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EFFECT OF SCORPION TOXIN ON THE ENTEROCHROMAFFIN-LIKE CELLS IN NORMAL AND Trypanosoma cruzi-INFECTED RATS: A MORPHOLOGICAL STUDY[1].

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SUMMARY

Intravenous injection of scorpion toxin (**Tityus serrulatus**) in normal and **Trypanosoma cruzi** infected rats did not cause ultrastructural morphologic changes on enterochromaffin-like (ECL) cells of the stomach, although it induced a significant increase of the gastric secretion.

Our data seem to indicate that gastric ECL cells structure is not affected by stimulation with scorpion toxin or by acute infection with **T. cruzi** in the rat.

KEY WORDS: Enterochromaffin-like cells; scorpion toxin; **Trypanosoma cruzi**; rat; gastric secretion.

INTRODUCTION

The ECL cells of the stomach present the basic morphological characteristics of the APUD cells[29, 30]. They are relatively numerous in oxyntic mucosa of rodents and could synthesize, store and secrete histamine, 5-hydroxytryptamine and peptides[3, 4, 17, 18, 19, 32, 39, 40]. The secretory activity of these cells seems to be regulated by the vagi nerves and by the hormone gastrin[20, 22, 23, 25]. Incubation of ECL cells in a medium containing catecholamines and acetylcholine has shown that epinephrine increases the volume of the secretory vesicles and decreases the density of its osmiophilic core[33].

The toxin from the Brazilian scorpion **Tityus serrulatus** induces gastric acid and pepsin secretion either "in vivo" and "in vitro"[9, 10, 16, 17]. This effect seems to be secondary to depolarization of autonomic post-ganglionic nerve endings induced by the toxin with consequent release of chemical mediators such as acetylcholine, catecholamines and histamine[13, 14]. On the other hand, the scorpion toxin enhances the gastric wall contents of histamine in normal[11] and **T. cruzi**-infected rats[1, 10]. Furthermore, the main source of histamine in the rat stomach is supposed to be the ECL cell[18, 19, 24].

Acute infection by **T. cruzi** leads to a decrease in the number of neurons and ganglia of the intramural plexuses of the digestive tract[36, 37, 38] and depletion of chemical mediators in rat

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heart and mouse colon[26, 27, 28]. These effects on rats were only observed when the experiments were performed around the 20th day of infection[26, 27]. A similar model has been used to study the effects of **T. cruzi** infection upon the morphological characteristics of ECL cells present in the rat stomach. In the present work we proposed to study the ultrastructure of ECL cells of the stomach of both normal and of **T. cruzi**-infected rats under the effect of scorpion toxin.

MATERIAL AND METHODS

Thirty-five to 45 days old, male albino rats (n = 42), with an average weight of 180 g were divided in 4 groups in the present study: I — rats injected with saline (n = 14); II — rats inoculated with **T. cruzi** and injected with saline (n = 14); III — rats injected with scorpion toxin (n = 9); IV — rats inoculated with **T. cruzi** and injected with scorpion toxin (n = 5). Nineteen days old rats of groups II and IV were inoculated with the Y strain of **T. cruzi** by intraperitoneal route (4000 trypomastigotes/g). The parasitism was confirmed two weeks later and the experiments conducted when the rats were 40 days old. Food, but not water, was withdraw 18-24 hours before the experiments. The animals were then anesthetized with an intraperitoneal injection of urethane (140 mg/100 g) and the trachea and right jugular vein were cannulated. A bolus injection of saline or scorpion toxin (25 μg/100 g) were injected through the jugular catheter. The animals were then killed 60 minutes later by section of abdominal segment of aorta and caval vein. The esophagus and pylorus were ligated and the whole stomach excised. The gastric juice was collected for the assessment of volume, acidity and peptic activity. To determine the sample acidity the gastric juice was transferred to a 10 ml beaker and titrated with 0.01 N NaOH to pH 7.4 using a tiny burette. The pepsin output was measured according to the modified method of ANSON[2, 6]. Fragments of oxyntic mucosa were fixed in 10% formol solution for 18 hours and were processed according to conventional histological techniques for optical microscopy studies. Sections of 5,0 μm were stained by hematoxylin and eosin and by silver impregnation. The ECL cells were counted in all sections. The area of each section was measured by a planimeter and the results were expressed as number of cells per mm^2. In the groups II and IV only the animals with lesions of the intramural plexuses were processed for ultrastructural studies of the ECL cells. To perform these studies slices of oxyntic mucosa from animals shown to have nerve damage were collected and fixed for 3 hours in 0,2 M phosphate buffered 3% glutaraldehyde, pH 7.2, followed by two hours fixation in 0,1 M phosphate buffered 1% osmium at 4 C. Semi-thin sections were obtained and stained for analysis of the tissue morphology. The ultra-thin sections were obtained at the level of one third of the basal portion of the mucosa in an ultra microtome Porter-Bloom MT1. Sections were stained with uranile acetate and lead citrate and examined in Zeiss EM-9 S2 electronic microscope.

A total of 253 ECL cells were analysed: 70 from group I; 91 from group II; 51 from group III and 41 from group IV. The photographs were taken and amplified, giving a final magnification of 20480 folds. The perimeter of all ECL cells, plasmatic and nuclear membranes as well as the granular and clear vesicles and dense core granules were reproduced on a transparent sheet. The areas of these structures were measured by a planimeter and expressed in μm^2. All granular and clear vesicles contained in the ECL cells were counted. Data were compared by the analysis of variance and differences were taken as significant when $P < 0,05$. Results were expressed as mean ± SEM.

RESULTS

The distribution and morphological aspects of the ECL cells in the oxyntic mucosa of rat stomach as demonstrated by optical microscopy were the same as described elsewhere[7, 25, 40]. The Auerbach's plexus of the stomach of acutely **T. cruzi**-infected rats showed the characteristic lesions: ganglionitis, periganglionitis and neuronal degeneration. On the other hand several other organs such as heart, liver, spleen and cecum also showed signs of severe parasitism. In spite of these alterations no morphological or numerical changes of the ECL cells were detected under these light microscopy studies (Figure 1 and Table 1).

In all groups, at the electron microscopic examination, the ECL cells were pyramidal or

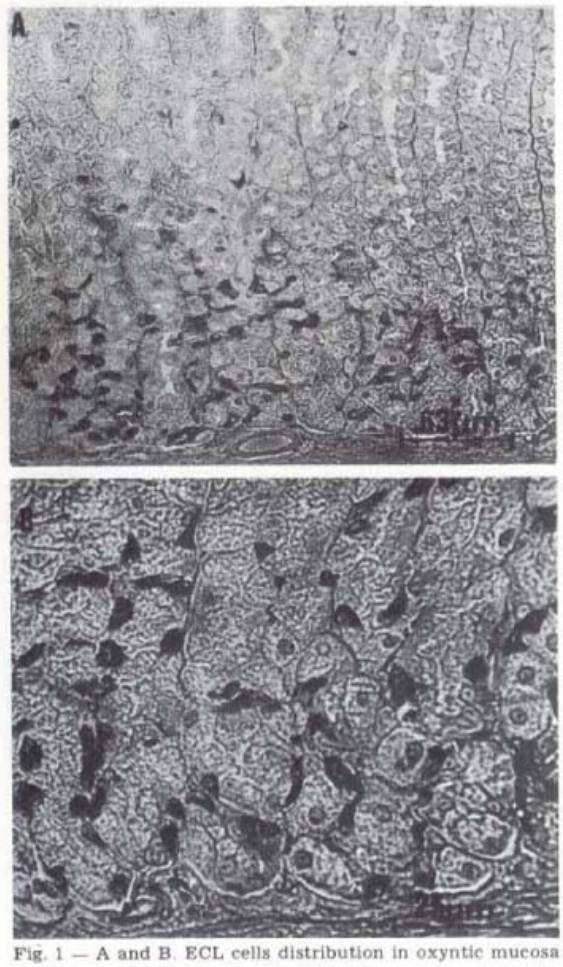

Fig. 1 — A and B. ECL cells distribution in oxyntic mucosa of rat stomach. The dark color is due to silver impregnation of ECL cells (Grimelius stain).

TABLE 1

ECL cells (number per mm^2) in oxyntic mucosa of rat stomach in control, **T. cruzi**-infected, scorpion toxin and **T. cruzi**-infected + scorpion toxin groups.

Groups (n)	Number of ECL cells/mm^2
I — Control (14)	103 ± 26
II — **T. cruzi**-infected (14)	126 ± 36
III — Scorpion toxin (9)	107 ± 19
IV — **T. cruzi**-infected + Scorpion toxin (5)	119 ± 32

Values represent the mean ± SEM

T. cruzi-infected rats (Y strain): 4000 trypomastigotes/g, i. p., 20 days before the experiments.

Animals from group I and II were injected with saline and from groups III and IV with scorpion toxin (25 μg/100 g).

Statiscally significant differences between groups were not observed.

irregular in shape and frequently presenting a thin cytoplasmic process which could be seen between parietal and zimogenic cells (Figure 2). Most of the ECL cells were adjacent to the basal membrane without communication with the glandular lumen (Figure 2). The granular vesicles presented an electron-dense, excentric core separated from the membrane by a wide clear halo (Figures 3 and 4). The agranular vesicles did not present any electron dense content. Among the vesicles, the endoplasmic reticulum, mitochondria, Golgi apparatus and free ribosomes were sparse. The nucleus of ECL cells was ovoid or irregular in shape. The chromatin showed a tendency to form clumps close to the nuclear membrane which presented a number of

TABLE 2

Average number of enterochromaffin-like cell secretory vesicles per μm^2 of cytoplasm in rat stomach.

Groups (n)	Number of ECL cells	Number of vesicles/μm^2	
		Granular	Clear
I — Control (14)	70	0,79 ± 0,06	0,61 ± 0,07
II — **T. cruzi**-infected (14)	91	0,93 ± 0,06	0,49 ± 0,05
III — Scorpion toxin (9)	51	0,74 ± 0,09	0,43 ± 0,07
IV — **T. cruzi**-infected + Scorpion toxin (5)	41	0,55 ± 0,04	0,42 ± 0,08

Values represent the mean ± SEM

T. cruzi-infected rats (Y strain): 4000 trypomastigotes/g, i. p., 20 days before the experiments.

Animals from group I and II were injected with saline and from groups III and IV with scorpion toxin (25 μg/100 g).

Statiscally significant differences between groups were not observed.

TABLE 3

Area (μm^2) of the enterochromaffin-like cell secretory vesicles (granular vesicles, clear vesicles and core)

Groups (n)	Granular vesicles	Clear vesicles	Core
I — Control (14)	0,15 ± 0,011	0,18 ± 0,011	0,02 ± 0,012
II — **T. cruzi**-infected (14)	0,17 ± 0,009	0,18 ± 0,013	0,03 ± 0,009
III — Scorpion toxin (9)	0,15 ± 0,009	0,17 ± 0,013	0,03 ± 0,019
IV — **T. cruzi**-infected + Scorpion toxin (5)	0,15 ± 0,01	0,18 ± 0,010	0,03 ± 0,012

Values represent the mean ± SEM for 5 to 14 experiments.
T. cruzi-infected rats (Y strain): 4000 trypomastigotes/g, i. p., 20 days before the experiments.
Animals from group I and II were injected with saline and from groups III and IV with scorpion toxin (25 μg/100 g).
Statiscally significant differences between groups were not observed.

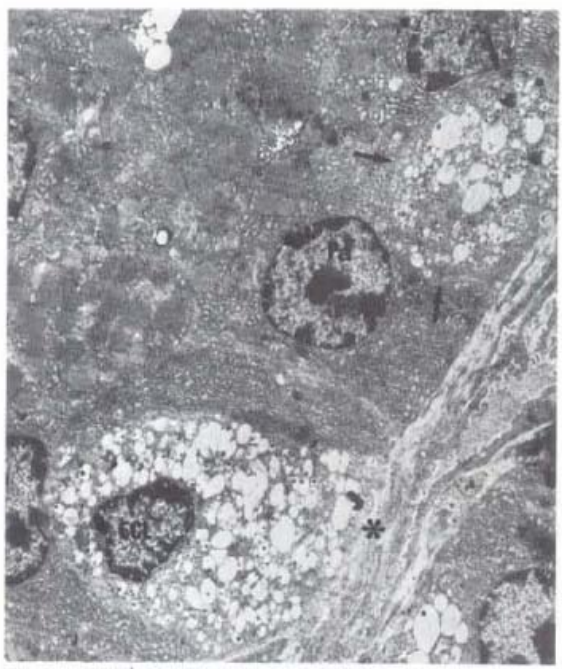

Fig. 2 — An enterochromaffin-like cell (ECL) and a cytoplasmic process (arrows) are lying on the basement membrane (✳) and adjacent to a parietal cell (PA) (X 7.780).

pores. Data showing the measurements of ECL cells vesicles and cores are presented in Tables 2 and 3, and no significant differences between the groups were detected. The dose of scorpion toxin used induced a great increase in volume, acidity and peptic activity of gastric juice when compared to controls (Table 4).

DISCUSSION

The intravenous injection of purified scorpion toxin from the Brazilian scorpion **Tityus serrulatus** in rats induces an increase on gastric secretion of acid and pepsin[9, 10]. This effect depends on the action of acetylcholine and histamine as it is blocked either by atropine or cimetidine[9]. On the other hand, the toxin induces a significant increase in the gastric content of histamine in the rat. It has been reported that the main source of histamine in the oxyntic mucosa

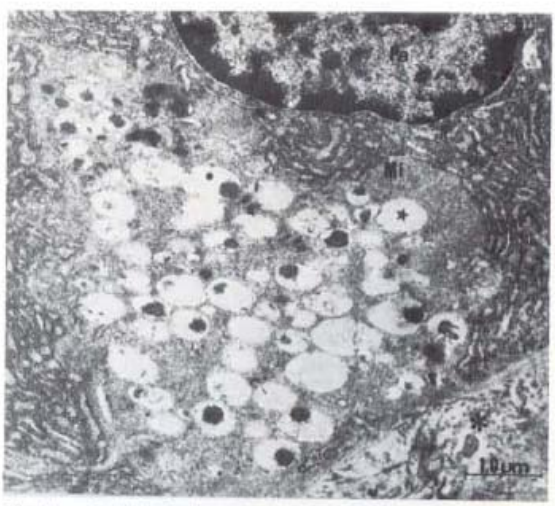

Fig. 3 — Detail of cytoplasmic process of an enterochromaffin-like cell presenting granular (*) and clear (★) vesicles PA = nucleus of an adjacent parietal cell; Mi = mitochondria; (✳) basement membrane (X 20.480).

TABLE 4
Effect of scorpion toxin on gastric secretion of acid and pepsin in the rat

Groups (n)	Volume (ml)	Acid output μEq/h	Pepsin output μmol/h
I — Control (6)	0,4 ± 0,05	11,5 ± 1,9	0,6 ± 0,1
II — **T. cruzi**-infected (6)	0,5 ± 0,1	23,0 ± 4,0	3,7 ± 0,6
III — Scorpion toxin (6)	2,1 ± 0,3*	128,0 ± 8,4*	16,6 ± 0,9*
IV — **T. cruzi**-infected + Scorpion toxin (6)	1,9 ± 0,2	139,6 ± 26*	18,5 ± 3,0*

Values represent the mean ± SEM for 8 experiments.
T. cruzi-infected rats (Y strain): 4000 trypomastigotes/g, i. p., 20 days before the experiments.
Animals from group I and II were injected with saline and from groups III and IV with scorpion toxin (25 μg/100 g).
* Statistically different from control group injected with saline and from chagasic rats injected with saline $P < 0,05$.

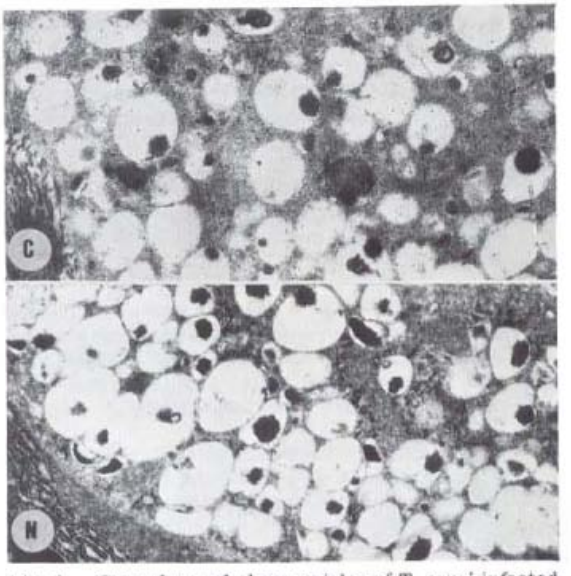

Fig. 4 — Granular and clear vesicles of T. cruzi-infected (C) and normal (N) rats after scorpion toxin (25μg/100g). There are no morphologic differences. (X 20 480).

of the rat are the ECL cells[18, 19, 35]. The morphologic characteristics of those cells make them easily recognized at electron microscope due to their typical secretory granules. In the present work the ultrastructural analysis of ECL cells has shown the same morphological pattern described elsewhere[7, 12, 21, 24, 30, 31, 34, 40]. Although the scorpion toxin has induced an increase in gastric secretion and in the histamine content of the rat stomach[11], our data has not shown ultrastructural changes in the ECL cells in both normal and **T. cruzi**-infected rats under its effect. If the scorpion toxin induces an increase of histamine content in the rat stomach, one should expect for modifications in the ultrastructure of the ECL cells in the animals inoculated with toxin once these cells are said to be the main source of gastric histamine in rats. However, this was not found. Some hypothesis can be raised to explain the results: a — it might well be that the secretory granules of ECL cells in the stomach contain other chemical mediators, for example peptides. In that way, only the release of histamine would not be enough to induce changes in the morphological characteristics of the cells; b — the histamine present in ECL cells would not participate in the process of gastric secretion. In this case the main source of histamine could be the mast cells. This possibility is reinforced by the fact that either normal and **T. cruzi**-infected rats, when inoculated with scorpion toxin, showed an increase in the number of mast cells present in the gastric wall of the rat in both, glandular and membranous regions[1, 10]; c — the scorpion toxin would not have acted on the ECL cells in the condition of the present experiment, although it had induced a substancial increase of volume, acidity and peptic activity of gastric juice; d — the 60 minutes, time elapsed between the injection of the toxin and the collection of the gastric juice, would have been sufficient for restoration of the original components of the ECL cells. For example, in spite of the release

of histamine the intracellular stores might have been replaced by means of a rapid turnover[5].

The acute infection with **T. cruzi** was employed in order to obtain an intrinsic denervation. This in fact has occurred as morphological studies of the Auerbach plexus of the stomach under ordinary light microscopy was done and all the animals studied have shown a ganglionitis and a partial destruction of neurones and nerve endings.

The ECL cells have shown similar ultrastructural characteristics either in normal rats or in **T. cruzi**-infected animals inoculated with saline or with scorpion toxin. In the present model we do not know if the denervation has been sufficient to impair the functional integrity of ECL cells as described by other authors[20, 25]. On the other hand, the gastroenteric neurons constitute an heterogenous population[8, 15] and if the ECL cells are influenced by a definite type of neuron an experimental model with selective denervation would be useful to clarify the present question.

RESUMO

Efeito da toxina escorpiônica sobre as células enterocromafins-like de ratos normais e infectados pelo Trypanosoma cruzi

A injeção intravenosa de toxina escorpiônica (**Tityus serrulatus**) em ratos normais e infectados pelo **Trypanosoma cruzi** não causou alterações morfológicas ultra-estruturais das células enterocromafins-like (ECL) do estômago, embora tenha induzido a aumento significativo da secreção do suco gástrico.

Nossos resultados parecem indicar que a estrutura das células ECL do estômago de ratos não é afetada pela estimulação com a toxina escorpiônica ou pela infecção aguda pelo **T. cruzi.**

REFERENCES

1. ALMEIDA, A. P.; GOBBI, H.; TOPPA, N. H.; CHIARI, E.; GONZAGA, H. M. S.; GOMEZ, M. V.; FREIRE MAIA, L. & CUNHA-MELO, J. R. — Gastric Acetylcholine and histamine content in normal and **Trypanosoma cruzi**-infected rats. **Braz. J. med. biol. Res.**, (submitted).

2. ANSON, M. L. — The stimulation of pepsin, trypsin, papain and cathepsin with hemoglobin. **J. gen. Physiol.**, 22: 79-89, 1938.

3. AURES, D. W. & HAKANSON, R. — Histidine decarboxylase and DOPA decarboxylase in the stomach of developing rat. **Experientia, 24**: 666-667, 1968.

4. AURES, D. W.; HAKANSON, R. & SCHAUER, A. — Histidine decarboxylase and DOPA decarboxylase in the rat stomach. Properties and cellular localization. **Europ. J. Pharmacol.**, 3: 217-234, 1968.

5. BEAVEN, M. A. — Factors regulating availability of histamine at tissue receptors. **In:** GANELLIN, G. R. & PARSONS, M. E., ed. — **Pharmacology of histamine receptors.** Bristol Wright PSG, 1982. cap. 3, p. 103-145.

6. BERSTAD, A. — A modified hemoglobin substrate method for the stimulation of pepsin on gastric juice. **Scand J. Gastroent.**, 5: 343-348, 1970.

7. CAPELLA, C.; VASSALLO, G. & SOLCIA, E. — Light and electron microscopic identification of the histamine-storing argyrophil (ECL) cell in murine stomach and of its equivalent in others mammals. **Z. Zellforsch., 118**: 68-84, 1971.

8. COOK, R. & BURNSTOCK, G. — The ultrastructure of Auerbach's plexus in the guinea pig. I. Neuronal elements. **J. Neurocytol.**, 5: 171-194, 1976.

9. CUNHA-MELO, J. R.; GONZAGA, H. M. S.; ALZAMORA, F. & FREIRE-MAIA, L. — Effects of purified scorpion toxin (tityustoxin) on gastric secretion in the rat. **Toxicon, 21**: 843-848, 1983.

10. CUNHA-MELO, J. R. — **Efeito da toxina purificada do** escorpião *Tityus serrulatus* **(tityustoxina) sobre a secreção gástrica de ratos normais e chagásicos.** Belo Horizonte, 1985. (Tese de doutoramento — Faculdade de Medicina da Universidade Federal de Minas Gerais).

11. CUNHA-MELO, J. R.; ALMEIDA, A. P.; GONZAGA, H. M. S.; GOMEZ, M. V. & FREIRE-MAIA, L. — Effect of scorpion toxin on gastric histamine and acetylcholine content in the rat. **Braz. J. med. biol. Res., 20**: 393-401, 1987.

12. FORSMANN, W. G.; ORCI, L.; PICTET, R.; RENOLD, A. E. & ROVILLER, C. — The endocrine cells in the epithelium of the gastrintestinal mucosa of the rat. **J. Cell. Biol., 40**: 692-715, 1969.

13. FREIRE-MAIA, L.; CUNHA MELO, J. R.; FUTURONETO, H. A.; AZEVEDO, A. D. & WEINBERG, J. — Cholinergic and adrenergic effects of tityustoxin. **Gen. Pharmacol. 7**: 115-121, 1976.

14. FREIRE-MAIA, L.; ALMEIDA, H. O.; CUNHA-MELO, J. R.; AZEVEDO, A. D. & BARROSO, J. — Mechanism of the pulmonary edema induced by intravenous injection of the scorpion toxin in the rat. **Agents and Actions, 8**: 113-118, 1978.

15. GABELLA, G. — **Structure of the autonomic nervous system. London,** Chapman and Hall, 1976.

16. GONZAGA, H. M. S.; ALZAMORA, F.; CUNHA-MELO, J. R. & FREIRE-MAIA, L. — Gastric secretion induced by scorpion toxin. **Toxicon, 17**: 316-318, 1979.

17. GONZAGA, H. M. S. — **Ação da tityustoxina sobre a secreção gástrica "in vitro" e "in vivo"**. Belo Horizonte, 1980. (Dissertação de mestrado — Instituto de Ciencias Biológicas da Universidade Federal de Minas Gerais).

18. HAKANSON, R. & OWMAN, Ch. — Concomitant histochemical demonstration of histamine and catecholamine in enterochromaffin-like cells of gastric mucosa. **Life Sci., 6**: 759-766, 1967.

19. HAKANSON, R.; OWMAN, Ch. & SJÖBERG, N. O. — Cellular store of gastric histamine in the developing rat. **Life Sci., 6**: 2535-2543, 1967.

20. HAKANSON, R. & LIEDBERG, G. — The role of endogenous gastrin in the activation of gastric histidine decarboxylase in the rat. Effect of antrectomy and vagal denervation. **Europ. J. Pharmacol., 12**: 94-103, 1970.

21. HAKANSON, R.; OWMAN, Ch.; SPORRONG, B. & SUNDLER, F. — Electron microscopic identification of histamine-storing argyrophil (enterochromaffin-like) cells in the rat stomach. **Z. Zellforsch., 122**: 460-466, 1971.

22. HAKANSON, R.; LARSSON, L. I. & SUNDLER, F. — Endocrine-like cells in rat stomach: effects of 6-hydroxydopa on amine acid decarboxylase activities. A chemical, fluorescence histochemical and electron microscopic study. **J.Pharmacol. exp. Ther., 191**: 92-101, 1974.

23. HAKANSON, R.; LARSSON, L. I.; LIEDBERG, G.; OSCARSON, I.; SUNDLER, F. & VANG, J. — Effects of antrectomy and portal shunting on the histamine storing endocrine-like cells in oxyntic mucosa of rat stomach. A fluorescence, histochemical, electron microscopic and chemical study. **J. Physiol., 259**: 785-800, 1976.

24. KUBOTA, H.; TAGUCHI, Y.; TOHYAMA, M.; MATSURA, N.; SHIOSAKA, S.; ISHIHARA, T.; WATANABE, T.; SHIOTANI, Y. & WADA, H. — Electron microscopic identification of histidine decarboxylase-containing endocrine cells of the rat gastric mucosa. An immunohistochemical analysis. **Gastroenterology, 87**: 496-502, 1984.

25. LUNDELL, L. — The role of cholinergic excitation in the formation of histamine within the rat gastric mucosa. **Acta physiol scand., 97**: 145-149, 1976.

26. MACHADO, A. B. M.; MACHADO, C. R. S. & GOMES, C. B. — Depletion of heart norepinephrine in experimental acute myocarditis caused by *Trypanosoma cruzi*. **Experientia, 31**: 1202-1205, 1975.

27. MACHADO, C. R. S.; MACHADO, A. B. M. & CHIARI, C. A. — Recovery from heart norepinephrine depletion in experimental Chagas disease. **Amer. J. trop. Med. Hyg., 27**: 20-24, 1978.

28. MACHADO, A. B. M.; MACHADO, C. R. S. & GOMEZ, M. V. — *Trypanosoma cruzi* acetylcholine content and cholinergic innervation of the heart in rats. **Exp. Parasit., 47**: 107-115, 1979.

29. PEARSE, A. G. E. & TAKER, T. T. — Neuroendocrine embriology and the APUD concept. **Clin. Endocr., 5** (suppl.): 229-280, 1976.

30. RUBIN, W. & SCHWARTZ, B. — An electron microscopic radioautographic identification of the "enterochromaffin-like" APUD cells in murine oxintic glands. Demonstration of a metabolic difference between rat and mouse gastric A-like cells. **Gastroenterology, 76**: 437-449, 1979a.

31. RUBIN, W. & SCHWARTZ, B. — Electron microscopic radioautographic identification of the ECL cells as the histamine-synthetizing endocrine cell in rat stomach. **Gastroenterology, 77**: 456-467, 1979b.

32. RUBIN, W. & SCHWARTZ, B. — Identification of the serotonin-synthetizing endocrine cells in the rat stomach by electron microscopic radioautographic and amine fluorescence. **Gastroenterology, 84**: 34-50, 1983.

33. SANO, M.; HOLAN, O.; BOMBECK, T.; NYHUS, L. M. & DAS GUPTA, T. K. — Effect of acetylcholine and catecholamines on the enterochromaffin-like cells of the stomach. **Surg. Gynec. Obstet., 153**: 337-340, 1981.

34. SOLCIA, E.; CAPELLA, C.; VASSALLO, G. & BUFFA, R. — Endocrine cells of the gastric mucosa. **Int. Rev. Cytol., 42**: 223-286, 1975.

35. SOLL, A. H.; LEWIN, K. J. & BEAVEN, M. A. — Isolation of histamine-containing cells from rat gastric mucosa: biochemical and morphological differences from mast cells. **Gastroenterology, 80**: 717-727, 1981.

36. TAFURI, W. L. — Pathogenesis of lesions of the autonomic nervous system of the mouse in experimental acute Chagas disease: light and electron microscope studies. **Amer. J. trop. Med. Hyg., 19**: 405-417, 1970.

37. TAFURI, W. L. — Light and electron microscope studies of the autonomic nervous system in experimental and human american trypanosomyasis. **Virchows Arch. Abt. A. path. Anat., 376**: 353-360, 1971.

38. TAFURI, W. L. — Alterações ultra-estruturais dos componentes muscular, intersticial e nervoso do coração, esofago e intestino na Doença de Chagas experimental e humana. Belo Horizonte, 1974. (Tese de Professor-Titular — Faculdade de Medicina da Universidade Federal de Minas Gerais).

39. THUNBERG, R. — Localization of cells containing and forming histamine in the gastric mucosa of the rat. **Exp. Cell. Res., 47**: 108-115, 1967.

40. VASSALLO, G.; SOLCIA, E. & CAPELLA, C. — Light and electron microscopic identification of several types of endocrine cells in the gastrointestinal mucosa of the cat. **Z. Zellforsch, 98**: 333-356, 1969.

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