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Solution CM-95 Magnetically Treated Effects over Nitrogen Metabolism in Balb / C Mice in Fasting and Non-Fasting Condition.

Clara Esther Martínez Manrique[1], Nelson Licea Beatón[2], BiganHung Guzmán[2], Ana Román Montoya[1]. Juan Francisco Infante[3], Juan René Martínez Manrique[4] Gustavo Sierra González.[5]

[1,4]*Department of Science and Innovation National Center for Applied Electromagnetism. Universidad de Oriente. Cuba*
[2,3]*Department of Biology. Faculty of Natural Science .Universidad de Oriente. Cuba*
[5]*Center of Production Serum and Vaccinia.Finlay Institute. Havana. Cuba*
[6]*Department of Alergy. Hospital Clínico Quirúrgico. Calixto García. Cuba*
[7]*Biotechnology Industry Farmaceuticals Group. Biocubafarma. Cuba*

Abstract: *In this work, the effects of the CM-95 Solution on nitrogen metabolism in Balb / c mice are evaluated. An experiment is developed, in conditions of not fasting and the other in fasting conditions. In both cases, the animals were inoculated with the CM-95 Solution orally and 24 hours later, different parameters of nitrogen metabolism in plasma, urine and faeces were determined in both nutritional conditions. The lowest values were recorded in fasting. It was demonstrated that Solution CM-95 varied in a general way the behavior of the nitrogen metabolism maintaining an adequate balance between the processes of synthesis and degradation, where the processes of energy production could be favored. The levels recorded do not imply metabolic damage by the magnetically treated solution for liver and kidney function compromised with these processes. These results allowed us to evaluate the effects of the CM-95 Solution on nitrogen metabolism in Balb / c mice.*

Key Words: *CM-95 Solution ,magnetically Treatment, nitrogen metabolism, Balb/C mice*

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I. Introduction

Nitrogen metabolism comprises a series of events that aim at the formation of nitrogenous compounds, among which amino acids are found, as fundamental pillars for the synthesis of proteins and also for their high energy value, due to the fact that nitrogenous molecules are little abundant in nature. During the nitrogenous metabolism, enzymes such as transaminases and other compounds such as creatinine, urea, uric acid, among others, are produced. Many of them can be excreted in the urine. The nitrogen that is excreted comes from the protein turnover that is established from the balance between the synthesis and the degradation of some compounds in others(Nelsonetal.2015)

A current focus for science is the application of the magnetic field and aqueous systems magnetically treated in the magnetotherapy of many human diseases. This has been possible due to the biostimulating and repairing effects of the magnetic field. Among them, its remarkable influence on many cellular processes is highlighted, stimulating the synthesis of energy, proteins and enzymatic activation, the ionic flow through the cell membrane, the production of prostaglandins as a preamble to its anti-inflammatory action, the repair Tissue reported by numerous national and international scientists (Borroto, 2016). The biological effects demonstrated of the CM-95 TM Solution towards a modulated stimulation process of the immune response, Martinez et al (2009), Martinez et al., (2011), Fernández. 2016, are closely related to the nitrogen metabolism, by the need to synthesize large amount of proteins with important functions for the defense of the organism.

The experiment was designed to evaluate the effects of the magnetically treated CM-95 solution on nitrogen metabolism in Balb / c mice in the non-fasting and fasting condition.

II. Material And Methods

All the tests were carried out according to the Good Laboratory Practice for Non Clinical Laboratory Studies (BPL) and the established in the guides for the handling of Laboratory Animals, according to the Canadian Council on Animal Care (1984), National Research Council of the National Academics Animals

(2010) The test protocol was reviewed and approved by the Ethics Committee for the Care and Use of Laboratory Animals of the National Center for Applied Electromagnetism.

Experimental Biomodels:

Mice of the Balb / c line, females 6 to 8 weeks old, with a body weight between 19 and 22 g were supplied by the National Center for the Production of Laboratory Animals (CENPALAB) ®, Havana, Cuba). Five animals were kept in the vivarium by plastic box of Microlon after quarantine, in conventional sanitary conditions at a temperature of 22 ± 2 ° C and a relative humidity of 60 and 65%, in a regime of 12 light hours and 12 dark hours. The animals were fed during all that time with ratonine and water acidulated ad libitum until they were individually transferred to acrylic boxes with dimensions of 18.25x13.5 cm, base of 13.5 x 9 cm and 16 cm high, for the development of the experiments in non-fasting and fasting., The feeding conditions and selection of the groups according to each experiment were maintained as shown in table 1.

Table 1. Experimental Groups

Experiment 1 n 10 Of not fasting condition with food withdrawal for 24 h and water ad libitum	Experiment 2 n 10 Fasting condition without removing the food in 24 h water maintenance ad libitum
GTM: Treatment group with Magnetically treated solution. (CM-95)	GTM1: Treatment group with Magnetically treated solution. (CM-95
GSTM: Control group the solution without magnetic treatment	GSTM1: Control group the solution without magnetic treatment
GCN: Control group without any treatment	GCN1: Control group without any treatment

Magnetic Treatment Solution CM-95:

Solution CM-95 formed by the salt of chloride and sodium at a physiological concentration (0.9%) was prepared, which was magnetically treated with an inhomogeneous permanent magnet device with magnetic induction between 0.01-0.16 T. This was built and certified by the National Applied Electromagnetism Center (CNEA) Santiago de Cuba. Calibrated according to the national standard and the standards referred to in certificate N004-001. Cuba.

Administration of the CM-95 Solution:

The dose of Solution CM-95, with magnetic treatment (groups GTM and GTM1) and without magnetic treatment (GSTM and GSTM1) was 1.8mg / kg in a volume of 0.2mL administered orally with gastric probes.

Extraction of blood.

At the end of 24 hours of inoculation of the mice with the magnetically treated solution and without magnetic treatment under the conditions of not fasting (Experiment I) and fasting (Experiment II), they underwent a total bleeding by cardiac puncture. The blood obtained was centrifuged in a centrifuge (JanetzkiT Germany) at 1800 g for 15 minutes. Then in the supernatant the plasma was obtained for the determination of the parameters of the blood biochemistry: total proteins, transaminases (ALAT and ASAT), creatinine, urea and uric acid.

Obtaining Urine and Stool:

Animals from experiments 1 and 2 were transferred to acrylic boxes, with a plate of the same material with holes three cm from the floor, which served as the basis for placing each mouse individually. The holes allowed the passage of urine but not of stool, avoiding contamination of samples. The urine of each mouse was collected for 24 hours in a sterile glass container located at the bottom of the acrylic box, under the plate at a temperature of 22 ± 20 C. The faeces were collected per animal during this same time. Then they were added in sterile knobs with 50% sulfuric acid to prevent microbial growth. Both types of samples were kept at 40 C, until their use in the corresponding tests.

Determination of Parameters of Nitrogen Metabolism:

Total proteins:

For the determination of total proteins, the technique of Wiener, (1995), was applied with the use of the commercial Kit (HELFA) ®. To 50 µL of serum or urine of each sample per group of experiments I and II, of the Reference solution with albumin (40.0-80g / L) and of the blank with distilled water was added 3mL of Biuret Solution. The protein concentration was determined at an optical density of 540 nm in HITACHI 902

spectrophotometer, China according to the expression: Cm = Am x Cr / Ar. Where: Cm concentration of total proteins in the sample (g / l); Am: absorbance of the sample; Ar: absorbance of the reference. Cr: concentration of the albumin reference (g / l). The presence of total proteins in urine was determined by the detection of the color contributed by the colored complex and expressed as positive (+), or negative (-) in the case of absence of color.

Determination of transaminases:

It was carried out applying the enzymatic method, where the reaction of Alanine amino transferase (ALAT) Bergmeyery col, (1980) and of aspartate aminotransferase (ASAT) Vassault et al, (1986), present in plasma samples, whose activity is proportional to the oxidation of NADH that is quantified by spectrometry. With the use of the HELFA ® commercial kit, the specific working reagent for each transaminase was prepared in 1/10 ratio by mixing ten volumes of reagent 1 with one volume of reagent 2. Then 2mL of this reagent was mixed with 200µL of each sample and with 2mL of the blank with distilled water and incubated at 37 ° C, for 1 minute. The variation of the optical density per minute (Δ DO / min) was measured during 3 minutes at 37 ° C against white at 340 nm in HITACHI 902 spectrophotometer, China. The concentration was calculated by the expression Activity (U / L) = Δ DO / min x 1746. Where: Activity (U / L): ALAT activity of the sample, Δ DO / min: variation of optical density obtained in the 3 minutes, 1746: factor of calculation of enzymatic activity.

Determination of creatinine in plasma and urine:

The method of the reaction of picric acid in alkaline medium Jaffe', (1986) was used. To develop this technique, the commercial kit for creatinine (HELFA) ® was used. The working reagent was prepared with the mixture of 1 mL of sodium hydroxide with 9 mL of picric acid which was mixed with 0.1 mL of creatinine as reference and with the same volume of each sample according to the experimental group and experiment. The determination of absorbance was performed at a wavelength of 510 nm and at a temperature of 37 0C in spectrophotometer ITACHI 902, China. To calculate the concentration in each sample the formula was used: Cm = (A2-A1) sample / (A2-A1) reference x Cr. Where: Cm: concentration of the sample (µmol / L); Cr: reference concentration (µmol / L); A2-A1: difference between the absorbance readings for the sample and the reference.

Determination of urea in plasma and urine:

The analytical method was used where the intensity of the coloring complex is measured by spectrophotometry Henry et al; (1980). The commercial kit for urea (HELFA) ® was applied. These

Were mixed according to the indications referred to in table E and incubated for 5 min at 37 ° C. At the end of this time the optical density of each sample and the blank reference at 620 nm were read with the use of an ITACHI 902 spectrophotometer, China. Initially, the working reagent formed by the lyophilised urease (enzyme) was prepared, to which 100 mL of color reagent was added. The concentration of the restored enzyme was 7 U / mL. Then 2mL of this reagent and the alkaline reagent were mixed with 20 µL of each sample, the blank with distilled water and the reference solution with urea. To calculate the behavior of the urea in each sample Lambert Beer's law was used: Cm = Am x Cr / Ar. Where: Cm: concentration of urea in the sample (µmol / L); Am: absorbance of the sample; Ar: absorbance of the reference; Cr: concentration of the reference (µmol / L).

Determination of uric acid:

Uric acid was determined by the enzymatic method where the hydrolysis of hydrates with urate oxidase forms a colored complex that is quantified by spectrophotometry at 550 nm Triverdi et al (1978). The analytical technique was developed with the Kit for uric acid (HELFA) ®. The mixture was mixed with 2 mL of the working reagent in 20 µL of each sample, the target with distilled water and the reference solution with uric acid. The concentration of uric acid in plasma was determined by the expression: Cm = Am x Cr / Ar. Where: Cm: concentration of the sample (µmol / L); Am: absorbance of the sample; Ar: absorbance of the reference. Cr: concentration of the reference. (µmol / L).

Determination of total nitrogen in stool:

The Kjeldhal method was applied to determine the nitrogen in feces of the different experimental groups, with modification of the technique using formaldehyde López, (1994). (13). 0.5 g of the stool samples were weighed in the Owa Labor Technical Scale with an accuracy of 0.1 mg. Then they were transferred to a Kjeldhal digestion balloon, and 0.1 mg of mixed catalyst (potassium sulfate and copper sulfate II pentahydrate) was added, then 0.1 mg of selenium and 5 to 6 drops of 30% hydrogen peroxide were added. , 2 to 3 drops of antifoam and 10 mL of concentrated sulfuric acid. The balloon with the different samples after shaking, was placed in the digester until boiling. Once the samples were transparent with a blue-green color, they continued

boiling for 30 minutes. Once the digestion was finished, it was allowed to cool to room temperature, then its content was transferred to a 100 mL volumetric flask and made up to the mark with distilled water. An aliquot of 6 mL was taken and 1 or 2 drops of mixed indicator (methyl methylene blue red) were added, it was evaluated with 2.9777 mol / L sodium hydroxide solution. Then, 10 mL of 35% formaldehyde was added and it was evaluated with sodium hydroxide solution at a concentration of 0.0496 mol / L. The concentration of total nitrogen in faeces was determined according to the expression: (C1V1 + C2V2) x M (N) x Vt / 1000 x Vmxm (g) Where: C1V1 + C2V2: concentration and volume of the samples before and after valuation, M (N): atomic mass of nitrogen, Vt: total volume used, Vm: sample volume, m (g): mass of the sample in grams, 1000: to take the nitrogen concentration to percent

Statistical analysis:

The Kolmogorov Smirnov test was used to verify if the data in the experiments fulfilled a normal distribution. To compare the experimental values, a simple classification analysis of variance (ANOVA) was applied. The comparison of the means when there were differences of statistical significance was evaluated with the Tukey test. For all tests, the level of statistical significance was $p < 0.05$. All the calculations were made with the SPSS program for Windows, version 12.0 (2003).

III. Result

Results of nitrogen metabolism

Biochemical parameters in non-fasting conditions in blood plasma

The behavior of the biochemical parameters in blood plasma of Balb / c mice for the three experimental groups, in non-fasting conditions are shown in table 2. The total protein values were similar for the GSTM and GCN groups, who presented statistically significant differences ($P < 0.05$) with respect to the GTM). But all are within the normal range for the species ($P < 0.05$)

The highest values for plasma ALAT in non-fasting conditions corresponded to GCN followed by GSTM and GTM. The GCN presented differences of statistical significance with respect to the GSTM and GTM and in turn the GSTM with respect to the GTM ($p < 0.05$).For the ASAT, the highest values corresponded to the GCN and GTM groups followed by GSTM. There were no statistically significant differences between the GTM and GCN groups ($p < 0.05$); but of these with respect to the GSTM ($p < 0.05$.) While in the creatinine (Table 2), the GTM showed the higher values in this parameter and the GSTM the lower ones. The differences of statistical significance were of the GTM with respect to the GSTM and the GCN and the GSTM with respect to the GCN ($p < 0.05$). In the case of the urea in plasma (table 2), the highest value corresponded to the group GCN followed by the GSTM and GTM group, there were statistically significant differences between all ($p < 0.05$).

Table 2. Values of plasma parameters of Balb / c mice inoculated with the CM-95 Solution magnetically treated under non-fasting conditions. n = 10

Parámetros	GTM	GSTM	GCN
Totals proteins (g/L)	62,18 ± 0,23 b	70,66 ± 0,42 a	0,86 ± 0,33 a
(ALAT) (U/L)	2,40 ± 1,4 c	2,80 ± 1,3 b	3.20 ± 1,3 a
(ASAT)(U/L)	1,60 ± 0,2 a	1,53± 0,9 b	1,63 ± 0,17 a
Creatinine (µmol/L	164,20 ± 1,02 a	151,04 ± 0,28 c	161,60 ± 0,69 b
Urea (mmol/L)	14,93 ± 0,41 c	15,95 ± 0,31 b	25,37 ± 0,31 a
Uric Acid (µmol/L)	262,8 ± 6,05 b	313,2 ± 14,78 a	307,80 ± 9,98 a

Diferent letters denote significant differences * ($p < 0.05$). Multiple Ranges.Tukey

When analyzing the levels of uric acid in plasma (table 2), a behavior similar to that registered for urea metabolism is shown. Differences in statistical significance were recorded for the GSTM and GCN groups with respect to the GTM ($p < 0.05$), but not between them ($p > 0.05$)

Biochemical parameters in plasma under fasting conditions.

The behavior of biochemistry parameters in blood plasma of Balb / c mice for the three experimental groups under fasting conditions are shown in Table 3. For total proteins, groups GSTM1 and GCN1 presented statistically significant differences with respect to group GTM1 ($p < 0.05$). But between GSTM1 and GCN1, the differences were not statistically significant ($p > 0.05$).

Table 3. Values of the plasma parameters of Balb / c mice inoculated with the CM-95 solution magnetically treated under fasting conditions .n = 10

Parámeters	GTM1	GSTM 1	GCN 1
Totals proteins (g/L)	53,58 ± 0,60 b	58,3± 0,80a	58,5± 0,53 a
(ALAT) (U/L)	1,1 ± 0,14 c	1,90 ± 0,17a	1,4 ± 0,6 b
(ASAT)(U/L)	1,43 ± 0,12a	1,48 ± 0,7a	1,31 ± 0,1 b
Creatinine (µmol/L)	159,2 ± 2,05 c	165,3 ± 0,83 a	163,6± 1,89 b
Urea (mmol/L)	7,53 ±0,38b	8,01 ± 0,10 a	5,44 ± 0,31 c
Acid Uric (µmol/L)	99,8 ± 13,08 a	99,4 ± 11,7 a	50,4 ± 8,50 b

Diferent letters denote significant differences * ($p <0.05$). Multiple Ranges.Tukey

In the ALAT there were statistically significant differences between the GSTM1 group with respect to the GCN1 and GTM1 group ($p <0.05$), and also the GCN1 with respect to the GTM1 ($p <0.05$). For aspartate aminotransferase (ASAT), differences of statistical significance were presented for the GTM1 and GSTM1 groups with respect to GCN1 ($p <0.05$). Between the groups GTM1 and GSTM1 there were no statistical differences ($p> 0.05$) for the concentration of this enzyme in plasma.

The creatinine quantified in the plasma of Balb / c mice in fasting conditions showed for the GSTM1 statistically significant differences with respect to GTM1 and GCN1 who also had statistical differences among them ($p <0.05$). In the case of urea levels, statistically significant differences were found between the groups GsTM1 and GTM1 with respect to the GCN and between them ($p <0.05$). While uric acid showed higher levels in the groups GTM1 and GSTM1 with respect to the group GCN1. With statistically significant differences between GTM1 and GSTM1 with respect to GCN ($p <0.05$). Between the groups GTM1 and GSTM1 there were no differences of statistical significance ($p> 0.05$).

Metabolism of nitrogen in urine

Biochemical parameters in urine in non-fasting conditions

Table 4 summarizes the behavior of different nitrogen metabolism compounds excreted in the urine in the non-fasting condition.In the GTM group the highest creatinine values were reported, with differences of statistical significance ($p <0.05$) with respect to the GCN and GSTM as well as between these two groups ($p <0.05$). For the urea excreted in the urine of the Balb / c mice, the highest values were reported for the GCN, followed by the GTM and GSTM group, which presented statistical differences with respect to the rest (GTM and GSTM) ($p <0.05$). In turn, the GTM presented statistically significant differences with respect to GSTM ($p <0.05$).

Table 4. Values of the urine parameters of Balb / c mice inoculated with the CM-95 Solution under non-fasting conditions. n = 10

Parámetros	GTM1	GSTM1	GCN1
Creatinine (µmol/L)	8,4± 0,55a	4,1± 0,36 c	5,4 ± 0,34b
Urea (mmol/L)	15,27± 1,46 b	12,14± 2,0 c	17,8 ± 1.76 b
Proteins	(-)	(-)	(-)

Diferent letters denote significant differences * ($p <0.05$). Multiple Ranges.Tukey

Table 5 summarizes the values of nitrogen compounds excreted in the urine of Balb / C mice in the fasting condition. The experimental groups GTM1, GSTM1 and GCN1 presented statistically significant differences among them ($p<0.05$). For creatinine, the lowest values were for GTM1 and for urea in GCN1. No proteins were detected in the urine of the mice for the non-fasting and fasting condition

Table 5. Values of the urine parameters of Balb / c mice inoculated with the CM-95 Solution magnetically treated under non-fasting conditions. n = 10

Parámeeters	GTM1	GSTM1	GCN1
Creatinine (µmol/L)	2,6 ± 0,68 c	3,7 ± 0,46 a	3,2 ± 0,44 b
Urea (mmol/L)	6,90 ± 0.31 a	7,58 ± 0,78 a	5,18 ± 0.76 b
Proteíns	(-)	(-)	(-)

Diferent letters denote significant differences * ($p <0.05$). Multiple Ranges.Tukey

Total nitrogen in faeces in conditions of no fasting and fasting

The total nitrogen values decreased in relation to the controls in the two conditions of non-fasting and fasting appear in Table 6. The lowest values corresponded to the groups with the CM-95 Solution (GTM and GTM1) and in the condition of fast.

Table 6.Total nitrogen content in stool of Balb / c mice in non-fasting and fasting n = 10

Experiments	% total Nitrógen		
	GTM	GSTM	GCN
No fasting	5.58 ± 0.31b	6.42± 0.23 a	6.38± 0.10 a
Fasting	GTM1	GSTM1	GCN
	4.50± 0.22 b	5.27± 0.14 a	5.70± 0.13 a

Diferent letters denote significant differences * (p <0.05). Multiple Ranges.Tukey

IV. Discussion

The use of Balb / c mice as experimental biomodels was adequate to evaluate the effects of the magnetically treated CM-95 solution on different parameters of nitrogen metabolism in plasma, urine and faeces. In general, the parameters evaluated for the non-fasting condition remained normal, although as expected in the fasting condition some of them were below this.

Despite the small volume of sample provided by the mouse, the use of analytical methods with the use of specific KIT, where small proportions of samples are used, made possible the development of the tests with a behavior according to the experimental conditions. On the other hand, the metabolism of nitrogen in this animal species has an analogy with the rest of mammals, being possible to interpolate the results to a similar behavior in humans.

For the non-fasting condition, the decrease of the total proteins in the GTM with respect to the rest of the groups can be interpreted as the effect that induced the CM-95 Solution magnetically treated on the cellular metabolism and specifically on the hepatocytes, possibly mobilizing amino acids to a greater extent for the synthesis of intracellular proteins. The nitrogen of dietary origin, incorporated mainly with food proteins, becomes part of body proteins that fulfill important biological functions (enzymes, hormones, antibodies, among others), or constitute cellular and tissue structures such as skeletal, among others. The amino acids incorporated into blood from the gastrointestinal tract are used not only for tissue protein synthesis, but also in Novo's production of glucose, or the direct generation of energy when they serve as substrates of oxidative metabolism. (García et al, 2015)

Martínez et al., (2010) reported higher values in the concentration of blood plasma proteins and glucose levels, when using a dose of the CM-95 solution magnetically treated in Balb / c mice administered intraperitoneally. However, when two doses were used, the concentration of proteins decreased as did glucose. The latter behavior could be explained by the possible mobilization of amino acids towards other metabolic pathways (gluconeogenesis), to counteract the effects of glucose reduction in blood, recorded in the trial of other experiments and which in turn may explain the decrease in total plasma proteins; that coincidentally happened in this work and that other authors discuss Nelson y col, 2015); Everything seems to indicate that the route of administration different from the oral route used in this work had a different effect on the behavior of this parameter when a single dose of the magnetically treated solution was administered, but not for ALT transaminase. Martínez et al; (2010) also evaluated the effects of the magnetically treated CM-95 solution on the levels of ALAT transaminase, inoculating a single dose of it and there was a coincidence with the decrease of

The behavior obtained in creatinine, with higher values in the GTM group, seems to indicate that the CM-95 solution upon receiving the active magnetic treatment 'to a greater extent this metabolic pathway from the hepatocytes with greater creatine formation, which could be available in muscle tissue of mice to be stored in the form of phosphocreatine and promote the formation of ATP and affect the formation of creatinine as has been proposed by other scientists (Palacios, 2012, Nelson et al, 2015).

The fact that the magnetically treated CM-95 Solution has decreased the values of uric acid in plasma, with respect to the control groups, does not mean that it is a negative effect for nitrogen metabolism. The uric acid that is produced from the degradation of the purine bases adenine, guanine not only comes from the diet, but also from the endogenous nitrogenous bases. In turn, nitrogenous bases are an essential raw material for the formation of other compounds such as nucleic acids, but participating in anabolic pathways. (Diaz et al, 2015). Therefore, it can be assumed that low levels of this compound in plasma could be due to a higher efficiency of the CM-95 solution with the magnetic treatment, to favor more the synthesis processes from the nitrogenous bases, more that the catabolic processes as precursors for the formation of uric acid.

In the non-fasting condition, the diet incorporates nitrogen elements (proteins), which constitute the immediate source of exogenous energy. Thus the body can count on an adequate balance of nutrients and energy to develop all the processes of metabolism.

In the fasting condition, the different experimental groups had a decrease in the values of the total proteins in the plasma of the mice, with respect to the non-fasting condition and to a greater extent the GTM1 group. Possibly the solution Cm-95 magnetically treated favored as a priority the catalytic processes of proteins to obtain energy via glucose, with the most efficient use of the few available a In transaminases all the experimental groups in the fasting condition also decreased their values with respect to the non-fasting condition below the normal values for the species. However, it does not represent diagnostic value indicative of toxicity

with liver disease, or heart and skeletal muscle disorders, as if it occurs when their levels increase above normal. In correspondence with what the literature points out, the condition of fasting minimizes the synthesis processes, since there is a rearrangement of the metabolism at the expense of the maintenance of homeostasis, where degradation processes are indispensable for obtaining energy (Murray et al. ; 2012, Nelson et al; 2015).,

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The nitrogenous compound creatinine formed by dephosphorylation presented the lowest levels in the group inoculated with the magnetically treated CM-95 Solution. In this case, it must be remembered that in fasting conditions there is a decrease in the available energy and the levels of phosphocreatine in the muscle depend on the availability of ATP and creatine, since it is synthesized from amino acids. Under these conditions, creatinine formation becomes more critical

We can defend the hypothesis that Solution CM-95 treated magnetically in this condition of fasting, could have diverted the obtaining of energy in other processes such as for example the synthesis of glucose, to compensate the cellular metabolism. Although anyway a part it will be used in the formation of creatinine by a readjustment of the metabolism, in the path of an energy stability in a general way in the organism.

It is known that approximately 5 g of free purines per day are normally produced in humans, however only 0.5 g of uric acid is excreted (Nelson et al, .2015). In the GTM1 group, the levels of this compound in the plasma of the mice experienced a slight increase with respect to the GCN1 group. It is possible that the solution has not exerted an effect, capable of recovering the free purines present in the plasma, for its use in nucleotide and nucleic acid synthesis processes, since high levels of energy are needed for this, in fasting not available. Therefore, they could be used to a greater extent in the formation of uric acid. The levels of this compound for the experimental groups do not represent any adverse effect for the processes of excretion, since only when it accumulates in large quantities precipitates in the form of crystals causing kidney damage.

The excretion of compounds by the urine depends on the behavior of the nitrogen metabolism (Martínez G et al 2015), as a result of which the creatinine and urea of the plasma in the mice must be excreted almost entirely in the urine. Therefore, the behavior obtained in the non-fasting condition was expected, for the GTM with higher values in the creatinine and the GCN in the urea, with respect to the rest of the groups. Even so, the values of creatinine and urea were normal for all groups without anomalies that made this physiological process impossible.

In the fasting condition, although the creatinine and urea values were lower than the non-fasting condition, the behavior trend was similar depending on the concentration recorded in plasma. According to Guyton, 2015 (1988) these concentrations depend not only on blood plasma levels but also on the intensity of glomerular filtration. It must be taken into account in the observed values, that this concentration is the result of a 24-hour metabolism, which is in constant readjustment due to fasting conditions. Apparently, the CM-95 solution treated magnetically has some effect on the processes that allow the excretion of urea, compared to the group inoculated with the same solution without magnetic treatment, although both contain sodium at physiological concentrations. Sodium is one of the ions that influences the physiological processes for glomerular and specific urea filtration. (Diaz et al, 2015)

Coincidentally, the results obtained for the total proteins in the condition of not fasting and fasting in the urine were not detected by the qualitative techniques applied. This result confirms the above-mentioned, about the low probability of renal damage in Balb / c mice inoculated or not with the CM-95 Solution magnetically treated. The absence of proteins in urine in both cases (Tables 4 and 5) is an expected result, when the loss of these in the urine is a sign of possible kidney damage (Salabarria et al, 2012)

The lowest values of fecal nitrogen excretion corresponded to the fasting condition, although in both conditions (fasting and non-fasting), this behavior coincided for the GTM and GTM1 groups with the magnetically treated solution. As already mentioned, possibly this solution with magnetic treatment, led to a better reuse of nitrogen elements, including amino acids for the performance of synthesis functions, which may explain the low excretion of nitrogen in faeces, making possibly more efficient these processes.

Nitrogen as an essential element for protein synthesis undergoes various types of changes in the body. The amount of nitrogen excreted by different routes is an example of the importance of it and is indicative of the way in which the metabolism was developed. Normally the excretion of nitrogen is regulated by many factors, among which stand out the protein availability by the diet and the biological value of it (Diaz et al, 2015, Brandan et al, 2015).

The results obtained in this research support the possibilities provided by the magnetic treatment of the CM-95 Solution, to achieve a more efficient balance between the processes of synthesis and degradation of nitrogen compound

V. Conclusion

It was possible to evaluate the effects of the CM-95 solution on the nitrogen metabolism of Balb /c mice, with a normal behavior of the nitrogen parameters in plasma, urine and faeces, corresponding to the condition of no fasting and fasting. Which could be interpreted as an adequate renal and hepatic function, where metabolic damage must not be compromised with the processes of synthesis and degradation

References

[1]. Bergmeyer HU, Horder MJ. Técnica para la determinación de la alaninaaminotransferasa.ClinChem.Clin. Biochem. 1980; 18: 52

[2]. BorrotoV.Campos electromagnéticos pulsados y ozono en el tratamiento de la artrosis de rodilla en el adulto mayor. Revista Española de Ozonoterapia 2016; 6(1): 27-393- *Brandan NC, Aispuru G. Metabolismo de compuestos nitrogenados .Universidad Nacional del Nordeste cátedra de Bioquímica . Facultad de Medicina UNNE. Internet 2013 [citado Enero 2015] pp1-26 Disponible en:* https://med.unne.edu.ar/sitio/multimedia/imagenes/ckfinder/files/files/.../nitro.pdf

[3]. Canadian Council on Animal Care (CCAC). Hematology and Clinical Biochemistry Reference Values, In: Guide to the care and use of experimental animals. Ottawa (Canadá): CCAC Publishing House,1984; T1: 86-8.

[4]. Díaz ZJ, Juárez OA. Bioquímica un enfoque básico Aplicado a las Ciencias de la Vida . Editorial McGraw- Hill Edición: 1ra Internet 2012 .*[citado 16 Noviembre 2015]* . Disponible en https://www.pinterest.com/pin/692921092637837025

[5]. García A , Santana PS. Nitrógeno ureico urinario estimado del índice de Excreción urea-creatinina construido para una muestra única de orina. Rev.Cub. Alimentación y Nutrición. 2015; 25(2) : 314-326

[6]. Guyton CA, Laso FJ. Tratado de FisiologiaMédica .12ªEdición . Editorial Elservier. España .2015

[7]. González S, Román A, Martínez C, Batuti M, Fernández I. Solución CM-95 tratada magnéticamente como profilaxis y terapia de la radiodermitis en pacientes con cáncer. MEDISAN. 2016; 20 (11):5105-12

[8]. Henry RJ, Cannon DC, Winkelman JW. Técnica de déterminacióndе urea.QuímicaClínica. Bases y Técnicas. Editorial Jims. 2da Edición. Tomo II. 1980

[9]. Jaffé M. Técnica de determinación de creatinina.ZtschPhysiolChem.1986 ;10:391.

[10]. López CJ. Determinación de nitrógeno total por Kjeldhal. Revista Técnica de Laboratorio, Tomo No. 191. Valencia. AquaTécnica. S. A.1994

[11]. Martínez G, Bravo A, Escadilla WC, Limon PD, LunaMF. Diferencias en la respuesta de lafurosemida entre ratas cepas Wistar y Spraguedawley. Rev Divulgación Científica de la FESAHANCCCAL.2015;1: 6-16

[12]. Martínez MC, Toledano Marlene, Rodríguez G, Andinas L, Lassalle I, Sierra G. Solución adyuvante CM- 95 tratada magnéticamente en comparación con el adyuvante de Freud para la obtención del suero de Coombs en conejo. VacciMonitor, 2009 : 18(3): 5-12

[13]. Martínez CE. Ramón CJ Belén, Favier RP, Tamayo OV, Sierra GG.Efectos de la solución CM-95 tratada magnéticamente sobre parámetros hematológicos y bioquímicos en ratones Balb/c. MEDISAN 2010; 14(9):2164

[14]. Martínez MC, Tamayo V, Favier P, Castillo JR, Hernández R, Sierra G. Efecto inmunomodulador de la Solución CM-95 tratada magnéticamente en ratones Balb/c inoculados con 5-fluorouracilo. VacciMonitor. 2011; 20: 13-21.

[15]. Murray Robert, Bender D, Bothan KM, Kenelly PJ, DodwellVN, WeilPA. BioquimicaHarper. en sección III Metabolismo de proteínas y aminoácidos pp27-31. 28 Edicion Editorial MC Graw Gill Lange.Interamericana .2012

[16]. National Research Council of the national Academics. Wide for the Care and use of animals laboratory. The National Academics Press Eighth Edition. Washington, D.C. 2010

[17]. Nelson LD .Cox M M. Lehninger. Principios de Bioquimica. 6ta Edicion Editorial OMEGA .Barcelona. España. 2015

[18]. Palacios G. M. Ayuda Ergogénica Nutricional. Federación Española de Medicina del Deporte. España. 2012.

[19]. Salabarría GJR, Santana PS. Laboratorio clínico y función renal. Madrid: Editorial EAE Académica Española. 2011

[20]. Triverdi RC, RebarL ,Berka E, Strong L. Técnica de determinación de ácido úrico. Clin. Chem. 1978; 24

[21]. Vassault A, Gafmeyer, D, et. al. Técnica para la determinación de Aspartatoaminotransferasa. Biol. Clin. 1986; 44:686.

[22]. Wiener V. Técnica de determinación de proteínas totales. Reactivos para Laboratorios Clínicos. Catálogo. Argentina. 1995:1-3.

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