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Mémoire.

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MULTINUCLEAR FORMS IN A DYSKINETOPLASTIC STRAIN OF *TRYPANOSOMA EVANSI* IN MICE

Z. R. LUN*, K. VICKERMAN**

SUMMARY

The production of short stumpy and multinuclear trypanosomes in a Chinese strain of dyskinetoplastic *Trypanosoma evansi* maintained in rabbits and mice is described. Production of multinuclear trypanosomes was increased following passage through a reptile (gecko), in which the trypanosomes did not multiply, and transfer back to mice. The multinuclear trypanosomes showed more nuclei than flagella indicating that disruption of the normal cell cycle had taken place and not simply inhibition of cleavage. A Chinese kinetoplastic *T. evansi* treated similarly rarely produced stumpy or multinuclear forms.

RÉSUMÉ : **Production, par une souche dyskinétoplastique de *Trypanosoma evansi*, de formes multinucléées chez la souris.**

La production de formes trypanosomes courtes, trapues et plurinucléées, par une souche diskinétoplastique de *Trypanosoma evansi*, maintenue par passages chez lapins et souris, est décrite. La production de trypanosomes plurinucléés a augmenté à la suite du passage par un gecko (chez lequel les trypanosomes ne se sont pas multipliés), puis transfert à nouveau sur souris. Les trypanosomes plurinucléés présentent plus de noyaux que de flagelles, ce qui indique une anomalie du cycle cellulaire et non une simple inhibition de la bipartition. Une souche chinoise kinétoplastique de *T. evansi* ayant subi les mêmes manipulations ne produit que rarement des formes trapues ou plurinucléées.

INTRODUCTION

Trypanosoma evansi which causes the disease Surra is one of the most economically important parasitic protozoa infecting domestic animals such as camels, horses, buffalo and deer in the People's Republic of China. *T. evansi* is generally recognized as monomorphic as only « slender forms » are observed in the blood of an infected host (Bruce, 1911; Liu, 1980). There are numerous reports, however, of « short-stumpy » and « intermediate » forms in old strains which have been maintained by serial passage in laboratory animals for several years (Lavier, 1933; Hoare, 1956, 1972; Miles, 1970; Lun, 1988). Recently, De Diego *et al.* (1985) reported multinuclear forms and stumpy-like forms in division in *T. b. brucei* from infected mice. Our study reveals that multinuclear forms are also present in the dyskinetoplastic and normal kinetoplastic strains of *T. evansi*. When we tested the survival time of *T. evansi* in different lower animals other than mammals we found that numbers of such forms can be increased if healthy mice are inoculated with blood containing trypanosomes from geckoes that have been previously inoculated with the dyskinetoplastic strain.

* Parasitology Laboratory, Department of Biology, Zhongshan University, Guagnzhou 510275, People's Republic of China.
** Department of Zoology, University of Glasgow, Glasgow G12 8QQ, Scotland, U. K.

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MATERIALS AND METHODS

The dyskinetoplastic strain of *T. evansi* was obtained from the Department of Veterinary Medicine, South China Agriculture University, 1979 and stored in liquid nitrogen. This strain was isolated from a horse in the outskirts of Guangzhou, in 1962 and maintained by continuous passage monthly alternating between rabbits and mice. No multinuclear forms in this strain were observed in rabbits. Cryostabilates were thawed at 37° C and inoculated into mice. For the present work, the trypanosome was passaged in mice. Gecko lizards *(Gekko japonicus)* (collected on campus) were inoculated intraperitoneally with 0.5 ml mouse blood containing 10^8 trypanosomes/ml. Blood smears of geckos indicated that there were no blood parasitic protozoa in the peripheral blood of these lizards before they were infected with *T. evansi*. Geckoes were kept at 35°-37° C in cages made of wood and nylon net for a week before and after inoculation, and fed with *Culex* or *Aedes* mosquitoes. Blood from the tails of infected geckoes was inoculated into mice every day for 7 days after infection of the gecko. Two days after inoculation, blood smears of infected mice were made every day until the mice died. A normal kinetoplastic strain of *T. evansi* isolated from a buffalo in Yang Jiang County, Guangdong Province, in 1982, was used as control. Both kinetoplastic and dyskinetoplastic strains were highly virulent in experimental animals killing mice 3 to 5 days after inoculation with 10^5 parasites. All blood smears were fixed in methanol and stained with 10 % Giemsa for examination by light microscopy.

RESULTS AND DISCUSSION

Short stumpy and multinuclear forms *(Fig. 1)* were found in the blood smears of mice infected with dyskinetoplastic and normal kinetoplastic strain of *T. evansi,* especially in the dyskinetoplastic one *(Table I).*

All Chinese strains of *T. evansi* hitherto isolated from buffalo, horses and horse-deer were claimed by their investigators (Liu, 1980; Gu, 1982) to be monomorphic as defined by Bruce (1911). Inconstant pleomorphism of *T. evansi* has been reported however in African and Indian strains (Hoare, 1952, 1956; Miles, 1970). Some strains proved to be highly pleomorphic (50 percent stumpy forms) even in clones (Miles, 1970). Miles (1972) also demonstrated that the mitochondrial marker enzyme, NAD diaphorase, was found in the short form of *T. evansi* SAK strain suggesting that the stumpy forms in this species correspond physiologically to those of *T. brucei* (Vickerman, 1965).

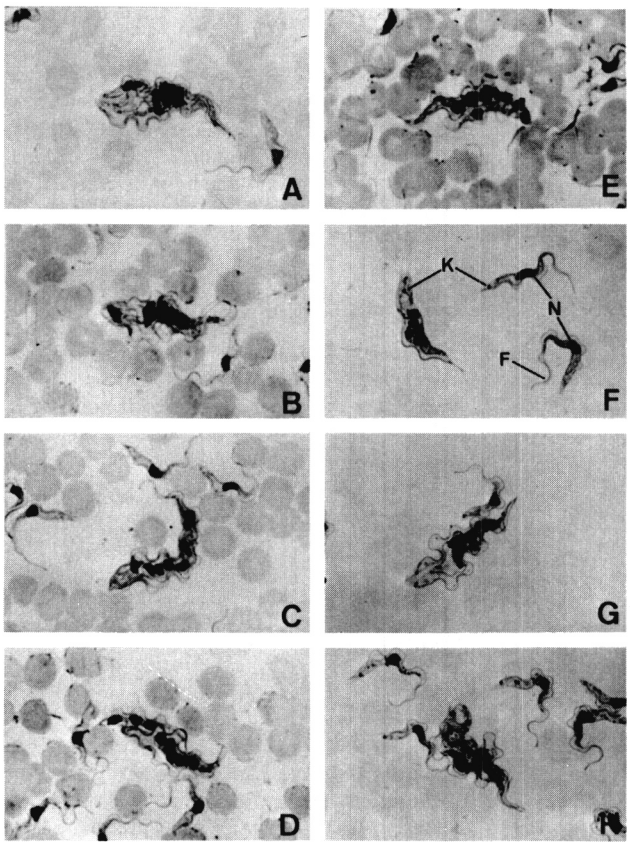

FIG. 1. — The morphology of multinuclear forms of two strains of *Trypanosoma evansi* in mice. F = flagellum; K = kinetoplast; N = nucleus. × 1 500.

A, B, C, D, E: Dyskinetoplastic strain on 5th day after being infected with the blood containing trypanosomes form the geckoes. In C, D and E note disparity between number of nuclei and number of flagella.

F, G, H: Kinetoplastic strain. In F, note 5 nuclei, 2 kinetoplasts and single flagellum.

TABLE I. — *Percentage of different morphological forms of* Trypanosoma evansi *in blood smears of mice. Taken at fifth day after inoculation* (Number of trypanosomes is sample = 3 000).

Dyskinetoplastic strain		*Normal kinetoplastic strain*	
Slender	94.6 -92.0	Slender	97.6 -94.5
Intermediate	4.5 - 6.1	Intermediate	2.1 - 4.7
Short-stumpy	0.65- 1.1	Short-stumpy	0.15- 0.5
Multinuclear	0.5 - 0.7	Multinuclear	0.2 - 0.4

In our present work, it was very interesting to find peculiar multinuclear forms (with 6 to 10 nuclei, *Fig. 1 C, D, E*) in the blood smears of mice inoculated with trypanosomes from geckoes. These lizards, when inoculated with the strains of *T. evansi* will harbour the parasites for about 7 days, though the trypanosomes do not multiply in the poikilothermic host. Most multinuclear forms were observed in the mouse blood smears taken on the 4th to 6th day after inoculation. What was peculiar about the multinuclear trypanosomes was that the number of nuclei was in excess of the number of flagella, shawing that the normal sequence of events in the cell cycle (replication of flagellum, replication of kinetoplast-mitochondrion, nuclear division, cleavage) had been disrupted *(Table II).* Recent work using *in vitro* isolation of such bizarre trypanosomes has demonstrated that they are viable *i. e.* can give rise to normal trypanosomes once more.

TABLE II. — *Number of flagella and nuclei in 50 multinuclear* Trypanosoma evansi *(dyskinetoplastic) after passage through gecko.*

Number of flagella	*Number of nuclei*							
	3	4	5	6	7	8	9	10
1	—	4	2	—	—	—	—	—
2	—	5	3	1	1	—	—	—
3	—	—	—	2	4	2	—	—
4	—	2	1	7	—	2	—	1
5	—	—	—	6	—	1	—	—
6	—	—	—	2	1	2	—	—
7	—	—	—	—	—	1	—	—

Multinuclear forms were rarely found in the kinetoplastic strain when it was treated in the same way. Results from the stocks of cloned trypanosomes were similar to those for uncloned ones. Previous investigators have noted that pleomorphism is more readily induced in dyskinetoplastic strains of *T. evansi* (Hoare, 1972).

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