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A comparative pathological study of three strains of *Trypanosoma cruzi* in an experimental model

J.A. de Diego[1], P. Penin[1], J. del Rey[1], R. Mayer[1] and C. Gamallo[2]

[1]Parasitology's Unit, department of Preventive Medicine and Public Health, Medical School, "Universidad Autónoma" of Madrid and [2]Department of Pathology, C.S.S.S. "La Paz", Madrid, Spain

Summary. *Trypanosoma cruzi*, the etiological agent of Chagas' disease, shows a wide variation in its biological behaviour depending on the geographical distribution of different strains. Moreover, some strains can show variations with the course of time. We have studied the tissular tropism of three strains of *T. cruzi*, Cali, Bolivia and Y, from different geographical origins (Colombia, Bolivia and Brasil respectively) on Swiss mice in order to detect any possible modification in their behaviour attributable only to parasite but not to host variations.

The anatomopathological study of sections from heart, brain, liver, spleen, lymphatic ganglion, skeletal muscle and colon from Swiss mice infected with these strains has evidenced the presence of some important discrepancies between the tissular tropism expected from their former descriptions, and classical typification and then observed lesions. The greatest variations were found in the Y strain which had been described as eminently reticulotropic but presented lesions in all the organs except the spleen and lymphatic ganglion. We consider that the variations found in our study can only be explained in terms of changes in the properties of the strains considered, and conclude that the classic typification techniques based on the constancy of the characteristics of the parasite are not fully reliable for the description and clinical management of some evolving strains.

Key words: *Trypanosoma cruzi*, Pathology, Microscopy

Introduction

Trypanosoma cruzi is the protozoan hemoflagellate which causes Chagas' disease. This disease affects about 24.7 million people in the American Continent with a risk population of 60 million people (Schofield, 1985). The disease is mostly circumscribed to Latin America, although there has been a recent increase in the incidence of *T. cruzi* seropositive patients in the USA in the last years mainly due to a corresponding increase of the increase of immigrants (Theis et al., 1985, 1987). Chaga's disease is the leading cause of heart disease in endemic countries, accounting for one quarter of deaths in the 24 to 45 years age group, causing the death of 10% of the patients in the acute phase of infection because of cardiac pathology. Moreover, this disease can affect in principle any body organ or tissue (Fife, 1977) and causes a considerable risk for blood transfusions.

Different strains of *T. cruzi* vary widely in their host preference, geographic distribution, virulence and tissular tropism (Bice and Zeledón, 1970; Lumsden, 1974; Morel et al., 1980; Braun and Titto, 1985). In addition, there are strains that greatly modify their biological behaviour, including their tissular tropism, with the course of time, while other strains only show slight variations or none at all.

The preferential involvement of one specific organ or tissue by strains of *T. cruzi* from a given geographical origin can direct the diagnostic study and therapy of the patient in that direction, relieving important efforts from the search for lesions in other organs that very probably will not be affected.

The acute phase is the most life-threatening because of the cardiac pathology in all *T. cruzi* strains, the chronic phase being decisive for disease prognosis and control. The chronic infection decisively marks the different spectra of histopathological involvement observed between strains or isolates of *T. cruzi* of different geographic origin and depends on the organs affected during the acute phase of the infection. These patterns of histopathological alterations have been considered to depend on the characteristics of both the host and the parasite (Postan et al., 1987).

A study of the early phase of tissular tropism can lead

Offprint requests to: Dr. José Antonio de Diego, Unidad de Parasitología, Departamento de Medicina Preventiva y Salud Pública, Facultad de Medicina, Universidad Autónoma de Madrid, C./ Arzobispo Morcillo, 4, 28029 Madrid, Spain

to a better knowledge of the organs that will most probably become involved in the later chronic phase, therefore simplyfing the diagnostic studies as well as reducing the costs of enhancing patient follow-up.

We have studied the tissular tropism of three strains of *T. cruzi* from different geographic origins (Bolivia, Brasil and Colombia) that show very different morphological parameters. It has been demonstrated that the number and localization of parasites in tissues of mice experimentally infected with *T. cruzi* may present variations in function of the parasite strain employed (Bice and Zeledón, 1970).

This study has been carried out using as host one of the most well characterized strains of mice, a strain of Swiss mice maintained inbred with the aim of comparing our results with those previously described to detect any possible modification in the tissular tropism properties of these strains which could be attributed only to parasite but not to host variations.

Materials and methods

T. cruzi strains

For our study we have used the following three strains:

T. cruzi Cali strain. It was isolated from a patient from Cali (Colombia) in 1968, maintained at our Laboratory of Parasitology of the School of Medicine of the "Universidad Autónoma" of Madrid (Spain) from February, 1970. This strain has been characterized morphologically and histopathologically at our Laboratory (submitted for publication). It has been maintained in our Laboratory through successive passes in Swiss mice at 30 day intervals.

T. cruzi Bolivia strain. This strain was isolated from a specimen of *Triatoma vitticeps* captured in Vitichi (Bolivia) in 1971 and was kindly given by the Faculty of Pharmacy of the "Universidad Complutense" of Madrid (Spain) in December, 1986. Thereafter, it has been maintained in our Laboratory by passes in Swiss mice at 30 day intervals.

T. cruzi. Y strains. It was isolated by Pereira das Freitas in 1950 in São Paulo, was obtained from the Faculty of Pharmacy of the "Universidad Complutense" of Madrid in October 1987 and then maintained in our laboratory by passes in Swiss mice at 7 day intervals.

In all the cases the doses of *T. cruzi* used to infect the mice with the corresponding strain were of 10^5 trypomastigotes injected intraperitoneally.

Host animals.

We have studied *Mus musculus* of the strain Swiss Ico (OF1; IOPS Caw) that are maintained inbred in standard conditions in the animalarium of the School of Medicine of the "Universidad Autónoma" of Madrid. These mice are the same as used in the previous descriptions that we have used as reference, and because of their well-characterized breeding and animalarium conditions it is very difficult that any diserepancy in our study should be due to changes in the host.

Histopathological study

We sacrificed sets of three mice at intervals of 5 days during the acute infection from the 7^{th} to the 65^{th} day post-inoculation (p.i.) in the strains Cali and Bolivia and from the 7^{th} to the 30^{th} days p.i. in the strain Y, as in this strain the acute phase only last lasts this time.

Each mouse was sacrificed in a hermetic chamber containing ethyl ether and then the following organs were removed: heart, brain, liver, spleen, inguinal lymphatic ganglion, skeletal muscle (quadriceps) and descending colon (Andrade, 1974). These organs were subsequently introduced into a vial containing a buffered solution of 10% saline formaldehyde where they were fixed for 15 days before being studied.

Each organ was subjected to a macroscopic study before making the sections to be embedded in paraffin.

From the sections embedded in paraffin, 5 µm slides were made with a microtome and three slides from each organ were placed on a microscopic slide and stained with H/E. The slides from each organ of every examined animal were separated on a different slides, each one receiving its unique protocol number.

The microscopic slides were examined successively using a ocular and a x10 objective to locate the areas affected by the infection and then x 20 and x 40 objectives were used for a more detailed study of the affected tissue, noting the area of inflammatory lesions and the eventual presence of pseudocysts. In heart sections we also noted the percentage of lesions in each cardiac cavity. We have used a conventional system to evaluate the importance of inflammatory lesions and the presence of pseudocysts considering the percentage area of tissular damage ranging it in three different categories of less than 25% affected area, between 25% and 50% and greater than 50% of affected area.

Due to the possible existence of non-specific inflammatory lesions, we also used a control group of six unifected mice which were sacrificed by the same procedure used with the infected mice. The epitomized heart was fixed in a 10% buffered formol saline solution. It was sectioned and embedded in paraffin. A set of these heart sections of mice belonging to the control groups (unifected mice) was stained with Green-methyl-pironine. This stain evidences the activation of lymphocytes with the presence of a great number of intense red-stained ribosomes in the cytoplasm of these cells. This staining makes possible a differentiation between the lymphocytary proliferation produced by an infection by *T. cruzi* in infected mice from any other of non-specific character.

Results

Macroscopic study

We only observed macroscopic lesions in heart and descending colon, the former in the strains Bolivia and Y and the latter in the strain Cali. The heart lesions found in the strains Bolivia and Y were more pronounced in

Table 1. *T. Cruzi:* Comparative percentage of the observed lesions in the studied organs.

STRAIN		CALI	BOLIVIA	Y
HEART	Inflammation	86.71	84.00	66.75
	Pseudocysts	–	68.00	70.75
BRAIN	Inflammation	–	11.11	38.46
	Pseudocysts	–	–	30.76
LIVER	Inflammation	71.42	88.00	92.30
	Pseudocysts	–	–	30.76
SPLEEN	Inflammation	90.47	–	–
	Pseudocysts	–	11.11	–
GANGLION	Inflammation	–	–	–
	Pseudocysts	–	–	–
SKELETAL MUSCLE	Inflammation	61.90	44.44	53.84
	Pseudocysts	38.09	33.33	23.07
COLON	Inflammation	47.61	–	30.76
	Pseudocysts	47.61	16.16	7.69

Table 2. *T. Cruzi:* Percentage of mice which presented lesions in the different cavities of the heart (Cali, Boliva and Y strains).

STRAIN	CALI	BOLIVIA	Y
INFLAMMATION			
R.A.	–	–	30
L.A.	12	28	20
R.V.	46	8	40
L.V.	72	56	56
PSEUDOCYSTS			
R.A.	–	–	20
L.A.	–	–	16
R.V.	–	4	60
L.V.	–	68	66

R.A. = Right auricle, L.A. = Left auricle, R.V. = Right ventricle, L.V. = Left ventricle.

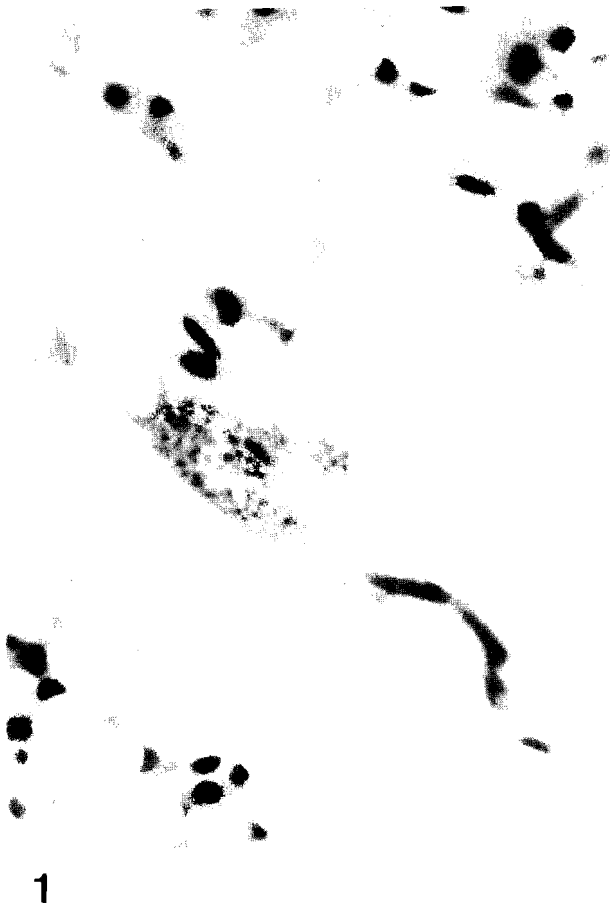

Fig. 1. Pseudocyst of *T. cruzi* in the perimuscular mouse fat. H/E. × 400

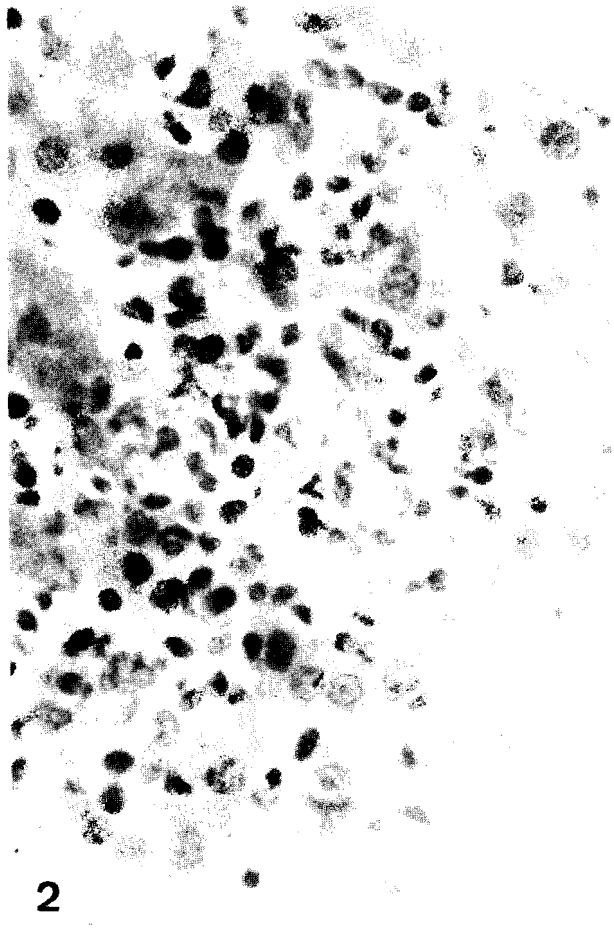

Fig. 2. Amastygotes of *T. cruzi* in hepatic parenchyme. H/E. × 400

mice sacrificed 23, 26 and 28 days p.i. showing a homogeneous brownish colouration of the cardiac tissue and a dilatation and thinning of the wall in both ventricular cavities constituting a pattern of dilated miocardiopathy. In the large intestine of mice infected with the Cali strain we observed a dilatation of the descending colon.

II. Microscopic study

IIa.– Cali Strain.

Cardiac Tissue.– The Cali strain showed inflammatory lesions in 86.71% of infected mice. These lesions appeared between the 14th and the 65th day p.i. corresponding to a + and only to ++ in hearts from mice in the 60th day p.i. None of the mice inoculated with this strain presented a miocarditis affecting more than 50% of its cardiac surface. The most affected cavity was the left ventricle (72%). We did not see any inflammation in the right auricle, and pseudocysts in any of the heart samples examined.

Brain.– No inflammatory processes or pseudocysts were observed.

Liver.– 71.42% of the studied mice presented inflammatory lesions at the hepatic level, corresponding to less than 25% of the area. No pseudocysts were observed in this tissue.

Spleen.– We observed inflammatory processes during all the period of the histopathological study, in 90.47% of the studied mice, affecting less than 25% of the area in all cases. The studied tissue was negative with respect to the presence of pseudocysts.

Lymphatic ganglion.– This tissue was negative for inflammatory processes and pseudocysts.

Skeletal muscle.– Inflammatory lesions were present in the quadriceps muscle in 61.90% of the studied mice. This miositis was present from the 14th day p.i., persisting until the 60th day. The inflammatory processes did not surpass 25% of the surface.

Pseudocysts were present from the 28th to the 60th day p.i. in a proportion of 38.09% of the studied mice. In all cases the affected area did not surpass 25% of the tissue.

Colon.– Inflammatory lesions appeared from the 21st day

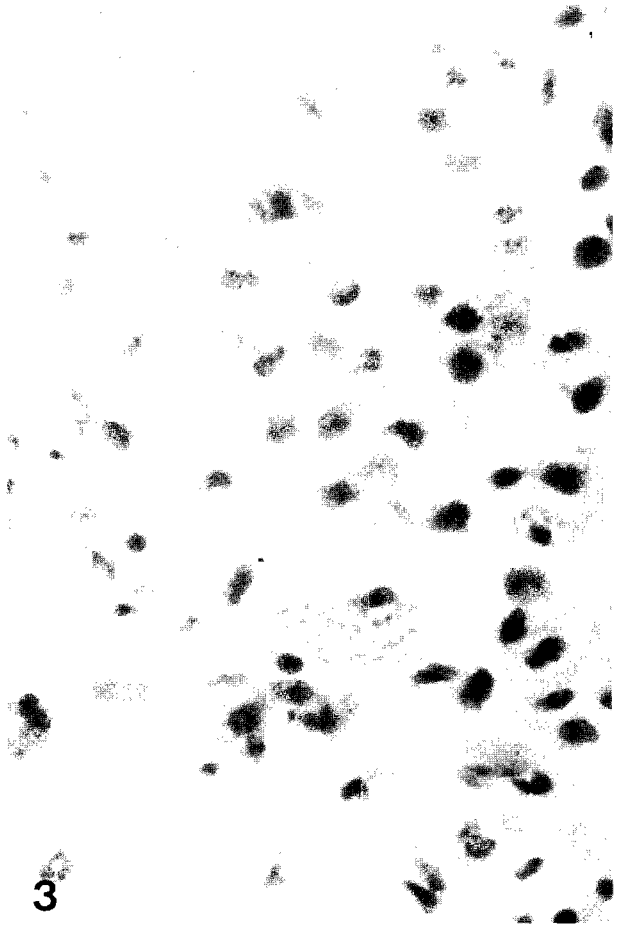

Fig. 3. Pseudocysts of *T. cruzi* in the mouse brain. H/E. × 400

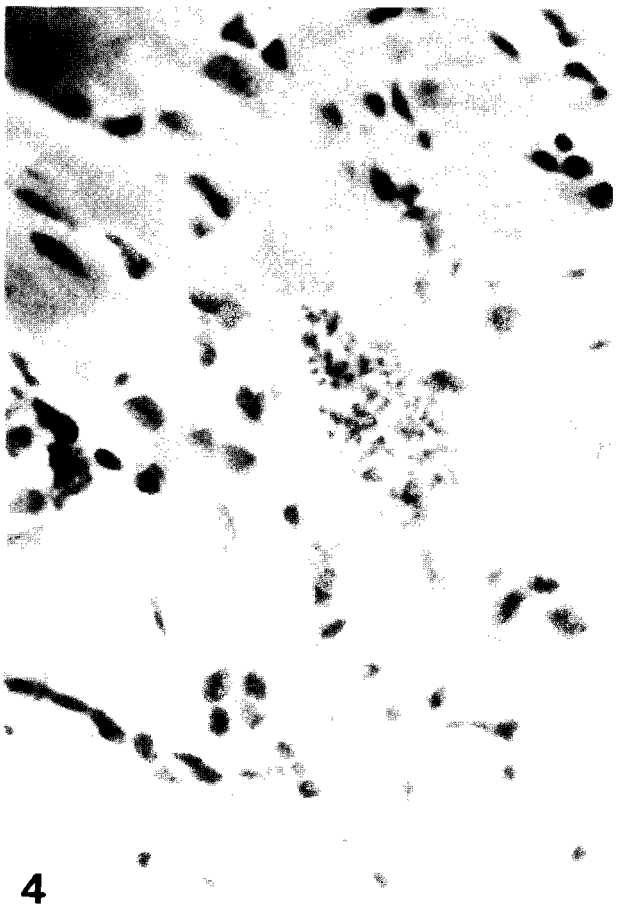

Fig. 4. Myocardic pseudocysts of *T. cruzi* in mouse. See the greast amount of amastygotes. H/E. × 400

p.i. to the 60th. These lesions were observed in 47.61% of the studied pieces. In no case was the inflammation greater than 25% of the studied area. 47.61% of the studied mice presented pseudocysts occupying a tissular surface of less than 25%. Both types of lesions coincided in the same time intervals.

IIb.– Bolivia strain

Cardiac tissue.– Miocarditis was evident from the 22nd to 65th days p.i. in 84% of the sacrificed mice. 20% presented an area of more than 50%, the major inflammation being observed between the 54th and the 59th days p.i.

The pseudocysts appeared on the same days as the inflammatory processes. Pseudocysts were present in 68% of all the observed samples. More than 50% of the tissular area was affected on the 35th and the 59th days p.i.

Necrosis of both ventricles was observed in 4% of mice.

The most affected cardiac cavity was the left ventricle in 56% of the cases. The right auricle did not present visible lesions in any of the studied samples.

Brain.– Cerebral inflammation was observed only in 11.11% of mice, at the 65th day p.i., affecting an extension of less than 25% of the tissue. The infiltrates were observed in perivascular, parenchymatous and meningeous locations. No pseudocysts were found in the brain.

Liver.– The presence of inflammatory focuses was evident during all the studied period in 88% of the studied mice the affected area being inferior to 25% in all the cases. In 28% of the cases, the granuloma with neutrophils was present and 11% showed in the hypertrophy of the Kuppfer cells. Pseudocysts were absent from all studied sections.

Spleen.– We did not observe inflammatory lesions in the whole study. Pseudocysts were present in 11% of the studied mice occupying small areas of less than 25% of the studied tissue.

Lymphatic ganglion.– No inflammatory lesions or pseudocysts were observed.

Skeletal muscle.– The inflammation was observed from the 22nd day p.i., reaching an area of more than 50% from the 59th day. This lesion was visible in 44% of the

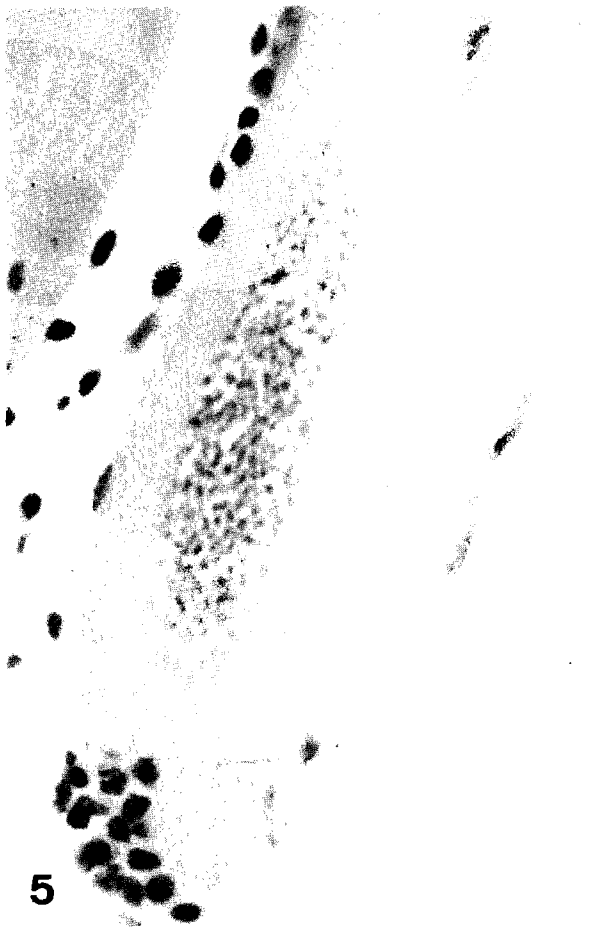

Fig. 5. Pseudocysts of *T. cruzi* in the striated mouse muscle H/E. × 400

studied mice, among which 30% presented infiltrates in areas of less than 25% of the tissue and 14% extended to more than 50% of the examined tissue. The interstitial infiltrate appeared in 16% of mice.

Pseudocysts were observed from the 17th day p.i., affecting 33% of the studied mice. In 19% of these, pseudocysts occupied less than 25% of affected surfaces, in 14% the pseudocysts occupied between 25% and 50% of the studied tissue. In the perimuscular fat zone pseudocysts were evident in 11% of the studied mice.

Colon.– No inflammatory lesions were found. Pseudocysts were present in 17% of infected mice.

II.c.– Y Strain

Cardiac tissue.– Miocarditis could be demonstrated from the beginning of the study on the 7th day p.i. in 67% of inoculated mice. This inflammation was more extensive between the 12th and 22nd days with an area of more than 50%. The inflammatory damage was greater in the left ventricle (56%).

Cardiac pseudocysts were observed in 70% of mice during all the examination period up to the 22nd day, when the extension of the inflammatory lesions diminished. They occupied areas of more than 50% of the tissue in 56% of the studied mice. The parasite was more frequently visualized in the left ventricle, appearing in 66% of the examined sections. Intracardiac calcifications and necrosis appeared in 18% of the studied mice.

Brain.– Focuses of lymphocyte infiltration were observed at the end of the acute period of infection, in all cases its extension being less than 25% of the studied tissue and affecting 38% of all the studied mice.

Pseudocysts were observed in brain sections after 17 days of infection. The percentage of mice presenting pseudocysts was 30% of all the studied animals.

Liver.– Inflammatory focuses were observed in 92% of the studied mice during all the acute phase, occupying areas of less than 25% of the visualized tissue. Close to the multifocal infiltrates of round cells we have also observed in many cases multifocal necrosis focuses. 9% of the mice presented granuloma. Pseudocysts were present in 31% of the studied animals, occupying areas of less than 50% in all the cases.

Spleen.– No inflammatory lesions nor pseudocysts were observed in the examined spleen tissue.

Lymphatic ganglion.– Inflammatory lesions and pseudocysts were absent at this level.

Skeletal muscle.– Inflammatory lesions were observed from the 12th day p.i. till the end of the acute phase, being evident in 54% of all the studied mice with an extension of less than 25% of the tissue. In 10% of the cases granuloma were evident in the studied muscle. Near the miositis, we observed inflammatory infiltration and pseudocysts in the perimuscular adipose tissue.

We could observe the presence of pseudocysts in 23% of the examined mice, occupying an area less than 25% of the studied tissue.

Colon.– Inflammatory focuses appeared at the muscular layer of the intestinal tissue of 31% of the studied mice. These focuses became patent from the 22nd day p.i., in all cases the invaded area being less than 25%.

Pseudocysts were present in 8% of the studied mice.

All the observed results are summarized in Tables 1 and 2.

Discussion

Different strains of *T. cruzi* tend to invade preferentially different organs or tissues (Köberle, 1968; Csete et al., 1985; Braun and Titto, 1985), however, in spite of this predilection, they also have the ability to invade virtually all the organs or tissues of the body (Fife, 1977). The preferential alterations of certain tissues or organs has been related to different geographic areas (Lumsden, 1974; Braun and Titto, 1985).

Andrade (1974) made a classification of *T. cruzi* that could allow the characterization of a given strain based on its morphological, histopathological and physiopathological characteristics. This classification has been widely used as a key tool for the description of many strains of *T. cruzi*.

The Bolívia strain should be included in group III of Andrade because of its high parasitemia, low mortality and high myotropic character. Ribeiro et al. (1988) studied the characteristics of this strain using Swiss mice, finding that it is capable to invade all types of cells. In our study, we have found a predominant parasitation of heart, skeletal muscle and colon without presence of the parasite in brain, liver and lymphatic tissue.

In experimentally infected mice the Cali strain shows a blood pleomorphism that ascribe it to group II of Andrade. This group has a predilection to invade cardiac muscle fibres. However, it did not show any preference of this kind in our study, appearing only in skeletal muscle and the smooth muscular layer of the colon.

The Y strain could be classified as belonging to group I of Andrade considering its blood pleomorphism pattern. It should be therefore eminently reticulotropic, as previously described by other authors (Brener, 1973; Andrade, 1974; Abrahamsohn, 1983; Pereira et al., 1987). Strikingly, we found evidence of inflammatory processes in all the organs studied except the spleen and lymphatic ganglions, the number of animals presenting cardiac and hepatic affections being greater. Pseudocysts were also observed in all the organs except lymphatic ganglions and spleen. It is really surprising the lack of reticulotropism found in this strain.

Some authors have pointed out the possibility that some strains of *T. cruzi* may change their biological characteristics with the course of time, including their tissular tropism, the severity of some lesions seeming to depend both upon the host and the parasite (Vianna, 1911; Fife, 1977; Postan et al., 1987).

It seems that a change has occurred in the tissular tropism characteristics of the strains of our study. Since we have used the same host strain used by other authors in previous reports, and have given the standarized tipification of this mouse and the careful stabulating and breeding conditions under which they have been maintained, we consider very improbable that these changes are due to a corresponding change in the host characteristics. It follows then that they can be most probably ascribed to a change in the parasite.

The most striking variations have been observed with the Y strain. This strain was first described by Andrade (1974) as mainly reticulotropic with early presence of lesions in the Mononuclear Phagocytic System (M.F.S.). Later on, Andrade and Freitas (1987) described the presence of a moderate number of intracellular parasites in cardiac fibres after 10 days of infection. Our results have shown a marked evolution in the tropic properties of this strain with a great involvement ($> 50\%$) in heart tissues as early as the 7^{th} day p.i. and absence of involvement in spleen and ganglions.

Bice and Zeledón (1970) and Clinton et al. (1975) described the different ability of different strains to invade specific tisues, the reticulotropic and myotropic strains being extremes of their spectrum. The properties we have observed in the Y strain seem to join these two extremes previously considered so distant.

The Bolivia strain was previously described as markedly capable of invading all tissues with preference for muscle fibres. We have confirmed this preference for muscular tissues, but have not been able to detect its presence in some major organs (brain, liver and lymphatic ganglion). This raises the possibility that this strain is also undergoing changes in its tropic properties.

The Cali strain has recently been characterized in our laboratory, and therefore it can be compared only with other strains of the same geographical origin. As happens with the Colombia strain, described by Andrade et al. (1985) our results show an intense inflammatory reaction due to macrophages and lymphocytes with focal infiltration with polinuclear neutrophils, as well as a marked myotropism. However, we have not observed the parasitism of cardiac tissue with fibre rupture they have reported. These differences could be attributed to a possible evolution of the properties of parasites isolated from this zone, although we cannot discard the idea that they are due to the fact that we are using a different strain, though they both have the same geographical origin.

Bice and Zeledón (1970) have established a neat correlation between the parasitaemia and the presence of pseudocysts. In this study we have observed that the major parasitic levels observed in a strain are not always coupled with major tissular lesions. In fact, we confirmed that the Bolivia strain, which presented the greatest parasitaemia, did not show a great level of tissular lesions, while the Y strain, with fairly lower levels of parasitaemia, affected a major number of organs and produced more intense lesions.

Because of the data we have described, we consider that the strains of *T. cruzi* subjected to study are probably experimenting a change in their tissular tropism due to parasite factors with the course of time. This supposes that the widespread classifications used for strain typification that rely on the constancy of the characteristics of the parasite cannot be considered to absolutely define the biological properties of every given strain, since some of them are susceptible to change with time.

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