

## INVOLVEMENT OF THE AUTONOMIC NERVOUS SYSTEM IN CHAGAS HEART DISEASE

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*The autonomic nervous system and especially the intracardiac autonomic nervous system is involved in Chagas' disease. Ganglionitis and periganglionitis were noted in three groups of patients dying with Chagas' disease: 1) Those in heart failure; 2) Those dying a sudden, non violent death and; 3) Those dying as a consequence of accidents or homicide. Hearts in the three groups also revealed myocarditis and scattered involvement of intramyocardial ganglion cells as well as lesions of myelinic and unmyelinic fibers ascribable to Chagas' disease. In mice with experimentally induced Chagas' disease we observed more intensive neuronal lesions of the cardiac ganglia in the acute phase of infection. Perhaps neuronal loss has a role in the pathogenesis of Chagas cardiomyopathy. However based on our own experience and on other data from the literature we conclude that the loss of neurones is not the main factor responsible for the manifestations exhibited by chronic chagasic patients. On the other hand the neuronal lesions may have played a role in the sudden death of one group of patients with Chagas' disease but is difficult to explain the group of patients who did not die suddenly but instead progressed to cardiac failure.*

Key words: Chagas' disease. Trypanosomiasis cruzi. Chagas' heart disease. Autonomic nervous system.

Lesions of the autonomic nervous system (ANS) and especially of the intracardiac autonomic nervous system are an almost constant occurrence in human *Trypanosoma cruzi* infection in Brazil<sup>7, 8</sup>. These lesions appear both during the acute phase of the disease in human patients<sup>10</sup> and under experimental conditions<sup>14</sup> and persist throughout its evolution. They are commonly found in cases which evolve towards congestive heart failure (CHF) which represents the most clinically important group in Chagas' disease. Less studied pathologically are hearts from patients with disturbances of the cardiac rhythm, accompanied or not by CHF and in cases without apparent symptoms and meeting with sudden death due to the disease or to other, often violent causes unrelated to Chagas disease such as accidents and homicide. In the present study we compare hearts of Chagasic patients who: 1) died

in congestive heart failure; 2) died suddenly, presumably from the disease or 3) experienced a violent death. In order to appreciate better the fine structure of the automatic nervous system in Chagas disease, the human lesions were also compared to those produced experimentally in mice.

### MATERIALS AND METHODS

Forty five hearts are available for study. There were 15 patients in each category of the three types of death. The 15 cases dying with heart failure derived from two University Hospitals, whereas the cases of sudden and violent deaths came from the Coroner's office. All hearts were removed at autopsy and the pericardial fluid was collected fresh and assayed by immunofluorescence, complement fixation and hemagglutination for *T. cruzi* infection<sup>11</sup>. Each heart was fixed by the instillation of 10% formalin into the chambers and tying the major blood vessels. They were weighed and sectioned coronally, after which histological samples were taken. The 4 sections were stained with hematoxylin and eosin, Giemsa, Masson's trichrome and Weigert - Van Gieson. In 5 cases the intracardiac conduction system was examined, as previously described by Andrade<sup>2</sup>; in 15 cases the number of neurons within the heart were counted by the technique of Lopes<sup>9</sup>.

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Chagas' disease was reproduced by injecting mice with *T. cruzi*, as described by Tafuri<sup>1,4</sup>.

Hearts of mice with experimentally induced Chagas' disease (18 with acute infection and 11 with chronic infection) were examined as with the human cases. In addition, fragments of subepicardial ganglia and atrial and ventricular myocardium were fixed in glutaraldehyde, embedded in plastic, sectioned and examined by microscopy following treatment by lead citrate and uranyl acetate.

The Student's test was used to evaluate human hearts weight differences to a 5% level of significance.

## RESULTS

Tables 1 – 3 summarize clinical and patholo-

gic data in the three groups of human cases under investigation. All cases had at least one of the three serologic tests for Chagas' disease positive in the pericardial fluid. The number of neurons in control cases (non Chagasics) varied from 1872 to 2099 (mean: 2001).

Gross Pathology – In the group of patients dying in congestive heart failure there was cardiomegaly in all but two cases. The heart weight ranged from 260 to 640 g. (Mean = 506g). The heart without cardiomegaly weighed 260 g but revealed epicarditis and neuronal loss. Another was only slightly dilated and hypertrophic (360 g) but showed epicarditis and a left ventricular apical aneurysm.

Table 1 – Morphologic findings of hearts from 15 Chagas patients who died in heart failure

Case no	Age (yrs)	Sex	Heart, Wt (g)	Macroscopy			Microscopy					Serology				
				Epicarditis	Lymph node	Apical Lesion	Inflammation		No. of Neurons	Conduction System				Pericardial fluid		
							Atrium	Ventricle		S.A.N.	A.V.N.	B.H.	L.B.	C.F.R.	I.F.R.	H.A.R.
01	28	M	640	+	—	+	000	000	154		N/A			R	R	N/A
02	22	M	360	+	—	+	00	00	1707		N/A			R	R	N/A
03	32	M	410	+	—	—	0	0	363		N/A			R	R	N/A
04	14	M	450	+	—	—	000	000	zero		N/A			R	R	N/A
05	30	M	600	+	—	—	000	000	170		N/A			R	R	N/A
06	80	M	260	+	—	—	000	000	311		N/A			R	R	N/A
07	40	M	620	+	—	—	00	00	N/A		N/A			A/C	R	R
08	65	M	500	+	—	—	000	000	N/A		N/A			R	R	R
09	38	M	550	+	—	—	000	000	N/A		N/A			R	R	R
10	42	M	460	+	—	—	—	—	N/A		N/A			R	R	R
11	43	M	560	+	—	+	0	0	N/A		N/A			R	R	R
12	42	M	630	+	—	+	000	000	N/A		N/A			R	R	R
13	46	M	450	+	—	—	000	000	N/A		N/A			I	R	R
14	27	M	640	+	—	+	000	000	N/A		N/A			R	R	R
15	50	M	460	+	+	+	00	00	N/A		N/A			R	R	R

S.A.N.	= Sino-Atrial Node	0	= mild
A.V.N.	= Atrio-ventricular node	00	= moderate
B.H.	= Bundle of His	000	= severe
L.B.	= Left branch	C.F.R.	= Complement fixation reaction
N/A	= Not available	I.F.R.	= Immunofluorescence reaction
+	= present	H.A.R.	= Hemoagglutination reaction
—	= absent	R	= Reactive
		A/C	= Anticomplementary

As a group, patients experiencing sudden death had hearts varying in weight from 290 to 470 g (Mean = 360 g). The cardiomegaly in 9 cases of the group which experienced sudden

death ranged from 340 to 470 g. Six of the fifteen cases had heart weights ranging from 290 to 320 g but all showed epicarditis and one had an apical aneurysm of the left ventricle.

Table 2 – Morphologic features of hearts from 15 Chagas patients who died suddenly

Case No	Age (yrs)	Sex	Heart Wt (g)	Macroscopy			Microscopy					Serology Pericardial fluid					
				Epicarditis	Lymph Node	Apical Lesion	Inflammation		No. of Neurons	Conduction System					C.F.R.	I.F.R.	H.A.R.
Atrium	Ventricle	S.A.N.	A.V.N.	B.H.	R.B.	L.B.											
01	30	M	300	+	-	-	000	0	zero	N/A	N/A	N/A	N/A	N/A	R	R	NR
02	19	M	400	+	-	-	000	0	141	N/A	N/A	N/A	N/A	N/A	R	R	NR
03	28	M	320	+	-	-	00	00	193	N/A	N/A	N/A	N/A	N/A	R	R	NR
04	34	F	N/A	+	+	+	00	00	1003	N/A	N/A	N/A	N/A	N/A	R	R	R
05	30	M	340	+	+	-	0	00	676	N/A	N/A	N/A	N/A	N/A	R	NR	NR
06	38	M	400	+	-	-	00	00	N/A	N/A	N/A	N/A	N/A	N/A	A/C	R	R
07	49	M	420	+	+	-	0	0	N/A	Alt	Alt	Alt	Alt	Alt	R	R	R
08	39	M	400	+	+	+	N/A	N/Alt	N/Alt	N/Alt	N/Alt	N/Alt	N/Alt	N/Alt	R	R	R
09	-	M	290	+	-	-	-	-	N/A	N/Alt	Alt	Alt	Alt	Alt	R	R	NR
10	45	M	300	+	+	-	0	00	N/A	N/A	N/A	N/A	N/A	N/A	NR	R	R
11	45	F	300	+	-	+	00	00	N/A	N/A	N/A	N/A	N/A	N/A	NR	R	R
12	33	M	440	+	-	-	000	000	N/A	N/A	N/A	N/A	N/A	N/A	R	R	NR
13	56	M	470	+	-	+	0	00	N/A	N/A	N/A	N/A	N/A	N/A	R	R	NR
14	27	F	N/A	+	+	+	0	0	N/A	N/A	N/A	N/A	N/A	N/A	R	R	NR
15	40	M	300	+	-	-	0	0	N/A	N/A	N/A	N/A	N/A	N/A	R	R	NR

S.A.N. = Sino-Atrial node  
A.V.N. = Atrio-ventricular node  
B.H. = Bundle of His  
R.B. = Right branch  
L.B. = Left branch  
N/A = Not available  
Alt = Altered  
N/Alt = Not altered  
+ = present  
- = absent  
0 = mild  
00 = moderate  
000 = severe  
C.F.R. = Complement fixation reaction  
I.F.R. = Immunofluorescence reaction  
H.A.R. = Hemoagglutination reaction  
R = Reactive  
NR = Non-reactive  
A/C = Anticomplementary

As a group the hearts of patients expiring from a violent death ranged from 240 to 390 g (Mean = 300 g). Only three of these hearts weighed

over 320 g, the lower limit of cardiomegaly for our region.

Table 3 – Morphologic features of hearts from 15 Chagas patients who suffered a violent death

Case No	Age (yrs)	Sex	Heart Wt (g)	Macroscopy			Microscopy					Serology Pericardial fluid			Cause of Death			
				Epicarditis	Lymph Node	Apical Lesion	Inflammation		No. of Neurons	Conduction System						C.F.R.	I.F.R.	H.A.R.
Atrium	Ventricle	S.A.N.	A.V.N.	B.H.	R.B.	L.B.												
01	24	F	280	+	-	-	00	-	126	N/A	N/A	N/A	N/A	N/A	R	N/A	N/A	HT
02	39	M	260	+	-	-	000	-	zero	N/A	N/A	N/A	N/A	N/A	R	N/A	N/A	IH
03	24	M	270	+	-	-	00	0	167	N/A	N/A	N/A	N/A	N/A	R	N/A	N/A	IH
04	23	M	310	-	-	-	00	0	N/A	N/A	N/A	N/A	N/A	N/A	R	N/A	N/A	MT
05	25	F	300	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A	R	N/A	N/A	AP
06	20	M	300	+	-	-	0	0	N/A	N/A	N/A	N/A	N/A	N/A	R	N/A	N/A	IH
07	19	M	280	+	-	-	00	00	N/A	N/A	N/A	N/A	N/A	N/A	R	N/A	N/A	IH
08	17	M	240	+	-	-	0	0	N/A	N/A	N/A	N/A	N/A	N/A	R	N/A	R	HT
09	39	M	290	-	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A	R	N/A	N/A	IH
10	35	F	390	-	-	-	0	0	N/A	N/A	N/A	N/A	N/A	N/A	R	N/A	N/A	IH
11	25	F	250	-	-	-	0	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	R	IH
12	44	M	360	+	-	-	0	0	2921	N/A	N/A	N/A	N/A	N/A	R	N/A	N/A	IH
13	35	M	300	+	-	-	0	0	N/A	N/Alt	Alt.	Alt.	Alt.	Alt.	A.C.	R	R	MT
14	38	F	N/A	+	-	-	0	0	N/A	N/Alt.	Alt.	Alt.	N/Alt.	Alt.	A.C.	R	NR	MT
15	29	M	380	+	-	-	00	000	N/A	N/A	N/A	N/A	N/A	N/A	R	R	R	MT

S.A.N. = Sino-atrial node  
A.V.N. = Atrio-ventricular node  
B.H. = Bundle of His  
R.B. = Right branch  
L.B. = Left branch  
N/A = Not available  
Alt = Altered  
N/Alt = Not altered  
+ = present  
- = absent  
0 = mild  
00 = moderate  
000 = severe  
HT = head trauma  
IH = internal hemorrhage  
AP = accidental poisoning  
MT = Multiple trauma  
C.F.R. = Complement fixation reaction  
I.F.R. = Immunofluorescence reaction  
H.A.R. = Hemoagglutination reaction  
R = Reactive  
N/R = Non-reactive  
AC = Anticomplementary

The means of the control group and the chagasics who died accidentally were not significantly different ( $t = 0,16; p > 0,05$ ) but the difference was significant when controls were compared with the chagasics who died suddenly ( $t = 9,26; p < 0,001$ ), and with chagasics with cardiac failure ( $t = 10,5; p < 0,01$ ). The means were also significantly different when the accidental and sudden death chagasic groups ( $t = 6,8; p < 0,01$ ) and sudden death and cardiac failure groups ( $t = 5,5; p < 0,01$ ) were compared. Hearts of the mice inoculated with *T. cruzi* were within normal limits.

**Histopathology** – Histologically, changes are indistinguishable whether in hearts from patients in heart failure or the ones who died suddenly or suffered a violent death. Microscopically, the myocarditis is characterized by chronic inflammation and fibrosis, with foci in the myocardium containing lymphocytes, plasma cells, granulocytes frequently in the vicinity of degenerating fibers which show no evidence of the parasites except in two cases. Similar inflammatory foci are noted in the epicardium, where they extend into the adipose tissue and the autonomic ganglia (Fig. 1).

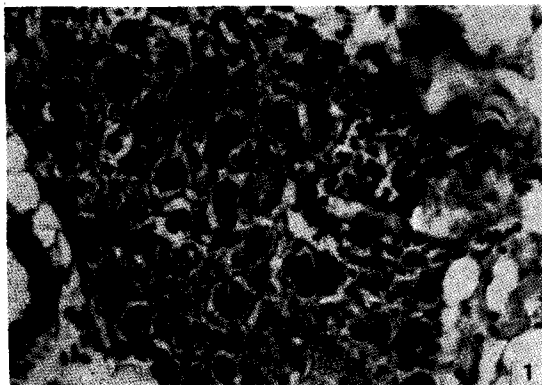


Fig. 1 – Subepicardiac ganglia of a chagasic patient who died with cardiac failure. Note the inflammatory reaction in the epicardiac adipose tissue (cellulitis) that extended to the ganglia (periganglionitis and ganglionitis). HE x 200.

As the periganglionitis and ganglionitis progresses, perineural tissue, nerve fibers and ganglion cells are replaced by fibrosis and chronic inflammation (Fig. 2 and 3).

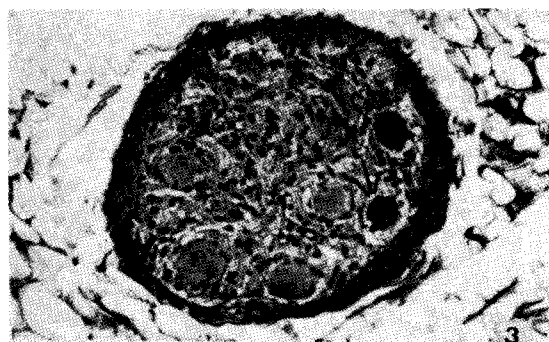


Fig. 2 and 3 – Superepicardiac ganglia of a chagasic individual who died due to accident without symptoms or signs of Chagas' Disease. Note the inflammatory reaction in the ganglia and in the nerve. Observe the neuronal lesion (arrow). HE x 400.

Eventually, only the remnants of degenerating ganglion cells can be identified, surrounded by a mononuclear inflammatory infiltrate (Fig. 4). The loss of neurons in the ganglia is better appreciated by counting the number of such cells in serial sections. By this approach, reduction of their number or total disappearance of such cells can be documented<sup>8,9</sup>.



Fig. 4 – Subepicardiac ganglia of a chagasic patient. The field illustrates a damaged neurone with surrounding mononuclear cells (probably lymphocytes). HE x 400.

The lesions elicited in mice in the optical microscopy were similar to that of the three groups of patients dying with Chagas' disease. Ultrastructurally, subepicardial ganglia revealed in the acute phase lesions of neurons (Fig. 5), satellite cells, myelinic and unmyelinic fibers, and interstitial and periganglionar connective tissue. The lesions were diffuse and related to the presence of degenerated amastigote forms of *T. cruzi* inside groups of cells, with or without inflammation as described formerly<sup>1,4</sup>. In the chronic phase the subepicardial ganglion lesions were similar but of diminished intensity; fibers of the parasympathetic autonomic nervous system revealed marked degeneration characterised by mitochondrial swelling, lysis of neurotubules and neurofilaments and accumulation of glycogen (Fig. 6).

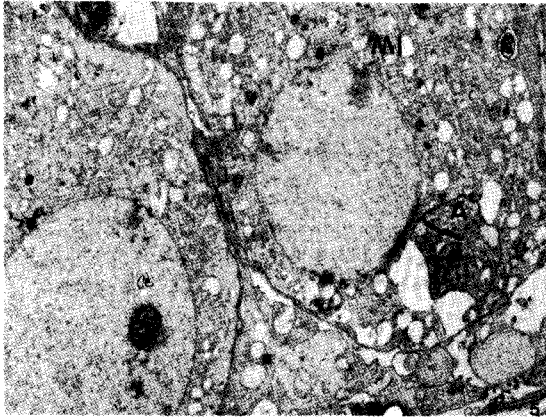


Fig. 5 - Acute Chagas' Disease in the mouse. Electron micrograph of neurones of subepicardiac ganglia. There is mitochondrial vacuolization (MI) and degenerating amastigotes (A) x 7200.

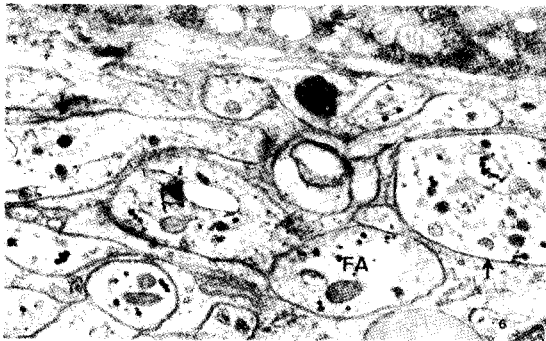


Fig. 6 - Chronic Chagas' Disease in the mouse. Electron micrograph of parasympathetic autonomic nervous system. Note preserved (arrows) and degenerated unmyelinated fibers (FA). Also noted is mitochondrial swelling, lysis of neurotubules and neurofilaments and presence of glycogen particles within the fiber. x 28.000.

## DISCUSSION

The salient finding of this study is the similarity of the lesions affecting the parasympathetic autonomic nervous system of the hearts from Chagas disease patients dying under different circumstances and experimental animals inoculated with *T. cruzi*. The lesions have a definite predilection for the subepicardial nervous ganglia but they affect the myelinic and amyelinic ganglion cells in the myocardium as well<sup>1,4</sup>.

Qualitative and/or quantitative evidence of neuronal loss could be demonstrated in practically every heart examined. In the subepicardial area, because of the larger concentration of neurons the loss seemed most intense, with some cases where all detectable neurons had disappeared. In the myocardium, quantitation of this loss is more difficult due to the diffuse distribution of neurons, but virtually all nerve fibers of the experimental animals examined ultrastructurally revealed degeneration.

The pathogenesis of ANS lesions is not clear at present and seems partially different at the level of the subepicardial ganglia and in the interior of the heart muscle<sup>1,4</sup>. The mechanism of formation of lesions in the ganglia seems to be complex, depending on: 1) ganglionitis and periganglionitis; 2) parasitosis and damage to the Schwann and satellite cells produced by any number of causes; 3) neuron parasitosis; 4) damage to the endothelial cells of capillaries and venules, with narrowing of the vascular lumen, alterations in the basal membrane and fundamental substance, all leading to metabolic alterations in neighboring cells; 5) degeneration and destruction of nerve fibers, caused, in turn, by multiple factors, and 6) immune mechanisms.

Damage to the intracardiac nervous network is caused by 1) damage to the nerve cell body produced by the previously mentioned mechanisms; 2) alterations in the Schwann cells produced directly by parasitic infection or indirectly by inflammation; 3) inflammation itself, which directly and violently attacks the nervous network throughout the entire myocardium, and 4) alterations in the capillary endothelium, in the basal membrane and fundamental substance.

The functional consequences of denervation have been termed: "neurogenic parasympathetic

cardiopathy<sup>7</sup> or "catecholaminergic cardiopathy"<sup>1,2</sup> but there is also evidence of neuronal loss in the sympathetic chains; therefore in patients with Chagas disease the cardiopathy can not be explained purely on an intracardiac denervation basis. The vorticular lesions (apical lesions) for instance, do not seem to be a direct consequence of denervation<sup>1</sup>. On the other hand, Chagas' disease is by no means the only cause of damage to the intracardiac autonomic nervous system in man. Similar lesions, although more focal and less severe can be found among other conditions such as endomyocardial fibrosis<sup>4</sup>, South African endomyocardial fibroses<sup>5</sup> and rheumatic heart disease<sup>9</sup>.

The role played by damage to the ganglion cells of the heart in the sudden non-violent deaths of patients with Chagas disease needs elucidation<sup>3</sup>. Similar lesions, in the sinus node of non-chagasic young women who died suddenly have been linked to electrical instability which may have had a fatal course<sup>6</sup>. However, the autonomic involvement in Chagas disease is more widespread and found equally in sudden death and in patients who progress instead to heart failure. On the other hand, ganglionic lesions have not been noted in hearts with Chagas' disease in other parts of South America<sup>1,3</sup>. Whether the involvement of the autonomic nervous system of the heart is a phenomenon peculiar to Central Brazil is not clear and its exact significance in the pathogenesis of Chagas' disease remains a challenge.

## RESUMO

*O sistema nervoso autônomo e especialmente o sistema nervoso autônomo intracardiaco são lesados na doença de Chagas. Ganglionite e periganglionite foram observadas em 1) chagásicos com insuficiência cardíaca; 2) em tripanossomóticos, assintomáticos ou paucissintomáticos, em vida, falecidos subitamente em consequência da doença de Chagas e 3) nos chagásicos, também assintomáticos, e que faleceram devido a acidentes ou homicídios. Em todos os três grupos os corações mostraram miocardite e lesões das células ganglionares intramiocárdicas bem como das fibras nervosas mielínicas e amielínicas. Em camundongos com infecção chagásica experimental, observamos que na fase aguda da doença as lesões são mais graves e intensas. A denervação talvez desempenhe algum papel na patogênese da cardiopatia chagásica. Entretanto, baseados em nossa experiência pessoal e em outros dados da literatura concluímos que a*

*perda de neurônios não é o principal nem o fator exclusivo pelas manifestações apresentadas pelos pacientes chagásicos crônicos. Por outro lado, as lesões dos neurônios talvez possam ter algum papel no mecanismo da morte súbita do chagásico mas é difícil explicar porque tripanossomóticos, com lesões similares, não faleceram subitamente.*

Palavras Chaves: Doença de Chagas. Tripanossomose cruzi. Cardiopatia chagásica. Sistema nervoso autônomo.

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