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Interspecific differentiation of *Trypanosoma cruzi*, *Trypanosoma conorhini* and *Trypanosoma rangeli* by lectins in combination with complement lysis

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Summary

Four-day-old epimastigote culture forms of *Trypanosoma cruzi*, *Trypanosoma rangeli* and *Trypanosoma conorhini* were tested with 21 lectins. Furthermore *T. conorhini* was incubated with the following sera: rat, Wistar HAN, germ free; normal fresh hen, rat and human serum. *T. rangeli* was agglutinated only by the D-mannose specific lectins from *Canavalia ensiformis* and *Pisum sativum*. *T. cruzi* and *T. conorhini* could be distinguished by the lectin from *Tridacna crocea*. The epimastigote culture forms of *T. conorhini* were not lysed by normal fresh rat, hen and human sera. Therefore, *T. cruzi*, *T. conorhini* and *T. rangeli* can be distinguished interspecifically by lectins and by the different lytic effect of rat, hen and human sera. It is possible to seperate each of the species by complement lysis. The lysis-resistant species can be cultivated for further examinations.

Key words: lectins; complement; *T. cruzi; T. rangeli; T conorhini.*

Introduction

Trypanosoma cruzi, the agent of Chagas' disease, and *Trypanosoma rangeli* have been found in man and animals in Latin America (Barretto, 1979). Both flagellates are transmitted by triatomine bugs. *Trypanosoma rangeli* seems to be non-pathogenic for man and vertebrates although it has some medical interest

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because its geographical distribution overlaps that of *T. cruzi* (Hoare, 1972; D'Alessandro, 1976). The natural hosts of *Trypanosoma conorhini* are rats (Hoare, 1972). These vertebrates can be infected with *T. cruzi* as well. As triatomine bugs also transmit *T. conorhini* it can happen that these vectors infect themselves with both trypanosome species by blood-sucking. Infections with *T. cruzi* and *T. rangeli* are often latent in man. Therefore, xenodiagnosis is made to isolate these blood parasites. Certain developmental stages from the intestine of the triatomine bugs and certain culture forms of *T. cruzi*, *T. rangeli* and *T. conorhini* are morphologically indistinguishable. Mühlpfordt (1975) was able to distinguish between these trypanosomes by the ultrastructure of their kDNA. Ebert et al. (1978) could characterize these flagellates with conventional electrophoresis. Mühlpfordt and Schottelius (1977) described differences between *T. cruzi*, *T. rangeli* and *T. conorhini* by the Aaptos II agglutination test. Schottelius (1982b) described that *T. cruzi* and *T. rangeli* can be distinguished by the different lytic effect of complement from different sera. A prerequisite for the Aaptos test is the permanent presence of this agglutinin but this lectin is not available on the market. Therefore, it was desirable to test more common lectins to find such an agglutinin which can be used for the interspecific differentiation of these trypanosomes. *T. conorhini* was examined by the complement lysis test to see whether we have differences relative to *T. cruzi* and *T. conorhini* (Schottelius, 1982b).

Material and Methods

The following strains of trypanosomes were tested in this study: *Trypanosoma rangeli* strain DA-3412 was isolated from naturally infected *Tamandua tetradactyla* in Carmagua, Meta, Colombia. This strain was a gift from Dr. D'Alessandro, Cali, Colombia. *T. rangeli* strain V was isolated from a patient by Prof. Dr. Pifano, Instituto de Medicina Tropical, Caracas, Venezuela. *Trypanosoma conorhini* strain Hawaii was isolated from *Triatoma rubrofasciata* by Dr. Tobie, Hawaii. This strain was a gift from Prof. Jadin and Dr. Le Ray, Instituut voor Tropische Geneeskunde, Antwerpen. *Trypanosoma cruzi* strain Y was received from Prof. Z. Brener, Centro de Pesquisas Rene Rachou, Belo Horizone, Minas Gerais, Brazil. This strain was isolated from a patient, São Paulo State, Brazil.

The parasites were cultured on Brain Heart Infusion Agar (Difco) in addition to undiluted rabbit blood at +26° C. In order to get a homogeneous parasite culture 3 passages of 4 days were made. These cells were centrifuged (1400 *g*/5 min) and washed once in NaCl 0.9%. The culture forms were resuspended in NaCl 0.9% at a concentration of 10^8 cells/ml. Autoagglutinations were not observed. Lectins (50 µl) and parasite suspension (50 µl) were incubated together for 15 min at 26° C. The agglutination reactions were controlled under a microscope using a slide with cover slip. The following lectins were used: *Canavalia ensiformis* (500 µg/ml) (Difco Lab, Detroit), *Ricinus communis*-120 (1:10) (Miles Yeda, Frankfurt/Main), *Pisum sativum* (1000 µg/ml) (Sigma, München), *Abrus precatorius* (1000 µg/ml) (Sigma, München), *Soja hispida* 10,000 µg/ml) (Pharmacia Fine Chemicals, Freiburg), *Triticum vulgaris* (10,000 µg/ml) (Sigma, München), *Tridacna crocea* (1000 µg/ml) (Prof. Dr. G. Uhlenbruck, Medical University Clinic, Köln), *Dolichos biflorus* (Dr. Fresenius KG, Bad Homburg), *Lotus tetragonolobus* (1000 µg/ml) (Sigma, München), *Aaptos papillata* II (1:128) (Dr. H. Bretting, Univ. Hamburg), *Arachis hypogaea* (2000 µg/ml) (Boehringer, Mannheim), *Phaseolus vulgaris* (10,000 µg/ml) (Difco Lab, Detroit), *Cytisus sessilifolius* (1000 µg/

ml) (Medac, Hamburg), *Ulex europaeus* I and II (1000 µg/ml) (Medac, Hamburg), *Euonymus europaeus* (1000 µg/ml), (Medac, Hamburg), *Wistaria floribunda* (1000 µg/ml (Medac, Hamburg), *Robinia pseudoacacia* (1000 µg/ml) (Medac, Hamburg), *Limulus polyphemus* (1000 µg/ml) (Medac, Hamburg), *Iberis amara* (1000 µg/ml) (Medac, Hamburg).

The agglutination tests were controlled by inhibition reactions using the following carbohydrates: *Canavalia ensiformis* plus 20 mg D-mannose (Merck, Darmstadt), *Ricinus communis*-120 plus 10 mg D-galactose (Merck, Darmstadt), *Soja hispida* plus 10 mg N-acetyl-D-galactosamine (Fluka, Buchs), *Triticum vulgaris* plus N,N',N'-triacetylchitotriose (Sigma, München), *Arachis hypogaea* plus 10 mg D-galactose (Merck, Darmstadt), *Aaptos papillata* II plus 10 mg N-acetyl-D-glucosamine (Serva, Heidelberg). As an additional control, the parasites were incubated in a volume of NaCl 0.9% equal to the volume of lectins to search for an eventual tendency of the cells to autoagglutinate.

Samples of serum of the following species were examined: rat, Wistar HAN, germ free (Central Institute for Laboratory Animals, Hannover), normal fresh rat, hen and human serum (Dr. Müller, Central Institute for Blood Transfusion, Eilbek, Hamburg). 50 µl of the culture forms of *T. conorhini* and 100 µl of the sera were incubated for 1 h at room temperature.

Culture forms of *T. cruzi* Y were put together with the culture forms of *T. conorhini* Hawaii and *T. rangeli* V for 24 h at 26° C. Then the cells were washed and incubated in normal fresh rat serum as described. The non-lysed cells were transfered to test tubes with the described culture medium to examine whether it was possible to separate a certain species of trypanosomes by complement lysis. The test tubes were controlled after 48 h.

Results

Common reactions of the trypanosome species

The strains of *T. rangeli, T. cruzi* and *T. conorhini* were agglutinated by *C. ensiformis* and *P. sativum.* The same flagellates did not agglutinate with *D. biflorus, U. europaeus* I, *U. europaeus* II, *R. pseudoacacia, L. polyphemus, P. vulgaris, M. pomifera* and *I. amara* (Table 1).

Interspecific differentiation of the trypanosome species

While the *T. rangeli* strains react only with *C. ensiformis* and *P. sativum, T. conorhini* is agglutinated by *R. communis*-120, *S. hispida, A. precatorius* and *A. hypogaea* but not by *T. vulgaris, A. papillata* II and *T. crocea. T. cruzi* strain Y shows agglutination reactions with *R. communis*-120, *S. hispida, A. precatorius, A. papillata* II, *T. crocea* and *T. vulgaris* but not with *A. hypogaea* (Table 1).

Inhibition tests

The agglutination reactions of the trypanosome species with *C. ensiformis, A. papillata* II, *R. communis*-120, *S. hispida, T. vulgaris* and *A. hypogaea* could be inhibited by the specific carbohydrates. Autoagglutinations were not observed. The lytic effect of the sera can be inhibited by inactivation or by EDTA (Schottelius, 1982b).

Table 1. Comparative lectin typing of *T. cruzi*, *T. rangeli* and *T. conorhini*

Lectins	Carbohydrate specificities	*T. rangeli*		*T. cruzi* Y	*T. conorhini* Hawaii
		DA 3412	V		
Canavalia ensiformis	αD-Man, αD-Glu	+	+	+	+
Pisum sativum	αD-Man, αD-Glu	+	+	+	+
Ricinus communis-120	βD-Gal	Ø	Ø	+	+
Soja hispida	αD-GalNAc=βD-GalNAc>α, βD-Gal	Ø	Ø	+	+
Abrus precatorius	D-Gal	Ø	Ø	+	+
Tridacna crocea	anti-β-galactosyl	Ø	Ø	+	Ø
Triticum vulgaris	N-acetylneuraminic acid Chitopentaose>Chitotetraose>Chitotriose>Chitobiose>D-GluNAc	Ø	Ø	+	Ø
Arachis hypogaea	anti-T, D-Galβ(1–3)D-GalNAc D-Galβ(13)D-GluNAc, βD-Gal, D-Galβ(1–4)D-GluNAc	Ø	Ø	Ø	+
Aaptos papillata II	Chitotriose>Chitobiose>D-GluNAc N-acetylneuraminic acid	Ø	Ø	+	Ø
Dolichos biflorus	anti-A_1, αD-GalNAc>βD-GalNAc	Ø	Ø	Ø	Ø
Ulex europaeus I	anti-H, αL-fucose	Ø	Ø	Ø	Ø
Ulex europaeus II	Chitobiose	Ø	Ø	Ø	Ø
Euonymus europaeus	anti-(H,B,A_2)	Ø	Ø	Ø	Ø
Lotus tetragonolobus	anti-H, αL-fucose	Ø	Ø	Ø	Ø
Wistaria floribunda	D-Gal, D-GalNAc	Ø	Ø	Ø	Ø
Robinia pseudoacacia	Di-sialoglycoprotein	Ø	Ø	Ø	Ø
Limulus polyphemus	Glucuronic acid, sialic acid	Ø	Ø	Ø	Ø
Phascolus vulgaris	D-GalNAc	Ø	Ø	Ø	Ø
Maclura pomifera	D-Gal	Ø	Ø	Ø	Ø
Iberis amara	anti-M	Ø	Ø	Ø	Ø
Cytisus sessilifolius	anti-O>A2, A1	Ø	Ø	Ø	Ø

+ = agglutination reaction Ø = no agglutination reaction

Table 2. The effect of different sera on the lysis of epimastigote culture forms of *T. conorhini*, *T. rangeli* and *T. cruzi*

Parasites	Origin	Rat serum	Human serum	Hen serum
T. conorhini				
strain Hawaii	Hawaii	∅	∅	∅
T. rangeli				
strain DA 3412	Colombia	∅	∅	+
strain V	Venezuela	∅	∅	+
T. cruzi				
strain Y	Brazil	+	+	+

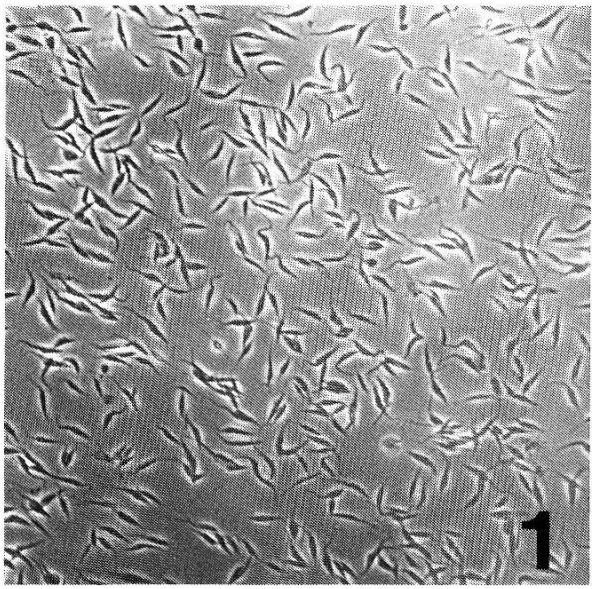
∅ = no lysis of epimastigote culture forms
+ = lysis of epimastigote culture forms

Complement lysis test

Incubation in normal fresh rat, hen and human serum did not lyse the epimastigote culture forms of *T. conorhini* (Table 2, Figs. 1, 3, 5). After complement lysis the surviving cells from the twofold cultures *T. cruzi*/*T. rangeli* and *T. cruzi*/*T. conorhini* were transfered to test tubes. The microscopical examination of the test tubes demonstrated that all transfered cells – *T. rangeli* and *T. conorhini* – were living. The culture forms of *T. rangeli* and *T. conorhini* were not damaged by the rat serum. Both species could be separated by complement lysis from the epimastigote culture forms of *T. cruzi*.

Discussion

These results clearly show that carbohydrate-specific agglutinins can be used for the differentiation between *T. cruzi*, *T. rangeli* and *T. conorhini*. The culture forms of *T. rangeli* were only agglutinated by lectins which belong to the D-mannose type (Table 1). Lectins specific for galactose and their derivates and for L-fucose did not react. Furthermore, reactions with lectins specific for N-acetylneuraminic acid, *T. vulgaris* and *A. papillata* II were also not observed. Mühlpfordt and Schottelius (1977) as well as Bretting and Schottelius (1978) discussed the different reactions of *T. cruzi*, *T. rangeli* and *T. conorhini* with the *A. papillata* II lectin. Using lectins it was not possible to distinguish intraspecifically between the tested *T. rangeli* strains (Table 1). As *T. rangeli* reacts only with D-mannose specific lectins it could be that *C. ensiformis* and *P. sativum* specific carbohydrates belong to a glycoconjugate which represents the high mannose type of simple composition containing N-acetyl-glucosamine, mannose and occasionally glucose (Rothenberg and Boyse, 1979). The epimastigote culture forms of *T. rangeli* can therefore easily be distinguished from the culture forms of *T. cruzi* because these cells react with lectins specific for galactose,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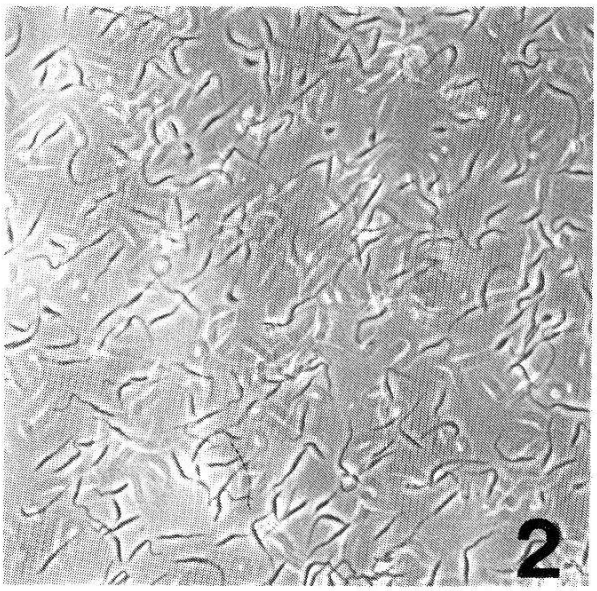
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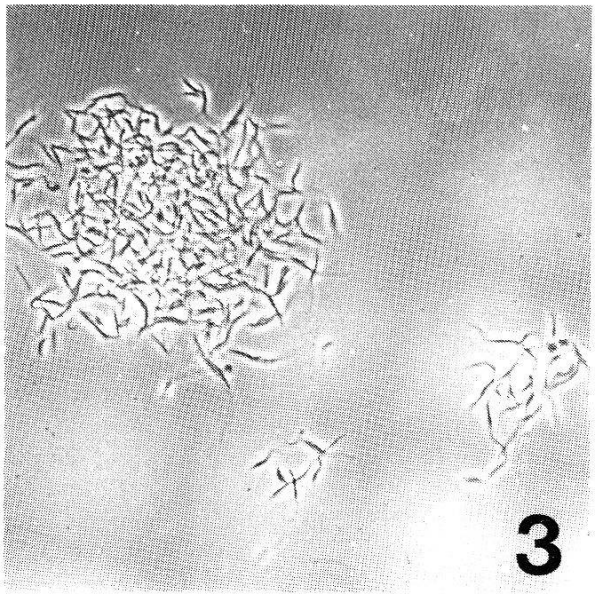
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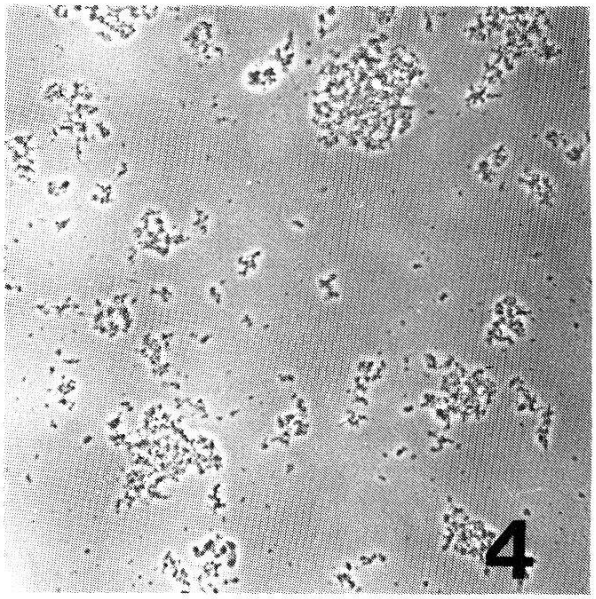
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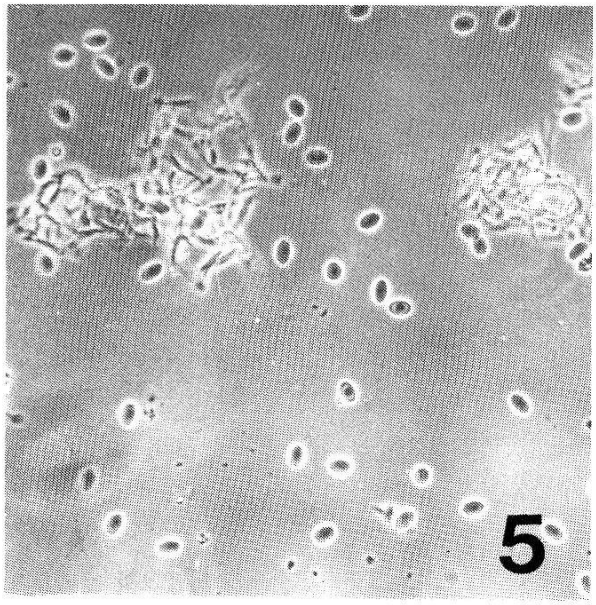
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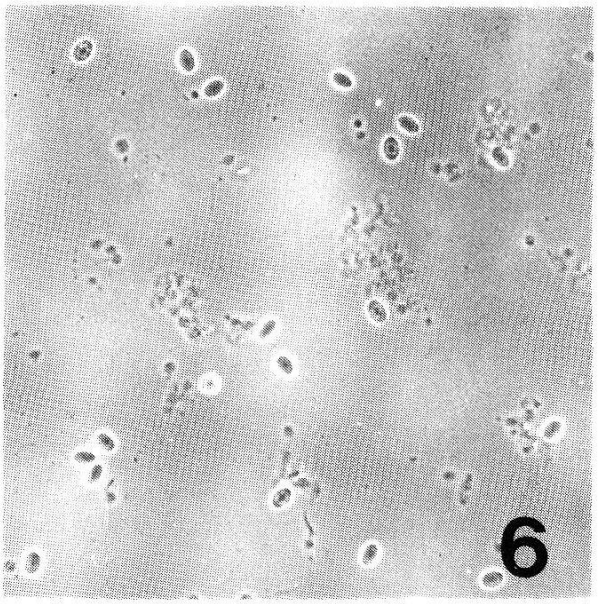
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glucose, mannose, N-acetyl-galactosamine, N-acetylneuraminic acid, N-acetyl-glucosamine (Gonçalves and Yamaha, 1969; Alves and Colli, 1974; Gottlieb, 1977; Mühlpfordt and Schottelius, 1977; Schottelius and Raabe, 1978; Schlemper and Schottelius, 1979; Vivas et al., 1979; Katzin et al., 1979; Pereira et al., 1980; Schottelius, 1982a). *T. cruzi* and *T. conorhini* can be differentiated by the lectin from *T. crocea.* With the lectins from the indo-pacific clams *T. crocea* and *T. maxima* (Uhlenbruck et al., 1975, 1979) it was possible to distinguish between *T. cruzi* and *T. vespertilionis* (Schottelius et al., 1983) because only the *T. cruzi* strains were agglutinated. *T. cruzi* and *T. conorhini* differ themselves in the reaction with *T. vulgaris* and *A. hypogaea* (Table 1). But these two lectins cannot be used for the differentiation because there are *T. cruzi* strains which react with *A. hypogaea* (PNA-type) and not only with *T. vulgaris* (WGA-type) (Schottelius, 1982a). The comparable lectin test for the differentiation between *T. cruzi* and *T. rangeli* can be substituted by other lectins which are available on the market.

Furthermore, *T. cruzi* and *T. rangeli* can be distinguished by their different complement sensitivity (Schottelius, 1982a). This demonstrates that these flagellates cannot only be distinguished by their different carbohydrates. The cell surface membranes themselves also differ relative to complement activating components. As *T. cruzi* and *T. conorhini* differ themselves only in their agglutination behaviour with the *T. crocea* lectin which is not available on the market, the rat trypanosome was tested with different sera as well. It could be demonstrated that *T. cruzi* and *T. conorhini* can be distinguished by their different complement specificity. These two flagellates can also be differentiated from *T. rangeli* (Schottelius, 1982b) (Table 2, Figs. 1–7). The complement lysis by the alternative pathway can be activated by various polysaccharides, endotoxins (Barkas, 1978) and by lipopolysaccharides (LPS) (Allen and Scott, 1980; Pangburn et al., 1980). Polysaccharides (Gonçalves and Yamaha, 1969) and lipopolysaccharides (Goldberg et al., 1979) were isolated from *T. cruzi.* Thereby substances are found on the surface of the epimastigotes of *T. cruzi* which are able to activate complement by the alternative pathway. As the metacyclic forms of *T. cruzi* and *T. conorhini* will not be lysed – these stages from *T. rangeli* were not observed – we probably can say that the cell surface components, responsible for the complement activation will be eliminated or changed by the transformation of the epimastigotes to the metacyclic trypomastigote forms. Rowley (1954) and Lüderitz et al. (1971) demonstrated that the so-called smooth strains of *Escherichia coli* and *Salmonella typhimurium* are relatively resistant to serum whereas rough strains in which the LPS lack peripheral carbohydrate residues

Fig. 1. *T. conorhini*-culture forms, 4 days; 160×.
Fig. 2. *T. cruzi*-culture forms, 4 days; 160×.
Fig. 3. *T. conorhini*-no cell lysis by rat serum; 160×.
Fig. 4. *T. cruzi*-cell lysis by rat serum; 160×.
Fig. 5. *T. conorhini*-no cell lysis by hen serum; 160×.
Fig. 6. *T. cruzi*-cell lysis by hen serum; 160×.

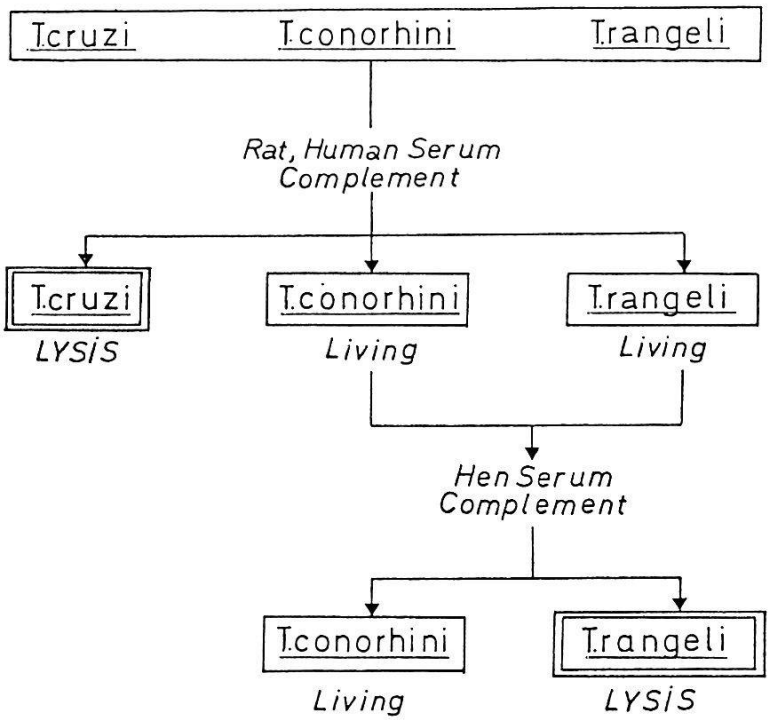

Fig. 7. Differentiation of *T. cruzi*, *T. conorhini* and *T. rangeli* by their different complement sensitivity.

are more susceptible to the lethal action of complement. Therefore, it possibly could be that the tested trypanosomes perhaps have different LPS on their surfaces. It cannot be said whether the lipopeptidophosphoglycan (Lederkremer et al., 1976) is able to activate complement by the alternative pathway.

T. rangeli and *T. conorhini* can be separated from *T. cruzi* by the complement lysis test, *T. rangeli* from *T. conorhini* as well. Therefore, the possibility is available to culture such separated cells for further investigations. As metacyclic forms of *T. cruzi* are also lysis resistant, it will be necessary to clone *T. rangeli* or *T. conorhini* for further tests.

It could be demonstrated that some of the used lectins simplify the differentiation between *T. rangeli* and *T. conorhini* without an additional treatment with pronase (Mühlpfordt and Schottelius, 1977). Differences between the tested trypanosome species exist relative to their lectin binding sites and to cell surface components for the activation of complement.

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