THE EFFECT OF BOTH PROTEIN AND VITAMIN B COMPLEX DEFICIENCY ON THE MORPHOQUANTITATIVE FEATURES OF THE MYENTERIC PLEXUS OF THE ASCENDING COLON OF ADULT RATS

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ABSTRACT: This study was performed in order to study the effects of protein desnutrition and vitamin B complex deficiency on the myenteric plexus of the ascending colon of Rattus norvegicus. Twenty rats were divided into two groups; one had been fed with a 22%-protein-level ration, and the other with a 8%-protein-level without vitamin-B-complex supplementation, for 120 days. The whole-amounts of the ascending colon were stained with either Giemsa or NADH-diaphorase technique. The disnurtured rats showed a 14.8% smaller body weight than the control group, and the area of colon of the sample group was 54.2% smaller. The average neuronal density was 26.7% greater with the Giemsa technique and 27% greater with the NADH-diaphorase technique. As the decrease in area was not accompanied by an inversely proportional increase in neuronal density, it is suggested that the experimental condition led to a myenteric neuron loss.
KEYWORDS: Enteric neurons. Protein desnutrition. Ascending colon. Vitamin B. Myenteric plexus.

EFEITO DA CARÊNCIA DE PROTEÍNAS E VITAMINAS DO COMPLEXO B SOBRE ASPECTOS MORFOQUANTITATIVOS DO PLEXO MIOENTÉRICO DO COLO ASCENDENTE DE RATOS ADULTOS

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RESUMO: O objetivo deste estudo foi estudar os efeitos da desnutrição protéica e da carência de vitaminas do complexo B sobre o plexo mioentérico do colo ascendente de Rattus norvegicus. Vinte ratos foram divididos em dois grupos, sendo que, para um dos grupos foi oferecida ração com teor protéico de 22% (controle) e, para outro, ração com teor protéico de 8% com menor teor de vitaminas do complexo B, durante 120 dias. Coramos os preparados de membrana do colo ascendente pelo método de Giemsa e pela técnica da NADH-diaforase. Os ratos desnutridos apresentaram peso corporal 14,8% menor que o grupo controle, média da área do colo 54,2% menor, e a média da densidade neuronal foi 26,7% maior com a técnica de Giemsa e 27% com a técnica da NADH-diaforase. Como a redução da área não foi acompanhada por um aumento inversamente proporcional na densidade de neurônios, sugere-se que a condição imposta causou perda de neurônios mioentéricos.
PALAVRAS-CHAVE: Neurônios entéricos. Desnutrição protéica. Colo ascendente. Vitamina B. Plexo mioentérico.

EFECTO DE LA CARENCIA DE PROTEÍNAS Y VITAMINAS DEL COMPLEJO B SOBRE ASPECTOS MORFO-CUANTITATIVOS DEL PLEXO MIOENTÉRICO DEL REGAZO ASCENDIENTE EN RATONES ADULTOS

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RESUMEN: El objetivo de este estudio fue analisar los efectos de la desnutrición proteica y de la carencia de vitaminas del complejo B sobre el plexo mioentérico del regazo ascendiente de Rattus norvegicus. Veinte ratones fueron divididos en dos grupos, siendo que, para uno de los grupos se ofreció ración con contenido proteico de 22% (control) y, para el otro, ración con contenido proteico de 8% con menor contenido de vitaminas del complejo B, durante 120 días. Coloreamos los preparados de membrana del regazo ascendiente por el método de Giemsa y por la técnica de la NADH-diaforasis. Los ratones desnutridos presentaron peso corporal 14,8% menor que el grupo control, promedio del área del regazo de 54,2% menor, y el promedio de la densidad neuronal fue 26,7% mayor con la técnica de Giemsa y 27% con la técnica de la NADH-diaforasis. Como la

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reducción del área no fue acompañada por un aumento inversamente proporcional en la densidad de neuronas, se cree que la condición impuesta causó pérdida de neuronas mioentéricos.
PALABRAS CLAVE: Neuronas entéricos. Desnutrición proteica. Regazo ascendiente. Vitamina B. Plexo mioentérico.

Introduction

Recent investigations demonstrated that protein desnutrition together with deficiency in vitamin B complex in adult rats induce changes in the body mass composition, decreasing in the plasma albumin values and lesser evidentiation of nitrergic neurons among myenteric neurons (SANT`ANA et al., 2001).

The study of protein deficiency on the morphology and physiology of the nervous system is based on the fact that it is composed of a tissue having low rates of cellular renewal, and the decrease in the amount or functions of its cells might lead to marked changes (DEO, 1978).

Many authors have verified the effects of desnutrition through the use of rations containing protein levels below those recommended: 20-24% (NATIONAL RESEARCH COUNCIL, 1995), such as 8% (MELLO et al., 1995; NATALI; MIRANDA-NETO, 1996; SANT`ANA et al., 1997b); 4% (SHRADER; ZEMAN, 1969) and 1% (PATRICIO et al., 1984), while others used protein-free diets (AMORIN, 1985; WAITZBERG et al., 1989; CASTELUCCI, 1999). Despite these variations, it is observed that the restricted protein ingestion causes, among other things, a decrease on the size of the organs and body weight (GIACOMELLI; NATALI, 1999).

As for the effects of the desnutrition on the myenteric plexus, it is observed that low-protein diets lead to smaller intestinal areas with greater clustering of the enteric neurons (MELLO et al., 1995; NATALI; MIRANDA-NETO, 1996; SANT`ANA et al., 1997b; TORREJAIS et al., 1995; NATALI et al., 2000) and reduced numbers of these neurons (SANT`ANA et al., 1997b; CASTELUCCI, 1999; SANTER; BAKER, 1988; MOURA, 1994).

This study was performed with the purpose of evaluating the results of the ingestion of a starch-rich, but deficient protein and vitamin B-complex diet upon the morphology and number of the myenteric neurons.

Material and methods

We used 20 male rats (*Rattus norvegicus*) of the Wistar strain aging 90 days (298±66g body weight). The animals were kept and killed according to the rules regarding Ethics on animal experimentation[1]. The rats were divided into two groups, control and experimental (disnurtured). Both groups were kept in individual cages with controlled temperature and light-dark cycles of 12 hours. Water was offered ad libitum. The control group has been fed with an average 22%-protein-level NUVILAB® ration, and the experimental one with a reduced 8%-protein-level ration, for 120 days.

The experimental group diet was obtained by reducing the protein level of the NUVILAB® ration through the addition of corn starch and a mixture of mineral salts, but without vitamin supplementation (SANT`ANA et al., 2001). This procedure was based on the models described in the literature (NATALI; MIRANDA-NETO, 1996; SANT`ANA et al., 1997b; NATALI et al., 2000; MOURA, 1994; LEPRI et al., 1994).

The body weight was accompanied weekly, as well as the food and water ingestion o. During the experimental period, we evaluated the face, tail and motor behavioral features of all the animals in both groups. After 120 days, they were killed through the inhalation of excessive ethylic ether. Laparotomy was carried out and the colon was removed. Its length and width were measured by using a millimeter ruler.

The ascending colons of five animals from each group were washed and filled with Krebs buffer solution (pH 7.3), washed twice in the same solution (10 minutes each), and immersed for five minutes in a 0.3%-Triton-X-100 solution dissolved in Krebs. Then, they were washed again twice (10 minutes each) in Krebs solution, and incubated for 45 minutes for NADH-diaphorase enzyme evidentiation. There were in 100 ml of this incubation medium: 25 ml of 0.5% stock solution of Nitro Blue Tetrazolium (NBT, Sigma, St Louis, USA); 25 ml of 0.1 M phosphate buffer pH 7.3; 50 ml of distilled water; 50 mg of β-NADH (Sigma, Steinheim, Germany), according to the technique described by Gabella (1969). After incubation, the segments were opened at the mesocolic margin and immersed in 10% buffered formol solution.

The intestinal segments of the other five animals of each group were washed in 0.9% saline solution, filled and immersed in fixative solution of acetic formol for 48 hours, then dissected and stained in staining solution of Giemsa (methylene blue) in Sorensen phosphate buffer (pH 7.0) (BARBOSA, 1978).

The whole-mounts were dissected under stereomicroscope with transillumination through the removal of the mucosa and the submucosa. Next, they were dehydrated in ascending series of ethylic alcohol, diaphanized in xylene and mounted between slide and coverslip with the synthetic resin Permount® (Fischer Chemical, New Jersey, USA).

The quantitative analysis was done on both techniques by using an Olympus BX40 microscope with a 40X objective. In each whole-mount, 80 microscopic fields had their neurons counted, half-seen neurons being considered in alternate fields. The area of each microscopic field was of 0.1735 mm^2. The quantitative analysis was carried out in the intermediate (60^o-120^o and 240^o-300^o) and antimesocolic (120^o-240^o) regions of the intestinal circumference (SANT`ANA et al., 2001; SANTA`ANA et al., 1997a).

The morphologic analysis of the myenteric neurons was carried out in an Olympus CBB microscope with a micrometer ruler coupled to the lens, and a 40X objective.

[1]BRASIL, Lei n. 6.638, 8 de Maio de 1979. Normas para a prática didático científica da vivissecção de animais e determinação de outras providências. Lex 1979; 43: 416.

The major longitudinal and transverse axes of the cell bodies were measured and the cytoplasmic basophily was analyzed in 500 neurons of each group (SANT`ANA et al., 1997a).

Five-hundred neurons of the ascending colon of the control rats were measured to allow neuronal classification according to size. It was considered medium neurons those whose sum of the axes resulted in values between the valid intervals of the mean. Neurons whose sum resulted in values below the mean minus the standard deviation were considered small. Large neurons were those whose sum yielded values greater than the mean plus the standard deviation. The morphometric analysis of the neurons was made in the whole-mounts stained with Giemsa.

The mean, standard deviation and variation coefficient of the data obtained were calculated. The variation coefficient indicated that the spread of the data was small and thus the mean was a good representative of the values. In this way, the means were compared using Student's t test for non-paired data at the significance level of 5%. The chi-squared test was employed for the comparisons of the neuronal size and basophily, at the significance level of 5%.

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

Results

At the end of the experimental period, the animals of the control group had an average body weight of 456 ± 33.48 g and the experimental group of 388.6 ± 46.3 g. This difference was statistically significant (t=2.74; c.v.=1.67; $p<0.05$).

Table 1 shows the data regarding length, width, and the area of the colon of both groups. Table 2 presents the results of the quantitative analysis on the whole-mounts of the control and experimental groups stained with Giemsa or NADH-diaphorase.

Table 1 - Mean of the length, width and area of the colon of adult rats from the control and experimental groups. The area of colon was based on the multiplication of the measures of length and width.

Group	Total colon length (cm)	Colon width (cm)	Total colon area (cm^2) (length x width)
Control (n=7)	18.34 ± 1.0 a	1.66 ± 0.21 a	30.1 ± 4.35 a
Experimental (n=7)	13.6 ± 0.8 b	1.04 ± 0.13 b	13.78 ± 2.46 b

Means followed by the same letter in each variable do not differ statistically at the level of 5%.

Table 2 - Incidence of neurons in the myenteric plexus of the ascending colon of adult rats from both the control and experimental groups, evidenced by the techniques of Giemsa and NADH-diaphorase in an area of 6.94 mm^2 (40 microscopic fields) in the antimesocolic and intermediate regions. Mean ± standard deviation.

Group	GIEMSA (n=5)		NADH-d (n=5)	
Region	Antimesoc.	Intermed.	Antimesoc.	Intermed.
Control	2015.8 ± 17.53 a ♠	2149.2 ± 314.9 a ♠	854.2 ± 96.13 a ♣	610.6 ± 1499.6 a ♦
Experimental	2756.6 ± 739.6 b ♣	2929.2 ± 399.3 b ♣	1145.4 ± 342. 08 b ♥	861 ± 239.23 b ♥

Means followed by the same letter in the same column do not differ at the level of 5%.

When these neurons were plotted by cm^2, we verified that the Giemsa-stained neurons average, regardless the circumference region, is 30007.2 neurons/cm^2 in the control animals, and 40963.9 neurons/cm^2 in the experimental group. A difference of 26.7% between the groups (Figure 1A, and 1B). In the same way, in the whole-mounts stained with NADH-diaphorase, we found an average concentration, regardless of region, of 10553 neurons/cm^2 in the control group, and 14455.3 neurons/cm^2 in the experimental group, yielding a difference of 27% (Figure 1C and 1D).

The morphometric analysis of the neurons was made through the sum of the major longitudinal and transverse axes of the cell bodies; small neurons had from 6.25 µm to 17.71 µm; medium neurons had from 17.72 µm to 38.35 µm and large neurons were larger than 38.36 µm. Table 3 presents the incidence of the these neuronal sizes in both groups.

Table 3 - Incidence of small, medium and large neurons in the myenteric plexus of the ascending colon of adult rats from both the control and experimental groups. Morphometry carried out in whole-mounts stained with Giemsa.

Group / size	Small Neurons (%)	Mean neurons (%)	Large Neurons (%)
Control (n=5)	80 (16)	346 (69.2)	74 (14.8)
Experimental (n=5)	148 (29.6)	289 (57.8)	63 (12.6)

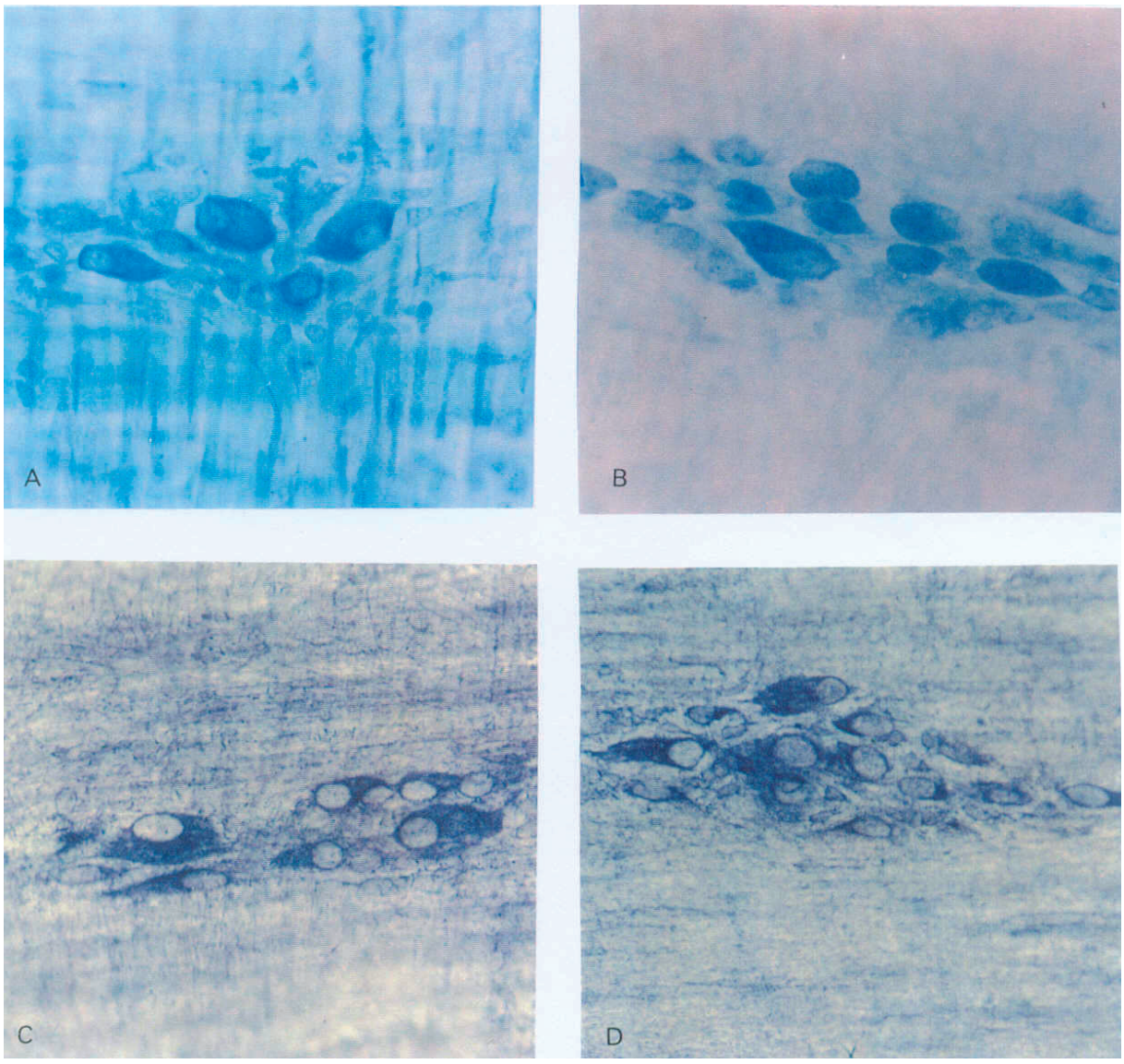

Figure 1 - Whole-mounts evidencing neurons of the myenteric plexus of the ascending colon of adult rats. A) Whole-mounted obtained from a control animal and stained with Giemsa. B) Whole-mounted obtained from an experimental animal and stained with Giemsa. C) Whole-mounted obtained from a control animal and stained with NADH-diaphorase. D) Whole-mounted obtained from an experimental animal and stained with NADH-diaphorase. 408 x.

Applying the chi-squared test to the data of table 3 we verified the existence of a greater amount of small neurons and a smaller amount of large neurons in the experimental group, as compared to the control.

As for the basophilic affinity of the enteric neurons, we verified a predominance of strongly basophilic neurons in both groups, but the chi-squared text did not demonstrate a significant difference, as observed in table 4.

Table 4 - Incidence of strong, weak and intermediate basophily in the myenteric plexus of the ascending colon of adult rats from both the control and experimental group. Evaluation carried out in the whole-mounts stained with Giemsa.

Group / basophily	strong (%)	intermediate (%)	Weak (%)
Control (n=5)	326 (64.6)	136 (27.2)	41 (8.2)
Experimental (n=5)	330 (66.0)	123 (24.6)	47 (9.4)

Discussion

In this study, we have carried out neuronal quantification in two regions of the intestinal circumference, the antimesocolic and the intermediate regions. The mesocolic region was not considered because it is highly vascularized and rich in adipocytes, making dissection and whole-mount preparations difficult (SANT`ANA et al., 1997b).

The differential counting around the intestinal circumference was already suggested (SANT`ANA et al., 1997a,b; IRWIN, 1931; GABELLA, 1973; ALI; McLELLAND, 1979; SANTER, 1994). Like these authors, we observed numerical differences between these regions in both groups and in either technique. Giemsa-stained neurons are more concentrated in the intermediate region of the colon in both groups, a result similar to that previously found (SANT`ANA et al., 1997a). In spite of these results demonstrating no statistically significant differences, they have been observed repeatedly in our investigations. Possibly the greater density of neurons is due to the greater thickness of the muscle layer in the lateral regions of the intestinal circumference, which would thus need a larger neuronal population to be innervated (MELLO et al., 1995; SHRADER; ZEMAN, 1969; SANT`ANA et al., 1997a).

While observing the neurons evidenced by the NADH-diaphorase technique, we noticed their predominance in the antimesocolic region of both groups. The smaller density in the lateral (intermediate) regions of the intestinal circumference may be related to the fact that the ganglia are located deep in the thick muscle layer, which would act as a barrier to the diffusion of the reagents. In the Giemsa technique, on the other hand, the segments, already dissected, are exposed to the stain for 18-24 hours, and certainly there is a better diffusion (PATRICIO et al., 1984). The possibility remains, however, that the diminished evidentiation resulted from distinct functional demands, which could be the reason why less NADH-diaphorase positive neurons were seen in the intermediate region.

We observed that, in either region, the number of Giemsa-stained neurons was more than two-fold that of NADH-diaphorase positive neurons in both groups. This is compatible with the findings in adult rats subjected to protein desnutrition and vitamin supplementation (SHRADER; ZEMAN, 1969). It is suggested that the Giemsa technique evidences all neurons because of its affinity for Nissl corpuscles, a ubiquitous element in neurons, while the NADH-positive neurons are those with greater concentration and activity of this enzyme (SANT`ANA et al., 1997b).

The analysis of our results demonstrated, in the experimental animals, a number of neurons greater than that in the control with both staining techniques. The density of neurons in the experimental animals with the Giemsa technique was 26.7%, and with the NADH-diaphorase technique 27%, greater than in the control animals.

We believe that the desnutrition does not lead to an increase in the number of neurons (SANT`ANA et al., 1997b). The control group gained more body weight during the experimental period, as observed in the results, making its final weight significantly different from that of the experimental group (14.8%). Differences were also observed in the large intestine, whose area in the control group was 54.2% greater than that in the experimental group.

It was expected that, if there was a decrease in intestinal area (length x width) of 54.2% in the experimental group relative to the control, there should be a neuronal density 54.2% greater, on average, in that group. However, this difference with the Giemsa technique was of 26.7%. These results indicated that about 27.5% of the neurons were lost. In the same segment, protein deficiency alone caused a mean loss of 13.25% of the neurons (SANT`ANA et al., 1997b).

In this experiment, the NADH-diaphorase technique indicated a 27.2% decrease in this neuronal population, while previous experiments with rats receiving diets 8%-protein-level diets, but with vitamin-B-complex supplementation, the decrease in the number of NADH-diaphorase positive neurons was only 0.9% (SANT`ANA et al., 1997b).

These data demonstrate that 8%-protein-level diets and vitamin-B-complex supplementation in adult rats are able to preserve the metabolic activity of the NADH-diaphorase positive neurons needed for their evidentiation, as well as prevents the loss of such neurons. On the other hand, the same protein level together with the absence of vitamin B complex was the cause of the significant reduction in the evidentiation of this neuronal population, demonstrating that these vitamins are fundamental for the metabolism of these neurons. In an experiment carried out in these animals, we verified a 50.5% decrease in the number of NADPH-diaphorase positive neurons (SANT`ANA et al., 2001). We believe that the decrease in the number of both NADH- and NADPH-diaphorase positive neurons is due to the fact that the vitamins of the complex B, which were ingested in amounts about 20% smaller than the recommended, participate of the metabolic pathways of carbohydrate usage (WIDDOWSON, 1966; SANT`ANA et al., 2001).

When analyzing the neuronal size, we verified a significant increase in the number of small neurons and a decrease of large neurons in the experimental group compared to the control. Nevertheless, there were no significant differences in the basophilic intensity between the two groups.

These results contrast with those of the authors who evaluated enteric neurons in rats disnurtured during gestation and lactation and then recovered with diet by having normal protein and vitamin levels (MELLO et al., 1995; NATALI; MIRANDA-NETO, 1996). These authors found increases in the proportion of large neurons as well as increases in the basophilic intensity which were attributed to a possible adaptive mechanism, which would make the neurons store nutrients during the recovery period, in this way increasing their cytoplasmic levels of protein and the volume of their cell bodies.

On the other hand, our results are similar to those of other authors, who also found greater numbers of small neurons in adult rats subjected to protein desnutrition with vitamin supplementation and in the absence of a recovery period (WIDDOWSON, 1966; SANT`ANA et al., 1997b).

The decrease in body weight, intestinal area and number of enteric neurons demonstrate that the reduction in the protein level in the diet impaired the supply of essential

aminoacids for the synthesis of structural proteins, resulting in a smaller physical development of the animal. Likewise, the shortage of these proteins may have interfered with the repair and renewal of cytoplasmic organelles of the neurons, an event that could lead to acceleration of the processes of atrophy, aging and cell death. On the other hand, a deficiency of B1 vitamin leads to diminished activity of three essential RNA-producing enzymes, reinforcing the hypothesis that the reduced metabolic rate in the enteric neurons is the cause of their smaller cell size (CHAVES, 1978). The deficiency in complex B vitamins - as they are elements of adenine nucleotides - may have led to large decreases in the intracellular concentration of NAD+, whose effect may be neuronal degeneration (COTRAN et al., 1993). In the specific case of evidentiation of enteric neurons through the NADH-diaphorase technique, the decreased metabolic activity of this enzyme would add up to the degeneration, causing less evidentiation of the NADH-diaphorase positive neurons.

References

ALI, H.A.; MCLELLAND, J. Neuron number in the intestinal myenteric plexus of the domestic fowl (*Gallus gallus*). **Zbl Vet. Med. C. Anat. Histol. Embryol**. v. 8, n. 3, p. 277-283, Sept.1979.

AMORIM, C. S. C. **Estudo morfológico da mucosa intestinal de ratos submetidos a desnutrição protéico-calórica experimental**. 1984. 92 f. Tese (Doutorado) - Universidade de São Paulo, Ribeirão Preto, 1984.

BARBOSA, A. J. A. Técnica histológica para gânglios nervosos intramurais em preparados espessos. **Revista Brasileira de Pesquisas Médicas e Biológicas**, São Paulo v. 11, p. 95-97, 1978.

CASTELUCCI, P. **Análise morfoquantitativa do plexo mioentérico do intestino grosso de ratos submetidos a desnutrição protéica pré e pós-natal e à renutrição pós-natal**. 1999. 125 f. Tese (Doutorado) - Instituto de Ciências Biomédicas da Universidade de São Paulo, São Paulo, 1999.

CHAVES, N. **Nutrição básica aplicada**. Rio de Janeiro: Guanabara Koogan, 1985. p. 37.

COTRAN, R. S.; KUMAR V.; ROBBINS S. L. **Patologia estrutural e funcional**. 4. ed. Rio de Janeiro: Guanabara Koogam, 1993, p. 359-387.

DEO, M. G. Biology of protein-calorie malnutrition. **World Rev. Nutr. Diet**. London, v. 32. p. 49-95, 1978.

GABELLA G. The number of neurons in the small intestine of mice, guinea-pigs and sheep. **Neurosciense**, São Paulo, v. 22, n. 2, p. 737-52, Aug. 1987.

GABELLA, G. Dettection of nerve cells by histochemical technique. **Experientia**, v. 25, p. 218-219, 1969.

GIACOMELLI, F. R. B.; NATALI, M. R. M. A utilização de ratos em modelos experimentais de carências nutricionais. **Arq. Ciênc. Saúde Unipar**, Umuarama, v. 3, n. 3, p. 239-249, set./dez. 1999.

IRWIN, D. A. The Anatomy of auerbach's plexus. **Am. J. Anat**. v. 49 n. 1 p. 141-65, 1931.

LEPRI, E. R.; BRUSCHII, L. C.; MOURA, A. S. Efeitos da desnutrição protéica durante os períodos pré e pós natal no desenvolvimento cerebelar. **Rev. Bras. Ciênc. Morf**. São Paulo, v. 11, n. 1, p. 23-27, jan./jun. 1994.

MELLO, E. V. S. L.; STABILLE, S. R. MIRANDA-NETO, M. H. Effect of maternal protein deprivation on morphological and quantitative aspects of the myenteric plexus neurons of proximal colon in rats. **Arq. Neuropsiquiatry**, São Paulo, v. 55 n.1, p. 106-113, Mar. 1997.

MOURA, A. S. **Estudo em ratos do processo de adaptação a dietas com níveis variados de proteínas com e sem restrição energética**. 1994. 182 p. Tese (Doutorado) - Instituto de Ciências Biomédicas da Universidade de São Paulo, São Paulo, 1994.

NATALI, M. R.; MIRANDA NETO, M. H.; ORSI, A. M. Effects of hypoproteic diet supply on adult Wistar rats (*Rattus novergicus*). Acta **Scientiarum**, Maringá, v. 22, n. 2, p. 567-571, 2000.

NATALI, M. R. M.; MIRANDA NETO, M. H. Effect of maternal proteic undernutrition on the neurons of the myenteric plexus of the duodenum of rats. **Arq. Neuropsiquiatry**, São Paulo, v. 54, n. 2, p. 273-279, Jun. 1996.

NATIONAL Research Council. Nutrient requeriments of laboratory animals. 4. ed. Washington: National Academic press, 1995, p. 11-79.

PATRICIO, F. R. S.; NOBREGA, F. J.; TONETE, S. S. Q. Desnutrição intra-uterina em diferentes períodos de gestação em ratas: estudo do intestino delgado proximal ao nascimento e durante a recuperação nutricional. **Rev. Pau. Pediatria**, São Paulo, v. 2, n. 8, p. 43-52, abr./jun. 1984.

SANTANA, D. M.G.; MOLINARI, S. L.; MIRANDA NETO, M. H. Effects of protein and vitamin B deficiency on blood parameters and myenteric neurons of the colon of rats. **Arq. Neuropsiq**. São Paulo, v. 59 n. 3a, p. 493-498, Sept. 2001.

SANTANA, D. M. G. et al. Morphological and quantitative study of the myenteric plexus of the ascending colon of rats subjected to proteic desnutrition. **Arq. Neuropsiquiatry**, São Paulo, v. 55, n. 4, p. 687-695, Dec. 1997.

_____. Neuron number of the ascending colon of plexus of the ascending colon of rats a comparative study using two staining thecniques. **Arq. Neuro-Psiquiatry**, São Paulo, v. 59, n.3a, Sept. 2001.

SANTER, R. M.; BAKER, D. M. Enteric neuron numbers and sizes in Auerbach's plexus in the small and large intestine of adult and aged rats. **J. Auton. Nerv. Syst**. v. 28, p. 59-67, Nov. 1988.

SANTER, R. M. Survival of the population of NADPH-diaphorase stained myenteric neurons in the small intestine of aged rats. **J. Auton. Syst**. v. 49, n. 2, p. 115-121, Oct. 1994.

SHRADER, R. E.; ZEMAN, F. J. Effect of maternal proteic deprivation on morphological and enzimatic development of neonatal rat tissue. **Journal of Nutrition**, Bethesda, v. 99, n. 4, p. 401-412, Dec. 1969.

TORREJAIS, M. M. et al. Effect of protein malnutrition after breast-feeding on the morphology of the intestinal wall and myenteric neurons of the ileum rats. **Rev. Unimar**, Maringá v.17, n. 2, p. 315-327, 1995.

WAITZBERG, D. L. et al. Resposta da composição corpórea a desnutrição e a repleção nutricional experimental. **Acta Cirurg. Bras**. São Paulo, v. 4, n. 2, p. 61-69, abr./jun. 1989.

WIDDOWSON, E. M. The place of experimental animals in the study of human nutrition. In: .Mc CANCE, R.A.; WIDDOWSON, E.W. **Calorie deficiencies and protein deficiencies**. London: Churchill,1966. p. 225-236.