Cyclical Transmission of in vitro Cultivated Bloodstream Forms and Procyclic Trypomastigotes of *Trypanosoma brucei brucei* by *Glossina morsitans morsitans*

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**Abstract.** In vitro cultivated bloodstream and procyclic forms of *Trypanosoma b. brucei* STIB 247 were cyclically transmitted by *Glossina m. morsitans*. The tsetse flies were infected artificially on a silicon membrane. Metacyclic trypanosomes from mature salivary gland infections were used to initiate bloodstream form cultures. They transformed into slender bloodstream forms and gave rise to established cultures that proved to be infective for the vector. The metacyclic forms retained the strain-specific basic set of variable antigen types.

**Introduction**

Bloodstream forms of pleomorphic *Trypanosoma brucei* stocks can be grown in vitro at 37°C (Brun et al. 1979, 1981) retaining their infectivity for mammalian hosts as well as the capacity to exhibit pleomorphism in culture. Cultivated bloodstream forms transform in vitro at 28°C to procyclic trypomastigotes in medium SDM-79 (Brun and Schönenberger 1979). The potential to undergo transformation to procyclic trypomastigotes suggests that culture bloodstream forms should be capable of infecting tsetse flies, ultimately resulting in mature salivary gland infections. However, this has not yet been demonstrated, although there are reports on salivary gland infections in tsetse flies fed with procyclic culture forms (Gordon and Miller 1961; Gray 1966; Evans 1979).

The variable antigen types (VAT) of metacyclic trypanosomes obtained from flies infected with cultivated trypanosomes have not been analyzed so far. Reversion of the metacyclic forms to a strain-specific basic set of VATs after cyclical passage through tsetse flies has been demonstrated for several strains of *T.b. gambiense* (Gray 1975) and *T.b. brucei* (Jenni 1977).
Antigenic variation also occurs in continuous bloodstream form culture (Doyle et al. 1980; Jenni and Brun 1981), in a manner similar to that in the mammalian host. Therefore we wanted to find out whether maintenance of the trypanosomes in culture influences the strain-specificity of metacyclic forms obtained from flies infected with culture trypanosomes. The aims of the present study were to show the transmissibility of culture forms of a *T. b. brucei* stock by the vector and to demonstrate the reversion of the metacyclic forms to the basic set of VATs.

Materials and Methods

**Trypanosome Stock and Trypanosome Cultivation.** *Trypanosoma b. 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 procyclic cultures bloodstream forms from mice were taken on days 43 and 29 post infection and transformed into procyclic trypomastigotes in the medium SDM-79 (Brun and Schönenberger 1979). Bloodstream forms were cultivated in a system consisting of a feeder layer of rabbit fibroblast-like cells in a modified Eagle’s MEM with Earle’s salts supplemented with 15% rabbit serum and penicillin 100 IU/ml (Brun et al. 1979, 1981). These cultures were initiated with bloodstream forms from female Swiss-ICR mice on day 32 post infection or with metacyclic forms from a positive tsetse fly.

**Tsetse Flies.** Freshly deposited pupae of *Glossina m. morsitans* were obtained from the Institut d’Elevage et de Médecine Vétérinaire des Pays Tropicaux; Maisons-Alfort (France) and from the International Laboratory for Research on Animal Diseases (ILRAD), Nairobi (Kenya). Pupae were kept on sterile sand at 29°C and 70–80% relative humidity until eclosion. Newly emerged flies were transferred to clean Geigy cages and kept in a separate incubator at 26°C and 70–80% relative humidity.

**Membrane Feeding.** The membrane feeding system as described by Bauer and Wetzel (1975) was used for the infection and the maintenance of the flies. Freeze-dried pig blood, prepared by Dr. H. Wetzel, IAEA (Vienna, Austria), was reconstituted in sterile distilled water and stored frozen in batches at −26°C. Procyclic forms from log phase cultures were offered to the flies in a cell density of 10^7 cells/ml either in medium SDM-79 or in a 1:1 mixture of medium and pig’s blood. In bloodstream form cultures the cell density was much lower (approx. 10^6 cells/ml). Therefore, supernatants from several cultures were concentrated by centrifugation to about 10^7 cells/ml and mixed with an equal volume of pig blood. Teneral flies were offered a single infective blood meal within the first 20 h after eclosion. Thereafter they were fed daily on the membrane except for Sundays and the days before the flies were salivated.

**Detection of Infective Flies and Harvest of Metacyclic Forms.** The flies were salivated on glass slides at 3 or 4 day intervals beginning with day 10 after the infective blood meal. Flies which extruded trypanosomes (trypomastigote and epimastigote or mature metacyclic forms) with their saliva were transferred to separate cages and further fed at 2-day intervals on clean ICR mice. Blood from the tails of mice was examined daily for trypanosomes. In addition, all dead flies were dissected and examined for midgut, proventricular and salivary gland infections. Metacyclic forms required for the initiation of bloodstream form cultures or for neutralization of infectivity tests (NIT) were obtained by allowing infective flies to salivate into a drop of warmed medium (MEM with 15% rabbit serum at 37°C) on a leucocyte migration plate.

**Serological Tests.** Metacyclic forms from positive flies infected with cultivated trypanosomes were tested using the neutralization of infectivity test (NIT) according to Schlümp and Jenni (1977). Metacyclic forms from flies initially fed on infected mice were used as controls. The antisera used for all tests was obtained from a rabbit immunized against irradiated metacyclic trypanosomes of the same stock according to Jenni (1979).