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GROWTH AND DIFFERENTIATION ON A TRYPANOSOME OF THE SUBGENUS *SCHIZOTRYPANUM* FROM THE BAT *PHYLLOSTOMUS HASTATUS*

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The effects of temperature, pH, osmolarity and aeration on the growth and differentiation of a trypanosome of the subgenus Schizotrypanum *isolated from the bat* Phyllostomus hastatus *were studied. In general, the growth characteristics of the flagellate were similar to those of* Trypanosoma (Schizotrypanum) cruzi. *However, the parasite did not growth at 33 or 37C. Increase in the osmolarity and aeration promoted growth at 33C. Significant metacyclogenesis was detected only in the growth condition where maximal growth occured (28C, pH 7.3, 380mOs/kg, in tissue culture flasks), at the end of the exponential growth phase. The begining of the metacyclogenesis process was coincident with most glucose utilization and lowest pH. During metacyclogenesis both culture medium pH and osmolarity increased steadly.*

Key-words: Schizotrypanum *from* Phyllostomus hastatus. *Growth. Differentiation.*

Trypanosoma (Schizotrypanum) cruzi the causative agent of Chagas' disease in Latin America can be found in several mammalian orders including bats. The latter are also hosts of cosmopolitan trypanosomes of the subgenus *Schizotrypanum* which are almost indistinguishable morphologicaly from each other[12]. Thus, in regions where they coexist, knowledge whether isolates are *T. (S.) cruzi* or not, is of public health intertest.

Several reports have been described the influence of the physichochemical conditions, such as temperature, pH, osmolarity and aeration surface on the growth and differentiation (metacyclogenesis) of trypanosomes[2 4 7 11 13 17]. Althought non-infective stocks of trypanosomes of the subgenus *Schizotrypanum* isolated from brazilian bats have been cultivated in axenic cultures[1 8 9 14], few informations is available about the growth characteristics and metacyclogenesis among these flagellates.

In the present study we investigate the effects of temperature, pH, osmolarity and aeration surface on growth and metacyclogenesis of a trypanosome of the subgenus *Schizotrypanum* isolated from the bat *P. hastatus*. This isolate was previously unable in produce detectable parasitemia in mice[15].

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MATERIAL AND METHODS

Parasite. The trypanosome studied was isolated from a *Phyllostomus hastatus* bat collected in Serrania, Minas Gerais, Brazil. isolation was performed by hemoculture in Brain-Heart-Infusion (BHI) medium supplemented with 10% (v/v) heat inactivated fetal calf serum (FCS) and 2% of a 10% rabbit hemoglobin solution. After isolation the flagellate was plating in the same medium supplemented with 10% rabbit blood (v/v) and 0.75% agar. The flagellate wae maintained by serial passages every 10 days. It was also cryopreservated in liquid Nitrogen after adding 10% glycerol to the culture.

Cell growth and differentiation. Experiments were carried out in the above referred culture medium and the following growth conditions were assayed: temperature (25, 28, 33, 37°C) pH (6.5, 6.3, 7.3, adjusted with 1N HCl or KOH), osmolarity (380, 520, 620mOm/kg H_2O, no adding or by adding 2 or 4% sorbitol) and aeration surface (culturing in 18x180mm) screw-capped tubes or in 24cm^2 surface area tissue culture flasks). Culture medium final volume was always 5.0ml. Inoculation was performed with mid-log phase cells kept at 28°C to give the start number of 2.0x10^{5}cells/ml. Growth was estimated by counting cells in a hemocytometer after different periods of incubation (4, 8, 12, 16 days). To determine percentages of evolutive stages of the parasite in cultures, Giemsa-stained were prepared. At least 200 organisms were examined on each preparation.

Analytical determinations. Glucose, pH, and osmolarity were monitotred in the culture medium where both maximal growth and differentiation occured. After different periods of incubation (4, 8,

12, 16 days), cells were removed by centrifugation (2.500r.p.m/min) and the suppernatant store at -20°C until used. Glucose content was determined by the glucose oxidase method using a Beckman DB-Gt Spectrophotometer. pH and osmolarity were monitored with a digital pH meter (Micronal B384) and a osmometer (Advanced Wide-Ring Osmometer), respectively.

RESULTS

Cell growth. Figure 1 represents growth curves of the parasite when cultivated under different physicochemical conditions. Maximal growth was detected after 16 days with cells incubated at 28°C, pH 7.3, 380mOsm/kg H_2O in 25cm^2 tissue culture flasks. Lower aeration surface decreased the final

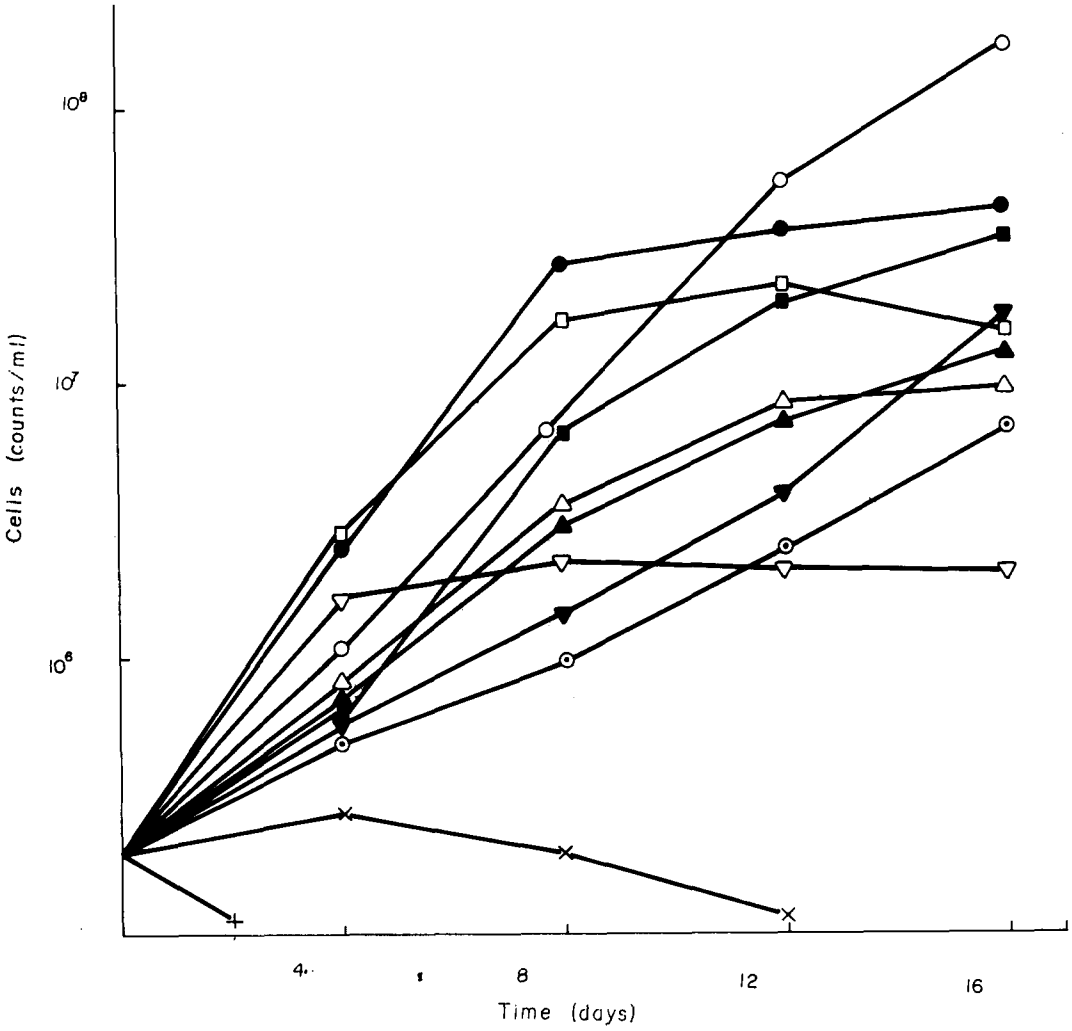

Figura 1 - Growth of a trypanosome of the subgenus *Schizotrypanum* isolated from the bat *P. hastatus* when incubed under different physicochemical conditions. The parasite was cultivated in BHI medium supplemented with 10% (v/v) fetal calf serum (FCS) and 2% of a 10% rabbit hemoglobin solution. The physicochemical conditions were as follow: (O) -28°C; pH 7.3; 380mOsm/kg H_2O in 25cm^2 tissue culture flasks; (●) -28°C, pH 6.3, 380mOsm/kg H_2O in 25cm^2 tissue culture flasks; (□) -28°C, pH 6.3, 380mOsm/kg H_2O in 18x180mm screw-capped tubes; (■) -28°C, pH 7.3, 380mOsm/kg H_2O in screw-capped tubes; (Δ) -28°C, pH 7.3, 520 mOsm/kg H_2O in 18x180mm screw-capped tubes; (▲) -28°C, pH 7.3, 620mOsm/kg H_2O in 18x180mm screw-capped tubes; (∇) -28°C, pH 5.3, , 380mOsm/kg H_2O in screw-capped tubes; (▼) -33°C, pH 7.3, 620mOsm/kg in 25cm^2 tissue culture flasks; (⊖) -25°C, pH 7.3, 380mOsm/kg H_2O in screw-capped tubes; (X) -33°C, pH 7.3, 380mOsm/kg H_2O in 18x180mm crew-capped tubes; (+) -37°C, pH 7.3, 380mOsm/kg H_2O in screw-capped tubes. Values are means of three replicates.

number of cells. Initial pH 6.3 readly acelerated growth where sharp stationary growth phases arose in the 8th day of incubation. Also here, higher aeration surface promoted better growth. Lower pH (5.3) reduced drastically the final number of cells, in which the stationary growth phase arose early on the day four of incubation. Increases in the osmolarity by adding sorbitol, caused a sligh inhibition of growth. Incubation at 25°C reduced markedly the growth rate. The parasite did not growth at 33 or 37°C, however, increase in the osmolarity and aeration promoted growth at 33°C.

Cell differentiation. Figure 2 summarizes observations on the percentages of the parasite stages in cultures after 16 days of incubation. Increase in the percentage of trypomastigote forms was only detected with cells cultivated at 28°C, pH 7.3, 380mOsm/kg H_2O in tissue culture flasks. In this condition, the percentage of trypomastigotes increased steadly after 12 days of incubation, reaching about 51% at the end of the observation time (Figure 3)

Changes in the culture medium during growth and differentiation. During the exponential growth phase, glucose utilization was parallel to increases in the epimastigote number and decrease in pH. In this growth phase the percentage of this proliferative stages remained practicaly unalterated. The start of the metacyclogenesis process was concomitant with most glucose utilization, lowest pH, and the end of the exponential growth phase. During differentiation, the culture medium pH increased from 6.7 to 7.4 and the osmolarity changed from 380 to 422 mOsm/kg H_2O. These results are shown in Figure 3.

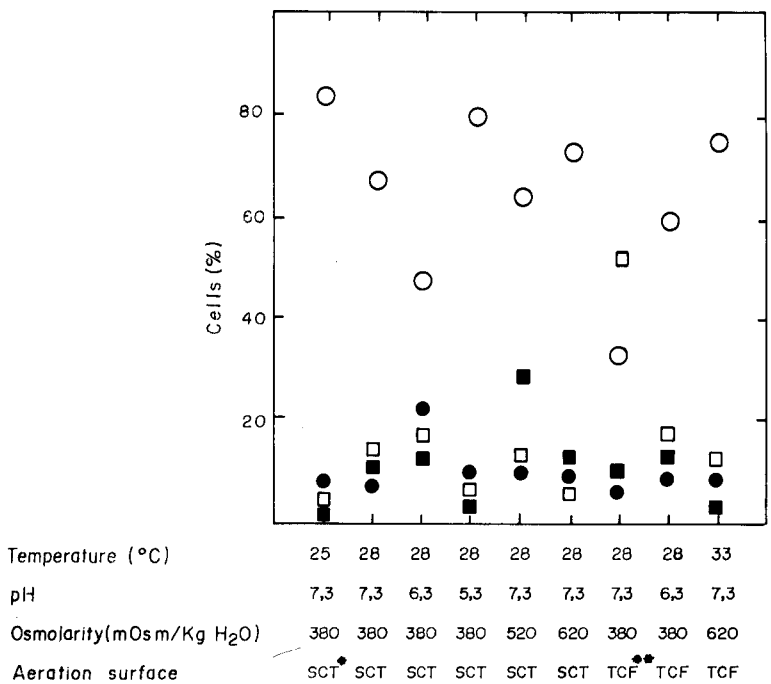

Figure 2 - Percentage of (O) epimastigote, (●) transitional epimastigote, (□) trypomastigote and (■) spheromastigote forms in the culture of a trypanosome of the subgenus *Schizotrypanum* isolated from the bat *P. hastatus*, after 16 days of incubation under different physicochemical conditions. The parasite was cultivated in the BHI medium supplemented with 10% (v/v) fetal bovine serum (FCS) and 2% of a 10% rabbit hemoglobin solution. Values are means of three replicates.

* Screw-capped tubes; ** Tissue culture flasks.

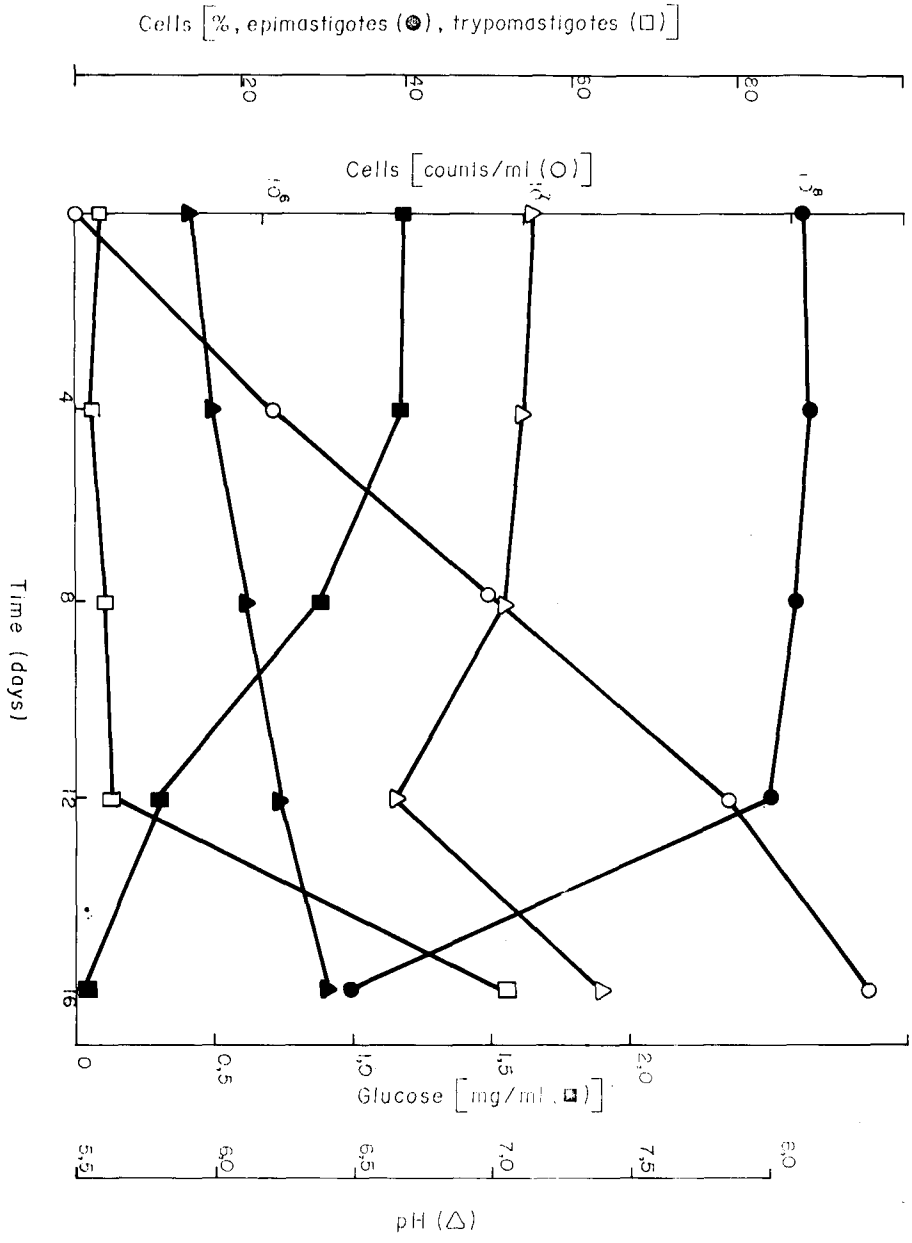

Figure 3 - Number of cells, percentage of epimastigotes and trypomastigotes, as well as glucose, pH and osmolarity changes in the culture medium during growth and metacyclogenesis of a trypanosome of the subgenus *Schizotrypanum* isolated from the bat *P. hastatus*. The parasite was cultivated in the BHI medium supplemented with 10% (v/v) fetal bovine serum (FCS) and 2% of a 10% rabbit hemoglobin solution at 28°C, pH 7.3, 380mOsm/Kg H_2O in tissue culture flasks. Values are mean of three replicates.

DISCUSSION

In general, the growth characteristics presented by the parasite studied when cultivated under different physicochemical conditions were similar to those of *T. (S.) cruzi*[2 10 13 17]. However, in our study the flagellate did not growth at 33 or 37°C. In previous report the parasite studied was unable to infect normal or irradiated C3H mice[15]. The sensibility to higher temperatures may be a characteristic widely distributed among trypanosomes of the subgenus *Schizotrypanum* from bats and migth be useful to discriminate wheter isolates from bats are *T. (S.) cruzi*, or not.

Metacyclogenesis is believed to be a process that preadap the trypanosomes to the vertebrate host. Inour study, this morphogenetic process only occured in the growth condition where maximal growth occured. Our results are in agreement with the Steinert statement that a precise period of growth is critical before metacyclogenesis process start[16]. In addition, the physiological events detected during differentiation were very similar to those found for *T. (S.) cruzi*[11]. At the present time, it is well known that epimastigotes of *T. (S.) cruzi* during the exponential growth phase, utilize glucose via glucolisis, and succinate, acetate and CO_2, are the main end products excreted[5]. After most of glucose is consumed a shift to amino acid catabolism with CO_2 and amonia production, occurs[3]. Aditionally, some of these compounds or other excretion products may acts as osmoactive substances. It was found that *Leishmania major* promastigotes excrets this kind of substances in the culture medium, mainly alanine[6].

More studies about on the growth characteristics and metacyclogenisis of trypanosomes of the subgenus *Schizotrypanum* isolated from bats collected over a large geographic area, are needed. Finally, the enigma why trypanosomes of this subgenus from bats, other than *T. (S.) cruzi*, are host restricted to bats only, remains to be answered.

RESUMO

Foram estudados os efeitos da temperatura, do pH, da osmolaridade e da areação sobre o crescimento e a diferenciação de um tripanosoma do subgenêro Schizotrypanum, *isolado do morcego* Phyllostomus hastatus. *Em geral, as características do crescimento do flagelado foram semelhantes àquelas do* Trypanosoma (Schizotrypanum) cruzi. *Entretanto, o parasita não de desenvolveu a 33 ou 37°C. O aumento na osmolaridade e areação estimulou o crescimento a 33°C. Metaciclogênese sifnificativa foi detectada somente na condição de crescimento, onde ocorreu desenvolvimento máximo (28°C, pH 7.3, 320mO/kg H_2O, em frascos de cultura de tecido), no final da fase do crescimento exponencial. O início do processo de metaciclogênese coincidiu com maior utilização de glucose e menor pH. Durante a metaciclogênese, o pH do meio de cultura e a osmolaridade aumentaram constantemente.*

Palavras-chaves: Schizotrypanum *do morcego* Phillostomus hastatus. *Desenvolvimento. Diferenciação.*

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