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IMMUNOCYTOCHEMICAL REACTION OF SERA FROM CHAGASIC PATIENTS AGAINST *TRYPANOSOMA CRUZI,* INTESTINE OF *TRIATOMA INFESTANS* AND NORMAL HUMAN HEART

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Abstract Chagas disease has been considered by some authors as an autoimmune pathology and denied by others. In this paper we present by means of immunocytochemical reactions with sera of chagasic patients, evidence in favor of the presence of similar antigens in the parasite, vector and non chagasic human heart. The immunocytochemical technique used permits the localization by electron microscopy of the antigens in the peritrophic membrane of the parasite and basement membranes of the vector's midgut and of the myosin band of the normal human heart. These observations support the assumption of an autoimmune response in Chagas disease.

Resumen ***Reacción inmunocitoquímica de sueros de enfermos chagásicos contra el* Trypanosoma cruzi*, el intestino medio del* Triatoma infestans *y el corazón humano normal.*** La enfermedad de Chagas ha sido considerada por algunos autores, como una patología autoinmune y negada por otros investigadores. En el presente trabajo aportamos evidencias, mediante el empleo de suero de pacientes chagásicos, a favor de la existencia de antígenos semejantes en el parásito, el vector y el corazón humano no chagásico. La técnica inmunocitoquímica empleada permite la localización de los antígenos a nivel ultraestructural por microscopia electrónica; en el parásito, la membrana peritrófica del intestino medio del vector, en las membranas basales y en las bandas de miosina del miocardio. Estas observaciones apoyan la existencia de una respuesta autoinmune.

Key words: Chagas, autoimmune disease

Chagas disease is a chronic parasitic disease which affects 18 million people in Latin America[19]. It is caused by infection with *Trypanosoma cruzi,* involving a hematophagous insect vector *Triatoma infestans,* or by transfusion of contaminated blood.

When the trypanosoma is incorporated into the insect by sucking blood of mammals infected with the parasite (trypomastigote), the parasite multiplies as epimastigotes in the midgut of the insect. Finally, they are released outside of the gut coincident with the blood sucking, as a rosette of trypomastigotes enclosed in a quitinous envelope[11]. This deposition with mature and active parasites on the skin or mucous membrane, is accepted as one of the main sources of human and animal contamination[18]. Once the parasite enters, the ma-crophages of the organism are usually the first carrier involved and the site of initial reproduction of trypa-nosomes. Antibodies obtained from the serum of Cha-gas patients bind to common antigens in the parasite and in the insect vector[12].

In the present paper we have detected by immunocytochemistry the localization of antigens in the parasite, in the vector and in the human heart of non chagasic patients, using as antibody the blood serum of chagasic patients.

Material and Methods

Samples of non chagasic human myocardium were obtained from patients with valvular implants and from hearts removed during transplantation. Samples of midgut from vector *Triatoma infestans,* with and without parasites (*Trypanosoma cruzi*) in the intestine, were fixed in PAF (picric acid 0.1%, formaldehyde 1% in 0.1 M phosphate buffer pH 7.2) for 1 hour, washed in buffer, dehydrated in gradient concentrations of alcohol (50° to 90°) and embedded in plastic LR-White. Ultrathin sections were mounted on aluminum grids and exposed to the immunocytochemical reaction.

Serum of chagasic patients with a high titer 1/500 (diluted 1:25, 1:50 and 1:100 in PBS-BSA) was used as first antibody.

The antigen-antibody reaction was revealed by protein A-gold (Taab Laboratories). Controls without the first antibody were also treated with the protein A-gold.

The ultrastructural study was carried out in other sections of the same human myocardium, vectors and parasites. Samples were fixed in 5% glutaraldehyde in 0.1 M buffer cacodylate pH 7.2, washed in the same buffer and refixed in

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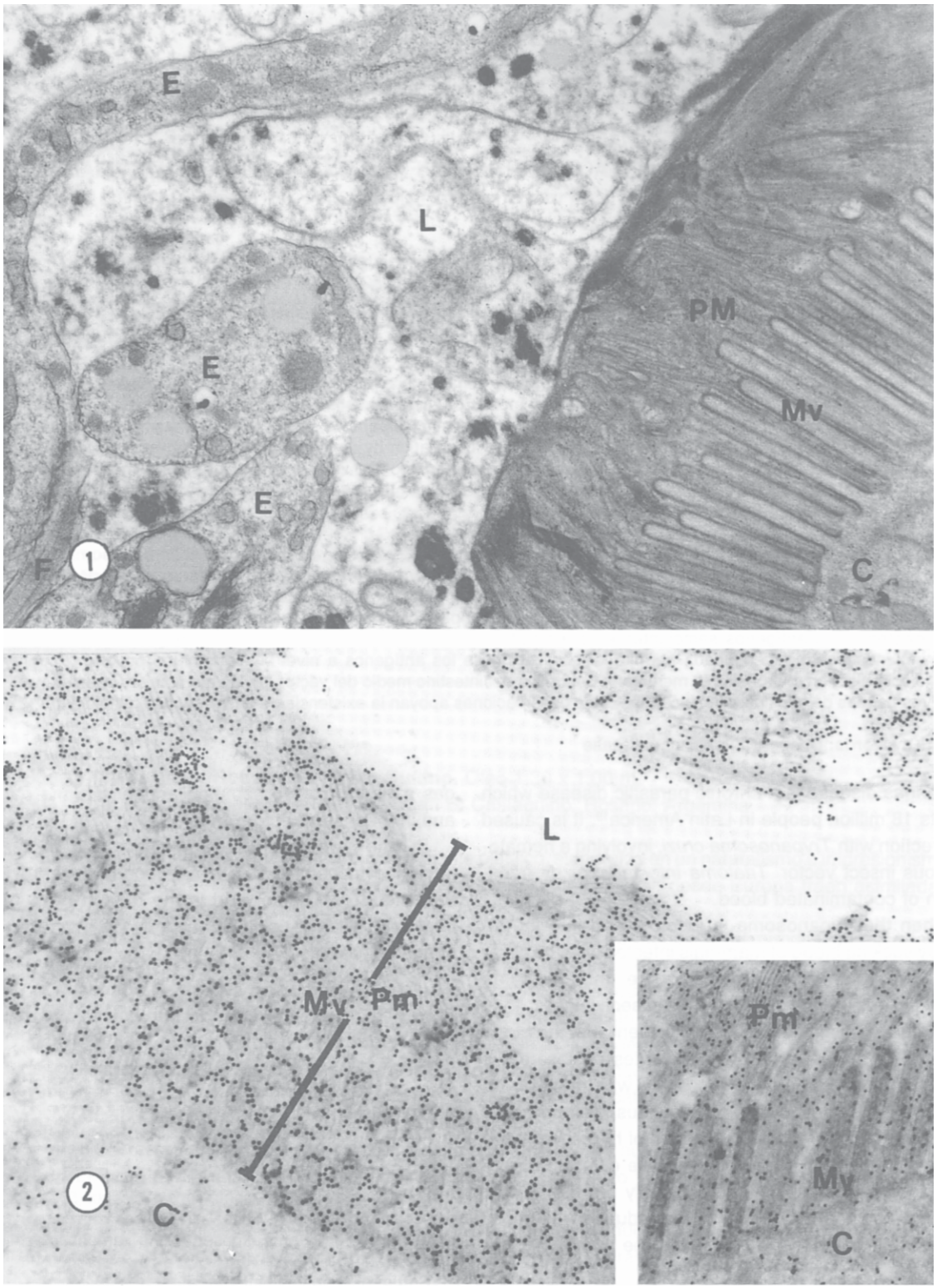

Fig. 1-2.– 1. (Trasmission Electron Microscopy TEM) Ultrastructural observation (without immunocytochemistry). Section of the apical portion of triatoma midgut epithelial cell and intestinal lumen show microvilli (Mv), cut in longitudinal orientation. It appears in palisade form. The peritrophic membrane covers the microvilli and proyects into the intestinal lumen (L). In this space appear epimastigotes (E) cut with different orientation (F) flagellum x 25.000. 2. Immunocytochemical reaction with chagasic patient serum as first antibody, labeled with colloidal particle gold, the positive reaction appears on peritrophic membrane (Pm) of triatoma midgut microvilli (Mv), lumen (L), cytoplasm (C) x 40.000.
Inset: show at low magnification the microvilli (Mv), cut in longitudinally orientation. Peritrophic membrane (Pm) with positive reaction, the cytoplasm (C) present poor reaction. x 25.000.

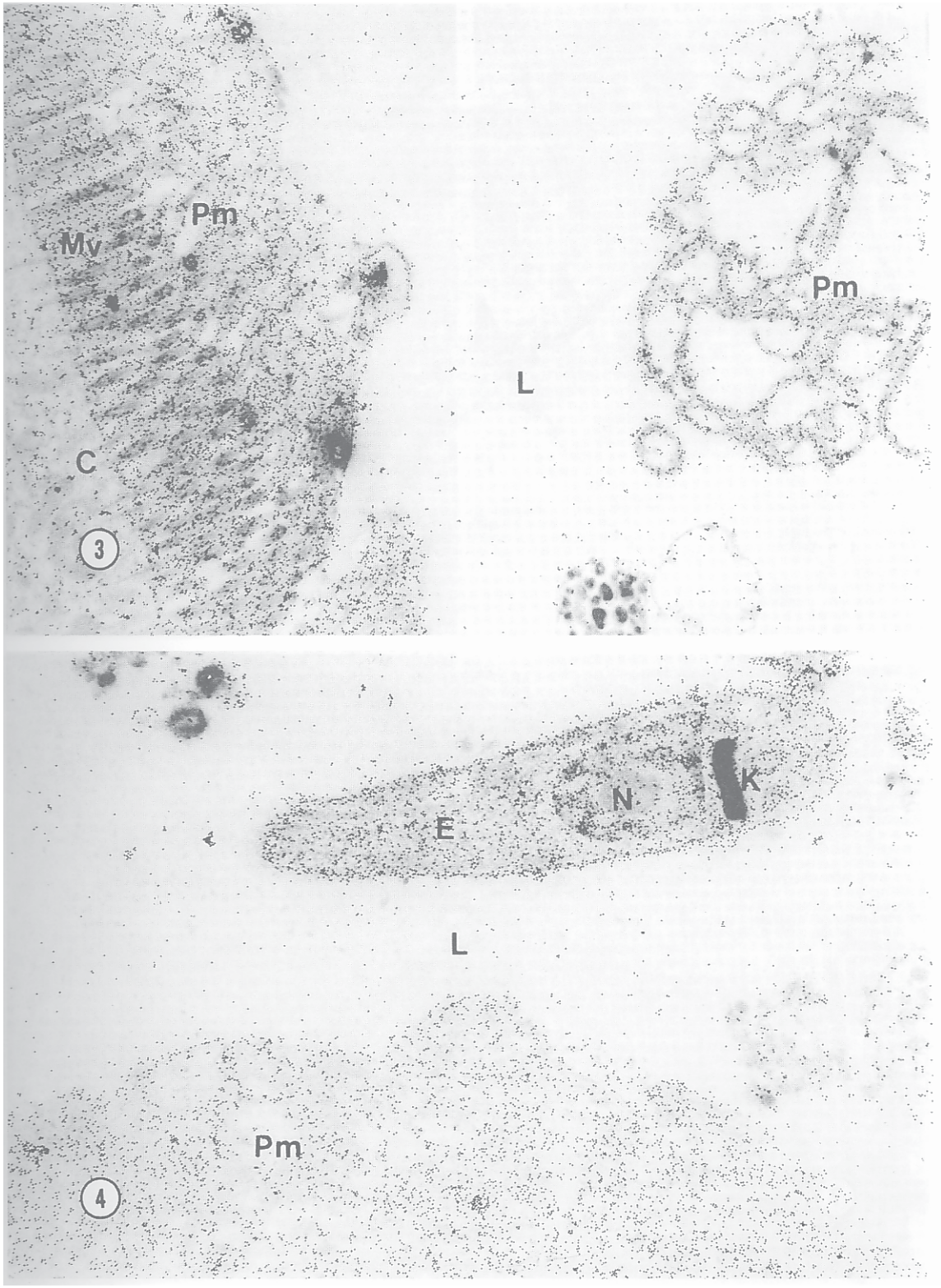

Fig. 3-4.– 3. Immunocytochemical reaction; in the apical region of the intestinal epithelium and in the lumen a portion of peritrophic membrane appear with intense reaction. x 20.000. 4. Vector midgut surface shows a portion of peritrophic membrane (PM) with intense presence of colloidal particles. The lumen (L) presents an epimastigote (E), with important reaction on the surface, the nucleus heterochromatin (N) and kinetoplast (K). x 40.000.

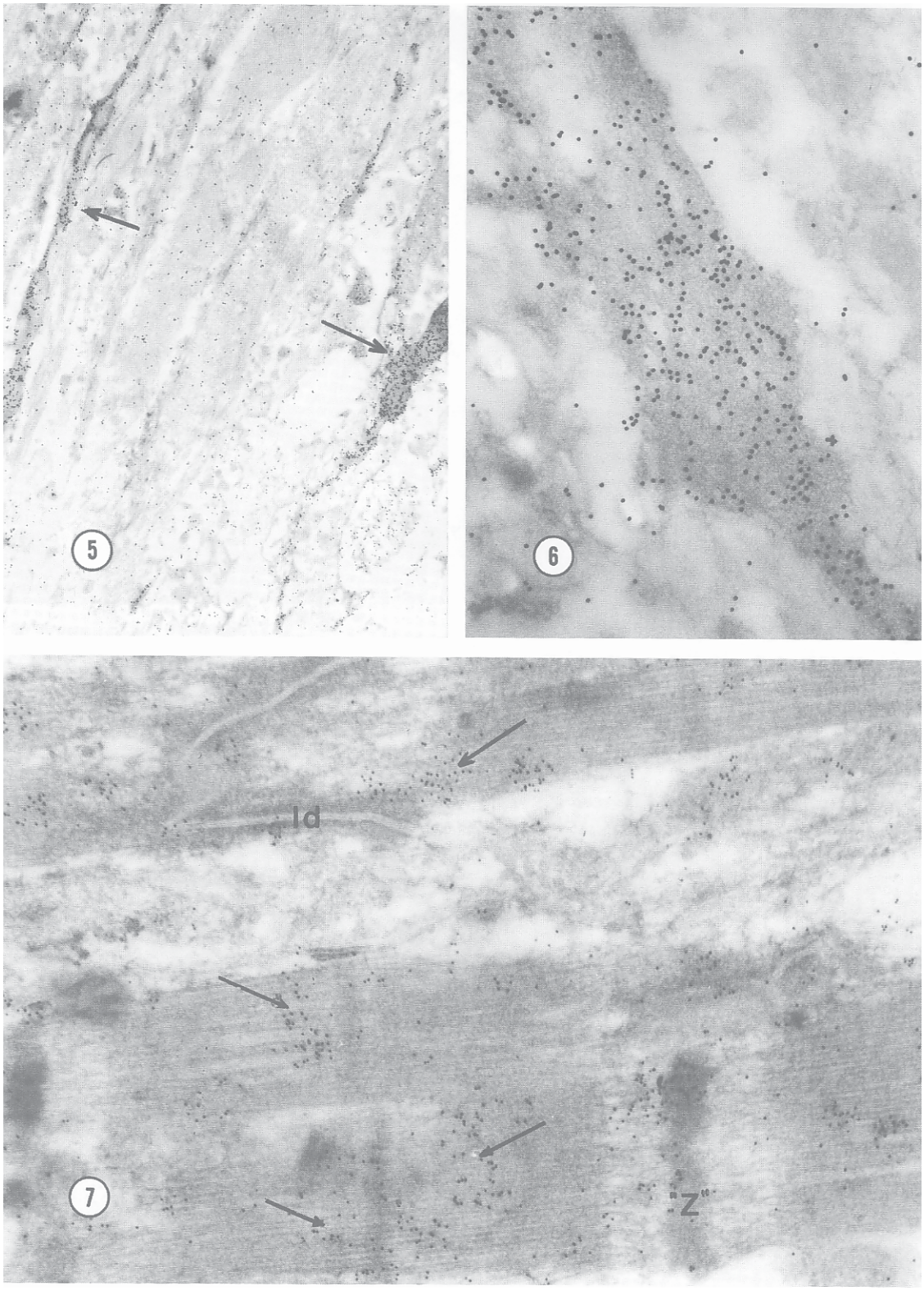

Fig. 5-7.– 5. Low magnification shows two basement membranes (arrows) with intense positive reaction, between collagen fiber of connective tissue of the heart. x 8.000. 6. High magnification of basement membrane with the positive reaction. x 35.000. 7. Ultrathin section of human cardiac muscle show a longitudinal sarcomere, with the reaction principally on "Z" line and in "A" band, the gold particles appear around miofilament (arrows). Intercalated disk (id). x 25.000.

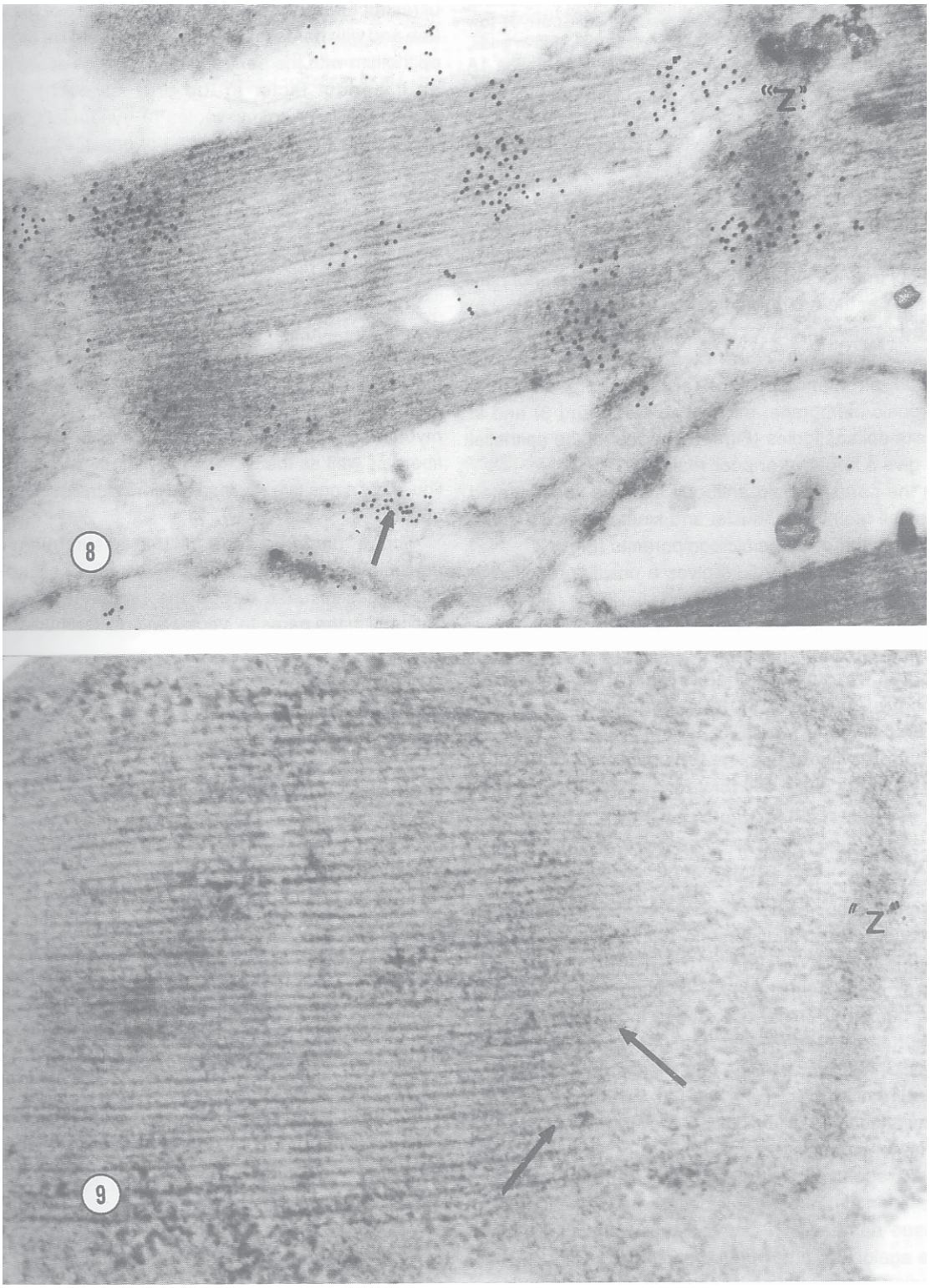

Fig. 8-9.– 8. Sarcomere at high magnification, the reaction appear in similar form of figure 7, it shows reaction in a portion of basement membrane (arrow). x 40.000. 9. Portion of sarcomera (control without immune reaction) shows at high magnification miofilaments, line "Z" and sarcoplasmic reticulum (arrows) x 60.000.

1% osmium tetroxide in 0.1 M buffer cacodylate (2 h). Finally, all samples were washed in buffer, dehydrated in progressive concentration of acetone and embedded in Spurr of low viscosity (Ted-Pella). Ultrathin sections were mounted in grids, stained with lead-uranyl and examined with a Siemens 1A electron microscope.

Results

Ultrastructure of midgut of *Triatoma infestans* shows the presence of a columnar epithelium with abundant microvilli covered by the peritrophic membrane. In the lumen of the infected vector are usually present abundant parasites in the epimastigote stage (Fig. 1).

Under immunocytochemical reaction with the antibodies present in the blood serum of chagasic patients, an intense positive reaction is present in the peritrophic membrane, microvilli (Figs. 2 and 3) and in parasite epimastigotes (Fig. 4). The rest of the epithelial cells give a negative or poor reaction (Fig. 2).

In the parasite, gold antibody appears concentrated in the cell membrane, nuclei and kinetoplast with some reaction in the cytoplasmic components (Fig. 4).

The human myocardium gives a positive reaction to the gold-antibody inside the myocardial fibers (Fig. 7 and 8), and in the basal membrane (Fig. 5, 6).

Inside the myocardial fiber, a positive reaction was observed in band "A" mainly where the myosin is concentrated (Fig. 7, 8). Some reaction was also observed in the "Z" line (Fig. 8).

Controls without the first antibody present no gold particles in the entire cell (Fig. 9).

Discussion

Chagas disease has been considered in many of the last publications as an autoimmune pathology[1, 13, 14, 15]. It is considered as a reaction of the human organism to antigens of the parasite which are homologous to some of the human proteins, specially to those of the heart[10] and gut associated disease (antigenic mimicry)[2, 6, 8]. In chronic Chagas cardiomyopathy, a basement membrane thickening of cardiac myocytes has been reported[7, 9] as well as antibodies to laminin[16]. But all these results need a clear confirmation.

In a recent paper, it has been reported that the parasitation of heart tissue is necessary for the induction of tissue damage in Chagas disease and the authors argue against an autoimmune etiology[19].

Facing this controversial situation we have studied – by immunocytochemical reactions at the level of the electron microscope– the localization of the antibodies of human Chagas positive serum in the parasite (*Trypanosoma cruzi*), the vector (*Triatoma infestans*) and human myocardial biopsies of non chagasic patients.

In previous publications we have studied the ultrastructural characteristics of the midgut of the vector, free and with parasites[3, 12]. The apical region of the midgut epithelium with the peritrophic membrane appears to be an important factor in the process of the parasite maturation from epimastigotes to trypomastigotes. The addition of midgut extracts free of parasites to a culture of epimastigotes in Warren medium causes a significant increase in the number of trypomastigotes[4].

In a previous publication we had found that the blood serum of chagasic patients recognize antigens in the parasite and in the apical epithelial region of midgut[12].

In the present report we have repeated that experiment and we have used the same pool of chagasic serum to detect antigens in the normal human myocardial biopsies. The results show again the presence of positive reactions in the parasite, in the vector midgut and in the myocardium. The basement membrane of myocardial fibers as well as the "A" band rich in myosin and the "Z" line show a positive reaction to the chagasic blood serum antibodies.

These findings support the assumption of an autoimmune response in the myocardial complications of Chagas disease due to the presence of common antigens in the parasite, vector and normal human heart.

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There is no point in looking back with regret at older, simpler days when the image of the ideal physician was relatively clear and the ways of attaining this ideal well structured. We have already entered a world where well-practiced medicine can only result from consensual actions by partners from different backgrounds, medical experience and basic interests. It is a fact, however, that a consensus on medical and public health policies is very far from being achieved. All too often, narrow and private interests lead to conflict, very much to the detriment of the only people everyone is claiming to act for, namely, our patients.

No tiene sentido rememorar viejos tiempos cuando la imagen del médico ideal era relativamente clara y la manera de alcanzar ese ideal era bien estructurada. Ya hemos entrado en un mundo en que la buena asistencia médica sólo puede ser el resultado de acciones consensuadas de partenaires de distintos orígenes, experiencia médica e intereses básicos. Es un hecho, sin embargo, que se está lejos de un consenso en política de salud. Demasiadas veces, intereses privados y mezquinos llevan a conflictos, muy en detrimento de las únicas personas para quienes todos pretenden actuar, es decir, nuestros pacientes.

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