The affinity of the lectins "Ricinus communis" and "Glycine maxima" to carbohydrates on the cell surface of various forms of "Trypanosoma cruzi" and "Trypanosoma rangeli", and the application of these lectins for the identification of "T. cruzi" in the ...

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The affinity of the lectins *Ricinus communis* and *Glycine maxima* to carbohydrates on the cell surface of various forms of *Trypanosoma cruzi* and *Trypanosoma rangeli*, and the application of these lectins for the identification of *T. cruzi* in the feces of *Rhodnius prolixus**

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Summary

Flagellates of *Trypanosoma cruzi* (stock Molino 1), obtained from the intestine of experimentally infected *Rhodnius prolixus*, grown in cellular or acellular culture, as well as from the blood of infected mice, were examined by a direct fluorescence test using the lectins RCA *(Ricinus communis*-120) and SBA (soy bean agglutinin; *Glycine maxima*), conjugated with fluorescein isothiocyanate, for the detection of β-D-galactose and α,β-N-acetyl-D-galactosamine on the membranes of the flagellates. The same reactions were carried out using *Trypanosoma rangeli* (stock San Agustin), obtained from the intestine, hemolymph or salivary glands of experimentally infected *R. prolixus*, as well as from cultures and from the blood of experimentally infected CFW mice. The results indicate that the membrane of *T. rangeli* in the salivary glands of the vector contains β-D-galactose, but that this sugar is absent from all other developmental stages of this trypanosome. All stages of intestinal and cultured. *T. cruzi*

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presented positive reactions with RCA-FITC and SBA-FITC. The high specificity of this technique makes it useful for the examination of *R. prolixus*, previously used in xenodiagnosis of Chagas' disease and for the examination of intradomiciliary or sylvatic vectors in epidemiological surveys in areas where *T. cruzi* and *T. rangeli* coexist. Formaldehyde fixed samples can be examined months later and false reports due to *T. rangeli* can be avoided.

Key words: *T. cruzi; T. rangeli;* vector feces; lectin identification.

Introduction

Chagas' disease, caused by *Trypanosoma cruzi*, is present in all Latin American countries. It is estimated that the number of people in potential risk of infection is more than 60 million, and that at least 20 million are infected (WHO, 1983).

Trypanosoma rangeli is non pathogenic for vertebrates, but infections have been found in man and numerous mammals in different Latin American countries (Hoare, 1972). In Colombia, Venezuela and some other countries, the principal vector of *T. cruzi* and *T. rangeli* is *Rhodnius prolixus*, and mixed trypanosomal infections are frequent (Marinkelle, 1972; D'Alessandro, 1976).

The interpretation of xenodiagnosis is often difficult because certain developmental stages of the two trypanosomes in the intestine of *R. prolixus* are indistinguishable from each other, whether in fresh or stained preparations (D'Alessandro, 1976; Schottelius, 1982; Schottelius and Müller, 1984).

The use of lectins has become an important tool in recent years for the differentiation of certain membrane glycoproteins and glycolipids found in protozoan membranes because they allow selective recognition and reversible binding to carbohydrates. Bretting and Schottelius (1978) using fluoresceine-labeled *Aaptos papillata* II lectin, differentiated cultured epimastigotes of *T. rangeli* by fluorescent techniques (FT). Recently, Schottelius and Müller (1984) established a method to differentiate between culture forms of *T. cruzi*, *T. rangeli* and *T. conorhini* by the use of lectins in combination with complement mediated lysis without antibodies. *T. cruzi* and *T. rangeli* can also be distinguished by their different complement sensitivity (Schottelius, 1982) and on the basis of their sialic acid content (Schottelius, 1984a).

The differentiation of the two trypanosome species is important during the examination of triatomines captured during epidemiological surveys, and those used in xenodiagnostic tests. To obtain cultures from the intestinal tract of all the triatomines would be a tedious and impractical method. The objective of this study was to investigate whether the use of fluoresceine-labeled lectins could be adapted to the direct examination of the intestinal content of *R. prolixus* infected with one or both trypanosome species and to investigate whether

the various developmental stages of the trypanosomes present in different tissues of the vector could be identified by this method.

Material and Methods

Parasites. T. cruzi (stock Molino 1) which belongs to the PNA-type (Schottelius et al., 1986), was isolated by mouse inoculation from the feces of an intradomiciliary *R. prolixus* from the region of Choachi in Central Colombia. The stock is being maintained in CFW mice by intraperitoneal (ip) inoculation of approximately 5×10^5 blood trypanosomes at intervals of 20 days. *T. rangeli* (stock San Agustin), was isolated from the salivary glands of *R. prolixus*, previously used in xenodiagnosis of a child from the region of Choachi. The stock is cyclically maintained by three-monthly passages through Tobie's culture medium (Taylor and Baker, 1978), *R. prolixus* and mouse. *T. cruzi* (stock Tulahuen), is being maintained in CFW mice by weekly ip inoculation of approximately 1×10^5 blood trypanosomes. This stock was used exclusively for production of the tissue trypomastigotes by infection of MRC-5 cell cultures.

Lectins (E. Y. Lab., San Mateo, Calif., USA / Medac, Hamburg). The following lectins were used: *Ricinus communis*-120 (1 mg/ml), *Glycine maxima (Soja hispida)* (1 mg/ml). *R. communis*-120 has a selectivity for β-D-galactose, whereas *G. maxima* reacts with α,β-N-acetyl-D-galactosamine and has a lower affinity for α,β-D-galactose (Goldstein and Hayes, 1978).

Experimental intestinal infection of R. prolixus. Fifty-five nymphs of *R. prolixus*, free of flagellate infection, raised in the laboratory, were fed in groups of 3 insects on CFW mice, which were inoculated ip with approximately 5×10^5 blood trypomastigotes of *T. cruzi* (stock Molino 1).

Infection of hemolymph and salivary glands of R. prolixus with T. rangeli. R. prolixus were inoculated intrafemorally with 0.01 ml Tobie's culture medium containing approximately 1×10^5 flagellates of *T. rangeli* (stock San Agustin) using a 27 G hypodermic needle. From 15 to 30 days after inoculation, abundant epimastigotes were present in the hemolymph. Few epimastigotes and numerous short metacyclic trypomastigotes were present in the salivary glands.

Preparation of the intestinal content of R. prolixus. Between the second and the tenth week after inoculation, the content of the rectum of 5 *R. prolixus*, infected with *T. cruzi*, and the posterior midgut of 5 *R. prolixus*, infected with *T. rangeli*, were obtained by dissection at weekly intervals. According to D'Alessandro and Mandel (1969), D'Alessandro (1976), Vallejo (1984), *T. cruzi* is more abundant in the rectum, while *T. rangeli* is more abundant in the posterior midgut. The content of each dissected intestinal section was placed in 10 ml PBS pH 7.2, cleaned by 3 successive centrifugations at 600 *g* for 15 min each, and the final sediment resuspended in 0.3 ml PBS pH 7.2 and centrifuged at 80 *g* for 2 min. The tube was then left for 10 min and the flagellates collected from the surface of the supernatant. This procedure permitted a high recovery of flagellates and eliminated most of the other fecal contaminants.

Direct fluorescence test with RCA-FITC and SBA-FITC (E. Y. Lab., San Mateo, Calif., USA/Medac, Hamburg). Twenty μl of washed flagellates were placed on slides, air dried, and ringed with blue waxpencil. The marked spots were covered with 20 μl fluorescein-marked lectins in a dilution up to 1:10 and incubated for 1 h at 26 °C in a humid chamber. The slides were then washed twice for 10 min with PBS pH 7.2, dried with filter paper, and after addition of a drop of buffer-glycerol pH 8.9, examined with a fluorescent microscope. The same procedure was used with *T. rangeli* obtained from Tobie's culture medium, blood trypomastigotes, epimastigotes from the hemolymph of *R. prolixus*, epimastigotes and trypomastigotes from the salivary glands of the triatomines, as well as with *T. cruzi* obtained from Tobie's culture medium, blood trypomastigotes from infected CFW mice, and trypomastigotes obtained from tissue culture of MRC-5 cell line. In another experiment 100 μl of RCA-FITC was incubated for 1 h at 26 °C with 40 macerated and uninfected salivary glands of *R. prolixus*. The gland tissues were then removed by centrifugation at 600 *g* for 15 min, and the supernatant containing the marked lectin, reincubated with the flagellates obtained from *T. rangeli* infected salivary glands. Finally the preparations were examined under the microscope.

Indirect fluorescence test with antibodies against RCA and SBA (E. Y. Lab., San Mateo, Calif., USA / Medac, Hamburg). The flagellate spots were covered with 20 µl lectins in a dilution 1:2 and incubated for 1 h at 26 °C in a humid chamber. The slides were then washed twice for 10 min in PBS pH 7.2 and dried with filter paper. The marked antigen spots were next covered with 20 µl fluorescein conjugated anti-lectins in a 1:2 dilution and incubated for 30 min at 26 °C in a humid chamber. Washing and drying procedure was repeated, and slides then examined as described.

Control reactions. For negative controls, fluorescence was inhibited using a 0.1 M solution of the respective sugar for each lectin. The sugar was added to the PBS pH 7.2 before the lectin solution was prepared. Incubations and washing were the same as in the binding experiments. For additional negative controls, the antigen spots were incubated with PBS pH 7.2 without lectin or anti-lectin solutions. For positive controls, cultured epimastigotes of *T. cruzi* and trypomastigotes of *T. rangeli* were used in the RCA-FITC tests.

Results

At the beginning of the study the labeled lectins were used undiluted, which resulted in an intense fluorescence of the positive flagellate forms. Table 1 shows the results of the FT with the lectins RCA-FITC and SBA-FITC for the different

Table 1. Results of the direct fluorescence reaction with the lectins SBA-FITC and RCA-120-FITC and the developmental stages of *T. cruzi* and *T. rangeli*

Lectins	*Trypanosoma cruzi* flagellates obtained from:				*Trypanosoma rangeli* flagellates obtained from:				
	culture	feces[2]	blood	tissue-culture[1]	culture	feces[2]	blood	hemo-lymph[2]	salivary glands[2]
SBA-FITC	+	+	+	+	∅	∅	∅	∅	∅
RCA-FITC	+	+	+	+	∅	∅	∅	∅	+

+ = fluorescence present on surface membrane and flagellum

[1] *T. cruzi* stock Tulahuen grown in human fibroblast MRC-5 cell line

[2] obtained from experimentally infected *Rhodnius* prolixus

Fig. 1a. *T. cruzi* Tulahuen, trypomastigotes from fibroblast culture with *R. communis*-120-FITC, pos. fluorescence (×570).

Fig. 1b. *T. cruzi* Tulahuen, trypomastigotes from fibroblast culture with *G. maxima*-FITC, pos. fluorescence (×570).

Fig. 2a. *T. cruzi* Molino 1, epimastigote with *R. communis*-120 FITC, pos. fluorescence (×570).

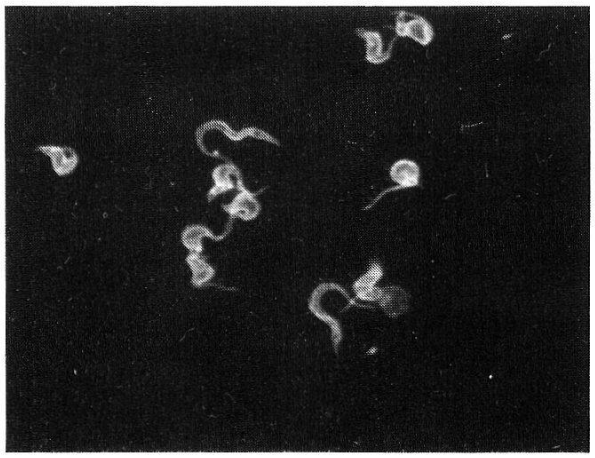
Fig. 2b. *T. cruzi* Molino 1, epimastigote and metacyclic trypomastigote forms from rectal ampul of *R. prolixus* wih *R. communis*-120–FITC, pos. fluorescence (×570).

Fig. 3a. *T. rangeli* San Agustín, salivary gland forms with *R. communis*-120-FITC, pos. fluorescence (×570).

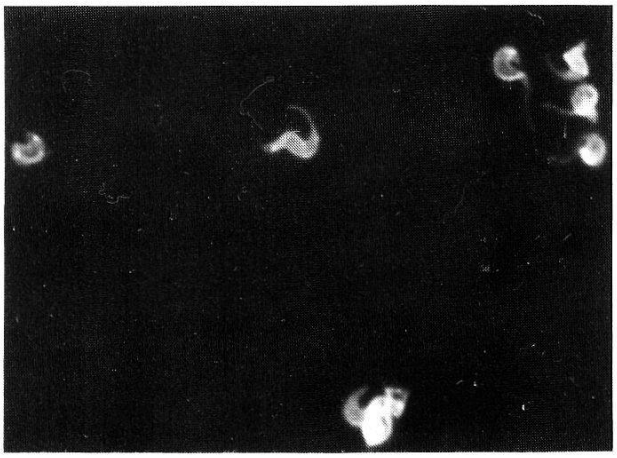
Fig. 3b. *T. rangeli* San Agustín, salivary gland form, metacyclic trypomastigote with *R. communis*-120-FITC, pos. fluorescence (×570).

Fig. 4a. *T. cruzi* Molino 1, blood form with *R. communis*-120-FITC, pos. fluorescence (×570).

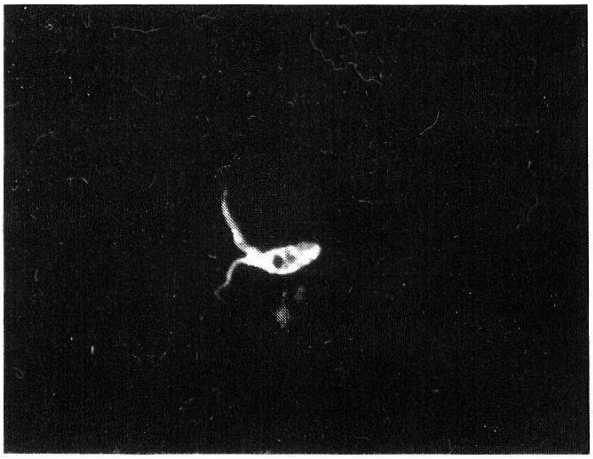
Fig. 4b. *T. cruzi* Molino 1, epimastigotes, unlabeled, no fluorescence-control reaction (×570).



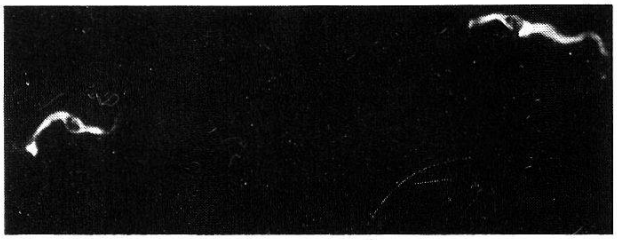
1a



1b

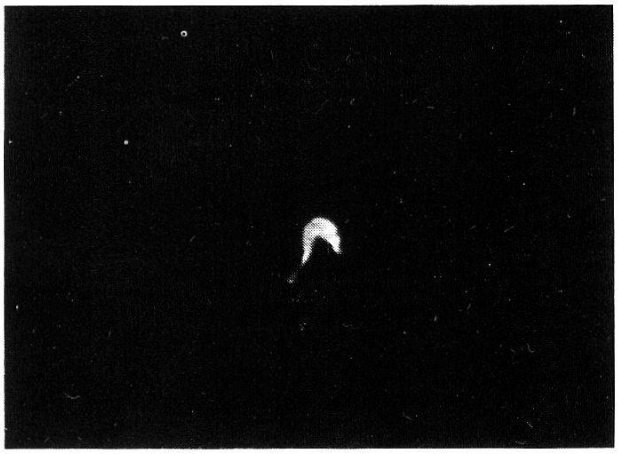


2a

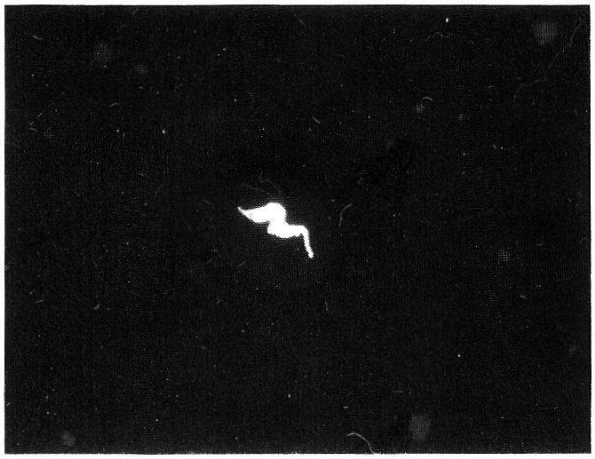


2b

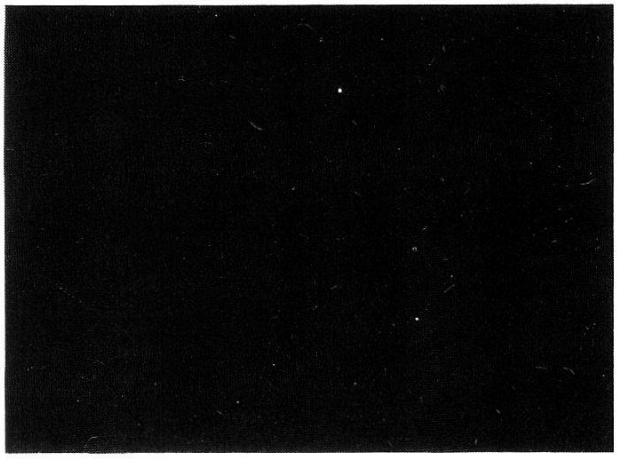
3a



3b



4a



4b

stages of *T. cruzi* (stock Molino 1), trypomastigotes of *T. cruzi* (stock Tulahuen) from fibroblast culture MRC-5 and the different stages of *T. rangeli* (stock San Agustin).

All stages of *T. cruzi* reacted positively with both lectins (Figs. 1a, 1b, 2a, 2b, 4a). Fluorescence was absent from the various forms of *T. rangeli*, with the exception of the epimastigotes and trypomastigotes obtained from the salivary glands when incubated with RCA-FITC (Figs. 3a, 3b). Incubation with SBA-FITC did not produce fluorescence.

All intestinal flagellates of *T. cruzi* produced fluorescence from the second to the tenth week while the intestinal forms of *T. rangeli* never showed any fluorescence. *T. rangeli* disappeared from the intestine after the sixth week. Serial dilutions from 1:2 to 1:10 were tested. Fluorescence of intestinal *T. cruzi* was clearly visible when a dilution of 1:2 was chosen. Similar fluorescence was obtained when direct or indirect fluorescence techniques were applied. When the developmental stages of *T. cruzi* were fixed with 1% formaldehyde in PBS pH 7.2 for 12 h, no alteration of the results was observed on slide preparations kept at –20°C during 8 months.

The intensity of fluorescence observed in the direct fluorescence test was similar to that observed in the direct test. As the direct fluorescence test requires only one step, this test was used in the majority of the experiments.

Discussion

Membrane carbohydrate composition of 3 *T. cruzi* stocks from Brazil (Y, C1, MR) was investigated by Araujo et al. (1980). Eight different FITC labeled lectins were used, showing differences in membrane carbohydrate composition. For instance, the epimastigotes of stock Y and C1 presented negative reactions with SBA-FITC lectin, but those of stock MR showed positive reaction. The epimastigotes and trypomastigotes of stocks Y, C1 and MR showed positive reactions with RCA-FITC lectin. The same authors reported different reactions when lectins such as concanavalin A *(Canavalia ensiformis)*, PNA *(Arachis hypogaea)* and WGA *(Triticum vulgaris)* were used. In contrast, Schottelius and Raabe (1978) and Schottelius (1982) found that the *T. cruzi* stocks Y and C1 reacted as well with the lectin from *Ricinus communis*-120 as with the *Glycine maxima* lectin. The same was reported for the Y stock by Pereira et al. (1980). Our results from the study of *T. cruzi* (Molino 1 stock) were similar to those obtained by Araujo et al. (1980) when RCA-FITC was used. The result of SBA-FITC lectin reaction with Molino 1 stock was similar to that obtained with the stocks Y and C1 (Araujo et al., 1980), since epimastigotes from culture and intestinal tract of the vector presented positive results (Tab. 1).

Our results for *T. rangeli* from culture and from the intestine an hemolymph of infected *R. prolixus*, demonstrate the absence of β-D-galactose and α,β-N-acetyl-D-galactosamine. The presence of fluorescence on the surface of

T. cruzi Molino 1 stock with RCA-FITC and SBA-FITC and the absence of fluorescence on the culture forms of *T. rangeli* San Agustin stock are in agreement with the results of Schottelius and Mühlpfordt (1984). These authors described that *T. cruzi* reacted with RCA-120 and SBA coupled with FITC or colloidal gold (Au = 13,8 nm), but the culture forms of *T. rangeli* did not. However, the epimastigotes and metacyclic trypomastigotes of *T. rangeli* from the salivary glands of *R. prolixus* presented fluorescence with RCA-FITC, which indicated the presence of a β-D-galactose component (Table 1) on the surface membrane. The intensity of fluorescence of the salivary gland epimastigotes was considerably weaker than that of the metacyclic trypomastigotes (Figs. 3a, 3b). Since the epimastigotes from the hemolymph did not produce fluorescence with RCA-FITC before entering the salivary glands, this could be an indication that the salivary glands induce the parasites to change their receptor structures on the surface membrane. Another possibility could be the presence of β-D-galactose in the salivary glands, with a capacity to adhere to the flagellate membrane. To exclude the latter possibility, the salivary gland flagellates were washed three times in PBS pH 7.2 and centrifugated at 600 *g* for 15 min. However, the RCA-FITC reaction produced the same intensity of fluorescence after the washing procedure. As no decrease of fluorescence intensity was observed in the flagellates incubated with RCA-FITC treated macerated glands, it is unlikely that free β-D-galactose is present in the salivary glands of uninfected *R. prolixus*. This would leave only the possibility of the salivary glands inducing the parasites to change the receptor structures on the surface membrane. We therefore believe that a surface coat containing β-D-galactose exists on the salivary gland forms of *T. rangeli*.

Membrane-bound lectins are known to be widely distributed on many eukaryotic cells (Hart, 1980; Monsigny et al., 1979; Simpson et al., 1978). Therefore, if a galactose specific lectin exists in the epithelium of the salivary glands of *R. prolixus*, the presence of β-D-galactose on the surface of the salivary gland flagellates would enable them to attach themselves to the epithelium of the glands. Steiger (1973) mentioned that the epimastigote forms of *T. brucei* in the salivary glands of the tsetse flies are closely attached to the epithelium by the flagellum. This close attachment could be due to the interaction between salivary gland lectins and carbohydrates on the surface of the flagellates. Various investigators have mentioned the presence of lectin-like substances in the intestine of *Glossina* (Ibrahim et al., 1984) and of *Rhodnius* (Pereiera et al., 1981).

The surface coat of bloodstream forms of *T. cruzi* protects them from lysis by complement activation through the alternative pathway (Kipnis et al., 1981; Nogueira et al., 1975; Schottelius, 1984b). Salivary gland flagellates of *T. brucei* subspecies also possess a surface coat which reacts with certain lectins (Seed et al., 1976; Renwrantz and Schottelius, 1977). The presence of the surface coat is considered a protective adaptation for survival in the bloodstream of mammals. Our experiments demonstrate that β-D-galactose on the surface of the salivary

gland forms of *T. rangeli* also reacted with a lectin. The selective glycoconjugates which are integrated into the surface membrane on the salivary gland stages of *T. rangeli* might be responsible for their survival in the mammalian host.

The results indicate that certain fluorescein-marked lectins are of practical use for specific detection of *T. cruzi* in the feces of *R. prolixus* and solve the problems of false positive results for *T. cruzi* due to misidentification. Samples fixed with formaldehyde can be prepared in the field and examined months later in a central laboratory.

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