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Studies on *Trypanosoma (Nannomonas) congolense*

II. Observations on the cyclical transmission of three field isolates by *Glossina morsitans morsitans**

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Summary

Teneral flies of *Glossina morsitans morsitans* were fed on mice infected with cloned and uncloned derivatives of three recent field isolates of *Trypanosoma (Nannomonas) congolense.* Flies with mature infections were identified by the warm-slide probe method and phase-contrast microscopy. High infection rates were achieved when such flies were fed on mice at peak parasitaemia. The infection rates were low when flies were fed on mice prior to or late after peak parasitaemia.

The duration of the developmental cycle of *T. congolense* in the tsetse fly varied from 7 to 40 days: in 45% of the infective flies the developmental cycle was completed within 12 days; and in 76%, within 18 days.

Key words: Trypanosoma (Nannomonas) congolense; cyclical transmission; *Glossina morsitans morsitans;* developmental cycle.

Introduction

Since the demonstration by Kleine (1909a, b) that tsetse flies are vectors of the pathogenic trypanosomes, several investigations have been undertaken to elucidate various factors that influence the infectivity of these parasites to tsetse flies. Such factors include temperature at which pupae and adult flies are kept (Burtt, 1946a; Fairbairn and Culwick, 1950), and age of the flies at the time of infection (Wijers, 1958). In addition, the host species from which the infective bloodmeals are taken (Corson, 1935; Van Hoof et al., 1937) and the morphology

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and number of trypanosomes ingested by the flies (Robertson, 1912; Wijers and Willet, 1960) are also apparently important in this type of vector-parasite relationship. However, most of these studies have been undertaken using the *T. brucei*-complex trypanosomes. *Trypanosoma (Nannomonas) congolense* has received comparatively little attention (Godfrey 1958, 1959; Dipeolu, 1975).

To study variant antigens of *T. congolense* metacyclics, it was necessary to standardize the conditions under which the flies were infected. These conditions together with the techniques that were used to infect and identify the infected flies are described in this paper. In addition, we present some observations on the transmissibility of these isolates and the length of their developmental cycle in the tsetse.

Materials and methods

Trypanosomes

The original *T. congolense* isolates – STIB 228, STIB 212, and STIB 249 – were isolated from 3 different lions in the Serengeti area, Tanzania (Geigy and Kauffmann, 1973). The cloned and uncloned derivatives of these isolates have been described elsewhere (Schläppi and Jenni, 1977; Nantulya et al., 1978a).

Infection, maintenance and identification of infected flies

ICR white female and C57/BL/6 male mice were each inoculated intraperitoneally with 10^5 trypanosomes of each stabilate and the course of the first parasitaemic wave was monitored daily using tail blood. Peak parasitaemia in mice varied from 10^7 to 10^8 trypanosomes per millilitre of blood. Batches of 10–15 teneral flies in Geigy cages were fed on infected mice within 8 h after emergence and kept at 25° C and 80% humidity. Ten batches of flies (154) were fed on mice during the log phase of rising parasitaemia; 19 batches (207 flies) were fed at peak parasitaemia; and 17 other batches (207 flies) were fed on the falling phase of the parasitaemic wave. Thereafter flies in each cage were maintained by feeding 3 times a week on normal mice. One mouse was used to feed the flies in each cage every Monday; the second, every Wednesday; and the third, every Friday, until the mice became infected. Mature infections in the flies were identified by the presence of metacyclics in the saliva using the warm-slide probe method (Burtt, 1946b) and phase-contrast microscopy. For each batch of flies the infection rate was determined by the number of flies with mature infection related to the total number of flies in the batch that survived the first 10 days after the infective bloodmeal.

The mean prepatent period in cyclically infected mice

Thirty-seven normal and 15 lethally irradiated (900 R) mice were used in this experiment. Each mouse was fed upon by a fly which showed mature infection. Beginning on the third day after this infective bite, a daily sample of tail blood from each mouse was taken and infection was determined by the haematocrit centrifuge technique (Woo, 1971).

Period of development of T. congolense in Glossina morsitans morsitans

The length of the developmental cycle of *T. congolense* in the tsetse fly was estimated in two ways. At first, flies were probed only after parasitaemia developed in the corresponding mice on which they had been maintained. The length of period of development of *T. congolense* in such flies was estimated by subtracting the mean prepatent period of cyclically infected non-irradiated mice from the day when parasites were first detected in one of the mice used to feed the flies in that particular cage.

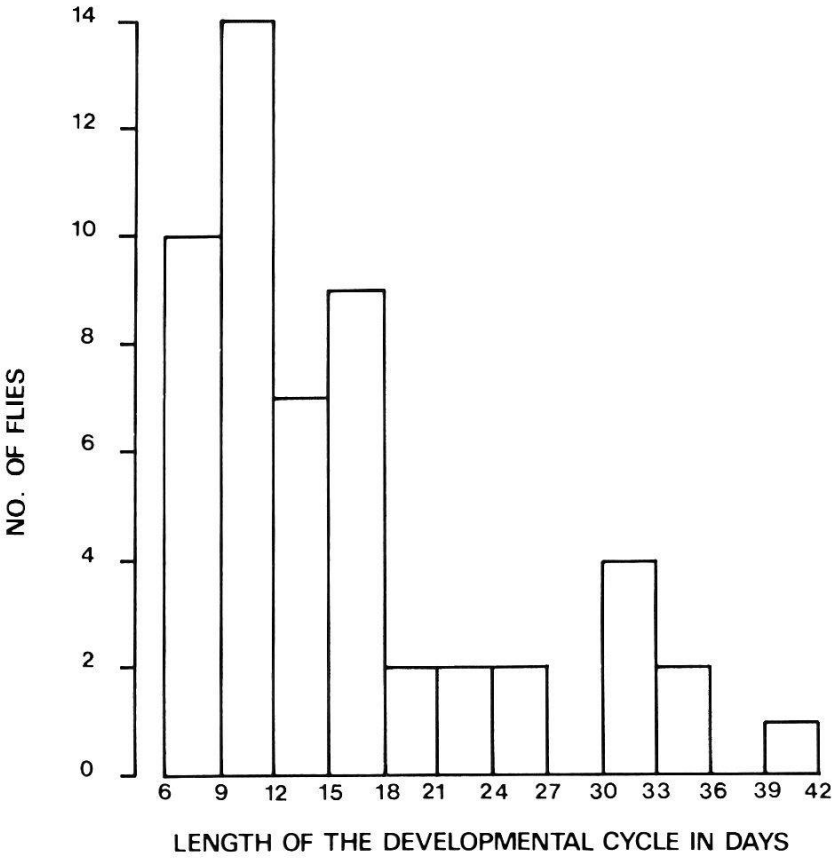

Fig. 1. Number of *Glossina morsitans morsitans* with mature *T. congolense* infection in relation to the duration of the developmental cycle.

Later, flies in each cage were probed 7, 10 or 15 days after the infective bloodmeal. The flies that had demonstrable metacyclics in their saliva were isolated, and those with negative saliva probes were returned into the original cage and maintained by feeding on new mice. If the new mice showed parasitaemia within 15 days after the first feed, infective flies in the corresponding cage were deemed to have been present although not identified at the time of the first probing. Since *T. congolense*-infected flies sometimes extrude no metacyclic forms in the saliva probes (Moloo and Kutuza, personal communication), it is possible that such flies were not identified as infected by the initial microscopic screening of the probes.

Results

Fly infection rates

Fifty-five (12%) of 457 teneral flies which were fed on mice infected with cloned and uncloned derivatives of STIB 228 developed mature infections as demonstrated by the warm-slide probe method (Burtt, 1946b) and phase-contrast microscopy. The infection rate was 7.1% (11/154) in flies fed on infected mice during the log phase of rising parasitaemia, 22.4% (34/152) in flies fed at peak parasitaemia, and 6.6% (10/151) in flies fed on the falling phase of the parasitaemic wave. The infection rates among different batches of flies fed on mice during the log phase of parasitaemia varied from 0 to 15%, the rates in flies fed at peak parasitaemia varied from 15 to 50%, while the rates among batches

infected by feeding on the falling phase of the parasitaemic wave varied from 0 to 12%. Significant differences in infection rates were not observed among the different clones of STIB 228.

The number of flies infected with the other two isolates (8/81 and 5/30) for STIB 212 and STIB 249, respectively was too small to allow comparison with STIB 228.

The developmental cycle of the isolates in Glossina morsitans morsitans

The period of development of *T. congolense* in the fly is shown in Fig. 1. These results show that in 45% (24/53) of the infected flies the developmental cycle was completed within 12 days after an infective bloodmeal; and in 76% (40/53) of the flies the infection matured within 18 days. The shortest cycle of development was 7 days and the longest was 40 days. There was no significant difference among the 3 isolates of *T. congolense* with respect to the length of the developmental cycle in the tsetse fly.

The prepatent period in cyclically infected mice

The mean prepatent period in 37 cyclically infected normal mice was about 10 days. In lethally irradiated (900 R) mice the prepatent period was about 5 days.

Discussion

In these preliminary studies high infection rates were achieved when teneral flies were fed on mice showing the first peak parasitaemia. The overall infection rate in flies fed on mice during the log phase of rising parasitaemia was lower than that at peak parasitaemia. These results would suggest that trypanosomes from the parasitaemic peak may have a higher capability to infect the tsetse fly than those obtained from the same mouse prior to the peak as reported for *T. brucei* (Jenni, 1977). This could be related to the morphology of *T. congolense* in that the long and/or intermediate forms which predominate at peak parasitaemia may be more infective to the vector than the short form which predominates the rising phase of parasitaemia (Nantulya et al., 1978b, in press). However, this aspect needs further investigation as the number of flies used was not large enough. Furthermore, since flies in these studies were infected by feeding on infected mice, the difference in the infection rates could have been due to the larger number of trypanosomes ingested by flies at peak parasitaemia compared to that ingested by flies before this period (Wijers and Willet, 1960).

The flies fed on the falling phase of parasitaemic wave showed a low infection rate. This low infection rate may have been a result of degenerative changes in the parasites prior to ingestion by the flies, possibly influenced by the host defense mechanisms, since many organisms from this phase were swollen and vacuolated (Nantulya et al., 1978b, in press).

The present study has also demonstrated that the length of the shortest cycle of development of *T. congolense* in the fly is about 7 days while the longest is about 40 days, and in 76% of the infected flies the developmental cycle was completed within 18 days. The developmental cycle of *T. congolense* in *Glossina morsitans morsitans* thus appears to take a much shorter time period than the 19–53 days previously reported by Bruce et al. (1915). We do not know the exact explanation for this difference but it is possible that the cycle was shorter in our studies because we used different strains of *T. congolense*. The species of host on which flies were fed may also account for this difference: in the study by Bruce et al. (1915) flies were fed on goats and dogs.

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