

HISTOLOGICAL AND HISTOQUANTITATIVE STUDY OF THE RAT PAROTID GLAND AFTER *TRYPANOSOMA CRUZI* INFECTION

SILVA R.C.*, CARDOSO J.E.**, SILVA G.A.B.***, MOREIRA A.*** & ALVES J.B.****

Summary :

The present study deals with the morphology of the rat parotid gland and its changes after *Trypanosoma cruzi* infection. The glands of control and infected animals were analyzed by histologic and histoquantitative methods. After 18 days of infection with *T. cruzi*, a significant reduction of the density of the volume of the acini and duct system, as well as a significant increase in the amount of connective tissue was noted. In addition, these animals displayed an increase in the number of cells undergoing mitosis. In the 45 day infected rats, there was return to the normal pattern. It is suggested that in the infected animals the decrease in body weight could be responsible for retarded sexual maturity, leading to the lower level of testosterone. It can be assumed that decreased levels of epidermal growth factor (EGF) and neural growth factor (NGF) caused by the lack of testosterone in infected animals also contribute to the atrophy of the parotid gland and to the proliferation of the connective tissue.

KEY WORDS : parotid gland, *Trypanosoma cruzi*, Chagas' disease.

Résumé : ÉTUDE HISTOLOGIQUE ET MORPHOLOGIQUE DE LA GLANDE PAROTIDE DU RAT APRÈS INFECTION PAR *TRYPANOSOMA CRUZI*

Cette étude concerne la morphologie de la glande salivaire parotide du rat et ses altérations après l'infection par *Trypanosoma cruzi*. Les glandes des animaux normaux et infectés ont été analysées par des méthodes histologiques et histoquantitatives. Après 18 jours d'infection par *T. cruzi*, il y a eu une réduction significative de la densité du volume des acini et du système de canaux glandulaires, ainsi qu'une augmentation significative de la quantité de tissu conjonctif. En outre, les animaux ont présenté une élévation du nombre des cellules en division. Au 45^{ème} jour d'infection, les animaux ont retrouvé un état normal. On peut suggérer que, chez les animaux infectés, l'abaissement du poids est responsable d'un retard de la maturité sexuelle, avec une baisse du taux de testostérone. L'abaissement des taux du facteur épidermique de croissance (EGF) et du facteur de croissance nerveux (NGF), dus au manque de testostérone chez les animaux infectés, pourrait aussi participer à l'atrophie de la glande parotide et à la prolifération du tissu conjonctif.

MOTS CLÉS : glandes salivaires, *Trypanosoma cruzi*, maladie de Chagas.

INTRODUCTION

Chagas' disease (South American trypanosomiasis), caused by the hemoflagellate *Trypanosoma cruzi* (Chagas, 1909), is one of the most frequent and dangerous diseases in South America. It affects several organs mainly in the cardiovascular and digestive system (Koberle, 1968), including the salivary glands (Vieira, 1961). The parotid are most often involved, but not exceptionally the enlargement of submandibular and even sublingual glands may be observed (Correia Neto, 1935). Patients with enlarged salivary glands frequently show amylasemia and increased amylase content in parotid tissue (Vieira, 1961).

* Faculty of Odontology of Diamantina, MG, Brazil.

** Institute Research Renné Rachou, Belo Horizonte, MG, Brazil.

*** Institute of Biological Science, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

**** University of Uberaba, Uberaba, MG, Brazil.

Correspondence: José Bento Alves, Department of Morphology, Institute of Biological Science, Federal University of Minas Gerais, P. Box. 486, 31270.010, Belo Horizonte, MG, Brazil.

Tel.: (0) 31-499 28 11 – Fax: (0) 31-499 27 71.

E-mail: alvesjb@mono.icb.ufmg.br

The observations that in Chagas' disease there occurs an enlargement of the submandibular gland in rats at 18 and 32 days after infection with *Trypanosoma cruzi* was first reported by Alves & Machado (1980). On the other hand, during the acute phase of experimental Chagas' disease, in spite of the appreciable inflammatory process in the submandibular glands of *T. cruzi*-infected rats (Alves & Machado, 1980), these glands undergo a severe reduction in sympathetic (Machado *et al.*, 1984) and parasympathetic innervation (Alves & Machado, 1984) and exhibit enlarged acini on day 18 of infection (Alves & Machado, 1980). At the histological level, the main changes observed were accelerated acinar development and delayed ductal maturation, characteristics that are more evident in the developing granular ducts (Alves & Machado, 1980). The enlargements induced by *T. cruzi* infection (acute phase) is mediated through β -receptors since it is blocked by propranolol (Alves & Machado, 1986).

It has been suggested that in the acute phase of experimental Chagas' disease increased levels of catecholamines may accelerate acinar maturation (Alves & Machado, 1986) and stimulate synthesis of proteins (Alves *et al.*, 1994). An increase in the circulating

levels of catecholamines would be expected as a result of large-scale destruction of adrenergic nerve terminals in the acute phase of Chagas' disease, as occurs in the heart (Machado *et al.*, 1975), in the submandibular gland (Machado *et al.*, 1984) and in the parotid gland (Alves, 1990). It appears, therefore, that the circulating catecholamines may act as a β -adrenergic agonist, directly on the submandibular acinar cells (Alves & Machado, 1986). As in isoproterenol-treated animals, the submandibular glands of infected rats exhibit glandular hypertrophy, acceleration of the acini development and alterations in the secretory granules morphology (Alves *et al.*, 1995) as well as synthesis of specific proteins. In fact, cystatin S has been described in the saliva (Alves *et al.*, 1994) and in granular fractions of the submandibular glands of infected rats (Silva *et al.*, 1995).

The rat parotid gland, like the rat submandibular gland is richly innervated by both branches of the autonomic nervous system. During the acute phase of experimental Chagas' disease there is sympathetic denervation of the parotid gland (Alves, 1990). However, alterations in the structure of the parotid gland is unknown. The present study was performed in order to verify if any histological changes occur in the parotid gland during the acute phase of experimental Chagas' disease.

MATERIALS AND METHODS

ANIMAL INOCULATION

Forty male Holtzman rats, aged 27-29 days, were inoculated intraperitoneally with 0.15 ml of mouse blood containing about 300,000 trypomastigotes of Y strain of *Trypanosoma cruzi*. This strain, isolated from a patient with Chagas' disease (Silva & Nussenzweig, 1953) and has been maintained in mice by repeated blood passages every seven days. The infection was confirmed by the presence of living trypomastigotes in the blood of all inoculated animals 10 days after inoculation.

HISTOLOGICAL AND HISTOQUANTITATIVE STUDY

Two groups of infected and 18 littermate control rats were killed by cervical dislocation and exsanguination, 18 and 45 days after inoculation. The right and left parotid gland were dissected out, fixed in Bouin's fluid for seven hours, briefly washed in running tap water, dehydrated, embedded in paraffin wax and sectioned at 6 μ m. The sections were stained with hematoxylin and eosin and then used to determine the proportional volume of the components in the whole gland. Microscopic examination was a magnification of X 400 using a Zeiss integration eyepiece graticule. All

counts were made by one observer (RCS) counting 2,000 test points per animal, 25 per microscopic field. Points were allocated to acini, intercalary duct, striated duct, excretory ducts and connective tissue (comprising fibrous, adipose, neural and lymphatic tissue) (Scott *et al.*, 1986). The number of points per component, expressed as a proportional of the total points, indicated the proportional volume of the component in the whole gland (Aherne & Dunnill, 1982). Wilcoxon non parametric method was used to compare the results from control and infected animals.

In order to verify the occurrence of mitosis, four infected and four control rats were submitted to a treatment with colchicine (Sigma, 6 mg/kg) three hours before being killed. Samples of parotid gland were prepared as outlined above, sectioned at 7 μ m and then stained using the Dominicki (0.2 % Eritrosin Plus, 1 % Orange G and 0.5% Toluidine blue). Mitosis counts were made using a calibrated eyepiece micrometer and comparisons between infected and control animals made using the Wilcoxon non parametric method.

RESULTS

The body weight of 18 day infected rats was significantly lower than that of controls, whereas the 45 day infected rats did not show differences when compared to controls (Table I). The histological examination of control and 18 day infected parotid glands revealed that changes in size of acinar cells comprised the major histologic alteration associated with *Trypanosoma cruzi* infection (Fig. 1a and 1b). Mitotic cells were not evident in controls (Fig. 1a), but were prominent in 18 day-infected rats (Fig. 1b and 2). The results from the volume density measurements of the various components of the parotid gland in control and infected rats are shown in Tables II and III. In 18 day infected rats there was a significant decrease of the volume density of the acini and an increase of the connective tissue and stroma ($p < 0.05$). The ducts were not appreciably altered. The number of mitotic cells was higher in the acini, ducts and stroma of the

Days after inoculation	Treatment	Body weight (g) Mean $\pm$ SD
18	Control (n = 8)	116.99 $\pm$ 30.27
	Infected (n = 16)	96.70 $\pm$ 24.39*
45	Control (n = 6)	192.95 $\pm$ 32.90
	Infected (n = 12)	175.22 $\pm$ 32.33

* Significantly different in the Wilcoxon test at $P < 0.05$; n = number of animals.

Table I. – The effect of *Trypanosoma cruzi* infection on body weight (g).

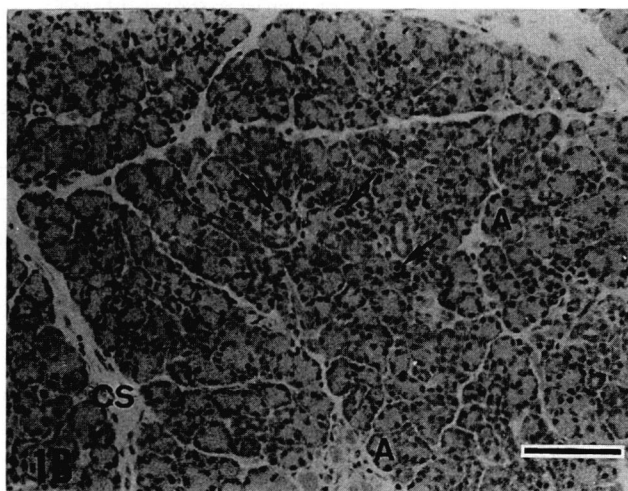
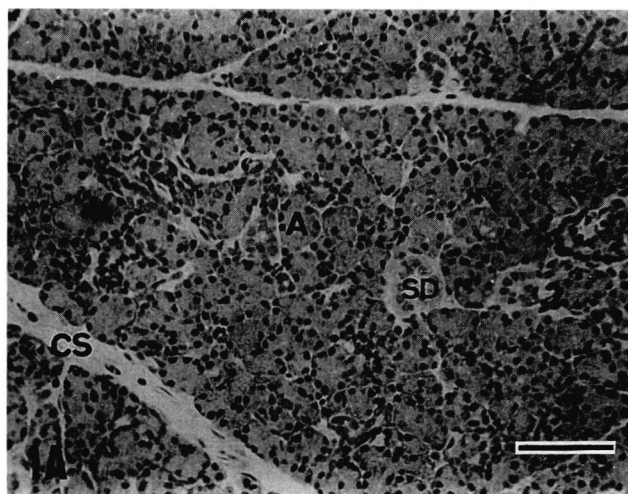


Fig. 1. – Sections of control and 18 day infected rats treated with colchicine. (a) Control. (b) Infected. Note reduction in size of acinar cells. Mitosis (arrows). Acini (A); Striated duct (SD); Connective stroma (CS). Dominici. Bar: 50 μ m.

infected animals, in contrast to the lower number in the controls (Table IV and Fig. 1a, 1b and Fig. 2). The histological aspect as well as the number of mitotic cells in the parotid glands of 45 day infected rats returned to the normal pattern (Table IV and Fig. 3a and 3b).

DISCUSSION

In 18 day infected rats, a gradual reduction of the volume of the acini and concomitant increase of the volume of stroma and connective tissue could be also observed (Table II). These findings are comparable to light-microscopic observations on the parotid gland of rats after liquid feeding (Hall & Schneyer, 1964; Scott *et al.*, 1990; Scott & Gunn, 1994). Our results are also

Tissue components	Treatment	
	Control (n = 8)	Infected (n = 16)
Acini	87.20	83.19*
Intercalary ducts	0.37	0.23
Striated ducts	3.67	3.04
Excretory ducts	1.64	1.96
Connective tissue	7.12	12.58*

Data presented as mean n = number of animals.

* Significantly different in the Wilcoxon test at $P < 0.05$.

Table II. – Volume density of the various components of the rat parotid gland of control and 18 days of infection.

Tissue components	Treatment		
	Control (n = 6)	Infected (n = 6)	
Acini	79.50	79.30	NS
Intercalary ducts	0.56	0.56	NS
Striated ducts	5.66	5.36	NS
Excretory ducts	2.76	3.13	NS
Connective tissue	11.53	11.63	NS

Data presented as mean n = number of animals.

* Significantly different in the Wilcoxon test at $P < 0.05$.

Table III. – Volume density of the various components of the rat parotid gland of control and 45 days of infection.

Tissue components	Treatment	
	Control (n = 4)	Infected (n = 4)
Acini	10.50	72.00*
Striated ducts	1.75	5.75
Excretory ducts	1.50	4.50*
Connective tissue	3.50	15.00*

Data presented as mean n = number of animals.

* Significantly different in the Wilcoxon test at $P < 0.05$.

Table IV. – Mean of mitoses number observed in rat parotid gland of control and 18 days infection.

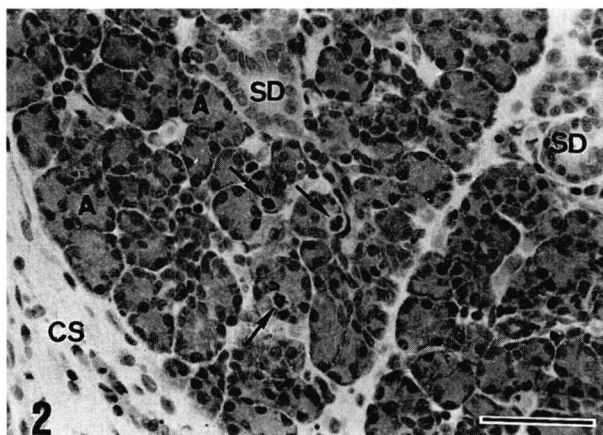


Fig. 2. – Section of 18 day infected rats treated with colchicine. Mitoses (arrows) were observed in acini, duct and connective tissue. Dominici. Bar: 50 μ m.

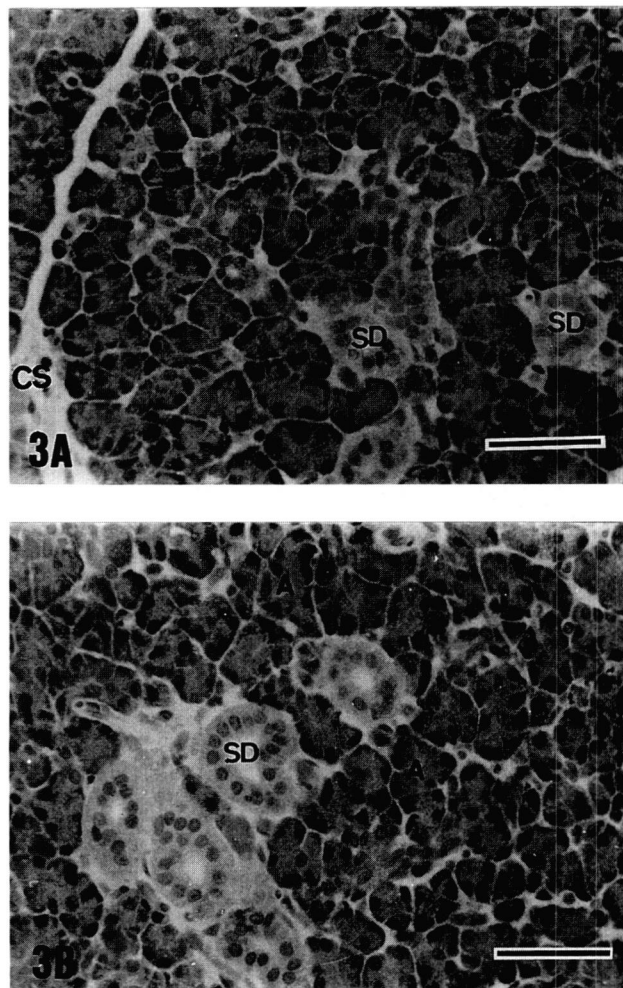


Fig. 3. – Sections of control and 45 day infected rats treated with colchicine.

(a) Control. (b) Infected-rats. Control and infected animals exhibit the same feature histologic. Dominici. Bar: 50 µm.

similar to those observed on the rat parotid gland after orchiectomy (Jezek *et al.*, 1996).

In general, testosterone stimulates anabolic processes in various cells (Wilson & Griffin, 1980). According to some biochemical investigations, the parotid gland binds more testosterone than the prostate or seminal vesicles, which largely depend on this hormone (Di Mangoni & Stefano, 1990). Moreover, it is known that testosterone stimulates the acini and ducts of salivary glands to secrete certain growth factors such as epidermal growth factor (EGF) (Schneyer & Humphreys-Beher, 1990) and neural growth factor (NGF) (Schneyer & Humphreys-Beher, 1990; Hazen-Martin *et al.*, 1974). These factors influence the renewal and the growth of the acinar and ducts cells (Barthe *et al.*, 1974; Walker, *et al.*, 1981; Thesleff *et al.*, 1988). In orchiectomized animals 30-60 days after castration, a significant reduction of the volume of the acini and the duct system as well as a significant increase of the connective tissue volume

were noted. This results suggest that orchiectomy affects the rat parotid gland, and suggests the existence of a hormonal interaction between the testis and the mammalian parotid gland (Jezek *et al.*, 1996).

During the acute phase of experimental Chagas' disease there is a significant decrease in body weight (Table I). Since body weight is more important than age to sexual maturity (Courout *et al.*, 1970), we expect that infected rats may exhibit a delay in testes development during the acute phase of the disease. Furthermore, we postulate that in these animals there is probably a reduction in the level of testosterone. In fact, a significant decrease was observed in the seminal vesicle and ventral prostate of chagasic rats, which also presented a decrease in plasma testosterone levels (Tavares *et al.*, 1994).

One can postulate that decreased levels of EGF and NGF caused by the lack of testosterone in infected-rats may lead to the atrophy of the gland and proliferation of the connective tissue.

Alternatively, it has also been suggested that in the acute phase of experimental Chagas' disease increased levels of plasma catecholamines could accelerate acinar submandibular gland maturation or stimulate protein synthesis (Alves & Machado, 1986; Alves *et al.*, 1994; Silva *et al.*, 1995). Increases in the circulating levels of catecholamines would be expected as a result of large scale destruction of adrenergic nerve terminal in the acute phase, as occurs in the heart (Machado *et al.*, 1975) in submandibular gland (Machado *et al.*, 1984), and in the parotid gland (Alves, 1990). It appears, therefore, that the circulating catecholamines may act as a β -adrenergic agonist and induce histological alterations. However, it has been noted that there is a marked decrease in the number of muscarinic and β -adrenergic receptors in parotid glands of rats maintained on a liquid diet, or after autonomic denervations of the glands (Schneyer *et al.*, 1987). Although there is an increase in the circulating levels of catecholamines, the parotid gland may not respond in a manner similar to the submandibular gland. Thus, the hypothesis that the acinar atrophy in the parotid gland induced by Chagas' disease may be related to the lack of testosterone gains credence from the rapid reversal that occurs on 45 days infected animal, when body weight, histological aspect and the number of mitoses return to normal pattern. This will be the subject for future studies dealing with a more detailed characterization of biochemistry and immunocytochemistry of the cell gland.

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