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Effects of hypoproteic diet supply on adult *wistar* rats (*Rattus norvegicus*)

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ABSTRACT. In this study the effect of the supply to adult rats of a diet of 8% protein for a period of 120 days was assessed, where body weight gain, dosage of total protein and proteic fractions, and thickness of the wall and muscle layer of the duodenum were analyzed. It was verified that the supply of isocaloric, yet low proteic-level diets, led to body weight stabilization, and decrease of total protein and globulins in malnourished rats. Histomorphometric analysis of the intestinal wall of disnurtured rats evidenced impairment of the hyperplasic and hypertrofic growth of its layers, verified by the decreased thickness of the duodenal wall.

Key words: proteic malnutrition, duodenum, adult rats.

RESUMO. Efeitos da imposição de dieta hipoprotéica em ratos *Wistar* adultos (*Rattus norvegicus*). Neste estudo foi avaliado o efeito da imposição de uma ração com 8% de proteínas por um período de 120 dias a ratos adultos, quando se analisaram a evolução do peso corpóreo, a dosagem de proteínas totais e frações protéicas, e as espessuras da parede total e da túnica muscular do duodeno. Verificou-se que para ratos adultos, a oferta de rações isocalóricas, porém com redução do teor de proteínas, levou à estagnação do peso corpóreo e à redução das proteínas totais e globulinas em animais desnutridos. A análise histomorfométrica da parede intestinal dos ratos desnutridos evidenciou comprometimento dos crescimentos hiperplásico e hipertrófico de suas túnicas, verificados pela menor espessura da parede duodenal.

Palavras-chave: malnutrição protéica, duodeno, ratos adultos.

Several are the descriptions found in the literature on the variations of the type of diet offered to the studied animals, on the mode of malnutrition induction, in addition to variations of species and age of these animals. The organic responses change according to the model used, this changes being of fundamental importance. Thus, Viteri and Schneider (1974) called attention to the direct relation between the type and degree of nutritional deficiency and the severity of the morphological, biochemical and functional alterations observed.

The effect of the supply of low-proteic level diets on the varied organs, in periods regarded as critical for the cellular development, that is, during gestation and lactation, was verified, among others, by Deo, 1978; Young *et al.*, 1987; Natali and Miranda-Neto, 1996; and Meilus *et al.*, 1998. The need for proteins, however, does not diminish with aging, and the requirement for some aminoacids may even increase so as to "compensate" for the morphofunctional alterations of the organism occurring as a consequence of the aging process (Mitchell *et al.*, 1978).

As for the morphological alterations, Deo (1978) stressed that the organs are not affected at the same time nor to the same extent, so that the tissues with high rates of cellular turnover are affected earlier than those of lower rates.

The small intestine is among those organs of high cellular turnover rates and has priority in the absorption and usage of nutrients. Some studies have reported morphologic and morphometric changes on the intestinal layers caused by proteic deficiencies (Takano, 1964; Shrader and Zeman, 1969; Natali *et al.*, 1995; Torrejais *et al.*, 1995; Brandão, 1998).

Thus, in this investigation the purpose was to verify the effects of a hypoproteic diet supply for a period of 120 days on adult Wistar rats. In this ration, prepared with a 8% proteic level, the following parameters were evaluated: body weight gain during the experimental period; levels of total protein, albumin and globulins in the plasma; and histomorphometry of the duodenal wall and its smooth muscle layer.

Material and methods

In this work, 26 male rats (*Rattus norvegicus*) from the Wistar[1] strain with 90 days of age were used. The animals were healthy, weighing on average of 296 g and were kept in individual cages at constant temperature and light-dark cycles of 12-12 hr, for 120 days. The rats were divided in two groups:

Control group (N): composed of 13 animals fed for 210 days with standard rodent chow - Nuvilab (recommendend by the National Research Council and National Health Institute, USA), with 22% proteic level. Chow and water were offered *ad libitum*.

Malnourished group (D): composed of 13 rats that from the 90th day of age received ration with 8% protein for 120 days. The desired proteic level was obtained adding corn starch to Nuvilab ration (Table 1). This chow was supplemented with hydrosoluble vitamins of complex B and saline mixture (American Institute of Nutrition, 1977; Lepri *et al.*, 1994; Natali and Miranda-Neto, 1996).

The hypoproteic ration was pelletized and had its proteic levels measured by the Semimicro Kjedahl method (Silva, 1981). Total calories of normoproteic chow were 468.27 Cal/100g and of the hypoproteic chow were 501.56 Cal/100g, being these values obtained through the calorimetric pump.

Table 1. Components used in preparing 100g of hypoproteic rations (8% protein) for rats

Nuvilab ration (22%)	38,3 g
Corn starch	30,2 g
Sugar	13,5 g
Soy oil	13,5 ml
Saline mixture*	2,7 g
Mixture of complex B vitamins**	1,8 g

*Saline mixture (in 2.7 g): potassium acid phosphate 1.04 g; calcium carbonate 0.73 g; calcium acid phosphate 0.24 g; sodium chloride 0.41 g; magnesium sulphate 0.25 g; tetra-hydrated ferrous citrate 0.02 g; potassium iodine 0.002 g; manganese sulphate 0.002 g; zinc chloride 0.0008 g; copper sulphate 0.0008 g; **Hydrosoluble vitamins from complex B (1.8 g): thiamine mononitrate (Vit. B1) 72 mg; riboflavine (Vit. B2) 72 mg; pyridoxine chlorhydrate (Vit. B6) 48 mg; Nicotinamide (Vit. PP) 240 mg; Calcium pantotenate (Vit. B5) 120 mg; Excipient q.s.p. 1248 mg

All the rats had their body weight assessed every 15 days until the 210th day. Five animals of each group had their blood collected for dosage of total protein (Biuret method - Labtest); albumin (Bromocresol Green - Labtest) and globulins.

Histomorphometric study of the duodenal wall. Samples of duodenal tissue of five animals of each group were washed, fixed in 10% formolin, dehydrated in ascending series of alcohol, diaphanized in xylene and included in paraplast plus for the obtention of transverse sections of 5μm thick stained with hematoxilin-eosin.

The intestinal circumference measured in four histological sections per animal was divided at eight points with 45° on from each other, beginning at the mesenteric border, yielding 320 measured points.

Through micrometric lens coupled to an Olympus microscope with a 10X objective, measurements of the total thickness of the duodenal wall from the villi apex to the mesotelium were performed, and with a 40X objetive the muscle layer was measured.

Statistical treatment. For comparison of body weight means between control and experimental rats, the profile analysis was carried out (Morrison, 1990). For comparison of the mean thickness of the duodenal wall and muscle layer between groups, of total protein, albumin and globulins, the test "t" of Student was applied.

Results

Body weight. The means of body weight of the albine rats studied, obtained every 15 days from the 90th day of age and during the following 120 days are presented in Table 2 and Figure 1. The animals receiving hypoproteic diet did not gain weight. From the 105th day of age on, the weight of the control animals became greater than that of the malnourished rats. The profile analysis revealed that these differences are statistically significant.

Table 2. Mean (in grams) of body weight of the control (N) and malnourished (D) groups assessed from the 90th to the 210th day of ages (n=13 in each group)

	Days								
Groups	90	105	120	135	150	165	180	195	210
C	301.8 a(1)	327.1 a	346.9 a	371.2 a	383.0 a	398.5 a	406.0 a	404.3 a	412.0 a
D	290.9 a	290.2 b	284.6 b	276.5 b	266.9 b	274.1 b	270.6 b	280.4 b	284.5 b

(1) Arithmetic means followed by the same letter, for each period, do not differ statistically, at the 5% significance level (profile analysis)

Dosage of total protein and proteic fractions. The amount of total protein and globulins collected from

[1] According to the ethical principles adopted by the Cobea and approved by the Ethical Comission in Animal Experimentation (CEEA) from the Institute of Biosciences/Unesp Botucatu, SP.

the malnourished rats was lower than that observed in the animals from the control group. The result of the analysis through the test "t" of Student revealed that this difference is significant, except for the albumin fraction, which did not show a significant difference. These values are presented in Table 3.

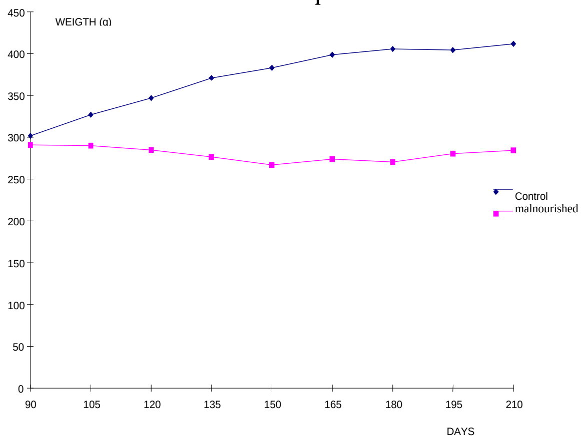

Figure 1. Means of body weight of the rats from the control and malnourished groups from the 90th to the 210th day of age

Table 3. Dosage of total protein, albumin and globulins (g/100 ml) from rats of the control (N) and malnourished (D) groups. Arithmetic mean ± standard deviation (n=5)

Variable	Total protein	Albumin	Globulin
C	6,49 ± 0,44 a(1)	3,06 ± 0,27 a	3,43 ± 0,31 a
D	5,88 ± 0,31b	3,35 ± 0,32 a	2,52 ± 0,19 b

(1) Means followed by the same letter, for each variable, do not differ statistically, at the 5% significance level

Histomorphometric analysis of the duodenal wall. The total thickness of the duodenal wall, from the apex of the villi of the mucosa to the mesothelium of the serosa, was decreased in the malnourished animals. Applying the test "t" of Student, it was verified that these differences were statistically significant. The thickness of the smooth muscle layer, despite being smaller in the malnourished animals, was not different at the significance level of 5%, and the values were presented in Table 4.

Table 4. Total thickness of the intestinal wall (μm) and thickness of the smooth muscle layer (μm) in the duodenum of rats from the control (N) and malnourished (D) groups. Mean ± standard deviation (n=5)

Groups	Intestinal wall	Muscle layer
C	886,11 ± 170,98 a (1)	86,71 ± 15,85 a
D	649,59 ± 119,86 b	74,50 ± 9,61 a

(1) Means followed by the same letter, for each variable, do not differ statistically at the significance level of 5%

Discussion

In this study, the nutritional element offered at inadequate levels to the rats in their diet was of proteic nature. Thus, to a comercially prepared chow for rodents, whose proteic level is 22%, was added corn starch until it had only 8% protein. This new chow to which were supplied mineral salts and vitamins thus assured a good development of the rodents, once none of them died or developed any pathology during the experimental period. On the other hand, it could be assertained that the possible changes observed would stem fundamentally from the proteic deficiency, as verified in previous studies by Lepri *et al.* (1994), Natali *et al.* (1995), Natali and Miranda Neto (1996) and Meilus *et al.* (1998).

The rats receiving chow with 22% protein had, at the end of the experiment, a body weight significantly higher than the rats receiving chow with 8% protein, although the hypoproteic diet had shown a higher caloric value. Our results demonstrated that the proteic level in the chow is essential for body weight gain, surpassing the importance of the level of starch.

The smaller body weight gain or even the decrease of body weight in rats receiving hypoproteic diet were reported by Takano (1964), Campana *et al.* (1975), Maffei *et al.* (1980), Amorim (1984), Natali *et al.* (1995), Natali and Miranda Neto (1996) and Brandão (1998).

Our results evidenced that in rats, much as in humans, the calories from carbohydrate, although essential for the energy-yielding processes, are "empty" for the purposes of synthetic processes. These latter promote the growth of cells and tissues and their predominance is reflected in the physical development of the organism, being in accordance with the considerations of Marcondes (1976).

In unbalanced nutritional states, specially those of a long-term nature, the lack of some groups of substances has a more pronounced effect on the normal dynamic balance, demonstrating a subtle biochemical hierarchy when the organism is in homeostasis. In this hierarchy the proteins are highlighted, because of their importance on the structures of support of organs and tissues, and their role as intermediates (proteic hormones and enzymes) which put into motion most of the metabolic machinery (Marcondes, 1976).

In the specific case of the rats used in this study, the caloric level of the chow supplied by the corn starch, in addition to compromising the growth, did not even assure storage in the adipose tissue. Nevertheless, the rats fed with hypoproteic ration did not need to consume their storage tissue or their muscle mass, once their body weights were kept unchanged. In this way we evidenced that the calories provided by the starch supplied the energy, while the proteins probably were spared and used, preferentially, for the maintenance and repair of tissues and production of enzymes.

The levels of total proteins and globulines present in the blood of the malnourished animals were significantly smaller when compared with those of the controls. Similar results were obtained by Campana *et al.* (1975) and Maffei *et al.* (1980), using aproteic ration, and by Amorim (1984) using 0% and 4% protein in the chow of rats.

The albumin fraction did not show significant alterations when compared between groups. A significant fall in the albumin fraction was verified in rats by Campana *et al.* (1975) and Maffei *et al.* (1980) with supply of aproteic ration during 28 to 32 days and during 84 days, respectively, and by Amorim (1984) also with aproteic ration.

The proteic level of 8% present in the chow offered to the rats in this experiment, in our view, was enough for the maintenance of the albumin fraction in the plasma. However, literature reports demonstrate that even in diets with proteic levels of 4% this fraction could remain unchanged (Amorim, 1984).

We consider that the maintenance of the levels of plasma albumin are related to a steady hemodynamic condition, preventing the formation of edema and aiding in a good performance of the circulatory and renal functions.

According to Marcondes (1978), the low proteic ingestion determines: immediate decrease of the synthesis of albumin, small decrease of the plasma levels of albumin, compensatory mechanisms such as albumin shunting from the extravascular to the intravascular compartments and decrease of its breakdown.

As for the thickness of the duodenal wall, this had a significant reduction in the malnourished animals, while the muscle layer, despite being smaller in the malnourished rats, was not statistically different at the level of 5%.

The atrophy of the small intestine wall is a frequent observation in investigations analyzing the effects of nutritional deficiencies on the morphology of this organ, and among them were cited Takano (1964), Hill *et al.* (1968), Viteri and Schneider (1974), Natali *et al.* (1995) and Brandão (1998).

The existence of the differences observed in our study would result from the changes in the development of the muscle layer and the other layers, specially the mucosa of the duodenal wall. This, being a tissue of high rate of cellular turnover during the whole life, could be easily affected by nutritional deficiencies, thus compromising its hyperplasic processes and consequently leading to atrophy of the wall thickness.

Supporting this last statement, Natali *et al.* (1995), Torrejais *et al.* (1995) and Brandão (1998) observed a decrease in the thickness of the duodenal wall after provoking proteic malnutrition in rats during gestation or in the post-birth period, that is, extremely critical periods in the intestinal development of rats. According to Winick (1970), the incidence of proteic deficiencies in these phases would affect both the hyperplasic and the hypertrophic growth of cells and tissues, resulting in smaller organs, our results lead to this same conclusion.

The occurrence of this decrease during adulthood, as verified in our study, was also observed by Takano (1964), and Hill *et al.* (1968), allowing us to suggest that, independently of the stage of development to be critical or not, some organs may have their development impaired or even decrease in size. We consider that the results obtained with the animals receiving 8% protein for a prolonged period may account for the smaller thickness of the duodenal wall.

Consequently, it can be inferred that some major factors would be related to the appearance of the response observed, such as impairment of the ability for intestinal cellular renewal (hyperplasic growth), storage of intracellular material (hypertrophic growth), and prolonged period of proteic deficiency to which the animals were subjected.

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