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Factors influencing the survival of mice infected with *Trypanosoma equiperdum*

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Introduction

When *Trypanosoma equiperdum* is used in studies employing large numbers of experimental animals, such as mice, and when standardization of the number of parasites to be injected per unit of transfer solution is required, a number of problems can adversely affect the experimental results. The most important of these problems is the time interval between taking the blood from the donor animal, such as a rat, until all counts and dilutions have been made and the required amount of blood has been injected into the experimental mice. Another factor is the temperature at which the diluted blood is maintained during this interval. The degree of parasitemia in the donor rat may be important, especially should the donor be in the terminal stages of infection. The solutions to these problems have not been reported in the literature. However, of value is the report of Chen, *et al.* (1945) which deals with the relationship of the number of parasites injected and the death time of mice. Morrell, *et al.* (1937) reported on the effect of age, weight, sex and dose on the death time in rats. Both accounts show great individual variations in death time in mice and rats.

For the purpose of preparing an experimental design and in order to clarify conflicting data obtained in experiments using *Trypanosoma equiperdum*, the above stated factors were tested. In addition, the effects of the ages and weights of mice to be injected were tested.

The results of the investigations show the importance of the time and temperature factors on planning experiments utilizing *T. equiperdum*, and in interpreting data from such experiments.

Materials and Methods

The NIH strain of *Trypanosoma equiperdum* was maintained at −70°C. Prior to use the trypanosomes were thawed, injected into mice and transferred into the donor rats which would supply adequate amounts of blood. Blood from these donor rats was then collected by cardiac puncture and diluted with a transfer solution (Chen, *et al.* 1945). The mice used in the following experiments were inoculated intraperitoneally with 0.2 ml, each receiving approximately 150,000 trypanosomes. Trypanosome counts were made prior to injection for each time interval.

Females CF_1 mice were divided into three test groups:

Test I: This group was to be used to determine the effects of intervals of time between removal and injection of the trypanosomes from the donor rats to mice, and the effects of temperature at which the blood was maintained. The group was sub-divided into two

groups of 150 mice weighing between 20 to 22 grams. Group A mice were to be inoculated with trypanosomes maintained at 37°C and Group B mice were to be inoculated with trypanosomes kept at ambient temperature (25°C). Each of the two groups was further divided into nine smaller series, the first seven were composed of twenty mice each, and the last two of five mice each. The mice of these series were injected at the following intervals from the time the blood was drawn from the donor rat: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, 8.0, and 11.5 hours.

Test II: The purpose was to determine the effects of the degree of parasitemia in donor rats, including terminal and post-terminal rats on infections in mice. The mice were divided into six sub-groups. The first five were composed of fifteen mice each, and the last sub-group of ten mice. Male Sprague-Dawley rats weighing between 250 to 270 grams and inoculated with ca. 1.5×10^6 trypanosomes were used as donors when the durations of their infection were respectively: 48, 60, 72, 84 hours, a few minutes prior to death of one donor rat at 90 hours, and 10 minutes after another donor rat had died at 93 hours. The transfer of the trypanosomes from the rats to mice was completed within one hour after the blood was withdrawn from the rats.

Test III: In order to study the effects of age on infection five groups of mice were used. Twenty-five mice were utilized in each group at the following ages: 5, 7, 9, 11, and 13 weeks. The transfer of the trypanosomes from the donor rats to mice was completed within one hour after the blood was withdrawn from the rats. In this test donor rats with an infection duration of 56 hours were used.

Results

The effects of the time interval between removal and injection of the parasites into mice, and temperature of the inoculum during the interval are depicted in Table I and Figure 1. Trypanosomes in blood kept at 25°C for 0.5, 1.0 and 1.5 hours killed the mice at the average time established for the controls (88.2 hrs.). At 2 hours and longer, the death times were extended, and some mice were killed by parasites kept for as long as 11.5 hours. However, those trypanosomes maintained at 37°C had somewhat longer death times at each 0.5 hour interval, with an average of 93.8 hrs. for the first 3 intervals. At 2 hours it took an average of 140.8 hrs., and only 13 of the 20 mice were

Table 1 Effects of temperature and the time interval between removal and inoculation on virulence of *T. equiperdum* in mice

Series no.	Time interval (hours)	Hours of survival: Group A (Inoculum kept at 37°C) Sample size	Range	Mean	S.D.	S.E.	Group B (Inoculum kept at 25°C) Sample size	Range	Mean	S.D.	S.E.	*t* value
1	0.5	20	78–105	83.45	7.00	1.57	20	74–115	85.85	12.74	2.85	0.74
2	1.0	20	85–112	92.10	5.76	1.29	20	74–176	92.00	21.60	4.83	0.02
3	1.5	20	95–123	106.05	7.17	1.60	20	75–106	86.70	6.82	1.53	8.74*
4	2.0	20	127–164	140.85	11.08	2.48	20	109–149	122.00	10.00	2.24	5.65*
5	2.5	20	(169–454)	13/20 dead at 223.92 hrs.			18	127–157	142.16	8.52	2.01	
6	3.0	20	None dead				20	137–239	161.25	20.17	4.51	
7	5.0	20	None dead				20	155–190	165.25	8.71	1.95	
8	8.0	5	None dead				5	174–192	182.40	6.50	2.91	
9	11.5	5	None dead				5	(175–211)	3/5 dead at 189.70 hrs.			

* Significant at the 0.01 level.

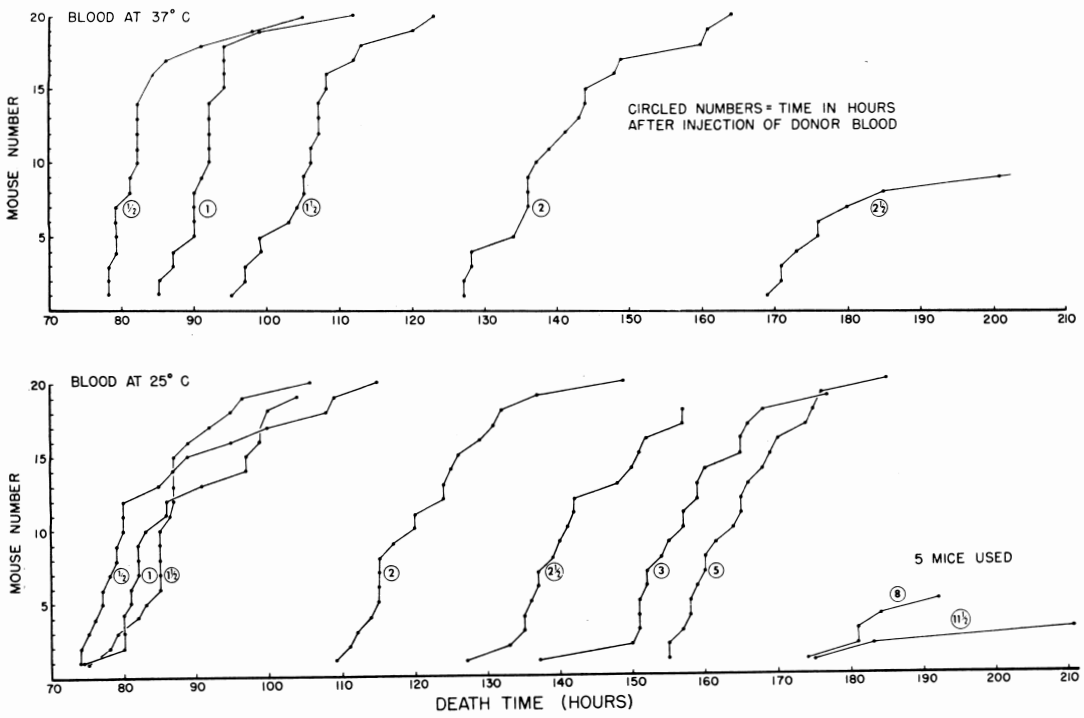

Fig. 1 Death times in mice due to *Trypanosoma equiperdum* infections as affected by temperature (25°C and 37°C) and time intervals between removal and inoculation of the donor blood

killed at an average of 223.9 hrs. at 2.5 hours. No mice were killed in any of the transfers of blood kept longer than 2.5 hours, and periodical tail snips from each mouse revealed no parasitemia.

In Test II the mice infected with trypanosomes in rat blood withdrawn 10 minutes after the death of the donor rat (post-terminal) lived an average of 124.1 hours, which is approximately 28.5 hours longer than the mice which were inoculated with trypanosomes withdrawn from a terminal convulsing donor rat. The parasitemia durations of 48, 60, 72, and 84 hours killed mice in an average of 93 hours.

Test III shows no significant correlation between the age and weight of the mice and death times. There are no significant differences between the means of the survival hours (average=91.1 hours) in the five groups.

Discussion

It has not been determined as to what factors play a part in the death times of mice inoculated with blood containing trypanosomes kept at 25°C or 37°C. Possibly a reduced metabolism at the cooler temperature permits the parasites to live longer, retain greater reproductive capabilities, virulence and/or infectivity. It is assumed by some, without supporting evidence, to be due to reduced viability.

According to Chen *et al.* (1945) under normal conditions (ambient temperature and transfers made within 50 minutes), death in mice is related directly to the number of trypanosomes injected. Their findings are: 5 trypanosomes per mouse; all mice died within a period of 30 hours, starting at 129 hours. When the infective dose was 500,000 per mouse, all mice died within 30 hours, starting at 62 hours. In our study all mice received the same number of trypanosomes. Apparently, in those cases where no deaths occurred in the mice, the trypanosomes died after being injected, or had lost their ability to reproduce, as they

were alive and motile at the time they were injected.

It is doubtful that any type of immunity could have played a protective role in these tests. Soltys (1964) demonstrated that it was possible to produce immunity in animals with repeated injections of dead *Trypanosoma brucei*. This bears no relationship to these conditions studied.

Poindexter (1933) found that the number of trypanosomes was decreased in terminal rats and suggested that death in rats was caused by hemolysis of red corpuscles and acidosis. A number of workers have given various theories regarding the cause of death due to trypanosomes. Most are refuted in a thorough discussion by von Brand (1966) who gives evidence that toxins most likely play a major role and that the liver is involved. We have demonstrated that trypanosomes from post-terminal rats are less virulent or infective than trypanosomes from non-terminal and terminal donors. The reason for this decrease is not known; however, it may be related to certain unknown factors associated with the death of the donor rat.

Morrell *et al.* (1937) reported that there was no correlation between the age or weight of rats and their length of survival after infection with trypanosomes. We have obtained similar evidence in mice.

Summary

Both the temperature at which the rat donor blood containing *Trypanosoma equiperdum* was kept, and the length of time the blood was thus maintained until injected into mice affected the host death times. Normal death times (ave. hrs. 88.1) resulted from transfers made into mice 0.5, 1.0, and 1.5 hours after the donor blood kept at 25°C had been taken from the rat. However, at the 2 hour interval, this time was increased by 34 hours (ave. hrs. 122). These increments continued as the time intervals were increased. The parasites kept at 25°C for as long as 11.5 hours killed some of the mice. When the donor blood was kept at 37°C the death times were increased with each additional half hour time interval. Only parasites injected into mice at 0.5 and 1.0 hour intervals killed mice within the normal range (83.5, 92 hrs.). At 1.5 hours the time was increased to 106 hours and at 2 hours to 141 hours; about 53 hours above normal. Only some of the mice injected with donor blood kept for 2.5 hours at 37°C died (at 224 hrs.), and none died at the time intervals above 2.5 hours. In other tests, it was found that there were no significant differences in survival hours when blood was taken at different durations of parasitemic development in the donor rat, including periods of 48, 60, 72, 84 hours and terminal. There was a slight loss in parasite virulence at 10 minutes post terminal. The ages and weights of mice used did not affect survival.

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