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Miscellanea

Influence of Bloodmeals from Different Donors on the Infection Rates of *Trypanosoma brucei* in *Glossina* *

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AMREIN, GEIGY & KAUFFMANN (1965) showed that the restitution of infectivity for the mammalian host in cultures of *T. brucei* partly depends on the individual human donor of the blood used in these cultures. Similar results were obtained by AMREIN & HANNEMAN (1969) with blood from domestic and zoo animals. There are more or less suitable as well as unsuitable donors.

It was supposed that the development of *brucei-subgroup* trypanosomes in the vector *Glossina* and the formation of metacyclic forms in the salivary glands also partly depended on the quality of the donor blood used for feeding the flies subsequently to the initial infective meal. This was the basic idea for working with tsetse flies in order to study these questions in the vector.

It is well known that the species of mammal from which the infective meal is derived affects the infection rates of *T. rhodesiense* in *Glossina* (CORSON, 1935, 1936 and 1938). Similar results were reported by VAN HOOF, HENRARD & PEEL (1937) working with *T. gambiense* in a monkey and *Glossina palpalis*. VAN HOOF (1947) stated that the monkey *Cercocebus* gives increased infection rates of *T. gambiense* and *T. brucei* in *G. palpalis*. FAIRBAIRN & BURTT (1946) showed with *T. rhodesiense* that the period for developing salivary gland infections in *G. morsitans* also partly depends on the species of the mammalian host.

Higher infection rates in *G. morsitans* after the infective meal on suidae were reported by VAN DEN BERGHE et al. (1963).

JORDAN (1965) studied the relationship between the natural hosts of tsetse flies and infection rate in different localities in Nigeria. He found that the total trypanosome infection rate is highest, where a large proportion of meals derived from bovids; lower, specially in regard to *T. vivax,* where the flies feed mainly on suids. He was working under field conditions on total trypanosome infection rates in several species of *Glossina.*

BURTT (1946) accepts the view that the host animal has an influence on the infection rates. However, FAIRBAIRN & CULWICK (1950) object that the conditions in the latter experiments have been unequal. This is an objection which may also be attributed to other experiments. In our experiment we use therefore standardized material working under equal conditions.

Material and Methods

In August and September 1970 about 7,000 newly hatched flies, i.e. 4,596 *G. morsitans,* 2,058 *G. fuscipes* and 338 *G. pallidipes,* were fed (first = infective bloodmeal) on infected rats showing a good parasitaemia of *T. brucei* (++++ and more than 40% stumpy forms). The rats were used from the 7th up to the 12th day after inoculation. As regards the origin of the flies, *G. morsitans* pupae were supplied from Singida/Tanzania and some from the Langford colony (Dr. T. A. M. Nash), *G. fuscipes* pupae were derived from wild caught flies from Lugala/Uganda and *G. pallidipes* originated from E.A.T.R.O.'s fly colonies. All pupae

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were kept up to emergence in E.A.T.R.O.'s fly rooms. Newly hatched tsetse flies fed with the infective meal within 24 hours of emergence acquire the highest infection rates as has already been proved for *T. gambiense* in *G. palpalis* by WIJERS (1958). Also the high amount of stumpy blood forms during the parasitaemia of the rat is important (WIJERS & WILLETT, 1960).

Out of quantitative reasons male and female flies were not separated. We got similar and statistically not significantly different infection rates in both sexes in an analogous experiment in 1969 (unpublished observations). The flies were kept in Geigy cages (15–20 each) at constant temperature (25°C) and humidity (75–80% rel.h.) in E.A.T.R.O.'s fly rooms.

Only one strain of *T. brucei* was used: Ulanga I 1093 (first passage; for the history of the strain see GEIGY, KAUFFMANN & BEGLINGER, 1967). The strain was kept deep-frozen in dry-ice and liquid nitrogen at the E.A.T.R.O./WHO reference bank in Tororo. This strain gave the highest infection rates in flies compared to other *T. brucei* strains as it was shown in the preliminary experiment (unpublished observation). One capillary tube of the strain was diluted with saline. Six rats were inoculated 0.5 cc each intraperitoneally.

One third of the infected flies were subsequently fed daily on *suitable donor blood* (SD), one third on *unsuitable donor blood* (UD) and the other third of them as a control on *defibrinated bovine blood* (Bovine) after the E.A.T.R.O. membrane-feeding technique by COCKINGS (1959). ATP as a feeding stimulant was added (10^{-3} M) according to the method described by GALUN & MARGALIT (1969).

The citrated and inactivated human donor blood came from Basle and was taken from the suitable human donor 21 (SD 21) and the unsuitable human donor 23 (UD 23). Both blood qualities were checked on cultures. The processed blood portions were transported in a cool-box at 6–10°C to Tororo and stored there up to use.

The bovine blood was taken daily fresh from three cows and one ox alternatively. The blood was defibrinated with glass-beads.

The flies were checked twice a day. All freshly dead and weak flies were dissected from the 10th day after infection (ID 10) on. The crop, proventriculus, midgut, hindgut, proboscis and salivary glands were examined under phase-contrast.

From heavy infections thin smears were made, fixed in 100% methanol for 10 to 20 minutes, and stained thereafter in Romanowsky-Giemsa for 40 minutes at pH 7.1.

Salivary gland infections were checked by slide probes and mouse inoculation. Thus, two substrains of *T. brucei* Ulanga I could be isolated (STIB 100 and 101). Established infections of midgut, proventriculus and salivary glands were recorded. Besides infected fly organs were fixed and processed for Electron Microscopy. This material is used for an ultrastructural study of the cycle of *T. brucei* in *Glossina* (STEIGER, in preparation).

The infection rates for midgut, proventriculus and salivary glands were calculated. Statistical evaluation of the results was carried out by comparing the ratios in a χ^2-test (chi-square; $P \leq 0.05$ or 0.01).

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Results

1. Survival Rate of the Flies:

The mortality of the flies used for our experiment was quite high. Only 527 out of 4,596 *G. morsitans,* i.e. 11.5%, and 1,060 out of 2,058 *G. fuscipes,* i.e. 51.6%, survived the 10th day after infection (ID 10), thus getting into consideration for our work. *G. pallidipes* were only available in very low numbers and they could so be omitted.

Only 100 *G. morsitans,* i.e. 2.2%, and 540 *G. fuscipes,* i.e. 26.3%, survived the important 18th day after infection (ID 18). The maximal age of the flies used for dissection was 28 days. See Table 1.

Table 1. Total numbers of flies and survival rates

Flies	*G. morsitans*			*G. fuscipes*			*G. pallidipes*			Total
	SD 21	UD 23	Bovine	SD 21	UD 23	Bovine	SD 21	UD 23	Bovine	
Total numbers of flies used	1,185	1,207	2,204	811	827	420	106	97	135	6,992 100%
Flies surviving ID 10	162	96	269	409	457	194	27	36	35	1,685 100%
Flies surviving ID 18	32	7	61	180	206	154	13	8	19	680 100%

2. Infection Rates of *T. brucei* in *G. morsitans* and *G. fuscipes:*

a. *Total Midgut Infections (ID 10 to ID 28)*

Each proventiculus or salivary gland infection involves an established midgut infection. This means occurrence of a great number of trypanosomes with a high division rate in the ectoperitrophic space. The data are given in Table 2.

Table 2. Infection rates

Fly organs	*G. morsitans*			*G. fuscipes*		
	SD 21	UD 23	Bovine	SD 21	UD 23	Bovine
Midgut	48/162 29.6%	31/96 32.3%	52/269 19.3%	42/409 10.3%	44/457 9.6%	28/194 14.4%
Proventriculus total (ID 10 to ID 28)	28/162 17.3%	10/96 10.4%	22/269 8.2%	24/409 5.9%	17/457 3.7%	22/194 11.3%
Proventriculus (ID 18 to ID 28)	6/32 18.8%	2/7 28.6%	8/61 13.1%	19/180 10.6%	12/206 5.8%	21/154 13.6%
Salivary glands	3/32 9.4%	0/7 –	6/61 9.8%	15/180 8.3%	9/206 4.4%	16/154 10.4%

In both fly species there is no significant difference in the infection rates of the flies fed on suitable human donor blood (SD 21) or unsuitable human donor blood (UD 23). Infection rates of *G. morsitans* after bovine blood feeding were significantly lower than after human blood feeding.

The overall infection rates in *G. morsitans* are higher than in *G. fuscipes,* but only after feeding with human blood.

In bovine blood there is no significant difference between the species.

b. *Total Proventriculus Infections (ID 10 to ID 28)*

The earliest infected proventriculus was found on ID 10. Only when the trypanosomes were seen in the lumen of the proventriculus we defined it as a proventriculus infection. The ratios are given in Table 2.

Again, there is no significant difference in the infection rates between SD 21 and UD 23 in both fly species (χ^2-value for *G. morsitans* being 2.25; $P \leq 0.05$).

Bovine blood gives a lower infection rate (not significant) compared to the human blood in *G. morsitans.* In *G. fuscipes* the ratio for bovine blood is significantly higher than for the *human blood* ($P \leq 0.05$).

For the human blood the infection rates in *G. morsitans* are significantly higher than in *G. fuscipes.*

c. *Proventriculus Infections (ID 18 to ID 28)*

No significant difference between SD 21 and UD 23 in the infection rates of both fly species was found.

Bovine blood compared to the human blood, gives the following results: in *G. morsitans* the infection rates are not significantly decreased, whereas in *G. fuscipes* they are not significantly increased ($\chi^2 = 0.05$ and $\chi^2 = 3.36$ respectively).

After feeding on human blood the infection rates for *G. morsitans* and *G. fuscipes* are different. The value for the unsuitable blood (UD 23) is statistically highly significant ($\chi^2 = 10{,}18$; $P \leq 0.01$). See Table 2.

d. *Salivary Gland Infections (ID 18 to ID 28)*

The earliest salivary gland infection was found on the 18th day after infection (ID 18). It has to be said that the trypanosome density in the infected glands fluctuated very much. On the whole the glands of *G. morsitans* were heavier infected than those of *G. fuscipes.* All infections, i.e. also "cryptic" infections with only a low number of trypanosomes in the gland duct, were considered.

Only a small number of the flies reached ID 18 (see Table 1). It is therefore not possible to give a reasonable statistical evaluation.

The salivary gland infection rates in our laboratory experiment coincide very well to ratios cited in literature (e.g. FAIRBAIRN & CULWICK, 1950). For our values see Table 2.

Discussion

In this experiment it could be demonstrated that the infection rates of midgut, proventriculus and salivary glands seem not to be affected by the human blood quality.

The high death rate of the flies, possibly due to the artificial membrane-feeding technique, leads to the fact that only small numbers of infections, especially salivary gland infections, could be taken into consideration for a reliable statistical evaluation.

The infection rates in our experiment may have been affected by several factors: possibly by the length of the trypanosome cycle, which is perhaps not only influenced by the sex of the fly (FAIRBAIRN & CULWICK, 1950), but also by

the species of the fly. Maybe the flies did not live long enough to develop a mature proventriculus or salivary gland infection, as in our case *G. morsitans* (Table 2).

As seen from our data it seems that the different fly species behave differently towards the same blood sort. This is well shown by *G. morsitans,* which have low infection rates with bovine blood compared to the human blood, and by *G. fuscipes,* which show higher infection rates in the same case. It is suggested, finally, that not the individual, but rather the species of mammal, from which the blood meals are derived, induces differences in the infection rates. It would be quite useful to extend analogous experiments to various mammalian species.

It is not clear what components of the blood affect infectivity in cultures. Blood groups, different amounts of plasma proteins, cholesterol, urea and glucose seem not to be determining factors (GEIGY & KAUFFMANN, unpublished observations). However, OGADA & NGULI (1968) assume influences of the different human blood groups on *T. rhodesiense* infections in sleeping sickness patients. AMREIN (1970) suggests that it is the donor's serum which determines infectivity in cultures. Using serum fractions without either albumines or gamma-globulines instead of whole serum does not induce production of infective metacyclic trypanosomes.

As it was pointed out in the introduction, we used for our work standardized material working under equal conditions. This means that the well known influences of temperature, relative humidity and differences in the strains of *T. brucei* on the fly and the cycle of the trypanosome in the fly can be ignored.

Yet, the processed human donor blood used for this experiment and stored sometimes up to two months may have had some influence on the infection rates. The anti-coagulant may also be of some importance.

Summary

In laboratory experiments working under equal conditions the authors show that the infection rates of *T. brucei* in *G. morsitans* and *G. fuscipes* are not affected by subsequent bloodmeals derived from a suitable or unsuitable human donor.

This is incompatible with results from culture experiments, where the restitution of virulence partly depends on the individual human blood donor.

Feeding the flies on bovine blood as an experiment control gave increased infection rates for *G. fuscipes* and decreased ones for *G. morsitans* compared to the human blood.

It is supposed that not the individual but rather the species of mammal and the species of the fly are decisive.

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