Histopathology of the Hematopoietic Bone Marrow in the Temporomandibular Joint of Rats Subjected to Undernutrition and to Mandibular Condyle Fracture

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Summary
Undernutrition can cause important functional and morphological alterations in the hematopoietic bone marrow (HBM). Degeneration of the HBM in malnourished individuals has been observed in the long bones, but none has been described in the cranial bones. Mandibular condyle fracture can lead to determine nutritional effects due to the high catabolism needed for the bone healing added to the difficulties of mastication. The aim of this study is to describe the histological aspect of HBM in the fractured mandibular condyle and in the temporal bone of malnourished rats. Thirty adult rats suffered unilateral mandibular condyle fracture and were divided into well-nourished (FG) and malnourished (MG) groups. In the MG the animals received a hypoproteic diet during the experiment. Histological sections of the temporomandibular joint were stained to visualize and quantify the HBM in this region at 24h, and 7, 15, 30, and 90 days post-fracture. At 24 hours, FG and MG showed hypocellularity and ischemic degeneration in the mandibular condyle and in the temporal bone. At 7 days, FG exhibited high cellularity in comparison with MG in the condyle; the temporal bone of both groups presented hypocellularity and degeneration. At 30 and 90 days, FG exhibited similar characteristics to those of the control; MG maintained the degeneration level mainly in the temporal bone. Malnutrition prejudices the regeneration of the HBM during a fracture healing in the temporomandibular joint. This fact contributes to a complete modification of the bone structure as well as to an impairment of the healing process.

Introduction
Diet restriction causes severe alterations in hematopoietic bone marrow (HBM), such as hypocellularity, necrosis, and extracellular matrix modifications (Travlos 2006; Vituri et al. 2000). These alterations can reflect the hematological disturbances observed in this situation, such as decrease in the erythrocyte and leukocyte count.

Mandibular condyle fracture is considered a severe trauma (Silvennoinen et al., 1992). Experimental models in animals have demonstrated a significant weight loss during the fracture healing of condyle (Luz & Araújo, 2001; Long & Goss, 2007) caused by the intense catabolism associated with the repair process, as well by the masticatory difficulties. Nutritional deficiencies during the healing of the fractured mandibular have been mentioned, but little is known about the hematological alterations in the different phases of this healing.

During development of the human temporomandibular joint (TMJ), the bone marrow in this region is formed by osteoprogenitor cells and hematopoietic cells. Hematopoietic marrow is seen at low levels in adult human TMJs, especially in the mandibular condyle and mandibular ramus, due to important
maturational changes that occur during and after cessation of the growth (Luder, 1998). In animals, high cellularity and low frequency of fat tissue are observed in the small marrow spaces of cranial bones in adult rats (Cline & Maronpot, 1985). Despite the limited amount of HBM in the TMJ, the bone marrow in the human mandibular condyle is frequently analyzed by magnetic resonance imaging (MRI) to detect TMJ dysfunction, such as osteoarthritis (Sano, 2000).

There are few studies analyzing the HBM of TMJ, and they are restricted to the embryological process, mainly as regards TMJ formation. The impact of undernutrition on the tissue, as well as the association of this disturbance with fracture healing, are also unknown. Information about the regeneration process of HBM and about the influence of undernutrition on this process could elucidate some of the aspects of hematological alterations detected during these situations.

The objective of this study is to describe the histological modifications of HBM of the TMJ with a fractured mandibular condyle in well-nourished rats and to compare these findings with those observed in malnourished rats previously submitted to a protein restricted diet. Our purpose is to observe the morphological alterations in the HBM that signal malnutrition during the different phases of fracture healing.

**Materials and Methods**

This methodology was approved by the Ethics Committee on Animal Experimental Research of the Dental School, University of São Paulo.

**Animals maintenance and experimental groups**

Thirty male Wistar rats (5 and 7 months of age, medium body weight of 200g) were obtained from the Central Biotery of the Biosciences Institute, University of São Paulo, and quarantined 15 days before the study in the Biotery of Oral Surgery Department, School of Dentistry, University of São Paulo. They were randomly divided into two groups: a) well-nourished fractured group (FG) – fifteen animals submitted to standard diet (Purina®, Labina, São Paulo, Brazil) and to unilateral condylar fracture; b) malnourished fractured group (MG) – fifteen animals submitted to low-protein diet during 30 days and to unilateral condylar fracture. The animals were housed 1 per cage with free access to water and to standard or low-protein diet. Water and food ingestion were weekly measured. Environmental conditions were temperature 20º-22ºC, relative humidity 50±5%, 12 hours light/day cycle.

**Low-protein diet regimen and malnutrition monitoring**

The animals of MG ingested a diet in which 23% of the proteins (presented in standard food) were reduced to 8% by the addition of carbohydrates, but vitamins and minerals were maintained in accordance with the American Institute of Nutrition AIN-93G recommendations for rodent diets (Reeves et al., 1993). This food was prepared in our laboratory using the diet composition described by Santos et al. (2004). Similar models of protein restriction are also reported in Gleason et al. (1982) and Prestes-Carneiro et al. (2006). The animals received this diet for 30 days previously to the mandibular fracture. Malnutrition was confirmed by the low weight these animals showed during the experiment, as well as by the total serum protein and serum albumin tests. In this case, after euthanasia, blood was collected by aortic puncture and the serum was submitted to blood protein measurements. The low-protein diet was maintained in this group up to the time of euthanasia after the fracture.

**Mandibular condyle fracture**

All the animals were anesthetized with an intraperitoneal injection of ketamine and xilazine (0.1 ml/kg and 0.01 ml/kg, respectively), for the surgical access to the right mandibular condyle. The condylar process was exposed and fractured (extracapsular fracture) using mosquito-(Halsted) forceps (Luz & Araújo, 2001). Suture with nylon was performed in layers at the conclusion of surgery.
Euthanasia and tissue processing
A lethal dose of anaesthetic was used for euthanasia at 24h, and 7, 15, 30, and 90 days after the fracture (3 animals per experimental group). After this the heads were cut off, fixed in formalin for 24h, and then sectioned sagittally. Left hemiheads, with non-fractured TMJ, were washed with water for 30 min and immersed in EDTA 10% solution, pH 7.4, at 8°C, for 2 months, for decalcification. After this, the specimen was sagittally sectioned in the TMJ region and submitted to histological processing. Histological sections 4 µm thick were stained with hematoxylin-eosin (HE) and with other stains to visualize blood cells (Giemsa technique), and reticular fibers (silver impregnation method).

Histological analysis
In the histological analysis under light microscopy, the articular regions of mandibular condyle and temporal bone, articular disc, and the marrow of both bones were observed. When the largest width of the mandibular condyle was not visible, additional serial sections were performed. The HBM of the subcondral region of mandibular condyle and of the superior region of the fibrocartilage in the temporal bone was analyzed by two pathologists without identifying the experimental periods. The pathologists described the constituents of the extracellular matrix in the marrow spaces, the level of cellularity, and the main cellular population presented, which were recognized by their morphology and histochemical properties. A semiquantitative analysis was performed for these parameters.

TMJs of three well-nourished animals without fracture and of the same age served as control. They were submitted to the same methodological steps.

Measurements of Cellular Nuclei
The visible cellular nuclei were quantified in order to obtain objective data about the cellularity of the HBM. The visible nuclei were counted by one pathologist without identifying the experimental group. Morphometry software (ImageLab 2000, São Paulo, Brazil) and a digital system were used to digitize the histological images, and the examiner electronically marked all the visible nuclei presented in the bone marrow space. A software tool automatically counted the marked nuclei. All the HBM spaces in the mandibular condyle identifiable in the HE-stained histological sections were quantified at 400X magnification. As the number of HBM spaces differed between the groups, the number of microscopic fields was variable. The 15-day post-fracture experimental group was not quantified because of the absence of HBM (see Results). Data were submitted to statistical tests. In addition to comparing the test groups, data of the 24h and 90 day experimental periods were also compared with the control values.

Statistical Analysis
Statistical analysis was performed with SPSS® (Statistical Package of Social Sciences) software for the number of cellular nuclei. The Mann-Whitney test was applied in order to verify the statistical differences between the groups. For the statistical analysis between the experimental periods, Friedman’s test was used. The differences were considered statistically significant when the P value was less than 0.05.

Results
Body weight and biochemical tests
The values of body weight, serum total protein, and serum albumin are listed in the Table 1. MG showed big reduction in body weight gain, serum total protein, and serum albumin in relation to FG. Analyzing each experimental period, the biggest reduction in these values was for MG at 15 days after the fracture. Additionally, at 7 and 15 days the body weight gain was negative in this group. FG did not show negative values for body weight gain. MG showed lower levels of serum total protein and serum albumin than FG from the first to the last experimental period (data of each experimental period is not shown).
**Table 1.** Medium values ± standard deviation of body weight gain, serum total protein, and serum albumin detected in each group.

<table>
<thead>
<tr>
<th>Animal data</th>
<th>Well-nourished fractured group (FG)</th>
<th>Malnourished fractured group (MG)</th>
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<tbody>
<tr>
<td>Total body weight gain (g)*</td>
<td>124.7±26.0</td>
<td>50.3±29.7</td>
</tr>
<tr>
<td>Serum total protein (g/dL)</td>
<td>8.63±0.43</td>
<td>4.84±0.75</td>
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<tr>
<td>Serum albumin (g/dL)</td>
<td>2.56±0.41</td>
<td>2.04±0.52</td>
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</table>

* Calculated at the latest experimental period, i.e., 90 days.

**Table 2.** Semiquantitative analysis of the histopathological characteristics of the bone marrow in the mandibular condyle in the different groups and experimental periods.

<table>
<thead>
<tr>
<th>Experimental period</th>
<th>Cellularity</th>
<th>Erythroid lineage</th>
<th>Myeloid lineage</th>
<th>Megakaryocytic lineage</th>
<th>Extracellular matrix</th>
<th>BM space area</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>FG</td>
<td>MG</td>
<td>FG</td>
<td>MG</td>
<td>FG</td>
<td>MG</td>
</tr>
<tr>
<td>24h</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
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<tr>
<td>7 days</td>
<td>+++</td>
<td>++</td>
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<td>++</td>
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<td>+</td>
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<tr>
<td>15 days</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>30 days</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>90 days</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

FG = well-nourished group; MG = malnourished group; BM = bone marrow.
(−) = absence; (+) = mild; (++) = moderate; (+++) = intense.

**Table 3.** Semiquantitative analysis of the histopathological characteristics of the bone marrow in the temporal bone in the different groups and experimental periods.

<table>
<thead>
<tr>
<th>Experimental period</th>
<th>Cellularity</th>
<th>Erythroblastic lineage</th>
<th>Megakaryocytic lineage</th>
<th>Extracellular matrix</th>
<th>BM space area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FG</td>
<td>MG</td>
<td>FG</td>
<td>MG</td>
<td>FG</td>
</tr>
<tr>
<td>24h</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>7 days</td>
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<td>15 days</td>
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<tr>
<td>30 days</td>
<td>−</td>
<td>−</td>
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<tr>
<td>90 days</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
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</tbody>
</table>

FG = well-nourished group; MG = malnourished group; BM = bone marrow.
(−) = absence; (+) = mild; (++) = moderate; (+++) = intense.
Histological analysis

Table 2 and Table 3 contain the degree of cellularity, type of cell population, extracellular matrix, and area of bone marrow space for each group and experimental period, respectively for mandibular condyle and temporal bone.

Control TMJ

The articular surface in the mandibular condyle of control TMJ had characteristics of articular cartilage, with an uppermost layer composed of fibrous connective tissue and subsequent layers of proliferating undifferentiated cells and well-differentiated hypertrophic condrocytes. The subchondral region is formed of compact bone with small marrow spaces. HBM-containing erythroid and myeloid lineage cells was observed in these spaces. Large sinusoid spaces with a high number of erythrocytes were also frequently observed. Erythroblasts, metamyelocytes, and mature granulocytes were the most frequent cells, which were scattered throughout the marrow space without cluster formation (Figure 1A). No adipocytes or fat tissue were observed. Reticular fibers and some mesenchymal cells resembling bone cells were observed in the extracellular matrix. An evident osteoblast line in the peritrabecular region was present (Figure 1A), as well as osteoclasts in marrow near the hypertrophic region.

In the temporal bone, the articular surface was formed by a fibrous connective tissue that was wider in the glenoid fossa. In the articular space the articular disk exhibited fibrous tissue with mature fibroblasts. In the temporal bone HBM was observed in a large marrow space with high cellularity. Here it was possible to distinguish megacaryocytes scattered throughout the space, as well as erythroid cells forming clusters in the peripheral marrow (Figure 2A). Mature granulocytes, mainly eosinophils and neutrophils, were seen predominantly in the perivascular space. Isolated fat cells resembling adipocytes were also detected. Reticular fiber networks were observed more clearly, forming microspaces filled with cells. In this bone the osteoblast line was not visible, whereas in the mandibular condyle, it was.

Figure 1. Histological sections of the HBM in the fractured mandibular condyle. A: Control group. B and C: 24 hours after the fracture; D to F: 7 days; G to I: 30 days; J to L: 90 days. A: High number of erythroblasts and myeloblasts, osteoblasts in the peripheral marrow and sinusoid spaces with erythrocytes (Hematoxylin-eosin, X400). B: Well-nourished group (FG), with low cellularity and extracellular matrix degeneration and necrosis (Hematoxylin-eosin, X400). C: Malnourished group (MG) showing marrow spaces with low cellularity and fibrous extracellular matrix (Hematoxylin-eosin, X100). D and E: FG exhibiting high cellularity (erythroblasts, myeloblasts, and megakaryocytes near the vascular spaces) distributed according to the microenvironments formed by the reticular fiber networks (D – hematoxylin-eosin, X400; E – silver impregnation, X100). F: MG with high cellularity (erythroblasts, myeloblasts, and mast cells) without cluster formation (Hematoxylin-eosin, 400X). G and H: FG with a high number of erythroblasts and sinusoid spaces, and some signs of cellular necrosis (arrow) in a reticular extracellular matrix with fibrosis (G – hematoxylin-eosin, 400X; H – silver impregnation, X100). I – MG exhibiting viable cell population formed by clusters of erythroblasts near the sinusoids (arrow) and immature cells with signs of degeneration (arrow head) in the same marrow space (Hematoxylin-eosin, X400). J: FG with high cellularity, mainly of mast cells (arrows), erythroblasts, and myeloblasts (Giemsa, X400). K and L: MG showing large marrow spaces with extracellular matrix degeneration, high number of congested sinusoids, and absence of line of osteoblasts at the periphery (Hematoxylin-eosin, X100 and X400). Bar – 20 and 100 µm.
Histological findings were similar in both the FG and in the MG. The condylar fracture was evident in the histological sections. The articular disc accompanied the dislocation of the fractured bone portion. The articular surfaces in the mandibular condyle and in the temporal bone were preserved. HBM spaces were seen in the fractured portion of the condyle and in the temporal bone (Tables 2 and 3, and Figure 1C). Cells with pyknosis and acidophilic cytoplasm were suggestive of ischemic necrosis in the HBM in both anatomical sites (Figures 1B, 1C, 2B, and 2C). Occasional erythroblasts and myeloblasts were visible, mainly in the mandibular condyle of FG (Figure 1B and Table 2). The extracellular matrix exhibited basophilic degeneration with some signs of reticular fiber networks (Figures 1B, 1C, 2B, and 2C). Granular eosinophilic debris without the presence of cells was frequently seen, mainly in the temporal bone (Figure 2B, 2C, and Table 3) in both groups. No sign of hemosiderin pigment was observed. The cellularity of MG was significantly lower than those of FG and control (Table 4).

7 days
New-formed bone originating a greater callus in the fractured portion and in the mandibular ramus was observed.

In the mandibular condyle, HBM was present in the callus, exhibiting high cellularity in the larger marrow spaces in both the FG and MG (Table 2 and Table 4). Erythroblasts, myeloblasts, mature granulocytes, and occasional megakaryocytes were observed adjacent to the sinusoids, mainly in the FG (Figure 1D). Mast cells were frequently seen scattered throughout the marrow (Figure 1F). Cells with large cytoplasm with amorphous material and round nuclei were also frequent, being compatible with macrophages. Double lines of osteoblasts and multiple osteoclasts were frequently seen. No sign of necrosis was noted in the extracellular matrix, which was well-formed by a reticular fiber network (Figure 1E). In the pre-existing bone of the mandibular ramus and of the condylar process, the HBM showed a morphological aspect similar to that in the previous period, with degeneration and necrosis. This tendency was mainly observed in the MG.

**Figure 2.** Histological sections of the HBM in the temporal bone. **A:** Control group. **B and C:** 24 hours after mandibular condyle fracture. **D:** 7 days; **E and F:** 30 days; **G to I:** 90 days. **A:** Control with high number of erythroblasts forming clusters, myeloblasts and granulocytes grouped around the sinusoids, and megakaryocytes with a degenerative aspect (Hematoxylin-eosin, X400). **B and C:** Malnourished (MG) and well-nourished (FG) group, respectively. Absence of cellularity and of peripheral osteoblast line, high degree of extracellular matrix degeneration, and sinusoid spaces with viable erythrocytes (Hematoxylin-eosin, X400). **D:** MG with total absence of cellularity and extracellular matrix, with maintenance of a marrow empty space (Silver impregnation, X400). **E:** MG with low recovery of cellularity and discontinuous osteoblast line (Hematoxylin-eosin, X400). **F:** FG showing dense network of reticular fibers, tissue debris, and myeloblasts with an aspect of degeneration adjacent to the sinusoids (Hematoxylin-eosin, X400). **G:** FG exhibiting intense cellularity of the erythroblast and myeloblast lineage, abundant mast cells, and large sinusoid spaces with erythrocytes (Giemsa, X400). **H and I:** MG showing metachromatic granulation (arrows) suggestive of mast cells and macrophages adjacent to the basophilic extracellular matrix (I) (H - Giemsa, X400; I- Silver impregnation, X100).

24 hours
Histological findings were similar in both the FG and in the MG. The condylar fracture was evident in the histological sections. The articular disc accompanied the dislocation of the fractured bone portion. The articular surfaces in the mandibular condyle and in the temporal bone were preserved. HBM spaces were seen in the fractured portion of the condyle and in the temporal bone (Tables 2 and 3, and Figure 1C). Cells with pyknosis and acidophilic cytoplasm were suggestive of ischemic necrosis in the HBM in both anatomical sites (Figures 1B, 1C, 2B, and 2C). Occasional erythroblasts and myeloblasts were visible, mainly in the mandibular condyle of FG (Figure 1B and Table 2). The extracellular matrix exhibited basophilic degeneration with some signs of reticular fiber networks (Figures 1B, 1C, 2B, and 2C). Granular eosinophilic debris without the presence of cells was frequently seen, mainly in the temporal bone (Figure 2B, 2C, and Table 3) in both groups. No sign of hemosiderin pigment was observed. The cellularity of MG was significantly lower than those of FG and control (Table 4).
In the HBM of temporal bone, there was basophilic degeneration with hypocellularity in both groups (Table 3), similar to that observed in the 24 hour period. The extracellular matrix of HBM in this bone was completely altered, with absence or disorganization of the reticular fiber network (Figure 2D).

15 days
The bone callus was larger and the union of the bone fragments was almost concluded in the FG. In the MG the bone union was delayed. A high number of osteoclasts was present in the peritabecular area of the new bone, which showed numerous vascular spaces filled with erythrocytes. In both of the groups, the marrow in the condylar process was composed predominantly of fibrous tissue and bone cells, mainly osteoblasts. Erythroblasts were occasionally seen in some spaces without the organization typical of haematopoietic tissue (Table 2). In the temporal bone the characteristics of degeneration observed in the previous periods were present again (Table 3).

30 days
An abundant bone callus was evident in the condylar process and the union was completely concluded in the FG. The articular disc of this group was thick and correctly positioned in the articular region, but this was not observed in the MG. In this group, there was a well-formed callus but the condyle showed no reposition in the articular fossa, and the articular disc was dislocated or completely absent. The condylar HBM of FG showed a discrete increase in cellularity (Table 4) with the presence of erythroblasts, metamyeloblasts, and mature granulocytes – mainly eosinophils (Figure 1G and Table 2). Occasional plasma cells were also observed, as well as megacaryocytes. Cells similar to the macrophages described in the period of 7 days were also seen in this phase (Figure 1G). The reticular fiber network presented a moderate aspect of an arrangement of niche spaces (Figure 1H). These characteristics were restricted to the bone callus. In the MG, degeneration was noted in the HBM of the condylar process. Despite this degeneration, viable erythroid and myeloid cells were also present and exhibited a tendency towards cluster formation (Figure 1I). There was statistical difference between the cellularity of FG and MG (Table 4).

Temporal bone in both groups showed no modifications in the articular surface. The HBM in this bone was similar to that in the previous period, with intense degeneration and few signs of extracellular matrix regeneration both in FG and MG (Figures 2E, 2F, and Table 2).

90 days
In the FG, the condyle head was correctly positioned in the glenoid fossa with the articular disc interposed in this region. The bone callus was in the remodelling phase. In MG, the articular disc and the articular surface of the condyle were not visible.

### Table 4. Mean values (± standard deviation) of the number of cellular nuclei presented in the hematopoietic bone marrow of the different fractured groups.

<table>
<thead>
<tr>
<th>Experimental periods</th>
<th>Well-nourished group (FG)</th>
<th>Malnourished group (MG)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td>111.00±16.09</td>
<td>43.67±22.19*</td>
<td>0.049</td>
</tr>
<tr>
<td>7 days</td>
<td>381.00±176.19</td>
<td>175.33±133.42</td>
<td>0.127</td>
</tr>
<tr>
<td>30 days</td>
<td>138.67±137.09</td>
<td>17.00±10.54</td>
<td>0.049</td>
</tr>
<tr>
<td>90 days</td>
<td>126.33±102.79</td>
<td>164.33±139.74</td>
<td>0.165</td>
</tr>
</tbody>
</table>

Statistically significant when p < 0.05.
* Also significant in relation to the control (p = 0.025).
HBM in the mandibular condyle of FG was composed mainly of erythroblasts, mature granulocytes, and mast cells (Figure 1J) distributed in large marrow spaces (Figure 1K). Macrophages were scattered throughout the scantly matrix. In the MG, degeneration, macrophages and some haematopoietic cells were present in the bone callus (Figure 1L and Table 1). Large sinusoid spaces with viable erythrocytes were also observed. There was no statistically significant difference between the groups as regards cellularity (Table 4).

The HBM of temporal bone in the FG showed high cellularity (Figure 2G and Table 3). In this group, some macrophages and mast cells were present (Figure 2G). In general, the HBM of the FG in this site had a normal aspect that resembled that of the control. On the other hand, the temporal bone of the MG showed HBM with a basophilic amorphous matrix (Figure 2I), with spaces similar to small fat spaces (Figure 2H). Hematological cells had a degenerative aspect but viable erythrocytes were seen in the sinusoid spaces.

**Discussion**

This study focused on the regeneration process of the HBM in the fractured mandibular condyle and temporal bone in well (FG group) and malnourished rats (MG group). Important similarities and differences were observed between the two groups, mainly in the later stages of the bone repair, as well as between the mandibular condyle and temporal bone.

Similarities were observed between the groups in the initial phases of fracture repair. Both in the mandibular condyle and temporal bone, degeneration and hypocellularity were observed at 24 hours. In the mandibular condyle this degeneration could be associated with ischemia in the fractured bone. The influence of malnutrition in the haematopoietic tissue of the MG was not visible in this experimental period. On the other hand, in the mandibular condyle, hypercellularity was also present at 7 days in both of the groups, probably due to the intense inflammatory response to the bone repair in this phase.

Furthermore, at 15 days, in both of the groups, the HBM was completely replaced by fibrous or non-haematopoietic tissue in both the mandibular condyle and temporal bone.

However, important differences were observed at 30 and 90 days after the fracture. The FG showed a recovery of cellularity in the mandibular condyle, with normal establishment of the M:E ratio mainly in the new-formed bone. This recovery was not observed in the MG, which showed high degrees of basophilic degeneration in the extracellular matrix and hypocellularity. In this group, the HBM was not replaced by fibrous tissue and empty bone marrow spaces were observed. This finding could be associated with the effects of malnutrition on the HBM, as well as with the poor bone repair observed in the histopathological analysis. In the literature, experimental studies have shown the strong influence of malnutrition on the femur and sternum HBM, such as diminished blood cell count (Fried et al., 1978), impaired myeloid cell differentiation (Borelli et al., 1995), alteration in the components of the extracellular matrix (Vituri et al., 2000), and ineffective erythropoiesis (Borelli et al., 2007). In the present study some morphological alterations resembling those mentioned in the literature were observed in both the mandibular condyle and temporal bone, confirming the susceptibility of these bones as well to malnutrition.

The normal re-establishment of the HBM in the mandibular condyle of the FG is particularly interesting, considering the age of the animals. In this experiment, adult animals were used, in which the HBM spaces are significantly reduced in comparison with young animals both in long bones and in the skull (Cline & Maronpot, 1985). Despite the differences between adults and young individuals as regards HBM morphology and repair capacity, the animals in this study showed that mandibular condyle and HBM healing were not affected by age. During callus formation, concomitant formation of hematopoietic tissue with high numbers of erythroblasts, myeloblasts, and even megakaryoblasts was observed in the recently formed marrow spaces.
of the FG and MG. This finding demonstrates the interaction between mandibular condyle bone and hematopoietic tissues and the preservation of the embryological memory of HBM formation in this region. Furthermore, it has been demonstrated that osteogenesis and hematopoiesis are strongly linked. Some hypothesis for this interaction are that osteoblasts secreted cytokines that acted on the differentiation of stem cells, or that bone cells are responsible for the creation of microenvironments in the marrow that regulate this differentiation (Taichman, 2005).

The tendency towards HBM regeneration presented in the mandibular condyle was not observed in the temporal bone. Surprisingly the HBM in this bone exhibited strong degeneration at 24 hours after the fracture, and up to 30 days in both the FG and MG. In the FG, only at 90 days did the HBM show recovery of cellularity, mainly of erythroblasts and myeloblasts. The marrow spaces exhibited vascular spaces with viable erythrocytes and no replacement by fibrous tissue was observed. In the MG the HBM basophilic degeneration in the temporal bone persisted throughout all the experimental periods. At 90 days the animals of this group showed the same morphological characteristics in other cranial bones, probably due the effects of chronic malnutrition on the entire skeletal system. In addition to this effect of malnutrition, the absence of articular function due to the fracture may be involved in the non-recovery of the HBM in the temporal bone. The pathogenesis of HBM degeneration is considered multifactorial (Böhm, 2000), and the impairment of TMJ articular movements without bone formation stimulus may be considered one of these factors. Further investigations must be conducted to elucidate this histological finding in the temporal bone.

HBM regeneration has recently been studied in bone marrow transplanted patients. Some steps of this process include proliferation of endothelial cells, invasion of progenitor cells and stem cells, erythroid differentiation around the restored fat cells, recovery of megakaryopoiesis, establishment of a reticular network, and myeloid differentiation mainly in eosinophils and neutrophils (van Marion et al., 2006). In the present study these steps were partially recognizable, such as the predominance of erythroid differentiation under the myeloid differentiation in order to maintain the M:E ratio. However, megakaryocyte differentiation was frequently absent, particularly in the MG. The effects of malnutrition, added to anatomical particularities of HBM in the mandibular condyle and temporal bone, may have influenced these differences. With regard to the anatomy, some differences in the HBM of the mandibular condyle and temporal bone in comparison with the femur in adult rats, were observed during this histopathological analysis. These include absence of fat cells and a high number of macrophages and mast cells in TMJ bones, low number of megaryocytes in the mandibular condyle, and absence of a typical reticular network, forming microenvironments both in the mandibular condyle and temporal bone.

In conclusion, malnutrition exerts a strong influence on the regeneration of HBM in the fractured mandibular condyle, mainly in the later experimental periods. Despite the adult age, the capacity of HBM regeneration in the mandibular condyle of the well-nourished individuals was maintained. Important morphological and chronological differences in HBM regeneration were observed between the mandibular condyle and the temporal bone, which must be further investigated.

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References
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