Blood pressure, heart rate and its autonomic modulation in malnourished rats at different ages

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ABSTRACT. Malnutrition is responsible for a number of alterations that occur in the organism, including alterations in the cardiovascular system. We hypothesize that long periods of malnutrition occurring after weaning may also be responsible for alterations in the cardiovascular system. Wistar rats were separated into the two following experimental groups: a labina® diet-fed control group (CG) and a malnourished group (MG), fed a multi-deficient diet. Results showed that the mg presented significantly lower body weight (p < 0.001) in rats of all ages, higher heart rate (HR) (p < 0.05) when rats were 3, 4 and 5 months old and lower HR in rats from 6 to 9 months of age, in relation to the CG. Spectral analysis showed a significant increase in the low frequencies (LF) components in the MG compared to the CG from the third month onwards (p < 0.05), showing evidence of sympathetic predominance greater cardiac autonomic balance in the MG versus that of CG indicated that, although aging influences autonomic behavior, it may also be altered by nutrition. Morphologically, an increase in the ventricle weight/body weight ratio was seen in the MG. These results show that the hearts of rats in the MG were not spared from malnutrition age-related detrimental cardiac effects associated with malnutrition.

Keywords: aging, multi-deficient diet, electrocardiography, spectral analysis, morphology of the ventricles.

Introduction

Malnutrition is a complex phenomenon caused by various etiologies (OLIVARES et al., 2005) and is responsible for a number of alterations that occur in the organism, including alterations in the cardiovascular system. Various authors have reported an association between malnutrition during the pre-natal period and the appearance of cardiovascular diseases in adult life in humans (BARKER, 1998; BARKER, 2004; VIJAYAKUMAR et al., 1995); and experimental animals (LANGLEY-EVANS et al., 1994; MONTEIRO et al., 2001; PAIXÃO et al., 2001; SILVA et al., 2011; TONKISS et al., 1998; VIEIRA FILHO et al., 2014). Diverse studies have shown that malnutrition, not only during the fetal period, but even during other
periods of development, may be responsible for alterations in the cardiovascular system, such as the following: increase in blood pressure (MURÇA et al., 2012; OLIVEIRA et al., 2004) and heart rate (OLIVEIRA et al., 2004), electrophysiological alterations (OLIVARES et al., 2005; PISSAIA et al., 1980; SARAIVA et al., 1992), morphological and histological modifications of the heart (PISSAIA et al., 1980), change in the contractile function of the myocardium (NUTTER et al., 1979; PINOTTI et al., 2010) and maybe even alterations in the regulatory mechanisms of the cardiovascular system as, for example, in the autonomic nervous system (ANS) (PENITENTE et al., 2007; TROPIA et al., 2001). The principal cause of chronic malnutrition is the socioeconomic status of an individual; however, it may also arise when adequate nutrition is available in qualitative terms, if associated with diseases like cystic fibrosis, chronic kidney disease, Aids and alcoholism, among others (OLIVEIRA et al., 2004), or even due to voluntary dietary restriction for various reasons, including psychological reasons such as anorexia nervosa (SHETTY, 2006). Few studies have investigated malnutrition using a protocol of undernourishment for prolonged periods. We believe that malnutrition occurring after weaning, especially for long periods, can also be responsible for changes in the cardiovascular system. Thus, the aim of this study was to examine heart rate (HR), systolic blood pressure (SBP), and spectral analysis of the HR of rats subjected to malnutrition from 21 days to 9 months of age, seeking to compare the effects of malnutrition on the process of aging and the mechanism of cardiovascular disease.

Material and methods

Animals

Male Wistar rats were maintained in cages in a room with an ambient temperature of ±21°C under adequate experimental conditions, including a controlled climate, an adequate air circulation system and a photoperiod that provided 12 of light and 12 hours of darkness. Previously, female breeding rats were mated with male rats and monitored daily throughout gestation and suckling phase. During these phases, the female breeding rats were fed with a standard Labina® diet. Offspring were weaned at 21 days of life, and only males were chosen for further research. Male rats were separated into two experimental groups: a control group (CG, n = 32) with rats fed the standard Labina® diet, and another group, malnourished (MG, n = 31), with rats fed a multi-deficient diet, from 21 days (weaning) up until 9 months of age. Both groups received their diet and water ad libitum.

Body weight and SBP (recorded indirectly) were measured and electrocardiograms (ECG) performed weekly in rats from both groups, in accordance with the procedures described below. SBP behavior, spectral analysis of HR variability and changes in heart morphology were examined.

Ethical considerations

All experimental procedures involving animals in this study were approved by the Ethics Committee for Animal Experimentation of the Center of Biological Sciences of the Federal University of Pernambuco and were carried out in accordance with the Committee’s guidelines.

Diet

Malnutrition was induced through a deficient diet as previously described (TEODÓSIO et al., 1990). The diet (g g⁻¹) was comprised of beans — *Phaseolus vulgaris* (18.34), manioc flour — *Manihot esculenta* (64.81), beef jerky (3.74) and sweet potato — *Ipomea batatas* (12.76). They were cooked, then dehydrated at 60°C and, finally, pulverized. All components were mixed with water. Meat fat (0.35) was added and the mixture was shaped into pellets that were dehydrated at 60°C for 24 hours. The contents of the main nutrients (g g⁻¹), as determined by the Pernambuco Federal University’s Laboratory for Food Experimentation and Analysis, are shown in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>LABINA®</th>
<th>Multideficient®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>20.5</td>
<td>8.59</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>56.35</td>
<td>81.52</td>
</tr>
<tr>
<td>Lipids</td>
<td>6.18</td>
<td>1.72</td>
</tr>
<tr>
<td>Vitamin suplement</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.110</td>
<td>0.109</td>
</tr>
<tr>
<td>Ashes</td>
<td>7.09</td>
<td>3.68</td>
</tr>
<tr>
<td>Kcal</td>
<td>363.02</td>
<td>375.00</td>
</tr>
</tbody>
</table>

Table 1. Diet composition (g g⁻¹).

As indicated by the manufacturer (Purina Agrícola, Paulinia, São Paulo State, Brazil).

According to the Laboratory for Food Experimentation and Analysis of the Nutrition Department at the Federal University of Pernambuco.

Measurement of systolic blood pressure

SBP was measured by the indirect method and measurements were taken weekly by means of a tail-cuff plethysmograph (Model ITTC 31, Woodland Hills, CA - USA) using software supplied by the manufacturer. For this purpose, the animals were confined and simultaneously acclimated by warming them for 3 to 5 min. to a temperature of 37°C (through a 100 W bulb placed in the upper
Heart rate and malnourished

A previously calibrated sensor was placed in the region near the tail to obtain SBP values. The instantaneous values of this parameter were recorded in files and later analyzed. On average, 3 measurements were performed on each animal per week, taken 5 after heating and at 3 min. intervals, for a total of 3 measurements per animal. The individual values for each animal were calculated through the mathematical average of the measurements made. In our study, animals from both groups were evaluated at 3, 5, 7, and 9 months of age.

Electrocardiogram and heart rate

ECG recordings were obtained using a non-invasive method in accordance with a methodology adapted by our laboratory (COLUGNATI et al., 2005). In this procedure, non-anesthetized animals were placed in a polyethylene box in a resting position supporting their paws on electrodes (silver rods) located on the floor of the box in which the animals were kept. The ECG signal was picked up, amplified and recorded in a computer using BioMed® data acquisition software system (João Pessoa, Paraíba State Brazil). After placement of the animal into the box, there was a period of approx. 5 min. to allow the animal to adapt to its surroundings. Then 3 ECG recordings of 1 min. each were taken per animal, in the DI derivation, at intervals of 2 min., for approximately 10 min. total. Records of at least 20 consecutive cycles were considered for the analysis. HR was quantified through analysis of the RR interval (RRi). In our study, animals from both groups were evaluated from 3 to 9 months of age. In order to assess HR responses (relatives values), the difference of each HR measurement was calculated in reference to the baseline, i.e. that of the third month.

Spectral analysis of the heart rate

The good signal quality obtained from the ECG made it possible for us to study HR variability in the animals, to evaluate the role of the autonomic nervous system (ANS) in the modulation of the cardiac autonomic tonus.

To undertake spectral analysis, we used the software developed in the Pre-Clinical Science Department, University of Milan - Italy, which uses the autoregressive model for analysis in the frequency domain of the spectral components. Each heart beat was identified through the use of an algorithm implemented in the BioMed® system, which automatically detected the RRi of the ECG. After the automatic reading, the recording was visually inspected to identify and/or correct any incorrect markings. Then, the temporal series of the cardiac interval (tachogram) was created. When necessary, we used an interpolation of the tachogram series to remove undesired distortions. The data were stored in files and used later for determination of the spectral analysis. The band for the frequency of interest for spectral analysis in rats is found in the interval from 0 to 3 Hz.

The spectral power was integrated into 3 frequency bands of interest: high frequencies (HF) between 0.8 and 3 Hz, low frequencies (LF) between 0.2 and 0.8 Hz, and very low frequencies (VLF), less than 0.2 Hz. The sympathetic-vagal or autonomic balance was also calculated by determination of the ratio between the components of LF and HF (PAGANI et al., 1986). In our study, animals from both groups were evaluated at 3, 5, 7 and 9 months of age.

Morphology

Animals were anesthetized with 60 mg kg⁻¹ sodium pentobarbital (Cristália Produtos Químicos Farmacêuticos, Itapira, São Paulo State, Brazil) by intraperitoneal (IP) injection. The right femoral vein was dissected and animals perfused with saline solution (0.9%) and subsequently killed by overdose of anesthetic. The heart was removed, washed with saline solution, dried on filter paper and weighed for calculation of wet weight (wW) using a digital scale (model AL204, METTLER-TOLEDO GmbH, Greifensee, Switzerland) with precision of 0.1mg. For measurement of dry weight (dW), the ventricles (v) were dehydrated in a laboratory drying and sterilizing oven (FANEM- Model 315SE, São Paulo State, Brazil), which was maintained at 70ºC for 48 hours. The wW coefficient of the ventricle (g) body⁻¹ weight (g) x 100 (wW bW⁻¹ x 100) was calculated in all cases. The water content of the tissues was evaluated by the dW wW⁻¹ ratio of the ventricles. Three animals from each group (CG and MG) were used for each reading.

Statistical analysis

Statistical analysis of weight gain in rats from both experimental groups (CG and MG) at all ages studied and comparative analysis of the cardiovascular parameters examined were performed using one-way ANOVA followed by Dunnett test and two-way ANOVA followed by the Bonferroni...
test. The results are expressed in mean ± SEM and relatives values. Values of p < 0.05 were considered statistically significant.

Results

Weight gain

Analysis of changes in weight gain (Figure 1) in our study showed that, with the exception of rats at weaning (CG: 46 ± 1 g vs. MG: 44 ± 1 g), rats from the MG weighed significantly less at all ages when compared to rats from the CG (two-way ANOVA p < 0.001).

Figure 1. Evolution of weight of Control Group (CG) and Malnourished Group (MG) with age. The values are mean ± SEM. Statistical analyses were performed by two-way ANOVA followed by Bonferroni test. *p < 0.001 compared with CG, w = weaning.

Systolic blood pressure

SBP levels did not present any statistically-significant difference between the groups at any age two-way ANOVA; p > 0.05). The values for rats from the CG and MG at 3, 5, 7, and 9 months were: 128.6 ± 1.89 (n = 32) and 129.5 ± 2.17 mm Hg (n = 32); 124.0 ± 1.35 (n = 24) and 127.2 ± 1.6 mm Hg (n = 24); 122.5 ± 1.96 (n = 16) and 118.5 ± 0.95 mm Hg (n = 16); 125.0 ± 4.54 (n = 8) and 116.7 ± 0.54 mm Hg (n = 8), respectively. However, the MG presented a significant reduction in SBP at advanced age (one-way ANOVA, p < 0.05).

Heart rate

HR levels and their responses are shown in Figures 2 and 3, respectively. HR was significantly higher in the MG compared to the CG for rats at 3, 4, and 5 months of age (two-way ANOVA, p < 0.05). Similar to what was observed for SBP, the MG presented a significant reduction of HR with advanced age (one-way ANOVA, p < 0.05).

HR responses were significantly decreased in the MG compared to the CG for 5-, 6-, 7-, 8-, and 9-month-old rats (4 ± 2 bpm vs. 3 ± 2 bpm, -3 ± 2 bpm vs. 3 ± 2 bpm and -6 ± 2 bpm vs. 3 ± 2 bpm, respectively; p = 0.01 for all comparisons).

Spectral analysis

Values of components of the spectral analysis are shown in Table 2. The normalized LF component (nu) was significantly greater (p < 0.05) in the MG when rats were 3, 5, and 7 months old. In addition, for both the CG and the MG, the normalized LF components increased with advanced age, although a significant difference was not observed in rats between the ages of 7 and 9 months. The normalized HF component was significantly less in the MG (p < 0.05) in 3-, 5-, and 7-month-old rats. The normalized HF component was reduced in both the CG and the MG with advanced age (one-way ANOVA, p < 0.05).

The cardiac autonomic balance (LF HF−1) was significantly greater in the MG compared to the CG when rats were 3, 5, and 7 months old, but not in 9-month-old rats (two-way ANOVA, p < 0.05), showing predominance of sympathetic tonus.
Table 2. Spectral Analysis of the Heart Rate at different ages in Control Group (CG) and Malnourished Group (MG).

<table>
<thead>
<tr>
<th>Ranges</th>
<th>CG (n = 3)</th>
<th>MG (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF (ms²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.23 ± 0.04</td>
<td>0.49 ± 0.00*</td>
</tr>
<tr>
<td>5</td>
<td>0.38 ± 0.03</td>
<td>0.71 ± 0.04*</td>
</tr>
<tr>
<td>7</td>
<td>0.67 ± 0.06*</td>
<td>1.13 ± 0.05*</td>
</tr>
<tr>
<td>9</td>
<td>1.52 ± 0.13*</td>
<td>1.15 ± 0.19*</td>
</tr>
<tr>
<td>LF HF-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
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</tbody>
</table>

Morphological analysis of the heart

The vwW bW⁻¹ ratio was significantly greater in the MG at all ages examined (Table 3, two-way ANOVA, p < 0.05). In the ventricles, the dW wW⁻¹ ratio was not statistically different between the groups at any of the ages studied.

Table 3. Ratio between ventricle wet weight/body weight (vwW bw⁻¹) and ventricle dry weight/ventricle wet weight (vdW vwW⁻¹) in the Control Group (CG) and Malnourished Group (MG).

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>CG (n = 3)</th>
<th>MG (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.242 ± 0.0247</td>
<td>0.228 ± 0.0038</td>
</tr>
<tr>
<td>5</td>
<td>0.202 ± 0.0127</td>
<td>0.212 ± 0.0034</td>
</tr>
<tr>
<td>7</td>
<td>0.202 ± 0.0070</td>
<td>0.212 ± 0.0024</td>
</tr>
<tr>
<td>9</td>
<td>0.243 ± 0.0167</td>
<td>0.2141 ± 0.0060</td>
</tr>
</tbody>
</table>

Discussion

Literature relating malnutrition induced by a multideficient diet to cardiovascular alterations in rats of different ages is scarce. In addition to malnutrition, aging also produces structural and functional changes in the cardiovascular system, such as loss of elasticity of the arteries and increased blood pressure. The use of a multideficient diet as an experimental model of malnutrition aids in the study of some biological aspects of nutrition in the population of Northern Brazil. The experimental model used in this paper is based on a previously described multideficient diet (TEODOSIO et al., 1990). The diet presently used is deficient in several important nutrients, different from other undernourishment models, such as reduction of protein content (OLIVEIRA et al., 2004; PENITENTE et al., 2007; TROPIA et al., 2001) or quantitative restriction using balanced diets (OKOSHI et al., 2001; SUGIZAKI et al., 2005). In the present study, the results show that an extended period of malnutrition (9 months) in rats does not cause hypertension, but physiological and morphological aspects of the heart do suffer adverse effects. In the MG, HR differed in two ways. It was elevated in relation to the CG when rats were 3, 4, and 5 months old, and it was significantly reduced in the MG as the malnourished rats grew older (at 6, 7 and 8 months of age).

A great deal of epidemiological and experimental evidence exists regarding the involvement of fetal malnutrition as the origin of various cardiovascular diseases, such as hypertension, in adult life (BARKER, 1990; LANGLEY-EVANS et al., 1994; SUGIZAKI et al., 2005; TONKISS et al., 1998), suggesting that the alterations found in the cardiovascular system may be related to the stage of development in which undernourishment occurs. Clinical and experimental studies that have investigated the effects of post-natal malnutrition on hemodynamic variables are controversial. Rats submitted to malnutrition after weaning did not show differences in SBP and HR compared to normally nourished rats (BARKER, 1992; BENABE et al., 1993; FIOROTTI et al., 2002; TROPIA et al., 2001). Similar results were also seen in studies with humans (OLIVARES et al., 2005). In these studies, nevertheless, chronic malnutrition, represented by the clinical state of Kwashiokor and marasmus, led to hypotension and bradycardia (OKOSHI et al., 2004; TALNER, 1990). Thus, in our malnutrition model, in which malnutrition occurred throughout the life of the animal (9 months), it is likely the chronic nature of the malnutrition is responsible for modifications in SBP and HR due to age in the MG compared to the CG. Although the age-dependent decrease in SBP and HR we observed may not be characterized as a hypotensive and bradycardic state, as seen in cases of Kwashiokor and marasmus, it showed a trend in the same direction, i.e., reduction in SBP and HR. Thus, because these parameters reflect cardiac function, in all probability, this diet significantly influences performance of the heart. In the CG, however, no age-related difference in HR and SBP was observed. These results are in agreement with those of a previous study (GROVER; EE, 2009), which did not observe differences between the levels of mean arterial pressure (MAP) and HR between young rats (70 days) and old rats (24 months).

Modifications in nutrient intake may have an important influence on the central nervous system (CNS), which in turn exerts an important influence
on regulation of the cardiovascular system (TROPIA et al., 2001), as the latter is modulated by both the nervous system and hormonal regulation (PENITENTE et al., 2007). Studies on rats fed a low-protein diet (7%) showed an increase in sympathetic nervous system (SNS) activity in these animals (IRIGOYEN et al., 2000). Another study showed that undernourishment in Fisher rats, induced by a low-protein diet (6% of protein), resulted in greater variability of HR and MAP when compared to rats fed a normal diet (OLIVEIRA et al., 2004), suggesting an increase in sympathetic tone.

The high quality of the ECG signals obtained in our study permitted us to examine HR variability in these animals. In our spectral analysis of the HR, we found an increase in the LF component and an increase in autonomic balance in the MG, indicating greater sympathetic activity in rats at 3, 5 and 7 months of age. According to Kaufman et al. (1986), it is accepted by many authors that an increase in the LF component occurs along with, and is considered a marker for, an increase in SNS activity. According to these findings and reports in the literature, we suggest that the increase in the LF component in the MG maybe related to changes in the SNS.

Based on these results, we expected that an increase in sympathetic activity would lead to elevated SBP and HR in the MG when compared to the CG. However, because HR only increased in rats between 3 and 5 months of age, and decreased after wards along with the SBP, a response of the adrenergic receptors to increased sympathetic activity may have occurred in the 3rd to the 5th month. Evidence of adrenergic receptor involvement comes from studies showing preserved (OKOSHI et al., 2001) or increased (DROTT et al., 1986; YOUNG et al., 1985) beta-adrenergic responsiveness. In general, this information provides evidence that SBP and HR levels may not be influenced by autonomic regulation as consequence of modifications either in the expression or in the sensitivity of the adrenergic receptors resulting from the nutritional state. These findings suggest that in the MG, in addition to modifications arising from aging, malnutrition also influences cardiac autonomic behavior.

According to Notarius and Floras (2001), the predominance of the LF component in the spectral analysis of the HR has been observed in patients with class II Congestive Heart Failure (CHF) (NYHA), but it diminishes or becomes absent in classes III and IV. This reduction, however, does not necessarily represent a reduction in sympathetic activity in advanced CHF which is accompanied by sympathetic hyperactivity, as observed by Negrão et al. (2001), by means of the microneurography of the peroneal nerve, and high levels of circulating catecholamines found in these patients as observed by La Rovere et al. (2003). A state of CHF in malnourished animals is not strange, since some studies on the effects of malnutrition in humans reported CHF and cardiomyopathies in malnourished children (MAISCH et al., 2002; TALNER, 1990). Thus, the behavior of the sympathetic activity, through the spectral analysis of the HR in malnourished animals in the present study, is similar to the autonomic state found in CHF.

Modifications in the autonomic modulation of rats from the CG were also observed. An increase in the LF component was observed as a function of age; however, these modifications are similar to those seen in response to aging, as previously described (KLEBANOV et al., 1997). However, the increase in the LF component is often not accompanied by alterations in the SBP and HR, as it has been shown that responsiveness to beta-adrenergic stimuli in the heart decreases with age (NARKIEWICZ et al., 2005). Another interesting finding was that SBP values were maintained in the MG even with oscillations in HR present (Figures 1 and 2), suggesting that peripheral vascular resistance may influence cardiac behavior and the possible involvement of other systems like the renin-angiotensin system (RAS).

Data in the literature in relation to the preservation of the cardiac muscle in malnutrition are conflicting. According to Fioretto et al. (2002), the heart weight/body weight ratio may vary in accordance with the duration and model of undernourishment adopted. Thus, for a normal individual submitted to a period of food privation for a short time, the heart may be spared, relatively speaking, from undernourishment due to the catabolism which occurs in other tissues, such as the adipose tissue and the glycogen reserves; whereas in chronic calorie-proteinic undernourishment, due to the inhibited growth of the animal, the heart will never reach its normal size.

For other authors, the heart weight/body weight ratio reflects a cardiac function; for Talner (1990), if the demand is increased, as occurs in severe anemia or in the overloading of volume, the size of the heart might increase and, according to Nutter et al. (1979), the cardiac atrophy observed in undernourishment probably occurs as a result of the decrease in the work of the heart.

In our study, the ventricle weight/body weight ratio was greater in the MG compared to the CG,
indicating a greater reduction in body weight in relation to ventricle weight in the MG. Other authors have obtained similar results in studies on rats (OKOSHI et al., 2001) and humans (KAWAMURA et al., 2007; SARAIVA et al., 1992), even when both ventricles (right and left) were weighed separately (OKOSHI et al., 2001).

On the other hand, the relative preservation of ventricle weight found in our study can be explained by edema in the myocardium, for there were no significant differences in the dry weight/wet weight ratio of the ventricles in either of the groups (CG and MG) nor at any specific age. In contrast to Nutter et al. (1979), who investigated, through the dry weight/wet weight ratio of the heart, the effects of caloric/proteinic undernourishment after weaning in rats. They found reduced heart weight in undernourished animals and, in the histological study, they did not observe pathological alterations, nor edema, in the left ventricle.

The aging processes, which in our study was from 21 days to 9 months of age, appeared to affect the autonomic behavior of the heart in both groups, as indicated by the increase in the sympathethic-vagal balance (Table 3); however, aging did not give rise to worsening or modifications of morphological characteristics of the heart in the animals from the MG.

Our studies were concluded when rats reached 9 months of age due to the high incidence of mortality in malnourished animals past this age. Also, malnourished animals older than 9 months were very fragile and many would not survive when subjected to invasive procedures such as autonomic blockade with drugs and anesthetics.

Conclusion

In conclusion, our data confirms that blood pressure parameters are maintained in the MG similar to the CG; however, the HR in the MG was increased compared to the CG, but showed a decrease with age. An increase in the sympathetic component was found in the MG suggesting a cardiac autonomic dysfunction. Further studies are necessary to elucidate the molecular mechanisms that are involved and modified in the cardiac effects seen in malnourished rats.

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References


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