MORPHOLOGICAL AND QUANTITATIVE STUDY OF THE MYENTERIC PLEXUS OF THE ASCENDING COLON OF RATS SUBJECTED TO PROTEIC DESNUTRITION

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ABSTRACT - We carried out this study with the purpose of contributing on the effects of the proteic desnutrition on the morphological aspects and quantitative analysis of the neurons in the myenteric plexus of the ascending colon of adult *Rattus norvegicus*. Twenty adult rats were divided into two groups; in one of them, we offered a normal ration with proteic level of 22% (control group) and in the other, a ration with a proteic level of 8% (experiment group) during 120 days. We did the whole-mount preparations for the ascending colon and stained them with the Giemsa technique and the histochemical technique of NADH-diaphorase. The rats with proteic desnutrition showed a body weight, on average, to be 35.1% less than those of the control group, and the colon was on average, 26.8% shorter and 6.7% narrower. Thus, it was to be expected that the colon of animals with proteic desnutrition had a neuronal density 31.62% greater than the rats of the control group. Nevertheless, the difference with the Giemsa technique was on average 18.4%, demonstrating a mean neuronal loss of 13.25%.

KEY WORDS: ascending colon, myenteric neurons, myenteric plexus, proteic desnutrition.

Estudo morfológico e quantitativo do plexo mientérico do colo ascendente de ratos submetidos a desnutrição protéica

RESUMO - Realizamos este estudo com o objetivo de analisar os efeitos da desnutrição protéica sobre os aspectos morfológicos e quantitativos dos neurônios do plexo mientérico do colo ascendente de *Rattus norvegicus* adultos. Vinte ratos adultos foram divididos em 2 grupos, sendo que para um dos grupos ofertamos ração normal, com teor protéico de 22% (controle) e para o outro ração com teor protéico de 8% durante 120 dias. Elaboramos os preparados de membrana do colo ascendente e coramos pelo método de Giemsa e pela técnica histoquímica da NADH-diaforase. Os ratos com desnutrição protéica apresentaram peso corporal em média 35,1% menor que os do grupo controle, e o colo era em média 26,8% mais curto e 6,7% mais estreito. Esperava-se que o colo dos animais desnutridos possuíssem uma densidade neuronal 31,62% maior que o dos animais normonutridos; no entanto, a diferença com a técnica de Giemsa foi em média de 18,4%, demonstrando uma perda média de 13,25% dos neurônios.

PALAVRAS-CHAVE: colo ascendente, neurônios mientéricos, plexo mientérico, desnutrição protéica.

Proteic-caloric desnutrition is one of the major problems of worldwide public health representing, in underdeveloped nations, about 30-40% of hospitalized patients[3]. Considering that all normal metabolic processes need proteins, it is believed that all tissues will be affected by a state

of poor protein nutrition, although not showing the same rate and type of modification. The first tissues to suffer alterations with proteic loss are those that exhibit a high rate of cellular turnover, like the intestinal mucosa; and the last are those that show a low rate of cellular renewal, such as the nervous system[7]. Many authors studied the consequences of proteic desnutrition on the central nervous system (CNS)[25]. Chase et al.[4] described cellular alterations in the neurons of the CNS of developing animals, subjected to proteic desnutrition. Beyond CNS alterations, nutritional deficiencies bring about alterations in the peripheral nervous system and in the digestive tract, which usually result in impaired absorption and change of intestinal motility.

Conboy et al.[5] described the decrease in the size of neurons and ganglia of animals subjected to proteic desnutrition during intra-uterine life. Santer & Conboy[23], analyzing whole-mount preparations stained with histochemical techniques, demonstrated a decrease on the sympathetic innervation in the rats subjected to proteic desnutrition during gestation, and a 27% decrease in the number of jejunal enteric neurons of these animals.

We verified through the literature that the authors who carried out experiments in animals subjected to desnutrition, demonstrated some concern with the incidence of this condition during periods of gestation and lactation, mentioning its effects on the CNS and the peripheral nerves[4,25]. Articles related to the enteric nervous system are rare, and this fact prompted the researchers from our department to develop a research line where they initially studied the effects of proteic desnutrition on the myenteric plexus of animals subjected to this condition during gestation and lactation[18,20]. Following that we carried out the present work with the purpose of contributing on the effects of proteic desnutrition in the morphological aspects and quantitative analysis of the neurons of the myenteric plexus of adult rats.

MATERIAL AND METHODS

We used in this study the ascending colon of 20 laboratory animals *Rattus norvegicus*, Wistar strain, from the Central Biotery of the Universidade Estadual de Maringá.

We selected male rats with 90 days of age (290±20.93 g of body weight). The animals were divided in two groups, control and experiment. We raised these animals in individual cages, with constant temperature and light-dark cycles of 12 hours. Water was offered *ad libitum*. The animals were offered NUVILAB (Recommended by the National Council of Research and National Institute of Health, USA) ration with a proteic level of 22% to the control group and of 8% to the experiment group, during 120 days.

Diet of the experiment group was obtained reducing the proteic level of NUVILAB to 8% with the addition of corn starch. Rations were administered in pellets. The proteic level was tested using the dosage of nitrogen by the Kjedahl semimicro method[24]. Hypoproteic diet was supplemented with hydrosoluble vitamins and mixture of mineral salts[17,19,20].

After 120 days animals were weighed and killed with ether. We performed the laparotomy, removed the large intestine, measured the colon length with a millimetred ruler, and the colon width was 1 cm from the cecocolic junction. We chose this region because it does not present constrictions or dilatations due to feces. We opened up the colon with an incision on the mesocolic region, and gently distended and measured it with a millimetred ruler. The interval between death and the beginning of the staining or fixation was about one minute and thirty seconds.

We subjected samples of the ascending colon of five animals from each group to whole-mount preparations and stained them according to the Giemsa method of Barbosa[2]. The colon of the other five animals from each group were subjected to the histochemical technique through the activity of the NADH-diaphorase enzyme[9] for quantitative analysis.

We carried out the quantitative analysis in the antimesocolic region, an area of circumference that included between 120° and 240°, and the intermediate region, an area of circumference that included between 60° and 120° or 240° and 300°, considering that 0° was the region of insertion at the mesocolon[10,21] (Fig 1). The neurons present in 40 microscopic fields on the antimesocolic and intermediate regions were counted in each animal, for each
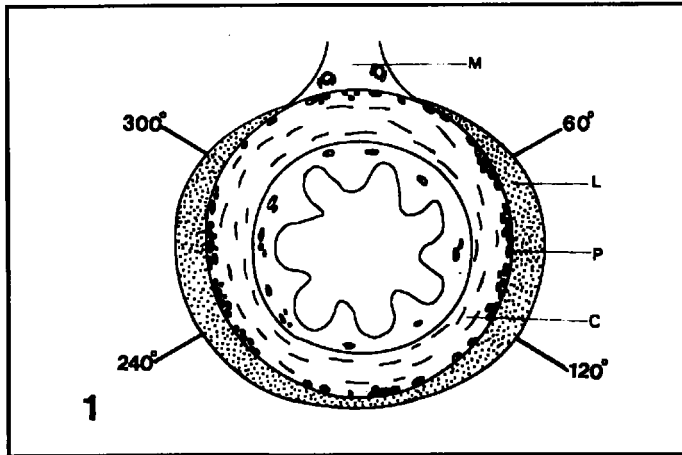
Fig 1. Schematic representation of a transverse section of the ascending colon, depicting the mesocolon (m), mesocolic (0°-60° and 300°-360°), intermediate (60°-120° and 240°-300°) and antimesocolic (120°-240°) regions. Longitudinal (L) and circular (c) layers of the muscular tunica; myenteric plexus (p).

technique. We considered all of the neurons of each field, discarding half of the neurons in a given field and considering half of the neurons of another. The area of each microscopic field with 40X objective was $0.1735mm^2$.

The morphological analysis of the myenteric neurons was carried out with an Olympus CBB microscope (coupled with a micrometer disc), with 10X lens and 100X objective. We measured the major transverse and longitudinal axes of the cell bodies and analyzed the cytoplasmic basophily of 500 neurons of each group. To classify the neurons as small, medium and large, the average and the standard deviation of the data resulting from the sum of the major axes of 500 neurons, from the ascending colon of rats from the control group, were calculated. It was considered a medium neuron from which the sum of the major axes resulted in values between the valid interval of the average. Neurons whose sum yielded values inferior to the average minus the standard deviation, were considered as small. Large neurons were those whose sum yielded values greater than the average plus the standard deviation.

We carried out the statistical analysis through average, standard deviation and variation coeficient of the number of neurons found in each region. We applied the Student's T test to compare the differences between the averages, and the Chi-square test for comparison of the incidences of the studied variables. The significance level adopted in both cases was 5%.

We subjected samples of the proximal portion of the ascending colon of three rats from each group to fixation in 10% formaldehyde solution, dehydration in an ascending series of alcohol, diaphanization in xylene, inclusion in paraffin and the histological sectioning of 10 and 15 μm thickness. We stained these sections with the technique of hematoxilin-eosin.

Photographic documentation was obtained with photomicroscope BX50 and photographic equipment PM 10AK.

RESULTS

On the beginning of the experiment the selected rats weighed on average, 287.6±17.01 g (control group) and 291.6±23.8 g (experiment group). After 120 days the average weight of the control rats was 429.1±35.2 g and of the experiment rats 278.5±35.4 g. In the control group, the total colon length was 19.5±1.8 cm and width 1.48±0.18 cm. In the experiment group, the length was 14.3±1.15 cm and the width was 1.38±0.16 cm.

Through the analysis of the hematoxilin-eosin stainings, we verified that in both groups the ganglia of the myenteric plexus showed different locations in the intestinal circumference. In the mesocolic region, we found myenteric neurons and ganglia between the muscle fibers and the subserous connective
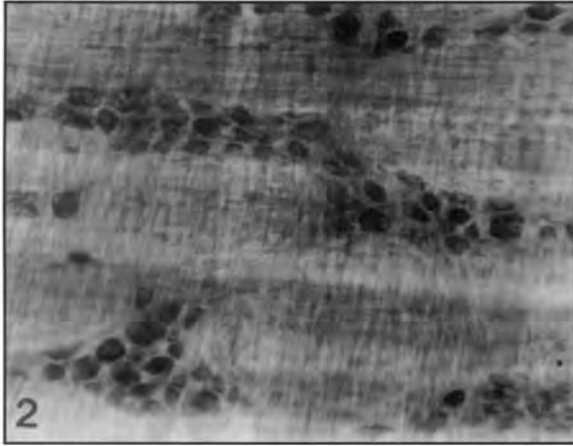
Fig 2. Whole-mount of ascending colon by Giemsa method displaying a group of nerve cells forming a ganglion of the myenteric plexus in the antimesocolic region. Control group. Green filter. 183.7 X.
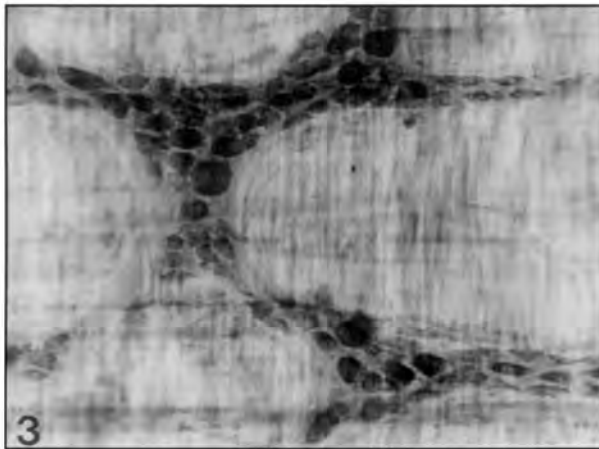

Fig 3. Whole-mount of ascending colon by Giemsa method displaying a group of nerve cells forming a ganglion of the myenteric plexus in the intermediate region. Control group. Green filter. 183.7 X.

tissue. In the intermediate and antimesocolic regions, we again found myenteric neurons and ganglia between the circular and longitudinal muscular layers, and sometimes among the muscle fibers of the circular layer.

In both groups the neuronal density shows differences when the antimesocolic (120°-240°) and intermediate (60°-120° or 240°-300°) regions were compared both in the Giemsa (Fig 2 and 3) and in the NADH-diaphorase techniques (Fig 4 e 5).

Table 1 presents the number of neurons found in the antimesocolic and intermediate regions of the control and experiment animals. Comparing the averages of neurons found with Giemsa in both groups, significant values were found for the antimesocolic region (t = 2.42; c.v.= 2.31) but not in the intermediate region (t = 2.28; c.v. = 2.31). When we compared the averages of neurons found with the NADH-diaphorase technique, the results attained a significance in the intermediate (t = 3.02; c.v. = 2.31) but not in the antimesocolic regions (t = 2.23; c.v. = 2.31).

Fig 4. Whole-mount of ascending colon stained by NADH-diaphorase histochemical technique displaying a group of nerve cells forming a ganglion of the myenteric plexus in the antimesocolic region. Control group. Green filter. 183.7 X.
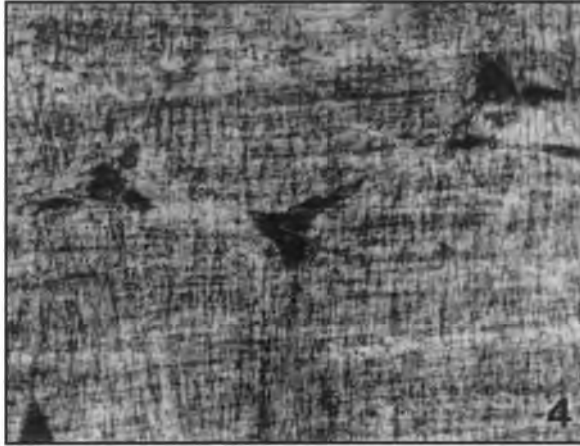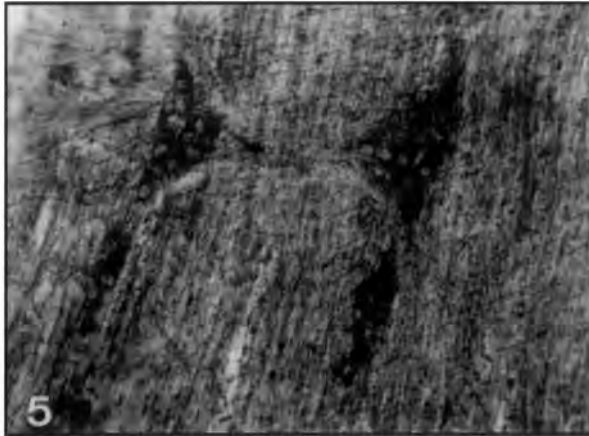

Fig 5. Whole-mount of ascending colon stained by NADH-diaphorase histochemical technique displaying a group of nerve cells forming a ganglion of the myenteric plexus in the intermediate region. Control group. Green filter. 183.7 X.

Through the quantitative analyses of the whole-mount preparations stained with Giemsa, we verified in 13.88 mm^2, referent to the 80 microscopic fields studied (40 in the antimesocolic region and 40 in the intermediate region), an average of 4165±429.8 neurons (30007.2 neurons/cm^2) in animals of the control group and 5102.4±487.3 neurons (36760.8 neurons/cm^2) in animals of the experiment group, with a difference of 18.4% existing between both groups. In whole-mount preparations stained with NADH-diaphorase, we found in an equal area of 13.88 mm^2, an average of 1464.6±233.8 neurons (10551.9 neurons/cm^2) in the control animals and of 2172.4±555.14 neurons (15651.3 neurons/cm^2) in the experiment animals, making up a difference of 32.6 %.

The sum of the major longitudinal and transverse axes of the measured neurons in the control group varied from 6.25 μm to 59.39 μm, with an average of 27.87 μm and a standard deviation of

Table 1. Incidence of neurons of the myenteric plexus found in an area of 6.94 mm² of whole-mount preparations of the ascending colon of rats with seven months of age, using the staining technique of Giemsa and the histochemical technique of NADH-diaphorase, in the antimesocolic and intermediate regions.

	Control				Experimental			
	Antimesocolic		Intermediate		Antimesocolic		Intermediate	
Technique	Giemsa	NADH	Giemsa	NADH	Giemsa	NADH	Giemsa	NADH
Animals								
1	2226	895	1776	631	2595	904	3285	808
2	2030	961	2521	833	2297	1489	2265	1151
3	2306	721	2353	540	2559	821	2427	705
4	2104	902	1879	624	2201	1211	2714	981
5	1413	792	2217	425	2570	1531	2599	1261
X	2015.8	854.2	2149.2	610.6	2444.4	1191.3	2658	981.2
s	353.46	96.13	314.9	149.6	182.04	325.6	389.73	230.98

Antimesocolic region: 120° - 240° considering 0° the region of insertion of the mesocolon. Intermediate region: 60° - 120° and 240° - 300° considering 0° the region of insertion of mesocolon.

Table 2. Incidence of small, medium and large neurons on the myenteric plexus of the ascending colon of control rats and rats with proteic desnutrition.

Variable Groups	Small Neurons (% Lin.)	Medium Neurons (% Lin)	Large Neurons (% Lin.)	Total (% Lin.)
Control	80 (16%)	346 (69.2%)	74 (14.8%)	500 (100%)
Experimental	102 (20.4%)	351 (70.2%)	47 (9.4%)	500 (100%)

10.48 µm. Small neurons ranged from 6.25 µm to 17.71 µm, medium neurons varied from 17.72 µm to 38.35 µm, and large neurons were those larger than 38.36 µm. Table 2 shows the incidences of small, medium and large neurons found in the two groups studied. Analyzing the incidences shown on Table 2, we obtained significant differences when applying the Chi-square test ($\chi^2 = 8.67$; c.v. = 5.99), indicating greater incidence of small neurons in the experimental group than in the control group, while large neurons were more frequently on the control group than in the experiment group.

Comparing the basophilic affinity, we observed a predominance of the strongly basophilic neurons, 323 neurons (64.6%) from the control group and 317 neurons (63.4%) from the experiment group. These neurons exhibited cytoplasm intensely and homogeneously stained. They were followed by neurons of intermediate basophily, 136 (27.2%) from the control animals and 149 (29.8%) from the experiment animals, whose cytoplasm showed multiple basophilic granules on a slightly basophilic background. Finally there were neurons of weak basophily; 41 (8.2%) and 34 (6.8%) from the control and experiment groups, respectively. The cytoplasm of these neurons showed few basophilic granules. When the groups were compared statistically by the Chi-square test, a significant difference was not found ($\chi^2 = 1.32$; c.v. = 5.99).

DISCUSSION

Our findings about the location of the myenteric ganglia are in accordance with those of Mello[18], who also found them in the mesocolic region, between the circular layer of the muscular

tunica and the subserous tissue. In the intermediate and antimesocolic regions, we found most of the neurons and ganglia of the myenteric plexus between the circular and longitudinal layers of the muscular tunica, as it is described for different intestinal regions in animals[8,11,12,14] and humans[6,16].

Nevertheless, we also found neurons and ganglia among the muscle fibers of the circular layer, agreeing with Iwanov[15] and Mello[18]. For this reason we avoided removing the circular layer during the whole-mount preparations, so as not to alter the number of neurons.

For the quantitative analysis, we used 40 microscopic fields in the intermediate region and 40 in the antimesocolic region. Our observations confirmed the findings which state that differences do exist in the neuronal density of the intestinal circumference[1,10,11,14,21]. We did not carry out countings of the mesocolic region because it was highly vascularized and rich in adipocytes, which makes the whole-mount preparation, staining and analysis, difficult to do.

When we compared the results obtained on the antimesocolic and intermediate regions with the Giemsa technique we did not find statistically significant differences, although in both groups studied we observed a greater number of neurons in the intermediate region, in accordance with the reports of Gabella[11] and Irwin[14].

With the NADH-diaphorase technique, the smaller amount of neurons in the intermediate regions attained significance. We believe that, with this technique, the intermediate region exhibits a smaller number of neurons because the ganglia are between thick layers of muscle fibers that would act as a mechanical barrier to the diffusion of reagents towards the neurons. With the Giemsa technique, exposure to the stain varies from 18 to 24 hours. Therefore, there was a long period of time for the reagents to diffuse and reach all of the neurons in the myenteric plexus. Another possibility which deserves investigation, is that in this region, the NADH-positive neuronal population could be smaller than in other regions.

In general, we verified that, independently of the region considered, the number of neurons found with the Giemsa technique is greater than that found with the NADH-diaphorase technique. In the literature, discussions are found about this issue, making it clear that, when quantifying neurons, the authors shall consider the animal species, the intestinal segment, the regions of the intestinal circumference, the animal age, the experimental condition, and the technique employed[1,11,22,26].

We think that in this experiment, both techniques are valid: the Giemsa technique because, for its affinity for Nissl's corpuscles, is supposed to stain all of the neurons, and the NADH-diaphorase technique for evidencing neurons that contain this enzyme. With both techniques, we can compare if an alteration occurs with the general frequency of neurons or with the frequency of NADH-positive neurons in conditions of proteic desnutrition.

As mentioned in our results, we verified that animals subjected to desnutrition have greater neuronal density than control animals; on average 18.4% with the Giemsa technique and 32.6% with the NADH-diaphorase technique, both of which are statistically significant. However, we understand that, from a biological point of view, the proteic desnutrition would not cause increase on the total number of neurons. Indeed, what occurred was that the control animals gained body mass during the experiment, altering their average weight from 287.6±17.06 g to 429.12±35.2 g, while the experiment animals reduced their average body weight from 291.6±8.15 g to 278.5±35.44 g. The difference of the final body weight of the groups was 35.1%.

Also, the intestine of the experiment group was smaller than the control group, on average of 26.8% for the length, and 6.7% for the width. Starting with the hypothesis that an animal is born with a total number of enteric neurons, which from early life, these neurons are densely packed due to the smaller area of the digestive tract and that as the animal grows its intestine gains more surface and the neurons become more sparse. It should be expected that the control group, in which width

and length increased were 31.62% greater than that found in the experimental group, should exhibit a neuronal density on average 31.62% smaller. Nevertheless, the difference from the control to the experiment group, as already mentioned, was on average 18.4% on the whole-mount preparations stained with Giemsa and 32.5% on those stained with NADH-diaphorase. Although an increase on the neuronal density existed in the intestine of the experimental animals (18.4% evidenced with the Giemsa technique), this was less than that expected in function from the intestinal size, which was reduced in 31.62%. This was indicative that about 13.25% of the neurons of these animals were lost because of the proteic desnutrition.

Among the NADH-diaphorase positive neurons, the estimate was that only 0.9% of the neurons were lost. Here is the reason why on the control group, the neurons seen with the NADH-diaphorase technique represent 35.2% of the number of neurons found with the Giemsa technique. While on the experiment group, the incidence of NADH-diaphorase positive neurons represents 42.6% of the number of neurons found with Giemsa, confirming that most of the neurons that were lost were not NADH- diaphorase positive. Similar data were verified by Hope & Vincent[13] in relation to the NADPH-diaphorase positive neurons, which, according to the authors, are less susceptible to damage than the others.

This decrease on the number of neurons from the animals subjected to proteic desnutrition can be attributed to the long period during which the animal received a hypoproteic diet, leading to the lack of essential aminoacids for protein synthesis. In this way the reparing and replacement of cytoplasmic organelles of these neurons of which they would be compromised, could even enhance the processes of atrophy, aging and cellular death. In this sense, Deo[7] considered that the tissues with a high rate of cellular renewal are the first to suffer changes with the protein loss, while those which show a low rate of cellular renewal are the last to be affected. Our findings concerning the decrease in the number of neurons from the experimental animals are in accordance with those of Santer & Conboy[23], which observe a 27% decrease on the number of myenteric neurons of the jejune of rats subjected to proteic desnutrition.

Natali & Miranda-Neto[20], in studies carried out with rats subjected to desnutrition during gestation and lactation, later recovered with normoproteic diet, until the 60th day of age, observed predominance of large neurons and of neurons with strongly basophilic cytoplasm. The authors ascribe these findings, to a possible adaptive mechanism where the animals, after being subjected to desnutrition, would have accumulated proteic material in their cells, including the enteric neurons, during the period of normal diet.

In the present experiment, we verified among the animals subjected to desnutrition a greater incidence of small neurons than in the control animals, with no significant statistical difference in the cytoplasmic basophily. We assume that the divergences between the results of these two studies are related to the fact that the animals employed in the experiment of Natali & Miranda-Neto[20] had a period of nutritional recovery, while those employed in the present research were killed after 120 days of hypoproteic diet and with no period of nutritional recovery. Also Conboy et al.[5] observed a decrease in the size of neurons from animals subjected to proteic desnutrition.

CONCLUSIONS

1. In the ascending colon of rats, subjected to proteic desnutrition, the number of neurons evidenced with the NADH-diaphorase technique was much smaller than that observed with the Giemsa technique.

2. The regions of the intestinal circumference located between 60°-120° or 240°-300°, with 0° being the insertion of the mesocolon, possess smaller neuronal density as evidenced with NADH-diaphorase than the region located between 120°-240°.

3. The ascending colon of animals with proteic desnutrition possessed a greater incidence of small neurons and a smaller incidence of large neurons than the animals of the same age but normally fed.

4. The ingestion of hypoproteic diet for a period of 120 days resulted in animals with body weight on average of 35.1% less than its control group, and colons on average of 26.8% shorter and 6.7% narrower.

5. The decrease of the intestinal size with the experimental animals is on average 31.62% in relation to the controls, accompanied by an increase in the neuronal density of 18.4%. This demonstrates that, on average, these animals lost 13.25% of the neurons of the myenteric plexus.

6. The increase in the proportion of NADH-positive neurons from 35.2% from the control group to 42.6% in the experiment group (in relation to the neurons stained with the Giemsa technique) demonstrates that the NADH-positive neurons are less susceptible to the damages caused by the proteic desnutrition.

REFERENCES

1. Ali HA, Mclelland J. Neuron number in the intestinal myenteric plexus of the domestic fowl (*Gallus gallus*). Zbl Vet Med C Anat Histol Embryol 1979;8:277-283.
2. Barbosa AJA. Técnica histológica para gânglios nervosos intramurais em preparados espessos. Rev Bras Pesq Med Biol 1978;11:95-97.
3. Bistrian BR, Blackburn GL Prevalence of malnutrition in general medical patients. JAMA 1976;235:1567-1570.
4. Chase HP, Sabiere CS, Welch NN, O'Brien D. Intrauterine undernutrition and brain development. Pediatrics 1971;47:491-500.
5. Conboy VB, Santer RM, Swift GL. Effects of prenatal undernutrition on prevertebral sympathetic neurons in the rats: a morphological and fluorescence histochemical study. J Anat 1987;154:47-53.
6. Cormack DH. Ham histologia. Ed. 9ª. Rio de Janeiro: Guanabara Koogan, 1995: 273-287, 384-404.
7. Deo MG. Biology of protein-calorie malnutrition. Wld Rev Nutr Diet 1978;32:49-95.
8. Furness JB, Costa M. The enteric nervous system. London: Churchill Livingstone, 1987.
9. Gabella G. Detection of nerve cells by a histochemical technique. Experientia 1969;25:218-219.
10. Gabella G. Fine structure of the myenteric plexus in the guinea-pig ileum. J Anat 1972;111:69-97.
11. Gabella G. On the plasticity of form and structure of enteric ganglia. J Auton Nerv Syst 1990;30:59-66.
12. Gershon MD. The enteric nervous system. Ann Rev Neurosci 1981;4:227-272.
13. Hope BT, Vincent SR. Histochemical characterization of neuronal NADPH-diaphorase. J Histochem Cytochem 1989;37:653-661.
14. Irwin DA. The anatomy of Auerbach's plexus. Am J Anat 1931;49:141-165.
15. Iwanow IF. Die sympatische Innervation des Verdaungstraktes einiger Vogelarten. Z Mikrosk Anat Forsch 1930;22:469-492.
16. Junqueira LCU, Carneiro J. Histologia básica. Rio de Janeiro: Guanabara Koogan, 1995:245-267.
17. Lepri ER, Bruschii LC, Moura AS. Efeitos da desnutrição protéica durante os períodos pré e pós natal no desenvolvimento cerebelar. Rev Bras Ciên Morfol 1994;11:23-27.
18. Mello EVSL. Estudo da morfologia da parede e dos gânglios do plexo mientérico do colo proximal de ratos. Dissertação de Mestrado, Centro de Ciências Biológicas da Universidade Estadual de Maringá, Maringá, 1995.
19. Moura AS. Estudo em ratos do processo da adaptação a dietas com níveis variáveis de proteína com e sem restrição energética. São Paulo. Tese de Doutorado, Instituto de Ciências Biomédicas da Universidade Estadual de São Paulo, São Paulo, 1994.
20. Natali MRM, Miranda-Neto MH. Effects of maternal proteic undernutrition on the neurons of the myenteric plexus of duodenum of rats. Arq Neuropsiquiatr 1995;54:273-279.
21. Santer RM. Survival of the population of NADPH-diaphorase stained myenteric neurons in the small intestine of aged rats. J Auton Syst 1994;49:115-121.
22 - Santer RM, Baker DM. Enteric neuron numbers and sizes in Auerbach's plexus in the small and large intestine of adult and aged rats. J Auton Nerv Syst 1988;28:59-67.
23. Santer RM., Conboy YB. Prenatal undernutrition permanently decreases enteric neurons and sympathetic innervation of Auerbach's plexus in the rat. J Anat 1988;168:67-72.
24. Silva DJ. Análise de alimentos (métodos químicos e biológicos). Viçosa: Imprensa Universitária da Universidade Federal de Viçosa, 1981,33-46.
25. Winick M, Noble A. Cellular response in rats during malnutrition at various ages. J Nutrition 1966; 89:300-306.
26. Young HM, Furness JB, Sewell P, Burcher EF, Kandiah CJ. Total number of neurons in myenteric ganglia of the guinea-pig small intestine. Cell Tissue Res 1993;272:197-200.