Quantitative Histological Data on Disuse Osteoporosis
Comparison with Biological Data

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The effect of immobilization on human bone was studied through a longitudinal, as well as cross-sectional, quantitative and dynamic histological analysis of 34 decalcified and undecalcified iliac crest biopsies. They were obtained at various times after the onset of immobilization in 28 patients of which 22 were suffering from post-traumatic spinal cord lesions. Trabecular bone volume, osteoid volume, trabecular osteoclastic resorption surfaces, size of the periosteocytic lacunae, thickness of iliac cortices and volume of the cell population of the marrow were measured. The histodynamic study was made by double tetracycline labeling in 19 patients. The histological data were compared with biological data from another group of 68 immobilized patients including 22 of the patients undergoing biopsy. Calcemia, phosphoremia, alkaline phosphatase, calciuria, phosphaturia and hydroxyprolinuria were measured. The decrease of the trabecular bone volume averaged 33% over 25 weeks and then stabilized. Immobilization also caused an early increase in the trabecular osteoclastic resorption surfaces and later in the size of periosteocytic lacunae, an early depression of osteoblastic bone formation and a thinning of the cortices. Calciuria was high, as was hydroxyprolinuria which correlates with resorption surfaces. The histological and biochemical changes suggest an histodynamic hypothesis according to which the global lifespan of the BMU (Basic Multicellular Unit from Frost) would be increased. These changes reflect a transient, leading to a new steady state: rarefied bone with a low rate of subsequent turn-over. They emphasize the importance of mechanical factors in the development of bone cells.

Key words: Bone — Histology — Morphometry — Osteoporosis — Immobilization.

Introduction

While numerous radiodensitometric (Mack and Lachance, 1967; Bruce and Wiebers, 1969) and biological studies (Semb, 1966; Lutwak et al., 1969; Sevastik and Mattson, 1971; Chantraine, 1971; Hardt, 1972) have been made of disuse osteoporosis, a scarcity of quantitative histological studies on this type of osteoporosis led us to study 34 iliac crest biopsies from 28 immobilized patients. The results of this quantitative histological survey were compared with biological data from another group of 68 immobilized patients. It was hope that such a comparison might further our understanding of the pathophysiology underlying the bone rarefaction occurring in immobilized patients. This study allowed a comparison of human histological and biological data, with experimental data obtained

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from previous studies in animals (Burckhart and Jowsey, 1967; Landry and Fleisch, 1964).

**Methods and Materials**

**Histology**

*a) Biopsy and Histological Preparation.* The samples were obtained by transfixing the iliac bone, at a point 2 cm behind the anterior superior iliac spine and 2 cm below the summit of the right iliac crest. Biopsy was performed under anesthesia, or, in case of cord lesions without it, using Bordier's trephine (inner diameter: 6 mm). The trocar provides a cylindrical bone sample limited by the two iliac cortices which protect the integrity of the spongy bone (Fig. 1). This integrity is an indispensable condition for valid morphometric analyses. In most cases (28 out of 34), two samples were taken, one for decalcification, the other for treatment without decalcification. The cylinders to be partially decalcified with nitric acid were fixed in Bouin's fluid. They were cut into 32 serial sections (5 \( \mu \) thick) which were stained with hematoxylin-phloxin-safranin. The undecalcified samples were fixed in 80% alcohol, and embedded by successive Bioplastic baths. They were then cut into 8 serial sections (8 \( \mu \) thick) using a Jung K microtome and then stained with solochrome cyanin and Villanueva's osteochrome. Four additional unstained sections (20 \( \mu \) thick) were used for observing tetracycline labels under fluorescence.

*b) Quantitative Reading Methods.* Six bone and medullary tissue parameters were measured:

1. **Trabecular bone volume (TBV).** This parameter represents the percentage of a given volume of iliac bone occupied by trabeculae, excluding medullary and vascular spaces, but including calcified and osteoid tissues. The measurement was performed either manually with a Zeiss I integrating eyepiece exposing 25 points or with an image analyzing computer (Quantimet 720).

2. **Relative osteoid volume of the cancellous iliac bone (R.O.V.).** This is the percentage of bone tissue occupied by osteoid tissue and was measured with a Zeiss integrating eyepiece exposing 100 points. From the relative osteoid volume it is possible to then calculate the osteoid volume related to the specimen sample volume, by means of the following formula:

\[
\text{Osteoid volume} = \frac{(\text{R.O.V.}) \times (\text{T.B.V.})}{100}
\]

3. **Trabecular osteoclastic resorption surfaces.** The resorption sites notch the edges of the bone trabeculae. Their surface were measured with the Zeiss II integrating eyepiece. This parameter was expressed as the percentage of the total trabecular surface.

4. **Mean size of the periosteocytic lacunae.** Periosteocytic lacunae generally appear on decalcified preparations as ellipsoidal cavities with clearly outlined contours. According to an original method (Meunier et al., 1973a) the larger and the lesser diameters of the ellipse were measured with a micrometric eyepiece. We consider the product of the two diameters as representing the surface of a rectangle within which the cross-section at cavity can be placed as an indication of the actual size of the lacuna. The measurement was performed on 50 periosteocytic lacunae selected at random in each bone sample. This was expressed in square microns. The actual cross-section areas of the periosteocytic lacunae are also measurable using the image analyzing computer (Meunier et al., 1973c).

5. **Thickness of iliac cortices.** This was measured with a micrometer eyepiece.

6. **Marrow adipose volume (M.A.V.).** This was measured with the Zeiss I integrating eyepiece and expressed as percentage of the total medullary volume. The absolute volume of the cell population of the marrow (V.C.P.M.) expressed as a percentage of the total bone volume, can be deduced from the marrow adipose volume by means of the following formula:

\[
\text{V.C.P.M.} = (100 - \text{M.A.V.}) \times \frac{\text{R.V.}}{100}
\]

\[
\text{R.V.} \cdot (\text{remaining volume}) = 100 - \text{Trabecular bone volume}.
\]