to 7.5 times that of control values within 2 hours of transfer, has been reported for other active nonoverwintering insects including crickets and locusts (*7*) and for diapausing silkworm pupae (*8*). Thus, the cold activation of phosphorylase and the ensuing rapid accumulation of glycerol may be a general adaptation of insects for protection against cold shock injury.

The rapid cold-hardening capacity we describe may be of considerable ecological importance in early spring and late autumn. Our results suggest that many insects, even those in nondiapausing stages, have the ability to quickly enhance their cold tolerance in response to a rapid temperature drop. Though such a mechanism, in the absence of diapause, may not enable an insect to survive the prolonged cold exposure characteristic of winter, it should permit the insect to adapt to diurnal changes in temperature and enable the insect to survive brief periods of exposure to low temperature. Our observation that cold shock injury can be reduced by brief exposure to 0°C suggests that methods can be developed for long-term cryopreservation and storage of *Drosophila* and other nondiapausing insects.

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Similarity of Cruzin, an Inhibitor of *Trypanosoma cruzi* Neuraminidase, to High-Density Lipoprotein

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A specific inhibitor of the neuraminidase of the protozoan parasite *Trypanosoma cruzi* was isolated recently and named cruzin. It is now shown that cruzin is similar to high-density lipoprotein by amino acid homology, by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, by immunoblot analysis, and by isoelectric focusing. Cruzin purified by ion exchange chromatography and high-density lipoprotein isolated by density gradient ultracentrifugation inhibited *Trypanosoma cruzi* neuraminidase to the same extent. Cruzin or high-density lipoprotein restores to normal the decreased multiplication rate of *Trypanosoma cruzi* epimastigotes grown in a medium depleted of lipoproteins, suggesting that it may be important for survival of the parasite in nature.

TRYPANOSOMA CRUZI, THE CAUSative agent of Chagas' disease, produces a developmentally regulated neuraminidase (E.C. 3.2.1.18) (*1*). The activity of the enzyme is greater in infective trypomastigotes than in epimastigotes (*1*), the parasite stage that multiplies in the gut of bloodsucking reduviid bugs. During infection in vitro, intact trypomastigotes desialylate erythrocytes and other blood cells (*1, 2*) as well as myocardial and endothelial cells (*3*). The neuraminidase is heterogeneously distributed among strains of *T. cruzi*, with some of the relatively low virulent ones possessing enzyme activity several orders of magnitude higher than the more virulent strains (*4*). Antibodies specific for *T. cruzi* neuraminidase augment parasite infection in vitro, and the enhancement is prevented by the addition of exogenous *Vibrio cholerae* neuraminidase (*5*). The *T. cruzi* neuraminidase may therefore participate in the association of *T. cruzi* with mammalian hosts through a negative control mechanism.

We recently isolated an inhibitor of the *T. cruzi* neuraminidase from human plasma and named it cruzin (*6, 7*). Purified cruzin is remarkably specific for *T. cruzi* neuraminidase, with 50% inhibition being reached at concentrations as low as $10^{-9}M$ (*6*). Cruzin inhibits trypanosome desialylation of cells, but not of soluble glycoconjugates; it is equally effective when the enzyme is present in a soluble form or on the outer membrane of living parasites (*6*). Molecular characterization of the purified material indicates that the native form has an M_r of 246,000 ± 20,000 with a major subunit of M_r 28,000 ± 2,000. Kinetics analysis of cruzin activity suggested a noncompetitive mechanism of inhibition (*6*).

Since many human plasma proteins have been identified and characterized, it was reasonable to expect that cruzin was already described in the literature. Initial attempts to match cruzin with a known human plasma component by using commercially available antisera to individual plasma proteins were unsuccessful. We therefore determined the sequence of the first 20 amino acids of the major protein component of cruzin and searched for matching sequences in a protein and nucleic acid sequence database. The sequence was identical in 18 of 20 positions with the amino terminus of human apolipoprotein A-I (apoA-I) (Fig. 1a), the major protein component of plasma high-density lipoprotein (HDL). The amino acids in positions 8 and 10 could not be positively identified during the sequencing of cruzin, because the former was obscured by diphenylurea, a by-product formed during the Edman degradation, and the latter was not present in sufficient amounts for detection.

This sequence similarity suggested that cruzin contained apoA-I and that it was HDL. This possibility was tested with the following results. In SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (*8*) (Fig. 1b), both cruzin and HDL displayed one major band of M_r 28,000 (apoA-I) (lanes A and B, respectively) whereas other lipoprotein fractions (*9*) such as low-density lipoprotein (LDL) (lane C) and very low density lipoprotein (VLDL) (lane D) showed a major band of M_r >200,000 (apolipoprotein B). In immunoblot analysis (*10*) with a monospecific rabbit antibody to cruzin (*11*), the antibody recognized equally well a major band of M_r 28,000 in cruzin and HDL preparations (Fig. 1c, lanes A and B). No cross-reactivity was observed with apolipoprotein B of LDL (Fig. 1c, lane C). Low levels of apoA-I were detected in both VLDL and LDL fractions (lanes C and D), indicating a cross-contamination during the purification procedure that was not revealed by Coomassie blue staining of the gel (SDS-PAGE) (Fig. 1b, lanes C and D). A similar contamination was reported previously (*12*). These results demonstrated that antibody elicited to cruzin cross-reacted with apoA-I present in the various lipoprotein preparations tested.

Additional evidence for the identity of cruzin with HDL was provided by a com-

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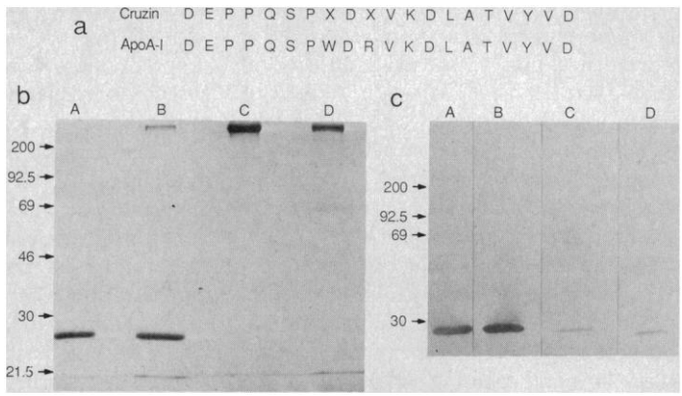

Fig. 1. (**a**) The 20 amino terminal amino acids of cruzin and apoA-I. The complete apoA-I sequence was obtained by translation from the DNA sequence (*29*) and from the protein residues (*30*). The amino acid composition of purified cruzin was determined by using a D-500 amino acid analyzer after hydrolysis in 6*N* HCl in vacuo for 24 hours at 110°C. The protein was sequenced by automated Edman degradation with a model 470A protein sequencer (Applied Biosystems) by P. Matsudaira (Massachusetts Institute of Technology). The amino acid sequence homology was verified by the Protein Identification Resource (National Biomedical Research Foundation, Georgetown University, Washington, D.C.) by scanning of the first 20 amino acids with the PSQ retrieval system. (The one-letter symbols for the amino acids are A, alanine; R, arginine; D, aspartic acid; Q, glutamine; E, glutamic acid; L, leucine; K, lysine; P, proline; S, serine; T, threonine; W, tryptophan; Y, tyrosine; V, valine; and X, unknown or "other.") (**b**) SDS-polyacrylamide gel (10%) electrophoresis analysis of cruzin and different lipoprotein subfractions. Samples (~3 μg of protein) were treated as described (*8*). Lanes are (A) cruzin, (B) HDL, (C) LDL, and (D) VLDL. Bands were visualized after staining with Coomassie blue. (**c**) Immunoblots showing the cross-reactivity of the major protein component of cruzin with apoA-I of HDL. Lanes are as in (b). Samples (~3 μg) were electrophoretically transferred to the zeta probe membrane (*10*) and probed with a monospecific rabbit antibody to cruzin. Bands were visualized by the alkaline phosphatase procedure. Molecular weight standards (Amersham) are indicated in kilodaltons.

parison of their isoelectric focusing profiles (Fig. 2). The HDL pattern displayed apoA-I with five bands having isoelectric points (pI) ranging from 5.4 to 5.85; the pIs of apoA-II, apoC-II, apoC-III-1, and apoC-III-2 were 5.0, 4.9, 4.7, and 4.5, respectively (*13*). The profile obtained for HDL was thus very similar to that of cruzin (Fig. 2). Moreover, cruzin was found to have a lipid and protein composition similar to that of HDL (approximately 25% phospholipid, 20% cholesterol, 5% triglyceride, and 50% protein by weight). Small amounts of carbohydrate were detected in our cruzin preparations (*6*); however, since apoA-I and apoA-II do not contain covalently bound carbohydrate (*12*), the sugar content of cruzin is probably due to glycoproteins such as apoC-II, apoC-III, apoA-IV, and apoE that are also present in HDL (*12, 14*).

If cruzin is HDL, the two substances should inhibit the *T. cruzi* neuraminidase to the same extent (*15*). As shown in Fig. 3, they both required approximately 1.5 μg of protein per milliliter to achieve 50% inhibition of neuraminidase activity. In contrast, normal plasma required 44 μg, VLDL 177 μg, and lipoprotein-depleted plasma 324 μg of protein per milliliter, respectively. The amount of apoA-I in these fractions was also determined (*16, 17*), and the residual inhibitory activity in the lipoprotein-depleted plasma could be accounted for by the small amount of apoA-I still present in those preparations. The lipid constituent of either cruzin or HDL does not seem to be essential for the inhibition of neuraminidase activity since delipidated apoA-I, obtained by electroelution from SDS-PAGE (*6, 18*), retained almost all of the lipoprotein inhibitory activity. Since these data indicate that cruzin is HDL, the procedure used to purify cruzin (*6*) (that is, polyethylene glycol precipitation, and chromatography on CM Affi-Gel Blue Sepharose and Ultrogel AcA 34) should serve as an alternative approach for isolating HDL uncontaminated with LDL and other lipoproteins (Fig. 2).

What is the role of HDL in the physiology of *T. cruzi*? Both HDL and LDL are cholesterol-carrying lipoproteins, and decreased plasma HDL cholesterol levels and increased LDL cholesterol levels have been associated with premature coronary artery disease (*13, 19, 20*). HDL stimulates cholesterol efflux from cells in vitro (*21*) and reverse transport of cholesterol in vivo (*22*). Moreover, the lipid constituent of both LDL and HDL enhances mitogen-stimulated growth of arterial smooth muscle cells (*23*). Thus the interaction of *T. cruzi* with HDL may serve some nutritional needs of the parasite. This hypothesis is supported by the following observation: the multiplication rate of epimastigotes in lipoprotein-depleted medium (LDM) (*24*) is at least two times less than in control cultures, and addition of purified human HDL to the medium completely restores normal growth (Fig. 4). Human HDL could mediate delivery of cholesterol and other lipids to epimastigotes and thus promote their growth, consistent with the observation that *T. cruzi* is apparently incapable of synthesizing cholesterol (*25*). This finding is also in accordance with the binding of ^{125}I-labeled HDL to intact epimastigotes, which follows a pattern of ligand-receptor interaction. Binding of ^{125}I-labeled HDL to *T. cruzi* is specific, because it was not prevented by other human plasma proteins. It is also stage- and strain-dependent and parallels the parasite neuraminidase activity (*26*).

Whereas HDL apparently enhances epimastigote multiplication (Fig. 4), it cannot have a mitogenic effect on trypomastigotes because *T. cruzi* trypomastigotes do not multiply. Instead, because neuraminidase is apparently involved in the in vitro infection of host cells by *T. cruzi* (*1–5*), HDL should have a direct influence on infection by virtue

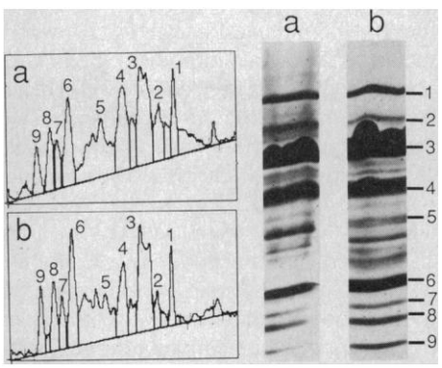

Fig. 2. Isoelectric focusing of cruzin (lane a) and HDL (lane b). The minigel methodology was used as described (*14*). The Ampholine range used was from *p*H 4 to 6.5. After destaining, the gels were scanned with an LKB Ultroscan laser scanning densitometer (insets a and b), and the isoelectric points were determined. The numbers 1 to 5 refer to apoA-I; 6, to apoA-II; 7, to apoC-II; 8, to apoC-III-1; and 9, to apoC-III-2.

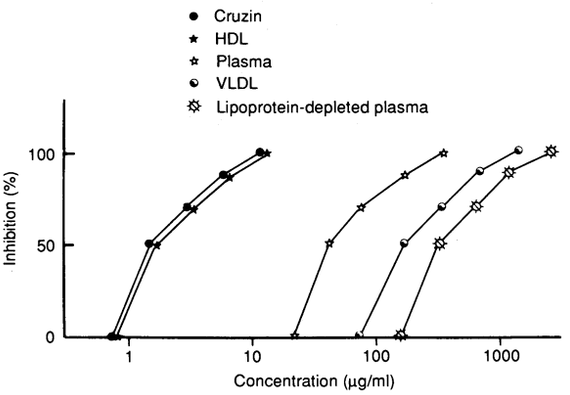

Fig. 3. Inhibition of the *T. cruzi* neuraminidase activity by cruzin, HDL, normal plasma, VLDL, and plasma depleted of lipoproteins. The inhibition assays were performed as described (*15*). The results are from a representative experiment. The amount of protein required to account for 50% inhibition did not vary more than 20% in each case.

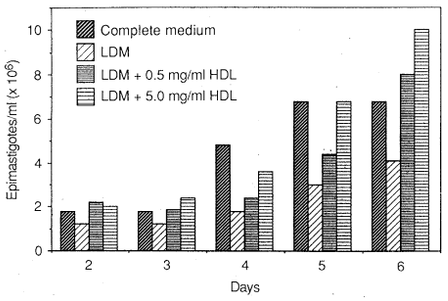

Fig. 4. Effect of HDL on the growth of *T. cruzi* epimastigotes (strain MV-13). Parasites were grown for the indicated time in a complete medium containing 10% fetal calf serum, in LDM, and in LDM reconstituted with human HDL at the indicated concentrations. The results shown are the average of duplicate tubes and representative of four experiments (standard deviation is less than 20% of the mean).

of its ability to inhibit neuraminidase activity of the infective trypomastigotes. Our observations support this concept (*27*). In this context, it is interesting that human HDL has been reported to have a specific lytic effect on trypomastigotes of the African trypanosome *T. brucei* and to be responsible for its host specificity (*28*). In view of these considerations, our working model is that HDL participates in the *T. cruzi* life cycle by promoting epimastigote multiplication in the insect vector and trypomastigote infection in the mammalian host. The results presented here raise the possibility that HDL may be one of the factors underlying the pathogenesis of Chagas' disease.

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11. Antibody to cruzin was elicited in rabbits as follows. Cruzin was purified from human plasma by polyethylene glycol precipitation, CM Affi-Gel Blue Sepharose (Bio-Rad) and Ultrogel AcA 34 (LKB) chromatography, and electrophoresis on SDS-polyacrylamide gel (*6*). Strips containing the cruzin bands (M_r 28,000) were excised, lyophilized, and ground to a fine powder with mortar and pestle. The powder containing approximately 30 μg of purified inhibitor was resuspended in 0.01*M* sodium phosphate–buffered saline (PBS), *p*H 7.2, and mixed with an equal volume of Freund's complete adjuvant for the first injection and with Freund's incomplete adjuvant for subsequent injections. Immune rabbit serum was collected after five injections 10 days apart. Preimmune and immune immunoglobulin G were purified by affinity chromatography on a protein A–Sepharose 4B column.
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15. Assays for inhibition of neuraminidase activity were performed as previously described (*6*). Water-soluble neuraminidase was harvested after the incubation of trypomastigotes for 72 hours at 4°C in the presence of serum-free medium containing protease inhibitors. Neuraminidase activity was assayed by incubating 50 μl of a fresh suspension of 10% human erythrocytes with 25 μl of the neuraminidase preparation for 2 hours at 37°C in a final volume of 100 μl of PBS. The erythrocyte suspension was then diluted to 2%. The degree of erythrocyte desialylation was determined by adding 25 μl of the suspension to a peanut lectin (PNA) solution diluted in microtiter plates. The hemagglutination titers were scored after incubation for 60 minutes at room temperature. Neuraminidase inhibition was assayed by incubating the proteins to be tested for inhibitory activity with neuraminidase for 60 minutes at 37°C before the addition of the red blood cells. One inhibition unit is defined as the amount of protein required to inhibit 50% of neuraminidase activity by the PNA hemagglutination method.
16. Human plasma apoA-I and apoB-100 were measured by a noncompetitive enzyme-linked immunosorbent assay (*17*). Polystyrene microtiter plates (Nunc Immunoplate 1, Denmark) were coated with affinity-purified polyclonal antibodies to apoA-I. Duplicate human plasma samples were diluted 1:3000 with PBS and added to designated wells in the microtiter plates, along with standards and controls obtained from the National Heart, Lung, and Blood Institute Lipid Research Clinics standardization program at the Centers for Disease Control (Atlanta, Georgia). After overnight incubation, immunopurified apoA-I polyclonal antibody conjugated to alkaline phosphatase was added. Color development was achieved with the addition of the substrate (0.1% *p*-nitrophenyl phosphate in 0.1*M* glycine). After 20 minutes at room temperature, the plates were read at 410 nm on a microtiter plate reader (Dynatech MR600), interfaced with an IBM XT microcomputer, and programmed with Immunosoft (Dynatech).
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Effect of Membrane Potential Changes on the Calcium Transient in Single Rat Cardiac Muscle Cells

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The mechanism that links membrane potential changes to the release of calcium from internal stores to cause contraction of cardiac cells is unclear. By using the calcium indicator fura-2 under voltage-clamp conditions, changes in intracellular calcium could be monitored in single rat ventricular cells while controlling membrane potential. The voltage dependence of the depolarization-induced increase in intracellular calcium was not the same as that of the calcium current (I_{si}), which suggests that only a small fraction of I_{si} is required to trigger calcium release from the sarcoplasmic reticulum. In addition, sarcoplasmic reticulum calcium release may be partly regulated by membrane potential, since repolarization could terminate the rise in intracellular calcium. Thus, changes in the action potential will have immediate effects on the time course of the calcium transient beyond those associated with its effects on I_{si}.

STUDIES ON CARDIAC MUSCLE HAVE shown that the amplitude of the calcium current (I_{si}) and the strength of contraction are closely linked (*1*), supporting the idea that the Ca^{2+} flux across the surface membrane during I_{si} directly trig-

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