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The Effects of Qualitative and Quantitative Protein Malnutrition on Electrolyte Homeokinesis in Serum of Wistar Rats

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ABSTRACT

Background**:** Proteins having structural and various regulatory functions are essential components of all unicellular and multicellular organisms. The only source of proteins and their building blocks, amino acids, for human and many animals are the proteins and amino acids in their foods. Although qualitative and quantitative protein malnutrition are common problems in animals and men, the impacts of dietary proteins on serum electrolytes are still controversial.

Materials, Methods & Results**:** Adult male Wistar rats were randomly divided into three groups of 6 or 7 animals serving as controls, and quantitative or qualitative protein malnutrition groups. Animals were held in metabolic cages individually in a conventional room with 12:12 h day/night cycle, 29°C temperature and 50-70% relative humidity. After a 10-day acclimation period controls (n = 7) were given rat chow diet consisting of 24% protein, while other groups received an almost N-free diet (quantitative malnutrition) or a diet containing 20% gelatin as protein source (qualitative malnutrition) for 35 days. Food and tap water were given animals *ad libitum* during acclimation period and throughout the experiment. At the end of experiment, blood samples were collected and Na^+, K^+ and Cl^- concentrations in serum were determined. Data were analyzed by ANOVA, ANCOVA and Pearson's correlation test. Dietary interventions had significant effects on mean body weights of animals ($P = 0.000$), but not on their food consumptions. However, in the last week controls consumed significantly more food than both malnourished groups ($P = 0.001$). If row data were used for statistical evaluation, it was seen that qualitative and quantitative protein malnutrition affected only the serum K^+, and Cl^- concentrations significantly ($P = 0.003$, $P = 0.000$). Controls had higher K^+ concentrations than those of gelatin-given group ($P = 0.002$) and N-free group had higher Cl^- concentrations than control and gelatin-given groups ($P = 0.001$). However, if body-weight corrected electrolyte values were considered, compared to controls a two-fold increase in mean concentrations of all three electrolytes in sera of both malnourished groups were seen ($P = 0.000$). There were certain negative or positive correlations between different variables interested.

Discussion**:** The findings of this study revealed that, on the basis of the row data, the dietary protein inanition increase serum Cl^- concentration, while gelatin in the diet decrease the K^+ concentration in sera of rats. Because of the body weights of malnourished animals decreased while controls continued to gain weight throughout the experiment, a substantial difference in body weights of malnourished animals and controls occurred. And, because of physiological variables are reflections of body weights, it was necessary to eliminate the possible effects of the body weights of animals. If the effect of the body weights of animals eliminated, a two-fold increase of all three electrolytes studied was seen. It was concluded that dietary proteins exert significant control on the homeokinesis of serum electrolytes. If physiopathological conditions are questioned, standardization for comparison of the results from control and experimental groups in a study as well as from different studies is indispensible.

Keywords: Dietary protein malnutrition, gelatin, N-free diet, serum electrolytes, male Wistar rats.

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INTRODUCTION

Food proteins, as only source, provide the amino acids for the synthesis of body proteins and other nitrogen-containing compounds [19]. They also have different regulatory functions in intestinal and systemic levels, and are very essential biological molecules in homeokinesis of almost all physiological processes or conditions and in survival of all forms of life [9,17,18]. For example, they provide a pathway for the transport of most molecules and all ions into and out of the cells [5]. Because proteins and amino acids are not stored within the organism in sufficient amounts, they have to be supplied continuously throughout the live. Thus, any dietary protein deprivation could be implicated in the genesis of disturbances in compositions and functions of various organs and systems of the body.

Malnutrition of different nature is still a very common health problem of men and animals. Very common forms of malnutrition are protein and calorie deficiencies [35]. Because of their widespread, several publications have concerned with the effects of malnutrition on wide-spectral life aspects in men and animals. However, information about electrolyte homeokinesis in a protein malnutrition state is gathered solely from a few clinical studies, also from data of patients with very different socio-demographic and ethiopathogenetic histories [21,27,30,36]. Although some findings indicate a possible effect of protein malnutrition on serum electrolytes [20,21,30], there are still discrepancies in this respect. Thus, information about definite protein malnutrition of quantitative or qualitative nature is still lacking.

This study aimed to investigate the effects of severe qualitative and quantitative protein malnutrition on the concentrations of certain electrolytes in sera of male rats.

MATERIALS AND METHODS

The Institutional Animal Ethics Committee approved the study. In this study, 19 adult male Wistar rats were used. The mean body weight of animals was 171 ± 19 g at the beginning of the experiment. Animals were randomly divided into 3 subgroups each consisting of 6 or 7 animals. Group I [C] served as control and was given a complete chow diet for mice and rats[1] including ca. 24% row protein (n = 7), while other two groups were given semi-synthetic diets[2], either an aproteineic or almost protein deficient diet, termed also N-free diet (group II [NF]) or a diet consisting of 20% gelatin, a protein of animal origin with lowest biological quality due to its very low contents of certain amino acids including tryptophan, as protein source (group III [G]). During the experiment, animals were held in individual metabolic cages *ad modum* Rufeger [28] in a semi-climate room with temperatures of 29 ± 1°C, a light/dark cycle of 12/12 h ($06.^{00}$-$18.^{00}$ and $18.^{00}$-$06.^{00}$), and humidity between 55-70%. Food and water were given to the animals *ad libitum*. Body weights and food consumptions of animals were recorded weekly. At the 35^{th} day of experiment, where a state of severe muscle wasting is arrived, blood was collected from tail into serum tubes under ether anesthesia between $9.^{00}$ and $11.^{00}$ h am. following a 12 h food restriction period. Serum samples were prepared and sodium (Na^+), potassium (K^+) and chloride (Cl^-) concentrations were determined by using of an ion selective electrode[3].

Data were analyzed by analyses of variance for repeated measures, one-way analysis of variance (ANOVA, for the data of the last week of experiment), covariance (ANCOVA) and Pearson's correlation tests with SPSS Version 19 for Windows®. Where the differences between groups were occurred, Tukey test was used to determine from which group they originated. Results were given as mean ± 1 SD. A probability value of < 0.05 was taken to indicate a significant difference.

RESULTS

Changes in Body Weights and Food Consumptions

Data relating to the body weights and food consumptions of animals are summarized in Figures 1 and 2, respectively. During the experimental period controls gained in mean 33% weight while malnourished animals in both groups lost ca. 34% of their initial body weights. Analyses of variance for repeated measures in factor time revealed a significant effect of dietary interventions on body weights of animals ($P = 0.000$) (Figure 1). There was also a significant interaction between diet and time in this respect ($P = 0.000$). Pairwise comparisons showed that the mean body weight of controls was significantly higher than that of both malnourished groups ($P = 0.000$).

Malnourished animals, especially those fed a diet containing 20% gelatin, refused consumption of protein deficient diets. However, analyses of variance for repeated measures in factor time showed that dietary interventions had no effect on the amount of consumed food. No interaction could also be found between diet consumption and time in this respect. However, the results of ANOVA confirmed the effect of the dietary proteins on food consumption in the last week of experiment ($P = 0.002$), and higher food consumption of controls than animals given gelatin-containing diet in the last week of experiment ($P = 0.001$). The difference between both malnourished groups could not be confirmed statistically ($P = 0.067$, 95% CI = -46.391 and 1.391) (Figure 2).

Relative food consumption, also the food consumed per g BW, was significantly affected by dietary interventions ($P = 0.012$). *Post hocs* revealed that in this case animals in N-free group consumed significantly more food than both controls and gelatin given animals ($P = 0.012$ and $P = 0.046$, respectively).

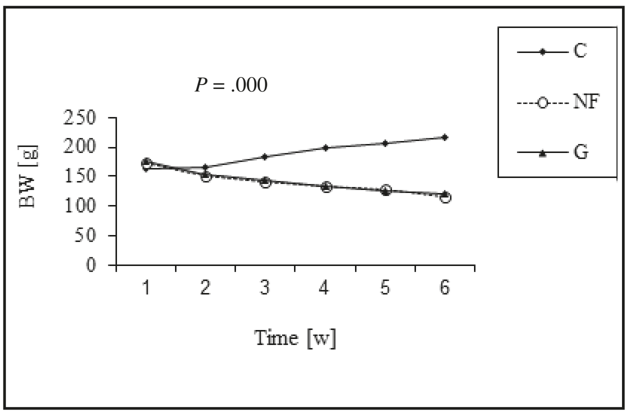

Figure 1. Mean body weight changes of animals fed diets with or without protein malnutrition.

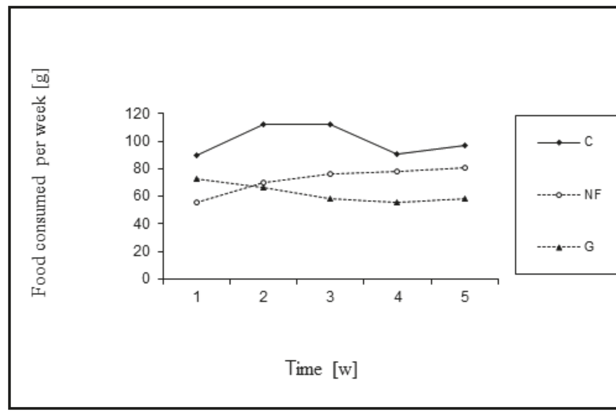

Figure 2. Mean food consumptions of animals given adequate or protein deficient diets.

Changes in Electrolyte Concentrations

The mean concentrations of electrolytes in serum samples from rats with or without protein malnutrition are summarized in Table 1. Evaluation of the row data revealed that dietary interventions had no effect on the serum concentrations of sodium. However, it influenced the concentrations of potassium and chloride in sera of rats ($P = 0.003$ and $P = 0.000$, respectively). *Post hocs* revealed that the mean serum potassium concentration of controls was significantly higher than that of animals given gelatin-containing diet ($P = 0.002$). The mean potassium concentration in serum of N-free group was also ca. 8% higher than that of gelatin group, but the difference between both malnourished groups was not confirmed statistically ($P = 0.052$, 95% CI = -.695 and -.00214). Besides, the mean chloride concentration of N-free group was significantly higher than that of both controls and gelatin-given group ($P = 0.001$).

Changes in Concentrations of Electrolytes in Relation to Body-Weights and Food Consumptions

Earlier studies suggested that physiological variables are related to the body weights of animals, and their changes could, at least partly, be a result of concomitant changes in body weights of animals seen during protein malnutrition [16-18]. In this study, a significant negative correlation was found between body weights of animals and only serum Cl^- concentrations among electrolytes studied (r = -.348, $P = 0.022$), while, although not significantly, Na^+ and K^+ concentrations correlated positively with body weights. There were also significant correlations between different variables interested (Table 2). A moderate, but significant negative relationship between chloride concentration with concentrations of total protein (TP) and albumin (ALB) in serum (data are not shown) was notable. On the other side, serum TP, ALB and globulin (GLOB) concentrations correlated positively with the body weights of animals (Table 2).

Table 1. Body weights, calculated daily food consumptions (DFC) and serum electrolyte values of Wistar rats with or without severe qualitative or quantitative protein malnutrition.

Variable	Group I [C] [n = 7] $\bar{X} \pm SD$ [Min - Max]	Group II [NF] [n = 6] $\bar{X} \pm SD$ [Min - Max]	Group III [G] [n = 6] $\bar{X} \pm SD$ [Min - Max]	*P* =
BW-35 [g]	217 ± 25[a] [187 - 249]	116 ± 15[b] [98 - 134]	120 ± 9[b] [109 - 135]	.001
DFC [g]	13.88 ± 1.66[a] [11.43 - 15.43]	11.55 ± 2.68[ab] [8.86 - 14.86]	8.33 ± 2.51[b] [4.86 - 11.43]	.001
Na^+ [mmol/L]	142.28 ± 1.09 [140.00 - 143.50]	141.14 ± 3.01 [137.10 - 145.60]	141.04 ± 2.08 [138.70 - 144.70]	n.s.
K^+ [mmol/L]	4.82 ± 0.22[a] [4.57 - 5.19]	4.62 ± 0.11[a] [4.50 - 4.78]	4.27 ± 0.33[b] [3.88 - 4.65]	.002
Cl^- [mmol/L]	102.60 ± 1.29[a] [100.90 - 104.50]	106.30 ± 1.69[b] [103.70 - 108.60]	102.66 ± 1.38[a] [100.60 - 104.30]	.001

Table 2. Correlation coefficients and two-tailed *p*-values for the body weight [BW in g], food consumption (FC in g), and serum total protein, albumin and globulin concentrations and electrolyte values (Na, K, Cl) of male Wistar rats.

Variable		BW	FC	TP	Alb	Glob	Na	K	Cl
FC	r =	.486							
	P =	.001							
TP	r =	.766	.188						
	P =	.000	.226						
ALB	r =	.659	.018	.788					
	P =	.000	.911	.000					
GLOB	r =	.521	.281	.757	.193				
	P =	.000	.068	.000	.215				
Na^+	r =	.154	.081	.221	.168	.174			
	P =	.325	.605	.154	.282	.264			
K^+	r =	.126	-.128	.206	-.048	.378	-.192		
	P =	.419	.412	.186	.760	.012	.217		
Cl^-	r =	-.348	.145	-.499	-.522	-.240	-.090	.210	
	P =	.022	.354	.001	.000	.120	.567	.176	

Because the body weights of all malnourished animals decreased and those of controls increased markedly during this experiment, a great difference between the mean body weights of control and experimental animals occurred in this respect. Similarly, compared to rat chow, consumption of qualitatively or quantitatively protein deficient diets were reduced profoundly. Thus, the necessity has arisen to eliminate the possible effects of the body weights and amount of daily food intake on serum electrolytes in the evaluating of the data. Table 3 summarizes the relative mean concentrations of electrolytes in serum; also concentrations of electrolytes as mmol per g body weight.

Table 3. Relative food consumptions (RFC) and serum electrolyte values of Wistar rats with or without severe qualitative or quantitative protein malnutrition.

Variable	Group I [C] [n = 7] $\bar{X} \pm SD$ [Min - Max]	Group II [NF] [n = 6] $\bar{X} \pm SD$ [Min - Max]	Group III [G] [n = 6] $\bar{X} \pm SD$ [Min - Max]	P =
RFC	0.45 ± 0.06[a]	0.71 ± 0.17[b]	0.49 ± 0.17[a]	.0012 0.046
Na^+ [mmol/L]	0.66 ± 0.08[a] [0.57 - 0.76]	1.24 ± 0.16[b] [1.09 - 1.46]	1.18 ± 0.09[b] [1.05 - 1.28]	.000
K^+ [mmol/L]	0.02 ± 0.002[a] [0.02 - 0.03]	0.04 ± 0.005[b] [0.03 - 0.05]	0.04 ± 0.002[c] [0.03 - 0.04]	.000
Cl^- [mmol/L]	0.048 ± 0.06[a] [0.41 - 0.55]	0.93 ± 0.13[b] [0.81 - 1.09]	0.86 ± 0.06[b] [0.75 - 0.93]	.000

[*]Significantly different from all experimental groups ($P < .001$).

If the relative concentrations of electrolytes were considered, all three electrolytes were significantly affected by dietary interventions ($P = 0.000$). *Post hocs* revealed that the mean serum Na^+, K^+ and Cl^- concentrations of both malnourished groups were significantly higher than that of controls ($P = 0.000$). No significant difference could be found between malnourished groups for Na^+ and Cl^-. Also, the higher K^+ concentration of N-free group than the gelatin-given group was not confirmed statistically ($P = 0.054$, 95% CI = -.00008 and .00991).

The results of ANCOVA indicated that both the body weights of animals and their food consumption had no effect on relative Na^+ concentrations in serum of rats. The interactions between BWs and the dietary intervention as well as those among dietary intervention, BWs and food intake could also not be confirmed statistically ($P = 0.070$, F = 3.990 and $P = 0.094$, F = 3.379). However, the effect of dietary intervention on the relative concentrations of K^+ and Cl^- in serum was significant if the possible effects of BWs and food consumptions are eliminated ($P = 0.039$ and $P = 0.013$, respectively). Pairwise comparisons showed that relative K^+ and Cl^- concentrations were higher in sera of N-free group than those of controls and animals given gelatin-containing diet ($P = 0.013$ and $P = 0.004$, respectively). No interactions could be found between the dietary interventions and BWs and food consumptions of animals ($P > 0.05$). Similarly, whether the effect of food consumed ($P = 0.098$, F = 3.635), nor the interactions between BWs and food consumptions and the dietary interventions could be confirmed statistically ($P = 0.097$, F = 3.678 and $P = 0.098$, F = 3.298). By contrast, the effects of BWs of animals on serum Cl^- concentrations proved to be significant ($P = 0.042$). However, the effects of food consumptions and the interaction between BWs and food consumptions could not be confirmed ($P = 0.053$, F = 5.391 and $P = 0.062$, F = 4.922, respectively).

If the body weights-corrected data were used for correlations it was seen that the relationships between different variables would be clearer. In this case, all three electrolytes were also found to be highly and negatively correlated with body weights of animals (Table 4).

Table 4. Correlation coefficients and two-tailed *p*-values for the body weight [BW in g], relative [corrected, c] food consumptions, and serum total protein, albumin and globulin and electrolyte concentrations of Wistar rats.

Variable		BW	DFC	c-DFC	c-TP	c-Alb	c-Glo	c-Na	c-K
DFC	r =	.586							
	p =	.008							
c-DFC	r =	-.512	.382						
	p =	.025	.107						
c-TP	r =	-.932	-.577	.422					
	p =	.000	.010	.072					
c-ALB	r =	-.927	-.695	.319	.965				
	p =	.000	.001	.184	.000				
c-GLOB	r =	.886	.486	-.543	-.692	-.748			
	p =	.000	.035	.016	.001	.000			
c-Na	r =	-.978	-.528	.570	.923	.925	-.906		
	p =	.000	.020	.011	.000	.000	.000		
c-K	r =	-.948	-.488	.583	.870	.869	-.893	.964	
	p =	.000	.034	.009	.000	.000	.000	.000	
c-Cl	r =	-.973	-.507	.589	.904	.906	-.916	.996	.977
	p =	.000	.027	.008	.000	.000	.000	.000	.000

Table 5. Body weight-corrected serum electrolyte values of Wistar rats with or without severe qualitative or quantitative protein malnutrition for 35 days.

	Group I [C] [n = 7]	Group II [NF] [n = 6]	Group III [G] [n = 6]
Variable	$\bar{X} \pm SD$ [Min - Max]	$\bar{X} \pm SD$ [Min - Max]	$\bar{X} \pm SD$ [Min - Max]
Na^+ [mmol/l]	0.65 ± 0.07 * [0.57 - 0.76]	1.26 ± 0.17 [1.09 - 1.46]	1.17 ± 0.10 [1.05 - 1.28]
K^+ [mmol/l]	0.02 ± 0.002* [0.02 - 0.03]	0.04 ± 0.006 [0.03 - 0.05]	0.04 ± 0.002 [0.03 - 0.04]
Cl^- [mmol/l]	0.47 ± 0.05* [0.41 - 0.55]	0.95 ± 0.13 [0.81 - 1.09]	0.85 ± 0.07 [0.75 - 0.93]

*Significantly different from all experimental groups ($P < .001$).

DISCUSSION

If proteins and their building blocks, amino acids, play a role in electrolyte metabolism and homeokinesis, changes in their concentrations in diet and plasma could theoretically be related with changes in electrolyte concentrations in circulating blood.

In this study, animals fed diets qualitatively or quantitatively deficient of proteins lost their initial body weights up to 34% with concomitant reduction of food consumptions during the experiment. Although animals in control group consumed more food than both malnourished groups, relative food consumption was significantly higher in N-free group than other two groups. These results relating to the body weights and food consumptions of animals, at the basis of raw data, are in agreement with preliminary literature, in general [1,12,29,32].

Several studies suggested that changes in different variables during protein malnutrition of qualitative or quantitative nature are more or less reflections of changes in body weights of animals [1,12,29]. The status of serum electrolyte concentrations differed also depending on raw or body-weight corrected data (Table 1 and Table 5, respectively). By considering raw data, the effects of dietary interventions on serum sodium concentrations was not statistically confirmed, while the concentrations of potassium and chloride were influenced remarkably ($P = 0.003$ and $P = 0.000$, respectively). Compared to controls, mean serum potassium concentration of animals given gelatin-containing diet was decreased ($P = 0.002$). In contrast, N-free nutrition increased the chloride concentration of animals significantly if compared to both controls and gelatin-given group ($P = 0.001$).

Related preliminary data orginate mainly from clinical studies on human beings with very different etiopathogenesis and are very discrepant in nature. Serum electrolytes of children and adults were found to be within normal ranges in some clinical studies [16,26,36]. Also, Howard *et al*. [10] suggested that fasting and low calorie-protein diet do not affect serum electrolyte concentrations. In contrary, Olowonyo *et al*. [21] found that serum Na^+ and K^+ concentrations of children with kwashiorkor were lower than those of their age-matched controls. Similar findings were also reported for children with cystic fibrosis [14]. Said *et al*. [30] found that plasma Na^+ concentrations were lower in all protein-calorie malnutrition cases except marasmic kwashiorkor, whereas plasma K^+ concentration was markedly lower than normal in all groups of malnourished subjects except those in 3rd grade marasmus, which revealed significantly higher plasma K^+ concentrations. Similarly, Patrick [22] reported significant increases in sodium and potassium concentrations in leukocytes of malnourished children in the presence of edema, but in marasmus sodium was increased while potassium decreased.

Experimental studies on animals have also given not always uniform results. Serum concentrations of sodium and potassium were significantly less in rats fed a low-protein diet than controls [36]. However, diets with protein contents varying between 8.2% and 44.4% did not influence serum electrolyte concentrations of dogs [24]. And, Pond *et al*. [25] observed significant decreases only in serum P, Ca and Mg concentrations of severely protein malnourished pigs in the absence of parasites, diarrhea and infection. Fiorotto *et al*. [7] suggested that after chronic protein deprivation no retention of Na and Cl was occurred until severe hypoalbuminemia and edema developed; at this time, however, Na and Cl were retained in extracellular fluid (ECF) in the same proportion as their molar ratios. Closa *et al*. [6] found an increase in intracellular Na (Na_i) concentrations and lower K_i/K_e, Na_e/K_e and K_i/Na_i ratios in skeletal muscles of rats fed an imbalanced protein diet containing low level of available lysin up to 50 days of age compared to corresponding control group fed a low protein balanced diet, but these differences were not seen in animals fed the same diets up to 90 days.

There are some evidences indicating that in protein deprivation an electrolyte influx into cells occurs in man and animals [22,32]. There was an increase in membrane leak to electrolytes in kwashiorkor resulting in increased Na and decreased K concentrations of the cells [8,11,22]. Possible, the severity and characteristics of malnutrition determines the influx of electrolytes between intracellular and extracellular spaces. Then, in a study the state of edema levels of protein malnourished children was associated with the intracellular electrolyte concentration, whereas the loss of edema was associated with its reduction [22].

In contrast to human beings, rats have long been considered as resistant to development of edema in case of a severe protein and/or calorie malnutrition [8], despite edema was reported also in rats rarely [7].

No signs of edema could be observed also in this study using almost protein free or gelatin-containing diets. If the row data are taken into consideration, dietary proteins seem to play an important role in the maintenance of serum potassium and chloride, but not in sodium homeokinesis (Table 1). However, if the concentrations of electrolytes were related to body weights of animals, a two-fold increase in concentrations of all electrolytes measured was seen. This increase was consistent for both qualitative and quantitative malnutrition (Table 3). In the case of concerning row data, the results of the present study support in great extent the findings of Vis *et al*. [36], Howard *et al*. [10] and Mauron & Antener [16].

Since serum electrolyte consentrations are so stringently regulated by a number of factors including neuro-endocrine system and renal functions [33] and intestinal microbiota [34], and qualitative or quantitative protein malnutrition affect among others the neuro-endocrine system and kidneys [4,13,15,23,28,31,37], and intestinal microbiota [3] as well, several mechanisms other than mentioned above could intervene with the changes in plasma sodium, potassium and chloride concentrations during a dietary protein and/or calorie malnutrition. This study examined not the neuro-endocrine and renal adaptations to qualitative and quantitative protein malnutrition. Thus, the exact mechanisms need still to be clarified *via* further detailed studies.

One of the main strategies in the treatment of malnourisheds is the correction of fluid and electrolyte imbalances. Both the raw and corrected data of this study revealed significant changes in electrolyte consentrations in serum of rats. However, corrected data seem to be most consistent, and could therefore be taken in consideration during the managment of malnourished patients.

CONCLUSIONS

The results of the present study indicate a very important role of dietary proteins in the homeokinesis of the electrolytes such as Na^+, K^+, and Cl^- in blood serum. However, the mechanisms remain still unclear. Furthermore, the results indicate that the methodical standardization is inevitable necessary to compare the data of control and experimental groups as well as the results of different studies especially when the data originate from healthy and disturbed subjects. These aspects neglect further study.

SOURCES AND MANUFACTURERS

[1]Complete chow diet for mice and rats: Best Yem, Gebze, Turkey.

[2]Almost N-free (Protein-free) and Gelatin-Containing foods: Altromine®, Lage, Germany.

[3]Ion-Selective Electrode: EasyLyte Plus®, Medica. Bedford, USA.

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