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THE NUMBER OF TRYPOMASTIGOTES OF TRYPANOSOMA CRUZI, REQUIRED TO INFECT RHODNIUS PROLIXUS

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SUMMARY

As few as 177 blood stream trypomastigotes of **Trypanosoma cruzi** successfully infected **Rhodnius prolixus,** but an accurate determination of the lowest limit could not be determined by present methods. There was an optimum number of ingested trypomastigotes for the heaviest infection of **R. prolixus.** This was 1 x 10^3 to 1 x 10^4 trypomastigotes and higher or lower numbers gave lower bug infections.

INTRODUCTION

The study of drug action against experimental infections with **Trypanosoma cruzi** often results in a situation where prolonged microscopical examination of the blood of the treated animal does not reveal any trypomastigotes. Such animals often have a significant titre of circulating antibodies, even if the treatment resulted in a prompt disappearance of trypomastigotes from the peripheral blood.

For our work, it was decided to rely on parasitological methods for detection of **T. cruzi** infection, rather than the reinfection techniques used by HABERCORN & GONNERT [2]. The parasitological methods which could be considered as having a degree of sensitivity were xenodiagnosis, as used successfully over many years by many Latin-American workers, and culture of blood.

In the first part of this investigation, various parameters of the xenodiagnostic procedure were studied and an estimate made of the numbers of trypomastigotes required to be ingested by a triatomid bug to give rise to a detectable infection.

Material, methods and preliminary experiments

Triatome colony and handling procedure

The **Rhodnius prolixus** strain was an old laboratory colony originally isolated in Venezuela (see MSHELBWALA & ORMEROD [4], for history). The **Triatoma infestans** bugs were from a colony maintained at the London School of Hygiene and Tropical Medicine since 1968. The colony originated from field material from Brasil.

The methods for rearing and maintaining the **Rhodnius prolixus** colony were similar in principle to previous workers (e.g. PHILLIPS [6]). Mixed age populations were maintained in glass bowls closed with nylon cloth of a known aperture size (500μ). For instars of known age, eggs were collected and reared separately. The bugs were fed on anaethetised guinea-pigs. All insects were kept at a temperature of 27-28°C and 50-60% humidity. Bugs were immobilized by chilling briefly and 3rd and 4th stage instars selected for feeding on animals infected with **T. cruzi.** Bugs for experiments were either housed singly or in groups of about 10.

Examination of bugs for T. cruzi

A fresh faecal smear was examined (4 mm dry objective and 6 x ocular) from each bug and the number of flagellates in 100 microscopic fields was determined. Unfed bugs, that is, those individuals without a weight increase were not included in data on infectivity. No normal bugs were found to have natural infections with flagellates (CERISOLA et al. [1]).

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Preliminary experiments showed that a greater proportion of **R. prolixus** bugs were infected when examined 22 days after feeding (12/22, 55%) than after 32 days (10/21, 48%). This is in agreement with PATTERSON & MILES [5] who also found that the shorter incubation period was better for **R. prolixus**.

Experimental vertebrate T. cruzi **infections**

The **T. cruzi** isolate **Peru** (for previous history see MSHELBWALA & ORMEROD [4] has been used throughout these studies. Guinea-pigs, Dunkin Hartley strain, were inoculated subcutaneously with 1-5 x 10^6 trypomastigotes/kg body weight. In the single experiment with the monkey host **Erythrocebus patas**, the animals were infected with 1 x 10^6 trypomastigotes/kg. The trypomastigotes were derived from infected mice in which the isolate was kept in the virulent stage by weekly passaging into clean mice. The course of infection in guinea-pigs and monkey was followed by microscopical examination of fresh blood taken from the ear, 1 to 3 times/week depending on the stage of the infection.

When bugs were fed, the white cell count on the guinea-pig or monkey was determined by the standard method using an improved Neubauer counting chamber and thick blood smears prepared. The unfixed thick blood films were stained with Giemsa stain. From the stained films, the proportion of trypomastigotes to 1000 or more white blood cells was determined microscopically.

From the white cell count and proportion of trypomastigotes to white blood cells, the number of trypomastigotes/ml in circulating blood was calculated. From the weight of ingested blood, the number of trypomastigotes ingested by each bug was determined.

RESULTS

1) **Number of trypomastigotes required to infect** R. prolixus

The correlation of the numbers of trypomastigotes ingested, with presence or absence of flagellates in the smear of rectal contents is presented by grouping the results according to numbers ingested (Tables I and II).

TABLE I

Infectivity of **T. cruzi** isolate **Peru** to **R. prolixus** after feeding on infected guinea-pigs

Group No.	No. of trypomastigotes ingested/bug	Proportion of infected bugs in different experiments				Total (%)
		1	2	3	4	
1.	1 — 1000	—	9/14	—	4/7	13/21 (62)
2.	1001 — 2000	—	38/43	—	1/1	39/44 (89)
3.	2001 — 3000	—	3/3	—	1/3	4/6 (67)
4.	3001 — 4000	—	—	—	0/4	0/4 (0)
5.	4001 — 5000	—	—	—	—	—
6.	5001 — 6000	—	—	0/1	—	0/1 (0)
7.	6001 — 7000	—	—	—	0/3	0/3 (0)
8.	7001 — 8000	—	—	0/2	0/1	0/3 (0)
9.	8001 — 9000	—	—	—	0/1	0/1 (0)
10.	9001 — 10,000	—	—	1/1	—	1/1 (100)
11.	1 — 10,000	—	49/60	1/4	6/20	56/84 (67)
12.	10,001 — 100,000	31/46	—	13/21	8/13	52/80 (65)
	Mean weight of blood ingested/bug mg	97	192	152	194	—

The overall infectivity shows that about a third of the fed **R. prolixus** remained uninfected. In about half of the bugs studied, massive numbers of trypomastigotes were ingested. Analysis of the results from bugs which had ingested smaller numbers of parasites, (1-10,000 parasites, Table I; 101-1000 parasites, Table II) shows that the number of bugs in each number band is often too small to draw detailed conclusions. However it is shown in Table I, that the percentage of bugs infected in group 1 was lower than that in group 2. Further analysis of group 1 (Table I) is shown in Table II and combined with results from bugs fed on an infected monkey **E. patas.** The results in Table II suggest that the bugs which had ingested 401 or more parasites, were infected with a percentage infection similar to the group with the larger number of ingested parasites (63 and 70%), whereas below 401 ingested parasites, the proportion of bugs infected was very much less. The lowest number of trypomastigotes to infect a bug was 177 in the monkey experiment and 320 from the guinea-pig experiment.

TABLE II

Analysis of numbers of positive **R. prolixus** after ingestion of an estimated less than 1000 trypomastigotes

Group No.	No. of ingested trypomastigotes	Proportion of positive bugs		Total	
		Monkey expt.	Guinea-pig expt.		
1.	101 — 200	1/2	—	1/2	
2.	201 — 300	0/3	0/1	0/4	2/8 (25%)
3.	301 — 400	0/1	1/1	1/2	
4.	401 — 500	2/5	1/1	3/6	
5.	501 — 600	5/7	1/3	6/10	15/24 (63%)
6.	601 — 700	3/3	3/5	6/8	
7.	701 — 800	—	2/2	2/2	
8.	801 — 900	—	1/3	1/3	7/10 (70%)
9.	901 — 1000	—	4/5	4/5	
	Total	11/21	13/21	24/42	

In order to investigate the ability of very low numbers of trypomastigotes to infect **R. prolixus,** 3rd stage instars were fed on five guinea-pigs with sub-patent infections. Microscopical examinations did not reveal any trypomastigotes in thick blood films. The stained films were examined until 10,000 white blood cells had been counted. From the white cell count, this was approximately equivalent to 140 μl of blood and therefore the circulating blood contained less than 7 trypomastigotes/ml. Bugs ingesting 100 mg of blood theoretically also ingested less than 0.7 of a trypomastigote. The results of examination of **R. prolixus** 4 weeks after feeding, showed 1/55 (2%) was positive.

2) **Numbers of flagellates observed in bugs**

The observations on feeding **R. prolixus** on infected guinea-pigs are divided in 3 groups (Table III).

TABLE III

Correlation between numbers of trypomastigotes ingested with numbers of flagellates observed in smears of rectal contents per 100 microscopic fields

No. of trypomastigotes ingested	No. of observations	Mean no. of flagellates
1 — 1,000	19	12.6
1,001 — 10,000	70	76.4
10,001 — 100,000	74	19.7

This shows that as expected, the lowest number ingested gave the lower infections in the bugs, but the heaviest infections were found after ingestion of 10^3 to 10^4 parasites. The mean number of flagellates in smears prepared from the bugs which had ingested from 10^4 to 10^5 blood stream trypomastigotes, was 27% of the middle group.

DISCUSSION

After the study of factors giving maximum infections with **R. prolixus,** the major objective was to determine the lowest number of trypomastigotes required to infect bugs. This had not been determined, since the lowest infection rate achieved of 177 trypomastigotes, was infective. However, at infection rates below about 400 trypomastigotes per bug, infections have been shown to be 2.5 times less frequent than with higher infection rates. Technical difficulties with the method have not allowed a more detailed conclusion. Lower rates are possibly infective as shown by the 2% (1/55) **R. prolixus** infected with blood which was microscopically negative. To pursue this objective, other techniques would need to be adopted such as feeding bugs through an artificial membrane, on blood containing a known dilution of trypomastigotes.

Two significant features of the **R. prolixus** infections have been observed throughout this investigation. The first is the failure to achieve highly uniform infection rates even with large numbers of ingested trypomastigotes. The second is the extreme variability of the level of infections in infected bugs.

One source of error in the calculation of numbers of parasites ingested by individual bugs, is due to defaecation during feeding. In the methods employed in the present study, the faecal drop was absorbed on to filter paper but in the time elapsing between feeding and reweighing (5-15 minutes) some or all of the water in the faecal drop must have evaporated. Therefore the calculated weight of ingested blood should be increased by the weight of the faecal drop. This latter is difficult to measure but is estimated to be in the region of 10 μl or about 10 mg. Thus the number of parasites ingested might be increased by 10 to 25%.

A further complicating factor is the assumption that the distribution of trypomastigotes in the circulating blood of guinea-pigs is uniform and that the numbers of parasites in ingested blood in samples of equal weight are identical. While this assumption may be approximately true in heavier infections, it is likely to be incorrect in very low grade infections. The major factors are likely to be the non-synchronous release of trypomastigotes from intracellular pseudocysts and the variation in the time that trypomastigotes circulate before penetration of new host cells, in addition to the effect of the immunological response of the host on the parasites.

The failure of the highest mouse infections to give the heaviest bug infections is difficult to explain. It is feasible to reject the results on the grounds of non-standardization of rectal smear preparation, but the difference between groups is quite large. Is the low grade bug infection due, perhaps, to competition for favourable sites for multiplication or to biochemical effects such as local depletion of growth factors or accumulation of toxic materials?

It is difficult to compare the present xenodiagnostic system, **Rhodnius prolixus** mouse infections with **T. cruzi** isolate **Peru,** with the systems used for detection of **T. cruzi** in man because of the absence of comparable data. The latter techniques have been developed with a large background of data, and it may be that the experimental system used in the present study has a lower sensitivity.

Most workers using xenodiagnosis as a diagnostic procedure use **Triatoma infestans** rather than **Rhodnius prolixus.** The former species is favoured due to the smaller skin reaction to the saliva as compared with the reaction to **R. prolixus** saliva. As regards infectivity to **T. cruzi,** MILES et al.[2] found that xenodiagnosis with **T. infestans** was better than with **R. prolixus.** However, they examined **R. prolixus** after 30 days incubation whereas it is known that this time is too long for this species of insect (PATTERSON & MILES[5] and above p. 178). In a smaller scale but similar experiment, no difference between the two insect species could be detected (NEAL, unpublished).

NEAL, R. A. & MILES, R. A. — The number of trypomastigotes of **Trypanosoma cruzi**, required to infect **Rhodnius prolixus**. **Rev. Inst. Med. trop. São Paulo** 19:177-181, 1977.

RESUMO

Número de tripomastigotas de Trypanosoma cruzi **necessário para infectar o** Rhodnius prolixus

Uma população de até 177 formas sanguícolas (tripomastigotas) de **Trypanosoma cruzi** mostrou-se suficiente para infectar o **Rhodnius prolixus,** mas, pelos métodos atuais, não foi possível estabelecer uma determinação acurada do número infectante mínimo.

Foi estimado o número ótimo de tripomastigotas ingeridas para se obter uma infecção mais intensa do **R. prolixus.** Esse número é de 1 x 10^3 a 1 x 10^4, verificando-se que números superiores ou inferiores a esse deram origem a infecções de nível mais reduzido.

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