HEMATOLOGIC AND BIOCHEMICAL PARAMETERS OF RATS SUBJECTED TO HYPOPROTEIC AND HIPERCALORIC DIET

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ABSTRACT: Malnutrition is a public health issue which still affects children and adults all over the world, especially in developing countries. Protein deficiency causing *Kwashiorkor* is the most prevalent type of malnutrition, because protein-rich foods are generally expensive. A hypoproteic diet causes metabolic alterations in an animal which are directly proportional to the degree of protein depletion, as well as to the duration of the malnutrition. In this sense, we proposed to evaluate the severity of a 4%-hypoproteic diet in young rats. We used 30 Wistar rats (90 days of age), divided in control (CG, n=15) and experimental (EG, n=15) groups. CG was fed with a normoprotein chow, while EG was fed with a diet having 4% protein, for 12 weeks. At the end of the experiment, blood was collected for determination of the hemogram, activities of alkaline phosphatase and alanine aminotransferase, and concentration of total and fractional proteins, total cholesterol, triglycerides, urea, uric acid, T3, T4 and plasma aminoacids. The animals from EG had lower activity of the alkaline phosphatase enzyme in blood, normochromic microcytic anemia, hypoproteinemia, hypoglobulinemia, decreased plasma triglyceride concentration, increased plasma concentrations of T3 and T4, and decreased plasma concentrations of the following aminoacids: methionine, phenylalanine, valine, leucine and isoleucine.
KEY WORDS: protein-energy malnutrition (PEM), hematologic and biochemical parameters

PARÂMETROS HEMATOLÓGICOS E BIOQUÍMICOS DE RATOS SUBMETIDOS À DIETA HIPOPROTÉICA E HIPERCALÓRICA

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RESUMO: A má-nutrição é um problema de saúde pública que ainda assola crianças e adultos no mundo inteiro, principalmente em países em desenvolvimento. A carência de proteínas, causando *Kwashiorkor*, é o tipo de má-nutrição mais prevalente, pois fontes de alimentos protéicos, geralmente, são mais onerosas. Uma dieta hipoprotéica causa alterações metabólicas num animal em intensidades diretamente proporcionais ao nível de depleção de proteínas, bem como o tempo em que o indivíduo permanece sob o estado de subnutrição. Nesse sentido, propõe-se avaliar a severidade de uma dieta hipoprotéica a 4% para ratos jovens. Utilizam-se 30 ratos Wistar (90 dias de idade), divididos em grupo controle (15) e experimental (15). O GC recebeu dieta nor0moprotéica, enquanto o GE recebeu dieta com 4% de teor de proteínas, ambos durante 12 semanas. No final do experimento, sangue foi coletado para realização de hemograma e dosagem de atividade de fosfatase alcalina, alanina aminotrasferase, além da concentração de proteínas totais e frações, colesterol total, triglicerídeos, uréia, ácido úrico, T3, T4 e aminoácidos plasmáticos. Os animais do GE demonstraram menor atividade defosfatase alcalina no sangue, anemia microcítica normocrômica, hipoproteinemia, hipoglobulinemia, reduação na concentração plasmática de triglicerídeos, aumento da concentração plasmática de T3 e T4 e diminuição da concentração plasmática dos seguintes aminoácidos: metionina, fenilalanina, valina, leucina e isoleucina.
PALAVRAS-CHAVE: Má-nutrição protéica-energética (MPE), parâmetros hematológicos e bioquímicos

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PARÁMETROS HEMATOLÓGICOS Y BIOQUÍMICOS DE RATONES SOMETIDOS A LA DIETA HIPOPROTÉICA E HIPERCALÓRICA

ARAÚJO[1*], E.J.A.; SANT'ANA[1], D.M.G.; MOLINARI[2], S.L.; NETO[2], M.H.M. Parámetros hematológicos y bioquímicos de ratones sometidos a la dieta hipoprotéica e hipercalórica. *Arq. ciên. vet. zool. UNIPAR*, 8(2): p. 139-146, 2005.

RESUMEN: La mala nutrición es un problema de salud pública que todavía aniquila niños y adultos en el mundo entero, principalmente en países en desarrollo. La falta de proteínas, causando *Kwashiorkor*, es el tipo de mala nutrición más común, pues fuentes de alimentos proteicos, generalmente, son más caras. Una dieta poco proteica causa alteraciones metabólicas en un animal en intensidades directamente proporcionales al nivel de depleción de proteínas, así como el tiempo en que el individuo queda bajo el estado de baja nutrición. En ese sentido, proponemos evaluar la severidad de una dieta de bajo contenido proteico al 4% para ratones jóvenes. Utilizamos 30 ratones Wistar (90 días de edad), divididos en grupo control (15) y experimental (15). El GC recibió dieta normoproteica, mientras el GE recibió dieta con el 4% de cantidad de proteínas, ambos durante 12 semanas. Al final del experimento, sangre fue recolectado para realización del examen de la sangre y cantidad de actividad de fosfatase alcalina, alanina aminotrasferase, además de la concentración de proteínas totales y fracciones, colesterol total, triglicerídeos, urea, ácido úrico, T3, T4 y aminoácidos plasmáticos. Los animales del GE demostraron menor actividad de fosfatase alcalina en la sangre, anemia microcítica normocrómica, hipoproteinemía, hipoglobulinemia, reducción en la concentración plasmática de triglicerídeos, aumento de la plasmática de T3 y T4 y disminución de la concentración plasmática de los siguientes aminoácidos: metionina, fenilalanina, valina, leucina y isoleucina.
PALABRAS CLAVE: Mala nutrición Proteica-Energética (MPE), parámetros hematológicos y bioquímicos

Introduction

The scientific and technologic development of the biological and health areas taking place these last years has been trying to favor, directly or indirectly, the longevity of the human being. The fields of investigations concerned with disease prevention and health improvement have been some of the most prominent. Within this scope it is important to consider that there are hundreds of thousands of people, including children, that are poor and/or under privation in economical terms, whose health becomes fragile due to the insufficiency of some food components (WORLD HEALTH ORGANIZATION, 2003).

It is known that the way malnutrition is established in animals (including man), leading to changes in the homeostatic mechanisms, is a time-dependent phenomenon. In this way, the studies aiming at assessing the metabolism at different moments of the malnutrition process in experimental animals subjected to controlled diets, allow the determination, with greater accuracy, of the mechanisms underlying the installation of malnutrition, as well as to contribute to the investigation of the factors related to the adaptation or not to adverse instances (WATERLOW, 1996), because it is known that pre-clinical malnutrition already presents biochemical alterations (NÓBREGA, 1996).

When there are protein and energy restriction in the diet of an animal, a scene of protein-energy malnutrition (PEM) is established. When there is a predominant protein deficit, the clinical picture is called *Kwashiorkor*, while when energy lack predominates; signs and symptoms of marasmus arise. PEM causes endocrine changes that aim at adapting the organism to the irregular availability of food, so as to conserve energy and nitrogen. In general terms, insulin, glucagons, growth hormone and corticosteroids either reflect or determine short-term metabolic alterations, and the thyroid hormones influence growth rate (WATERLOW, 1996). The thyroid-produced hormones (T3 and T4) stimulate the transcription of genes that influence the metabolic rate of all cells, thus the greater the level of these hormones, the larger the metabolism (GUYTON & HALL, 2002). The scientific community admits that PEM causes a certain degree of hypothyroidism, and as in this instance the availability of substrates is smaller, it is believed that this is adaptive (WATERLOW, 1996).

However, before growth retardation occurs in a disnurtured animal, metabolic adjustments arise with the goal of adapting the organism to ambient changes. This was demonstrated in some experimental studies with rats, that report: (a) increased liver concentration of lipids (ENWONWU & SREEBNY, 1970; CAMPANA *et al.*, 1975; EDOZIEN & SWITZER, 1978a); (b) diminished plasma levels of total protein (ENWONWU & SREEBNY, 1970; PHILBRICK & HILL, 1974; ANTHONY & EDOZIEN, 1975; CAMPANA *et al.*, 1975; EDOZIEN & SWITZER, 1977; HEARD *et al.*, 1977; AKINGBEMI & AIRE, 1994; DOS SANTOS *et al.*, 1997; NATALI *et al.*, 2000; SANT´ANA *et al.*, 2001; OBATOLU *et al.*, 2003); (c) diminished plasma levels of some aminoacids, especially the essential ones (ENWONWU & SREEBNY, 1970; PHILBRICK & HILL, 1974; ANTHONY & EDOZIEN, 1975; HERMELO & RODRIGUES, 1989) and (d) diminished level of hemoglobin (ANTHONY & FALOONA, 1974; EDOZIEN & SWITZER, 1977; OBATOLU *et al.*, 2003).

The scientific literature has varied results related to nutritional experiments in rats, although this is the most used biological model because of the rats small size, short gestation, adaptability to several diets and easiness of manipulation (NATIONAL RESEARCH COUNCIL, 1995). There are studies supplying protein diet to the rats (CAMPANA *et al.*, 1975; OBATOLU, 2003) or diets of different protein levels: 0.4% (ANTHONY & EDOZIEN, 1975), 0.5% (ENWONWU & SREEBNY, 1970; ANTHONY & FALOONA, 1974; PHILBRICK & HILL, 1974), 0.8% (ANTHONY & EDOZIEN, 1975), 2% (EDOZIEN & SWITZER, 1977; EDOZIEN & SWITZER, 1978a, b), 5% (EDOZIEN & SWITZER, 1977; EDOZIEN & SWITZER, 1978a,b), 6% (AKINGBEMI *et al.*, 1995; DOS SANTOS *et al.*, 1997), 8% (AKINGBEMI & AIRE, 1994; SANT´ANA *et al.*, 2001; ARAÚJO *et al.*, 2003; MELLO, 2004), 10 %

(EDOZIEN & SWITZER, 1977; EDOZIEN & SWITZER, 1978a,b), 12% (HEARD *et al.*, 1977; OBATOLU *et al.*, 2003) and 15% (ANTHONY & EDOZIEN, 1975; EDOZIEN & SWITZER, 1977; EDOZIEN & SWITZER, 1978a,b). In addition to the different protein levels, these studies also differ in the duration of the experiment and the age and strain of the rats. As for the protein level, commercial chows for rodents usually have a minimal protein level of 22%; however, some studies (NATIONAL RESEARCH COUNCIL, 1995) demonstrated that 12%-protein diets do not lead to metabolic changes in rats during the growth period.

In this investigation, we aimed at evaluating the severity of a 4%-protein diet, in the long run, on young adult rats, through the analysis of biochemical and hematologic parameters.

Material and Methods

We used 30 male Wistar rats (*Rattus norvegicus*) aging 90 days (291.52±38.24g), which were kept in individual metabolic cages in a biotery with controlled temperature (± 25ºC) and light/dark cycles (12/12 hrs). During the whole experimental period water and chow were supplied *ad libitum*.

The protocols for animal handling and killing were approved by the Committee on Ethics in Research Involving Animal Experimentation of Paranaense University (UNIPAR).

The animals were randomly allotted to two groups: control (n = 15) and experimental (n = 15). The control group (CG) was fed with NUVILAB® commercial chow for rodents. This chow was bromatologically analyzed and from the results the components were calculated to be added so as to decrease the protein level to 4% while keeping the vitamin and mineral balance. Thus, for each kg of the experimental chow it was used 153.85g of ground commercial chow, 53.10g of a mixture of mineral salts, 172.87g of sucrose as table sugar, 391.41g of commercial corn starch, 140.7g of vegetal oil as lipid source, and 18g of vitamins B complex as ground pills. The other vitamins were in excess in the initial formulation and the supplementation was not necessary. Water was added to allow mixture and pelletization of the chow. The pellets were dried in a stove at 55 degrees and then they had their percent composition analyzed (Table 1).

The experiment lasted 12 weeks, at the end of which the rats from both CG and EG were fasted for about 12 hours and then anesthetized with the following mixture, injected intramuscularly: Acepran (1.26 ml/Kg) + 10% Ketamine (1.26 ml/Kg) + 2% Xilazine (0.42 ml/Kg) and 1% Atropine (0.22 ml/Kg) (PACHALY *et al.*, 2003). We carried out the laparotomy and collected 4 ml of blood from each animal through cardiac puncture. The blood of each animal was split into two essay tubes (at the proportion of 1:3), one containing anti-clotting (EDTA) and the other wasn't; after complete hemolysis in this last tube, the serum was obtained through centrifugation and stored at –20ºC.

The non-clotted blood collected from eight animals of each group was used for the determination of the complete hemogram. All the values obtained were calculated from the Cell-Dyn 1400 Automatized System (Abbott Diagnostics).

From the serum of four animals of each group we dosed the activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and the concentration of total proteins, albumin, globulins, total cholesterol, triglycerides, urea, and uric acid through the Express-Plus Automatized System. From the serum of other four animals of each group, we dosed the concentration of the following aminoacids: alanine, histidine, phenylalanine, methionine, tyrosine, valine, leucine, isoleucine and tryptophan (Method: High-Performance Liquid Chromatography). From the serum of other four animals of each group we dosed the levels of T3, T4 and free T4 (Method: Enzyme-immunoassay by micro particles – Automation Axsym Abbott).

We removed the liver from the animals, which was immediately weighed to obtain the wet weight, dried in stove at 35 degrees for 24 hours and weighed again to obtain the dry weight. From the bromatologic analysis, the percentages of protein and lipids were determined, as well as the [protein]/[lipid] ratio.

To identify significant differences between CG and EG in each set of data, we employed Student's t-test at the significance level of either 5% or 1%. To detect any degree of dependence between variables, we calculated Pearson's Correlation Coefficient, to which the t-test was applied at $\alpha = 5\%$ or 1%. The results are presented as mean ±standard deviation.

Results

The erythrogram and the leukogram of rats from CG and EG are presented in tables 2 and 3, respectively. The erythrogram of the animals from EG showed that these animals had anemia of the microcytic normochromic type.

The serum activity of the alkaline phosphatase (ALP) and alanine aminotransferase (ALT) enzymes is shown in figure 1.

The biochemical analysis of the serum of both groups led us to the results presented in tables 4 and 5.

Table 6 shows that, on average, the liver weight of the CG rats is greater than the one from the experimental rats ($p<0.01$), as well as the protein concentration in this organ ($p<0.05$). On the other hand, the lipid concentration in the liver of EG rats was higher than in CG ($p<0.01$), and because of this there is a marked difference in the protein/lipid ratio between the groups ($p<0.01$).

Discussion

Anemia is a pathologic manifestation that can occur as a consequence of continuous ingestion of a hypoprotein diet. In this study, the rats from EG had, at the end of the experiment, a smaller amount of circulating hemoglobin than those from CG ($p<0.01$), and below the reference value for rats (HILLYER & QUESENBERRY, 1997), thus characterizing a picture of anemia. The type of anemia that the EG rats developed was microcytic normochromic, because the mean corpuscular volume (indicating the volume of the erythrocytes) was below normal and the mean cellular concentration of hemoglobin, taking into account the smaller volume of the erythrocytes, was within the recommended range (HILLYER & QUESENBERRY,

Table 1 – Percent composition of the chow offered to the Control Group (NUVILAB®) and of the hypoproteic chow prepared for the Experimental Group

Component	Control Chow - NUVILAB® (%)	Experimental Chow (%)
Humidity	8.58	8.9
Proteins (% N x 6.25)	26.02	4.07
Lipids	6.45	14.8
ashes	9.05	8.97
Fibers	7.24	2.34
Carbohydrates (by ≠)	42.66	60.92
Energy Value	332.77 Kcal/100g	402.60 Kcal/100g

Source: Percent composition carried out by the Laboratory of Physic-Chemistries Analyses of Paranaense University.

Table 2 – Erythrogram of rats subjected to balanced diet (CG) and of rats subjected to the 4%-hypoproteic diet (EG)

Observations	*CG*	*EG*	*Reference values*	*EG difference relative to CG*
Erythrocytes (millions/ml)	7.49±0,40**	5.75±0.49**	5.4 – 8.5	-23.23%
Hemoglobin (g/ml)	13.61±0.39**	9.61±0.93**	11.5 – 16.0	-29.39%
Hematocrit (%)	35.84±1.64**	25.19±2.83**	35 – 51	-29.72%
M. C. V. (μm³)	55.48±13.89*	43.73±1.97*	57 – 65	-21.18%
M. C. H. (pg)	21.01±4.81*	16.71±0.69*	15 – 22	-20.46%
M. C. H. C. (%)	38.09±2.57	38.23±0,.0	30 – 35	-0.36%

*p<0.05; **p<0.01. Values are given as mean±standard deviation; n = 8 for each group. M.C.V. = mean corpuscular volume; M.C.H. = mean corpuscular hemoglobin; M.C.H.C. = mean corpuscular hemoglobin concentration.

Table 3 – Leukogram of rats subjected to balanced diet (CG) and of rats subjected to the 4%-hypoproteic diet (EG)

Observations	*CG*	*EG*	*Reference values*	*EG difference relative to CG*
Leukocytes	3,328.57±652.47	4,400±1,927	4,000 – 10,200	-24.32%
Neutrophils	400.65±429.89	391.56±396.49	1,300 – 3,600	-2.27%
Eosinophils	43.19±44.06	46.43±30.92	30 – 500	-6.98%
Lymphocytes	2,270.43±564.24	3,185.43±1,489.97	5,600 – 8,300	+40.30%
Monocytes	103.88±138.02	79.14±40.75	100 –700	-23.82%
Basophils	3.38±9.55	0.00	0 – 50	----

*p<0.05; **p<0.01. Values are given as mean±standard deviation (per/mm³); n = 8 for each group.

Table 4 – Serum concentrations of total proteins, albumin, globulins, total cholesterol, triglycerides, urea, uric acid, T3, T4 and free T4 of rats subjected to balanced diet (CG) and of rats subjected to the 4%-hypoproteic diet (EG)

Observations	*CG*	*EG*	*Reference values*	*EG difference relative to CG*
Total protein (g/dl)	6.01±0.26**	4.67±0.28**	5.9 – 7.9	- 22.29%
Albumin (g/dl)	2.85±0.26	2.61±0.41	2.8 – 4.4	-8.42%
Globulins (g/dl)	3.16±0.23**	2.06±0.32**	2.6 – 3.9	-34.81%
Total cholesterol (mg/dl)	70±14.19	73±8.49	10 – 54	-4.11%
Triglycerides (mg/dl)	46.86±27.29**	28±12.18**	----	-40.25%
Urea (mg/dl)	33.75±4.92*	20.5±4.03*	----	-39.26%
Uric acid (mg/dl)	1.19±0.39	0.99±0.31	----	-16.81%
T3 (ng/dl)	0.38±0.05*	0.56±0.09*	----	+32.14%
T4 (μg/dl)	6.04±0.45**	7.98±0.48**	----	+24.31%
Free T4 (ng/dl)	1.54±0.09**	0.83±0.07**	----	-46.11%

*p<0.05; **p<0.01. Values are given as mean±standard deviation; n = 4 for each group.

Table 5 – Serum concentrations of some aminoacids of rats subjected to balance (CG) and of rats subjected to the 4%-hypoproteic diet (EG)

Observations	*CG*	*EG*	*EG difference relative to CG*
Alanine	5.35±2.48	3.70±0.80	-30.84%
Histidine	4.73±1.36	4.38±1.32	-7.39%
Phenylalanine	1.22±0.48**	0.69±0.19**	-43.44%
Methionine	1.04±0.81**	0.27±0.05**	-74.04%
Tyrosine	4.26±2.96	2.70±0.54	-36.62%
Valine	2.81±1.47*	1.55±0.37*	-44.84%
Leucine	2.93±1.55*	1.54±0.43*	-47.44%
Isoleucine	1.55±0.71**	0.74±0.18**	-52.26%
Tryptophan	1.11±0.89	0.67±0.31	-39.64%

*p<0.05; **p<0.01. Values are given as mean±standard deviation (mg/dl); n = 4 for each group.

Table 6 – Weight, percentage of protein and lipids, and protein/lipid ratio of the liver of rats with balanced diet (CG) and of rats receiving the 4%-hypoproteic diet (EG)

Observations	*CG*	*EG*	*EG difference relative to CG*
Liver weight (g)	10.60±0.61**	7.57±1.10**	-28.58%
Liver weight (mg)/body weight (g)	26.96±10.63	26.99±42.97	+0.11%
Protein (mg/g of liver)	416±32*	207±42*	-50.24%
Lipid (mg/g of liver)	14±11**	317±59**	+2,164.28%
Protein/Lipid (ratio)	29.71**	0.65**	-97.81%

*p<0.05; **p<0.01. Values are given as mean±standard deviation; n = 15 for CG e n = 12 for GE.

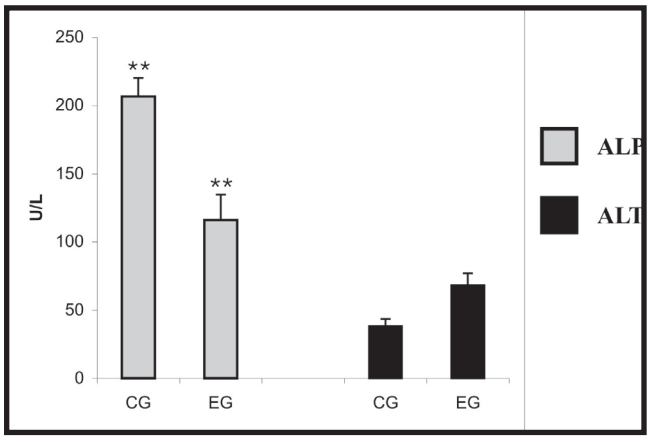

Figure 1 – Serum activity of alkaline phosphatase (ALP) and alanine aminotransferase (ALT) of rats fed with a balanced diet (CG) and of rats subjected to the 4%-hypoproteic diet (EG). ** p<0.01

1997). The smaller volume of the erythrocytes, as well as the smaller concentration of hemoglobin (p<0.05) of the EG rats relative to CG was probably due to the smaller availability of aminoacids for the synthesis of the proteins that make up this cellular type. We argue that the possibility that anemia was also caused by iron lack is negligible, because the diet offered to the EG rats had mineral salt supplementation, including iron. However, it must be considered that protein depletion, in the long run, can impair iron absorption, as well as its storage and transport in the organism; more detailed biochemical investigations are required about this aspect. The decreased number of erythrocytes in EG relative to CG (p<0.01), on the contrary, may have occurred because of lower erythropoietin production and/or secretion, perhaps as an adaptive mechanism to the hypoproteic diet. Since the first studies of nutritional experimentation, the importance of the protein level for the maintenance of the number and structure of erythrocytes has been appraised (ANTHONY & EDOZIEN, 1975; EDOZIEN & SWITZER, 1977; AKINGBEMI & AIRE, 1994; AKINGBEMI *et al.*, 1995; SANT´ANA *et al.*, 2001; OBATOLU *et al.*, 2003; MELLO, 2004). The literature shows that alterations in the number of erythrocytes and in hemoglobin concentration as well, are triggered only in experimental studies of protein deficiency, when the protein level of the diet is markedly reduced and when the experimental period is not too short, as it was observed by ANTHONY & EDOZIEN (1975). Their results are in agreement with ours, once they verified a reduction in the number of erythrocytes and in hemoglobin concentration in rats fed with a 3%-protein chow during 12 weeks. Researches show that an 8%-protein level in the diet of the rats, either for a short or a long time period, do not alter these hematologic parameters (AKINGBEMI & AIRE, 1994; SANT´ANA *et al.*, 2001; MELLO, 2004). Another investigation shows that even a protein diet, in the short run, do not change the parameters related to the erythrocyte (OBATOLU *et al.*, 2003).

We assessed the activity of the alkaline phosphatase (ALP) and alanine aminotransferase (ALT) enzymes to see if there were secondary effects of protein malnutrition on the liver, and observed that the animals fed with a lower protein level had a significantly lower level of ALP, but no significant change of ALT, when compared to the rats from CG (p<0.01 and p>0.05, respectively). Due to the lack of a basement membrane in the hepatic sinusoids, the enzymes

released by the parenchymatous cells from the liver have immediate access to the blood circulation (NOGUEIRA *et al.*, 1990) and thus above-normal levels of ALP and ALT in blood are indicative of hepatic dysfunction (OBATOLU *et al.*, 2003), and may be linked to hepatocyte lesion. Based on this, we can suggest that the hepatocytes of the disnurtured rats of this research were not damaged and, therefore, the hepatic functions of these animals probably were maintained. This is corroborated by the equal production of circulating cholesterol and albumin in both CG and EG ($p>0.05$), which are important substances produced by the hepatocytes. A significantly lower level of ALP was also encountered in the blood of rats subjected to 8%-protein (OBATLU *et al.*, 2003) and 6%-protein levels (AKINGBEMI *et al.*, 1995). A slight but significant reduction was observed for ALT activity in rats subjected to the 8%-protein diet (AKINGBEMI & AIRE, 1994) and no significant difference was observed when the rats were fed with the 6%-protein diet (AKINGBEMI *et al.*, 1995). As urea production in mammals occurs essentially in the liver (VOET *et al.*, 2000), the plasma concentration of this compound, resulting from aminoacid catabolism, could also be used as an indicative of hepatic function. In this study, we observed that the serum level of urea in EG rats was less (39.26%; $p<0.05$) than the one from the CG, but we suggest that this is not an indicative of hepatic dysfunction, but due to the smaller aminoacid catabolism, a phenomenon probably triggered by the hypoproteic and hipercaloric diet offered, in an attempt to keep the positive nitrogenous balance. In addition, NOGUEIRA *et al.* (1990) stress that reduced urea due to hepatic impairment only takes place when the damage is extensive, which does not seem to be the case in this investigation.

Another remarkable feature of animals subjected to protein malnutrition is the reduction in the levels of plasma proteins, and in this study this was also observed. Reduced concentrations of plasma proteins were reported for animals subjected to diet with protein levels of 8% (NATALI *et al.*, 2000; SANT´ANA *et al.*, 2001; MELLO, 2004), 7% (HEARD *et al.*, 1969), 4% (HEARD *et al.*, 1977), 3% (ANTHONY & EDOZIEN, 1975), 2% (ENWONWU *et al.*, 1973) e 0% (OBATOLU *et al.*, 2003). The major types of protein present in plasma are albumin, globulins and fibrinogen (GUYTON & HALL, 2002). There is a dynamic balance among the plasma proteins, the plasma aminoacids and the tissue proteins; then, when tissue proteins are depleted, plasma proteins can act as a rapid reposition deposit (GUYTON & HALL, 2002). In the EG rats of this study, we observed a slight decrease in the albumin level which, although insignificant relative to CG ($p>0.05$), was slightly below the reference values for rats (HILLYER & QUASENBERRY, 1997). This finding is similar to the one of Amorim *apud* NATALI (2000), who also fed rats with a 4%-protein diet. EDOZIEN & SWITZER (1977) evaluated the effect of diets differing in protein level on rats and observed that for albumin the maximal value is reached with 10%-protein diets. Albumin is not significantly altered as well when the rats are fed with 8%-protein (AKINGBEMI & AIRE, 1994) and 6%-protein level (AKINGBEMI *et al.*, 1995) in the short run, but it decreases significantly with protein diet (OBATOLU *et al.*, 2003). NATALI *et al.* (2000) demonstrated that a diet with 8% protein, in the long run, is not even capable of changing albumin concentration, while SANT'ANA *et al.* (2001) and MELLO (2004), employing the same diet with rats of the same strain, in the long run, verified a significant fall of the concentration of this type of plasma protein. The most intense effect of the diet employed in these two last investigations is that there was no vitamins B complex supplementation in the chow of the experimental group. As the major function of albumin is to establish the plasma colloidosmotic pressure so as to prevent plasma filtration through the capillaries (GUYTON & HALL, 2002), edema possibly did not form in EG animals because the decrease of this protein was slight. Rats seldom develop edema, which forms only when the amount of protein in the food is largely reduced, as demonstrated by ENWONWU & SREEBNY (1970), which supplied a 0.5%-protein chow to Sprague-Dawley rats and observed edema in 25% of the animals after 10 weeks of treatment. Nevertheless, it is believed that human *kwashiorkor* is rarely triggered by an aproteic or a 0.5%-protein diet, and that is why we developed this 4%-protein chow. As for the globulins of our animals, we observed a reduction of 34.81% in the EG animals relative to CG ($p<0.01$). Smaller amounts of globulins were also observed when animals were subjected to diets having protein levels of 8% (AKINGBEMI & AIRE, 1994; NATALI *et al.*, 2000) and 0% (OBATOLU *et al.*, 2003). The globulins in blood can be antibodies or complement, hemagglutinins, hormones, enzymes (such as ALP), blood clotting factors, hypertensinogen, carriers, and others (FERREIRA, 1994; GUYTON & HALL, 2002). Maybe the animals from EG of this study were immunodepressed due to the lack of aminoacids for immunoglobulin synthesis, but the leukogram did not point out to changes in the incidence of any of the types of leukocytes in the animals of either group. No significant alteration of leukocyte numbers was found in studies using 8%-protein (AKINGBEMI & AIRE, 1994; SANT´ANA *et al.*, 2001; MELLO, 2004), 6%-protein (AKINGBEMI *et al.*, 1995) e 0% protein diets (OBATOLU *et al.*, 2003).

We observed that the triglyceride level of EG decreased by 40.25% relative to CG ($p<0.01$). The control of plasma lipid levels is done primarily by the thyroid hormones (T3 e T4). The increased secretion of these hormones diminishes the plasma concentration of triglycerides (GUYTON & HALL, 2002). In this study, the level of T3 increased 32.14% in the blood of the animals from EG relative to those from CG ($p<0.05$) and the level of T4 increased 24.31% in EG relative to CG ($p<0.01$), although the level of free T4 had decreased 46.11% ($p<0.01$). Knowing that T4 is capable of increasing the rate of protein synthesis when there are adequate amounts of carbohydrates and lipids (GUYTON & HALL, 2002), this essential cellular process should be impaired in the animals from EG, especially in organs of greater cellular and/or protein turnover, due to the low level of the free hormone and also due to the presumptive lack of available aminoacids caused by the hypoproteic diet. Despite being notorious that hormonal changes are essential for mammals to adapt to a lower protein and/or calorie intake, experimental studies on animal nutrition are rare which present results of the effect of the hypoproteic diet on the blood level of thyroid hormones.

EDOZIEN *et al.* (1978) also observed an increased plasma level of T3 while T4 levels were reduced when the rats were fed with chow containing 2, 5 or 10% protein. Perhaps the differential effect over T4 in the experiment of these authors as compared to our results is due to the fact that they used rats from another strain and younger than ours.

Among the plasma aminoacids analyzed in this research, methionine was reduced the most (74.04%) in the rats from EG relative to CG ($p<0.01$). This essential aminoacid gives rise to cysteine and taurine, which make up key enzymes of the intermediary metabolism, especially cellular respiration (FERREIRA, 1994). In addition, methionine is a component of keratin and melanin, and this could explain why some animals from EG lost their fur. As methionine stimulates erythropoietin (FERREIRA, 1994), its lack leads to decreased red blood cells and hemoglobin, as also observed in this study. Several investigations concerned with hypoproteic diets demonstrate diminished plasma methionine (ENWONWU & SREEBNY, 1970; ENWONWU *et al.*, 1973; PHILBRICK & HILL, 1974; ANTHONY & EDOZIEN, 1978a). BROADBENT & HEARD (1973) suggested that methionine is an aminoacid which is always decreased at all levels of protein malnutrition. Another markedly affected aminoacid was phenylalanine, which was reduced by 43.44% relative to CG ($p<0.01$). This is probably due to a higher conversion of phenylalanine to tyrosine so as to promote the metabolic changes needed to keep the homeostasis. This finding is in keeping with those of ENWONWU & SREEBNY (1970), ENWONWU *et al.* (1973), PHILBRICK & HILL (1974) and ANTHONY & EDOZIEN (1975). We additionally noticed that the 4%-protein diet also reduced the plasma levels of tyrosine (36.62%) relative to CG animals. Tyrosine is a precursor of thyroid hormones and catecholamine (FERREIRA, 1994). As for the thyroid hormones, their significant increase was already discussed. As for the catecholamine, studies evaluating hormonal changes caused by ingestion of hypoproteic diets demonstrate that there is a negative correlation between the dietary protein level and the plasma levels of epinephrine and norepinephrine (EDOZIEN *et al.*, 1978), i.e., hypoproteic diets increase the levels of these hormones in blood. In this way we may suggest that the tyrosine decrease observed in the animals from EG probably was due to a greater consumption of this aminoacid for the production of T3, T4, epinephrine and norepinephrine. Branched-chain aminoacids can implement muscle and visceral protein synthesis and provide nitrogen for glutamine synthesis, which is used by the immune system cells and for system repair during periods of metabolic stress (RUFFER *et al.*, 2001), so we suggest that the low concentration of these aminoacids in the blood of EG animals is caused by their continuous flow towards the tissues to keep their homeostasis. Reductions in the plasma levels of valine, leucine and isoleucine were also reported in other studies of nutrition in rats (ENWONWU & SREEBNY, 1970; PHILBRICK & HILL, 1974; ANTHONY & EDOZIEN, 1975) and primates (ENWONWU *et al.*, 1973).

An increase in the lipid concentration in the liver is a characteristic of children with *kwashiorkor* (WATERLOW, 1996). For the rat, the literature demonstrates that an increased hepatic concentration of lipids depends on a diet with a very diminished amount of proteins, as demonstrated by ENWONWU & SREEBNY (1970) when they subjected Sprague-Dawley rats to a 0.5%-protein diet for eight weeks, and by EDOZIEN & SWITZER (1978a) when subjected Sprague-Dawley rats to diets of different protein levels for eight weeks and observed visible fat in the liver when the animals consumed 2% protein. This was also observed by CAMPANA *et al.* (1975) when subjected Wistar rats to aproteic chow during four weeks. In our investigation we also observed that the liver of the EG animals had a marked increase in the lipid concentration relative to CG ($p<0.01$). Considering that the liver is one of the organs carrying out intense turnover of cytoplasmatic proteins, the hepatocytes are rapidly impaired by a hypoproteic diet (DEO, 1978), become smaller (EDOZIEN & SWITZER, 1978a) and, accordingly, can exhibit greater lipid concentrations, not necessarily from an increased lipogenesis, because the energy intake of the EG animals was smaller. In this study we noticed that the liver from the EG rats had smaller absolute weight ($p<0.01$) and smaller amount of protein ($p<0.05$) relative to that of CG. Studies demonstrate that the protein level of the diet is prevalent on the liver weight and that in rats a minimum of 15% protein in the diet is needed for the liver to reach its maximal weight (EDOZIEN & SWITZER, 1978b). An adequate supply of exogenous aminoacids to the liver provides enough substrate for the turnover of its cytoplasmatic proteins, so that liver enzymes work properly and keep the balance of compounds that influence its internal osmolarity (such as glycogen) and hence its water content, a determinant factor which also needs to be taken into account when evaluating the hepatic weight.

The metabolic, physiologic and structural changes observed in the rats fed with hypoproteic chow (4%) demonstrate the adaptive potential that these species (*Rattus norvegicus*) have when challenged by dietary changes.

Therefore, we can conclude that the malnutrition caused by the ingestion of a diet having 4% protein by young (90-180 days) Wistar rats is severe enough to trigger: microcytic normochromic anemia, hypoproteinemia, hypoglobulinemia, reduced plasma concentrations of triglycerides, increased plasma concentrations of T3 and T4, decreased plasma concentrations of the aminoacids: methionine, phenylalanine, valine, leucine and isoleucine, decreased liver weight and increased lipid concentration in the liver.

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