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In Vitro Cultivation of *Trypanosoma brucei* Subspecies with Cells Derived from Brain and Muscles of New Born Mouse

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Abstract: Feeder layer cells derived from brain and muscles of ICR new born mouse proved to support continuous growth of bloodstream forms of *Trypanosoma brucei gambiense* Wellcome (W) strain. An attempt has been made to show how well these feeder layer cells can support continuous growth of more recent isolates of *Trypanosoma brucei* subspecies. Bloodstream forms of *T. b. gambiense*, *T. b. rhodesiense* and *T. b. brucei* obtained from infected ICR mice were cultured with the above feeder layer cells in Eagle's Minimum Essential Medium (MEM) supplemented with 20% foetal bovine serum. A maximum density of 8×10^6 parasites/ml was obtained from each culture of the subspecies after 8 −10 days. These cultured trypanosomes were identical to bloodstream forms from ICR mice and they were infective for mice.

Key Words: *Trypanosoma brucei* subspecies, *in vitro* cultivation, feeder layer, new born mouse.

Mammalian feeder layer cells have proven to be essential for the continuous cultivation of bloodstream forms of *Trypanosoma brucei* (Hirumi *et al.*, 1977a, b), but sometimes it is not possible for one type of feeder layer to support the growth of different subspecies or strains of *T. brucei* (Hirumi *et al.*, 1980). In addition, some strains require an additional adaptation phase before they could be efficiently maintained in culture for long periods (Baltz *et al.*, 1985). Thus, in the present work an attempt had been made to clarify the ability of these feeder layer cells in supporting continuous growth of *Trypanosoma brucei* subspecies.

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Parasites used in the experiments were kindly presented by Dr. V. M. Nantulya from International Laboratory for Research on Animal Diseases (ILARD), Nairobi, Kenya. *T.b. brucei* TC221 is a descendant of stock 427 cloned in tissue cultures and cryopreserved. *T.b. rhodesiense* ILRAD 1501 was isolated from human by Brun Walde of Walter Read 24. 12.1980. After one rat passage and one mouse passage it was cryopreserved. *T.b. gambiense* ILRAD 1582 was isolated in south Nyanza, Kenya in 1970 from human by WHO team. It was maintained by syringe passage in mice and rats and cryopreserved in liquid Nitrogen.

The above *T. brucei* subspecies were cultivated following the procedure described earlier (Mhando *et al.*, 1986). Briefly, trypanosomes were collected from blood of infected ICR mice, preferably when the infection was 10^8 parasites/ml of blood. Trypanosomes were separated from blood cells by chromatography method (Lanham and Godfrey, 1970). The parasites were washed once, resuspended in the culture medium and counted. The volume of 10 ml of culture medium containing 7.5×10^5/ml trypanosomes was introduced into each tissue culture dish (Falcon 100×20 mm) with confluent monolayer cells. The dishes were incubated at 37℃ in a 5% CO_2 air−atmosphere. Feeder layer cells which were obtained from new born mouse brain and muscles were prepared and maintained as previously described (Mhando *et al.*, 1986). MEM with Earle's salt (GIBCO) containing penicillin 200 U/ml and streptomycin 100 μg/ml, was supplemented with 20% foetal bovine serum before use in experiments.

After initiation of culture, half of the medium had to be replaced by fresh one once every day. Three to four days later when trypanosome population became established either on the surface or between the feeder layer cells, complete medium exchange was done after every 24 h. Trypanosomes were counted from the culture supernatant fluid and their morphology was assessed by examination of Giemsa−stained preparation. Infectivity test was carried out after 60 days of *in vitro* cultivation by infecting ICR mice intraperitoneally with 1×10^4 cultured trypanosomes.

A few hours after inoculation of bloodstream forms to feeder layer cells trypanosomes were seen moving actively between the cells. In case of *T.b. gambiense* the number of intercellular population steadily increased and after 4 days they formed clusters. These clusters were more prominent in brain cells than in muscle cells. Regarding other two subspecies (*T.b. rhodesiense* and *T.b. brucei)* no formation of clusters was observed, but most of the trypanosomes were seen adhered on or very close to the surface of feeder layer cells. Maximum density of 8×10^6 parasites/ml was obtained after 8−10 days of *in vitro* cultivation (Fig. 1). Culture forms of each of these subspecies were morphologically similar to bloodstream forms isolated from ICR mice and they retained their infectivity for mice for more than 60 days.

This result shows that newly prepared cells derived from a new born mouse brain and muscles can support continuous growth of bloodstream forms of *T. brucei* subspecies derived from infected mice.

Though there was a difference of trypanosome densities in the first week of *in vitro*

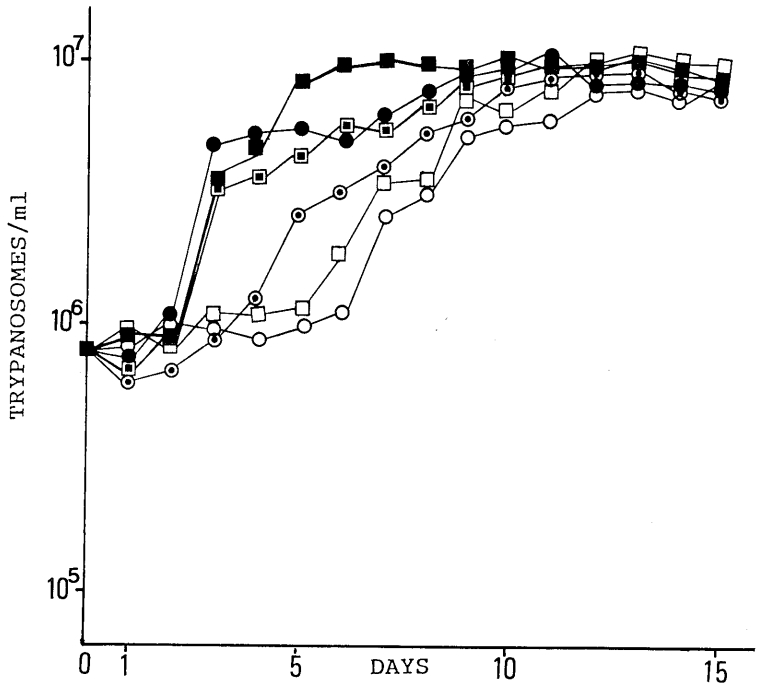

Fig. 1 Growth curves of bloodstream forms of *T.b. brucei* TC221, T.b. *rhodesiense* 1501 and *T.b. gambiense* 1852 in the presence of feeder layer cells derived from new born mouse brain (■, ▣, □, respectively) and muscles (●, ◉, ○, respectively).
Each point represents the mean trypanosoma densities of three experiments determined after every 24 h.

cultivation, all the strains attained almost the same maximum density by the second week. Note worthy however, is the fact that *T.b. gambiense* 1582 formed clusters between feeder layer cells resembling those of *T.b. gambiense W* strain, whereas *T.b. brucei* and *T.b. rhodesiense* attached on the surface of the feeder layer cells and grew in the medium.

The system can therefore provide a tool for analysing the mechanisms of these feeder layer cells in supporting the growth of trypanosomes, furthermore, the parasites density obtained every day is quite enough to be used for biological and immunological studies.

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新生仔マウスの脳および筋由来細胞存在下でのブルセイ亜種トリパノソーマ原虫の培養

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マウス新生仔の脳および筋由来細胞は，*Trypanosoma brucei gambiense* (Tg) Wellcome 株の培養をたすける．Tg でも新しく分離された株，あるいはブルセイ亜種に属する *T.b. brucei* や *T.b. rhodesiense* の分離株の培養についても，それらのマウス細胞が，侍養細胞（feeder cell）として有効かどうか検討してみた．感染マウスから得た血流型原虫を，牛胎仔血清加(20%) Eagle's Minimum Essential Medium で培養した．いずれの分離株も持続的に培養可能で，原虫密度は 8 $\times 10^6$/ml に達した．培養された原虫は，マウスからの血流型と酷似し，マウスに対する感染性も保持していた．

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