Gap Junction Distribution Is Altered Between Cardiac Myocytes Infected With *Trypanosoma cruzi*


Conduction disturbances frequently accompany both acute and chronic Chagas' disease. To explore the possibility that changes in gap junction distribution or abundance might play a role in these disturbances, we have investigated intercellular communication between rat neonatal cardiac myocytes in cultures infected with *Trypanosoma cruzi*. Contractile activity of infected cells was characterized by regional asynchrony within the culture as well as by irregular contraction patterns. Junctional conductance between infected cell pairs was found to be significantly lower than in uninfected cell pairs, and the rapidity and extent of intercellular transfer of the dye lucifer yellow was markedly reduced between infected cells. Immunocytochemical studies demonstrated that the parasitic infection significantly decreased connexin43 expression at junctional membrane regions, correlating with the detected functional uncoupling. These findings of reduced gap junction abundance and function in trypanosome-infected cells may provide important insight into the pathogenesis of the cardiac arrhythmias that attend Chagas' disease. (*Circulation Research* 1992;70:733–742)

KEY WORDS • heart • Chagas' disease • connexin43 • parasite • chagasic cardiomyopathy

Infection with *Trypanosoma cruzi*, a hemoflagellate protozoan parasite, causes Chagas' disease, one of the most prevalent causes of heart disease in Latin America. In Central and South America, 10–20 million people are infected with *T. cruzi*, and 50,000 deaths per year can be directly linked to the disease.1,2 The parasite exists in two forms in the mammalian host, trypomastigotes and amastigotes; trypomastigotes are found in the blood and penetrate cells, where they transform into intracellular amastigotes.

Chagas' disease exhibits both acute and chronic manifestations. The acute stage of infection is characterized by myocarditis and may be accompanied by tachycardia, congestive heart failure, and cardiomegaly; the appearance of arrhythmias, heart block, or progressive congestive heart failure indicates poor prognosis.3 During acute disease, pathological damage is associated with the presence of parasites and presumably follows as a consequence of host cell cytolysis.3 However, during acute and chronic disease, amastigotes coexist with intact host cells and may interfere with host cell metabolism even in the absence of gross pathological changes.4 For example, infection inhibits expression of muscle-specific mRNAs (thereby inhibiting myotube differentiation),5 alters G protein–mediated functions of myoblasts,6 and affects cAMP metabolism and Ca2+ mobilization in endothelial cells.7,8

Chronic chagasic cardiomyopathy generally appears after extended asymptomatic periods and is often accompanied by myonecrosis, myocyte lysis, and contraction band necrosis. Focal and diffuse areas of myocardial hypertrophy may be seen with or without inflammatory infiltrates. In other areas, focal fibrosis is evident, replacing previously damaged myocardial tissue.3

Spread of excitation through the heart may be affected in both acute and chronic Chagas' disease, leading to arrhythmias and other conduction disturbances.9–13 Because the mechanisms responsible for these conduction abnormalities are unclear, we have investigated effects of *T. cruzi* infection on intercellular current flow and the distribution of intercellular connections (gap junctions) in cultured cardiac myocytes.

Gap junctions are specialized regions of adjoining cell membranes that are composed of intercellular low resistance channels.14,15 In the heart, gap junctions mediate electrotonic current flow, thereby coordinating the spread of excitation and subsequent contraction throughout the myocardium.16 Recent computer mod-
eling studies strongly implicate changes in electrical coupling as a contributory mechanism in reentrant arrhythmias,17–20 and experimental studies demonstrate that arrhythmias can develop when junctional conductance (gj) is lowered by various manipulations.21–27 Moreover, there are indications that remodeling of gap junction connections in ventricular muscle with an overall decrease in gap junction expression occurs during aging and after injury to the heart, such as in myocardial infarction.28–33 Thus, it is apparent that changes in gap junction-mediated intercellular communication may play a fundamental role in the physiological dysfunction of cardiac tissue.

To assess the extent to which gap junction abnormalities might contribute to aberrant current flow between T. cruzi–infected cardiac cells, we have assayed intercellular communication by examining a variety of gap junction–related parameters including synchrony of contractions, gj, and dye coupling. In addition, we have compared the intracellular distribution of the cardiac gap junction protein (connexin43) in cultures of infected and uninfected neonatal rat cardiac myocytes. Our results demonstrate that infection of heart cells by T. cruzi leads to the loss of intercellular communication and that this loss of function is restricted to cells that are infected. These observations may provide a basis for understanding some aspects of the pathogenesis of the conduction disturbances and arrhythmias that attend acute Chagas’ disease.

Materials and Methods

Preparation and Infection of Cardiocytes

Neonatal rat cardiocytes were prepared according to a modification34 of the techniques of Harary and Farley.35 Briefly, the hearts of 0–2-day-old rats were removed, minced, and subjected to sequential 15-minute periods of digestion in pancreatin (GIBCO, Grand Island, N.Y.). The supernatant fraction containing single cells from each digestion period was centrifuged, resuspended in culture medium, and preplated into a large Petri dish for 90 minutes to allow the nonmuscle cells to attach. After this period the unattached muscle cells were plated (2×10^5 cells/cm^2) into 60-mm tissue culture dishes with or without glass coverslips. The cells formed a confluent monolayer that beat spontaneously after 2–3 days in culture. To obtain cell pairs, cells were plated at a lower density (1×10^5 cells/cm^2). Arabinosylcytosine (3 μg/ml) was added to the culture medium during the first 12 hours of culture to eliminate residual fibroblast contamination. Cells were maintained in 5% CO_2 in air at 37°C in a humidified incubator. Culture medium (RPMI 1640 with 5% fetal calf serum, GIBCO) was changed at 12 hours after arabinosylcytosine addition and every other day thereafter. One to 3 days after the cardiocytes were plated, they were infected with trypanosomes of the Tulahuen strain of T. cruzi that were propagated in L_Eb myoblasts5 at a multiplicity of infection of ~1:1. At day 2 of infection, the percent parasitism was determined by staining replicate dishes in situ with May-Grünwald–Giemsa.36 The percent parasitism was consistently 30–50% at the time of these studies. We have taken particular care not to examine overtly damaged cardiocytes; we routinely monitor these cultures by light and electron microscopy to assure that the cells studied are intact.

Contractility

Spontaneous contractile activity in cultures infected with T. cruzi was measured optically. An image analysis system (Image 1/AT, Universal Imaging Corp., West Chester, Pa.) was used to measure the brightness of six to eight small areas (3–25 μm^2, generally separated by >0.2 mm) of a field (×100 magnification) of confluent cells over time. Because of changes in refractivity of the phase-contrast image, contractions are detectable as changes in brightness, allowing evaluation of synchrony, rate, and variability of intercontraction intervals. Bathing solution for these studies contained (mM) NaCl 133, KCl 3.6, CaCl_2 1.0, MgCl_2 0.3, glucose 16, and HEPES 3.0, pH 7.2.

Junctional Conductance

Each cell of a pair was penetrated with brief, strong suction after high-resistance seals were obtained against the cell membrane using polished patch pipettes (3–5 MΩ, filled with [mM] potassium glutamate 120, KCl 10, EGTA 10, CaCl_2 1, MgCl_2 1, and HEPES 10 at pH 7.2, with or without MgATP 2–5). Bathing solution for these studies contained (mM) NaCl 133, KCl 3.6, CaCl_2 1.0, MgCl_2 0.3, EGTA 0.5, glucose 16, and HEPES 3.0, pH 7.2. Cells were voltage-clamped at holding potentials of −60 mV, and command steps were presented alternately to each cell of a pair. g_0 was measured as the current evoked in one cell by the voltage step in the other divided by the amplitude of the voltage step delivered to the other cell.37,38

Dye Coupling

Lucifer yellow CH (5% [wt/vol] in 150 mM LiCl) was injected through microelectrodes (~20 MΩ if filled with 3 M KCl) using short current pulses or brief overcompensation of the negative capacitance compensation on an electrometer (model M701, World Precision Instruments, New Haven, Conn.). Epifluorescence was examined on a Nikon Diaphot microscope equipped with xenon arc illumination and fluorescein isothiocyanate barrier and excitation filters. Fields of injected cells were photographed 1 minute after the injection was initiated by exposing Kodak TMAX 400 film for 30 seconds.

Antibodies

Antisera were prepared to bovine serum albumin conjugates of peptides corresponding to amino acids 241–260 (KGRSPDPYHATTTGPLPSKDC, antibody 16A) and 346–360 (KVAAGHELQPLAIVDM, antibody 18A) of the rat connexin43 sequence.39 A cysteine was added at the amino terminus of peptide 346–360 to facilitate coupling chemistry. For affinity purification of the antibody to peptide 346–360, peptide was conjugated to an Ultrafineity EP high-performance liquid chromatography column (Beckman Instruments, Inc., Fullerton, Calif.), and antiserum was passed over the column as per the manufacturer’s suggested procedure. Unbound antibodies and proteins were removed by an extended buffer wash, and antibody was eluted with 2 ml of 0.1 M glycine-HCl, pH 2.1. The eluted antibody was neutralized and dialyzed against Dulbecco’s phosphate-buffered sa-
Immunofluorescence represents infected cultures, cells within fixed were washed with Dulbecco’s PBS, frozen and washed with PBS, and incubated with secondary goat anti-rabbit immunoglobulin G conjugated to fluorescein isothiocyanate for 60–90 minutes at room temperature. For competition experiments, excess peptide antigen was preincubated with the antisera for 1 hour at room temperature.

Western Blots
Western blots were performed with cultured L<sub>6</sub>E<sub>6</sub> cells and isolated extracellular amastigotes that were obtained according to the method of Andrews et al. Cells were washed three times with cold PBS containing freshly added phenylmethylsulfonyl fluoride (1 mM) to inhibit proteolysis and solubilized in Laemmli sample buffer. For each sample, 50 μg protein, as determined by protein assay (Bio-Rad Laboratories, Richmond, Calif.), was loaded in 12.5% sodium dodecyl sulfate–polyacrylamide gels and then electrophoretically transferred to a nitrocellulose membrane. The membranes were incubated with antibody 18A and then incubated with 125I-labeled protein A. This antibody has been shown to react specifically with connexin43 in gap junctions through immunofluorescence and immunoelectron microscopy.

Results
Figure 1 shows examples of patterns of spontaneous contractions recorded from control and T. cruzi–infected cultures of neonatal rat cardiac myocytes, using the brightness-versus-time feature of the Image 1/AT system. This imaging approach takes advantage of the change in light scattering when individual myocytes contract to give continuous sampling of a large number of small regions within the same field. As is shown in this figure, contractions usually occur rhythmically, with little variation in intercontraction interval in the control group. In contrast, contraction patterns of T. cruzi–infected myocardial cells in culture were strikingly different from matched uninfected controls. When examined under low magnification, fields of parasitized cells exhibited slower spontaneous contractions, irregular intercontraction intervals, and frequent asynchronous regional contractions. In infected cultures, the rate of spontaneous beating was lower than in controls (decrease of 51±9% [mean±SEM] in matched experiments on control and infected cells from three separate preparations), the intercontraction interval was variable (top example), extra contractions were frequent (middle example), and rapid contractions occurred, followed by long periods of quiescence (lower tracings). The most common of these patterns, observed in virtually all the well-infected dishes examined, was that of asynchrony
or failure of contractions to spread uniformly throughout the dish.

To determine whether functional coupling was altered among the infected cells, lucifer yellow was injected into one cell of a cluster, and the rapidity and extent of dye spread to neighboring cells was monitored. In uninfected dishes, the injected dye spread rapidly to contiguous cells (Figure 2B), so that within 1 minute all contiguous and numerous second-order cells were stained. However, in infected dishes the dye was either totally or mainly confined to the injected cell with only occasional faint spread to adjacent myocytes (Figure 2D). Figure 3 shows that the presence of the parasite inside at least one of the cells was required for the reduction in dye coupling to take place. Low-density plated cardiac myocytes from control (Figures 3A and 3B) and infected (Figures 3C–3F) cultures were injected with lucifer yellow. Dye injected into uninfected cells spread readily to the neighboring cells in uninfected dishes (Figures 3A and 3B) and also between nonparasitized cells within dishes in which other cells were infected with the parasite (Figures 3C and 3D). By contrast, in the parasitized cells from infected dishes, dye was confined to the injected cell, as is illustrated in Figures 3E and 3F. Here, the middle cell, which was not parasitized, was injected with lucifer yellow, and there was no dye spread to either flanking cell, both of which were well parasitized. This finding of strong dye coupling in nonparasitized cells within infected dishes indicates that the uncoupling is linked to interaction of the parasite with the myocyte rather than to action of a soluble factor released by the parasite into the medium. In numerous other experiments on clusters of heart cells where some cells were infected and others were not, dye injection into infected cells led to little diffusion to other cells, whereas dye diffusion from uninfected cells was restricted to those cells that were not parasitized. This finding of dye spread among uninfected cells that were contiguous with parasitized cells further suggests that alterations in the extracellular matrix are not responsible for the altered distribution of gap junctions in these cells.

To quantify this apparent difference in junctional permeability between infected and noninfected cells, myocytes were cultured at low density so that pairs were available for electrophysiological study at 3 days after plating (at 48 hours after infection). In pairs where both cells were visibly infected, gj averaged 0.7±0.5 nS (mean±SEM, n=7 cell pairs, three experiments [where an experiment refers to an individual cardiocyte dissociation followed by infection]), and in pairs where only one of the cells was visibly infected, gj averaged 5.9±3.3 nS (mean±SEM, n=5 cell pairs, three experiments). These values do not differ significantly from one another, but averaged together, they are significantly lower (p<0.001, Student’s t test) than the average gj value of 24.6±5.6 nS for matched controls (mean±SEM, n=6, three experiments). The value obtained under control conditions is consistent with a mean gj of 19.2±0.7 nS seen in 68 other experiments under similar conditions,44 and in Figure 4 we used the pooled data from these 74 experiments to represent the gj of cell pairs under control conditions. In three cell pairs studied, strong coupling was also seen between uninfected cells in infected dishes (average gj of 17.8 nS).

The observed reduction in dye coupling and gj between infected cells could be due to either closure of operational junctional channels present in the membrane or decreased expression of the gap junction protein between the cells.16,44 To evaluate whether gap junction expression altered as a function of myocyte infection with T. cruzi, we used immunocytochemical techniques to localize gap junction protein within the infected cells. Immunostaining using connexin43–specific gap junction antibodies demonstrated striking dif-
ferences between controls and infected cells. In control cultures, typical gap junction immunoreactivity was seen at virtually every interface between the

cultured cells (Figures 5A and 5B). In contrast, gap junction immunoreactivity was seen only rarely at interfaces between infected cells, although adjacent uninfected cells showed normal staining of intercellular membranes (Figures 5C and 5D). Surprisingly, considerable staining in infected cultures was intracellular, corresponding to locations of the amastigote form of the parasite (Figure 5D). Similarly intense staining of amastigotes was obtained with connexin43-specific antibodies obtained from Dr. Eric Beyer (not illustrated).

These findings could imply that infection led to a redistribution of junction protein, so that it became confined to the parasite and was lost from the cell membrane. To further investigate the nature of this cross-reactivity, we performed Western blots of purified amastigotes, obtained as described in "Materials and Methods," and compared the staining pattern obtained with that from uninfected L6E9 cultures, both in the absence and presence of an excess of the peptide antigen (amino acids 346–360 of the rat connexin43) used to produce one of the connexin43 antibodies. A faint band could be identified in the control amastigote lane (AMA in Figure 6), which comigrated with the connexin43 proteins of L6E9 cells (arrowheads in Figure 6) and was not present after absorption with excess peptide antigen (+ peptide in Figure 6); this band is possibly due to minor contamination of the isolated amastigotes with L6E9 cellular debris. However, the immune serum labeled high molecular weight proteins most strongly. These high Mr proteins, as well as more rapidly migrating ~30-kd species, are not associated to

![Bar graph showing junctional conductance values in control and infected cell pairs studied under the dual whole-cell patch-clamp technique.](image)

FIGURE 3. Photomicrographs (left panels) with corresponding fluorescence micrographs (right panels) showing that reduced dye coupling in infected cultures is not due to the release of a soluble factor into the culture medium. Dye coupling is extensive in cell pairs from uninfected dishes (panels A and B) or from nonparasitized cell pairs obtained from infected dishes (panels C and D). In panels E and F, the middle cell in an infected culture dish was injected with dye, and there was no detectable spread of lucifer yellow to either of the parasitized neighboring cells.
Figure 5. Phase-contrast micrographs (panels A and C) and fluorescence micrographs (panels B, D, and F) showing localization of gap junctions in cardiac myocyte cultures using an antibody prepared against polypeptide 346–360 of connexin43. Immunocytochemically localized gap junctions are prominent at interfaces between cells under control conditions (panels A and B) but are less extensive between infected cells (panels C and D). Note that cells adjacent to infected cells have relatively normal gap junction staining (arrowheads in panel D), whereas punctate intercellular connexin43 immunoreactivity is almost entirely absent in infected cells. Intracellular connexin43 immunoreactivity in the infected cells is localized strikingly to intracellular forms of the parasites (amastigotes, arrows in panels C and D). Panel E, taken after competition of the antibody with the peptide antigen, indicates that the parasite is still stained, suggesting that the antigen recognized by the serum is not connexin43. Panel F, taken after immunocytochemical staining with preimmune serum, indicates that there is recognition of the amastigote by the preimmune serum. Scale bar in panel F, 50 µm.

Connexin43 as demonstrated by the persistence of the label in the presence of an excess of peptide antigen (Figure 6, + peptide). Figure 5E illustrates the competition experiment with immunostaining of infected cardiac myocytes. In the absence of the synthetic antigen, junctional regions as well as the amastigotes were recognized by the immune serum (Figure 5D). In the presence of peptide, there was total disappearance of the immunoreactive sites at cell interfaces (corresponding to the expected localization of the junctional protein), but the amastigotes inside the cells were still labeled by the serum (Figure 5E).
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The labeling in amastigotes the bands excess of proteins. labeled with the immune were patternsorescent the blots) other antibodies the connexin43 of 360 cells cross-reactivity and the parasite. To these circumstances and the nostaining of control and infected cultures. lacks reactivity of the antiserum with the intracellular parasite. To more rigorously exclude this possibility, we affinity-purified the antibody and repeated the immunostaining of control and infected cultures.

These results obtained using competition of synthetic peptide for connexin43 binding sites indicated that the apparent cross-reactivity of connexin43 antiserum with the amastigotes was probably due to the presence of other antibodies in the connexin43 antiserum. Thus, the observed cross-reactivity between the immune serum and the amastigotes was most probably not related to the association of connexin43 with the intracellular parasite. To more rigorously exclude this possibility, we affinity-purified the antibody and repeated the immunostaining of control and infected cultures.

Figure 7 illustrates a comparison of the immunofluorescent patterns obtained from uninfected (Figures 7A and 7B) and infected (Figures 7C and 7D) cultures using the affinity-purified antibody and also illustrates the efficacy of the competition control experiment under these circumstances (Figure 7E). Junctional protein lacks reactivity with preimmune serum, but the intracellular amastigotes are slightly labeled even by the preimmune serum (Figure 7F; see also Figure 5F). Note that the immunoreactivity in control cultures is striking at membrane borders and that in infected cultures this appositional staining is much reduced, although adja-
cent uninfected cells still display gap junction immunoreactivity. When these immunofluorescence micrographs of infected cells are compared with those performed with the same antibodies before affinity purification (Figure 5), the major difference is the lack of labeling of the intracellular amastigotes. Thus, amastigote labeling apparent in Figure 5 is attributable to binding of parasite-associated antigens recognized by several rabbit sera that are not homologous to connexin43 antigens (higher and lower molecular weight bands in the Western blot, Figure 6).

Discussion

The data presented here demonstrate that infection of rat neonatal cardiac myocytes with T. cruzi is accompanied by marked disturbances in intercellular communication. Dye permeance and g between infected cells are decreased, and synchrony and rhythmicity of contractions in infected myocyte cultures are decreased compared with controls; these functional alterations are associated with the disappearance of gap junction protein from the appositional regions of the cell membranes of infected cells. These findings would predict that infection of the heart with this parasite should result in a slowing of cardiac conduction and alter patterns of propagation as a consequence of decreased strength of electrotonic coupling.

Preliminary experiments indicate that similar changes in connexin43 distribution and disruption of intercellular communication occur in other cell types obtained from a variety of tissues, including leptomeningeal cells, astrocytes, corpus cavernosum smooth muscle, and endothelial cells in culture (A.C. Campos de Carvalho, J.A. Kessler, G. Christ, S. Morris, and D.C. Spray, manuscript in preparation). Because acute infection is associated with parasitism of other tissues as well as of heart, gap junction dysfunction in these other tissues could also contribute to the pathophysiological consequences of the acute disease. It is perhaps noteworthy that the major gap junction protein in each of the cell types examined is connexin43; whether trypanosome infection similarly affects the distribution of the other members of the connexin family that are expressed in other cell types is currently under investigation.

Nature of the Defect in Intercellular Communication

The disappearance of connexin43 and loss of functional coupling are confined to the infected cells; apparently normal intercellular connexin43 staining, g, and dye coupling persist in uninfected cells adjacent to those that are infected. This finding indicates that the change in expression and intracellular distribution of connexin43 as a consequence of infection is not due to soluble factors released into the medium or infection-related changes in the extracellular matrix. Thus, the previously reported effects of hormones and matrix composition on gap junction expression are unlikely to explain the changes reported here.

The cross-reactivity between the amastigotes and three distinct polypeptide antisera raised in two different laboratories was rather surprising. Immunoelectron microscopic localization studies using the non-affinity-purified sera showed intense labeling in the nucleus and kinetoplast regions of the amastigotes (data not shown),
FIGURE 7. Immunofluorescence of control and *Trypanosoma cruzi* (Tc)-infected cultures using affinity-purified antibody against polypeptide 346–360 of connexin43. Panel A shows the bright field and panel B shows the fluorescent label obtained in control cultures. Panels C and D show the corresponding records obtained in infected cultures. Immunolabeling localized to gap junctions is present between uninfected cells in the field (arrows in panels C and D) and is much less extensive between infected cells (amastigotes in one cell indicated by Tc in panels C and D). In contrast to results obtained with non-affinity-purified antibody, the amastigotes are not labeled by the affinity-purified antibody. Panel E shows the immunofluorescence obtained during competition of the labeling with the peptide antigen (note the lack of staining of either amastigotes or junctional membranes in this panel compared to Figure 5E). Panel F indicates the immunofluorescence obtained using the preimmune serum in infected cell cultures (note that staining of the intracellular parasites is evident). Scale bar in panel F, 50 μm.

which initially suggested that labeling might not be specific for connexin43. The Western blots of extracellular amastigotes showed only minor labeling of a connexin43 comigrating band, which could be due to contamination of the purified amastigotes with connexin43-containing cell debris. However, strong labeling was present in higher and lower molecular weight bands (M, >90, <30 kD). The competition with the peptide antigen revealed the non-connexin43-related nature of the interaction between the immune serum and the high molecular weight proteins of the amastigotes. Additionally, the faint band detected by Western blot analysis was indeed shown to be connexin43. Moreover, we detected antibody staining of intracellular parasites in cultured
SKHep1 cells (a highly metastatic human hepatoma cell line) in which connexin43 is not expressed (data not shown). Finally, the immunofluorescence line) in which connexin43 is responsible for the decreased expression of connexin43 at the surface of the infected cells.

**Specificity of Effects of Infection on Gap Junctions**

As already noted, intracellular amastigotes may reside within host cell cytoplasm for prolonged periods of time without altering cell or tissue structure but with profound alterations in host cell metabolism. Cell or tissue damage is thought to be related to disintegration of these intracellular forms. In the experiments reported here and at the times after infection that the cells were studied, there was little cytolysis, as evidenced by light and electron microscopic examination of the cultures. We have demonstrated that intracellular amastigotes can live for long periods in the cytoplasm of a variety of host cells and may alter aspects of host cell metabolism. For example, we have shown that infection of myoblasts results in inhibition of differentiation into myotubes and is associated with alterations of muscle-specific mRNAs, whereas mRNAs for other nonspecific proteins are unaffected. In addition, infection of endothelial cells influences several important cell functions such as the mobilization of intracellular calcium and the generation of cAMP and prostacyclin. In addition, we have recently demonstrated that infection of cells does not result in alterations in α-tubulin mRNA (H.B. Tanowitz, J.P. Gunpechht, D. Spurr, T.M. Calderon, M.C. Ventura, C. Raventos-Suarez, S. Kellie, S.M. Factor, V.B. Hatcher, M. Wittner, J.W. Berman, submitted manuscript). However, infection of endothelial cells resulted in the upregulation of gene expression for interleukin-1β, interleukin-6, and colony stimulating factor-1, whereas that for c-sis was decreased.

**Role of Gap Junction Changes in Chagas’ Disease**

Conduction disturbances are evident in both acute and chronic Chagas’ disease. We show in this study that gap junctions are reduced between infected cardiac myocytes, which could contribute to retarated conduction. In contrast to that observed in acute disease, the number of parasites bears little relation to chronic myocardial pathology. The pathological findings that accompany chronic chagasic cardiomyopathy often show few, if any, organisms and have led to the notion that the pathogenesis of chagasic cardiomyopathy has an autoimmune or neurogenic basis. More recently, however, a microvascular etiology has been suggested.

Recent clinical studies suggest that the cardiomyopathy associated with chronic Chagas’ disease is the result of a progressive, focal compromise in the integrity of both the myocardium and the conduction system. In chronic chagasic cardiomyopathy, the presentation of reentrant arrhythmias might be accelerated because of the decreased safety factor for intercellular communication as focal interstitial fibrosis, myocytolysis, and myocrosis add geometrically to effects of other factors that tend to split the aging heart into slowly conducting units.

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