Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 78 (3) :317-333, jul./set. 1983

A SIMPLE METHOD TO PURIFY BIOLOGICALLY AND ANTIGENICALLY PRESERVED BLOODSTREAM TRYPOMASTIGOTES OF *TRYPANOSOMA CRUZI* USING DEAE-CELLULOSE COLUMNS

MARIA AUXILIADORA DE SOUSA

A method to purify trypomastigotes of some strains of Trypanosoma cruzi *(Y, CL, FL, F, "Berenice", "Colombiana" and "São Felipe") from mouse blood by using DEAE-cellulose columns was standardized. This procedure is a modification of the Lanham & Godfrey methods and differs in some aspects from others described to purify* T. cruzi *bloodstream trypomastigotes, mainly by avoidance of prior purifications of parasites. By this method, the broad trypomastigotes were mainly isolated, accounting for higher recoveries obtained with strains having higher percentages of these forms: processing of infected blood from irradiated mice could be advantageous by increasing the recovery of parasites (percentage and/or total number) and elution of more slender trypomastigotes.*

Trypomastigotes purified by this method presented normal morphology and motility, remained infective to triatomine bugs and mice, showing in the latter prepatent periods and courses of parasitemia similar to those of control parasites, and also reproducing the polymorphism pattern of each strain. Their virulence and pathogenicity also remained considerably preserved, the latter property being evaluated by LD 50 tests, mortality rates and mean survival time of inoculated mice. Moreover, these parasites presented positive, clear and peripheral immunofluorescence reaction at titres similar to those of control organisms, thus suggesting important preservation of their surface antigens.

Several methods to isolate trypomastigotes of *Trypanosoma cruzi* from blood of vertebrate hosts have been described, although obtention of pure suspensions of this parasite stage had presented some difficulties. Differential centrifugation is the more commonly used procedure which isolated trypomastigotes from the majority of erythrocytes, but not from leucocytes and platelets; this procedure was also reported with adaptations in order to reduce the blood element contamination (Yaeger, 1955, 1960; Gutteridge, Knowler & Coombes, 1969; Kloetzel, Camargo & Giovannini, 1975). Differential centrifugations using substances to determine density gradients, such as sucrose (Williamson & Cover, 1966), ficoll-hypaque (Budzko & Kierszenbaum, 1974), dextran (Sanderson, Thomas & Towmey, 1980) and metrizamide (Loures, Pimenta & Souza, 1980), also have been described, although these methods include additional procedures for removing the remaining blood cells and platelets.

Instituto Oswaldo Cruz, Caixa Postal 926, 20000 Rio de Janeiro, RJ, Brazil.

Received for publication November 29th, 1982 and accepted January 6th, 1983.

Ion exchange chromatography using DEAE-cellulose columns was successfully introduced by Lanham (1968) to purify bloodstream trypomastigotes of several species of salivarian trypanosomes and *T. lewisi*. By this method, *T. cruzi* (Y strain) was the only species of trypanosome examined which could not be separated from mouse blood, this fact being related to the small difference in surface charge between the trypanosomes and blood cells (Lanham, 1968). For this reason, other authors employed prior purification of the parasite suspensions to eliminate the majority of blood cells before processing them through DEAE-cellulose columns (Gutteridge, Cover & Gaborak, 1978; Villalta & Leon, 1979; Mercado & Katusha, 1979).

This work presents a simple method to purify trypomastigotes of several *T. cruzi* strains from experimentally infected mice exclusively employing ion exchange chromatography on DEAE-cellulose columns. It differs in some essential aspects from others already described for isolating this stage of *T. cruzi* using this type of chromatography, mainly concerning the avoidance of prior purifications of the trypanosomes. Aspects of the biology and antigenicity of the purified parasites were also studied and showed that they did not present the alterations reported by some authors for trypomastigotes isolated on DEAE-cellulose columns (Souza et al, 1976; Villalta & Leon, 1979).

MATERIAL AND METHODS

Strains of *Trypanosoma cruzi*

The *T. cruzi* strains chosen were: Y (Silva & Nussenzweig, 1953), "Berenice" (Salgado et al, 1962), "Colombiana" (Federici, Abelmann & Neva, 1964), "São Felipe" (Andrade, 1974), F (Deane & Kloetzel, 1974), CL and FL (Brener & Chiari, 1963). The Y, "Berenice", "Colombiana", CL and FL strains were supplied by Dr. Zigman Brener (René Rachou Institute, Belo Horizonte), while the "São Felipe" and F strains were supplied, respectively, by Dr. Gabriel Grimaldi Filho and Dr. Maria P. Deane (Oswaldo Cruz Institute, Rio de Janeiro). The majority of these strains were received during 1978 in LIT medium, with exception of the "Colombiana" and F strains which were received in inoculated mice; the F strain was received in 1980. Thence these strains have been maintained by serial passages in 20 g male albino mice by intraperitoneal inoculation of 10^5 parasites/mouse.

Experimental infections for trypomastigote purification

Male albino mice weighing 20g were used in all experimental infections. Some of them received 600 rad of whole-body gamma-irradiation 24 h before inoculation of parasites. Both normal or irradiated mice were inoculated with 10^5 trypomastigotes/mouse by intraperitoneal route. Infected blood from non-irradiated mice necessary for trypomastigote purification was collected on parasitemic peaks of each strain, as follows: Y and "Berenice" – 7th day, CL – 14th day, FL – 12th day, "Colombiana" – 15th day, "São Felipe" – 16th to 18th day, F – 33th day. Infected blood from irradiated mice was collected on the above mentioned days or before 100 per cent mortality was reached in the group of inoculated mice, as follows: Y and "Berenice" strains – 7th day, CL and FL – 9th to 13th day; "Colombiana" – 15th day and "São Felipe" – 16th or 17th day.

Buffers and resin equilibration

Concentrated phosphate-saline buffer (PS), pH 8.0 and ionic strength (I) 0.362, was prepared according to Lanham & Godfrey (1970), as follows:

$Na_2 H PO_4 . 12 H_2 0$ (Merck or Carlos Erba *)	34.0 g
$Na H_2 PO_4 . H_2 0$ (J. T. Baker, U.S.A.)	0.69 g
Na Cl (Carlos Erba *)	4.25 g
$H_2 0$ q.s.p.	1000 ml

*orange label

When necessary, the pH of the PS buffer was adjusted with 5% (w/v) H_3PO_4 (Riedel-de Haën). Phosphate-glucose buffers (PSG) of the same pH and various ionic strengths were prepared by dilution of concentrated PS and adding glucose (anhydrous dextrose, Merck) as specified in Table I.

DEAE-cellulose (DE 52, Whatman), 200g, was initially washed various times with distillated water to eliminate the fines. Its equilibrium was started by two washes with 200ml of concentrated PS and followed by ten to twelve washes with 300ml of the PSG chosen to equilibrate the resin. Initially, some buffers were assayed to equilibrate the resin: PSG 3:7 (I = 0.109), PSG 5.5 (I = 0.181) and PSG 6:4 (I = 0.217). From the preliminary results, it was established to equilibrate the resin with PSG 5:5 for the purification experiments. The equilibrated resin was kept at 4°C until use; when it was stored during one week or more, the equilibration buffer was replaced. The slurry of DEAE-cellulose was degassed before packing the column.

Columns

Fifteen or sixteen ml of equilibrated DEAE-cellulose were packed in a common 20ml syringe on a layer of glass wool and filter paper. Subsequently, about 30ml of equilibration buffer were run through the column, leaving only a meniscus of buffer at the adsorbent surface. Eluate flow was controlled at 1.5–2.0ml/min by a screw-clip on the latex tube fitted to the column outlet. Columns correctly packed using well equilibrated resin were essential for sucessful purifications.

Standardization of the purification procedure

Infected blood for purification experiments was generally obtained from the orbital plexus of three mice with high parasitemias. The blood sample (1.5ml) was directly collected in equal volume of PSG 5:5 containing 10 U heparin/ml; the number of trypomastigotes in this sample was determined with a Neubauer hemocytometer according to the Hoff method (1974). Then, the diluted blood sample was gently applied on the top of the adsorbent and the column outlet was opened to allow its entry into the resin; subsequently, the eluent buffers were run through the column. During the preliminary experiments to determine the conditions for purification of the trypomastigotes, the following eluents were assayed: PSG 6:4 alone (I = 0.217), an increasing stepwise series from PSG 3:7 (I = 0.109) to concentrated PSG (I = 0.362) (as in Table I) and a stepwise series using PSGs 5:5 (I = 0.181), 6:4 (I = 0.217) and 7:3 (I = 0.253). The first eluent employed, was

TABLE I

Preparation of phosphate-saline-glucose buffers (PSG)

Buffer Solution	*Vol. PS*	*Vol. H_2O*	*Glucose % (w/v)*	*Ionic Strength*
PSG 3:7	3	7	1.5	0.109
PSG 4:6	4	6	1.0	0.145
PSG 5:5	5	5	1.0	0.181
PSG 6:4	6	4	1.0	0.217
PSG 7:3	7	3	0.8	0.253
PSG 8:2	8	2	0.8	0.289
PSG 9:1	9	1	0.5	0.326
PSG conc.	10	–	0.5	0.362

always that used for column equilibration. Thirty six ml of each buffer were consecutively run through the column at room temperature. Aliquots of eluates (9 ml) were directly collected in plastic tubes and centrifuged at 2000 or 2500 g, respectively, for 30 or 15 min. The supernatant fluids were discarded and samples of the remaining eluates (2-3 ml) examined in fresh preparations under light microscope. Since pure trypomastigote suspensions were obtained using columns equilibrated with PSG 5:5 and consecutive elutions with PSGs 5:5 and 6:4, this procedure was systematically repeated in order to quantify the yield of this method with various strains of the parasite. Subsequent elution with PSG 7:3 permitted the recovery of trypanosomes still adsorbed on the columns, however with a low erythrocyte contamination.

The number of parasites harvested during the running of each buffer was estimated with a Neubauer hemocytometer. The percentages of recovered trypomastigotes and purity of the parasite suspensions were also determined.

Morphology and motility of purified trypomastigotes

The morphology and motility of parasites eluted during the running of each buffer were initially observed in fresh preparations. Giemsa stained slides were prepared for a more accurate morphological study. The presence and predominance of different trypomastigote forms in the eluates were also observed.

Biological behaviour of purified trypomastigotes

The infectivity, prepatent periods, parasitemic curves, virulence and pathogenicity of parasites purified during the running of PSG 6:4 and their controls were studied in 20 g male albino mice. Throughout this study, the Y, CL, FL and "Berenice" strains were used. The control parasites remained in the original blood, 1:2 diluted in heparinized PSG 5:5, to be inoculated after the same time interval necessary for column processing of the trypomastigotes. Groups of six mice were used in all experiments, each being intraperitoneally inoculated with 10^5 trypomastigotes, except for LD 50 determinations of the FL strain, whose inocula were 10^6, 0.5×10^6, 0.25×10^6 and 0.125×10^6 parasites/mouse. Parasitemic curves were carried out using the arithmetic mean of the number of parasites/ml/mouse; parasite counts were made according to the Hoff method (1974) using a blood sample (5 μl) from the tail of each animal. For evaluation of infectivity, prepatent periods and virulence of purified trypomastigotes, the data of their parasitemias in mice were considered, since in this work infectivity was judged by evidence of parasite multiplication, while virulence was estimated by intensity of this multiplication, as suggested by Andrade (1974). Pathogenicity of these parasites was evaluated by their LD 50 (Reed & Muench, 1938) and by mortality rates and mean survival time of inoculated animals. The ability of the FL and "Berenice" strain purified trypomastigotes to reproduce their typical polymorphism pattern in mice (Brener & Chiari, 1963; Brener, 1965) was also verified.

The infectivity of purified parasites for the invertebrate host was studied using fifth instar nymphs of *Triatoma infestans*. Pure trypomastigotes of the FL strain were mixed with heparinized normal mouse blood and their concentration adjusted at 10^5 parasites/ml; this suspension was placed in an apparatus for artificial bug feeding (Garcia et al, 1975). Thirty days after feeding of the insects, their mid-and hind-guts were dissected and observed under light microscope.

Antigenicity of purified trypomastigotes

The indirect fluorescent antibody (IFA) reaction was chosen to verify whether trypomastigotes purified through DEAE-cellulose, both during the running of PSG 5:5 and 6:4, had preserved their ability to interact with specific antisera *in vitro*. The control parasites were isolated from the majority of blood cells by differential centrifugation (Kloetzel et al, 1975). Trypomastigotes of the F and CL strains were employed in the

IFA reactions, which were performed according to Coutinho et al (1981). Positive and negative control human sera, which are used in serological investigations on Chagas' disease (SUCAM-Brazil), were employed in these reactions.

RESULTS

Standardization of the purification method

Attempts to isolate *T. cruzi* trypomastigotes from mouse blood using DEAE-cellulose columns equilibrated with PSG 6:4 and elution with this same buffer, or columns equilibrated with PSG 3:7 and stepwise elution from PSG 3:7 to concentrated PSG, were unsuccessful because the parasites, when recovered, were eluted together with some blood cells.

The standard conditions for purifying *T. cruzi* bloodstream trypomastigotes were achieved using columns equilibrated with PSG 5:5 and consecutive elutions with PSGs 5:5 and 6:4. Although the running of PSG 5:5 had generally determined very low or no recovery of parasites, its initial use was maintained because it allowed the subsequent desorption of higher numbers of isolated parasites when PSG 6:4 was passed through the columns (Table II). All *T. cruzi* strains assayed could be purified using this procedure, although at different recovery percentages, which were higher with the CL, FL, F, "Colombiana" and "São Felipe" strains than with the Y and "Berenice" ones (Table II). Processing of infected blood from irradiated mice generally increased the number of recovered trypomastigotes of all strains tested (Table III), although a striking per cent increase occurred only with the FL and "São Felipe" strains (Table II).

TABLE II

Per cent recovery of bloodstream trypomastigotes of seven *T. cruzi* strains purified through DEAE-cellulose columns during consecutive running of PSGs 5:5 (I= 0.181) and 6:4 (I= 0.217), pH 8.0. The infected blood was from normal or irradiated mice

Strains	*Per cent recovery*					
	Normal mice			*Irradiated mice*		
	PSG buffers		*days of infection*	*PSG buffers*		*days of infection*
	5:5	*6:4*		*5:5*	*6:4*	
Y	0	1.9	7	0	8.1	7
	0	6.5	7	0	5.8	7
	–	–	–	0	4.1	7
	–	–	–	0	7.2	7
	–	–	–	0	2.6	7
"Berenice"	0	8.2	7	0	4.3	7
	0	2.0	7	0	3.4	7
	0	2.2	7	–	–	–
CL	0.2	64.3	14	0.2	35.8	13
	3.1	30.6	14	0	73.5	9
	2.0	51.8	14	0	56.6	12
	–	–	–	1.1	36.9	9
	–	–	–	4.7	61.5	13
FL	0.2	29.5	12	0.1	80.6	13
	0.1	30.3	12	1.0	65.3	10
	–	–	–	0.3	77.2	9
"Colombiana"	5.3	28.0	15	0.8	46.9	15
	2.2	39.3	15	0.2	42.9	15
"São Felipe"	0	18.4	18	0.7	46.9	17
	0	26.2	16	0	65.5	16
F	23.0	29.9	33	–	–	–

TABLE III

Number of bloodstream trypomastigotes (mean and range) of seven *T. cruzi* strains purified through DEAE-cellulose columns during elution with PSG 6:4 (I= 0.217, pH 8.0), after passage of PSG 5:5. The infected blood (1.5 ml) was from normal or irradiated mice

Strains	*No. of trypomastigotes x 10^5*	
	Normal mice	*Irradiated mice*
Y	2.2 (1.1–3.3)	5.5 (3.1–9.7)
"Berenice"	0.6 (0.4–0.7)	4.3 (2.3–6.2)
CL	32.7 (27.3–39.6)	41.5 (5.0–131.2)
FL	36.1 (8.1–64.1)	103.0 (75.2–124.6)
"Colombiana"	13.4 (12.4–14.5)	96.6 (17.4–175.9)
"São Felipe"	3.2 (1.6–4.8)	22.1 (10.8–33.4)
F *	224.2	–

*One experiment

The mean purity of trypomastigote suspensions recovered from the columns during the running of PSGs 5:5 and 6:4 were, respectively, 100 and 98 per cent, as regards the absence of blood cells; however, 100 per cent purity was always achieved with well equilibrated and packed columns. Platelets never occurred in the eluates.

Morphology and motility of purified trypomastigotes

The parasites harvested during elution with PSG 5:5 were exclusively broad forms, whereas those eluted during the running of PSG 6:4 were mainly broad forms, since intermediate, short and slender trypomastigotes could be also recovered (Fig. 1). An increase of slender forms in these eluates generally occurred processing blood from

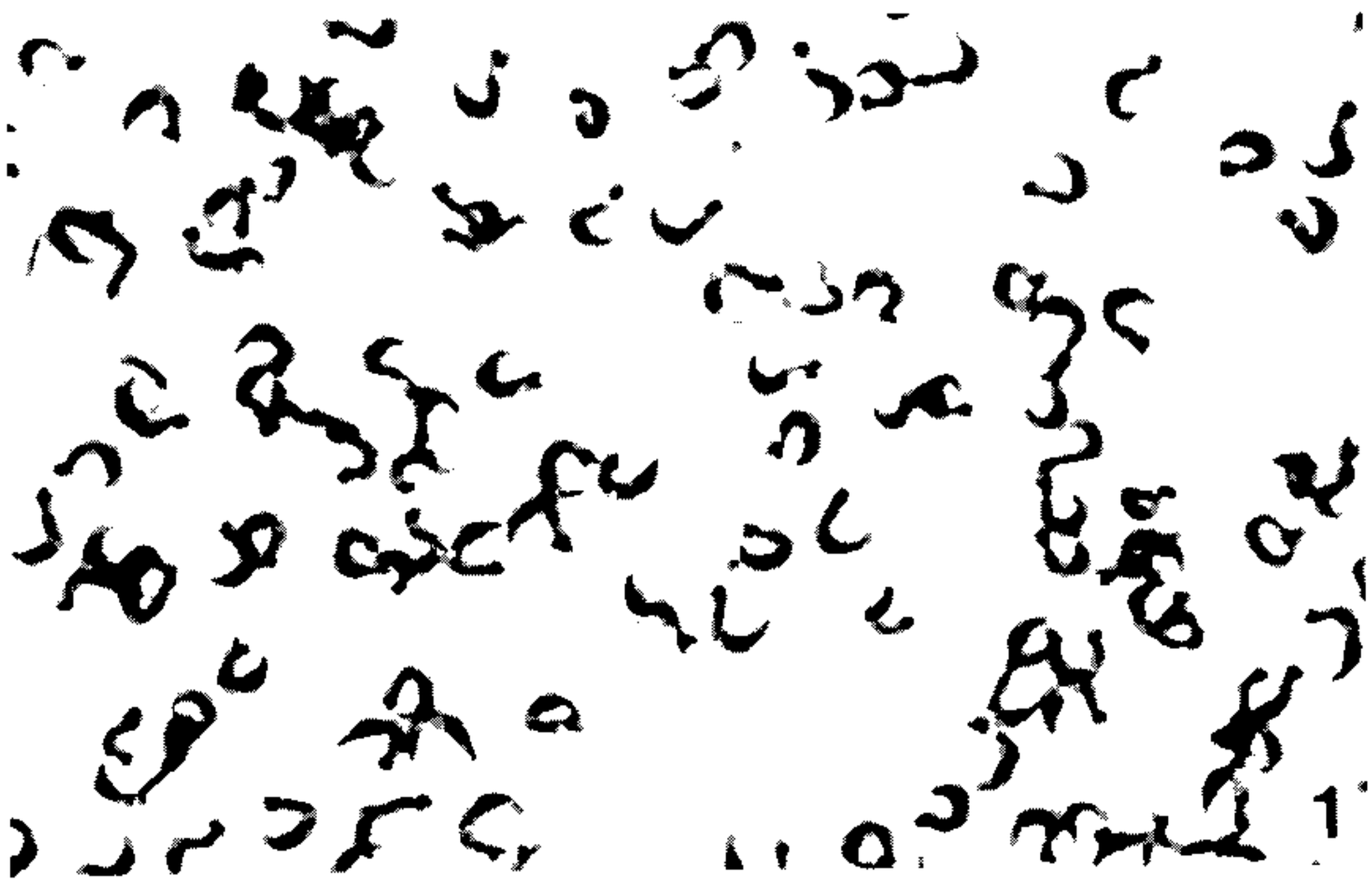

Fig. 1 – Bloodstream trypomastigotes of *T. cruzi* (FL strain) purified through DEAE-cellulose columns during elution with PSG 6:4. Giemsa strain, X 1,000.

irradiated mice. The motility of these parasites was normal and characteristic of each morphological type; they also presented well preserved morphology as evidenced in fresh and Giemsa stained preparations (Fig. 1).

Biological behaviour of purified trypomastigotes

Infectivity of purified parasites of the CL, FL and "Berenice" strains was initially shown by the presence of more bloodstream trypomastigotes than had been inoculated in each mouse. Additionally, the course of their parasitemias was similar to that of mice receiving control parasites, as regards prepatent periods, profiles and days of parasitemia peaks (Figs. 2-4). Moreover, the virulence of purified trypomastigotes of these strains was shown by parasitemic levels as high as those presented by control parasites (Figs. 2-4). However, on the 5th day of the mouse infection with purified parasites of the "Berenice" strains a lower parasitemic level was observed (Fig. 4), suggesting a lower initial multiplication, wich did not occur with purified parasites of other strains (Figs. 2-3).

Mortality rates of 100 per cent within the expected time occurred in groups of mice inoculated either with purified or non-purified parasites of the CL and FL strains. On the other hand, mortality rates of 33 and 67 per cent were observed with purified trypomastigotes, respectively, of the Y and "Berenice" strains, as compared with 83 and 100 per cent, respectively, for their controls. Mortality generally began one day later in mouse groups inoculated with purified trypomastigotes of the CL and FL strains, and one or two days later in groups inoculated with purified parasites of the Y and "Berenice" strains. Table IV presents these results.

LD 50 of column-processed parasites of the FL strain were similar to those of control organisms, being only 1.3 and 1.1 times higher for the former parasites, respectively, on the 12th and 13th days of mouse infection (Table V). Furthermore, the mean survival time of mice inoculated with purified trypomastigotes of this strain generally was not increased by more than one day with the majority of inocula used (Table VI).

Purified trypomastigotes of the FL and "Berenice" strains were able to reproduce the typical polymorphism pattern of each strain at different days of mouse infection, according to that described by Brener (1965).

Thirty days after ingestion of column-purified parasites of the FL strain, 100 per cent of the *T. infestans* nymphs were infected, presenting the evolutive stages of the parasite.

Antigenicity of purified trypomastigotes

Column-purified trypomastigotes, either eluted with PSG 5:5 or 6:4, presented a positive, clear and peripheral IFA reaction, similar in appearance and sensitivity to that of control parasites, even using highly diluted sera from Chaga's disease patients (Table VII; Fig. 5).

DISCUSSION

Column separation of trypomastigotes from blood introduced by Lanham (1968) is a simple and elegant method for isolating infective parasites for biochemical and immunological studies. However, its use to purify *T. cruzi* bloodstream trypomastigotes presented some difficulties on account of their highly negative surface eletrical charge, similar to that of mouse erythrocytes (Lanham, 1968). The present work reports a method to purify *T. cruzi* trypomastigotes from mouse blood only by anion exchange chromatography on DEAE-cellulose columns, since it avoids prior parasite purifications described in other methods (Gutteridge et al, 1978; Villalta & Leon, 1979; Mercado & Katusha, 1979), by establishing conditions which discriminated the parasite surface charge from that of the mouse erythrocytes.

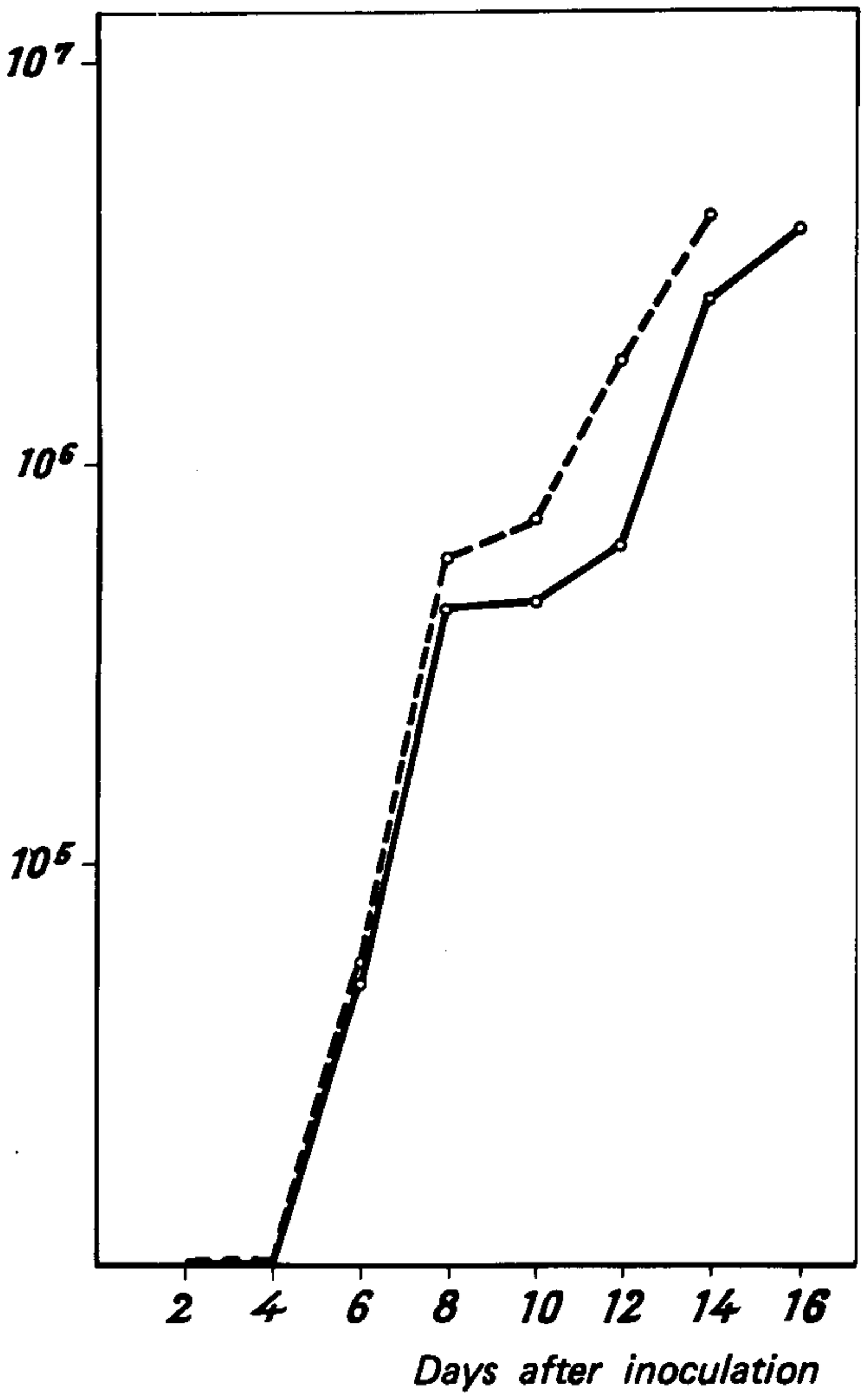

Fig. 2 – Course of parasitemia in groups of six mice intraperitoneally inoculated with 10^5 bloodstream trypomastigotes of the CL strain either purified (——) or non-purified (– – –) through DEAE-cellulose columns.

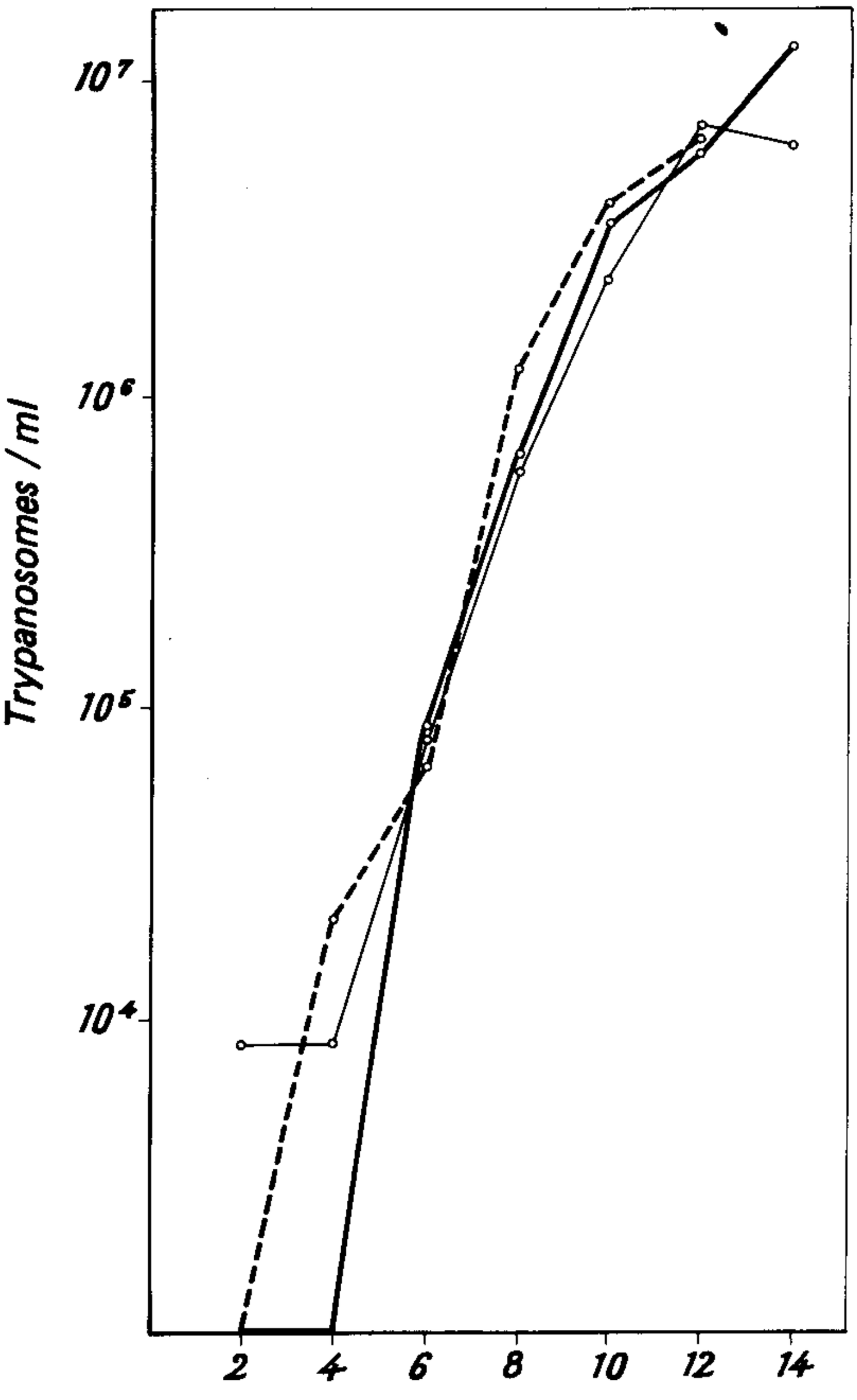

Fig. 3 – Course of parasitemia in groups of six mice intraperitoneally innoculated with 10^5 bloodstream trypomastigotes of the FL strain either purified (———; ———) or non-purified (– – – –) through DEAE-cellulose columns. The parasites of one experiment (———) were inoculated 4 h after purification.

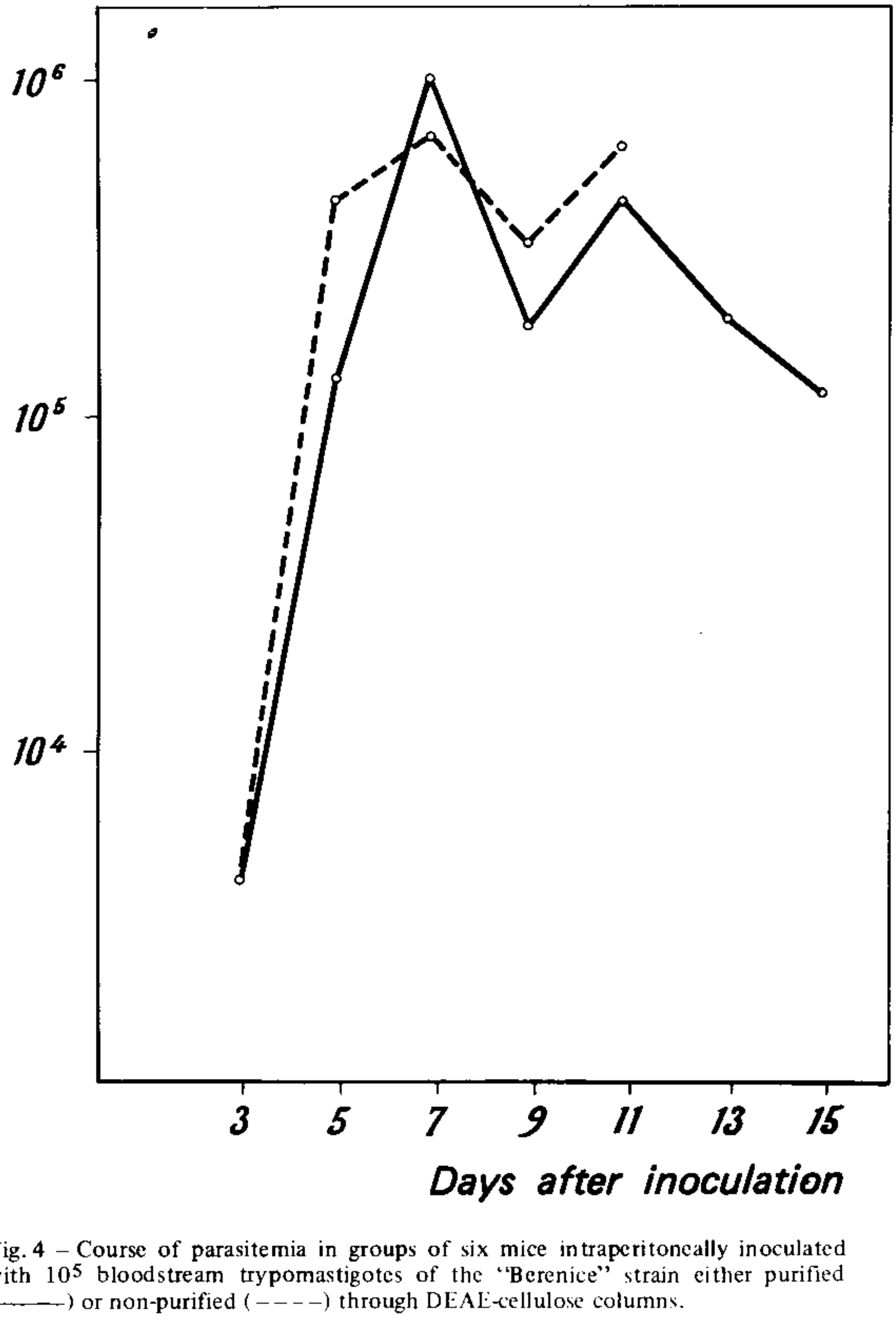

Fig. 4 – Course of parasitemia in groups of six mice intraperitoneally inoculated with 10^5 bloodstream trypomastigotes of the "Berenice" strain either purified (———) or non-purified (– – – –) through DEAE-cellulose columns.

TABLE IV

Mortality of 20 g male albino mice (groups of six) intraperitoneally inoculated with 10^5 bloodstream trypomastigotes of four *T. cruzi* strains purified or non-purified through DEAE-cellulose columns (respectively, experimental and control groups)

Strains	*Groups*	*Number of dead mice by day postinoculation*													*Total*
		11º	*12º*	*13º*	*14º*	*15º*	*16º*	*17º*	*18º*	*19º*	*20º*	*21º*	*22º*	*30º*	*dead*
CL	experimental	0	0	0	0	0	1	4	0	0	0	1	–	–	6
	control	0	0	0	0	2	3	1	–	–	–	–	–	–	6
FL	experimental	0	0	0	2	4	–	–	–	–	–	–	–	–	6
	experimental	0	0	0	3	2	0	1	–	–	–	–	–	–	6
	control	0	0	3	2	0	0	0	1	–	–	–	–	–	6
Y	experimental	0	0	2	1	0	1	0	0	0	0	0	0	0	4
	control	0	3	1	1	0	0	0	0	0	0	0	1	–	6
	control	3	0	1	1	1	–	–	–	–	–	–	–	–	6
"Berenice"	experimental	0	0	1	1	0	0	0	0	0	0	0	0	0	2
	control	0	1	3	0	1	0	0	0	0	0	0	0	0	5
	control	1	1	3	0	0	0	0	0	0	0	0	0	0	5

TABLE V

LD 50 of *T. cruzi* bloodstream trypomastigotes (FL strain) purified through DEAE-cellulose columns and of their controls. Determinations were made on 12th and 13th days postinoculation of 20g male albino mice

Trypomastigotes	*LD 50 (no. of trypomastigotes)*	
	12th day	*13th day*
Purified	5.0×10^5	3.3×10^5
Control	3.8×10^5	3.0×10^5

TABLE VI

Mean survival time of groups of 20g male albino mice intraperitoneally inoculated with different numbers of *T. cruzi* bloodstream trypomastigotes (FL strain) purified or non-purified through DEAE-cellulose columns (respectively, experimental and control groups)

Groups	*Mean survival time in days with each inoculum*			
	10^6	0.5×10^6	0.25×10^6	0.125×10^6
Experimental	12.5	12.5	14.3	14.7
Control	11.0	11.7	14.0	14.0

TABLE VII

IFA reaction titres of sera from chagasic or normal individuals using *T. cruzi* bloodstream trypomastigotes (F strain) either purified through DEAE-cellulose columns or isolated by differential centrifugation (control parasites)

Isolated trypomastigotes	*Titres of sera*		
	chagasic		*normal*
	serum 1	*serum 2*	
Through DEAE-cellulose (A)	1: 256	1: 1024	1: 16
Through DEAE-cellulose (B)	1: 256	1: 1024	1: 16
Control	1: 256	1: 512	1: 8

(A) Isolated during the running of PSG 5:5
(B) Isolated during the running of PSG 6:4

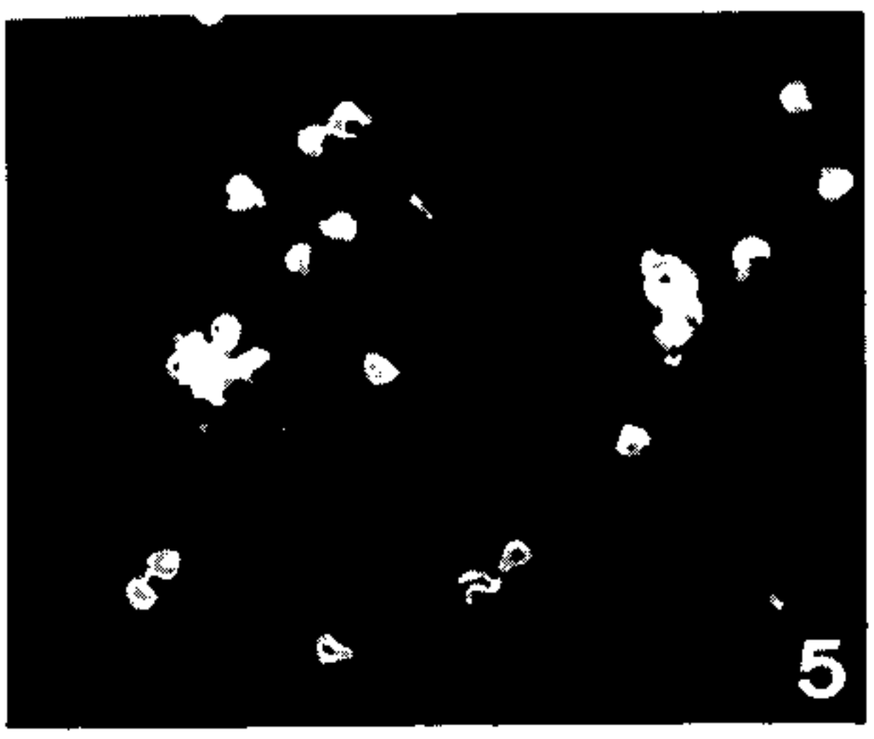

Fig. 5 - IFA reaction of *T. cruzi* bloodstream trypomastigotes (CL strain) purified through DEAE-cellulose columns during elution with PSG 6:4. X 400.

The present method follows principles of ion exchange chromatography for protein purifications (Sober et al, 1956; Sober & Peterson, 1958) and employs elements from the original works of Lanham (1968) and Lanham & Godfrey (1970). Initially, the infected blood to be processed was always collected in the same PSG buffer used to equilibrate the resin; this procedure had not been described in the other methods (Gutteridge et al, 1978; Villalta & Leon, 1979; Mercado & Katusha, 1979). The preferred anticoagulant was heparin, rather than sodium citrate used by other authors (Gutteridge et al, 1978; Villalta & Leon, 1979), because it does not alter the ionic strength of the buffer used to collect the infected blood. Stepwise elution using buffers of increasing ionic strengths, always starting with the column equilibration PSG, was the decisive condition for trypomastigote desorption from the columns free of blood cells and platelets; the buffer used, i.e. PSGs 5:5 and 6:4, had been also employed by Lanham & Godfrey (1970) for the salivarian trypanosome purifications. Additionally, no forced flow using vacuum system was applied to the column during the trypanosome elutions, as adopted by Villalta & Leon (1979), in order to prevent possible parasite damage due to a filtration procedure. Moreover, the collection of eluted trypomastigotes promptly into serum used in other methods (Gutteridge et al, 1978; Villalta & Leon, 1979) was not necessary, because these parasites were not easily lysed, even if centrifuged at higher

speeds than those mentioned by Gutteridge et al (1978), and could remain in the centrifuged eluates during at least 4 h. Thus, it is clear that the present method differs in essential aspects from those already described to isolate *T. cruzi* bloodstream trypomastigotes through DEAE-cellulose columns, probably accounting for the preservation of biological and antigenic properties of the purified trypomastigotes.

Trypomastigotes of all *T. cruzi* strains assayed were purified by the present method, although at different recovery percentages. As previouslly observed by Gutteridge et al (1978) with the Sonia strain isolated on DEAE-cellulose columns, with the present method the main morphological purified type of all strains processed was the broad trypomastigote, although other forms could be obtained, including the slender ones. Otherwise, the recovery percentages of purified parasites of various strains assayed (Table II) showed a clear correlation with the relative occurrence of broad trypomastigotes for each. This fact could explain the lower recovery rates of the Y and "Berenice" strains, in which the slender forms predominate on the 7th day of mouse infection, and the higher recovery rates of others with predominance of broad trypomastigotes, as CL, FL, F, "Colombiana" and "São Felipe" strains.

On the other hand, processing of infected blood from irradiated mice could be advantageous, mainly in the case of FL, "Colombiana" and "São Felipe" strains, by increasing the number and percentage of recovered parasites (Tables II and III) and elution of more slender trypomastigotes among the purified parasites.

Preservation of mouse infectivity of bloodstream trypomastigotes purified through DEAE-cellulose columns has been reported by some authors (Lanham, 1968; Taylor, Lanham & Williams, 1974; Gutteridge et al, 1978; Mercado & Katusha, 1979), excepting Villalta & Leon (1979). In these works, the concept of infectivity comprised several aspects which were examined separately in the present paper. Infectivity was demonstrated by parasite multiplication in the host, the virulence evaluated by the intensity of this multiplication (Andrade, 1974), and the pathogenicity verified only by lethality for the host. Trypomastigotes purified by the present method remained infective both for mice and triatomine bugs, displaying in the former prepatent periods and parasitemic profiles similar to those of control organisms. Their virulence was also considerably preserved, since they presented in mice parasitemic levels as high as those of unprocessed parasites (Figs. 2-4). However, column purified trypomastigotes of the CL and FL strains preserved their lethality to mice, while those of the Y and "Berenice" strains did not present similar preservation (Tables IV, V and VI). Taking into account that the control parasites of the Y and "Berenice" strains were mainly slender forms, and that the purified forms were mainly broad trypomastigotes, it is possible that the above mentioned results could be due to differences in the biological behaviour of these forms, which have been already suggested by some authors (Silva, 1959; Brener, 1965, 1969; Brener & Chiari, 1965; Howells & Chiari, 1975; Deane, 1979). Additionaly, this assumption could also explain the lower initial parasitemia of purified parasites of the "Berenice" strain as compared with that of control organisms (Fig. 4). On the other hand, it is possible that other similarities found between purified and non-purified trypomastigotes as regards their biology in mice had largely derived from the potentiality of the former to reproduce the typical polymorphism pattern of the original strain.

Villalta & Leon (1979) reported that *T. cruzi* bloodstream trypomastigotes (Y strain) purified through DEAE-cellulose columns were less infective to mice than unprocessed parasites based on data of LD 50 tests. Since these authors recovered high percentages of parasites of this strain, most probably they did not elute mainly broad trypomastigotes and so their results can not be explained by the biological competence of these forms; thus, it is possible that their method had impaired the viability of the purified parasites.

An important property of the trypomastigotes purified by the present method was revealed by their positive, clear and peripheral immunofluorescence reaction, at titres

comparable to those obtained with control parasites (Table VII; Fig. 5), since it suggests the preservation of their surface antigens, which make possible the use of these organisms in immunological studies. Positive IFA reactions of parasites purified through DEAE-cellulose columns had been already reported by Moser et al (1978) using *Plasmodium berghei* sporozoites.

There are some references to alterations in the membranes of *T. cruzi* metacyclic trypomastigotes isolated on DEAE-cellulose columns, which were shown by negative immunofluorescence reactions (Souza et al, 1976), modifications in kinetics of amino acid transport (Goldberg et al, 1976) and changes in surface eletrical charges (Souza et al, 1977). These results were obtained with parasites isolated according to the procedure described by Goldberg et al (1976), whereby the slurry of DEAE-cellulose was packed in Buchner funnels and the parasites filtered through the resin using a vacuum system. *T. cruzi* bloodstream trypomastigotes purified according to Villalta & Leon method (1979) were also filtered in a similar way, this being a common feature with the above mentioned procedure. Therefore, such elution could be a factor accounting for the alterations reported in the eluted parasites. Moreover, a critical revision should be undertaken as regards other operational conditions of methods whereby altered organisms were obtained (Goldberg et al, 1976; Villalta & Leon, 1979; Alvarenga & Brener, 1979, 1980; Schmatz & Murray, 1981), since there are references to changes in trypanosomes simply due to the pH and ionic strengths of saline solutions, even without processing them through ion exchange columns (Lumsden et al, 1965; Taylor et al, 1974; Jackson, 1975).

RESUMO

Usando colunas de DEAE-cellulose foi padronizado um método para purificação de tripomastigotas de várias cepas de *Trypanosoma cruzi* (Y, CL, FL, F, "Berenice", "Colombiana" e "São Felipe") a partir do sangue de camundongos. Este método é uma modificação daqueles descritos por Lanham & Godfrey e difere em vários aspectos de outros descritos para purificar as formas sanguíneas deste parasita, particularmente na dispensa de pré-purificações. Por ele foram isolados principalmente os tripomastigotas largos, o que certamente justifica as maiores percentagens de recuperação obtidas com cepas em que predominavam estas formas; por outro lado, o processamento de sangue infectado de camundongos irradiados podia ser vantajoso pela recuperação de maior número e percentagem de parasitas e eluição de mais formas finas.

Os tripomastigotas purificados por este método apresentavam morfologia e motilidade normais e continuavam infectantes para barbeiros e camundongos. Nestes últimos apresentaram período pré-patente e curvas parasitêmicas semelhantes aos dos parasitas controles, além de reproduzirem o padrão de polimorfismo típico da cepa. Sua virulência e patogenicidade também manteve-se consideravelmente preservada, sendo que esta última propriedade foi avaliada por testes de DL 50, taxas de mortalidade e tempo médio de sobrevida de camundongos inoculados. Além do mais, os tripomastigotas purificados apresentaram reação de imunofluorescência positiva, nítida e periférica, cujos títulos eram comparáveis aos dos parasitas controles, assim sugerindo uma importante preservação de seus antígenos de superfície.

ACKNOWLEDGEMENTS

I wish to thank Dr. Bernardo Galvão de Castro Filho for starting me in this research and Dr. Tomaz Langenbach for helpful suggestions. I also thank Dr. Sérgio Gomes Coutinho for cooperation in immunological studies and Dr. Sérgio Lannes Vieira for making possible the irradiation of the animals.

REFERENCES

ALVARENGA, N.J. & BRENER, Z., 1979. Isolation of pure metacyclic trypomastigotes of *Trypanosoma cruzi* from triatomine bugs by use of DEAE-cellulose column. *J. Parasitol., 65* :814-815.

ALVARENGA, N.J. & BRENER, Z., 1980. Virulence attenuation of *Trypanosoma cruzi* metacyclic trypomastigotes from triatomine bug faeces after passage through a DEAE-cellulose column. Presented in the "VII Reunião Anual sobre Pesquisa Básica em Doenças de Chagas", BI-49, Caxambu, MG, Brazil.

ANDRADE, S.G., 1974. Caracterização de cepas do *Trypanosoma cruzi* isoladas no Recôncavo Baiano. *Rev. Pat. Trop., 3* :65-121.

BRENER, Z., 1965. Comparative studies of different strains of *Trypanosoma cruzi*. *Ann. Trop. Med. Parasitol., 59* :19-26.

BRENER, Z., 1969. The behaviour of slender and stout forms of *Trypanosoma cruzi* in the bloodstream of normal and immune mice. *Ann. Trop. Med. Parasitol., 63* :215-220.

BRENER, Z. & CHIARI, E., 1963. Variações morfológicas observadas em diferentes amostras de *Trypanosoma cruzi*. *Rev. Inst. Med. trop. São Paulo, 5* :220-224.

BRENER, Z. & CHIARI, E., 1965. Aspects of early growth of different *Trypanosoma cruzi* strains in culture medium. *J. Parasitol., 51* :922-926.

BUDZKO, D.B. & KIERSZENBAUM, F., 1974. Isolation of *Trypanosoma cruzi* from blood. *J. Parasitol., 60* :1037-1038.

COUTINHO, S.G.; SOUZA, W.J.S.; CAMILLO-COURA, L.; MARZOCHI, M.C.A. & AMENDOEIRA, M.R.R., 1981. Resultados das reações de imunofluorescência (IgG) para toxoplasmose em 6079 indivíduos, durante os anos de 1971 a 1977. *Rev. Inst. Med. trop. São Paulo, 23* :48-56.

DEANE, M.P., 1979. Significance of Polymorphism in *Trypanosoma cruzi*. Presented in the "Congresso Internacional sobre Doença de Chagas" (pp. A-6, A-7), Rio de Janeiro, RJ, Brazil.

DEANE, M.P. & KLOETZEL, J., 1974. Lack of Protection against *Trypanosoma cruzi* by Multiple Doses of *T. lewisi* Culture Forms. A Discussion on Some Strains of "Lewisi". *Exp. Parasitol., 35* :406-410.

FEDERICI, E.E.; ABELMANN, W.B. & NEVA, F.A., 1964. Chronic and progressive myocarditis and myositis in C3H mice infected with *Trypanosoma cruzi*. *Am. J. Trop. Med. Hyg., 13* :272-280.

GARCIA, E.S.; MACARINI, J.D.; GARCIA, M.L.M. & UBATUBA, F.G., 1975. Alimentação de *Rhodnius prolixus* no Laboratório. *An. Acad. Brasil. Cienc., 47* :537-545.

GOLDBERG, S.S.; PEREIRA, A.S.S.; CHIARI, E.; MARES-GUIA, M. & GAZZINELLI, G., 1976. Comparative kinetics of Argentine and Lysine Transport by Epimastigotes and Trypomastigotes from Two Strains of *Trypanosoma cruzi*. *J. Protozool., 23* :179-186.

GUTTERIDGE, W.E.; COVER, B. & GABORAK, M., 1978. Isolation of blood and intracellular forms of *Trypanosoma cruzi* from rats and other rodents and preliminary studies of their metabolism. *Parasitology, 76* :159-176.

GUTTERIDGE, W.E.; KNOWLER, J. & GOOMBES, J.D., 1969. Growth of *Trypanosoma cruzi* in Human Heart Tissue Cells and Effects of Aminonucleoside of Puromycin. Trypacidin and Aminopterin. *J. Protozool., 16* :521-525.

HOFF, R., 1974. A Method for Counting and Concentrating Living *Trypanosoma cruzi* in Blood Lysed with Ammonium Chloride. *J. Parasitol., 60* :527-528.

HOWELLS, R.E. & CHIARI, C.A., 1975. Observations on two strains of *Trypanosoma cruzi* in laboratory mice. *Ann. Trop. Med. Parasit., 69* :435-448.

JACKSON, P.R., 1975. A New Column Design for the Isolation of Bloodstream Trypanosomes Using DEAE-cellulose. *J. Parasitol., 61* :963-965.

KLOETZEL, J.; CAMARGO, M.E. & GIOVANNINI, V.L., 1975. Antigenic Differences Among Epimastigotes, Amastigotes and Trypomastigotes of *Trypanosoma cruzi*. *J. Protozool., 22* :259-261.

LANHAM, S.M., 1968. Separation of Trypanosomes from the Blood of Infected Rats and Mice by Anion-exchangers. *Nature, 218* :1273-1274.

LANHAM, S.M. & GODFREY, D.G., 1970. Isolation of Salivarian Trypanosomes from Man and Other Mammals using DEAE-cellulose. *Exp. Parasitol., 28* :521-534.

LOURES, M.A.; PIMENTA, P.F.P. & SOUZA, W., 1980. Isolation of Bloodstream Trypomastigotes of *Trypanosoma cruzi* by a Gradient of Metrizamide. *J. Parasitol., 66* :1058-1059.

LUMSDEN, W.H.R.; CUNNINGHAM, M.P.; WEBBER, W.A.F.; VAN HOEVE, K.; KNIGHT, R.H. & SIMMONS, V., 1965. Some Effects of Hydrogen Ion Concentration on Trypanosome Numbers and Infectivity. *Exp. Parasitol., 16* :8-17.

MERCADO, T.I. & KATUSHA, K., 1979. Isolation of *Trypanosoma cruzi* from the blood of infected mice by column chromatography. *Prep. Biochem. 9* :97-106.

MOSER, G.; BROHN, F.H.; DANFORTH, H.D. & NUSSENZWEIG, R.S., 1978. Sporozoites of Rodents and Simian Malaria Purified by Anion Exchangers, Retains their Immunogenicity and Infectivity. *J. Protozool., 25* :119-124.

REED, L.J. & MUENCH, H., 1938. A simple method of estimating fifty per cent endpoints. *Am. J. Hyg., 27* :493-497.

SALGADO, J.A.; GARCEZ, P.N.; OLIVEIRA, C.A. & GALIZZI, J., 1962. Revisão clínica atual do primeiro caso humano da Doença de Chagas. *Rev. Inst. Med. trop. São Paulo., 4* :330-337.

SANDERSON, C.J.; THOMAS, J.A. & TOWMEY, C.E., 1980. The growth of *Trypanosoma cruzi* in human diploid cells for the production of Trypomastigotes. *Parasitology, 80* :153-162.

SCHMATZ, D.M. & MURRAY, P.K., 1981. *Trypanosoma cruzi:* selective isolation of pure trypomastigotes from cultured muscle cells. *J. Parasitol., 67* :517-521.

SILVA, L.H.P., 1959. Observações sobre o ciclo evolutivo do *Trypanosoma cruzi*. *Rev. Inst. Med. trop. São Paulo, 1* :99-118.

SILVA, L.H.P. & NUSSENZWEIG, V., 1953. Sobre uma cepa de *Trypanosoma cruzi* altamente virulenta para o camundongo branco. *Folia Clin. biol. São Paulo, 20* :191-207.

SOBER, H.A. & PETERSON, E.A., 1968. Protein chromatography on ion exchange cellulose. *Fed. Proc., 17* :1116-1126.

SOBER, H.A.; GUTTER, F.J.; WYCKOFF, M.M. & PETERSON, A.E., 1956. Chromatography of Proteins. II – Fractionation of Serum Protein on Anion-exchange Cellulose. *J. Am. Chem. Soc., 78* :756-763.

SOUZA, W.; CHIARI, E.; ROMANHA, A.J. & CHIARI, C.A., 1976. Agglutination by concanavalin A of trypomastigotes and epimastigotes of *Trypanosoma cruzi* separated on DEAE-cellulose column. Presented in the "III Reunião Anual sobre Pesquisa Básica em Doença de Chagas", pp. 12-13, Caxambu, MG, Brazil.

SOUZA, W.; ARGUELLO, C.; MARTINEZ-PALOMO, A.; TRISSL, D.; GONZÁLES-ROBLES, A. & CHIARI, E., 1977. Surface Charge of *Trypanosoma cruzi*. Binding of Cationized Ferritin and Measurement of Cellular Electrophoretic Mobility. *J. Protozool., 24* :411-415.

TAYLOR, A.E.R.; LANHAM, S.M. & WILLIAMS, J.E., 1974. Influence of Methods of Preparation on the Infectivity, Agglutination, Activity, and Ultrastructure of Bloodstream trypanosomes. *Exp. Parasitol., 35* :196-208.

VILLALTA, F.V. & LEON, W., 1979. Effect of Purification by DAEA-cellulose Column on Infectivity of *Trypanosoma cruzi* Blood forms. *J. Parasitol., 65* :188-189.

WILLIAMSON, J. & COVER, B., 1966. Separation of blood-cell-free trypanosomes and malaria parasites on a sucrose gradient. *Trans. R. Soc. Trop. Med. Hyg., 60* :425-427.

YAEGER, R.G., 1955. Studies on the isolation and "in vitro" metabolism of the bloodstream and intracellular stages of *Trypanosoma cruzi*. Dissertation, Tulane University Graduate School, New Orleans.

YAEGER, R.G., 1960. A method of isolating trypanosomes from blood. *J. Parasitol., 46* :288.