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INFLUENCE OF HEAT STRESS ON EXPERIMENTAL *TRYPANOSOMA BRUCEI BRUCEI* INFECTION IN MICE

by

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Summary — Influence of heat stress on parasitaemia, mortality rate and trypanosome morphology was studied in inbred C3H/HE mice following IP inoculation of 500 metacyclic forms of *Trypanosoma brucei brucei* (EATRO 1125/AnTAR 1). One groupe was kept at 35 °C, the other one at room temperature.

No significant differences were observed in mortality rate, neither in the evolution of the parasitaemia levels, nor in parasite pleomorphism. Both unstressed and heat stressed mice showed a sharp increase in long slender forms before death.

KEYWORDS: Trypanosomiasis, Experimental; *Trypanosoma brucei brucei;* Temperature; Survival Time; Parasitaemia; Pleomorphism.

Introduction

The temperature range to which animals are exposed can influence the course of trypanosome infection, by modifying the host's response or the parasite virulence, as observed by Kolodny (6) for *Trypanosoma cruzi* in rats and by Otieno (11) for *T. brucei* and *T. evansi* in mice.

As part of our studies on trypanotolerance, it was decided to undertake further experiments on *T. brucei brucei* infection in heat stressed mice.

Material and methods

Twelve weeks old female C3H/He mice (Bantin & Kingman, U.K.) were divided into two groupes of 12 animals. Three days before inoculation group I was put into an incubator (Curfew Appliances Ltd., Type 148, Ottershaw, U.K.) at a temperature of 35 ± 1 °C and group II was kept in the same room at ambiant temperature (25 ± 3 °C). During the experiment the two groups were provided with feed and water ad libitum.

The mice were inoculated intraperitoneally with 500 metacyclic forms of *Trypanosoma brucei brucei* extracted from salivary glands of experimentally infected *Glossina morsitans morsitans* (7) (8). The levels of parasitaemia of the inoculated mice were monitored three times a week by direct examination of peripheral blood according to Herbert and Lumsden (4). Smears of tail blood were stained with May-Grünwald Giemsa (phosphate-buffered diluent at pH 7.4) and examined unbiased for trypanosome morphology. The

trypanosomes were divided into 3 main types (slender, intermediate and stumpy forms) and 5 accessory types (filiformous, multinucleated, anucleated, dividing and abnormal forms) (2).

Results

Survival time:

Survival time ranged from 18 to 22 days in group I and from 16 to 22 days in group II (Fig. 1).

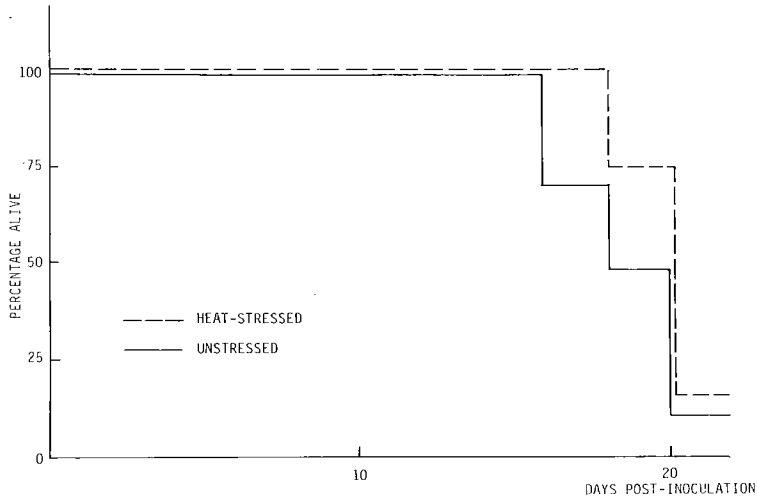

Figure 1.

Survival curves of heat stressed and unstressed female C3H/He mice inoculated with *T.b. brucei* EATRO 1125/AnTAR 1.

The comparison of the survival curves with the logrank test revealed no significant difference in mortality between the two groups ($\chi^2 = 0{,}402$; $\varphi = 1$; $p > 0.05$).

Parasitaemia:

At day 5 postinoculation, 8 mice out of 12 revealed positive in heat stressed group I, for 10 out of 12 in the unstressed group II.

From day 7 postinoculation on, parasitaemia levels were equal or lower in group I than in group II. However, none of the differences was significant at $\alpha = 0.01$; at $\alpha = 0.05$ only the difference on day 14 was significant (Fig. 2).

Individual parasitaemia curves in heat stressed animals usually showed two slight relapses before a third lethal peak. In group II relapses were less marked.

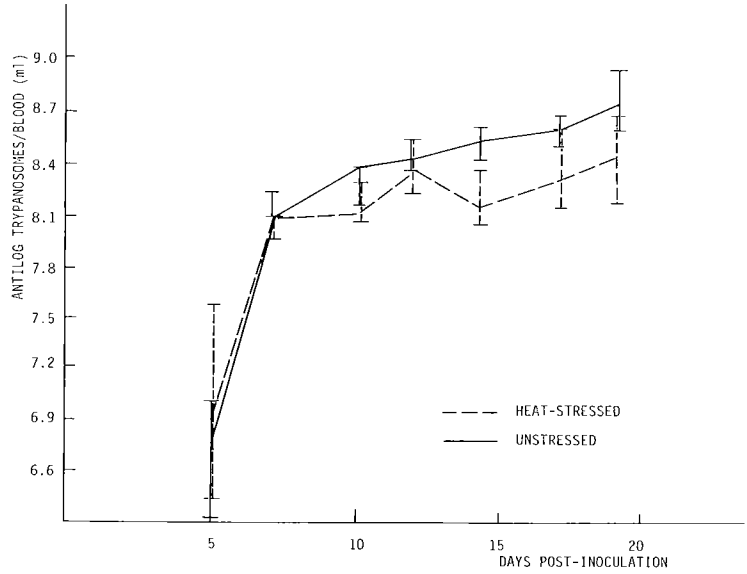

Figure 2.

Center parasitaemia levels and confidence limits of heat stressed and unstressed female C3H/He mice inoculated with *T.b. brucei* EATRO 1125/AnTAR 1.

Polymorphism:

Results of blood smears analysis are given in table 1.

Differences in main blood forms evolution were not significant. At day 19 all mice showed a rapid increase in slender forms (more pronounced in group II), a decrease in intermediate forms (more pronounced in group I) while in case of stumpy forms these decreased after a peak at day 14.

A lower proportion in stumpy forms at day 10, a higher one in slender forms and a lower one in stumpy forms at day 19 observed in unstressed mice are not significantly different when compared with the proportion of bloodforms observed with heat stressed mice.

Discussion

Ross and Williams (15) and Van den Branden (3) found that by lowering the ambiant temperature, the course of *T. brucei* infection in mice and rats became more chronic. Oehler (8) carried out serial passages of *T. brucei* in mice kept at 35 °C, and noticed a chronic remitting disease.

On the other hand, Klinger (5) found no effect of temperature on the course of *T. evansi* infection in rats. But in the experiments of Otieno (12), dyskinetoplastic *T. evansi* failed to develop in mice kept at 35 °C, while mice at 24-27 °C died during the first parasitaemia wave.

TABLE 1

Median percentages and confidence limits of bloodforms in heat-stressed and unstressed female C3H/Me mice inoculated with *T.b. brucei* EATRO 1125/AnTAR 1.

Day	Group	Number mice examined	Number trypanosomes counted	Main bloodforms			Accessory bloodforms				
				Slenders	Intermediates	Stumpies	Filiformous	Multi-nucleated	Anucleated	Dividing	Abnormal
10	25 ± 3°C	10	4465	12,50 ± 2,42	81,85 ± 4,37	5,53 ± 0,97	0,27 ± 0,63	0 ± 0	0,125 ± 0,27	0,44 ± 0,33	1,66 ± 1,0
	35 ± 1°C	8	3234	9,06 ± 4,71	81,29 ± 2,81	7,17 ± 4,04	0,86 ± 0,49	0 ± 0,2	0,18 ± 0,40	0 ± 0,21	1,07 ± 0,57
14	25 ± 3°C	10	4857	12,76 ± 1,82	65,55 ± 5,99	20,90 ± 4,49	0,56 ± 0,70	0 ± 0	0 ± 0,16	0 ± 0,28	1,12 ± 0,34
	35 ± 1°C	8	3365	12,03 ± 3,04	69,54 ± 3,51	18,54 ± 3,71	0,36 ± 0,43	0 ± 0	0,11 ± 0,31	0 ± 0	0,74 ± 0,69
19	25 ± 3°C	5	2740	33,49 ± 6,94	59,21 ± 3,56	7,59 ± 4,51	0,98 ± 0,54	0 ± 0	0,24 ± 0,43	0 ± 0	0,49 ± 0,25
	35 ± 1°C	6	2491	31,91 ± 7,52	59,43 ± 8,95	8,63 ± 1,49	0,43 ± 0,3	0,15 ± 0,52	0,38 ± 0,26	0,1 ± 0,17	1,13 ± 0,8

Kolodny (6), Trejos *et al.* (14), Amrein (1), Marinkelle and Rodriguez (9) all observed that a high ambiant temperature prolonges the life of animals and in some cases cures experimental *T. cruzi* infections in rodents.

Otieno (13) found that a virulent strain of *T. brucei rhodesiense* caused chronic infection when ambiant temperature of mice was raised to 35 °C. Under similar conditions a strain of *T.b. brucei* caused lower parasitaemia but survival time was not altered.

Also the phenomenon of heat stress on *Plasmodium berghei berghei* infection in Swiss mice was studied by Moyou-Somo *et al.* (10). They observed that infected mice maintained in permanence in a hot environmental temperature (35 °C) undergo a chronic infection whereas controls maintained at the laboratory temperature develop an acute and lethal infection.

In the present study, we found no significant difference in average time to death between the two groups, but difference exists regarding the start of mortality. Neither did we find any significant influence of the ambiant temperature on the level of parasitaemia.

Higher slender forms proportion at day 19 in unstressed mice is not significant, nor correspondig lower proportion in stumpy forms.

The widely varying effect of high environmental temperature on the course of a trypanosome infection in mice seems to be the result of a complex host-parasite relationship in which the strain of trypanosomes and the strain of mice are equally important.

L'influence du stress thermique sur l'infection expérimentale de la souris par *Trypanosoma brucei brucei.*

Résumé — L'influence du stress thermique a été étudiée chez des souris consanguines C_3H/He inoculées par voie intrapéritonéale avec 500 formes métacycliques de *T.b. brucei* (EATRO 1125/AnTAR 1).

Un groupe a été soumis à une ambiance thermique chaude (35 °C), un autre groupe a été maintenu à la température ambiante de laboratoire. Les souris stressées comme les non stressées montrent une forte augmentation du nombre de formes longues de *T.b. brucei* au cours de la période précédant la mort. Aucune différence significative n'a été observée entre les deux groupes en ce qui concerne la parasitémie, le temps de survie moyen et le pleomorphisme parasitaire.

Invloed van warmtestress op een experimentele *Trypanasome brucei brucei* **infektie bij de muis.**

Samenvatting — De invloed van warmtestress werd bestudeerd op een experimentele *T.b. brucei* infektie bij inteeltmuizen C_3H/He geïnoculeerd met 500 metacyclische vormen van *T.b. brucei* (EATRO/AnTAR 1).

Een groep werd gehouden bij een temperatuur van 35 °C., een andere groep bij laboratoriumtemperatuur. Onmiddellijk voor het moment van sterfte vertoonden beide groepen een aanzienlijke toename van lange vormen van *T.b. brucei.* Er werden echter geen signifikante verschillen gevonden betreffende parasitimie, gemiddelde overlevingstijd en parasitair pleomorfisme.

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