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Growth of infective forms of *Trypanosoma (T.) brucei* on buffalo lung and Chinese hamster lung tissue culture cells

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Summary

Infective cultures of *Trypanosoma (T.) brucei* (strain 427) have been initiated and maintained on Chinese hamster lung tissue culture cells and buffalo lung tissue culture cells. By changing daily one-third of the RPMI-1640 plus 20% fetal bovine serum medium, the cell numbers can be maintained at $2–4 \times 10^6$ cells/ml. The cultured trypanosomes on these two tissue culture cell types were infective to mice and morphologically similar to bloodstream slender trypomastigotes in having a subterminal kinetoplast and a surface coat. In addition, they possessed the L-α-glycerophosphate oxidase, the predominant steady state terminal oxidase identified in bloodstream trypomastigotes.

Key words: Trypanosoma (T.) brucei; cultured infective trypomastigotes; Chinese hamster lung tissue culture cells; buffalo lung tissue culture cells; L-α-glycerophosphate oxidase.

Introduction

The development of a medium and conditions under which infective trypanosomes, the causative agents of African sleeping sickness, can be maintained have been attempted for numerous years without significant success. However, the recent excellent description by Hirumi et al. (1977) of conditions which will allow the development of infective forms of *Trypanosoma brucei* in culture has stimulated further studies in this area. Using bovine fibroblast-like cells in HEPES-buffered Rosewell Park Memorial Institute (RPMI) 1640 medium plus 20% inactivated fetal bovine serum (FBS), these investigators were able to maintain trypanosomes infective for mammalian hosts which also retained the

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morphological characteristics of long slender bloodstream trypomastigotes with the surface coat (Hirumi et al., 1977).

Our studies have been directed towards identifying established tissue culture cell lines which may support the growth of *T. brucei* at high yield and developing simple procedures for establishing these infective trypomastigotes in culture. In addition, we want to determine the biochemical similarities between our infective trypomastigotes maintained in culture and slender bloodstream infective trypomastigotes isolated from rats or mice. In this paper we report that it has been possible to initiate and maintain infective forms of *T. brucei* 427 on buffalo *(Bison bison)* lung tissue culture cells (ATCC CCL 40, BU, IMR-31) or Chinese hamster *(Cricetulus griseus)* lung tissue culture cells (ATCC CCL 16 Don). The trypanosomes maintained on either of these tissue culture cells are infective to mammalian hosts and possess the L-α-glycerophosphate oxidase system characteristic of bloodstream forms.

Materials and methods

The Chinese hamster lung (ATCC CCL 16 Don) or buffalo lung (ATCC CCL 40, Bu, IMR-31) tissue culture cells were obtained from The American Type Culture Collection. They were maintained in RPMI-1640 with 20% FBS containing per 100 ml, penicillin – 10,000 U, fungizone – 25 μg, streptomycin – 10,000 mg and kanamycin – 12.5 μg (RPMI medium). The morphology of both tissue cell lines was fibroblast-like. An inoculum of 1.8×10^6 viable tissue culture cells in T-25 flasks or 3.0×10^6 tissue culture cells in T-75 flasks multiplied 3–5 fold in 7 days in the described culture medium at 37° C in an atmosphere of 5% CO_2 – 95% air. The tissue culture cells were used just prior to becoming confluent, usually 3–4 days after a flask was inoculated.

The preparation of the trypanosomes was simplified over the procedures described by Hirumi et al. (1977). Successive cultures were established with *T. brucei* 427 from lethally irradiated rats that were inoculated with 2×10^6 trypanosomes. Three to four days later, when the parasitemia reached a level of $1–5 \times 10^8$ trypanosomes/ml, the rats were bled by cardiac puncture. The trypanosomes were centrifuged at 1025 *g* at 25° C and the buffy coat removed. Special precautions were taken to prevent the removal of red blood cells below the buffy coat. The trypanosomes were washed twice with sterile Hank's balanced salt solution with 5% sodium citrate. The trypanosomes were then inoculated into a total of 5.0 ml of RPMI medium in T-25 flasks or 15.0 ml in T-75 flasks. Most successful experiments occurred in those initiated in the T-75 flasks. The steady state studies using the respirograph system were performed as previously described for *T. mega* (Hill and Degn, 1977).

Results and discussion

Two different tissue culture cell lines, Chinese hamster lung and buffalo lung, were used. The biochemical results were the same with either cell line. A typical experiment was performed by inoculating a T-75 flask containing a monolayer of buffalo lung cells with an initial concentration of 8×10^4 trypanosomes/ml. The cultures were incubated at 37° C in an atmosphere of 5% CO_2 – 95% air in the RPMI medium. Two days later, a third of the medium was changed. After a lag of four days, the cell numbers then began to increase. By day 8, the cell count was 5×10^5/ml. One-third of the medium was then changed

Table 1. Growth of *T. brucei* on Chinese hamster lung cells

Day	Cell numbers* (trypanosomes/ml)
0	1.5×10^5
1	4.0×10^5
2	1.2×10^6
3	2.1×10^6
4	1.9×10^6
5	1.2×10^6
6	1.6×10^6

* One-third of the RPMI medium was changed daily.

daily and on day 11 the cell count was 2.5×10^6 cells/ml. By changing one-third of the medium daily, the cells were maintained at a concentration of $2–4 \times 10^6$ cells/ml over the original buffalo lung cells for 30 days. However, in our routine procedures, the tissue culture cells are usually changed every 7–10 days. Experiments initiated on Chinese hamster lung cells were generally the same. At present, we have maintained the trypanosomes initiated on the Chinese hamster lung or buffalo lung cells for 60 days.

It was also possible to initiate successful cultures after incubation of the trypanosomes in the RPMI medium at 25° C for 24 h prior to adding the trypanosomes to the Chinese hamster lung or buffalo lung tissue culture cells at 37° C. Cells maintained for three days at 25° C on RPMI medium were as infective as controls for mice and rats. In addition, 20–30% of the cells were observed dividing on day 2 and day 3.

Additional cultures from buffalo lung or Chinese hamster lung flasks were initiated on either tissue culture cell. The flasks were usually initiated at 2×10^5 cells/ml. The results of a typical experiment on Chinese hamster cells are seen in Table 1. On day 3 after inoculation, with one-third of the cell medium changed daily, the cell count was 2.1×10^6 cells/ml, reflecting a division time of 12 h. The daily trypanosome count continued at $1.0–3.0 \times 10^6$ cells/ml until the tissue culture cells had to be changed.

The trypanosomes grow differently on the two tissue culture cells. On the buffalo lung cells, the trypanosomes grow not only in the medium but also in spaces between the tissue culture cells (Fig. 1). This is particularly true after the cells are established and growing at a concentration above 2×10^6 cells/ml. This was also reported by Hirumi et al. (1977) for trypanosomes growing on bovine fibroblast-like cells. In contrast, the trypanosomes growing on Chinese hamster lung cells remained in the medium and grew close to the tissue culture cells only in older cultures. Thus, the cell counts of the trypanosomes maintained over Chinese hamster cells are more accurate than those maintained on buffalo lung cells.

Trypanosomes maintained on buffalo lung cells or Chinese hamster lung cells have a subterminal kinetoplast, clearly distinguishing the trypanosomes as long, slender trypomastigotes. The ultrastructure of *T. brucei* maintained on buffalo lung cells or Chinese hamster lung cells can be seen in Fig. 2 and 3. The surface coat is evident as well as the mitochondrion with few cristae, both features characteristic of slender bloodstream trypomastigotes.

Experiments to test the infectivity of the cultures were performed with stabilates of bloodstream trypomastigotes of *T. brucei* 427 as controls. The mice were inoculated with 10^4, 10^5 or 10^6 cells. The results of an experiment are given in Fig. 4. Mice inoculated with trypanosomes from bloodstream stabilates or infective cultures from Chinese hamster lung or buffalo lung cells develop comparable parasitemias and death occurred after a similar period of time.

In order to determine the terminal oxidase present in the cultured infective forms, the steady state level of the terminal oxidases present was determined. Ninety ml of trypanosomes cultured in three flasks over Chinese hamster lung cells were harvested at a concentration of 2.5×10^6 cells/ml. The cells were collected by centrifugation and the steady state oxygen levels observed. The results are given in Fig. 5. Salicylhydroxamic acid (SHAM) inhibited 90% of the cell respiration. Azide had little effect, inhibiting 5% of the cell respiration. No significant azide and SHAM insensitive respiration was observed. These data are similar to previously published results for bloodstream trypomastigotes of *T. brucei* (Hill, 1977). They clearly indicate that the SHAM sensitive α-GP oxidase is present in these organisms. Identical results were obtained for trypanosomes grown on buffalo lung tissue culture cells.

The procedures presented in this paper provide a simplified technique for initiating and maintaining large quantities of bloodstream forms of *T. brucei* 427 in culture on established tissue culture cell lines. The trypanosomes have been stored as frozen stabilates at –90° C in a 1:1 mixture of 20% glycerol in Hank's balanced salt solution in the culture. The thawed stabilates are infective to mice and can continue to develop on Chinese hamster lung or buffalo lung tissue culture cells. The concentration of cells obtained in our experiments ($2–4 \times 10^6$ cells/ml) provide adequate numbers of trypanosomes to support further biochemical and immunological studies.

Fig. 1. *T. brucei* slender trypomastigotes growing in the spaces between the buffalo lung tissue culture cells. The cells had been growing in culture for 25 days. $\times 200$.

Fig. 2. Electron micrograph of *T. brucei* trypomastigote which was grown on Chinese hamster lung tissue culture cells for 45 days. The microtubules (mt), plasma membrane (pm) and surface coat (sc) are quite distinct. The surface coat also surrounds the flagellum (F). $\times$ 100,000.

Fig. 3. Electron micrograph of *T. brucei* trypomastigote which has been growing on buffalo lung tissue culture cells for 25 days. Note the presence of the surface coat and the absence of cristae in the mitochondrion (M). The kinetoplast (K) region of the mitochondrion is evident. The buffalo lung tissue culture cell (BL) is present near the trypanosome. $\times$30,000.

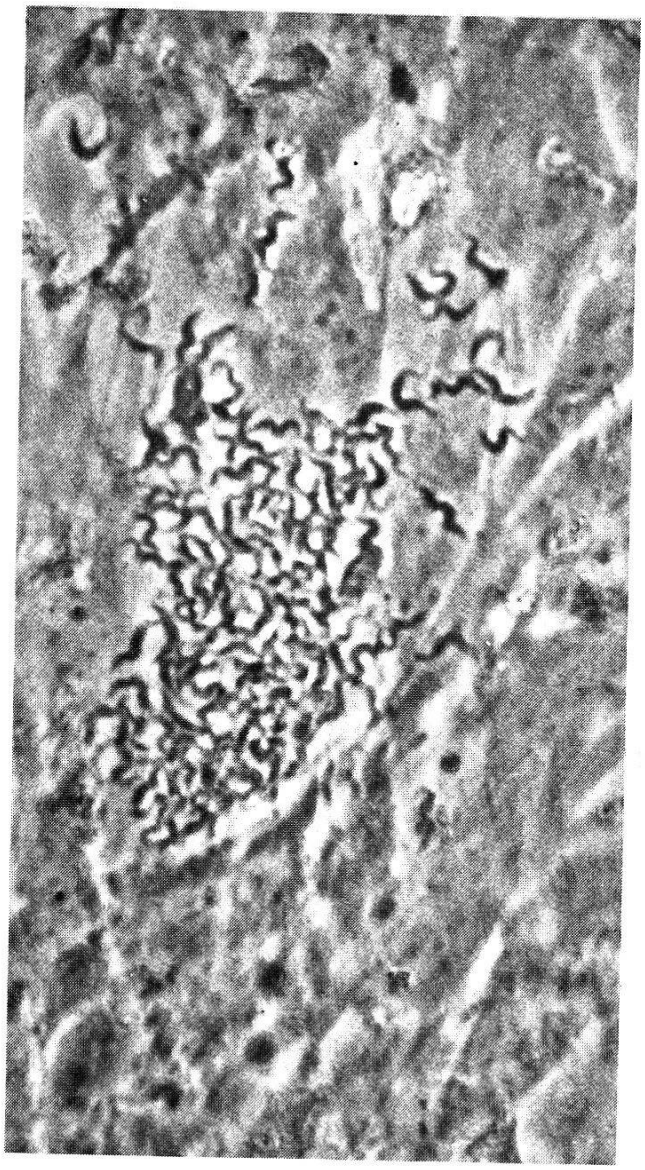

Fig. 1

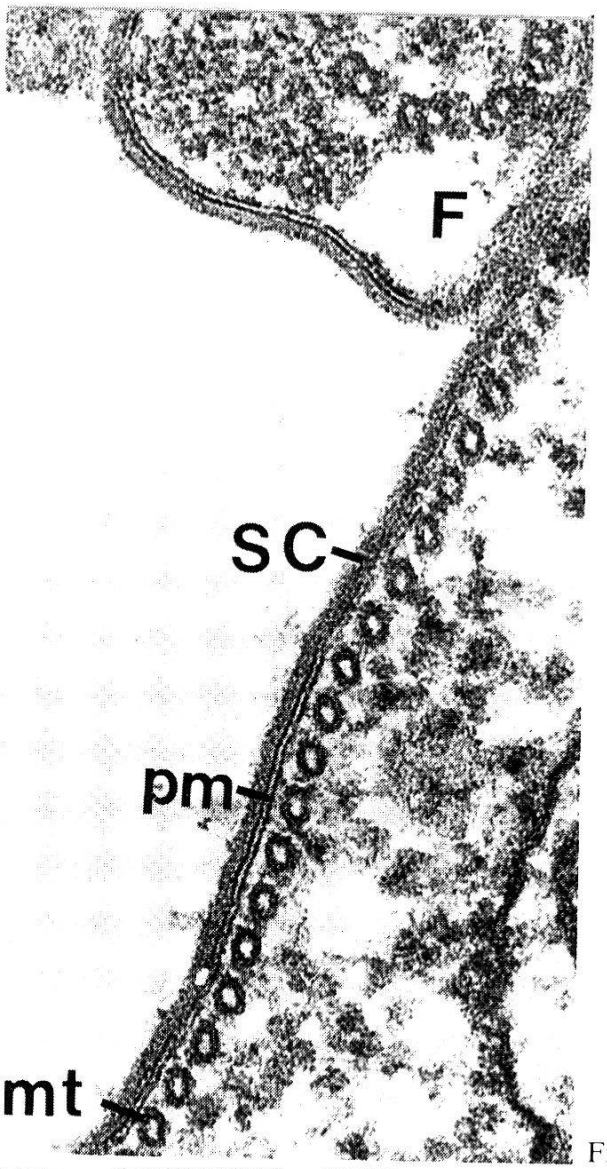

Fig. 2

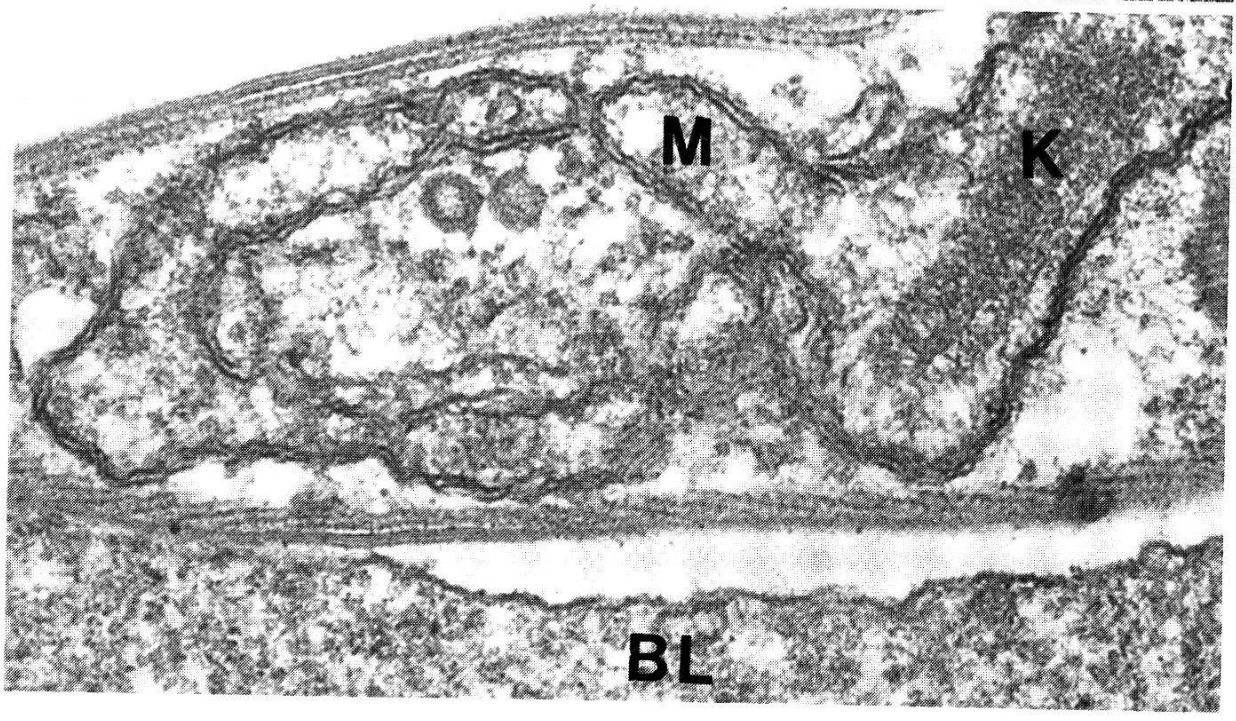

Fig. 3

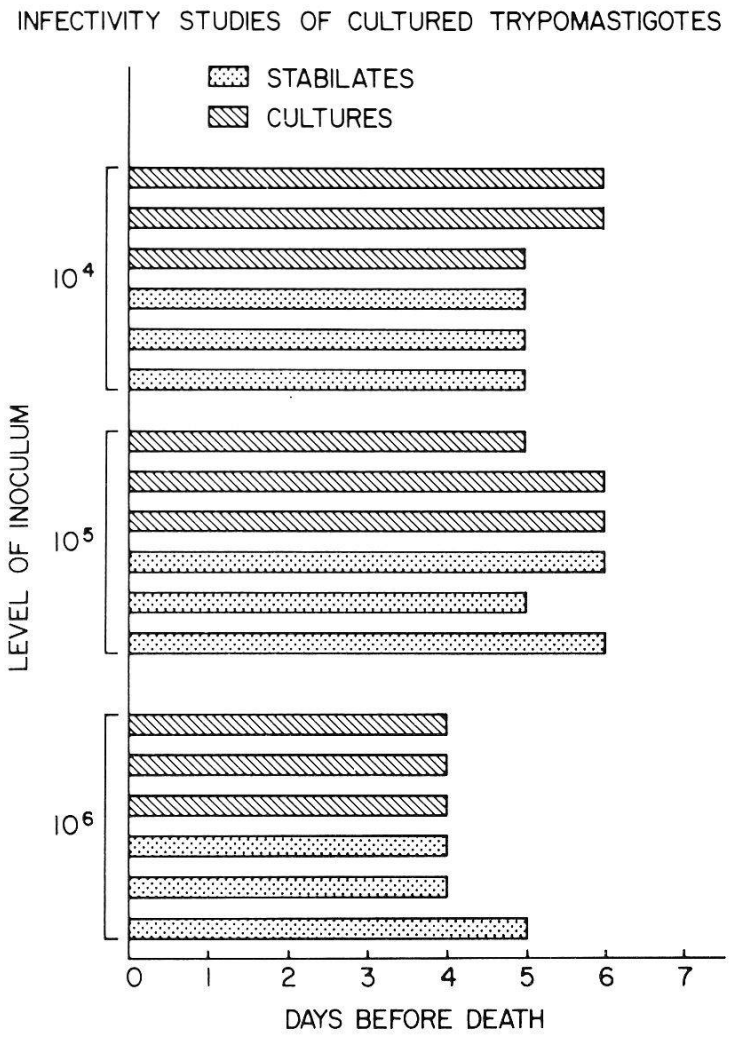

Fig. 4. Graph of infectivity test of cultured trypomastigotes of *T. brucei*. The control was a stabiliate of *T. brucei* bloodstream trypomastigote. The cultured trypomastigotes had been maintained in vitro for 25 days on buffalo lung tissue culture cells.

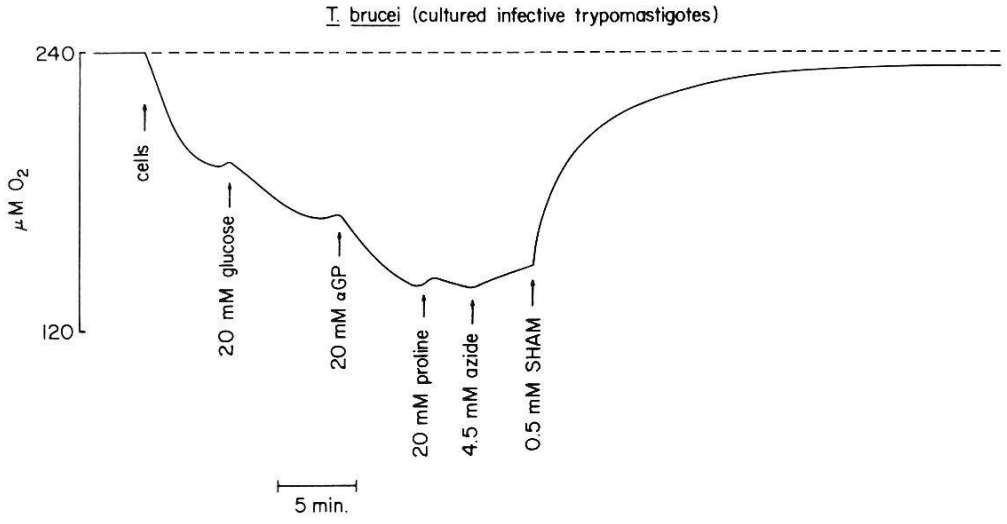

Fig. 5. Steady state oxygen trace of *T. brucei* trypomastigotes maintained in vitro for 45 days on Chinese hamster lung tissue culture cells. The concentration of cells was 1.7×10^8 cells.

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