Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 32 (1975)
Heft: 3

Artikel: Differentiation of newly isolated strains of "Trypanosoma (Schizotrypanum) cruzi" by agglutination and precipitation reactions

Autor: Ketteridge, D.

DOI: https://doi.org/10.5169/seals-312086

Department of Medical Protozoology,
London School of Hygiene and Tropical Medicine,
Keppel Street, London WC1E 7HT

Differentiation of Newly Isolated Strains of *Trypanosoma (Schizotrypanum) cruzi* by Agglutination and Precipitation Reactions

D. KETTERIDGE

Abstract

Serological relationships between new isolates of *Trypanosoma cruzi* from a single locality have been examined by direct agglutination and immunodiffusion, and compared with established strains. The strains were divided into three groups according to the absorption properties of their precipitinogens. The three groups were defined by the same characteristics as those in which strains of wide-ranging provenance and long laboratory history have been placed by previous workers. A lack of correspondence between the antigenic constitutions of the groups as described here and as described previously was demonstrated using reference strains. Relatedness values were calculated from agglutination titres, which indicated a complex heterogeneity of antigens, and from which a scheme of relationships was drawn.

Introduction

Trypanosomes assigned by morphological criteria to the species *Trypanosoma (Schizotrypanum) cruzi* CHAGAS 1909 exihibit remarkably little host specificity, occurring in over 75 species of natural host representing 40 genera among 8 orders of mammals. Degrees of infection range from those in which only low-grade transient parasitaemias are observed and where tissue stages are rare or not found, to those with severe parasitaemias and where extensive intracellular involvement and tissue damage develops, as with clinically evident Chagas' disease in man.

Attempts have been made to distinguish between strains using morphological criteria (FLOCH & DE LAJUDIE, 1943), differences in pathogenicity (DEANE et al., 1963; HAUSCHKA et al., 1950), differences in tissue affinity (DIAS, 1934; PIZZI, 1957; ANDRADE et al., 1970), and differences in antigenic constitution (HAUSCHKA et al., 1950; NUSSENZWEIG et al., 1963; NUSSENZWEIG & GOBLE, 1966; GONZALEZ CAPPA & KAGAN, 1969). These studies, together with cross-protection tests (NORMAN & KAGAN, 1960; NUSSENZWEIG et al., 1963; MARR & PIKE, 1967; SEAH & MARSDEN, 1969) have demonstrated the complex antigenic inter-relationships of strains and the spectrum of the variation. The studies of Nussenzweig and his co-workers and of Gonzalez Cappa and Kagan have indicated that the species may be subdivided according to the segregation of group-specific antigens. From a wide range of laboratory-established strains of *T. cruzi* of varied provenance, in reactions with heterologously absorbed antisera, three groups emerged:

1. Mutually absorbable strains comprising Group A.
2. Group B strains whose antisera were completely absorbed by Group A strains, but which gave incomplete absorption of Group A antisera.

3. Group C strains which gave incomplete absorptions of antisera to Groups A and B, and whose antisera were incompletely absorbed by A and B strains.

In the present study, agglutination and immune precipitation methods were used in an analysis of strains newly isolated from the field and maintained by techniques designed to restrict laboratory induced variation to a minimum. Two strains, Tulahuen and Peru, assigned by the previous authors to Group A, were included for reference.

Materials and Methods

The trypanosomes

With the exception of the Tulahuen and Peru strains, all strains have been recently isolated by Drs P. D. Marsden, M. A. Miles, D. M. Minter and E. Minter-Goedbloed from São Felipe, near Salvador, Bahia State, Brazil. Strains were isolated by xenodiagnosis in the vector. Bloodstream forms were raised by subcutaneous inoculation of bug faeces containing metacyclic trypanosomes into juvenile male mice (Theiler's Original, T.O.) which hade received 600 r total body irradiation (^{60}Co gamma source) 24 h previously. Stabilates were prepared from trypanosomes of the first parasitaemia, and of bug faeces containing metacyclic trypanosomes, by standard methods of cryopreservation (LUMSDEN et al., 1973), and banked according to the London University Medical Protozoology (LUMP) code. Stabilates of bloodstream forms were the routine source of the experimental material.

Cultivation of trypanosomes

Trypanosomes of each strain were cultivated at 28 °C on Maekelt's medium (MAEKELT, 1960) modified to contain 4 % v/v defibrinated rabbit blood in the agar phase, and Oxoid brain-heart-infusion with Oxoid liver infusion diluted to 1.4 % and 0.5 % w/v, respectively, with Hanks balanced salt solution pH 7.2, in the overlay. Antibiotic was excluded. The ratio of blood agar to overlay was 4 : 1, final volumes. Cultures were inoculated with bloodstream trypanosomes isolated aseptically from primary parasitaemia in groups of sublethally irradiated T.O. mice infected with stabilate of each strain. Cultures were passaged during exponential growth at 7-day intervals.

The strains

Strain	Origin	Xenodiagnosis	LUMP
J	1968 José, aged 2, acute infection	*Rhodnius prolixus*	709
A	1968 Antonio, aged 5, acute infection	*R. prolixus*	710
E	1971 Esmereldo, aged 8, acute infection, fatal	*R. prolixus*	643
Ph	1972 Phillippa, aged 17, chronic infection	*R. prolixus*	715
N	1972 Noel, aged 5, acute infection	*Triatoma infestans*	718
R	1972 Rita, aged 7, acute infection	*T. infestans*	754

Strain	Origin	Xenodiagnosis	LUMP
7	1965 Acute infection in man; established laboratory strain; 33 passages in mice	(–)	692
G	1971 Guinea pig (SFGP1), domestic	*R. prolixus*	753
Cat A	1972 Cat (FV22), domestic	*R. prolixus*	792
Cat C	1973 Cat (T82), domestic	*R. prolixus*	883
Rat B	1972 *Rattus rattus frugivorus* (WA 102)	*R. prolixus*	787
P	1963 (Peru strain) Acute infection in man (NUSSENZWEIG & GOBLE, 1966); established laboratory strain, passaged 157 times in mice	(–)	152
PB	Peru strain (above) cyclically transmitted through *R. prolixus* into mice	(–)	722
T	1946 (Tulahuen strain) from *Triatoma infestans,* Coquimbo, Chile (JARPA et al., 1950; lyophilized antigen prepared by Dra Stella M. Gonzalez Cappa, School of Medicine, Buenos Aires	(–)	(–)

Preparation of agglutination antigens

Trypanosomes were harvested from the 3rd subculture when they were consistently 98–100 % epimastigote (counts of 1,000). Organisms were washed 5 times in Hanks' solution (Oxoid) pH 7.2 by centrifugation at 1,500 g 15 minutes 4 °C and resuspension. The concentrations of the suspensions were adjusted to contain 1×10^7 trypanosomes/ml. Organisms in these preparations would remain in suspension for 24 h or, if settled, disperse by slight agitation.

Preparation of precipitation antigens

Strains were established in culture as described above in Roux flasks. Organisms were harvested from between 3rd–7th serial subculture. Blood was excluded from the agar phase of the harvest culture. Harvested organisms were washed as above. Washed packed trypanosomes were disrupted by 10 alternations between –79 °C (solid CO_2-methanol) and 25 °C (water bath), freeze-dried and stored at –20 °C. This was crude antigen (CA).

Aqueous extract (AE)

Freeze-dried cells were ground to a final concentration of 100 mg dry wt/ml in an all-glass homogeniser (Griffin) on ice using 4 vols distilled water, to which 1 vol × 5 concentrated isotonic phosphate buffered saline (PBS) (final concentration 0.15 M, pH 7.2) was added as grinding proceeded. The suspensions were centrifuged at 10,000 g for 30 min at 4 °C. The clear supernatants were each adjusted with PBS to a final concentration of 20 mg protein/ml, by the method of LOWRY et al. (1951) and stored at 120 °C.

Triton extract (TE)

Sediments from the above preparation were washed 6 times in PBS by centrifugation at 10,000 g for 30 min at 4 °C and resuspension. The washed sediments were finally resuspended in 1.5 ml 200 mg/ml Triton X-100 (BDH) and held at 4 °C for 4 h and 37 °C for 30 min. Suspensions were centrifuged at 10,000 g lh at 4 °C. The clear supernatants were stored at –20 °C.

Preparation of antisera

a) Agglutination antisera

Inocula were prepared from washed suspensions of bloodstream forms of each strain, isolated during rising parasitaemia. Groups of 10 normal mice for each strain were inoculated subcutaneously with 5×10^4 trypanosomes/mouse, with the exception of the highly virulent Peru strains, for which the inocula contained 50 trypanosomes/mouse. Five mice from each group were bled after two weeks and the remaining 5 were bled after 5 weeks. Sera from each group were pooled, filtered through Millipore membranes of 0.22 μm pore size, inactivated at 56 °C for 30 min and stored at –20 °C.

b) Precipitation antisera

For each strain 2 New Zealand male rabbits (c. 2.0 kg) were used. Antisera were raised against whole crude antigen (CA) ground from disrupted freeze-dried cells in PBS at 20 mg/ml, using the following regime: 10 mg in PBS emulsified in an equal volume of Freund's Incomplete Adjuvant (FIA) (DIFCO) was inoculated intramuscularly at 2 sites in each thigh; 1 week later a further 5 mg in FIA was inoculated in 4 subcutaneous nuchal sites; 7 weeks later 5 mg in PBS only was inoculated intramuscularly into 4 femoral sites. Rabbits were bled after a further 1 and 2 weeks. Sera were stored at –20 °C.

Absorptions

Precipitating antisera were absorbed in all heterologous combinations, at a concentration of 1.0 mg dry weight disrupted cells (CA) to 0.1 ml antiserum. Absorptions were incubated 1 h at 4 °C, then 1 h at 37 °C and overnight at 4 °C. Antisera were separated from the sediments by centrifugation at 5,000 g 15 minutes at 4 °C and stored at –20 °C. Absorbed antisera were tested by immune precipitation and if necessary reabsorbed with a further 0.5 mg CA per 0.1 ml antiserum.

Agglutination titrations

Titrations were performed on microtitre plates (Cooke Engineering Company) with round bottomed wells. 0.05 μl antisera in doubling dilutions were incubated at room temperature with 0.05 μl antigen in all heterologous and homologous combinations. The plates were gently agitated before being read after 30 min, 3 h and 12 h.

Relatedness values were derived from the ratios of combined heterologous and homologous titres of all strains considered in pairs, according to the method of ALLING (1967) as applied by HOLLINGDALE & LEMCKE (1970). The degree of serological relatedness, R, of two strains is expressed as the difference between the

sum of the products of the two heterologous titres and the sum of the products of the two homologous titres. Each titre takes the form c^{by}, where c is the reciprocal of the initial dilution and b is the dilution factor. The y values are calculated for each reaction, and the mean of the replicates is $\bar{y}$, then

$$\log_b R = \bar{y}_{het1} + \bar{y}_{het2} - \bar{y}_{hom1} - \bar{y}_{hom2}$$

$$CI = \pm \hat{o}\, t \text{ at } 0.05 \text{ probability.}$$

Immunodiffusions

Ouchterlony plates were prepared from 1% Ionagar No. 2 (Oxoid) in 0.15 M phosphate buffered saline pH 7.2 containing 0.05% sodium azide. Wells were cut 2.5 mm diameter, 2.0 mm deep, with 6.0 mm spacing. The antigens were tested by precipitation for optimum dilution and the antigen extracts tested in all homologous and heterologous combinations. Plates were examined after 48 h, 72 h and again after rinsing, drying and staining with 0.5% w/v Amido Black 10 B (Gurr Ltd.) in 5% v/v acetic acid containing 5% w/v mercuric chloride.

Results

1. Agglutination test

All strains of *T. cruzi* inoculated into mice to raise antisera for agglutination tests produced infections which ran a similar course. The inocula of the highly virulent Peru strains (strains P and PB) containing 50 organisms/mouse produced a pattern of parasitaemia resembling that produced by inocula of 5×10^4 organisms/mouse for all the remaining strains, which were less virulent. Control mice, in which the course of infection was observed for 75 days, developed parasitaemia which was positive by light microscopy (wet preparations, 100 fields $\times$ 40 objective) in 10 ± 2 days and persisted until 55 ± 5 days. Peak parasitaemia occurred on day 25 ± 4. All control mice survived into the chronic phase.

Antisera taken 2 weeks after infection cross-reacted throughout with all heterologous combinations of antigen. Titres were low but ranged over 2 dilutions only, from 10 to 40; thus although common interrelationships could be established, differences between titres were too narrow to permit statistically significant assessment of degrees of relationship and attempts to do so were equivocal.

Antisera taken 5 weeks after infection also cross-reacted throughout in all heterologous combinations. Titres were consistent in each triplicate reaction and differences between replicates never exceeded 1 dilution. Each antiserum distinguished between heterologous antigens by differential titres (Table 1). In some cases a higher titre was obtained in a heterologous reaction of a particular antiserum than in the homologous reaction (note J, A, Ph and G, antisera). The values $\log_b R$ are

Table 1. Titres of agglutinating antisera in triplicate cross-reactions between ten strains of *Trypanosoma cruzi:* Titres expressed as dilution multiple, where dilution factor = $^1/_2$, and initial dilution of 1/10 = 0

Antiserum	Agglutination antigen									
	E	R	J	N	A	Ph	G	7	P	PB
E	6	4	4	4	6	4	3	4	6	6
	6	4	4	4	6	4	3	4	6	6
	5	3	4	4	5	5	4	4	6	6
R	4	6	4	5	5	5	6	4	3	3
	4	6	4	5	5	5	6	4	3	3
	4	6	4	5	6	5	6	4	4	3
J	6	4	5	4	6	3	4	7	5	5
	6	4	5	4	6	3	4	7	5	5
	6	4	5	4	6	3	4	8	5	5
N	3	5	4	5	5	3	3	3	4	4
	3	5	4	5	5	3	3	3	4	4
	3	5	4	5	5	3	3	4	4	5
A	6	4	4	4	6	4	3	4	7	7
	6	4	4	4	6	4	3	4	7	7
	6	4	4	4	6	4	3	3	7	7
Ph	3	5	5	2	5	4	5	5	2	2
	3	5	5	2	5	4	5	5	2	2
	3	4	5	2	6	3	5	4	3	3
G	6	3	6	3	6	5	5	4	5	5
	6	3	6	3	6	5	5	4	5	5
	6	3	6	3	6	5	5	4	5	5
7	6	3	6	3	6	5	5	6	4	4
	6	3	6	3	6	5	5	6	4	4
	5	2	6	2	6	5	5	6	3	4
P	5	5	6	4	6	4	5	4	7	7
	5	5	6	4	6	4	5	4	7	7
	6	5	6	5	6	5	6	3	8	8
PB	5	5	6	4	6	4	5	4	7	7
	5	5	6	4	6	4	5	4	7	7
	5	5	5	5	7	4	5	4	7	7

Table 2. Relatedness values for ten strains of *Trypanosoma cruzi,* from the ratios of combined heterologous and homologous agglutination titres of all strains considered in pairs (ALLING, 1967; HOLLINGDALE & LEMCKE, 1970). Confidence interval = ± 1.217. Significant values italicised.

	E	R	J	N	A	Ph	G	7	P
R	–4.00								
J	*–0.67*	–3.00							
N	–3.67	*–1.00*	–2.00						
A	*0*	–3.33	*–1.00*	–2.00					
Ph	–2.00	*0*	*–0.67*	–3.67	*–0.33*				
G	*–1.00*	–2.00	*0*	–4.00	–2.00	*+1.33*			
7	*0*	–5.33	+2.33	–5.00	–2.67	*0*	–3.00		
P	–1.67	–5.00	–1.33	–3.67	*–0.33*	–4.00	–3.00	–3.00	
PB	–1.67	–5.00	–2.67	–3.00	*–0.33*	–4.00	–2.00	–5.00	*0*

given in Table 2. Strains wiht $\log_b R$ values within 95% CI of ±1.217 are significantly related. Strains at point 0 are statistically indistinguishable. Figure 1 shows the relatedness values calculated against each strain in turn. A number of anomalies appear in the system: when compared to E, strains A and 7 are indistinguishable from each other, and both are identical to E; but in the reciprocals, E is confirmed in its identity with A, but 7 is statistically dissimilar to A; E is likewise confirmed in its identity with 7. Similarly, although Ph is given as statistically indistinguishable from both 7 and R, R and 7 are themselves shown to be the most disparate strains. Figure 2 depicts the strain relationships which emerge from the above treatment.

2. *Immune precipitation*

Controls. Maximum definition and line number were obtained using AE at 20 mg protein/ml and TE at 10 mg protein/ml in reactions with antisera taken 14 days after the final immunising dose. No precipitation was given by any antiserum against culture medium antigen prepared from 0.1, 1.0 and 10.0 times physiological concentration of overlay. Antisera absorbed with homologous antigen gave no precipitation.

a) Precipitation with aqueous extract (AE)

(i) Before absorption. In direct precipitation with unabsorbed sera, a maximum of 6 arcs were observed in homologous reactions (Table 3). The arcs were designated 1–6 from their proximity to the antigen wells. Where only 5 arcs were discerned in homologous reactions, cross reactivity with heterologous sera revealed correspondance with arcs nos. 1–5. The apparent absence of arc no. 6 from AE of certain strains

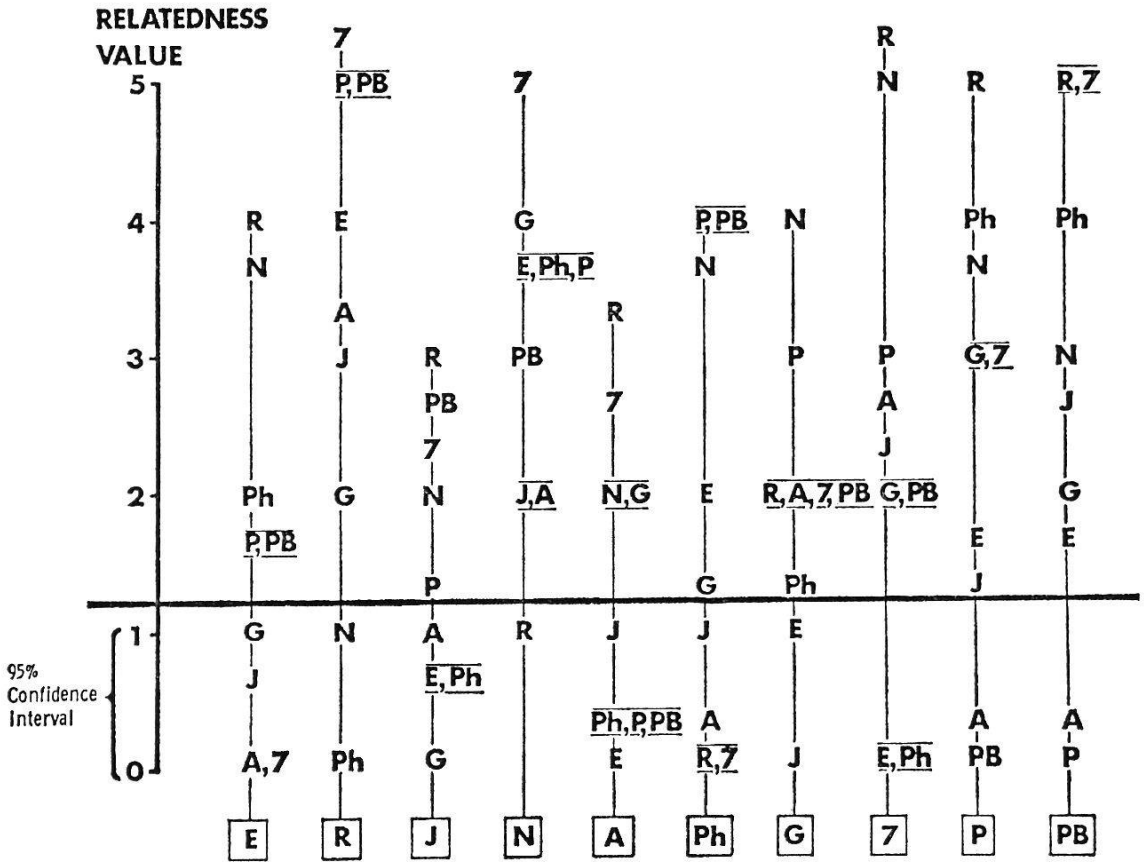


Fig. 1. Strain comparison of *Trypanosoma cruzi* by relatedness values (RV) from agglutination tests, taking each strain in turn as base reference (boxed). Strains with RV = O are statistically indistinguishable from the reference strain. Strains within 95% confidence interval are in significant relationships to the reference strain.

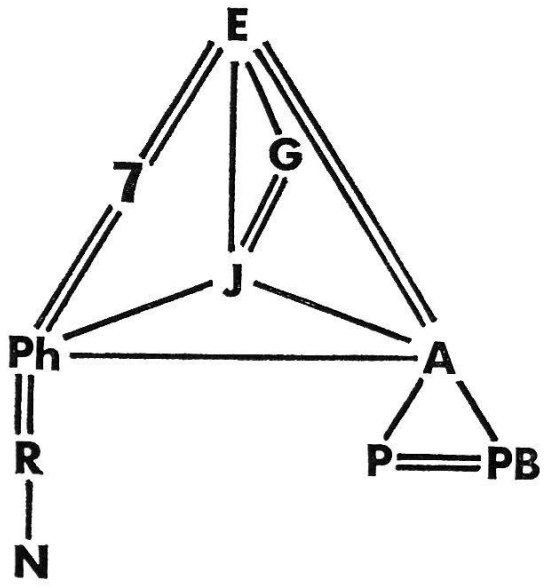


Fig. 2. Scheme of relationship by agglutination of strains of *Trypanosoma cruzi*. Double lines = strains statistically indistinguishable. Single lines = strains significantly related.

Table 3. Arcs of precipitation (1–6) against aqueous extract (AE) of 7 strains of *Trypanosoma cruzi* in homologous reaction, and the cross-reactivity with heterologous antisera

Anti Serum	Arc numbers precipitating with homologous and heterologous AE						
	Ph	E	N	A	7	T	P
Ph	123456	12345–	12345–	–2345–	123456	12–45–	12345–
E	123456	12345–	12345–	–2345–	123456	12–45–	12–45–
N	123456	12345–	123456	123456	123456	123456	12345–
A	123456	12345–	1–3456	123456	123456	123456	12345–
7	123456	12345–	12345–	12–456	123456	1–345–	–2345–
T	–234–6	12–45–	12–45–	12–456	–23456	–23456	12–45–
P	–2345–	12–45–	12–456	12–4—	–23456	1–3456	12345–

(e.g., E and P) appeared to be due to low sensitivity for the precipitinogen using the direct test, since the existence of an antigen implicated in reactions involving arc 6 was invariably demonstrated for all strains by reciprocal absorption (vide infra). Cross reactions occurred throughout in heterologous combinations for at least 3 and at most all factors. Strain homologues were difficult to establish owing to apparent anomalies of specificity: e.g., antisera to strain A and strain E each precipitated 5 antigens from strain E extract; but from A extract, E antiserum precipitated only 4 of the 6 antigens revealed by the homologous A antiserum. Relationships between precipitins were made clearer after absorption with heterologous strains.

(ii) After absorption. The numbers of arcs of precipitation persisting in homologous reactions after the absorption of antisera by heterologous antigen were between 3 and 0 and always involved arcs 1, 2 or 3 (Table 4). Arcs 4, 5 and 6 were removed throughout by all heterologous absorptions.

A first category of strains (7, P, Ph, E, G and Cat C) were mutually absorbable for all precipitations, with these exceptions: (i) absorption of Ph antiserum by P and G antigens did not affect the precipitation of arc no. 2; (ii) absorption of G antiserum with E antigen did not affect the precipitation of arc no. 1. Reciprocal absorption for each of the

Table 4. The effect of heterologous strain absorption on homologous precipitation by gel diffusion of aqueous extracts (AE) of eleven strains of *Trypanosoma cruzi*

Homologous precipitation arc no.		Resultant precipitation after absorption of antisera by homologous or heterologous antigen										
		Ph	E	N	A	Cat 7	Cat A	Rat C	B	G	T	P
Ph	1	–	–	–	–	–	+	–	–	–	–	–
	2	–	–	–	–	–	+	–	+	+	+	+
	3	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–	–
E	1	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–
N	1	+	+	–	–	+	–	–	–	+	+	+
	2	+	+	–	+	+	+	+	–	–	+	+
	3	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–	–
A	1	+	+	–	–	+	+	+	–	+	+	+
	2	–	–	+	–	–	–	–	–	–	+	–
	3	+	+	–	–	+	–	–	–	+	+	+
	4	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–	–
7	1	–	–	–	–	–	–	–	–	–	+	–
	2	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	+	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–	–
Cat A	1	+	+	+	+	+	–	+	+	+	+	+
	2	–	+	+	+	+	–	+	+	+	–	+
	3	–	–	–	–	+	–	–	–	–	+	–
	4	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–	–

Table 4 (continued)

Homologous precipitation arc no.		Resultant precipitation after absorption of antisera by homologous and heterologous antigen										
		Ph	E	N	A	Cat 7	Cat A	Rat C	B	G	T	P
Cat C	1	–	–	–	–	–	–	–	–	–	+	–
	2	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–	–
Rat B	1	+	+	–	–	+	–	+	–	+	+	+
	2	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–
G	1	–	+	–	–	–	+	–	–	–	+	–
	2	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–
T	1	–	–	+	–	–	+	+	+	+	–	+
	2	+	+	+	+	+	+	+	+	+	–	+
	3	+	+	+	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–	–
P	1	–	–	–	–	–	+	–	–	–	+	–
	2	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–

above were complete. The placement of such exceptions were determined by overall comparisons, and in relation to the following two groups.

A second category of strains (N, A and Rat B) removed all precipitating activity from antisera to the first category of strains (7, P, Ph, E, G and Cat C). Reciprocally, precipitating activity remained in the

antisera to N, A and Rat B strains for both or either arcs 1 and 2 after absorption with antigens from the first category of strains.

A third category was represented by strains T and Cat A. Antisera to these strains were incompletely absorbed by the antigens of all other strains tested, and precipitating activity remained to one, two or three antigens (of arcs 1, 2 and 3) according to the heterologous antigen used in absorption. Conversely, antigens of strains T and Cat A absorbed incompletely the antisera raised against all other strains, where precipitating activity remained for variously arcs 1 and/or 2 and/or 3. Exceptions were: (i) E antiserum from which T antigen removed all precipitating activity; (ii) E, Cat C and Rat B antisera from which Cat A antigen removed all precipitating activity. The reciprocals of these exceptions conformed to the properties of the group. In addition, Cat A and T strains differed mutually by the specificity of two precipitations.

b) Precipitation with Triton extracts (TE)

Before absorption. TE of each strain was tested against all heterologous antisera and compared with the homologous reaction. One major arc of precipitation developed which was common to all strains, and cross-reactions of identity were produced throughout each series of heterologous reactions. TE and AE of each strain were compared in the homologous reaction, and the major component precipitating from TE in each strain demonstrated serological identity with arc no. 4 precipitating from AE. Where subsidiary reactions could be discerned in TE preparations, these gave reactions of identity with arcs 5 and/or 6 precipitating with AE.

After absorption. Precipitating activity for TE components was removed from all antisera by heterologous absorptions, confirming that the antigenic identity of TE components was shared by all strains tested.

Discussion

1. Agglutination

The "complete antigenic cross-relationship with some quantitative differences in reactivity" obtained by HAUSCHKA et al. (1950) was also demonstrated for the present series of strains of *T. cruzi*. Hauschka saw the cross-reactivity as encouraging for purposes of serodiagnosis, and a good diagnostic agglutinating antigen has since been developed (VATTUONE & YANOVSKY 1971). The quantitative differences in serological titre have in the present study been used to calculate relatedness values. By this treatment, a closely inter-related complex of 6 strains (E, 7, A, Ph, G and J) was distinguished from the co-endemic R and N strains, by their relationship with Ph alone. All the Sao Felipe strains

were similarly distinguished from the extraneous Peru strains, by their relationship through A alone (Figure 2).

The heterogeneity of agglutinogens was shown by:

1. The occurrence of higher titres for the heterologous than the homologous reaction in some cases, suggesting differences in the antigenicity between different agglutinogens. Antigenic competition could permit higher titres of specific antibody against a highly antigenic determinant present in small proportions than against a determinant present in greater relative proportion but of less antigenic capacity. This antiserum would conceivably give a higher titre with an heterologous strain, should that strain carry a greater bruden than the homologous strain of the identical determinant of high antigenic capacity.

2. The anomalous inequivalents given by the statistical analysis, e.g. $E = 7$, $E = A$, but $7 \neq A$, could arise if the titres obtained represent the net result of a large number of different agglutinin-agglutinogen interactions, in which case E may equal 7 for, say, 50% of the determinants, and E may equal A for the remaining 50% determinants, without any necessary equivalence between A and 7.

3. Cross-reactivity with differential titres, indicating that trypanosomes of differing strains carry different burdens of common antigenic determinants.

Alternatively, differential titres may be the result not of the net difference between the activities of strain-specific and common antigens, but of a hierarchy of specificity involving differential avidity of antibody directed against a common antigen present in possibly modulated form according to the strain.

2. *Precipitation*

Of the 6 major precipitation arcs normally detectable by immunodiffusion using AE, three (nos. 4, 5 and 6) represented antigens which were common to all strains, and three (nos. 1, 2 and 3) represented antigens whose specificity varied from strain to strain. Of the common antigens, one (of arc no. 4) was the major constituent in the Triton extracts. Arcs nos. 5 and 6 were occasionally detectable with these Triton extracts. These simple extractions indicate that the common precipitinogens originated in membrane-bound, or particulate residues, although they may either leach into the aqueous extract, or share common determinants with water soluble components. It cannot yet be said whether these antigens are species-specific. Intraspecific antigens have already been described for trypanosomes (GRAY 1961), as well as intrageneric antigens (BARBOSA et al. 1973) for trypanosomatids.

The relationship between the three categories of strains in this study

is characterized by the same principles of precipitin absorption as the classification used by NUSSENZWEIG et al. (1963), NUSSENZWEIG and GOBLE (1966) and GONZALEZ CAPPA and KAGAN (1969), as defined in the Introduction.

These groups are, in this study:

Group A: Noel, Antonio, Rat B.

Group B: Esmereldo, Phillippa, Cat C, SFGPl, 7 and Peru.

Group C: Cat A and Tulahuen.

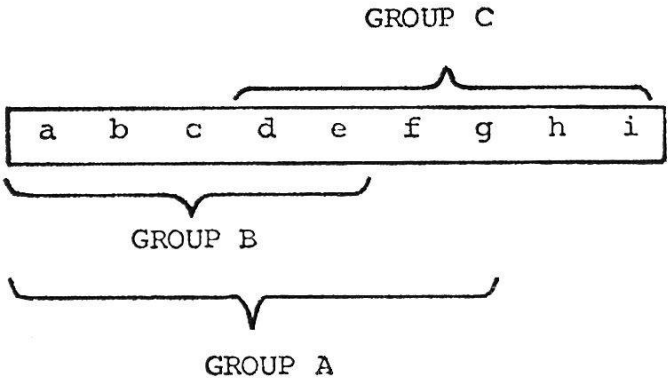
However, Nussenzweig's group have previously assigned Tulahuen and Peru strains to Group A. Both Tulahuen and Peru strains have a long history of laboratory passage. It is possible that antigenic properties become modified when strains are maintained in this way; otherwise it would appear that this classification does not reflect natural evolutive groupings, but is relative to the range of the antigen pool from which the combination of strains studied are constituted. The strain composition of the groups will therefore shift according to the normal distribution of segregated antigens and according to the particular combination of strains tested.

Strain specific antigens were evident by mutually exclusive absorptions. If these antigenic differences were strictly determinant-specific, group differences would be in the nature of Model 1 (figure 3). Since by absorption, all B is contained in A, but not all A in B, homologous immune precipitations in Group A would be expected to produce more arcs of precipitation than those of Group B. Since this was not the case, a hierarchy of specificity must exist, in which antibodies to Group A precipitinogens have a greater avidity for Group B precipitinogens than Group B antibodies have for Group A precipitinogens. This is represented in Model II (figure 3).

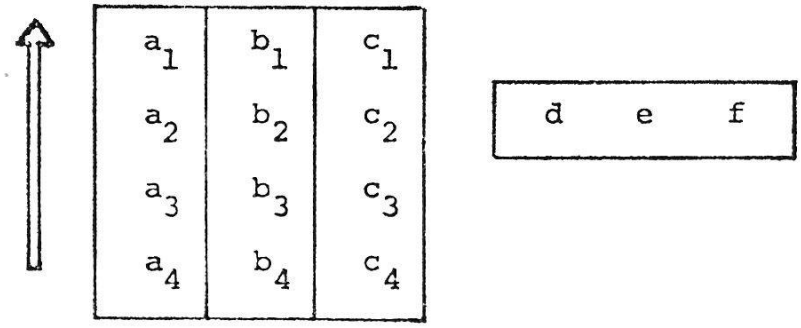
Differences between agglutination and precipitation relationships

The agglutination test described close relationship for some strains, e.g. E and A, which were separated by absorption characteristics of precipitation. Conversely strains not related by the agglutination test have been assigned to the same group of immune precipitation, e.g. E and P.

The agglutination test was believed to have some advantage since it separated from the rest the Peru strains whose history was greatly dissimilar. The agglutination test measured degrees of relatedness according to the net result of possibly a number of interactions; in contrast, immune precipitation divided strains by the presence or absence of unit characters. GONZALEZ CAPPA and KAGAN (1973] found that not all column chromatographic fractions of an aqueous extract of Tulahuen



Model 1



Model 2. Hierarchy of specificity

Fig. 3. Possible models in which antigenic constitution of strains differs. Model 1. Spectrum of antigenic possibilities within the species (a–i), and their mode of segregation. Model 2. Hierarchical cross-reactivity of antigenic modulations (a_1–a_4 etc.), and common invariable antigens (d–f).

strain which contained precipitinogens contained agglutinogens. NUSSENZWEIG et al. (1963) found that while whole trypanosomes absorbed all precipitating activity from specific antisera, agglutinating activity persisted in the antiserum after absorption by aqueous extracts of trypanosomes. Thus in addition to the group-specific agglutinogens described by Nussenzweig, the number of antigens detectable by agglutination exceed the number of antigens detectable by immune precipitation.

If the nature and identity of the groupings shifts relative to the antigenic constituents of the strains tested, the possibility of finding antigenic markers to identify strains of clinical significance becomes more elusive.

Acknowledgement

This work was supported by a grant from the Wellcome Trust. I am indebted to Dra Stella Gonzalez Cappa and Professor W. H. R. Lumsden for their interest and helpful discussion, to Mr. G. Newman for technical assistance, and to all my Department colleagues for their co-operation.

References

ALLING, D. W. (1967). Tests of relatedness. – Biometrika *54,* 459–469.

ANDRADE, S. G., CARVALHO, M. L. & FIGUERIA, R. M. (1970). Caracterizacão morfo-biológica e histopatológica de differentes cepas do *Trypanosoma cruzi.* – Gaz. med. Bahia, *70,* 32–42.

BARBOSA, W., PINHEIRO, Z. B. & DE OLIVEIRA, R. LOPES. (1973). Eletroimunodifusão no diagnóstico do calazar com os antigenos de *Leishmania donovani, Leishmania brasiliensis* e «*Leptomonas pessoai*». – Rev. Pat. trop. *2,* 377–386.

CHAGAS, C. (1909). Neue Trypanosomen. Vorläufige Mitteilung. – Arch. Schiffs- u. Tropenhyg. *13,* 120–122.

DEANE, M. P., BRITO, T. & DEANE, L. M. (1963). Pathogenicity to mice of some strains of *Trypanosoma cruzi* isolated from wild animals of Brazil. – Rev. Inst. Med. trop. São Paulo, *5,* 225–236.

DIAS, E. (1934). Estudos sobre o *Schizotrypanum cruzi.* – Mem. Inst. Oswaldo Cruz, *28,* 1–110.

FLOCH, H. & LAJUDIE, P. DE (1943). Schizotrypanosomiase humaine et Schizotrypanosomes. – Publ. Inst. Pasteur Guyane, No. 67.

GONZALEZ CAPPA, S. M. & KAGAN, I. G. (1969). Agar gel and immunoelectrophoretic analysis of several strains of *Trypanosoma cruzi.* – Exp. Parasit. *25,* 50–57.

GONZALEZ CAPPA, S. M. & KAGAN, I. G. (1973). Antigenicity of fractions of a somatic antigen of *Trypanosoma cruzi.* – J. Parasit. *59,* 1080–1084.

GRAY, A. R. (1961). Soluble antigens of *Trypanosoma vivax* and of other trypanosomes. – Immunology, *4,* 253–261.

HAUSCHKA, T. S., GOODWIN, M. B., PALMQUIST, J. & BROWN, E. (1950). Immunological relationships between seven strains of *Trypanosoma cruzi* and its application in the diagnosis of Chagas' disease. – Amer. J. trop. Med. *30,* 1–16.

HOLLINGDALE, M. R. & LEMCKE, R. M. (1970). Antigenic differences within the species *Mycoplasma hominis.* – J. Hyg. (Cambridge), *68,* 469–477.

JARPA, A., CHRISTEN, R., AGOSIN, M., PIZZI, T. & CORTES, G. (1950). Ensayos de quimioterapia en la enfermedad de Chagas experimental. IV. Compuesto de quinina – pentaquinina, cloroquina y quinina. – Bol. Inform. parasit. Chilenas, *5,* 19–21.

LOWRY, O. H., ROSEBURGH, N. J. FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin-phenol reagent. – J. biol. Chem. *193,* 265–275.

LUMSDEN, W. H. R., HERBERT, W. J. & MCNEILLAGE, G. J. C. (1973). Techniques with Trypanosomes. Edinburgh: Churchill Livingstone.

MAEKELT, G. A. (1960). Die Komplementbindungsreaktion der Chagaskrankheit. – Z. Tropenmed. Parasit. *11,* 155–166.

MARR, J. S. & PIKE, E. H. (1967). The protection of mice by "Corpus Christi" strain of *Trypanosome cruzi* when challenged with "Brazil" strain. – J. Parasit. *53,* 657–659.

NORMAN, L. & KAGAN, I. G. (1960). Immunologic studies on *Trypanosoma cruzi.* II. Acquired immunity in mice infected with avirulent American strains of *T. cruzi.* – J. infect. Dis. *107,* 168–174.

NUSSENZWEIG, V., DEANE, L. M. & KLOETZEL, J. (1963). Differences in antigenic constitution of strains of *Trypanosoma cruzi.* – Exp. Parasit. *14,* 221–232.

NUSSENZWEIG, V., KLOETZEL, J. & DEANE, L. M. (1963). Acquired immunity in mice infected with strains of immunological types A and B of *Trypanosoma cruzi.* – Exp. Parasit. *14,* 233–239.

NUSSENZWEIG, V. & GOBLE, F. C. (1966). Further studies on the antigenic constitution of strains of *Trypanosoma (Schizotrypanum) cruzi.* – Exp. Parasit. *18,* 224–230.

PIZZI, T. (1957). Immunologia de la enfermedad de Chagas. – Santiago.
SEAH, S. & MARSDEN, P. D. (1969). The protection of mice against a virulent strain of *Trypanosoma cruzi* by previous inoculation with an avirulent strain. – Ann. trop. Med. Parasit. *63,* 211–214.
VATTUONE, N. H. & YANOVSKY, J. F. (1971). *Trypanosoma cruzi:* Agglutination activity of enzyme treated epimastigotes. – Exp. Parasit. *30,* 349–355.

Zusammenfassung

Die serologische Verwandtschaft neuisolierter *Trypanosoma cruzi*-Stämme aus São Felipe bei Salvador (Brasilien) ist mit der direkten Agglutination und der Immundiffusion bestimmt und mit etablierten Stämmen verglichen worden. Aufgrund der Absorptionseigenschaften ihrer Präzipitine sind drei Gruppen gebildet worden. Diese Gruppen sind nach den Kriterien, wie sie in früheren Arbeiten mit Stämmen verschiedener geographischer Herkunft beschrieben worden waren, gebildet worden. Wie Referenzstämme gezeigt haben, korrelieren aber die antigenen Zusammensetzungen der Gruppen der neuisolierten Stämme nicht mit den beschriebenen. Aufgrund der Agglutinationstiter, welche auf eine sehr komplexe Heterogenität der Antigene schliessen liessen, wurde die Antigenverwandtschaft berechnet und schematisch dargestellt.

Résumé

Les relations sérologiques existant entre différentes souches de *Trypanosoma cruzi* provenant d'une même localité (Brésil) ont été étudiées par agglutination directe et immunodiffusion, puis comparées à des souches connues. Ces souches ont été divisées en 3 groupes, en fonction des propriétés d'absorption de leurs précipitines. Les 3 groupes ont été définés par les mêmes caractéristiques que celles retenues par d'autres chercheurs pour des souches d'origine très différente et entretenues depuis longtemps au laboratoire. Des différences de constitution antigénique entre les groupes ont été demontrées en utilisant des souches de référence, comme cela avait été décrit précédemment. Sur la base des titres d'agglutination, qui montre une grande hétérogénéité des antigènes, un tableau des relations antigéniques a été établi.