Pre- and Post-Natal Protein Deficiency in Albino Rats. I. Weight Progression Analysis

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Alterations in periodontal tissue were analyzed histologically in rats who had received a protein-deficient diet during intra-uterine life due to inadequate nutrition of the mothers and during the post-natal period due to the continuity of poor nutrition. The animals were weighed at sacrifice: 0, 1, 4, 11, 17, 21, 42, 50 and 105 days. Histological data comparing the undernourished and control groups will be the objective of a future publication. This first part reports the statistical analysis of weight progression of the two groups, concerning not only the mean weight, but also the individual weight of each animal at the various sacrifice periods. Weight progression was gradual and proportional in each group, despite the fact that the control group had accentuated weight gain, deviating, with time, from the weight of the undernourished group of the same age.

Key Words: nutrition, weight progression, protein deficiency.

Introduction

The relationship between periodontal disease and nutrition has been the objective of numerous investigations in the last two decades (Ferguson, 1969; Clark et al., 1970; Stahl, 1971; De Paola and Alfano, 1977; Alfano, 1979). These authors have affirmed that the inflammatory response of the gingiva, injured by micobic plaque and its products, is substantially influenced by the nutritional state of the patient.

Various types of malnutrition exist which can manifest their prejudicial action on the periodontium in diverse ways. Nutritional deficiencies are not rare, but their manifestations can remain in a subclinical state causing unobserved alterations in periodontal tissues which cause the periodontium to become more vulnerable to the action of local etiological factors.

The importance of diverse vitamins in the etiology of periodontal disease and their influence on the result of therapy has been proven in several studies which have shown that
an accentuated lack of various vitamins can lead to periodontal tissue modifications, and
even facilitate the formation or the deepening of a periodontal pocket. However, it has always
been emphasized that there is not a direct relationship of cause and effect, since the primary
action of the microbial factor is fundamental (Linghorne et al., 1946; Keller et al., 1963;
El-Ashirey et al., 1964; Jones et al., 1985).

Glickman and Stoller (1948) showed that in rats with vitamin A deficiency pockets
formed only with the presence of a local factor; however, an increasing vitamin deficiency
intensified the destruction of periodontal tissues.

Another form of malnutrition which has been considered as having a decisive
influence on general health, affecting various body tissues, and logically also periodontal
tissues, is that of protein deficiency. Despite the fact that acute forms of protein deficiency
are not common, if analyses of patients’ diets were made more frequently, a greater number
of cases of periodontal lesions with nutritional deficiency would probably be revealed.

Increased consumption of carbohydrates is the result of alimentary habits deficient
in protein, which has as a consequence the absence or reduction of several essential amino
acids. Thus, a moderate protein or vitamin deficiency can be an aggravating factor or
modifier of the periodontium, not manifesting as a specific form of periodontal disease, but
as a subtle, important role in resistance of the individual, who becomes susceptible to the
establishment of a pathological process. These facts have been demonstrated extensively in
medicine, showing vital organ alterations as a consequence of protein deficiency.

In dentistry, poor nutrition, and specifically protein deficiency in this study, has
also been amply studied, however, limited to only certain clinical situations due to the
existing difficulties in establishing appropriate experimental methods.

The study of alterations is more complex in research involving protein because
certain lesions are related to specific amino acids, which have complex interactions in order
to be a part of the vital activity of cellular metabolism.

Stahl et al. (1962), analyzing vascular modifications as a consequence of protein
deficiency, suggested that this interference in metabolism by protein causes debilitation in
patients and their vulnerability to pathological progress. Marginal gingival lesions can be
considered to be a type of lesion in which exists, to some degree, activities of destruction
and of repair, in which equilibrium will be affected by nutritional protein deficiency.

Stahl (1962, 1963, 1965, 1966) has studied the repair of periodontal tissue injuries
by establishing diverse clinical situations in animals, with degrees of protein deficiency
varying from low protein to total absence. Injuries caused by experimental surgery had their
repair hindered by a delay in connective tissue reconstruction, due to protein deficiency. The
inflammatory period of the connective tissue was lengthened, lasting until after
re-epithelialization of the area, a fact not expected under normal conditions.

Goldman and Gould (1965) and Goldman and Ruben (1963) reported that the
attachment apparatus in rats and monkeys, under experimental conditions with reduced
protein and profound debilitation, presented accentuated anatomic and biochemical
alterations in principal collagen fibers, on which the integrity of the periodontal ligament
depends.
The present study analyzes these alterations under other conditions. The accentuated reduction of protein, or even its elimination, did not cause immediate drastic alterations, as was expected. On the contrary, medium- and long-term deficiencies were more debilitating. Thus, the experimental models of Madi et al. (1970), which submitted rats to a prolonged low-protein, high-caloric diet to study the effect on the liver, pancreas, stomach and small intestine and of Campos and Madi (1975), who introduced malnutrition in rats during pregnancy and continued this malnutrition post-natally to study debilitated offspring under these conditions, were followed.

McLeod et al. (1972) and Stewart (1973) used this method of undernutrition during the prenatal period and reported that the fetus was greatly affected by the intra-uterine undernutrition, not recuperating despite correct nutrition after weaning.

Campos and Madi (1975), using a high-protein diet until the age of 220 days, tried to recuperate the rats with provoked undernutrition, but did not succeed in reaching the same levels as control rats. This strongly suggests that intra-uterine undernutrition provokes permanent sequela.

The effects of these models on the study of pre- and post-natal protein deficiency seem to suggest that a lack of protein can be an important aggravating and predisposing factor in the evolution of periodontal disease because this would probably cause damage to periodontal tissues which would be easily invaded and destroyed, or at least modified, when later exposed to etiological microbial action.

Thus, the objective of this study, which will be presented in parts, is to histologically analyze possible alterations in periodontal tissues of rats which received low-protein diets intra-uterine, due to the undernutrition imposed on the mother, and during the post-natal period, with the continuity of the low-protein diet. This first part analyzes statistically the weight progression of control and undernourished rats.

Materials and Methods

A total of 45 adult Wistar rats (15 male and 30 female; 4 months old; mean weight, 205 g) were used. The rats were from the Oswaldo Cruz Institute and were housed at the Department of Pathology of the Federal University of Rio de Janeiro.

Mating was allowed for 5 days with a rotation of the males on the 3rd day. Three weight measurements were made for weight progression to indicate the effectiveness of mating. The first measurement was made before mating, the second during mating and the third 5 days later when the rats were placed in individual cages and divided into groups according to the type of diet they would be fed.

Rats of the pregnant control group (group C) were fed normally with a commercial diet from “Moinho Fluminense” (Rio de Janeiro) which, according to manufacturer’s specifications, contains 18 g% proteins, a quantity considered good for the albino rat (Table 1).

Undernourished pregnant rats (group C) were fed low-protein diets. A diet with 6.7 g% protein was given from the 6th day after mating (diet A, Table 2). A diet even poorer in
proteins (3.3 g%) (Moinho São Cristóvão, Rio de Janeiro) was fed to the offspring after weaning (diet B, Table 2). A multivitamin supplement was added to the diet for groups C and D.

Table 2 - Diets fed to the experimental (undernourished) group of animals. Diet A was fed to the undernourished group of rats from the 6th day after mating. Diet B was fed to the offspring after weaning.

<table>
<thead>
<tr>
<th>Content</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>São Cristóvão ration</td>
<td>500 g</td>
<td>250 g</td>
</tr>
<tr>
<td>Manioc flour</td>
<td>60 g</td>
<td>60 g</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>150 g</td>
<td>250 g</td>
</tr>
<tr>
<td>Corn meal</td>
<td>150 g</td>
<td>300 g</td>
</tr>
<tr>
<td>Burned sugar cane</td>
<td>200 g</td>
<td>200 g</td>
</tr>
<tr>
<td>Soy bean oil</td>
<td>± 60 ml</td>
<td>± 60 ml</td>
</tr>
<tr>
<td>Protein (mean %)</td>
<td>6.7%</td>
<td>3.3%</td>
</tr>
</tbody>
</table>

The same standard diet was given to the Control group (C) throughout the entire experiment. On the other hand, the low-protein diet fed to group D was interrupted for a period of 3 days before the expected day of delivery until 5 days after delivery, during which a normal diet was fed to these animals. The possibility of cannibalism due to malnutrition, as well as the possibility of lack of milk for feeding, prevention against abortion and even death justify the interruption of prolonged malnutrition.

After weaning, group D animals, offspring of malnourished mothers, received diet B (3.3% protein) while the offspring of group C (control) continued with the normal diet. In this manner, pre-natal malnutrition, which affected the fetus intra-uterine, continued during the post-natal period, maintaining the protein-deficient condition.
A total of 3 or 4 animals were weighed and sacrificed after anesthesia, by ether inhalation, at 0, 1, 4, 11, 17, 21, 42, 50 and 105 days. The animals were decapitated, the maxillae were separated from the mandibles, and were fixed in 10% buffered formaldehyde. They were then cut in smaller blocks, decalcified, cut at a thickness of 7 μm and stained with H&E.

Results and Discussion

A total of 3 or 4 offspring were sacrificed from both the control and the malnourished groups giving a total of 62 samples.

Histological sections were analyzed with the objective of comparing, between groups, possible alterations in the formation and eruption of teeth, and to analyze alterations in periodontal tissues. These data will be reported in future publications.

Weight data showed a difference between groups C and D, with the malnourished offspring weighing more than the controls (5.6 g vs 7.5 g and 5.3 g vs 7.0 g, respectively) at the first 2 periods, 0 and 1 day. From this point on there was a progressive inversion as shown by the mean weights: 4 days (C = 9.1 g vs D = 6.0 g); 11 days (C = 14.8 g vs D = 10 g); 17 days (C = 15 g vs D = 9.0 g); 21 days (C = 34.5 g vs D = 18.5 g); 42 days (C = 70.0 g vs D = 16.5 g); 50 days (C = 91.4 g vs D = 17.5 g) and 105 days (C = 163.5 g vs D = 25.5 g). Weight loss was evident in the evolution of the malnourished group in relation to the control group.

Statistical analysis

The data were analyzed statistically, not only in terms of means but also the individual weight of each animal at each sacrifice period. The linear regression and correlation statistical tests adapt to this type of experiment, since the experiment reported the weight evolution of the animals over a long time period. Thus, the times of observation were placed along the axis of the abscissa and the animal weights along the axis of the ordinates in order to draw a line which represents the relationship between time and weight, and verify if there is a significant correlation between these variables. Table 3 shows the relationship of age, in days, and weight, in grams, of the animals used for the linear regression and correlation tests. Age of each nourished (control) and experimental (undernourished) group were analyzed and compared within groups.

The first test sought to determine which type of curve better showed this relationship, referring to the nourished group of rats. The most elevated value for r (in other words, the value closest to +1 or -1) which indicated this correlation showed that the geometric model which adapted better to the group of points was a straight line, with a value of $r = 0.9648$. The probability associated with this $r$ value, for the number of pairs of data used, is less than 1% for the hypothesis of not having a correlation; in other words, for the hypothesis of the inclination of the line to be more or less parallel to the horizontal (axis of the abscissa). In fact, if there was no proportional variation between time and weight, the
Table 3 - Paris of data used for the linear regression and correlation tests relative to the two groups of animals, control and undernourished.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Weight</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Undernourished</td>
</tr>
<tr>
<td>0</td>
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<td>7.0</td>
</tr>
<tr>
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</tr>
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</tr>
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</tr>
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</tr>
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<tr>
<td>11</td>
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<td>10.0</td>
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<tr>
<td>11</td>
<td>17.0</td>
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<td>15.0</td>
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<td>17</td>
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<td>26.0</td>
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<tr>
<td>105</td>
<td>148.0</td>
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</table>
tendency would be a line parallel to the x axis, which leads the test to point to low values of r, close to 0.

The same test provides the equation of this regression line \( y = 1.73158x + 0.12305 \) and calculates the limits of confidence for the value of the inclination of this line, which is the numerical value which, in the mathematical equation, is tied to x. These limits are between 1.55589 and 1.90727, which signifies that any line with values situated between these two extremes are considered statistically equivalent to it (Figure 1A).

The same test, carried out on the group of malnourished rats, showed a regression line with the equation: \( y = 0.16836x + 8.0555 \). The value of r was 0.86479, equally

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**Figure 1** - Linear regression and correlation between age and weight of the control nourished group (A) and the undernourished group (B) of rats.
significant at the level of 1% of probability for the hypothesis of equality of the inclination of the diet with the horizontal. The limits of confidence, situated between 0.13049 and 0.20623, indicated that the inclination of this line was completely different from that of the group of nourished rats, not having overlapping of the two intervals compared (Figure 1B).

A third test, performed to compare the two $r$ values (0.96484 and 0.86479), confirmed this difference between the inclinations of the two lines. The fact that both $rr$ were positive indicates that age and weight evolved in the same manner, that is, when one increased, the other increased proportionately.

The graph which unites the two lines of regression corresponding to the two groups in one tracing shows this difference of behavior in weight progression of the two groups of experimental animals. The upper position of the line which represents the nourished group reveals that the weight of these animals is always greater than the malnourished group of animals (lower line); the more abrupt inclination of the line which represents the nourished group indicates that the weight increase occurred throughout the experiment. The weight of the malnourished animals increased very slowly, such that the difference between the weights of the animals of the two groups increased with time (Figure 2).

Finally, if a graph was made representing the intervals of confidence for the inclinations of these two lines of regression (value $b$ in the mathematical equation of line $y = a + bx$), it would have the aspect shown in Figure 3, in which the intervals did not overlap, indicating mathematically and statistically unequal lines; in other words, not parallel.

**Conclusions**

Based on the statistical analysis of the data of weight progression, we conclude:

1) In each group, control and malnourished, weight progression was gradual and proportional.

![Figure 2 - Comparison of regression lines of control (N) and undernourished (U) groups, showing a difference between their angles of inclination.](image-url)
Figure 3 - Intervals of confidence for the inclination of the lines which are referred to in Figure 2; in other words, for the \( b \) values of the equations of the two lines of regression, referring to groups \( N \) and \( U \). The lack of overlapping indicates a statistical difference in the weight progression of the two groups.

2) The weight of the control animals increased emphatically, separating, with time, from the undernourished animals of the same age.

3) During the first days of life, the undernourished animals weighed more; however, this situation inverted with time, possibly due to alterations during gestation.

References


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