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Cultivation of vertebrate infective forms derived from metacyclic forms of pleomorphic *Trypanosoma brucei* stocks

Short communication

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The first success in the continuous cultivation of vertebrate infective forms of *Trypanosoma brucei* has been recently reported (Hirumi et al., 1977a, b; Hill et al., 1978a). These investigators used HEPES buffered RPMI 1640 (Rosewell Park Memorial Institute) medium supplemented with 20% inactivated foetal bovine serum over a feeder layer of bovine or other fibroblast-like cells. Le Page (1967) had earlier attempted similar experiments, using a feeder layer in combination with tissue culture medium but with little success. It has to be pointed out that these investigators were using the monomorphic *T. brucei* stock 427 which no longer exhibits pleomorphism in the vertebrate host and is not transmissible by the tsetse fly. Hill et al. (1978b) have reported the successful growth of a pleomorphic *T. rhodesiense* stock as vertebrate infective forms in vitro. In this note we present a new culture system for the long-term cultivation of vertebrate infective bloodstream-like forms of pleomorphic, tsetse fly transmissible stocks of *T. brucei* and *T. rhodesiense*.

The following trypanosome stocks were grown in vitro (Table 1):

- *T. brucei* STIB 247 was isolated in 1971 in the Serengeti National Park (Tanzania) from a hartebeest (*Alcelaphus buselaphus*) and cryopreserved after one rat passage. For the last 7 months it was cyclically passaged in our laboratory.
- *T. brucei* STIB 366A, a derivative of a stock which we received in 1974 as S42/030 from the Molteno Institute, Cambridge, England. It was isolated in 1966 in Tororo, Uganda from a warthog (*Phagochoerus aethiopicus*).
- *T. brucei* STIB 345A is a derivative of the stock EATRO 1529 which was isolated in 1969 from an infected *Glossina pallidipes* in Kiboko, Kenya and cryopreserved after 6 passages in mice. In 1973 this material was stabilized after 5 short passages in rats as STIB 345.
- *T. (T.) brucei* stock 427 was received in 1977 from ILRAD, Nairobi as cultured bloodstream-like forms in a bovine fibroblast culture (Hirumi et al., 1977a, b) and was kept for 2 years in culture in our laboratory.

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Table 1. Trypanosome stocks

Trypanosome stock	Source	Time in culture as bloodstream-like form*
STIB 247	metacyclic forms from <i>Glossina m. morsitans</i>	> 6 months
	mouse blood	> 3 months
STIB 366A	mouse blood	> 3 months
STIB 345A	mouse blood	> 3 months
427	cultured bloodstream-like forms from bovine fibroblast culture**	> 3 months
STIB 350	mouse blood	38 days***

* also remaining infective for mice

** see Hirumi et al. (1977a, b)

*** terminated due to fungal contamination

- *T. rhodesiense* STIB 350 is a clone of STIB 241 which was isolated in 1971 in the Serengeti National Park from a lion (*Panthera leo*). This stock was tested on man in 1973 and found to be infective for the human.

Our new culture system allows the propagation of pleomorphic *T. brucei* stocks and consists of a rabbit fibroblast-like cell line in a modified MEM (Minimum Essential Medium) supplemented with 15% rabbit serum. The rabbit fibroblast-like cells were isolated from blood of a young rabbit (New Zealand White) according to the method described by Hirumi et al. (1977b). The fibroblasts were grown in T-25 tissue culture flasks in the modified MEM plus 15% inactivated foetal bovine serum or newborn calf serum. Subcultures were made every 7–10 days. At passage 7 the cells were cryopreserved.

The modified MEM consists of Minimum Essential Medium (Eagle) with Earle's salts and Na-bicarbonate, without L-glutamine (Cat. no. 109; Gibco Bio-Cult Ltd.) and was supplemented with HEPES buffer (21 mM), glucose 1.5 g/l, L-glutamine (0.3 g/l), Na-pyruvate (1 mM) and 1% MEM nonessential amino acids (100×). This medium was further supplemented with 15% freshly prepared rabbit serum which could be stored at –20° C for several weeks. Five commercially available rabbit sera (Flow Laboratories Ltd., Gibco Bio-Cult Ltd., Microbiological Associates, North American Biologicals Inc., Seromed GmbH) have so far given disappointing results. Penicillin (100 U/ml) and streptomycin (100 µg/ml) were also added.

Cultures were initiated in 24-well tissue culture plates (Costar, Cambridge, USA) containing an almost confluent layer of rabbit fibroblast-like cells. Bloodstream forms from a mouse or metacyclic forms from infected *Glossina* were used as inoculum. Pupae of *Glossina m. morsitans* were obtained from Dr. A. M. Jordan (Langford, England) and teneral flies infected according to Jenni (1977).

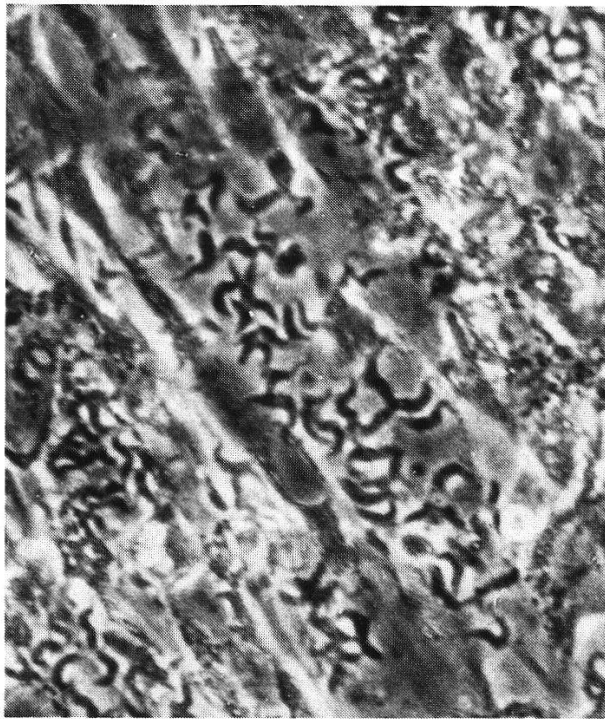


Fig. 1

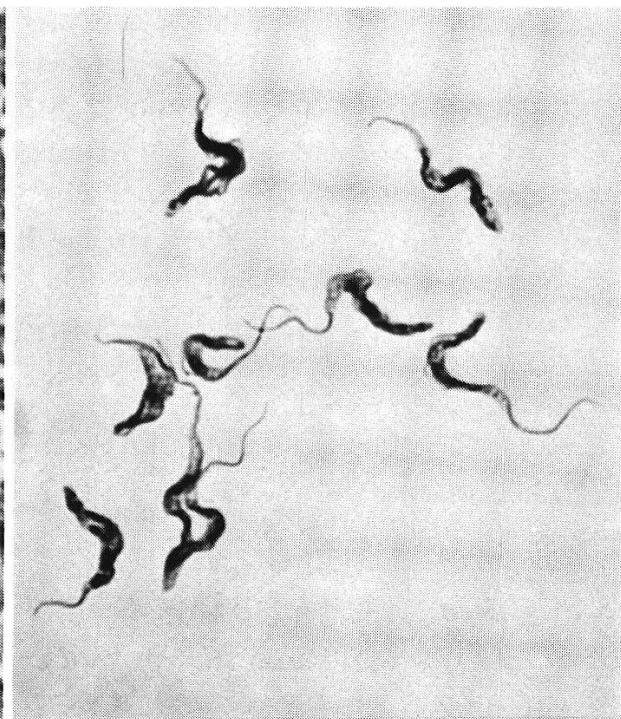


Fig. 2

Figs. 1 and 2. *Trypanosoma brucei* STIB 247 vertebrate infective forms in vitro.

Fig. 1. Formalin fixed feeder layer of rabbit fibroblast-like cells with intercellular clusters of trypanosomes, 3–4 days after subcultivation. 425 \times .

Fig. 2. Giemsa stained smear of pleomorphic forms growing in the supernatant of an established culture over the feeder layer. Slender, intermediate, stumpy and dividing forms are found at the same time. 750 \times .

Metacyclic forms were harvested by letting salivary gland infected tsetse flies salivate into a drop of warmed medium. After counting, the metacyclic forms were transferred into a well containing 1.0 ml medium. The number of trypanosomes extruded by one *Glossina m. morsitans* (3000 to 8000 salivary gland forms) was sufficient to initiate growth in a single well. The culture plate was incubated in 4% CO₂ in air at 36–37° C. After 24 h all metacyclic forms had transformed to long slender bloodstream-like forms and the first dividing forms could be observed. After 48 h half of the medium was exchanged by carefully removing 0.5 ml from the top of the well and adding 0.5 ml fresh medium. By that time the trypanosomes had formed clusters in the fibroblast layer (Fig. 1). Once an intercellular trypanosome population had been established the medium could be exchanged completely every day.

Cultures could also be initiated with bloodstream forms from mice. Female Swiss ICR mice were infected with the trypanosome stocks and the parasitaemia was daily examined. A rising parasitaemia with mainly slender and a high percentage of dividing forms turned out to be the most suitable trypanosome source. Citrated blood was collected aseptically from the heart and mixed with an equal volume of modified MEM. The blood cells were removed by a slow

centrifugation (15 min, 50 g) and the trypanosomes pelleted (7 min, 1000 g) from the supernatant. The pellet was resuspended in modified MEM, the trypanosomes counted and the wells of a tissue culture plate inoculated with various concentrations, ranging from 10^4 to 10^6 bloodstream forms per well. It was necessary to exchange the medium partially or completely every 24 h, depending on the trypanosome density. A subculture into a new fibroblast culture was carried out about every second week but the same feeder layer could normally be used for over one month (with some cultures for even more than 4 months). Established cultures reached in the supernatant final trypanosome densities between 1×10^6 and 2×10^6 per ml.

During the whole period of cultivation the bloodstream-like trypanosomes retained their infectivity for mice, in which a pleomorphic trypanosome population developed. The cultured bloodstream-like forms exhibited pleomorphism in vitro: slender, intermediate and also stumpy forms could be observed at the same time (Fig. 2). This observation was supported by the finding that the cultured bloodstream forms had the capability to transform to procyclic vector culture forms when transferred to SDM-79 (Brun and Schönenberger, 1979) at 27° C. Experiments are in progress to infect tsetse flies with cultured bloodstream-like forms by membrane feeding to demonstrate the full retention of the stocks original characteristics.

This system is currently being used to study in vitro the transformation of the metacyclic forms to the bloodstream forms as well as for comparative studies of initial antigenic variation in vitro and in vivo.

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