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Direct transmission of *Trypanosoma cruzi* between vectors of Chagas' disease

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Summary

Trypanosoma cruzi was transmitted directly between triatomines by cannibalism or coprophagy. Different conditions involving cannibalism that excluded coprophagy were studied in *Dipetalogaster maximus.* Infections occurred if an uninfected donor bug sucked infectious blood and if this blood was taken up from the stomach by a cannibalistic bug. If the donor was infected and sucked uninfected blood afterwards, the source of the uninfected blood determined the transmission rate: If the uninfected blood originated from mice, many cannibalistic bugs became infected because complement factors from mouse blood did not lyse *T. cruzi* in the stomach of the bug. If the uninfected bloodmeal originated from chickens, cannibalistic bugs occasionally became infected, even though chicken blood is known to lyse all stages of *T. cruzi* in the stomach. Experiments on coprophagy provided the first conclusive demonstration that transmission of *T. cruzi* occurs between individual *Triatoma infestans,* as a result of coprophagic behaviour alone, and excluding the possibility of cannibalistic transmission.

Key words: cannibalism; coprophagy; *Trypanosoma cruzi;* triatomines; Chagas' disease.

Introduction

Field infections of Chagas' disease vectors with *Trypanosoma cruzi* or other heteroxenous trypanosomatids result mainly from the uptake of infected blood from vertebrate hosts. Alternatively, direct transmission from insect to insect may result from two modes of behaviour, cannibalism and coprophagy.

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Cannibalism is performed by many bloodsucking insects (Hocking, 1971) and may be traced back to insectivorous ancestors which subsequently became haematophagous (Brumpt, 1914). Since the prey survives and only the stomach contents and/or haemolymph of the prey are ingested, the term «cannibalism» does not exactly describe the behaviour of the bugs, but has been retained despite several attempts to introduce new terminology (Añez, 1982). Cannibalism has often been observed in laboratory colonies of bugs, where hungry young larvae feed upon engorged bugs (e.g. Brumpt, 1914; Añez, 1982). Cannibalistic transmission of *T. cruzi* has rarely and only doubtfully been demonstrated (see discussion) and the effects of postinfective blood meals taken by the donor on transmission to the next bug have not been examined.

In the blood feeding hemiptera coprophagy serves primarily as a means for acquiring midgut-associated symbionts. Although Brumpt (1914) observed ingestion of an entire drop of faeces, uptake of the symbionts from the egg shell can be sufficient to allow the development of midgut populations (Brecher and Wigglesworth, 1944). If minor amounts of faecal or egg shell material are taken up, it is not possible to determine whether coprophagy has occurred or not, the presence of symbionts or parasites in the intestinal tract providing only an indirect proof.

All studies on but-to-bug transmission of *T. cruzi* by either cannibalistic or coprophagic means do not satisfactorily eliminate infection by the alternative route. In the present study, transmission of *T. cruzi* between bugs by each route is successfully demonstrated in isolation, and the effects of a postinfective bloodmeal on cannibalistic transmission are investigated.

Materials and Methods

Trypanosoma cruzi. – Strains "Chile 5" (zymodeme 1) and "Chile 7" (zymodeme 2) were isolated from *Triatoma infestans* originating from Cachiyuyu, Chile (Ebert and Schaub, 1983; Schaub and Schottelius, 1984) and were maintained cyclically between *T. infestans* (faeces of established infections) and mice (increasing parasitemia, 3–4 weeks p.i.).

Triatomines. – *Triatoma infestans* strains "Chile" and "Chile 11" also originated from Cachiyuyu and were started in 1979. *Dipetalogaster maximus* was obtained from the Tropical Institute, Hamburg, FRG, and the stock was presumably started in 1974 by P. Marsden, Brazil. Parallel studies on cannibalism using different triatomines demonstrated the extraordinary readiness of *D. maximus* to undergo cannibalistic behaviour (Schaub, unpublished), and this species was therefore chosen for the cannibalism studies. All bugs were fed on chickens and reared at ca. 26° C, 60–70% relative humidity and on a 16 h/8 h day/night rhythm.

Cannibalism experiment 1. – Uninfected first instars (L1) of *D. maximus* were fed on mice with a parasitemia of ca. 10^7 *T. cruzi* "Chile 5"/ml blood. Within 15 min after the donor bugs had finished feeding they were exposed to hungry L1. Defense reactions of the donors were prevented be seizing the bug behind the last pair of legs (Nicolle and Mathis, 1941). Some bugs were cooled for 15 min in a refrigerator to test the influence of temperature on the induction of cannibalism. The cannibalistic bugs were separated after they had stopped sucking. Ten days later ¾ of the not fully repleted cannibalistic larvae were fed individually on uninfected mice. Twenty-one days after cannibalism faeces of all cannibalistic bugs were studied for the occurrence of *T. cruzi.*

Cannibalism experiment 2. – L1 of *D. maximus* were fed on mice infected with one of the *T. cruzi* strains. After ecdysis, some L2 (3 weeks p.i.) were used as donors, the other were fed again on uninfected mice and used as L3 donors (6 weeks p.i.). Infection in the stomach of the donors was demonstrated by dissection of 2 L2 and L3, and Giemsa-stained smears were used to determine the percentage of the developmental stages of *T. cruzi.* The infected donor bugs (L2 or L3) were fed on uninfected mice or chickens, then fed upon by hungry L1. The rate of cannibalism in hungry L1 was enhanced by warming and holding the donors (Nicolle and Mathis, 1941). The cannibalistic larvae were reared separately for 3 weeks, fed individually on uninfected mice and examined 4 or 5 weeks after cannibalism (faeces or intestinal content if the faeces were negative for *T. cruzi*).

"In vitro cannibalism". – Two major unknown variables associated with the above experiments are the density of stomach populations and amount of blood imbibed by the donor bugs. Therefore, "in vitro cannibalism" studies were performed in order to better compare the influence of different blood sources on cannibalistic transmission. The stomachs from adults with established infections of *T. cruzi* "Chile 5" and starved for 4–6 weeks were dissected, disrupted and diluted in physiological saline, and the number of *T. cruzi* counted in a Neubauer haemocytometer. *T. cruzi* were diluted to concentrations of 10^6 and 10^4 flagellates/ml saline and both concentrations were diluted 1:10 in blood from laboratory mice or a brown leghorn chicken. The 4 mixtures were offered to the bugs at 36° C through a silicone membrane for 1 h and larvae, which had fed to capacity, were then individually maintained in an incubator. Since the L1 of *D. maximus* ingest 50–100 µl blood, infective doses of 5,000–10,000 or 50–100 *T. cruzi*/bug were achieved. The infective doses for the L1 of *T. infestans* were about $^1/_{10}$ those of *D. maximus.* After 4 weeks all larvae had moulted and were fed individually on uninfected mice. Four weeks later faeces and, if negative, the intestinal contents were examined for *T. cruzi.*

To examine the direct action of both blood types on *T. cruzi,* 300 µl of the stomach homogenate (10^6 flagellates/ml solution) were mixed with 300 µl blood from mice or chickens, incubated for 10 and 60 min, then examined under the light microscope for signs of trypanosome damage.

Coprophagy. – 9 groups, each consisting of 12 uninfected L4 of *T. infestans* (14 days after feeding) and of 4 *T. cruzi* "Chile 5"-infected L5 (established infection) were reared in 1 litre glass beakers.These were divided into 3 experimental series:

- Series Co I – Infected and uninfected bugs were maintained together for 2 weeks, then fed weekly for 1 h on a chicken for a further 8 weeks, after which previously uninfected bugs were dissected and examined for intestinal and rectal *T. cruzi* infections.
- Series Co II – Infected bugs were maintained above uninfected bugs by separating them with 2 layers of wire mesh. This prevented direct contact between the groups but allowed faeces from the infected bugs to fall into the area occupied by the uninfected bugs. First uninfected and then infected bugs were fed weekly on the same chicken and previously uninfected bugs examined for infection as above.
- Series Co III – Bugs were maintained as in series Co II. Only infected bugs were fed weekly on a chicken for 8 weeks. Faeces from primarily uninfected bugs were examined after 8 weeks, the bugs fed on a chicken, then examined 4 weeks later for infection as above.

Results

Cannibalism experiment 1

Cannibalism could easily be induced in L1 of *D. maximus* and often occurred without additional warming or handling of the donor bugs. In one experiment a chain of 3 cannibalistic bugs was observed. Sometimes the stomach of the donors was totally emptied, but when fed again 2 days later these bugs moulted normally. Donors, which had been cooled in the refrigerator, were never attacked. After donors had taken up infected blood, cannibalism always

Table 1. Cannibalism experiments 1 and 2: transmission of *Trypanosoma cruzi* "Chile 5" and "Chile 7" by cannibalism to uninfected first instars of *Dipetalogaster maximus*

Infection of donor bugs with	Feeding of donor bugs on	Number of cannibalistic larvae infected/uninfected	
		group A	group B
–	mice, infected with *T. cruzi* "Chile 5"	1/0	12/0
T. cruzi "Chile 5"	hen	1/3	0/5
	uninfected mice	3/3	0/1
T. cruzi "Chile 7"	hen	0/2	0/2
	uninfected mice	3/0	1/2

group A: larvae about half repleted
group B: larvae less than half repleted and therefore not able to moult

lead to an infection with *T. cruzi* "Chile 5" (Table 1). More flagellates occurred in the faeces of cannibalistic larvae after being fed with an additional blood meal from uninfected mice.

Cannibalism experiment 2

Infection could still result from cannibalism even after primarily infected donors had imbibed uninfected blood (Table 1). Sample specimens from L2 and L3 groups, which were used as infected donors, revealed a stomach population of both *T. cruzi* strains consisting of about 99% epimastigotes and 1% metacyclic trypomastigotes. The infection rates in the different combinations of *T. cruzi* strains and sources of blood were much higher (7 of 13 L1), if the infected donors were fed on uninfected mice rather than chickens (1 of 13 L1). Only 1 of 11 bugs which had been less than half repleted (group B) became infected.

"In vitro cannibalism"

The in vitro incubation also demonstrated the deleterious effect of chicken blood on *T. cruzi.* Ten minutes after mixing blood with the stomach population of *T. cruzi,* only 1 weak but live flagellate could be found in the chicken blood mixture. In the mouse blood mixture all trypanosomes were alive, but covered with adhering blood platelets. After 60 min, no surviving *T. cruzi* could be observed after incubation with chicken blood, while in mouse blood the platelets still covered the flagellates which showed reduced motility.

The infection rates of *D. maximus* and *T. infestans* L1 after "in vitro cannibalism" are shown in Table 2. With chicken blood, fewer bugs fed to capacity and only 1 *D. maximus* became infected at the highest infective doses. Mouse

Table 2. "In vitro cannibalism": infections using a mixture of blood from hen or mice with different numbers of *Trypanosoma cruzi* "Chile 5" from the stomach of *Triatoma infestans*

Source of blood	Bug species	Infection doses/bug	Number of larvae* infected/uninfected
Hen	*D. maximus*	>5000	1/4
		>50	0/5
	T. infestans	>500	0/3
		>5	0/5
Mice	*D. maximus*	>5000	5/1
		>50	0/14
	T. infestans	>500	12/0
		>5	1/37

* only fully repleted bugs were considered

blood mixtures were infectious for 5 of 6 *D. maximus* and for all *T. infestans* at the high infection doses, but no infected *D. maximus* and only one of 38 *T. infestans* were found at the lower infective doses.

Coprophagy

In addition to cannibalism, transmission of *T. cruzi* could also be demonstrated in the "coprophagy groups". A mixed maintenance of infected and uninfected larvae (Co I) led to one infection in the 36 larvae which were previously uninfected. In infected and uninfected larvae, that were kept separately but were both fed regularly (Co II), an infected bug occurred in 2 of the 3 groups. In series Co III, where feeding of the uninfected larvae started after removal of the infected ones, no infection could be found.

Discussion

According to Torres (1915), Machado first described cannibalism between triatomines. Many other authors have subsequently reported this behaviour to occur in different species (e.g. Brumpt, 1914; Phillips, 1960; Pippin, 1970; Añez, 1982), although in some studies, laboratory induction of cannibalism was not possible (Uribe, 1927; Schenone et al., 1969). However, transmission of *T. cruzi* by cannibalism has been difficult to demonstrate. In *Triatoma sordida, Triatoma sanguisuga,* and *Triatoma gerstaeckeri* transmission was unsuccessful as cannibalistic larvae only ingested haemolymph from donors (Torres, 1915; Hays, 1965; Pippin, 1970). Transmission of *T. cruzi* by cannibalism was first achieved by Chagas (mentioned in Dias, 1934) and Dias (1934), then later by several other workers using different hemipteran species (Dias, 1936; Phillips, 1960; Marinkelle, 1965). Although these infections with *T. cruzi* almost cer-

tainly resulted from cannibalism, none of the studies excluded the possibility of infection by coprophagy, and the different circumstances involving cannibalism recognized here were not mentioned in these 3 papers.

One possible pathway of cannibalistic transmission is the uptake of infectious blood by a previously uninfected bug, and Phillips (1960) attempted to demonstrate this using L1 of *Rhodnius prolixus* and *T. infestans.* Unfortunately, cannibalism was not practised during or after the first feeding, and the time allowed for development of *T. cruzi* in the donors (18 days) was sufficient to enable possible infection from both stomach or rectal populations. Intergeneric transmission was also demonstrated in this study.

Therefore, the results presented here represent the first unequivocal demonstration of cannibalistic transmission of *T. cruzi* by a hemipteran vector after an uptake of infectious blood. This transmission is not very surprising, since the very short stay of the blood in the stomach of the donors should not induce great alterations in *T. cruzi.*

The second possible route of cannibalistic transmission involves the uptake of an uninfected blood meal by a *T. cruzi*-infected donor bug, followed by cannibalistic feeding of initially uninfected bugs. Marinkelle (1965) demonstrated such transmission, but unfortunately no mention was made of the blood source for the uninfected meal. It is well known that complement factors of the blood can lyse insect-derived metacyclic trypomastigotes or epimastigotes from in vitro cultures, and that serum from closely related vertebrates can act differently on species of trypanosomatids (Muniz and Borriello, 1945; Rubio, 1956; Kierszenbaum et al. 1976, 1981; Lima and Kierszenbaum, 1984; Schottelius and Müller, 1984). The implications of such complement-factor action on the stomach populations of *T. cruzi* have never been considered, although it should be stressed that the intestinal populations always develop well (Urdaneta-Morales, 1973; Schaub, unpublished).

The results from the second mode of transmission by cannibalism reflect the different actions of mouse and chicken blood on populations of *T. cruzi* in the stomach of vector species. Good transmission resulted from cannibalism on bugs which had fed on mouse blood, while chicken blood almost completely prevented bug-to-bug transmission of trypanosomes. Microscopical examination showed the chicken blood to have a total lytic effect after 60 min incubation. Therefore, the incidences of transmission could be caused by some lysis-resistant epimastigotes, a phenomenon which has been reported for guinea pig serum (Schottelius, 1982; Marinkelle et al., 1985), or by survival of individuals sheltered in an infolding of the stomach wall, if the donor is not fed to repletion.

The *T. cruzi* strains "Chile 5" and "Chile 7" belonging to zymodemes 1 and 2, respectively, also differed in their transmission rates, which may be due to differences in susceptibility to complement-mediated lysis (Krettli et al., 1979). This must be studied with a greater quantity of bugs. Previous studies using the same strains have demonstrated that both strains are fully infective to the bugs

(Böker und Schaub, 1984; Schaub and Böker, 1986; Schaub, unpublished) in contrast to other *T. cruzi*/bug systems (e.g. Phillips and Bertram, 1967; Szekely et al., 1971).

A third mode of transmission by cannibalism was studied by Dias (1936) using L1 of *Panstrongylus megistus* in which infected bugs were attacked a number of days after feeding. Experiments of this kind were not included in the present study because only disproportionate efforts could exclude a coprophagic infection.

Whereas cannibalism can easily be recognized by the distension of the abdomen, proof for coprophagy is more difficult, the uptake of large amount of faeces being an exception. In *R. prolixus* and *T. infestans* only 1% of the L1 possessed a distended abdomen after a long starvation period (Phillips, 1960), and in addition, only 2% of starved *R. prolixus* L1 imbibed an eosin solution (Marinkelle, 1965). Such frequencies of coprophagy do not seem to be sufficient to provide a whole bug population with its symbionts. Phillips (1960) observed that some bugs merely extended their proboscises and probed the faecal fluid, but did not regard this as coprophagic behaviour. This behaviour was also often observed during the present study and has to be considered as real coprophagy, since contamination of the stylet with symbionts is sufficient to allow development of a midgut population (Brecher and Wigglesworth, 1944).

Due to such difficulties, transmission of *T. cruzi* by coprophagy was rarely investigated and could not be conclusively demonstrated. The experiments of Brumpt (1914), often cited as an example, are not sufficient. He injected a mixture of blood and infectious faeces into fresh meat, which was pierced by the bugs which then became infected. No transmission by coprophagy could be achieved by Dias (1936) or Phillips (1960) offering infectious faeces. As coprophagy might be induced by the behaviour of the defaecating bug, infected and uninfected *T. infestans* were kept and fed together in this study (Co I) which resulted in transmission of infection. Cannibalism could not be excluded, but was improbable because the bugs were fed regularly and L4 were used. More conclusive results were obtained from groups in which infected and uninfected bugs had no possibility for direct contact and in which the primarily uninfected bugs were fed (Co II). Here, 2 previously uninfected bugs possessed detectable infection levels after 8 weeks. Identical studies with the more virulent trypanosomatid, *Blastocrithidia triatomae,* also indicate the importance of feeding (Schaub, unpublished).

The factors which affect both methods of transmission are very similar, and include the quantity and virulence of the imbided flagellates and the susceptibility of bugs to *T. cruzi* infection. This direct, bug-to-bug transmission may be important in the epidemiology of Chagas' disease. While cannibalistic transmission may be common in aggressive species such as *D. maximus,* coprophagic transmission is probably more universally important since all bugs must obtain their symbionts by coprophagy at least once in their lifetime.

Further studies must therefore clarify the relative importance of coprophagy and cannibalism for the direct transmission of *T. cruzi* in the different species of triatominae.

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