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ANALYTICAL POLYACRYLAMIDE-GEL ELECTROPHORESIS OF AQUEOUS *TRYPANOSOMA CRUZI* EXTRACTS. I — CHEMICAL AND IMMUNOLOGICAL INVESTIGATION OF Y AND BRASIL STRAINS ANTIGENIC COMPONENTS

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SUMMARY

Analytical acrylamide-gel electrophoresis of *T. cruzi* soluble extracts, strains Y and Brasil, displayed 11 and 12 bands respectively. Staining of the gels with basic Fuchsin has shown the presence of glycoproteins in all extracts. Non-sonicated Y strain extract showed a stronger staining with basic Fuchsin than sonicated Y and Brasil strain extracts. Immunoelectrophoresis of the extracts, using rabbit and human antibodies, showed differences in the shape and position of arcs developed by the 2 types of immunesera.

INTRODUCTION

Trials to elucidate the antigenic composition of *T. cruzi* are not very numerous in the literature and were mainly concerned with the ability of the antigen(s) due to their chemical composition to give rise in immunized animals to antibodies which would react in different serological tests. Thus, FIFE & KENT [7], studied protein and polysaccharide *T. cruzi* fractions which were able to react with complement-fixing antibodies, and GONÇALVES & YAMAHA [8], isolated from *T. cruzi* a polysaccharide which was also able to give positive complement-fixation tests with sera from Chagas' disease patients. Double diffusion techniques were used to establish immunotaxanomic relationships between several strains of *T. cruzi* (NUSSENZWEIG et al. [14]; GONZALEZ-CAPPA & KAGAN [9]), among several trypanosomes including *T. cruzi* (BENEX et al. [3]), between *T. cruzi* and *L. tropica* (DUPOUEY & MARÉCHAL [5]). AFCHAIN & CAPRON [1], showed 19 precipitatin arcs corresponding to 19 soluble antigens in *T. cruzi* extracts, Tehuantepec strain, by means of immunoelectrophoresis, using rabbit anti-*T. cruzi* serum.

Chemical studies concerning *T. cruzi* were done by WILLIAMSON & DESOWITZ [21], while studying several strains of trypanosomes, mainly African ones. TARRANT et al. [20], described the chemical composition of *T. cruzi* exoantigen considering it to be a glycoprotein with antigenic activity, and GONÇALVES & YAMAHA [8], were able to elucidate the composition of the polysaccharide obtained from aqueous *T. cruzi* extracts.

In this paper we have tried to study the chemical composition of 2 strains of *T. cruzi* by means of analytical polyacrylamide-gel electrophoresis in order to search for differences which could be useful in the distinction between such strains and to verify the precipitation patterns of *T. cruzi* antigens in relation to human and rabbit antibodies.

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MATERIAL AND METHODS

In vitro grown *T. cruzi* epimastigotes, strains Y (PEREIRA DA SILVA & NUSSENZWEIG [15]) and Brasil (HAUSHKA et al. [11]) were used. After 8 days growth in LIT culture medium (FERNANDES & CASTELLANI [6]) the parasites were washed 3 times in saline (0.15M NaCl) and lyophilized.

Soluble extract — One gram of dry parasites were placed in a mortar with 1 ml of saline and dispersed. The slurry was placed in a separation funnel to which 5 ml of anhidrous ether was added and shaken vigorously for 30 seconds. After 5 minutes the ether fraction was decanted and the procedure repeated once more. The parasites were suspended in 5 ml of saline and sonicated twice in a Sonic Dismembrator (Quigley-Rochester, Inc.) in an ice-bath for 4 minutes with a rest period of 5 minutes between sonications, extracted overnight at 4°C, spun down at 6,000 g/15 min/4°C and the supernatant concentrated in dry sucrose followed by dialysis against saline. Protein content was assayed by means of the biuret reagent (GORNALL et al. [10]) using human-globulins as standard, determined by the Kjehldahl method.

Non-sonicated soluble extract — Procedure same as above, except that the parasites were not sonicated.

Analytical polyacrylamide-gel electrophoresis — Electrophoresis was carried out in a Canalco Model 1200 according to DAVIS, 1964 [4] with slight modification in sampling gel.

Sonicated Y and Brasil strains extracts had both a protein concentration of 17 mg/ml and non-sonicated Y-strains extract had a protein concentration of 9 mg/ml.

One drop (approximately 0.05 ml) of sample was added to 0.20 ml of a 1:6 acrylamide-bisacrylamide solution. All samples were run simultaneously as to have comparable migration in all tubes. Each tube was submitted to 4 mA until the ring of Bromophenol Blue reached the bottom of it.

After electrophoresis samples were stained blue for proteins with Amido Black 10 B, purple for glycoproteins with basic Fuchsin (ZACARIUS et al. [23]), blue for acid mucopolysaccharides with Toluidine Blue 0 (RENNERT [18]) and black for lipoproteins with Sudan Black B (PRAT et al. [17]). As standards, perchloric acid-precipitated normal rabbit serum was used for glycoproteins (WINZLER [22]), blood group substance AB (Central Laboratory of the Dutch Red Cross) for mucopolysaccharides and normal rabbit serum for lipoproteins.

Immunoelectrophoresis — Polyacrylamide gels were placed on microscope slides. One per cent agar in phosphate buffered saline pH 7.2 was pipetted over and around the gels as to obtain a 3 mm thick layer. After gelification, 2 parallel troughs were cut and rabbit anti-*T. cruzi* serum (Y strain) was put in one and human gamma globulin from

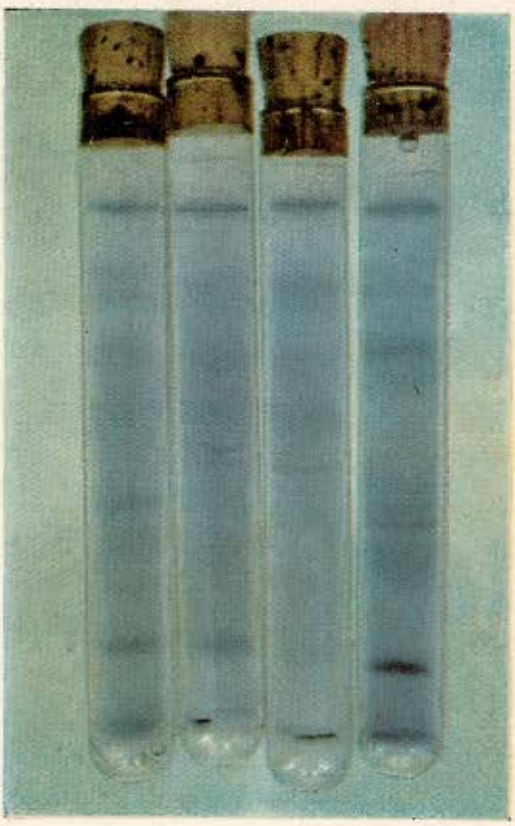

Fig. 1 — Gel electrophoresis — Protein staining (left to right) 1 — sonicated Y strain extract (standard extract at 20 mg/ml); 2 — sonicated Y strain extracts (17 mg/ml); 3 — non-sonicated Y strain extract; 4 — sonicated Brasil strain extract.

sera of chronic Chagas' disease patients in the other. Gamma globulin was 2.5 times more concentrated than the serum, which showed positive titers for anti-*T. cruzi* antibodies in previous immunofluorescence and hemagglutination tests. Diffusion was allowed to proceed in a moist chamber at room temperature until precipitin arcs were developed with human gamma globulin (7 days).

RESULTS

1 — *Electrophoresis*

1.1 *Proteins* — Sonicated and non-sonicated Y-strain extracts showed 11 protein bands (Fig. 1 tubes 2 and 3). Sonicated Brasil strain extract displayed the same number and positioning of bands plus one band of medium mobility (Fig. 1 tube number 4).

1.2 *Lipoproteins* — Sonicated Y-strain extract showed 2 bands. One of them, of fast mobility was also shown in non-sonicated Y-strain and Brasil strain extracts. The second band, of medium mobility appeared only in sonicated Y strain extract (Fig. 2).

1.3 *Acid mucopolysaccharides* — In all extracts at the above-mentioned concentrations only one fast moving band was observed (Fig. 3).

1.4 *Glycoproteins* — In all extracts there was development of colored reactions for glycoproteins. At the above-mentioned concentrations, non-sonicated Y strain and Brasil strain extracts showed stronger staining for

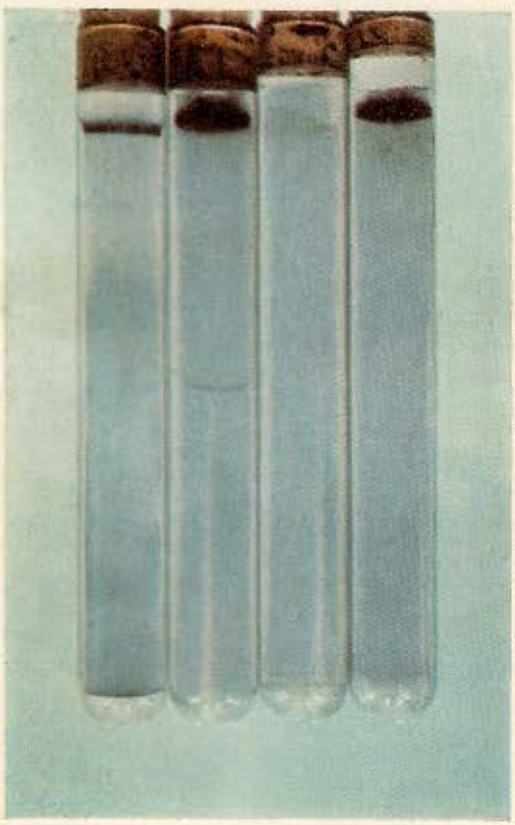

Fig. 2 — Gel electrophoresis — Lipoprotein staining (left to right) 1 — normal rabbit serum; 2 — sonicated Y strain extract; 3 — non-sonicated Y strain extract; 4 — sonicated Brasil strain extract.

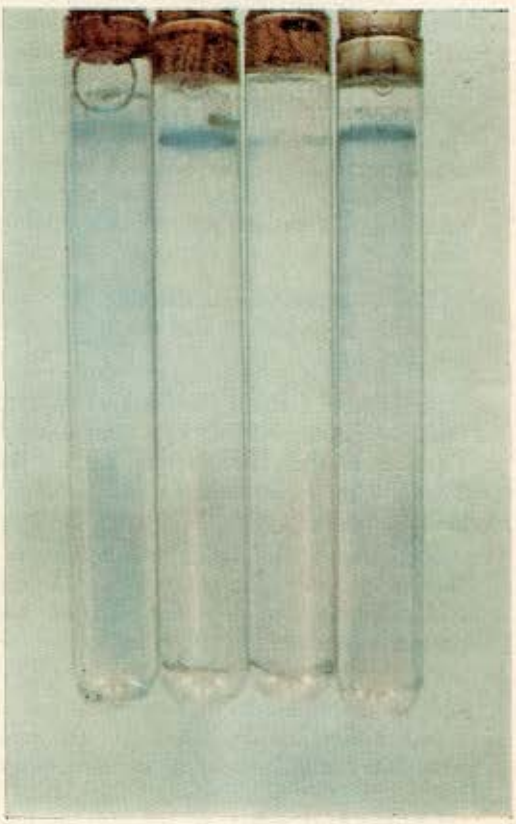

Fig. 3 — Gel electrophoresis — Acid mucopolysaccharides staining (left to right) 1 — blood group substance AB; 2 — sonicated Y strain extract; 3 — non-sonicated Y strain extract; 4 — sonicated Brasil strain extract.

glycoproteins than the sonicated Y strain one, although all 3 showed the same extent of band migration (Fig. 4).

2 — *Immunoelectrophoresis* — Sonicated Y strain extract showed 3 precipitin arcs with rabbit serum, distributed throughout gel length. Human gamma globulin showed 2 precipitin arcs (Fig. 5a).

Non-sonicated Y strain extract showed 2 precipitin arcs with rabbit serum and 3 with human gamma globulin 2 of which displayed total identity (Fig. 5b).

Sonicated Brasil strain extract showed 2 precipitin arcs with rabbit serum and 4 with human gamma globulin, of which 2 showed partial identity (Fig. 5c).

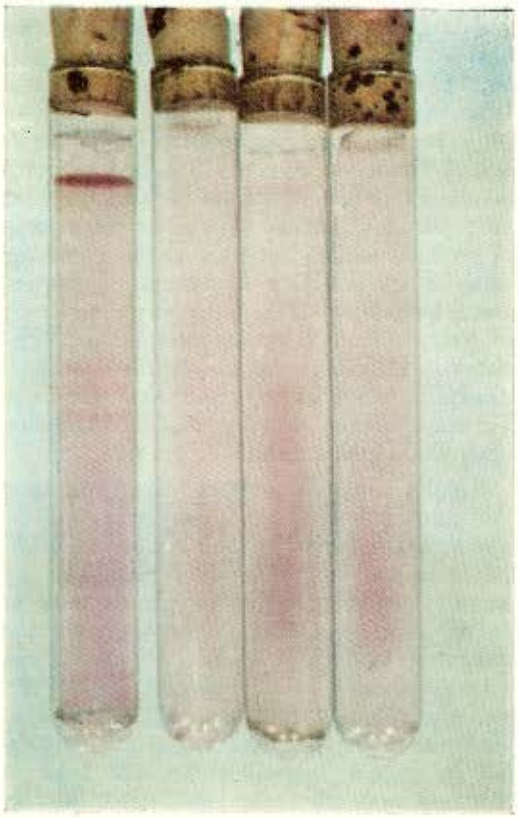

Fig. 4 — Gel electrophoresis — Glycoprotein staining (left to right) 1 — normal rabbit serum; 2 — sonicated Y strain extract; 3 — non-sonicated Y strain extract; 4 — sonicated Brasil strain extract.

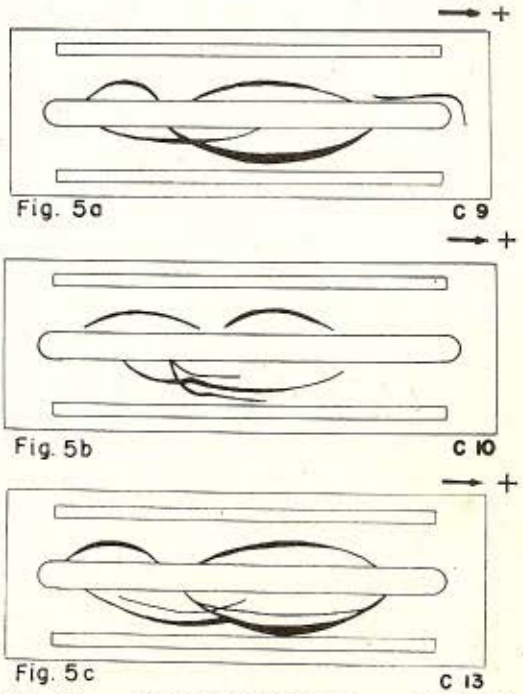

Fig. 5a — Immunoelectrophoresis — Sonicated Y-strain extract. Upper trough: Rabbit anti-*T. cruzi* serum. Lower trough human gamma globulin. Fig. 5b — Immunoelectrophoresis — Non-sonicated Y-strain extract. Upper trough: Rabbit anti-*T. cruzi* serum. Lower trough: Human gamma globulin. Fig. 5c — Immunoelectrophoresis — Sonicated Brasil-strain extract. Upper trough: Rabbit anti-*T. cruzi* serum. Lower trough: gamma globulin.

DISCUSSION

Non-sonicated Y strain extract showed equal number and position of stained bands as well as intensity of staining when compared with the sonicated one. Since these 2 differed in protein concentration as assayed by the biuret reagent, this seems to indicate that protein content of the parasite was readily soluble and disruption of the membrane was not necessary to remove them from the parasitic body. On the other hand, the higher protein content seen in sonicated Y strain extract could be only apparent and due to substances able to reduce copper in the biuret reagent, present in the cytoplasm of the flagellate or associated to cytoplasmics organelles, freed after disruption of the membrane but not proteic in composition,

which would account for the higher biuret measurement in the sonicated extract.

OSTMAN et al. [16], have found 23 protein stained bands in polyacrylamide-gel electrophoresis of *T. cruzi* (Peru strain). These findings were difficult to correlate with ours since the Authors did not mention protein concentration of the samples or the dye used to stain them. If one assumes that sample concentration was the same as in the present paper, this would suggest that the Peru strain possesses a more complex protein composition than Y and Brasil strains. However, since Y and Peru strains belong to group A trypanosomes (NUSSENZWEIG & GOBLE [13]) a greater chemical diversity would be expected between Y and Brasil (which belongs to group B) than between group A strains as it is the case. But, as already reported in the present paper sonicated Y and Brasil strains extracts did not differ except for one band when stained for proteins with Amido Black 10 B. In our laboratory a non-delipidized sonicated *T. cruzi* (Y strain) extract at a protein concentration of 12 mg/ml showed essentially the same number of bands as the previous ones (sonicated and non-sonicated *T. cruzi* extracts) except for a higher diffuse background staining throughout gel length.

Acid mucopolysaccharides — Although polysaccharides precipitated at the interfase between ether and saline and were decanted together with the ether fraction not all of them were removed from the slurry as shown by a fast moving band stained by Toluidine Blue 0 in all extracts. Sonicated extracts showed stronger staining than non-sonicated ones but this could be due to differences in concentration between extracts.

Glycoproteins — Non-sonicated Y strain extract showed stronger staining with basic Fuchsin than sonicated Y strain extract although the first was 2/3 less concentrated than the latter. Sonicated Brasil strain extract showed also stronger staining for glycoproteins than sonicated Y strain extract at the same concentration. TARRANT et al. [20] found that *T. cruzi* exoantigens contained 1:2 to 1:2.5 sugar to protein ratio in antigens exhibiting greater serologic activity as measured by C-fixation tests.

Glycoproteins are found in cell membranes and are normal constituents of tissues such as heart, aorta, liver, striated muscle, spleen, brain (SHIBATA & NAGASAWA [19]) and kidney glomerular membrane (MISRA [12]). Antigens chemically identified as glycoproteins have recently been implicated in the pathogenesis of nephrotoxic nephritis (SHIBATA & NAGASAWA [19]). In view of these findings and because of the high glycoprotein content of *T. cruzi* extracts it seems that further studies are necessary to try to elucidate the possible role of glycoproteins in the pathogenesis of Chagas' disease.

Lipoproteins — These substances were still present in all extracts. Differences in the intensity of staining of the fast moving band between sonicated and non-sonicated ones could be due to protein concentration.

Immunoelectrophoresis — Serum from chronic Chagas' disease patients is a poor source of antibodies as opposed to serum from hyperimmune animals as far as precipitin reactions are concerned. Human antibodies could be directed towards antigenic determinants different from the ones presented to rabbit's immune system, or the amount of secreted human antibody is small, making difficult its detection by means of conventional techniques. This second hypothesis was in part confirmed by AFCHAIN et al. [2], who showed by immunoelectrophoresis that 9 precipitin arcs could be observed in the chronic stage of the disease, with 3 times concentrated human serum.

Results from the present paper indicated that the first hypothesis was also involved. The arcs developed by rabbit antibodies differed in shape from the ones developed by human gamma globulin as far as total or partial identity reactions were concerned and were very noticeable in arcs developed with gamma globulin with non-sonicated Y and sonicated Brasil strains extracts, whereas in antigens, from the same extracts, precipitated by rabbit antibodies no such kind of reactions were observed. Comparison between such arcs when using as antigen, sonicated Y strain extract illustrates this point very clearly: precipitation by rabbit antibodies was initiated and terminated at a more anodic position than by human antibodies, so

much so, that the corresponding human antibodies' arc displayed non-identity reaction with the next arc.

Differences in the amount (or affinity) of antibodies present in human and rabbit antisera were relevant since in took 7 days for the arcs produced by human antibodies to became visible as opposed to 2 days for rabbit antibodies.

RESUMO

Eletroforese analítica em gel de poliacrilamida de extratos aquosos de Trypanosoma cruzi. *I — Investigação química e imunológica sobre componentes antigênicos das cepas Y e Brasil.*

Eletroforese analítica em gel de poliacrilamida de extratos solúveis de *T. cruzi*, cepas Y e Brasil, mostrou a presença de 11 e 12 bandas protéicas respectivamente em extratos sonicados e não-sonicados. Coloração do gel com Fucsina básica evidenciou a presença de glicoproteínas em todos os tipos de extratos com predominância, porém, naquele não-sonicado da cepa Y.

Imunoeletroforese dos extratos, usando anticorpos de coelho e humano mostrou diferenças na forma e posição dos arcos desenvolvidos pelos dois tipos de imunesoro.

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