (Figs. 1 and 3). The differentiation rate was, however, rather different in the cultures of Y strain: culture $Y_{77}$ consistently presented, on the 8th day of growth, about 30-35% of trypomastigotes metacyclic forms, where as PF cultures steadily presented a low proportion of metacyclic forms (~5%) (Fig. 2). Culture $MR_{44}$ showed a constant differentiation rate of 40-50% whereas in cultures $MR_{147}$ the proportion of metacyclic forms does not exceed 15-20% (Fig. 4). Cultures PF which steadily present a low differentiation rate in LIT medium, showed a 5-fold increase of metacyclic forms in HIL medium (Fig. 5). Cultures $Y_{77}$ and $MR_{44}$ after one passage through mice (identified as $Y^{II}_{77}$, $MR^{II}_{44}$) regained the usual capacity to form a high percentage of metacyclic trypomastigotes; culture $PF^{II}$, however, kept its low differentiation rate (Fig. 6).

## DISCUSSION

BICE & ZELEDON [1] reported that *T. cruzi* cultures long kept in the laboratory seem to produce larger number of flagellates than those maintained for a shorter period of time, in artificial media, which suggests a gradual adaptation of the parasite to the culture medium environmental conditions. In our experiments, however, no significant differences in the growth rate of the various cultures kept for different periods of time in culture was detected. It is worth emphasizing the importance, in such comparative studies, of using standardized inocula formed by flagellates in the exponential growth phase so that unpredictable delay and fluctuation in the parasite's multiplication are avoided.

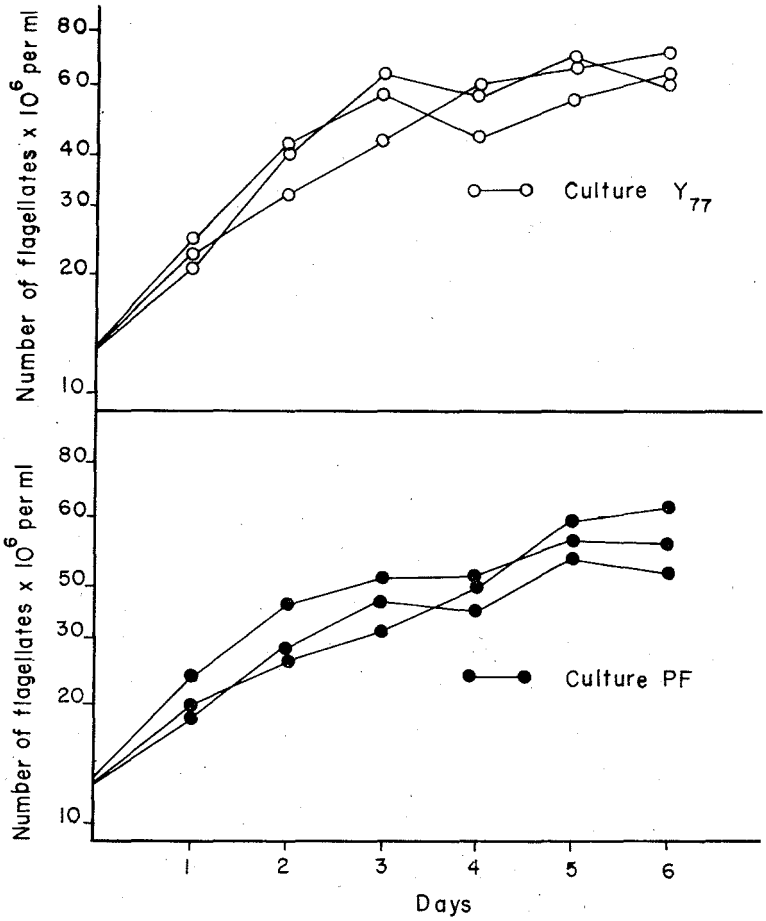


Fig. 1 — Growth curves of *T. cruzi* cultures $Y_{77}$ and PF in LIT medium.

On the other hand, a significant variation in the differentiation rate of the several cultures studied was observed. This finding shows, apparently for the first time, in cultures kept in laboratory for different periods of time after their isolation from vertebrate host, a large variation of the rate of metacyclic trypomastigote morphogenesis. The relative ability of those cultures to build up different proportions of metacyclic forms is rather constant and steady results are obtained in repeated experiments performed over large periods of time. This has been observed chiefly with the PF strain which, during 2.5 running years, produced a low differentiation rate (5%). The high differentiation rates of cultures $Y_{77}$ and $MR_{44}$ were repeatedly observed during 2 years; a gradual tendency of decrease in the proportion of metacyclic forms was thereafter detected. Nevertheless, the percentage of metacyclic forms in these cultures was never under 25-30%. The low differentiation rate of PF culture was kept after a single passage through the vertebrate host and subsequent gradual adaptation of the parasite to exponential growth in LIT medium. However, the usual capacity of cultures $Y_{77}$ and $MR_{44}$ to form a high percentage of metacyclic forms was suddenly regained by means of the passage, through albino mice. This suggests a correlation between the proportion of metacyclic forms in the cultures and the period of time they are kept in artificial medium. The regulation of the cyclic process of differentiation epimastigotes → try-

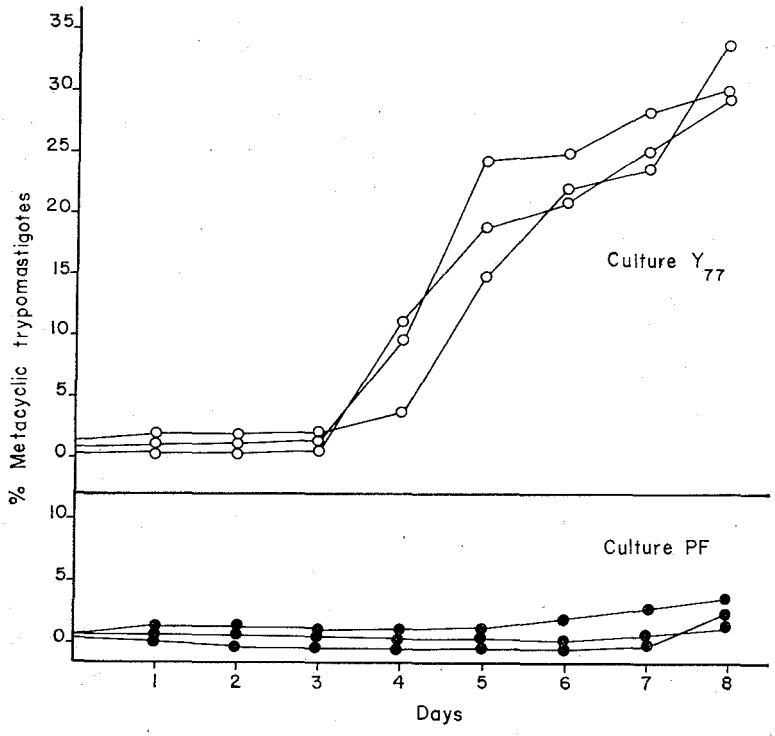


Fig. 2 — Differentiation curves of *T. cruzi* cultures $Y_{77}$ and PF in LIT medium

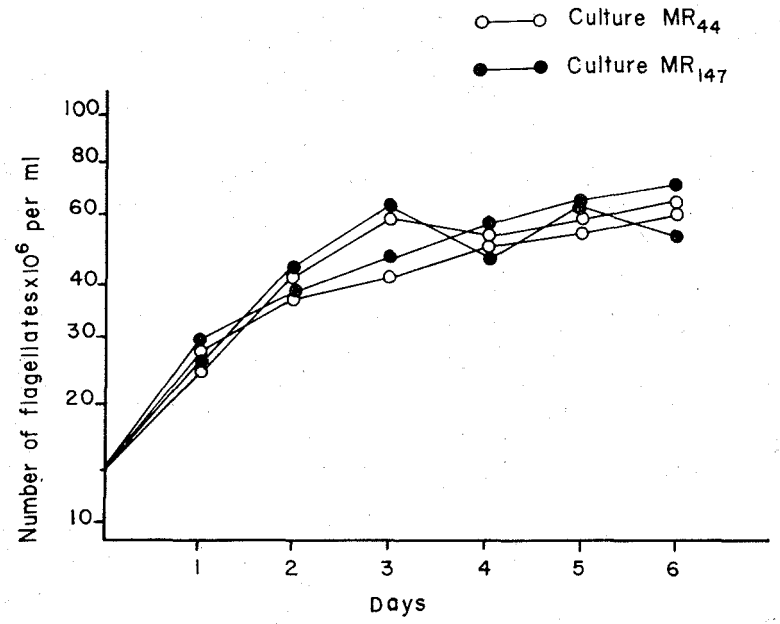


Fig. 3 — Growth curves of *T. cruzi* cultures $MR_{44}$ and $MR_{147}$ in LIT medium

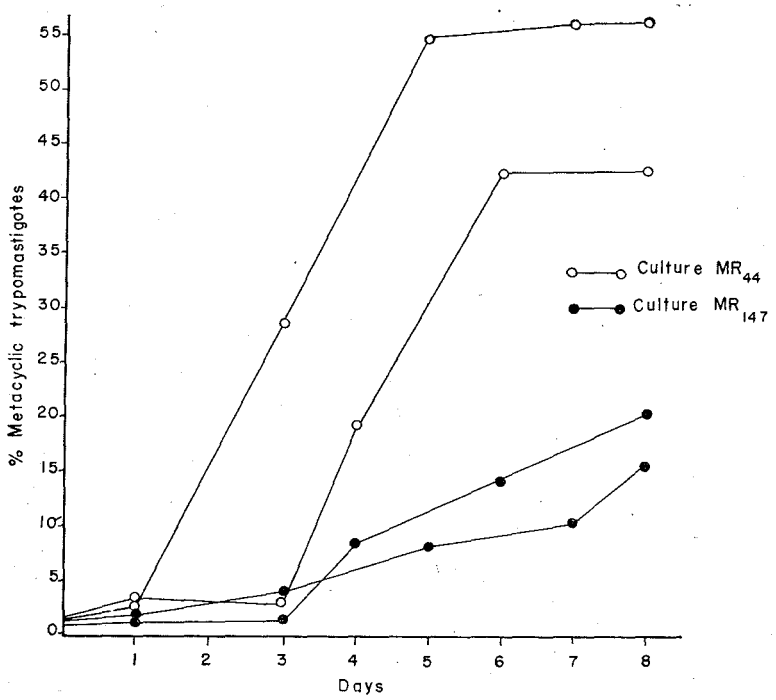


Fig. 4 — Differentiation curves of *T. cruzi* cultures $MR_{44}$ and $MR_{147}$ in LIT medium.

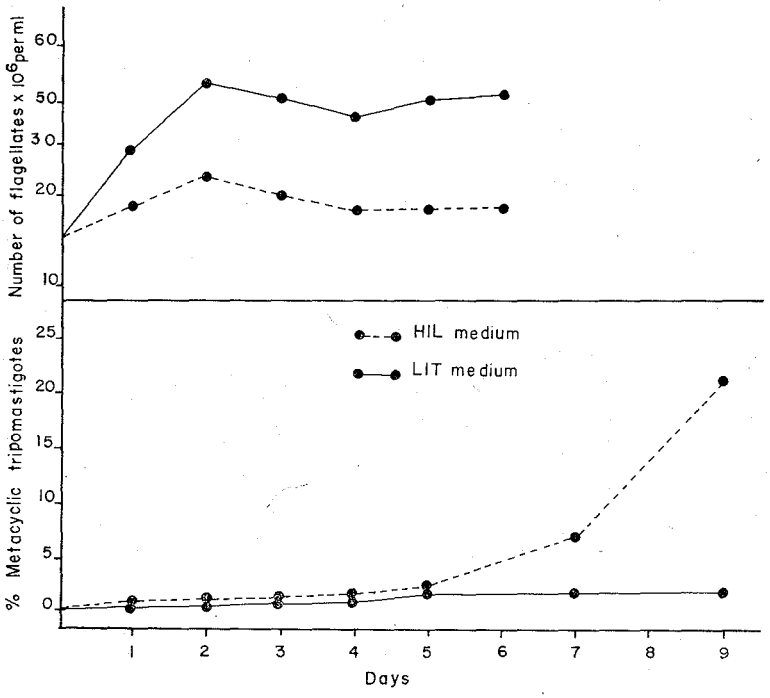


Fig. 5 — Growth and differentiation curves of *T. cruzi* culture PF in LIT and HIL media.

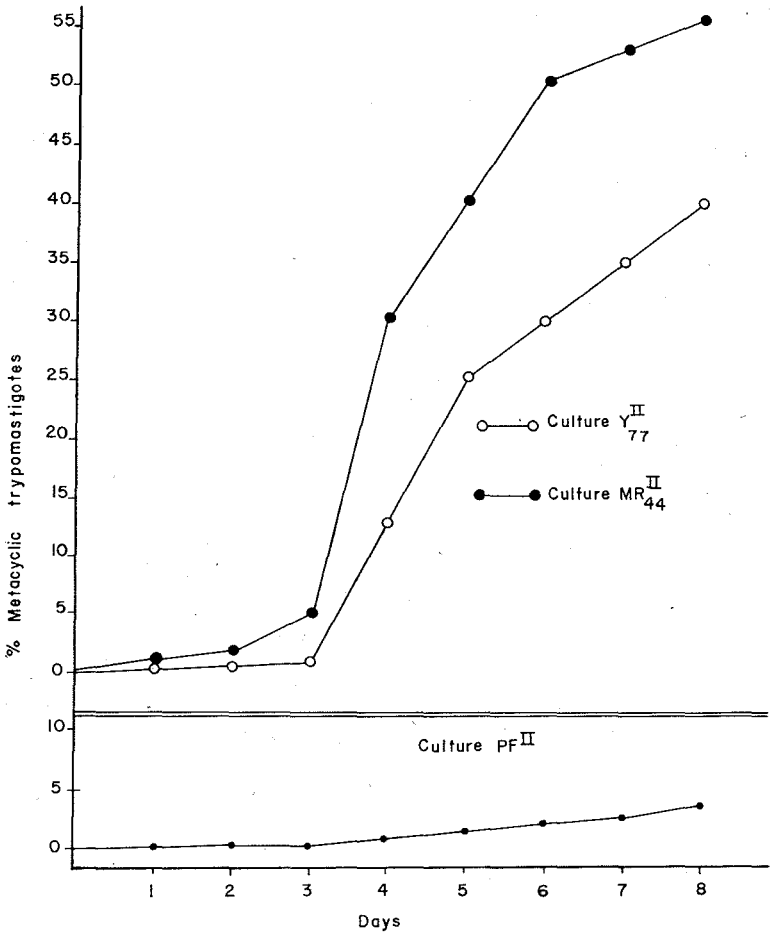


Fig. 6 — The differentiation curves of *T. cruzi* cultures $Y^{II}_{77}$, $MR^{II}_{44}$, $PF^{II}$ in LIT medium.

pomastigotes has been studied by CAMARGO [3] who showed that the proportion of metacyclic forms increases in aged cultures. This phenomenon has been explained either by a depletion of nutrients or by gradual accumulation of metabolites. The observations of STEINERT [9], who induced form transition in *Trypanosoma mega* by adding urea to the medium and of CASTELLANI et al. [4] who increased the proportion of metacyclic forms by replacing liver-infusion with heart-dog infusion in LIT medium, show that chemicals or biological substances are likely to stimulate morphogenetic processes otherwise under control of repressive gene mechanisms.

The present experiments show that besides repressing/stimulating factors present in the media some inherent parasites' characteristics probably selected by the prolonged maintenance in artificial media participate in the epimastigote → trypomastigote process of differentiation. The possibility of the occurrence of different strains showing a different behaviour as regards trypomastigotes morphogenesis soon after their isolation from the vertebrate host should be investigated. *T. cruzi* is now considered an extensive pool of populations presenting peculiar characteristics and the capacity to form infective forms may well be a further strain genoty-

pic distinguishing mark. From a practical point of view the study reported in this paper may provide a number of cultures which, under natural conditions, show a large range variation in the proportion of infective forms. This kind of material permitted to demonstrate that trypomastigotes from several cultures revealed differences in their capacity of infecting vertebrate hosts; those differences seemed also to depend on intrinsic characteristics of the metacyclic forms rather than on the number of culture forms inoculated CHIARI [5].

RESUMO

*Crescimento e diferenciação de formas de cultura do* Trypanosoma cruzi *mantidas em laboratório por diferentes períodos de tempo*

Culturas de *T. cruzi*, amostras Y e MR, mantidas em meio LIT por diferentes períodos de tempo após isolamento do hospedeiro vertebrado, apresentaram curvas de crescimento semelhantes. Entretanto a taxa de diferenciação (epimastigotas → tripomastigotas metacíclicos) na amostra Y mostrou acentuada diferença: 5% (cultura PF) e 30% (cultura $Y_{77}$). As culturas da amostra MR forneceram resultados semelhantes apresentando, no entanto, uma taxa de diferenciação mais alta do que as observadas com a amostra Y. Não pode ser identificado o fator responsável pelas diferenças relatadas, quanto a taxa de metaciclogenese.

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