

BIOCHEMICAL CHARACTERIZATION OF *TRYPANOSOMA* SPP. BY ISOZYME ELECTROPHORESIS

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Abstract. Isozyme patterns of 13 enzymes were compared for cultures of *Trypanosoma avium*, *T. vespertilionis*, *T. cruzi* and *T. rangeli*. The isozyme separation was made by cellulose acetate electrophoresis. Each of the species had distinctly migrating isozyme bands for glutamate oxaloacetate transaminase (GOT), isocitrate dehydrogenase (ICD), malic enzyme (ME), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), and malic dehydrogenase (MDH). For other enzymes, two or more species had identically migrating bands. In addition to these interspecific species differences, variability was observed among the strains of *T. cruzi* and *T. rangeli*. Among the *T. cruzi* strains, there were two different isozyme (possibly allozyme) types of the enzymes alanine aminotransferase (ALAT), fructokinase (FK), glucose-6-phosphate dehydrogenase (G6PDH), GOT, MDH and three types of ME. In the *T. rangeli* isolates two isozyme types for the enzymes ALAT, FK, G6PDH, GOT, ICD, and LDH, were observed. Among the eight strains of *T. cruzi* studied there were six isozyme types, and among the seven *T. rangeli* isolates there were four isozyme types. There was an indication that isozyme types were associated with geographical distribution.

In the past few years there have been increasing numbers of studies on isozyme electrophoresis of protozoan parasites.¹ Miles et al.² studied six enzymes from *Trypanosoma cruzi* stocks isolated primarily from a rural area of endemic Chagas' disease in São Felipe, Bahia, Brazil and reported two distinct combinations of isozyme patterns, one associated with domestic stocks and a second pattern associated with sylvatic stocks. In a second paper by Miles et al.³ the authors described a third type of *T. cruzi* with an isozyme pattern distinct from the other two patterns reported in the earlier paper. Baker et al.⁴ obtained data from buoyant density tests and electrophoretic patterns of six enzymes on certain species of *Trypanosoma* isolated from bats in Europe and Latin America. Using these data the authors were able to separate the species into three distinct groups. In these studies the authors report both intra- and inter-specific biochemical differences among trypanosome isolates. Certain World Health Organization (WHO) publications (TDK/WP/76.14 and 76.5) emphasize the necessity for developing simple diagnostic tests which are suitable for use under

field conditions and which will strengthen the biomedical capabilities in developing countries. Miles et al.² emphasize the potential of enzyme electrophoresis as a technique for the intrinsic identification of trypanosome isolates.

In the studies cited above gel electrophoresis was used for enzyme separation. Cellulose acetate electrophoresis requires no special training, can be run under field conditions, original zymograms can be sent through the mail,⁵ and a system using cellulose acetate has already been adapted to *Leishmania*.⁶ Therefore, an isozyme study was undertaken on trypanosomes by the cellulose acetate electrophoresis procedure.

MATERIALS AND METHODS

Trypanosome strains

Seven strains of *T. cruzi*, seven strains of *T. rangeli*, one strain of *T. avium* and one strain of *T. vespertilionis* were isolated at Gorgas Memorial Laboratory (GML), Panama, Republic of Panama. One strain of *T. cruzi* was originally obtained from Brazil. The source of each isolate is listed in Table 1. The strain of *cruzi*-like parasites from a Panamanian bat (*Artibeus lituratus*) was identified as *T. vespertilionis* because of its lack of infectivity to laboratory mice and poor development in *Rhodnius prolixus*. However, following recent findings of Baker et al.,⁴ the affinities

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of these parasites to *T. cruzi marinkellei* should be defined.

Cultivation

All strains were grown on modified Senekjic's diphasic blood medium at 25–27°C. Cultures were transferred every 15–30 days. Flagellates from 10- to 12-day-old subcultures were used to inoculate three stock tubes of Senekjic's medium with an overlay of saline (0.85% NaCl). Parasites were harvested on the 8th day following inoculation.

Preparation of extracts for electrophoresis

Parasites harvested from stock cultures of each strain were agitated and filtered through gauze into 35 ml centrifuge tubes. These were centrifuged for 15 min at 1,000 × *g*. The supernatant was decanted and the parasites were resuspended in saline. After three washings the pellet of epimastigotes was suspended in two parts of membrane buffer (14 ml distilled water: 1 ml 0.1 M Tris/Maleic Acid/EDTA/MgCl₂, pH adjusted to 7.4 with 40% NaOH) and quick-frozen in Dry Ice and alcohol. Disruption of the parasites was accomplished through freeze-thawing procedures, repeated three times, and confirmed through microscopy. The suspension was then quick-frozen and stored in a Revco freezer at -70°C until used for electrophoresis. Aliquots of the samples were taken from this suspension and were applied to the cellulose acetate membranes as described below.

Enzyme activity

The enzymes studied are listed in Table 2. The ALAT and MDH electrophoresis procedures were similar to those reported by Miles et al.,² Kilgour and Godfrey,⁷ Kilgour et al.,^{8,9} Godfrey and Kilgour,¹⁰ and Al-Taqi and Evans.¹¹ Isozymes of these enzymes were observed under ultraviolet light. Many of the other enzyme procedures were similar to those described by Shaw and Prasad¹² and Ayala.¹³ The PGM cellulose acetate procedure was described by Kreutzer et al.⁵ The electrophoresis procedures for the enzymes described by these authors were modified for trypanosomes and cellulose acetate electrophoresis.

Control membranes were run for each enzyme system. The membranes were run under identical

TABLE 2
Enzymes of Trypanosoma spp.

Enzyme	Enzyme Commission number 16	Enzyme abbreviation
Acid phosphatase	3.1.3.2	ACP
Alanine amino-transferase	2.6.1.2	ALAT
Fructokinase	2.7.1.11	FK
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PDH
Glutamate oxaloacetate transaminase	2.6.1.1	GOI
Hexokinase	2.7.1.1	HK
Isocitric dehydrogenase	1.1.1.42	ICD
Lactic dehydrogenase	1.1.1.27	LDH
Malic enzyme	1.1.1.40	ME
6-phosphogluconic dehydrogenase	1.1.1.44	6PGDH
Phosphoglucoisomerase	5.3.1.9	PGI
Malic dehydrogenase	1.1.1.37	MDH
Phosphoglucomutase	2.7.5.1	PGM

conditions as the experimental ones, but the specific enzyme substrate was omitted from the developing components. Bands were not observed on the control membranes.

Electrophoresis

An aliquot of 5 µl was taken from the specimen sample with a microdispenser and transferred to a well of a Zip Zone Well Plate (all electrophoresis equipment used in this study was manufactured by Helena Laboratories, Beaumont, Texas). Eight samples were run on each cellulose acetate membrane; however, under certain conditions more than one enzyme can be assayed on a single plate. Presoaked (20 min) Titan III cellulose acetate membranes were removed from the membrane buffer, blotted, and aligned on the Super Z Aligning Base. About 0.5 µl of the aliquot was transferred from the well plate to the membrane with a Super Z Applicator. Most of the enzymes in this study required a triple application of sample. Additional applications were made to other membranes from the original aliquot, and three or four enzyme systems were studied from one 5 µl aliquot. Two assays of all 13 enzymes were made from each trypanosome isolate. The membranes were placed in the Zip Zone chamber. Up to three membranes (or 24 samples) can be run in a single chamber. To prepare the cell, 100 ml of cell buffer (Table 3) were added to both buffer wells, and filter paper wicks were placed over the cell/mem-

TABLE 3
*Conditions for electrophoresis and components for developing zymograms**

Enzyme	Cell buffer	Voltage	Run time (minutes)	Reaction buffer	Developing components†
ACP‡	1	160	10	A	75 mg Naphthol as phosphoric acid; 50 mg Fast Garnet GBC
ALAT‡	2	150	15	B	30 mg α -Ketoglutaric acid (Na salt); 50 mg L-alanine; 30 units Lactic dehydrogenase (beef heart in phosphate buffer pH 7.5); 15 mg β -Nicotinamide adenine dinucleotide, reduced form (β -NADH) (Na_2 salt)
FK	2	150	15	C	100 mg β -D-(-)-Fructose; 15 mg MTT Tetrazolium (MTT); 15 mg β -Nicotinamide adenine Dinucleotide Phosphate (β -TPN) (Na salt); 10 mg Phenazine methosulphate (PMS); 40 mg Adenosine Triphosphate (ATP) (Na_2 , Salt from equine muscle); 20 mg MgCl_2 , 50 units Phosphoglucose Isomerase
G6PDH‡	2	170	15	C	75 mg D-Glucose-6-phosphate; 15 mg MTT; 15 mg β -TPN (Na salt); 10 mg PMS
GOT‡	3	180	15	C	100 mg L-Aspartic acid; 75 mg α -Ketoglutaric acid (readjust pH to 8.0). Then add 10 mg Pyridoxal-5-phosphate; 75 mg Fast blue BB
HK	2	150	15	C	50 mg α -D-(+)-Glucose; 20 units Glucose-6-phosphate dehydrogenase (Bakers yeast); 20 mg MgCl_2 ; 15 mg MTT; 15 mg β -TPN (Na salt); 10 mg PMS; 40 mg ATP (Na salt)
ICD	2	150	15	E	50 mg DL-Isocitric acid (Na_3 salt); 15 mg β -TPN (Na salt); 15 mg MTT; 10 mg PMS; 50 mg MgCl_2 ; 100 mg MnCl_2
LDH	2	160	15	F	2.5 ml DL-Lactic acid (Na salt); 15 mg β -DPN; 15 mg MTT; 10 mg PMS
MDH	4	200	15	B	15 mg Oxaloacetic acid; 15 mg β -NADH (Na_2 salt)
ME	3	180	15	E	270 mg DL-Malic acid (readjust pH to 7.5). Then add 15 mg MTT; 15 mg β -TPN (Na salt); 10 mg PMS; 25 mg MnCl_2
6PGDH	2	180	15	C	15 mg 6-Phosphogluconic acid (Na_3 salt); 120 mg MgCl_2 ; 15 mg MTT; 15 mg β -TPN (Na salt); 10 mg PMS; 60 mg EDTA (Na_2 salt)
PGI	3	180	15	C	20 mg Fructose-6-phosphate; 120 mg MgCl_2 ; 15 mg MTT; 15 mg β -TPN (Na salt); 10 mg PMS; 25 units Glucose-6-phosphate dehydrogenase (Bakers yeast); 60 mg EDTA (Na_2 salt)
PGM	3	200	10	C	200 mg α -D-Glucose-1-phosphate; 120 mg MgCl_2 ; 15 mg MTT; 15 mg β -TPN (Na salt); 10 mg PMS; 25 units Glucose-6-phosphate dehydrogenase (Bakers yeast); 60 mg EDTA (Na_2 salt); 1.2 mg α -D-glucose 1,6 diphosphate

* Cell buffers:

- 0.15 M Citric acid (Na_3) 0.24 M NaH_2PO_4 ; adjust to pH 6.1 with 40% NaOH.
- Add 0.2 M NaH_2PO_4 to 0.2 M NaHPO_4 to reach pH 7.0.
- 0.1 M Tris; 0.1 M Maleic acid; 0.1 M EDTA (Na_2); 0.1 M MgCl_2 ; adjust to pH 7.4 with 40% NaOH.
- 0.25 M Tris; 0.09 M Citric acid monohydrate; adjust to pH 7.0 with Tris.

Membrane buffers:

- ALAT, MDH—one ml (cell) buffer and nine ml distilled water.
 All other enzymes—one ml cell buffer and fourteen ml distilled water.

Reaction buffers:

- 1 part 0.1 M Citric acid (anhydrous) and 4 parts 0.1 M Sodium Citrate
- 0.025 M NaH_2PO_4 /0.08 M Na_2HPO_4
- 0.06 M Tris; adjust to pH 8.0 with 50% HCl.
- 0.1 M Tris; adjust to pH 8.0 with 50% HCl.
- 0.06 M Tris; adjust to pH 7.5 with 50% HCl.

† To make 60 ml of stain. All chemicals Sigma.

‡ These systems require cooling during electrophoresis.

brane contact areas. The Titan Power Supply was set for the appropriate voltage and time, and if cooling was necessary during electrophoresis (most enzyme systems do not require cooling), ice cubes were placed in the center wells. After electrophoresis the membranes were removed from the cell, trimmed with a scissors, and placed, enzyme side down, on a previously prepared substrate/stain. Zymograms were incubated at 37°C until the bands which indicate enzyme activity were dark enough to be observed (10–15 minutes for most of the enzymes in this study). The membranes were then removed from the reaction mixture, placed in 5% acetic acid to stop the reaction, washed in tap water, blotted, and allowed to air dry.

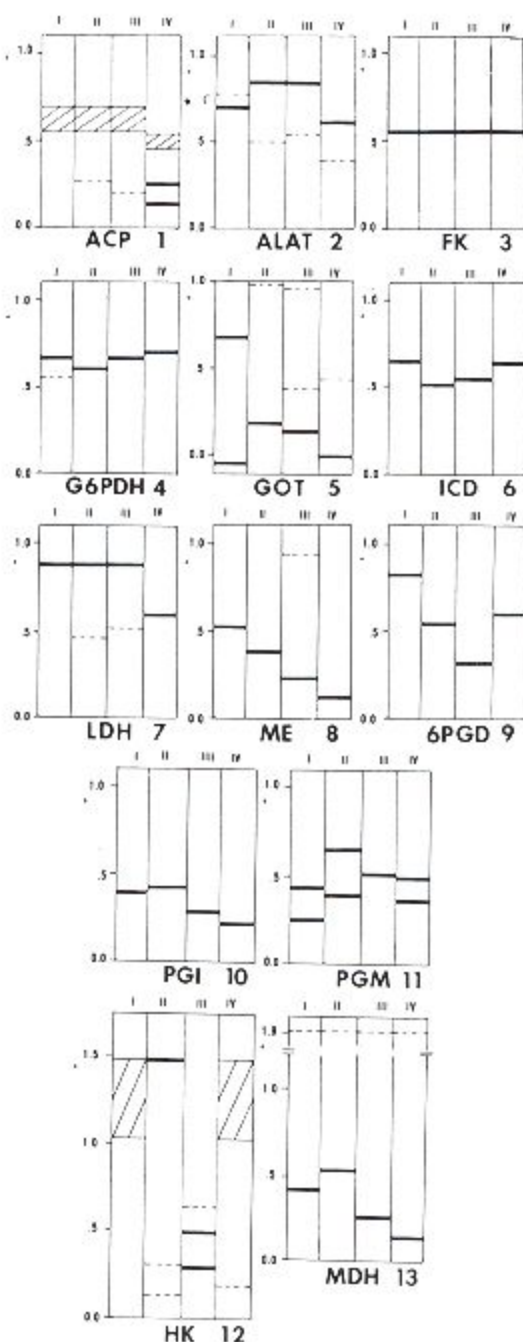
To prepare the staining dishes, the specific components (Table 3) were dissolved in 30 ml of reaction buffer, combined with 30 ml of 2% Noble Agar (50°C), 10 ml portions were placed in 100 × 15 mm petri dishes, and stored at 3°C until needed.

Controls

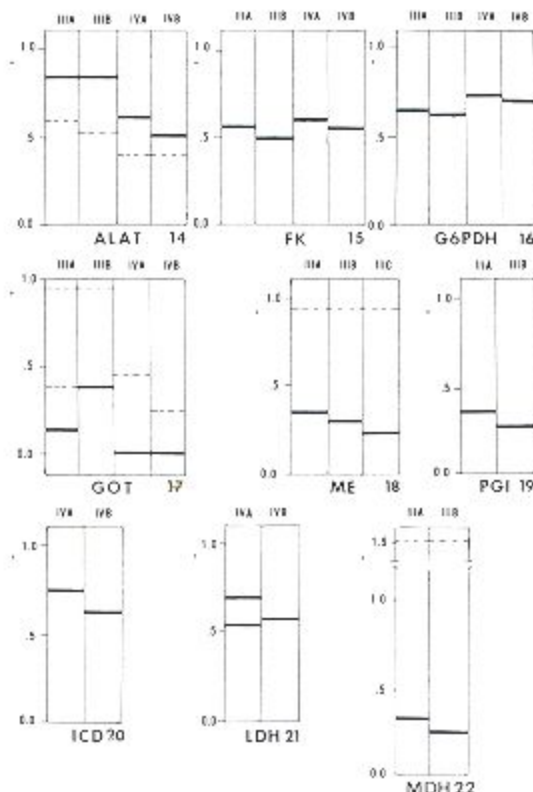
The 17 strains (three cellulose acetate plates) were run at the same time in separate cells attached to one power pack; therefore fluctuations due to current and length of electrophoresis time would expose all samples to identical experimental conditions. In addition an isolate of *Leishmania braziliensis* (GML-designation, L-10) was run with each of the groups of trypanosome isolates. No plate-to-plate variation was noted in the control band migration under these conditions. The relative band migrations of the trypanosome isolates and the control *Leishmania* band were then compared. A second run was made of these isolates. New isolates were prepared and these were also run twice. These four runs produced identical migrations on cellulose acetate. In Figures 1–22 the bands of enzyme activity are diagrammatically represented and their relative migrations are noted.

RESULTS

The results of multiple runs of separately isolated extracts of four species of *Trypanosoma* are shown diagrammatically in Figures 1–13. Intra-specific variability was noted in different isolates of *T. cruzi* and *T. rangeli* for certain enzymes, Figures 14–22. These bands which denote intra-



FIGURES 1–13. Diagrammatic representation of the electrophoresis patterns of 13 enzymes obtained from four species of *Trypanosoma*. See Table 2 for the complete names of the enzymes. Numbers at left represent relative mobilities of the isozymes; 1.0 is 1 inch from the origin (0,0) on the cellulose acetate plate. Anode is at the top of each diagram. I, *T. avium*; II, *T. vespertilionis*; III, *T. cruzi* (Panama); IV, *T. rangeli* (Panama).



FIGURES 14-22. Diagrammatic representation of possible allozymes recovered from strains of *T. cruzi* and *T. rangeli*. See Table 2 for the complete names of the enzymes. Numbers at left represent relative mobilities of the isozymes; 1.0 is 1 inch from the origin (0.0) on the cellulose acetate plate. Anode is at the top of each diagram. III, *T. cruzi*; IV, *T. rangeli*. The most anodally migrating band recovered from the species' strains has arbitrarily been designated as A. Therefore, Figure 15 shows the two isozymes (probably allozymes) which were obtained from the *T. cruzi* strains; these have been identified as FK IIIA and FK IIIB. Likewise, two isozymes (probably allozymes) have been isolated from the *T. rangeli* strains; they have been designated FK IVA and FK IVB.

specific differences have arbitrarily been designated with A, B and C (A is the most anodally migrating band). The isozyme types have been combined and tabulated in Table 1. More than one number indicates which of the species have an identically migrating isozyme for a specific enzyme. A photograph of six zymograms is presented in Figure 23.

The ACP isozyme patterns were different in each species but identical in each of the species' strains. *T. avium*, *T. vespertilionis*, *T. cruzi* each

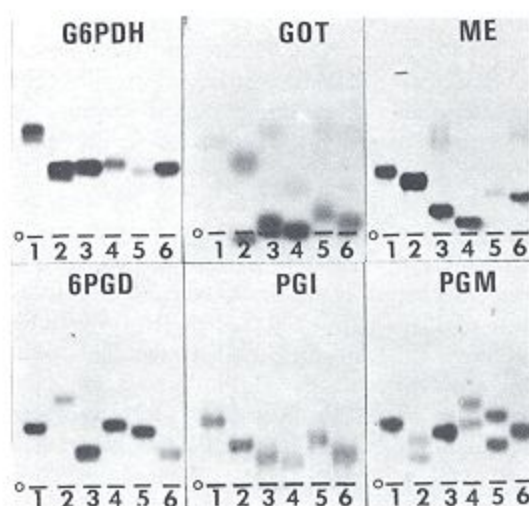


FIGURE 23. Photograph of zymograms of certain enzymes. See Table 2 for the complete names of the enzymes. 1, *Leishmania braziliensis* (Panama); 2, *Trypanosoma avium* (Panama); 3, *T. cruzi* (Panama); 4, *T. rangeli* (Panama); 5, *T. vespertilionis* (Panama); 6, *T. cruzi* (Brazil).

had a rapid anodally migrating wide band which had a certain amount of "bleeding"; however, *T. vespertilionis* and *T. cruzi* had an additional slower migrating light staining band. No additional light band was recovered from the *T. avium* isolate. The *T. rangeli* isolates had a slower migrating wide lead band than the other species, in addition each isolate had a pair of slow migrating dark staining bands (Fig. 1, Table 1).

The ALAT bands which were observed with UV were different in the four species. Each isolate had two bands of ALAT activity one light showing reduced activity on the substrate and one dark showing greater activity. The dark bands in *T. vespertilionis* and *T. cruzi* had identical migration (Fig. 2, Table 1). Two different isozyme types were observed in *T. cruzi* (III A & B) and *T. rangeli* (IV A & B) isolates. In the *T. cruzi* isolates all strains had an identically migrating dark staining band, but in *T. rangeli* all isolates had an identically migrating light staining band (Fig. 14, Table 1).

All species and most strains had an identically migrating band of FK activity (Fig. 3, Table 1); however, an additional and differently migrating band was recovered from the *T. cruzi* (III A & B) and *T. rangeli* (IV A & B) strains (Fig. 15, Table 1).

Both *T. avium* and *T. cruzi* had an identically migrating, dark staining band of G6PDH activity, but *T. avium* had an additional more cathodal lightly staining band (Figs. 4, 23, Table 1). The strains of *T. cruzi* (III A & B) and *T. rangeli* (IV A & B) each had two isozymes for G6PDH (Fig. 16, Table 1).

All isolates produced at least two bands of GOT activity, one lightly staining, the other dark staining (Figs. 5, 23, Table 1). Certain strains of *T. cruzi* (III A & B) produced three bands, two light and one dark. Others produced one dark and one light band, and the dark band in these stains had an identical migration as the middle light band of stains with three bands. All *T. cruzi* strains had one identical rapidly migrating light staining band. In the *T. rangeli* (IV A & B) isolates the dark staining band had an identical migration, but two types of light staining bands were recovered, one more anodal than the other (Fig. 17, Table 1).

ICD produced a single band of activity which was differently migrating in all species (Fig. 6, Table 1). All strains of *T. cruzi* had an identical ICD band, but two different bands were recovered from *T. rangeli* (IV A & B) isolates (Fig. 20, Table 1).

T. avium, *T. vespertilionis*, and all *T. cruzi* strains had an identically migrating dark staining band of LDH activity; however, *T. vespertilionis* and *T. cruzi* strains had an additional differently migrating light staining band. Two bands were observed in the *T. rangeli* (IV A & B) strains, but any one strain produced only a single band of activity (Figs. 7, 21, Table 1).

Each species and species' strain produced a different band of ME activity. Three differently migrating bands were recovered from the *T. cruzi* (III A, B & C isolates (Figs. 8, 18, Table 1).

One band of 6PGD activity was observed in each isolate. Each species had a differently migrating band, but the strains of *T. cruzi* and *T. rangeli* had no isozyme variability (Figs. 9, 23, Table 1).

The PGI isozymes were differently migrating in each species. No variation was observed among the *T. rangeli* strains, but two differently migrating bands were found in the *T. cruzi* (III A & B) strains (Figs. 10, 19, 23, Table 1).

The strains of *T. avium*, *T. vespertilionis* and *T. rangeli* each produced two dark bands of PGM activity. These bands were all differently migrat-

ing. The *T. cruzi* strains produced only one band of activity. No intraspecific variability was noted, and all bands produced whether single or double had different migrations (Figs. 11, 23, Table 1).

There was a wide band of HK activity produced by *T. avium* and the *T. rangeli* strains. Although this was the only band produced by *T. avium*, the *T. rangeli* strains produced another light staining band close to the origin (point of application). Three bands were recovered from the *T. vespertilionis* isolate. A dark one at the anodal end of the wide band in the *T. avium* and the *T. rangeli* strains and two light bands closer to the origin. All strains of *T. cruzi* produced a pair of dark bands and a third more anodal light band (Fig. 12, Table 1).

The MDH activity was observed by UV. All isolates had an identical rapidly migrating light staining anodal band as well as a single dark staining more cathodal band. The dark bands in each species and the strains of *T. rangeli* had a different migration, as did the two bands recovered from the *T. cruzi* (III A & B) strains (Figs. 13, 22, Table 1).

DISCUSSION

The data from the strains of *T. cruzi*, type III, and *T. rangeli*, type IV, reveal a high level of intraspecific isozyme (possibly allozyme) polymorphism. This same high level of intraspecific polymorphism has been reported by Miles et al.^{2,3} in *T. cruzi*. If high levels of intraspecific isozyme polymorphism are characteristic of trypanosome species, specific identification of isolates by enzyme profile analysis might prove to be more difficult due to allozyme migration overlap among species. As more enzyme systems become available for the study of trypanosomes the effect of allozyme overlap from species to species should be minimized or eliminated. On the other hand the strain polymorphism could provide some important information on geographical distributions, reservoir hosts, strain differences, etc. Miles et al.³ have identified three forms of *T. cruzi* infecting man in Brazil. In this study minor differences, two allozymes of ALAT (A & B) (Fig. 14), G6PDH (A & B) (Fig. 16), GOT (A & B) (Fig. 17), PGI (A & B) (Fig. 19), and MDH (A & B) (Fig. 22), three allozymes of ME (A, B, C) (Fig. 18) were noted among strains of *T. cruzi* (III) from humans isolated in Panama and Brazil. Also

strains of *T. rangeli* (IV) from humans isolated in Panama show minor differences, two allozymes of ALAT (A & B) (Fig. 14), FK (A & B) (Fig. 15), G6PDH (A & B) (Fig. 16), GOT (A & B) (Fig. 17), ICD (A & B) (Fig. 20) and LDH (A & B) (Fig. 21) (Table 1). Among both the *T. cruzi* (III) and *T. rangeli* (IV) strains there is a slight correlation between the location of strain location and the enzyme profile. Three isolates of *T. cruzi* (III), P-100, P-100a, and P9,443, from Chorrera, had identical enzyme profiles, and the profiles of two additional isolates from Chorrera, XRA-65 and P-11,689, differed by two or one isozymes, respectively (Table 1). Because so few isolates were examined, it is difficult to make any definitive statements concerning the type and locale.

There is some similarity in the basic pattern of bands from the strains of *T. cruzi* (III) reported here and the patterns obtained by Miles et al.^{2,3} That is, strains of *T. cruzi* (III) isolated from humans produced one PGM band, one PGI band, one G6PDH band, and two ME bands. In addition, there are also certain similarities in the number of ALAT and GOT bands produced by the strains in these studies.

There is a high level of enzyme heterogeneity among the species isolates which were studied. With minor exceptions each species produced bands of enzyme activity for each enzyme which had different electrophoretic mobility. The strains of *T. avium*, type I, and *T. vespertilionis*, type II, and most strains of *T. cruzi*, type III, and *T. rangeli*, type IV, produced an identically migrating FK band; however, the P-9,426 strain of *T. cruzi* (IIIB for FK) produced a more cathodal FK band and the P-18,131 strain of *T. rangeli* (IVA for FK) produced a more anodal FK band (Table 1). Also the ICD band of *T. avium* (I) and band produced by most strains of *T. rangeli* (IV) were identically migrating; however, the ICD band in the P-18,131 isolate of *T. rangeli* (IV A for ICD) was more anodal than the band in the other *T. rangeli* (IV B for ICD) strains (Table 1). A similar high level of interspecific variation was reported among species of *Trypanosoma* from bats.⁴ Although specific identification of the strains which were studied could be made by the isozymes produced by any one of the enzymes (except FK and ICD), multiple enzymes or an entire enzyme profile should be used to assure such identifications.

As emphasized by the data in Table 1 and in other studies, there are many biochemical variants among strains of trypanosome species, and any

parameters of trypanosome biology which are to be measured or defined by electrophoresis should necessarily be considered only preliminary until more comprehensive studies can be made of isolates.

In certain of the systems there were, in addition to the prominent major bands, other secondary lightly staining ones. These were evident in ACP, ALAT, G6PDH, GOT, LDH, ME, HK and MDH (Figs. 1, 2, 4, 5, 7, 8, 12, 13). These bands might be the "epigenetic"¹⁴ bands observed in many different types of organisms. A possible explanation of their origin in mosquitoes was proposed by Bullini and Coluzzi.¹⁵ These authors proposed that the light staining band might be derived from the primary, more intensely staining band by secondary modifications of the enzyme. It is also possible that certain of these bands are allozymes in a diallelic system. In this study all of the strains produced these bands.

These data indicate that enzyme electrophoresis has a great potential for rapid trypanosome isolate identification. After the samples have been prepared an enzyme profile of eight isolates for 13 enzymes can be produced in less than 2 hours. It should be possible for any laboratory capable of culturing *Trypanosoma* isolates to also characterize the isolates by cellulose acetate electrophoresis.

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REFERENCES

1. Chance, M. L., Gardener, P. J., and Peters, W., 1977. The biochemical taxonomy of *Leishmania* as an ecological tool. Pages 53-62 in *Colloques Internationaux du CNRS No. 239, Ecologie des Leishmanioses*.
2. Miles, M. A., Toyé, P. J., Oswald, S. C., and Godfrey, D. G., 1977. The identification by isoenzyme patterns of two distinct strain-groups of *Trypanosoma cruzi* circulating independently in a rural area of Brazil. *Trans. R. Soc. Trop. Med. Hyg.*, 71: 217-225.
3. Miles, M. A., Souza, A., Povoia, M., Shaw, J. J., Lainson, R., and Toyé, P. J., 1978. Isozymic heterogeneity of *Trypanosoma cruzi* in the first autochthonous patients with Chagas' disease in Amazonia, Brazil. *Nature*, 272: 819-821.
4. Baker, J. R., Miles, M. A., Godfrey, D. G., and Barrett, T. V., 1978. Biochemical characteriza-

- tion of some species of *Trypanosoma* (*Schizotrypanum*) from bats (Microchiroptera). *Am. J. Trop. Med. Hyg.*, 27: 483-491.
- Kreutzer, R. D., Posey, F., and Brown, P., 1977. A fast and sensitive procedure for identifying genetic variants of phosphoglucomutase in certain genera of mosquitoes. *Mosq. News*, 37: 407-409.
 - Kreutzer, R. D., and Christensen, H. A., 1980. Characterization of *Leishmania* spp. by isozyme electrophoresis. *Am. J. Trop. Med. Hyg.*, 29: 199-208.
 - Kilgour, V., and Godfrey, D. G., 1973. Species characteristic isozymes of two aminotransferases in trypanosomes. *Nature New Biol.*, 244: 69-70.
 - Kilgour, V., Gardener, P. J., Godfrey, D. G., and Peters, W., 1974. Demonstration of electrophoretic variation of two aminotransferases in *Leishmania*. *Ann. Trop. Med. Parasitol.*, 68: 245-264.
 - Kilgour, V., Godfrey, D. G., and Na'isa, B. K., 1975. Isoenzymes of two aminotransferases among *Trypanosoma vivax* in Nigerian cattle. *Ann. Trop. Med. Parasitol.*, 69: 329-335.
 - Godfrey, D. G., and Kilgour, V., 1976. Enzyme electrophoresis in characterizing the causative organism of Gambian trypanosomiasis. *Trans. R. Soc. Trop. Med. Hyg.*, 70: 219-224.
 - Al-Taqi, M., and Evans, D. A., 1978. Characterization of *Leishmania* spp. from Kuwait by isoenzyme electrophoresis. *Trans. R. Soc. Trop. Med. Hyg.*, 72: 56-65.
 - Shaw, C. R., and Prasad, R., 1970. Starch gel electrophoresis of enzymes—a compilation of recipes. *Biochem. Genet.*, 4: 297-320.
 - Ayala, F. J., 1975. Genetic differentiation during the speciation process. *Evol. Biol.*, 8: 1-78.
 - Markert, C. L., 1974. Biology of isozymes. Pages 1-9 in C. L. Markert, ed., *Isozymes*, Volume 1. Academic Press, New York.
 - Bullini, L., and Coluzzi, M., 1973. Electrophoretic studies on gene-enzyme systems in mosquitoes (Diptera, Culicidae). *Parassitologia*, 15: 221-248.
 - Commission on Biochemical Nomenclature, 1973. *Enzyme Nomenclature: Recommendations (1972) of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry*, 3rd ed. American Elsevier Publishing Co., Inc., New York, pp. 443.