

Trypanosoma cruzi: Paninfectivity of CL Strain during Murine Acute Infection

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LENZI, H. L., OLIVEIRA, D. N., LIMA, M. T., AND GATTASS, C. R. 1996. *Trypanosoma cruzi*: Paninfectivity of CL strain during murine acute infection. *Experimental Parasitology* **84**, 16–27. A systematic study of the distribution of intracellular parasites in the organs and tissues of mice acutely infected (15 days) with the CL strain of *Trypanosoma cruzi* was performed. Almost all tissues and organs were parasitized with different intensities, including several epithelial cell types. In addition to striated, cardiac, and smooth muscles a very high parasitism of fat cells, pancreas, and genital adnexa was observed. A smaller number of parasites was found in all other structures studied except in highly vascularized structures such as in the penile corpora cavernosa, pulmonary and renal parenchyma, islets of Langerhans, hepatic sinusoids, and in atrial endothelium. This paper also shows, for the first time in the literature, the parasitism of milky spots, cornea epithelium, cornea stroma, retroorbital fibroblasts, seminal vesicles, and coagulative, Cowper's, urethral, preputial, sebaceous anal, and clitoris glands. The results indicated that CL strain is highly invasive, being able to infect cells derived from the three embryonic layers (ectoderm, mesoderm, and endoderm), suggesting that the paninfectivity may influence the outcome of immunological and pathological events. © 1996 Academic Press, Inc.

INDEX DESCRIPTORS AND ABBREVIATIONS: Chagas' disease; *Trypanosoma cruzi*; protozoa; CL strain; infectivity; pathology; epithelial cells; adipose tissue; H&E, hematoxylin-eosin.

INTRODUCTION

Trypanosoma cruzi is a very polymorphic parasite and differences in infectivity and biological characteristics among the strains have been described (Dvorak *et al.* 1988; McDaniel and Dvorak 1993). Although trypomastigotes from *T. cruzi* can invade and proliferate in most of the vertebrate cells tested *in vitro*, differences in tissue tropism for the various strains have been described in experimental models (Taliaferro and Pizzi 1955; Bice and Zeledon 1970; Shoemaker *et al.* 1970; Melo and Brener 1978). In spite of that, independent of the strain, the pathology of the chronic phase involves mainly the cardiac and skeletal

muscles and the gastrointestinal tract. Maybe for this reason, investigation of pathological aspects induced by *T. cruzi* infection in experimental models has been directed to these structures, underestimating the importance of other organs and tissues on the pathogenesis of the disease. Indeed, as pointed out by Chagas (1910) and others (Watkins 1966; Bice and Zeledon 1970), *T. cruzi* can invade several organs and tissues whose commitment may contribute to the establishment of the disease and resultant immunologic response, pathology, or mortality.

This paper utilizes mice acutely infected with the *T. cruzi* CL strain to investigate the involvement of organs, other than muscle cells, on the pathology of experimental Chagas' disease. The CL strain, isolated from naturally infected *Triatoma infestans* (Brener and

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Chiari 1963), is described as a myotropic strain, which preferentially infects heart and skeletal muscle (Melo and Brener 1978). Our results show that this strain is ubiquitously infective, inducing extensive alterations on the lymphohematopoietic system (manuscript in preparation) and in almost all cells and tissues of mice. In addition of showing new places of parasitization by *T. cruzi*, this paper focuses on the diverse tissues and anatomical structures specially ignored or not pointed out by most researchers on *T. cruzi*, which emphasize mainly the reticulo- and myotropism of this parasite (Andrade *et al.* 1970).

MATERIALS AND METHODS

The CL strain of *T. cruzi*, isolated from naturally infected *T. infestans* collected from a house in Rio Grande do Sul, Brasil (Brener and Chiari 1963), was maintained by passages through BALB/c mice, every 14 days. Infection with this strain was achieved by intraperitoneal injection of 0.2 ml PBS-diluted blood containing 10^5 trypomastigotes.

Either male or female 6-week-old BALB/c mice, from our own animal facilities, were used throughout the experiments. The mice were separated according to sex and housed in groups of five to six per cage.

Twelve BALB/c mice from both sexes, weighing around 16–20 g, were sacrificed on Day 15 after infection. Tissue samples were taken from the heart, lymph nodes, thymus, spleen, milky spots from thoracic, abdominal, and testicular cavities, salivary glands, lung, trachea, thyroid, liver, tongue, nasal mucosa, eye, diaphragm, kidney, ureter, adrenal glands, pancreas, mesentery, urinary bladder, penis, testis, epididymis, vas deferens, vesicular, coagulating Cowper's, and preputial glands, uterus, uterine tube, ovaries, skin, striated muscles, bones, bone marrow (from several places), teeth and tail, esophagus, stomach, intestine, and brain. The intestines were processed according to the Swiss roll technique (Lenzi and Lenzi 1986). Samples were fixed in Formalin–Millonig buffer (Carson *et al.* 1973) and embedded in paraffin. Sections were stained with hematoxylin-eosin (H&E) and Lennert's Giemsa.

RESULTS

Almost all tissues and organs were parasitized with different levels of intensity (Table I). In general, the numbers of parasites were higher in the brown and white adipose tissue, atrial cardiac muscle, striated muscle of differ-

ent places, muscular layer of gastrointestinal tract, sebaceous anal glands (Fig. 1), pancreas, autonomic ganglions, wall of pulmonary and renal veins, and epididymis. The adipose tissue presented a universal panniculitis, with a large number of parasites, macrophages, lymphocytes, and necrotic cells (Fig. 3). Without involvement of epidermis, high numbers of parasites were found in the dermis of the skin close to the anus and external genitalia, including the prepuce. The parasites formed rows or small nests in the dermis without inflammatory infiltrate or caused only a little monocyte/macrophage reaction (Fig. 5). It was impossible by conventional microscopy to specify the type of cell or structure involved (fibroblasts, macrophages, dermal nerve cells?). Similar aspects were seen in the corneal stroma (Figs. 9 and 10), and parasites were also localized in the retroorbital fibroblasts between choroid and extraocular muscle (Fig. 11). Parasites were also detected in the interstitium of lachrymal glands (Fig. 12). Pancreas showed a diffuse interstitial pancreatitis, with edema, monocyte/macrophage infiltration specially around intralobular ducts, occasional lobular atrophy, and plenty of parasites inside macrophages or/and myoepithelial cells, excluding acinar cells and islets of Langerhans. Similar aspects, but less intense, were observed in the interstitium of parotid, submaxillary, and major sublingual glands. In the endocrine glands, the parasite was found mainly in the zona fasciculata, less frequently in the zona glomerulosa of the adrenal gland, in isolated macrophages in the thyroid interstitium, and in follicular granulosa of ovary. Parasites were searched for, unsuccessfully, in adrenal medulla, islets of Langerhans, theca layer of ovary, and testicular Leydig cells.

Isolated or small nests of parasites were detected in epithelial layer of trachea (Fig. 6), urinary bladder (Fig. 7), renal pelvis (Fig. 8), mammary ducts, cornea, epididymis, and in pale-staining cells of anal sebaceous and preputial glands. Only one mouse presented isolated subcapsular macrophages filled up by *T. cruzi* amastigotes in the liver. In general, in

TABLE I
Sites Where *Trypanosoma cruzi* Were Located after 15 Days of Infection by CL Strain

LOCATION	INTENSITY	LOCATION	INTENSITY
BLOOD VESSELS		FEMALE REPRODUCTIVE SYSTEM	
Pulmonary Veins	++	Vagina	+
Aorta (Adventitia)	+	Uterus	+/-
Jugular Vein	+	Oviduct	+
Renal Veins	+	Ovary (granulosa cells)	+
RESPIRATORY SYSTEM		Mesovary	+
Lung	+/-	Clitoris Glands	+
Trachea	+	Mammary Glands	+
GASTROINTESTINAL TRACT		MALE REPRODUCTIVE SYSTEM	
Tongue	+	Preputial Glands	++
Esophagus	+	Penis	+
Stomach	+	Testis - Seminiferous Tubules	-
Small Intestine	++	Testis - Albuginea	+
Large Intestine	++	Epididymis	++
MESENTERIUM		Vas Deferens	+
	++	Seminal Vesicles	+
NERVOUS SYSTEM		Coagulative Glands	+
Central	-	Ampullary Glands	-
Peripheral	+	Bulbo Urethral Glands	+
Autonomic Ganglia	++	Urethral Glands	+
Auerbach Plexus	+	Prostate	+
EXOCRINE GLANDS		KIDNEY	
Parotid Glands	++	Capsule	+
Submaxillary Glands	+	Parenchyma (close to Pelvis)	+/-
Sublingual Glands	+	URINARY TRACT	
Serous/Mucous Intralingual Glands	-	Renal Pelvis	+
Exocrine Pancreas	++	Ureter	+/-
Dermal Sebaceous Glands	+	Urinary Bladder	++
Anal Sebaceous Glands	+	Urethra	+
Lachrymal Glands	+	ADIPOSE TISSUE	
ENDOCRINE GLANDS		Brown	+++
Thyroid	+	Univacuolar	++++
Adrenals	+	EYE	
Endocrine Pancreas	-	Corneal Epithelium	+
Leydig Cells	-	Corneal Stroma	+
LYMPHOHEMATOPOIETIC ORGANS		Retroorbital Fibroblasts	+
Thymus	+	LIVER	
Bone Marrow	+/-	+/-	
Lymph Nodes	+	GALLBLADDER	
Spleen	+	N.E.	
Milky Spots	+	SKIN	
EPIPLOON		++	
		CARTILAGE	
		-	

Note. +/- to ++++ indicates levels of parasitism intensity and N.E. indicates not examined.

this organ, there were small aggregates of macrophages with few or no parasites inside. In the lymphohematopoietic organs, nests of parasites were observed in practically all animals in macrophages of the thymus, lymph

nodes (neck, renal hilus, justa-thymus, and justa-pancreas), splenic red pulp, peritoneal milky spots (Figs. 15 and 16), and rarely in bone marrow macrophages. In these omental structures and in mesenteric membranes the

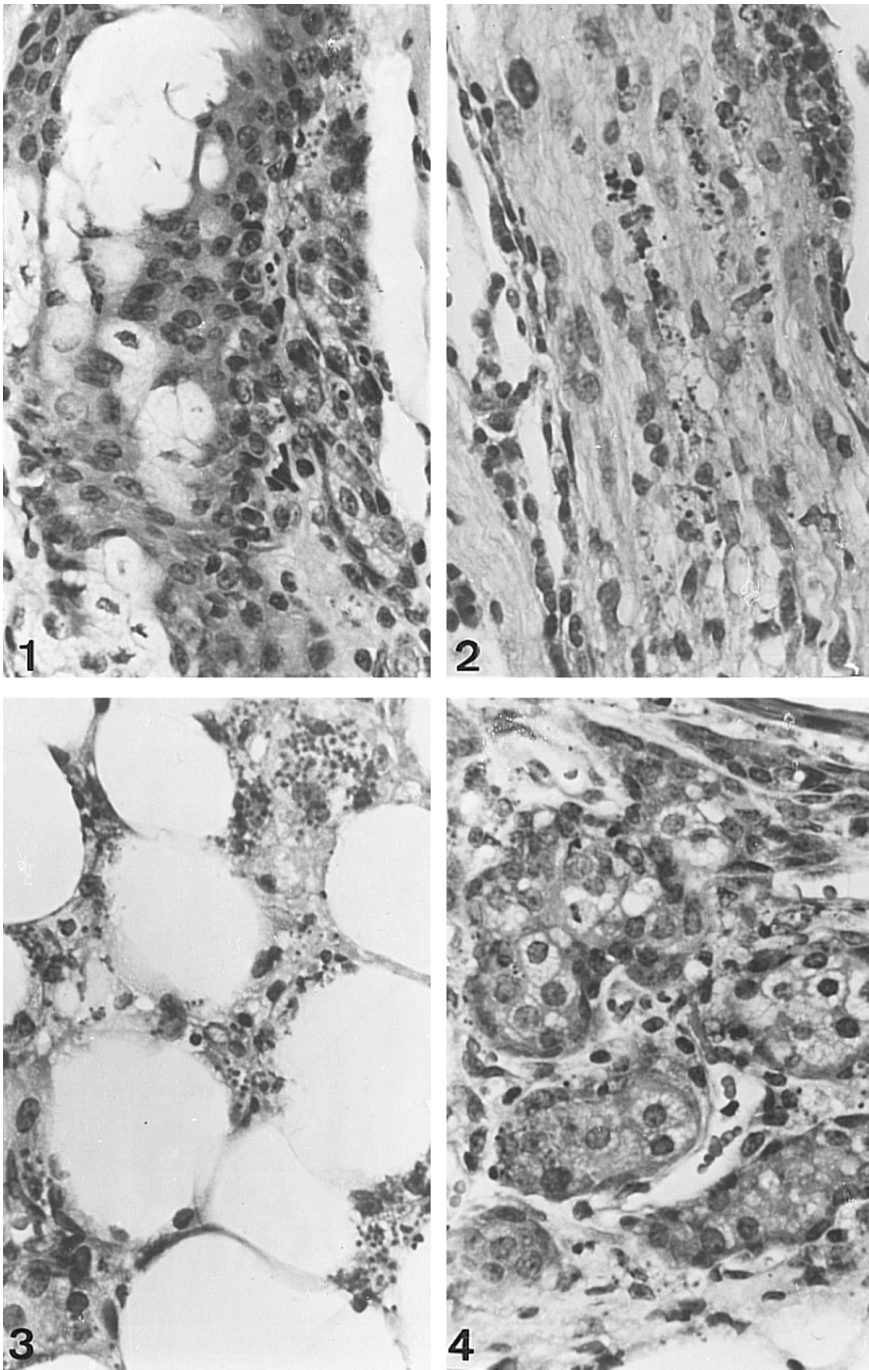


FIG. 1. Nests of *T. cruzi* in the interstitium of sebaceous anal gland. H&E. $\times 500$. FIG. 2. Peripheral nerve in mesenterium with several *T. cruzi* nests in Schwann cells. H&E. $\times 500$. FIG. 3. Univacuolated adipose tissue showing numerous and large *T. cruzi* nests. H&E. $\times 500$. FIG. 4. *T. cruzi* nests in interstitium and in sebaceous cell of preputial gland. H&E. $\times 500$.

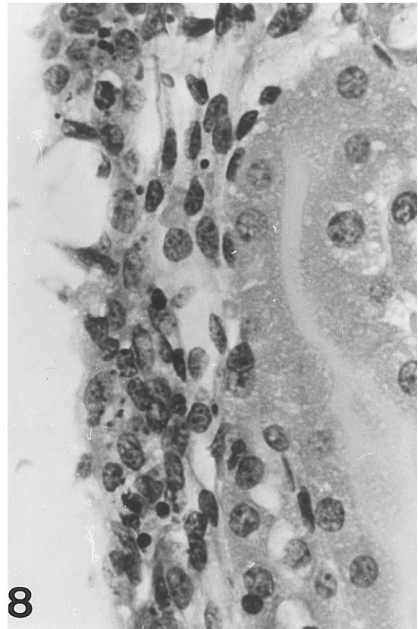
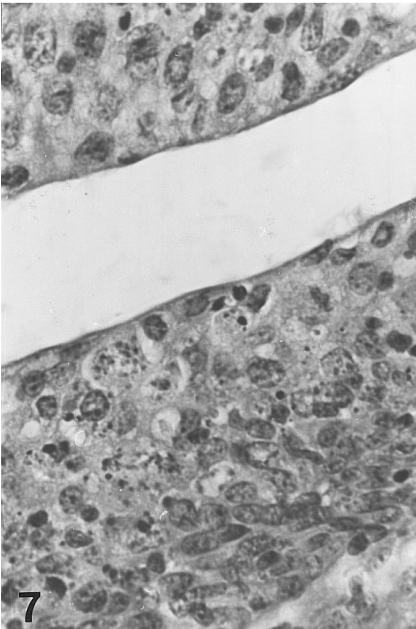
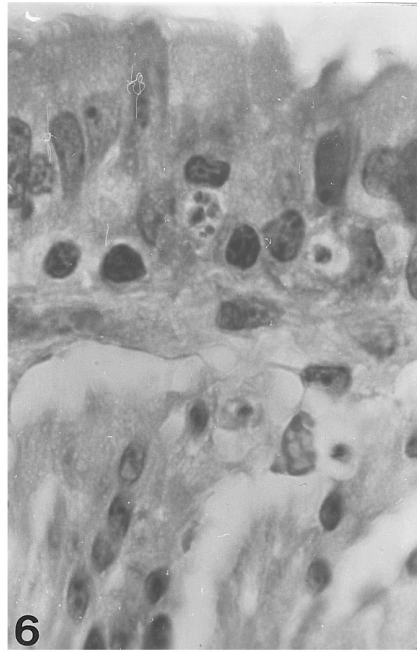
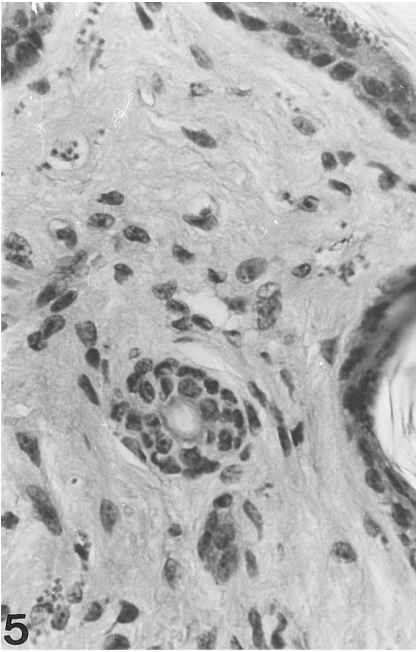


FIG. 5. *T. cruzi* nests and rows in the dermis, without inflammatory reaction. H&E. $\times 500$. FIG. 6. *T. cruzi* nests in tracheal epithelial cells. H&E. $\times 1250$. FIG. 7. Large numbers of *T. cruzi* nests in urothelial cells of the urinary bladder, some of them located very close to the lumen. H&E. $\times 500$. FIG. 8. *T. cruzi* nests in epithelial cells of the renal pelvis, close to a proximal tube. H&E. $\times 640$.

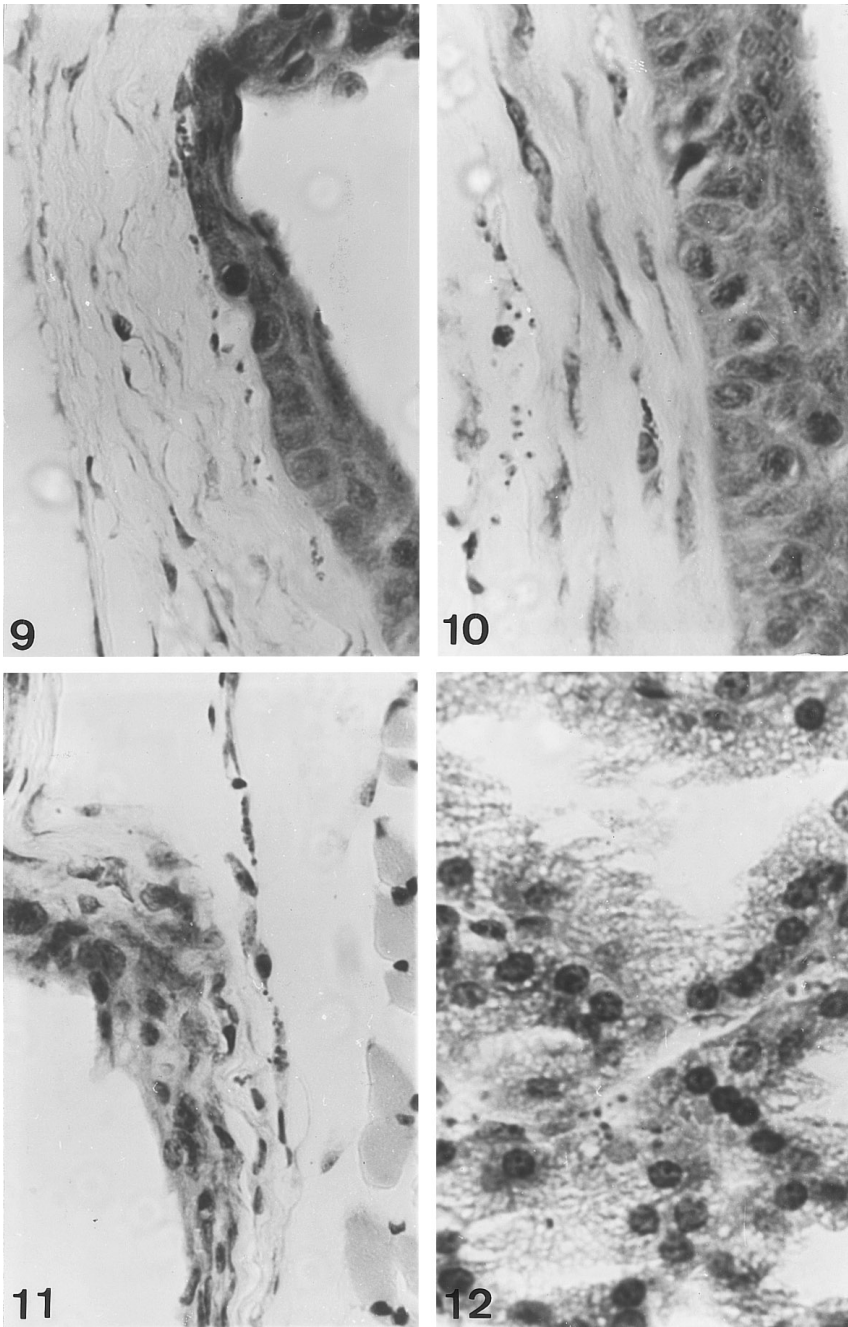


FIG. 9. Intraepithelial nest and rows of *T. cruzi* in the stroma of the cornea. Lennert's Giemsa. $\times 640$. FIG. 10. Rows of *T. cruzi* in corneal stroma. Lennert's Giemsa. $\times 800$. FIG. 11. *T. cruzi* nests in retroorbital fibroblasts, between choroid and extraocular muscle. H&E. $\times 640$. FIG. 12. Small *T. cruzi* nests in the interstitium of lacrimal gland. H&E. $\times 800$.

parasites were also found inside mesothelial cells (Figs. 13–16). The thymus exhibited severe cortical atrophy with thymocyte apoptosis. Parasites were not detected in brain. However, they were often present in peripheral nerves (Fig. 2), Auerbach plexus (Schwann cells), and in the interstitium of sympathetic and parasympathetic ganglia.

Both male and female reproductive systems were intensively involved by parasites, which were detected in the mesovarium; ovarian follicular granulosa; lamina propria and muscular coat of the oviduct; muscular and adventitia of the uterus; lamina propria, muscular coat and adventitia of the vagina; epithelium of excretory ducts and periductal adipose cells of mammary glands; and in periductal and interstitial fibroblasts of clitoris glands. In the male reproductive system, large numbers of parasites were detected in the interstitial tissue and epithelial cells of epididymis; lamina propria and muscular layer of prostate, seminal vesicles, coagulative glands, and vas deferens; interstitium and sebaceous cells of preputial glands (Fig. 4); interstitium and vacuolated epithelial cells of Cowper's glands; interstitium of urethral glands; connective tissue under the penile urethra and in the penile tunica albuginea surrounding the corpora cavernosa; in the dermis of the prepuce and in the testicular albuginea. In one ampullary gland analyzed, parasites were not identified. In the male reproductive system the parasites predominated in the epididymis and preputial glands.

DISCUSSION

Except in the opossum (Deane *et al.* 1984; Lenzi *et al.* 1984), *T. cruzi* is an obligate intracellular parasite. It is well established that, both *in vitro* and *in vivo*, *T. cruzi* can infect a great variety of cells types including fibroblasts, epithelial and endothelial cells, myocytes, and macrophages. Trypanosomes were also found in the cephalic end of a spermatozoon (Vianna 1911). The mechanisms by which *T. cruzi* invades cells is not completely

understood and recently Ming *et al.* (1995) proposed that *T. cruzi* invasion involves transsialidase/sialic acid, penetrin/heparan sulfate, and recognition and activation of the TGF signaling pathway.

In this paper we show that, during the acute phase, the CL strain of *T. cruzi* can cause an universal, ubiquitous, or septicemic infection, parasitizing almost all tissues and anatomical structures of mice (Table I), reproducing the findings on human autopsies (Vianna 1911; Chagas 1916a,b; Crowell 1923) and other immunocompromised experimental models (Calabrese *et al.* 1994; Gonçalves da Costa and Calabrese 1992; Gonçalves da Costa *et al.* 1986a, 1994).

The detection of parasites in the central veins of adrenal glands (Almeida *et al.* 1981, 1986) and in the myocardium of chronic chagasic patients (Almeida *et al.* 1984; Jones *et al.* 1993) and the recent observation that parasites can be reactivated in HIV patients (Rocha *et al.* 1994) and form subcutaneous nodules in recipients of cardiac transplants (Bocchi 1987; Stolf *et al.* 1987) indicate the *T. cruzi* capability to migrate to different organic compartments, changing its location according to the immune state of the host (Gonçalves da Costa *et al.* 1984). Indeed, in the 13th day of infection, a marked difference between nu/nu and nu/+ mice infected with *T. cruzi* was observed. While in nu/nu mice almost all tissues and organs were intensely parasitized, in nu/+ mice only some organs were parasitized with low intensity (Gonçalves da Costa *et al.* 1984).

In our material, we present some original anatomical aspects of the *T. cruzi* infection such as parasite involvement of mesenteric membranes, milky spots, intraorbital lachrymal glands, eyes, tracheal and corneal epithelia, anal sebaceous glands, and different genital adnexa (prostate, seminal vesicles, and coagulative, bulbourethral, preputial, and clitoris glands). We also stress the parasite invasion of dermis and adipose tissue and of different epithelial cells (sebaceous glands, mammary excretory ducts, mesothelium, and epididym-

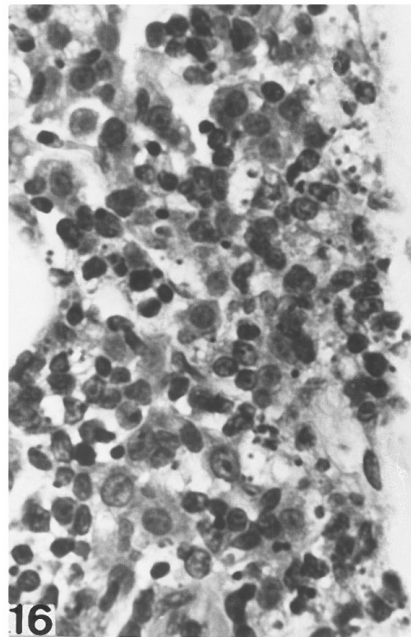
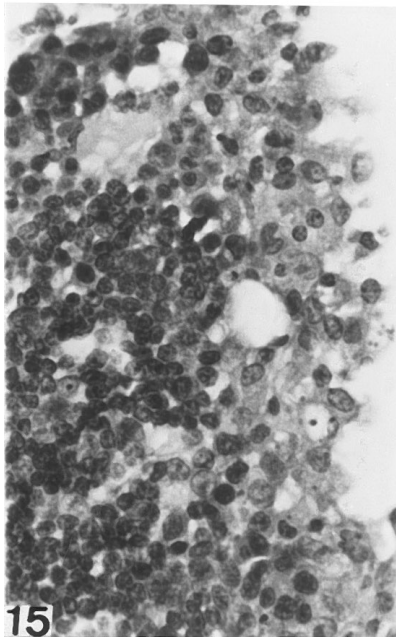
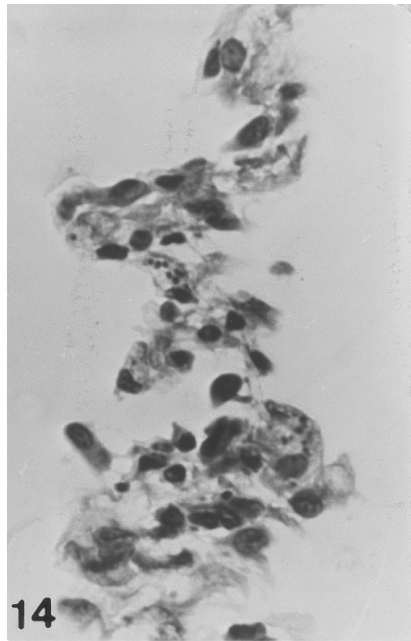
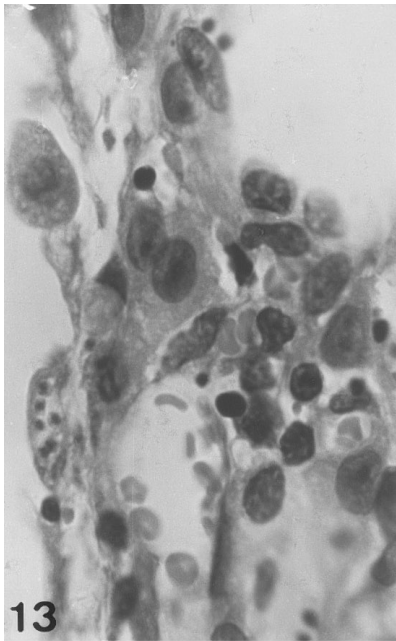


FIG. 13. Mesenteric membrane showing one *T. cruzi* nest in mesothelial cell. H&E. $\times 1250$. FIG. 14. Mesenteric membrane with several *T. cruzi* nests in fibroblasts and/or mesothelial cells. H&E. $\times 500$. FIG. 15. Peritoneal milky spot, predominantly of lymphocytic type, covered by parasitized mesothelial cells. H&E. $\times 500$. FIG. 16. Large number of *T. cruzi* in peritoneal milky spot rich in macrophages. H&E. $\times 640$.

mal, tracheal, corneal, urothelial, and mesothelial cells).

Brown and white adipose were two of the most heavily infected tissues in our material, presenting a systemic panniculitis with a large number of parasites (Fig. 3). Mazza *et al.* (1940) observed very similar lesions in *T. cruzi*-infected dogs and they referred to the fact that Meyer and Da Rocha Lima (1912) and Da Rocha Lima (1929) considered the adipocytes together with reticuloendothelial and glial cells as the second preference for the *T. cruzi* after heart and skeletal muscle. A tropism for adipose tissue by the Tulahuen strain of *T. cruzi* has also been described (Shoemaker *et al.* 1970). The occurrence of subcutaneous nodules (lipophagic granulomas) in recipients of cardiac transplants (Bocchi 1987; Stolf *et al.* 1987) has recently drawn attention to the parasitism of adipocytes. According to Andrade and Silva (1995), this situation suggests a link between *T. cruzi* adipocyte parasitism and immunosuppression. In our material, the parasitism was intense in brown and white fats, being more intense in the adipose tissue close to the male and female genital tracts. The fact that adrenal cortex (Vianna 1911; Shoemaker *et al.* 1970), myelin sheaths in peripheral nerves (this paper, Fig. 2), brain (Chagas 1910; Vianna 1911), ovarian theca interna (Calabrese *et al.* 1994) and follicular granulosa, dermal and genital sebaceous glands (Fig. 4), and anal glands of the opossum (Lenzi *et al.* 1984) are sometimes heavily infected reinforces the evidence for the preference of *T. cruzi* for places rich in lipids and steroids. Indeed, adipose tissue has an endocrine function and is active in the conversion of androstenedione to estrone, the major source of estrogen in men and postmenopausal women (Hirsch *et al.* 1989). Adipose tissue is also rich in stearic acid (Shoemaker *et al.* 1970), an essential growth factor for *T. cruzi* *in vitro* (Bone and Parent 1963), and is a source of apolipoprotein E, angiotensin, insulin-like growth factor I, adipin, and thermogenin which uncouples the oxidation of fatty acids from generation of ATP (Bouillaud

et al. 1983; Cunningham *et al.* 1985; Hirsch *et al.* 1989). Although a possible function of S-100 protein in *T. cruzi* growth is unknown, it is interesting to note that both fat cells and Schwann cells contain its B form (S-100-b) (Takahashi *et al.* 1984; Nakazato *et al.* 1985), which is a highly acidic calcium binding protein with a molecular weight of 21,000.

Our findings also emphasize the parasite migration to the dermis of the skin, probably in the interior of perivascular macrophages, fibroblasts, and/or small nerve fibers with little monocyte/macrophage reaction or without any inflammation. According to Chagas (1916b) cutaneous processes in humans with acute infections were rarely observed. One of his patients showed dark maculae of rather original appearance, intercalated by small erythematous vesicles full of a serous fluid. Another patient showed gangrenous cutaneous patches which sloughed off and laid bare the subjacent tissues. Mazza *et al.* (1941) detected parasites only in the skin of hematogenic chagomas and not in morbiliforme schizotrypanide. Medina and Pons (1974) otherwise did not detect parasites in metastatic chagomas and in trypanosomids but only in macrophages in reticular dermis and hypodermis of inoculation chagomas. More or less diffuse erythematous lesions with a great number of diffuse amastigotes in the dermis were observed in nude and newborn BALB/c mice acutely infected with Y and CL strains of *T. cruzi*. However, when the skin of adult susceptible C3H/HeN mice was studied, a small colonization of the dermis was seen associated with an intense inflammatory reaction (Gonçalves da Costa and Calabrese 1992). In our adult BALB/c mice we found large numbers of parasites in the dermis with few or no inflammatory infiltrates.

Chagas (1916b) frequently found inflammatory reactions of the eye, such as conjunctivitis, uni- or bilateral keratitis, and occurrence of postkeratitis and secondary panophtalmia among the clinical signs of the human acute form of Chagas' disease. This paper shows, for the first time in the literature, the parasit-

ism of cornea epithelium, cornea stroma, and retroorbital fibroblasts in a non-inoculation chagoma lesion (Figs. 9–11). We do not know if the parasites were inside nerve processes, which are difficult to detect anywhere in the cornea in standard tissue sections even with the use of special histological techniques.

We found parasites in almost all structures of male and female genitals but in the penile corpora cavernosa. The presence of *T. cruzi* in the genitals was frequently described in humans (Vianna 1911; Chagas 1916a,b) and in immunocompromised mice (Gonçalves da Costa 1986a; Calabrese *et al.* 1994). Their data and our observation suggest that *T. cruzi* can reach the sperm through the seminiferous tubules, epididymis, and genital adnexa.

Although we detected parasites through all the urinary tract, from the renal pelvis to the urethra, they were completely absent in the renal parenchyma. These results confirm the data of Melo and Brener (1978) which described low renal parasitism by Y, Berenice, and ABC *T. cruzi* strains and none by CL strain. Although plenty of parasites were observed inside macrophages or/and myoepithelial cells in the pancreas, no parasites were found in acinar cells and islets of Langerhans. The absence or low level of parasitism in very high vascular structures such as in the penile corpora cavernosa, pulmonary and renal parenchyma, islets of Langerhans, hepatic sinusoids, and in atrial endothelium is indicative of some inhibitory properties of tissues rich in endothelial cells and vascular structures. Indeed, parasitism of these structures was only observed in some specific situations. Thus, parasitized cells in the alveolar wall (pneumocyte II ?) and lumen, sometimes associated with the presence of amastigotes in the amniotic epithelium, were only observed in congenital Chagas' disease (Bittencourt *et al.* 1981). Colonization of Kupffer cells and hepatocytes was described in mice immunosuppressed with cyclophosphamide (Calabrese *et al.* 1991). Among different models of immunocompromised mice infected with Y and CL strains of *T. cruzi*, athymic mice showed the

most consistent colonization of islets of Langerhans (Calabrese *et al.* 1994). Changes in the α and β cells of the islets of Langerhans in infection with the Bolivian strain of *T. cruzi* were described by Albuquerque *et al.* (1990) in agreement with Oliveira *et al.* (1993), who suggested that the lower insulin response in chronic chagasic patients was probably due to denervation of, or injury to, pancreatic β cells.

This paper emphasizes the capacity of *T. cruzi* CL strain to invade quite unusual structures such as fat and skin and shows, for the first time in the literature, the parasitism of milky spots, cornea epithelium, cornea stroma, retroorbital fibroblasts, and different male and female adnexa. The infectivity of tissues not described here such as the lymphoid and lymphohematopoietic organs will be the subject of a specific paper (manuscript in preparation). Taken together, our data point to the CL as a paninfective strain of *T. cruzi*, being capable of invading almost all organs and tissues of mice. About 7 decades ago, Crowell (1923) concluded that *T. cruzi* can infect cells derived from the mesoderm (muscle, fat, suprarenal gland, ovary, uterus, testis, serous membranes, etc.), endoderm (thyroid, thymus, liver, etc.), and ectoderm (brain, ganglion cells, and peripheral nerves). These observations, which could also be applied to our results, raise two very interesting questions: (1) Does *T. cruzi* use the same mechanism to adhere to and invade so many different types of cells? (2) Why is the parasitism of some highly vascularized organs negative or very low?

ACKNOWLEDGMENTS

The authors thank Luzia Fátima Gonçalves Caputo, Deise de Andrade Costa, and José Avelino for technical assistance. This paper was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Financiadora de Estudos e Projetos (FINEP), Instituto Oswaldo Cruz (FIOCRUZ), Coordenação de Aperfeiçoamento do Pessoal de Ensino Superior (CAPES), Conselho de Ensino para Graduados da UFRJ (CEG-UFRJ), and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

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Received 31 January 1996; accepted 22 April 1996