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Studies on *Trypanosoma (Nannomonas) congolense*

I. On the morphological appearance of the parasite in the mouse*

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Summary

The pleomorphism of bloodstream *Trypanosoma (Nannomonas) congolense* was studied during the course of the first parasitaemic wave in mice using cloned and uncloned derivatives of three recent field isolates. The different morphological types were identified using the criteria described by Godfrey (1960). It was found that at any point of parasitaemia there were several morphological types of the parasite present, ranging from short to long forms. In the rising phase of parasitaemia, the short forms predominated, while at peak parasitaemia the parasites were highly pleomorphic, with significant proportions of short and "intermediate" forms although the long forms predominated. Pleomorphism was observed both in normal and in lethally irradiated (900 R) mice, even when the infection was initiated using a single organism. Such pleomorphism may result from physiological differences between the different forms of this parasite since these morphological types of *T. congolense* also differed in their ability to infect a new mammalian host.

Key words: Trypanosoma (Nannomonas) congolense; morphological types; mouse infectivity.

Introduction

In 1904 Broden discovered a small trypanosome in the blood of domestic ungulates from Congo (Zaire). He called it *Trypanosoma congolense.* Later in the same year Laveran and Mesnil (1904) created the name *Trypanosoma dimorphon* for an isolate from a horse in the Gambia which was characterized

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by the presence of both long and short *congolense*-type trypanosomes. This trypanosome species was believed to be different from *T. congolense* and was renamed *Trypanosoma confusum* by Montgomery and Kinghorn (1909), while, Laveran (1905) created yet another name (*Trypanosoma nanum*) for a small *congolense*-type bovine trypanosome isolated in the Sudan. In a review of these "species", however, Bruce et al. (1910) agreed that these trypanosomes were morphologically indistinguishable and so they proposed that all these "species" be considered as one species under a new name, *Trypanosoma pecorum.* However, the merging of *T. dimorphon* with *T. congolense* was not universally accepted and in a morphological and biometric revision by Hoare (1959) based on the original material studied by Laveran and Mesnil, *T. dimorphon* was restored to its original status of a distinct species on the basis that the mean trypanosome length of *T. dimorphon* (15.3–17.6 μm) was distinctly different from that of *T. congolense* (12.2–14.4 μm). However, Hoare (1959) pointed out that should strains with intermediate mean lengths be discovered, then it would be necessary to reconsider *T. dimorphon* as a minor variety of *T. congolense.*

The strains with intermediate mean lengths (12.98–13.85 μm and 12.45–13.85 μm) were subsequently described by Godfrey (1960) and Fairbairn (1962), respectively. Godfrey (1960) and Fairbairn (1962) then proposed that *T. congolense* should be sub-divided into 3 distinct sub-species or sub-groups: *T. congolense* proper, intermediate type, and *T. dimorphon*-type, which could be distinguished on the basis of morphology. Later, however, Huisenga (1969) reported that when measured exclusively in the initial stages of division, both *T. congolense* and *T. dimorphon* have the same mean lengths, implying that the two belong to one and the same species.

Thus the problem as to what represents a species or sub-species of *T. congolense*, as defined by morphology, has remained unsolved. In this paper we describe studies on three recent *T. congolense* field isolates which indicate that the conflicting results of previous investigations on the morphology of bloodstream forms of this trypanosome species were due to pleomorphism.

Materials and methods

1. Trypanosomes

The parent strains, STIB 228, STIB 212 and STIB 249 were isolated in the Serengeti in 1971 (Geigy and Kauffmann, 1973). STIB 68-0 is a clone raised in irradiated (600 R) mice from STIB 228. Further information on these isolates has been given elsewhere (Schläppi and Jenni, 1977; Nantulya et al., 1978).

2. Laboratory animals

White ICR female mice and C57/BL/6 males were used. All the trypanosome strains and clones used in this study were found to be equally infective to the two strains of mice.

3. Changes in morphology

A suspension of 10^5 trypanosomes of each stabilate was inoculated intra-peritoneally into a mouse. Thin films of tail blood were made daily, starting from the third day after infection up to the

Table 1. Distinguishing characteristics of the various morphological types of *Trypanosoma congolense* organisms

Characteristics	Morphological type		
	short	intermediate	long
1. Shape of posterior end	rounded	bluntly pointed	sharply pointed
2. Distance of kinetoplast from the posterior end	sub-terminal (<1 μm)	about 1 μm	about 2μm
3. Undulating membrane	indistinct	thrown into 1–2 weak folds	well-developed and thrown into 2–3 well-defined folds
4.* Trypanosome length (mean $\pm$ 1 S.D.) (range)	11.3 ± 1.09 (9–13) μm	14.14 ± 0.87 (12.5–16) μm	17.31 ± 1.03 (16–21) μm

* One hundred non-dividing trypanosomes of each form were examined and measured. The differences in mean lengths between all groups are statistically significant ($p < 0.001$).

time of relapse of the parasitaemia. In the case of very low parasitaemias, tail blood was taken into four haematocrit tubes and spun for 4 min in the haematocrit centrifuge. The buffy coats of the 4 tubes were pooled and then thin films made. Dried blood films were fixed in methyl alcohol for 20 min and then stained with Giemsa.

4. Identification of the different morphological types of the parasite

The different forms of the parasite were identified and counted using the criteria described by Godfrey (1960). These criteria are based on the shape of the posterior end of the parasite, the position of the kinetoplast in relationship to the posterior end, and the presence or absence of a distinct undulating membrane. These distinguishing characteristics are summarized (Table 1) together with measurements of the various morphological types of the parasite. The length of the trypanosomes was measured by drawing (with the aid of a camera lucida at $\times 1250$) a line running through the middle of the body of each trypanosome, from one end of the body to the other, and measuring this line with dividers set to measure the equivalent of 1 μm. The typical morphological types are shown (Fig. 1).

5. Infectivity of the various forms

A normal C57/BL/6 mouse was inoculated intraperitoneally with 10^5 trypanosomes of a *T. congolense* clone (STIB 68-0). This clone had initially been shown to be antigenically homogeneous by immunofluorescence.

Starting from the fourth day after infection 50 μl of tail blood was obtained daily for a total parasite count together with a titration of the infectivity of the parasites. The titration was performed as described by Lumsden et al. (1963) with some modification. Groups of 10 mice were inoculated with each log dilution. Tail blood from the test mice was examined for parasitaemia 8 days after intraperitoneal inoculations of the parasites by the haematocrit centrifuge technique (Woo, 1971) and phase-contrast microscopy, and the ID 50 of each daily parasite population was determined. This experiment was done twice.

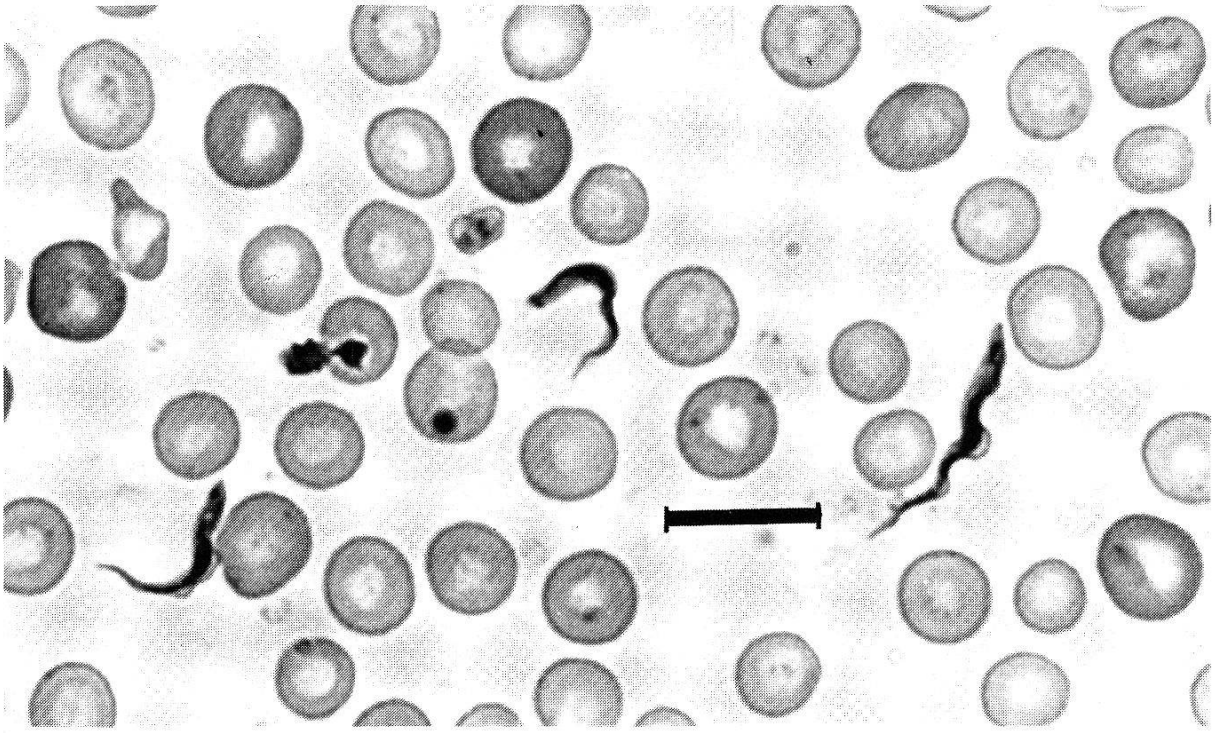

Fig. 1. Showing three different morphological types of bloodstream *Trypanosoma congolense:* intermediate (left), short (middle), and long (right). – Scale bar represents 10 μm.

Serial blood films were also made, stained with Giemsa and used to determine the proportion of the different forms in each blood sample. 300 trypanosomes in consecutive microscope fields of the blood film of each daily blood sample were counted.

In another set of 4 experiments the infectivity of the individual forms of the parasite was tested by cloning both in normal and lethally irradiated mice. Prior to inoculation into a mouse the morphology of each trypanosome was observed by phase contrast microscopy and classified as long or short or "intermediate" on the basis of size: The short form was less than twice the mouse red blood cell diameter, while the long form was about 3 times that diameter. Trypanosomes that did not fall into these categories were classified as "intermediate".

6. Indirect immunofluorescent antibody test (IFAT)

The surface variant antigenic type of the various morphological types of the parasite in the clones raised in lethally irradiated mice was examined using the IFAT. The IFAT was performed on formalin-fixed trypanosomes as described by Nantulya and Doyle (1977). Antiserum to each clone was made as described by Nantulya et al. (1978).

7. Conjugate

Fluorescein-conjugated IgG fraction of rabbit anti-mouse IgG (heavy and light chains) Lot 8864 was obtained from Cappel Laboratories, Inc., Downington, Pennsylvania, 19335, USA. This conjugate had a molar fluorescein: protein ratio of 3.1, and was used at a dilution of 1/80.

Results

The morphological appearance of all cloned and uncloned derivatives of the three isolates of *T. congolense* used in this study was identical. At the beginning of each parasitaemic wave there was a high proportion of short forms (Fig. 2). The overall morphological picture at this point corresponded to that previously attributed to "typical" *T. congolense* (Broden, 1904).

This morphological picture was observed to undergo a gradual change in the course of the parasitaemic wave such that at peak parasitaemia the trypanosome population was highly pleomorphic. At peak parasitaemia (days 7 and 8) the long forms constituted the predominant population (Fig. 2) and the overall picture was similar to that previously attributed to *T. dimorphon* (Laveran and Mesnil, 1904; Godfrey, 1960). A rapid syringe sub-passage at 3–4-day intervals of such a dimorphic trypanosome population in mice resulted into a shift to the short *T. congolense*-type appearance. During the late stages of the rising parasitaemia (day 5, Fig. 2) the morphological appearance of the parasite population was similar to the "intermediate" strains of *T. congolense* described by Godfrey (1960).

Pleomorphism of *T. congolense* was observed to occur both in normal and in lethally irradiated (900 R) mice even when the infection was initiated by a single trypanosome. The various morphological types of the parasite in each clone were identical with respect to the surface variant antigen as shown by the IFAT.

A representative picture of the infectivity of the parasites taken at different points of the parasitaemic wave in a normal mouse infected with a clone (STIB

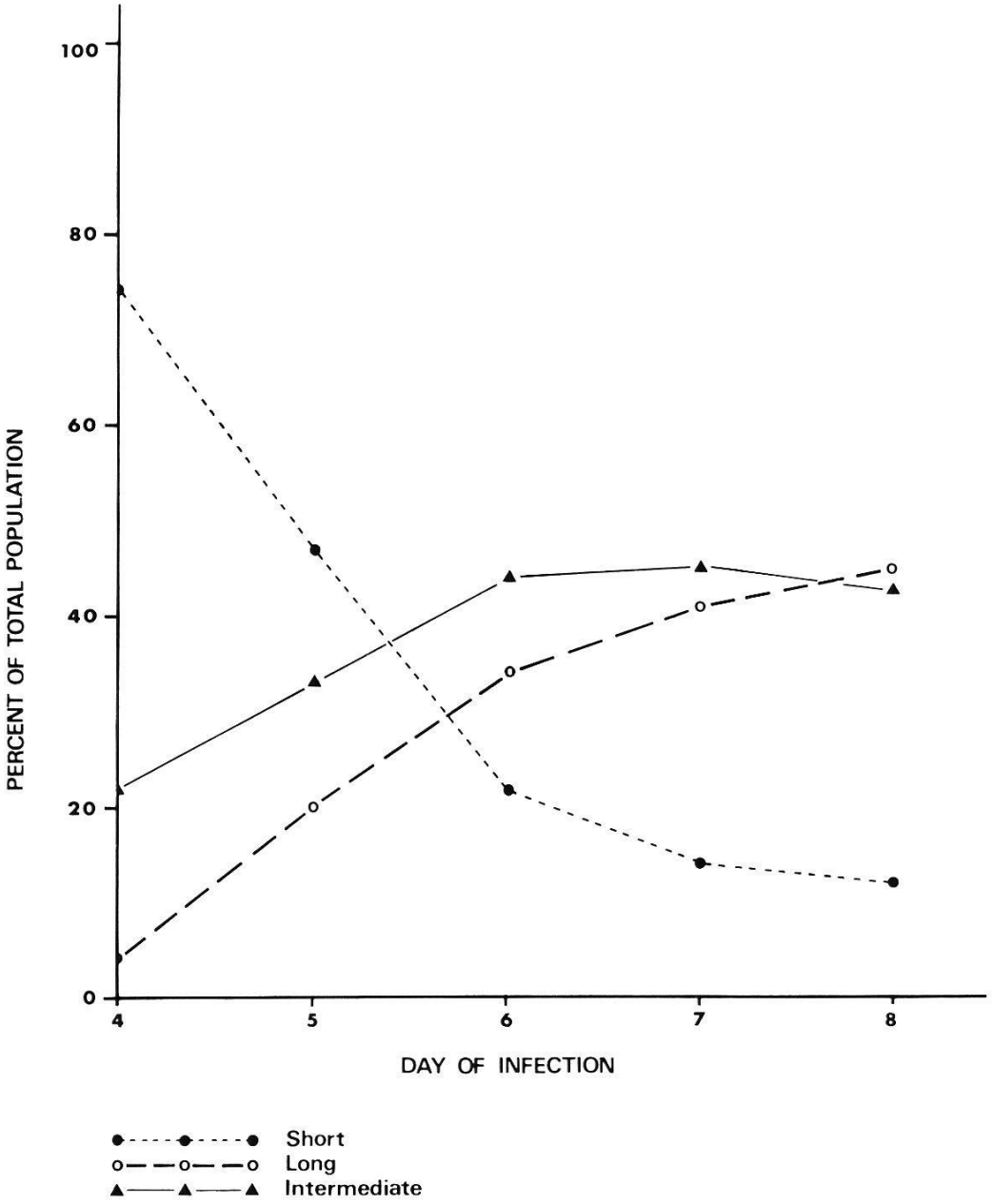

Fig. 2. Proportions of the various morphological types of *Trypanosoma (Nannomonas) congolense* related to the parasitaemic wave in the mouse.

68-0) as determined by ID 50's is given in Fig. 3. These results indicate that the infectivity of trypanosome populations obtained at peak parasitaemia is significantly reduced, compared to those from the rising phase of parasitaemia. The results obtained with cloned organisms (Table 2) suggest that this fall in infectivity may be related to the predominant morphological type of the parasite in each phase of the parasitaemic wave in that the short form which predominates the log phase of a rising parasitaemia is more infective to the mammalian host than the long form which predominates at peak parasitaemia. Prior to or during the falling phase of parasitaemia, however, antibody possibly reduces further the infectivity of all the morphological types of the parasite since tail blood films

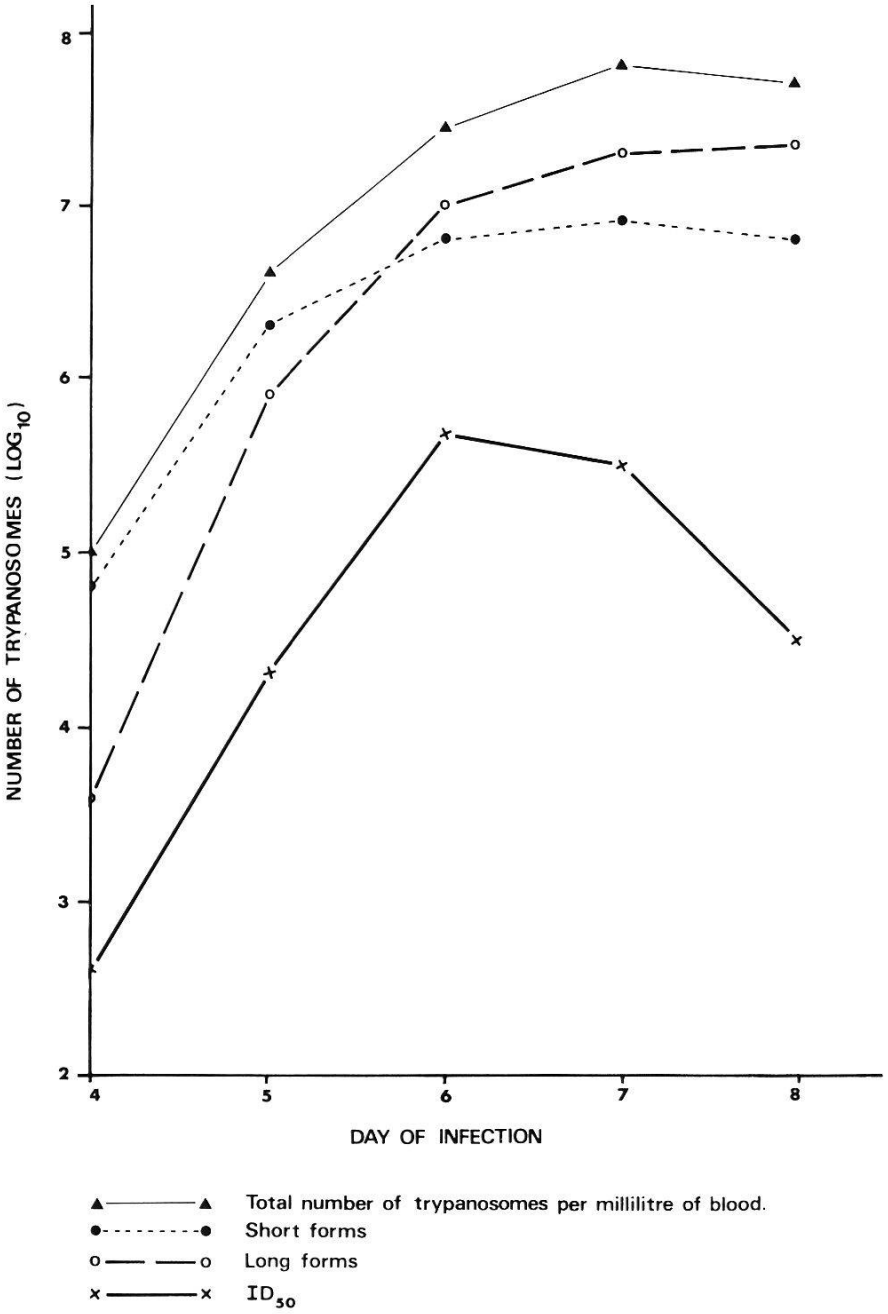

Fig. 3. The infectivity of *Trypanosoma (Nannomonas) congolense* related to parasitaemia and morphology in the mouse.

Table 2. The relationship between morphology and subsequent infectivity for mice of individual organisms of a clone of *T. congolense*.

Morphological type	No. tested	No. infective*
1. Long forms	19	3
2. Intermediate forms	6	3
3. Short forms	19	15

* Using the χ^2 test of association the following differences in infectivity were observed between groups 1 and 2 ($p < 0.5$), and groups 1 and 3 ($p < 0.001$).

from this phase of the parasitaemic wave contained many swollen and vacuolated (degenerating) parasites.

Discussion

The results of our work show that at any point of parasitaemia, there are several morphological types of the parasite ranging from short to long forms. In the rising phase of parasitaemia, the short forms predominate, while at peak parasitaemia the long forms predominate. The morphological appearance of the parasites at the beginning of the parasitaemic wave is identical to that previously attributed to the "typical" *T. congolense*, while at peak parasitaemia this appearance changes to that of "typical" *T. dimorphon*. Parasites with the appearance of the intermediate strains of *T. congolense* described by Godfrey (1960) were observed in each mouse as part of the transitional stage of this change in morphology.

It would, therefore, appear that the mean length of any bloodstream populations of *T. congolense* depends on the predominant morphological type, and varies significantly during the course of each parasitaemic wave. The results of an earlier study by Hoare (1959) show that this is indeed the case since in that study significant differences were noted in the mean length of parasite populations obtained on successive days from a rat infected with a strain of *T. dimorphon*. At the same time the mean length of trypanosomes obtained from different hosts infected with this same strain of *T. dimorphon* varied from that of "typical" *T. congolense* to that of "typical" *T. dimorphon*.

In previous studies where the mean length of the parasites was used to distinguish between strains and sub-species of *T. congolense*, this correlation of mean length and parasitaemia was not taken into account. It is therefore possible that the conflicting results from these earlier studies could in part be explained on the basis that different investigators might have measured parasites derived from different hosts at different points of parasitaemia.

Our work has also demonstrated that changes in parasite morphology do occur both in normal and in lethally irradiated (900 R) mice even when the infection is initiated using a single trypanosome. The various morphological types of the parasite in clones raised in lethally irradiated mice were identical with respect to their surface variant antigen as demonstrated by the indirect immunofluorescent antibody test. Therefore, the various forms of the parasites of this species may simply represent the stages of maturation and differentiation of the individual organisms. The physical characteristics described by Godfrey (1960) should therefore be used for description rather than classification of isolates of *T. congolense*.

Also, in the present study, the short forms of *T. congolense* were shown to be more infective to the mammalian host than the long forms. Since the long forms predominate during peak parasitaemia this suggests that the parasite

itself may be capable of limiting its number in each parasitaemic wave. This decrease in the infectivity of the parasite during the course of infection of the mammalian host might be advantageous to the parasite in that it could facilitate the parasite to establish a chronic infection in the mammalian host, as has been suggested for *T. brucei* (Ormerod et al., 1974).

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