

**Zeitschrift:** Acta Tropica  
**Herausgeber:** Schweizerisches Tropeninstitut (Basel)  
**Band:** 43 (1986)  
**Heft:** 4

**Artikel:** A strategy for the prevention of the transmission of Chagas' disease during blood transfusion  
**Autor:** Hammond, D.J. / Croft, S.L. / Hogg, J.  
**DOI:** <https://doi.org/10.5169/seals-313648>

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Department of Biochemical Microbiology, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, UK

## A strategy for the prevention of the transmission of Chagas' disease during blood transfusion

D. J. HAMMOND, S. L. CROFT, J. HOGG, W. E. GUTTERIDGE

### Summary

Our strategy for preventing the transmission of Chagas' disease during blood transfusion is discussed. In addition, the possibility that the Peru, Sonya, Tulahuen and Y strains of *Trypanosoma cruzi* show varying sensitivities to a series of amphiphilic cationic drugs in vitro at 4°C was investigated using a microscope lysis test. All 21 drugs tested at a concentration of  $10^{-3}$  M lysed Sonya bloodstream trypomastigotes, but Peru, Tulahuen and Y strains were affected by 17, 17 and 11 drugs, respectively. All four strains were most sensitive to the acridines; acranil, aminacrine and mepacrine. Although some variation was seen in their responses to certain drugs, no one strain was particularly insensitive to the series as a whole. The effects of gentian violet, maprotiline and mepacrine on the infectivity of Sonya trypomastigotes following incubation at 4°C for 24 h were evaluated. Mepacrine, at a concentration of  $2.5 \times 10^{-4}$  M greatly decreased the viability of trypomastigotes, while  $10^{-3}$  M concentrations of both maprotiline, mepacrine, and gentian violet (at low parasite densities only) apparently abolished all infectivity. Although the compounds we tested did not show a significant improvement over gentian violet, the compound currently used in some blood banks, other existing amphiphilic cationic drugs could be of use in preventing the transmission of Chagas' disease during blood transfusion.

**Key words:** *Trypanosoma cruzi*; blood transfusion; amphiphilic cationic drugs.

### Introduction

Although blood transfusion has considerable value to modern medicine, it has frequent serious complications. About 19% of adverse transfusion reactions

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Correspondence (present address): Dr. David J. Hammond, Bristol Meyers Company, 5, Research Parkway, Wallingford, Connecticut, USA

are due to the transmission of microbial agents present in the blood, e.g. hepatitis, AIDS and malaria (Barbara, 1983). The protozoan *Trypanosoma cruzi* is a particularly grave problem for transfusion in South America (Gutteridge, 1982), where it causes the debilitating, and sometimes fatal Chagas' disease for which there is still no effective chemotherapy (Brener, 1979). Since gentian violet is active at 4°C against blood forms of *T. cruzi* (Nussenzweig et al., 1953) it has been added to sterilise stored blood in some endemic areas and has successfully prevented the transmission of Chagas' disease. However, certain factors, including side effects, make it a far from satisfactory chemoprophylactic agent (Rezende et al., 1965). Thus, there is an urgent need for a replacement (Anonymous, 1980), but drug development for tropical diseases is prohibitively expensive. We report here our strategy for overcoming this problem by identifying existing drugs with appropriate activity which may be useful in preventing the transmission of Chagas' disease by blood transfusion.

First, a rapid screen for assessing drug activity against the infective blood trypomastigote forms of *T. cruzi* was developed (Cover and Gutteridge, 1982). This procedure identified compounds known to be active against trypomastigotes in vitro at 4°C, but was apparently free of false negative or false positive results. This screen was then used to assess over 500 drugs, with a wide range of therapeutic and chemical properties for possible trypanocidal activity. About 70 of these were active at a concentration of 1 mM or less (Hammond et al., 1984). Although these drugs have a wide variety of clinical uses, 64 were chemically

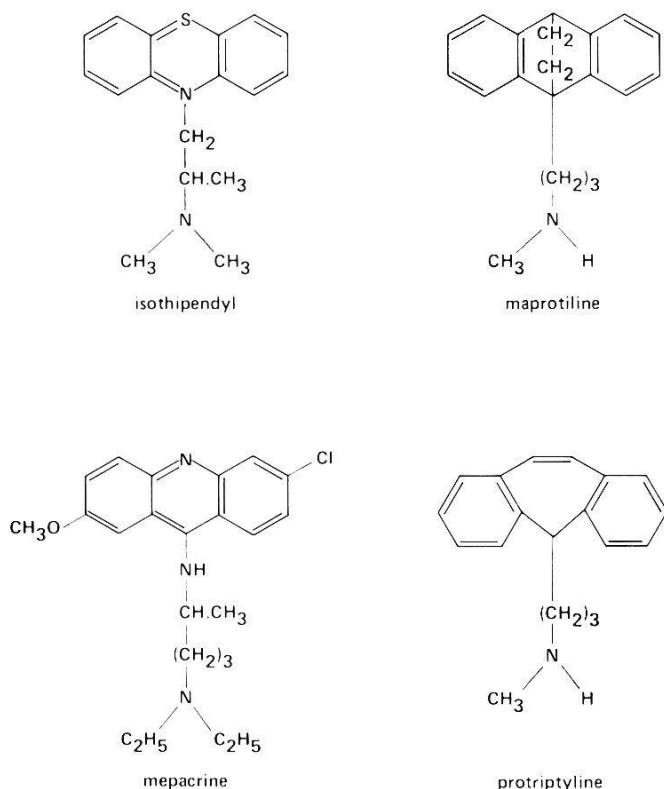


Fig. 1. Structures of some antitrypanocidal "amphiphilic cationic" drugs.

similar "amphiphilic cationic drugs" which were characterised by having hydrophobic ring moiety and an ionizable amine (Fig. 1). The selection of additional drugs was based on these chemical considerations and, at present, about 200 have been found to be active against the Sonya strain of *T. cruzi* (Hammond et al., 1985).

Since different strains of *T. cruzi* show variations in both their biological properties (Bice and Zeledon, 1980; Brener and Chiari, 1963; Watkins 1966), and their responses to drugs (Hauschka, 1947; Haberkorn and Gonnert, 1972; Schlemper, 1975; Cover and Gutteridge, 1981) we selected the 21 drugs found to be most active against the Sonya strain of *T. cruzi* (Hammond et al., 1985) and tested them for activity against the Peru, Tulahuen and Y strains of *T. cruzi*. These results are reported here. Furthermore, because our test system measured lysis, parasites which may be rendered uninfected at amphiphilic cationic drug concentrations below those necessary to cause lysis would not be identified. Consequently, in this paper we report also the effect of 2 amphiphilic cationic drugs, maprotiline and mepacrine, on the infectivity of trypomastigotes.

## Materials and Methods

### *Strains of T. cruzi and their maintenance*

The strains of *T. cruzi* used in this study were the Peru strain (Nussenzweig and Goble, 1963), Sonya strain (Garnham, 1956) and the Y strain (Silva and Nussenzweig, 1953) which were all originally isolated from human infections, while the Tulahuen strain was obtained from *Triatoma infestans* (Jarpa et al., 1950). Peru, Tulahuen and Y were routinely maintained in CDI mice, while Sonya was maintained in either CFLP or BALB/c mice.

### *Amphiphilic cationic drugs*

The following were all gifts: acranil from Bayer U.K. Ltd., aminacrine from Syntex Pharmaceuticals; aminopromazine from Specia; chlorpromazine, mepacrine and thioproperazine from May and Baker Ltd., clomipramine, desipramine and maprotiline from Ciba-Geigy Ltd.; diphenylpyraline from Smith, Kline and French Labs. Ltd.; dothiepin from Boots Co., Ltd.; flupentixol and litracene from Lundbeck and Co. Ltd.; isothipendyl from I.C.I. PLC; methixene and sulforidazine from Sandoz Products Ltd.; nortriptyline from Eli Lilly & Co. Ltd.; perhexiline from Merrel Dow Pharmaceuticals Inc.; prazosin from Pfizer Ltd.; protriptyline from Merck, Sharpe and Dohme Ltd.; and brompheniramine from A. M. Robins Co. Ltd. Gentian violet was obtained from Pharmazeutische Abteilung Berlin.

### *Lysis test*

The trypomastigote lysis test was performed by the method of Cover and Gutteridge (1982): infected mice were exsanguinated using heparin as anticoagulant and the infected blood was diluted 20 fold with new born calf serum (Gibco Europe, Paisley, Scotland) to give a trypomastigote density of  $2 \times 10^6$ /ml. Drugs were dissolved in Krebs' saline (Krebs and Eggleston, 1940), mixed with an equal volume of parasite suspension and loaded into a microslide tube (Camlab Limited, Cambridge, England). After 24 h incubation at 4°C the tests were examined directly under the microscope as described.

### *Infectivity test*

The susceptibility of CFLP mice to infection with *T. cruzi* was ascertained by exsanguinating infected mice 7 days after infection. Heparin was added as anticoagulant to the infected blood which

was mixed with non-infected blood to yield a parasite density of  $2 \times 10^6$ /ml as calculated from a haemocytometer counts. This was then diluted 1:1 (v/v) with Krebs' saline containing 11 mM glucose, and samples were further serially diluted with 50% new born calf serum (Gibco Europe, Paisley, Scotland) in Krebs' saline to give densities of  $10^5$ ,  $10^4$ ,  $10^3$  and  $10^2$  trypomastigotes/ml. These suspensions were incubated at 4°C for 24 h after which time 0.1 ml aliquots were inoculated subcutaneously into freshly weaned male CFLP mice. Tail blood smears were examined daily from 5 to 26 days after inoculation for the presence of trypomastigotes, then once every 3 or 4 days until day 60.

In order to test the effect of drugs on the viability of trypomastigotes, aliquots of the same parasite suspension as described above, initially at a density of  $2 \times 10^6$ /ml parasites/ml, were mixed with an equal volume of Krebs' saline containing drug. These samples were incubated at 4°C for 24 h, and the mice inoculated and examined as previously described. In subsequent experiments, parasites were diluted to  $10^2$  and  $10^1$  organisms/ml of blood before incubation with drug and groups of 10 mice were examined every other day from day 16 to day 45. Those mice which had not shown a positive parasitaemia by then were exsanguinated by cardiac puncture using aseptic techniques and their blood subject to haemoculture. This was carried out in Warren's medium at 26°C, the culture being observed after 3 and 6 weeks.

## Results

### *Effect of drugs on different strains of T. cruzi*

Tables 1–3 show the effects of 21 amphiphilic cationic drugs against the Peru, Sonya, Tulahuen and Y strains of *T. cruzi*. Most drugs gave reproducible activity in duplicate experiments on the same strain; both results are given only for those that did not. Since these drugs were selected for their activity against Sonya strain trypomastigotes, all 21 drugs lysed this strain at a concentration of

Table 1. Effect of acridines on 4 strains of *T. cruzi* trypomastigotes

Drug	Strain of <i>T. cruzi</i>	Final concentration of drug (×M)			
		$1 \times 10^{-3}$	$2.5 \times 10^{-4}$	$6 \times 10^{-5}$	$1.5 \times 10^{-5}$
Acranil	Peru	–	–	+	+++
	Sonya	–	+S	+++(+)	+++
	Tulahuen	–	–	++S(–)	+++
	Y	–	++	+++	+++
Aminacrine	Peru	–	–	–	+++
	Sonya	–	–	++S	+++
	Tulahuen	–	–	+S	+++
	Y	–	+(-)	+++(++ )	+++
Mepacrine	Peru	–	–	+	+++
	Sonya	–	–	+S	+++
	Tulahuen	–	–	+S	+++
	Y	–	++(-)	++	+++

Compounds were tested as described in Materials and Methods. The results are from two experiments expressed as +++ = the same number of trypomastigotes as the control; ++ = less than 20% of the control; + = less than 5% of control; – = no unlysed organisms were seen; S = the trypomastigotes present were severely deformed.

$<10^{-3}$  M, but trypomastigotes of the Peru, Tulahuen and Y strains, were affected by 17, 17 and 11 drugs, respectively, at the same concentration. Many of these drugs showed higher trypanocidal activity than the sample of gentian violet we tested, which is included in Table 2 for comparison.

Table 2. Amphiphilic cationic drugs that have some effect on all 4 strains of *T. cruzi* at a concentration of  $\leq 1$  mM

Drug	Strain of <i>T. cruzi</i>	Final concentration of drug ( $\times M$ )			
		$1 \times 10^{-3}$	$2.5 \times 10^{-4}$	$6 \times 10^{-5}$	$1.5 \times 10^{-5}$
Aminopromazine	Peru	–	+++(-)	+++	+++
	Sonya	–	+++(+)	+++	+++
	Tulahuen	–	+++(+)	+++	+++
	Y	–	+++(+)	+++	+++
Brompheniramine	Peru	+	+++	+++	+++
	Sonya	–	+++	+++	+++
	Tulahuen	–	+++	+++	+++
	Y	+	+++	+++	+++
Desipramine	Peru	–	+++(-)	+++	+++
	Sonya	–	+++	+++	+++
	Tulahuen	++S(-)	+++	+++	+++
	Y	–	+++S	+++	+++
Litracene	Peru	+	+++(+)	+++	+++
	Sonya	–	–	+++	+++
	Tulahuen	–	+++(-)	+++	+++
	Y	–	+++	+++	+++
Maprotiline	Peru	–	–	+++	+++
	Sonya	–	–	+++	+++
	Tulahuen	–	–	+++	+++
	Y	+	+	+++	+++
Nortriptyline	Peru	–	–	+++	+++
	Sonya	–	+++	+++	+++
	Tulahuen	–	+++	+++	+++
	Y	–	+++(+)	+++	+++
Protryptiline	Peru	–	–	+++	+++
	Sonya	–	+++	+++	+++
	Tulahuen	–	–	+++	+++
	Y	–	+++	+++	+++
Gentian violet	Peru	–	++(+++)	+++	+++
	Sonya	–	+++	+++	+++
	Tulahuen	++S(-)	+++(-)	+++	+++
	Y	+++S	+++	+++	+++

Compounds were tested as described in Materials and Methods. The results are from two experiments expressed as +++ = the same number of trypomastigotes as the control; ++ = less than 20% of the control; + = less than 5% of control; – = no unlysed organisms were seen; S = the trypomastigotes present were severely deformed.

Table 3. Amphiphilic cationic drugs that do not effect all 4 strains of *T. cruzi* at a concentration of  $\leq 1$  mM

Drug	Strain of <i>T. cruzi</i>	Final concentration of drug ( $\times M$ )			
		$1 \times 10^{-3}$	$2.5 \times 10^{-4}$	$6 \times 10^{-5}$	$1.5 \times 10^{-5}$
Chlorpromazine	Peru	+++	+++	+++	+++
	Sonya	–	+++	+++	+++
	Tulahuen	+++	+++	+++	+++
	Y	+++	+++	+++	+++
Clomipramine	Peru	+++	+++	+++	+++
	Sonya	–	+++(-)	+++	++
	Tulahuen	+++	+++	+++	+++
	Y	+++	+++	+++	+++
Diphenylpyraline	Peru	+++	+++	+++	+++
	Sonya	+	+++	+++	+++
	Tulahuen	–	+++	+++	+++
	Y	+++	+++	+++	+++
Dothiepin	Peru	–	+++	+++	+++
	Sonya	–	+++(++)	+++	+++
	Tulahuen	+++	+++	+++	+++
	Y	+++	+++	+++	+++
Flupentixol	Peru	+++(++)	+++	+++	+++
	Sonya	–	+++	+++	+++
	Tulahuen	+++	+++	+++	+++
	Y	+++	+++	+++	+++
Isothipendyl	Peru	–	–	+++	+++
	Sonya	–	+++	+++	+++
	Tulahuen	++	+++	+++	+++
	Y	+++	+++	+++	+++
Methixene	Peru	+++(-)	+++	+++	+++
	Sonya	–	+++	+++	+++
	Tulahuen	+++S	+++	+++	+++
	Y	+++S	+++	+++	+++
Perhexiline	Peru	+++(-)	+++(++)	+++	+++
	Sonya	–	+++	+++	+++
	Tulahuen	–	+++(-)	+++	+++
	Y	+++(-)	+++(-)	+++	+++
Prazosin	Peru	+++	+++	+++	+++
	Sonya	–	+++(+S)	+++	+++
	Tulahuen	+++	+++	+++	+++
	Y	+++	+++	+++	+++
Sulforidazine	Peru	+++(-)	+++	+++	+++
	Sonya	–	+++	+++	+++
	Tulahuen	–	+++	+++	+++
	Y	+++(-)	+++	+++	+++
Thiopropazine	Peru	–	+++	+++	+++
	Sonya	–	–	+++(-)	+++
	Tulahuen	++(-)	+++	+++	+++
	Y	+++(-)	+++	+++	+++

See Table 1 for legend



All four strains were most sensitive to the acridines: acranil, aminacrine, and mepacrine (Table 1). Mepacrine caused complete lysis of Peru, Sonya and Tulahuen trypomastigotes at a concentration of  $6 \times 10^{-5}$  M. However, the Y strain appeared to be slightly less sensitive to all 3 acridines.

Seven drugs, excluding acridines, showed some activity against the four strains of *T. cruzi* at a concentration of  $10^{-3}$  M (Table 2): aminopromazine, nortriptyline and protriptyline caused complete lysis of all strains at  $10^{-3}$  M. Brompheniramine, desipramine, litracene and maprotiline failed to clear all trypomastigotes from Peru, Tulahuen or Y strains. Our sample of gentian violet caused total lysis of Peru and Sonya, but not Tulahuen or Y trypomastigotes.

Table 3 shows those compounds at  $10^{-3}$  M which are inactive to one or more of the four strains. Chlorpromazine, clomipramine, flupentixol and prazosin are active against only the Sonya strain at a concentration of  $10^{-3}$  M. Diphenylpyraline, perhexiline and sulforidazine lyse both the Sonya and Tulahuen strains, while dothiepin and isothipendyl lyse only Peru and Sonya trypomastigotes at a concentration of  $10^{-3}$  M. Thioproperazine showed activity against three of the four strains, but the results for methixene and perhexiline were inconclusive.

#### *Effect of drugs on the viability of T. cruzi*

Fig. 2 shows the time required for trypomastigotes to be seen in tail blood of inoculated mice. Examination was delayed until 5 days after injection to ensure

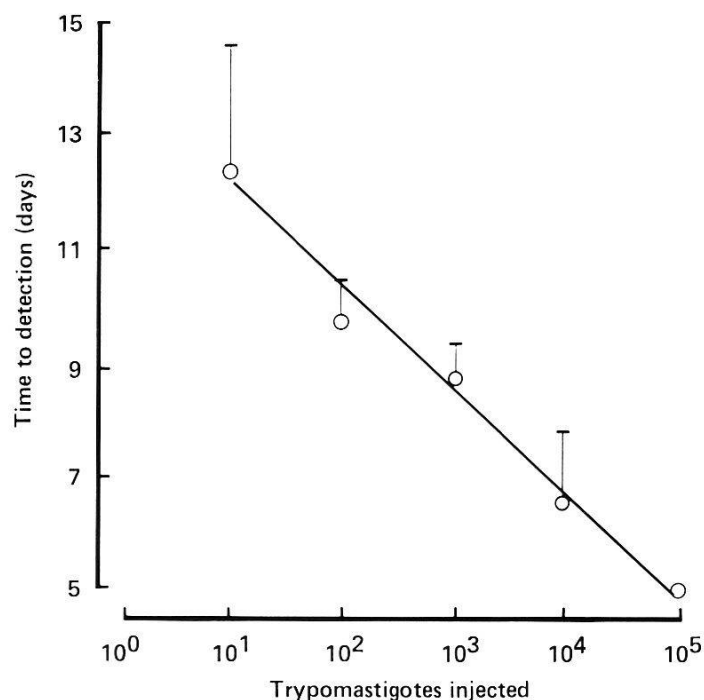


Fig. 2. Time required for Sonya to be seen in the tail blood of CFLP mice following subcutaneous inoculation. The experiment was performed as described in Materials and Methods and the results are given as the mean+standard deviation for the 10 mice inoculated per group. Only 8 of the 9 mice inoculated with 10 parasites gave a detectable parasitaemia.



Table 4. Time required for Sonya trypomastigotes to be seen in the tail blood of CFLP mice following pre-incubation with gentian violet, maprotiline and mepacrine

	Concentration of drug (M)				
	$10^{-3}$	$2.5 \times 10^{-4}$	$6.0 \times 10^{-5}$	$1.4 \times 10^{-5}$	0
Gentian violet . . . . .	$8.8 \pm 0.8$ (5/5)	$6.0 \pm 1.4$ (5/5)	$5.8 \pm 1.3$ (5/5)	$5.0 \pm 0.0$ (5/5)	$5.0 \pm 0.0$ (10/10)
Maprotiline . . . . .	(0/5)	$7.2 \pm 2.5$ (5/5)	$5.6 \pm 0.5$ (5/5)	$5.2 \pm 0.4$ (5/5)	$5.0 \pm 0.0$ (10/10)
Mepacrine . . . . .	(0/5)	$17.2 \pm 2.5$ (5/5)	$7.2 \pm 1.0$ (5/5)	$5.6 \pm 1.3$ (5/5)	$5.0 \pm 0.0$ (10/10)

Trypomastigotes ( $10^6/\text{ml}$ ) were incubated with drugs as described in Materials and Methods. The results are given as the mean  $\pm$  standard deviation of the time lag between inoculation of 0.1 ml of the incubation media and the detection of trypomastigotes in tail blood. The fraction of mice infected is given in brackets.

that only infective trypomastigotes were observed. Injection of 10 parasites caused a detectable parasitaemia in 8 out of 9 mice suggesting that CFLP mice are very susceptible to infection with this strain of *T. cruzi*. The time required for trypomastigotes to be seen in tail blood was directly dependent upon the logarithm of the number of parasites injected.

Trypomastigotes at a concentration of  $10^6/\text{ml}$  were incubated with gentian violet, maprotiline or mepacrine for 24 h at  $4^\circ\text{C}$ , then 0.1 ml samples were injected into CFLP mice. Table 4 shows the effect that these drug exposures had on the development of the subsequent blood parasitaemias. These results showed that pre-incubation with gentian violet at a concentration of  $10^{-3}$  M delayed the appearance of parasites from  $<5.0$  to  $8.8 \pm 0.8$  days suggesting a large reduction of infectivity. Both maprotiline and mepacrine at a concentration of  $10^{-3}$  M appeared to abolish totally the infectivity of the trypomastigotes since no parasites were found in any of the mice inoculated up to 60 days post infection, but concentrations of maprotiline of  $<2.5 \times 10^{-4}$  M did not adversely affect parasite viability. Exposure of trypomastigotes to  $2.5 \times 10^{-4}$  M mepacrine for 24 h at  $4^\circ\text{C}$  in vitro retarded the appearance of blood parasites, when injected into mice, from  $<5.0 \pm 0.0$  to  $17.2 \pm 2.5$  days. This delay is much greater than that observed for mice inoculated with only 10 parasites (Table 4) strongly suggesting that the vast majority of organisms (probably  $>99.99\%$ ) were destroyed by the drug treatment.

Finally, trypomastigotes at a concentration of  $10^2$  and  $10^1$  organism/ml blood were incubated with gentim violet or maprotiline at concentrations of  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  M. After incubation at  $4^\circ\text{C}$  for 25 h, 1 ml samples of the incubation mixture were injected i.p. into CFLP mice. Table 5 shows that gentian violet reduces infectivity to zero at  $10^{-4}$  M, but at  $10^{-3}$  maprotiline was

Table 5. The infectivity of 10 or 100 Sonya trypomastigotes/ml following pre-incubation with maprotiline or gentian violet

	Drug concentration	Number of mice +ve	
		10 parasites/ml	100 parasites/ml
Untreated . . . . .	—	3/10	4/10
Maprotiline . . . . .	10 <sup>-3</sup> M	0/10	0/10
	10 <sup>-4</sup> M	2/10	4/10
	10 <sup>-5</sup> M	2/10	5/10
Gentian violet . . . . .	10 <sup>-3</sup> M	0/10	0/10
	10 <sup>-4</sup> M	0/10	0/10
	10 <sup>-5</sup> M	1/10	5/10

The experiment was performed as described in Materials and Methods, and the results are given as the number of mice showing a positive blood parasitaemia over the total number of mice inoculated.

required to achieve the same result. Those mice, which had not shown a positive parasitaemia by day 45 were exsanguinated and their blood subjected to haemoculture in Warren's medium at 26°C. No new positive mice were detected.

## Discussion

Tables 1–3 show that our amphiphilic cationic drugs lyse all four strains of *T. cruzi* examined. The 21 drugs tested were selected because they lysed Sonya trypomastigotes in vitro within 24 h at 4°C, but Peru, Tulahuen and Y strains were affected by only 17, 17 and 11 drugs, respectively. Some significant variations in the responses of the different strains to individual drugs were found, e.g. trypomastigotes of the Peru strain were lysed by isothipendyl at a concentration of  $2.5 \times 10^{-4}$  M, while those of the Y strain were unaffected by  $10^{-3}$  M isothipendyl. Moreover, at a concentration of  $10^{-3}$  M, our sample of gentian violet, the present compound of choice for preventing the transmission of Chagas' disease by blood transfusion, cleared only trypomastigotes of the Peru and Sonya strains of *T. cruzi*, though different samples of gentian violet may be more active. Overall, there was comparatively little difference in the sensitivities of the four strains to amphiphilic cationic drugs. The acridines, acranil, aminacrine and mepacrine, which proved most effective against Sonya trypomastigotes (Hammond et al., 1985) were similarly effective against the Peru, Tulahuen and Y strains (Table 1).

In agreement with our test for parasite lysis, our infectivity studies showed that no viable parasites were detected after infected blood was incubated with either  $10^{-3}$  M maprotiline or mepacrine, though gentian violet at  $10^{-3}$  M had much less effect upon viability at high parasites densities. The infectivity studies did, however, show that trypomastigotes were less sensitive to these 3 com-

pounds than was observed in our lysis tests. This apparent discrepancy can be explained by the much greater sensitivity of the infectivity studies, where from 10 to 100,000 parasites could be detected, than is feasible using microscopic techniques.

It is not possible to quantify accurately what percentage of parasites survived the 24 h incubation with drug by measuring the time required for trypomastigotes to be seen in the blood of the inoculated hosts. This is primarily because the accumulation of the drug within the survivors may retard their initial development *in vivo*. However, the detection of very low levels of parasite survival, as seen, for example in the initial experiments following incubation with  $2.5 \times 10^4$  M mepacrine, would be of dubious clinical significance since the densities of trypomastigotes present in infected donor blood are very much lower than those used in our tests. Thus, this level of parasite kill would sterilise the blood. Moreover, in both lysis and infectivity tests, we used a drug exposure time of 24 h as a compromise between that necessary to meet transfusion demands and that required for trypanocidal activity. However, there is ample evidence to suggest that longer exposure times would result in even greater trypanocidal activity (Hammond et al., 1985), though this may also possibly alter the relative sensitivities of the different strains to amphiphilic cationic drugs. It is evident, however, that the parasites were not rendered uninfected at drug concentrations lower than that required for lysis.

The final decision to proceed with clinical trials in blood banks depends upon the establishment from existing data that intravenous infusion not only presents no safety hazards but also no adverse pharmacological side effects. Information was obtained by WHO on our behalf from the manufacturers of the 21 short listed compounds about safety and tolerance, plasma levels required for pharmacological activity and side effects of injectable formulations. This information was reviewed in December, 1984 by an expert committee (WHO report TDR/CHA/BS/84.3), and, while it was clear that complete data was not available for most of the compounds, further information was sought on maprotiline in particular. However, it was ruled by WHO in July, 1985 that not only must the drug be safe, but it must also be used at a concentration that, when transfused into the patient, did not provoke the pharmacological response for which it was originally marketed. This ruling eliminated our most promising compound, maprotiline, since it meant we could not exceed  $10^{-5}$  M in the blood bag, and this concentration has no effect on the infectivity of parasites present at low densities (Table 5). Yet, the need for a replacement for gentian violet remains, and although our list of drugs may not contain one suitable for the function envisaged it is expedient to emphasise that therapeutically inactive or rapidly metabolised isomers may have trypanocidal activity without the possible side effects of the pharmacologically active isomer.

Furthermore, the widespread occurrence of chloroquine and multi-drug resistant malaria underlines the problem of the transmission of other parasitic

protozoa, especially malaria, by blood transfusion (currently, chloroquine is given post-transfusion to “cure” possible malarial infections). Significantly, many amphiphilic cationic drugs are also active against other parasitic protozoa including malaria. Consequently, a similar strategy and other drugs may be useful in preventing the transmission by transfusion of other tropical diseases.

## Acknowledgments

We thank Drs. L. Hudson and R. A. Neal for their valuable assistance and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases for financial support.

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