bin-stimulated platelets (606 ± 24 cpm) compared to the unstimulated control (87 ± 7 cpm).

Discussion. We have shown by 3 different methods, that lipoxygenase present in the cytosol of human platelets is inhibited by KCN. Lipoxygenase activity (as well as cyclooxygenase activity) in intact platelets stimulated with thrombin was also inhibited. In intact platelets metabolism of AA by both the lipoxygenase and the cyclooxygenase enzymes was low although the total amount of AA released from phospholipids (and presumably available as substrate for these enzymes) was considerable. In conclusion, KCN behaves like 5,8,11,14-eicosatetraynoic acid (ETYA)[13] in that it inhibits both cyclooxygenase and lipoxygenase activities in platelets.

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Proteolytic activity on endogenous substrates in cell-free extracts of *Trypanosoma cruzi*

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Summary. Cell-free extracts of *Trypanosoma cruzi* contain proteolytic activity able to degrade endogenous substrates. The activity was maximal at pH 3 to 4, had an 'optimal' temperature of 65 °C, and was strongly inhibited by N-α-p-tosyl-L-lysine chloromethyl ketone.

The American trypanosomiasis, Chagas' disease, affects about 20 million people in Central and South America. The causative agent is a parasitic flagellate, *Trypanosoma cruzi*, which has a rather complex life cycle, involving bloodstream trypomastigotes and intracellular amastigotes in the mammalian host, and epimastigotes and metacyclic trypomastigotes in the insect vector[2]. Epimastigotes, apparently identical to the insect form of the parasite, are readily obtained in axenic culture, and have been employed in most biochemical studies on the parasite[3]. The epimastigotes have been shown to contain several proteolytic activities[4-8]. The study of the proteases in *T. cruzi* is interesting from several points of view. First, these enzymes initiate protein catabolism, which is probably intense in the parasite, and might explain, together with lipid oxidation, the high rate of endogenous respiration observed[3]. Second, proteases might be involved in the penetration of the nondividing trypomastigotes into mammalian cells[6], a process required for their transformation in intracellular amastigotes, able to multiply and thus essential for the perpetuation of chagasic infection. Finally, proteases are able to degrade proteins with enzymatic and/or antigenic properties during their isolation. It is very important from a practical standpoint to obtain knowledge about the response of these activities to inhibitors, in order to prevent their action.

Itow and Camargo[4] reported the presence in cell-free extracts of epimastigotes of *T. cruzi*, Y strain, of proteases acting on α-L-benzoyl-D-L-arginine-p-nitroanilide (BAPA), azocasein, and carbobenzoxy-L-tyrosine-p-nitrophenylester, and also of aminopeptidase activities. Torruella et al.[5] confirmed in 5 different strains of the parasite the presence of the proteases acting on azocasein and BAPA, and described an activity on casein, with a considerable difference in optimal pH with respect to that acting on azocasein. The protease acting on BAPA was purified and studied by Bongertz and Hungerer[6]. Avila et al.[7] detected in crude extracts of epimastigotes of the Y strain 3 activities which were classified as peptidases, and 2 classified as cathepsins. Recently Rangel et al.[8] purified a protease able to act optimally on casein at pH 6.0 and on hemoglobin at pH 3.0, and inhibited by thiol reagents. All these proteolytic activities have been detected using extraneous substrates such as casein, azocasein, hemoglobin and BAPA. From the practical standpoint of the prevention of proteolytic action on antigenic and enzymatic proteins of *T. cruzi* during isolation, it is perhaps more important to study the degradation of the proteins present in the cellfree extracts themselves. This paper presents the results of a study of the proteolytic activity on endogenous substrates in cell-free extracts of *T. cruzi*, including its response to pH and temperature, and the effects of different inhibitors.

Materials and methods. Culture epimastigotes of the Tulahuén strain, Tul 2 stock[5], were disrupted by compression-decompression in a Sorvall-Ribi cell-fractionator[9]. The homogenate was centrifuged at 1000 × g for 15 min; part of the supernatant (S 1000) was kept, and an aliquot was centrifuged at 105,000 × g for 1 h. Both supernatants (S 1000 and S 105,000) were used without further treatment for the experiments described. After incubation of the reaction mixtures (described in the legends to figures) the proteolysis was stopped by addition of 1 ml of 3% trichloroacetic acid (TCA), and the TCA-soluble peptides liberated were determined by reaction with Folin and Ciocalteu's phenol reagent[10]. Zero-time controls, to which TCA was added before the supernatant, were substracted in all cases. The reaction was linear with time for at least 15 min at 30 °C with both supernatants either at pH 5.0 or 7.2.

Results and discussion. The proteolytic activity on endogenous substrates, expressed per ml of supernatant, was

higher in S 1000; this might be due both to the presence of particulate proteases eliminated by the centrifugation at 105,000 × g, and to the higher protein (substrate) concentration of S 1000, which was usually twice as high as that of S 105,000. However, the specific activities of S 1000 and S 105,000 were similar. The proteolytic activity on endogenous substrates was quite thermostable; figure 1 shows that the 'optimal' temperature was 65 °C for both supernatants, at pH 5.0 and 7.2. It is noteworthy that the activity of S 105,000 at the latter pH was very low up to 45 °C, and increased considerably at higher temperatures. Figure 2 shows that the proteolytic activity was highest at pH 3–4 for both supernatants. S 1000 presented a 2nd, much lower, peak of activity at pH 6–7, which was not detected in S 105,000 (fig. 2). This suggests the presence of a particulate activity. The proteolytic activity on endogenous substrates was very sensitive to the inhibitor N-*a*-p-tosyl-L-lysine chloromethyl ketone (TLCK); even at 0.01 mM the inhibition was nearly complete, at pH 5.0. The inhibitory effect of 0.1 mM TLCK was observed over the whole range of pH investigated (fig. 2). Phenyl methyl sulphonyl fluoride (PMSF), another commonly used protease inhibitor, was much less effective; maximal inhibition at pH 5.0 was about 20% for both supernatants even at 5 mM PMSF. The inhibitor affected only the low pH optimum activity; the secondary peak presented by S 1000 at pH 6–7 was not affected (fig. 2). Thiol reagents were also studied as possible inhibitors. The low solubility of compounds such as p-chloromercurybenzoate and fluorescein mercuric acetate at low pH values made this study difficult. p-Chloromercuryphenylsulphonic acid, which is quite soluble at low pH, was ineffective at concentrations up to 0.1 mM, which inhibited completely the activity of thiol proteases[6,8]. Higher concentrations seemed to be inhibitory, but there was interference with the color reaction, and the apparent effect was therefore most likely an artefact.

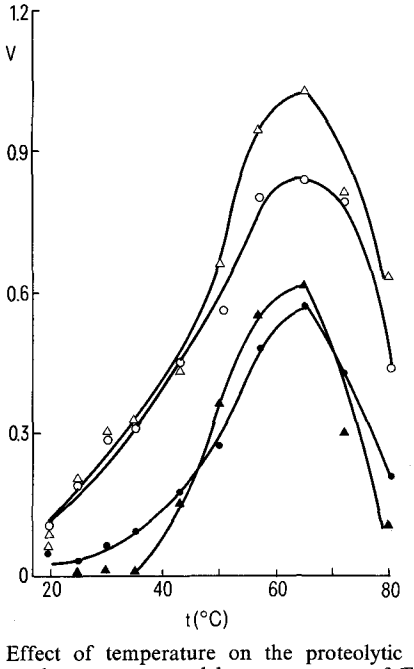

Figure 1. Effect of temperature on the proteolytic activity on endogenous substrates presented by supernatants of *T. cruzi*. Proteolytic activity was assayed in S 1000 (circles) and S 105,000 (triangles) at the temperatures stated on the abscissa. The reaction mixtures (0.4 ml) contained 0.2 ml of supernatant, and 0.2 ml of 0.1 M acetate-acetic acid buffer, pH 5.0 (open symbols) or 0.1 M Tris-acetate buffer, pH 7.2 (closed symbols). The incubations were conducted for 10 min. V is expressed as Δ_{A750nm}/h/mg of protein.

Although our results indicate the action of several enzymes in the proteolytic degradation of endogenous substrates in *T. cruzi*, the low pH optimum, the high 'optimal' temperature, and the pattern of inhibitory effects, suggest that the main enzyme involved may be the protease able to degrade azocasein at pH 5.0, previously described[4,5]. The enzyme reported by Rangel et al.[8] is not likely to be responsible for the proteolysis observed, since it was little affected by 1 mM TLCK, and sensitive to thiol reagents. The enzyme able to use BAPA as substrate[6] had a much higher optimum pH, and was very sensitive to thiol reagents. Our results might find application in immunological studies related to the obtaining of a vaccine. González Cappa et al.[11] assayed the immunoprotective effect on mice of homogenates of *T. cruzi* obtained at higher pressures than that used in the present work, and thus more likely to liberate hydrolytic enzymes situated within organelles. They found that the protective activity decreased with time, being maximal immediately after the homogenate was obtained. Such an effect might be due to the action of proteases, and it would be interesting to see whether an inhibitor such as TLCK might help to preserve the immunoprotective activity. Other authors[12] have used 2 mM PMSF in their solutions for the isolation of antigenic glycoproteins. Our study would show only gross proteolytic action on the bulk of protein present in the cell-free extracts, and would not necessarily correlate with the degradation of specific enzymes or antigens. Despite this fact, it seems reasonable to use TLCK as the inhibitor of choice for the isolation of proteins from *T. cruzi*.

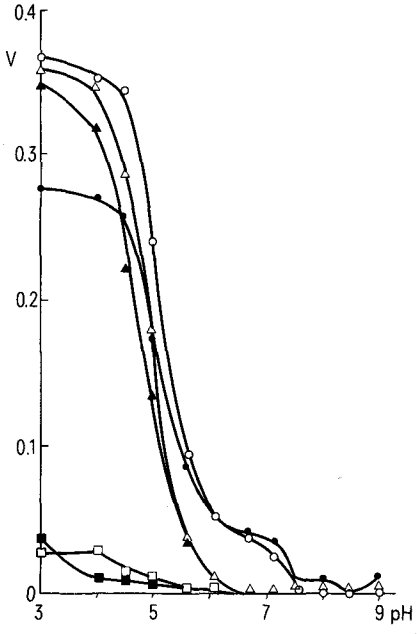

Figure 2. Effect of pH and inhibitors on the proteolytic activity on endogenous substrates presented by supernatants of *T. cruzi*. The reaction mixtures (0.4 ml) contained 0.2 ml of S 1000 (○, ●, □) or S 105,000 (▲, △, ■), 0.15 ml of the appropriate 0.1 M buffer (acetate-acetic acid in the range of pH 3.0–5.6; Tris-acetate in the range 6.1–7.2, and Tris-HCl in the range 7.6–9.0) and 0.1 mM TLCK (□, ■) or 2.5 mM PMSF (●, ▲). Since PMSF was added as a 100 mM solution in 90% ethanol, the controls (○, △) and the samples with TLCK were added 0.01 ml of 90% ethanol. The incubations were conducted for 15 min at 30 °C, after preincubation with the inhibitors for 1 h at 0 °C. V is expressed as Δ_{A750nm}/h/mg of protein.

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Cone mosaic in a teleost retina: No difference between light and dark adapted states[1]

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Summary. The mosaic arrangement of retinal cones in the eye of the African cichlid fish, *Haplochromis burtoni,* is the same in both light and dark adapted states. This is in contrast to Kunz' claim[2] that the retinal mosaic pattern changes from a square to a row type during dark adaptation, in the guppy *(Poecelia reticulata).* Kunz' histological procedure may account for this difference in results.

Cone photoreceptors in many teleost retinae are arranged in a highly regular mosaic pattern[3–6]. 2 basic types of mosaic patterns have been described; 'square' and 'row' types. In both patterns, a row of double or twin cones alternates with a row of single cones. The patterns differ in that in one case the double (twin) cones are arranged so that lines through the centers of each pair are at right angles with respect to one another ('square' pattern) whereas in the other case the axes of the double cones are parallel to one another ('row' pattern) (fig. 1,a). The 'square' mosaic (fig. 1,b) is thought to be associated with fish which are highly dependent on vision[3]. In some species, both patterns exist in different regions of adult retina[7,8] or occur at different times during ontogeny[9–11]. Rods are interspersed in the cone arrays throughout the extent of the retina[12].

In the African cichlid fish, *Haplochromis burtoni,* a highly ordered square type mosaic is present[13]. The chromatic organization of these cones, as determined by vital staining with nitro-blue tetrazolium di-formazan, is an alternating symmetry which serves to maximize the chromatic resolution available to the animal[12]. This is consistent with behavioral[14,15], developmental[16], and neuroanatomical[17] evidence that vision is the primary sensory input for *H. burtoni.*

In a recent report, Kunz[2] suggested that the cone mosaic, as reflected in outer segment organization in the guppy (*Poecilia reticulata* P.) changes from a square to a row type during dark adaptation. She removed adult fish retinae by microdissection in Ringer's solution with or without calcium and examined the tissue using light or electron microscopy. At the level of the outer segments, her micrographs show an apparent change in mosaic type from double cone zig-zag rows (fig. 1,b) to parallel rows (fig. 1,a). She suggests that this change supports the hypothesis that zig-zag rows of double cones are more suitable for high acuity vision and parallel rows more suitable for low-level light detection. If this hypothesis were true, such a change in mosaic type should occur in other teleost species dependent on vision during dark adaptation. For this reason, we examined the cone mosaic in the retina of *H. burtoni* under conditions of light and dark adaptation.

Materials and methods. Adult male *Haplochromis burtoni* (7–8 cm long) bred from wild-caught fish[14] were used for the experiments. The animals were maintained at 12:12 light-dark cycle characteristic of their natural habitat in central Africa[14]. Animals were anesthetized by immersion in tricaine methanesulphonate (MS222, Sandoz) at 03.00 h and one eye removed, and the procedure repeated at 15.00 h and the other eye removed. This allowed us to compare 2 eyes from the same animal, differing only in the time of day when they were prepared for histology. Enucleation during the dark portion of the light-dark cycle was performed using an infrared viewing device (FJW, Mt. Prospect, Ill.) so that there was no significant illumination below 750 nm, which is well above the photopigment sensitivity limit[13]. Following enucleation, the lenses were removed and the eyecups were immediately immersed in fixative (1% paraformaldehyde, 2.5% gluteraldehyde, 3% sucrose in 0.06 M PO_4 buffer) with dorsal margin of the eye

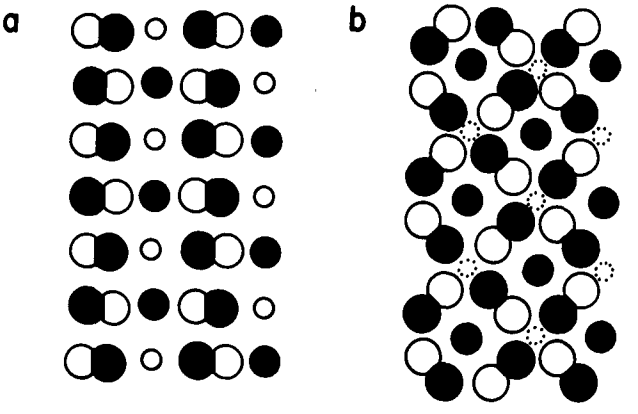

Figure 1. *a* A sketch of the 'row' type of cone pattern. The rows of single cones alternate with rows of double cones which are oriented to that lines drawn through the twin cones are parallel. *b* 'Square' type of cone pattern. The rows of double cones are oriented so that lines drawn through the twin cones are perpendicular. Dotted lines indicate single cones which are not present ind *H. burtoni* retina.