JOURNAL OF MULTIDISCIPLINARY SCIENCES AND INNOVATIONS
GERMAN INTERNATIONAL JOURNALS COMPANY
ISSN: 2751-4390
IMPACT FACTOR (RESEARCH BIB) : 9,08. Academic reserach index

MODIFICATION OF THE METHODOLOGICAL STUDY OF BONE TISSUE

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Abstract: The article describes the method of obtaining, staining and subsequent morphometry of non-decalcified bone sections.
Keywords: technique, histological examination, bone tissue.

INTRODUCTION

One of the conditions for solving these problems is the ability to distinguish mineralized and non-mineralized (osteoid) bone tissue on histological preparations, which is only possible on non-decalcified sections. It should also be emphasized that only on non-decalcified sections is it possible to conduct a reliable quantitative histomorphometric study of the static and dynamic parameters of volume, remodeling and mineralization of bone tissue.

MATERIALS AND METHODS

In most studies conducted abroad, these problems are solved in several stages. At the first stage, pieces of bone tissue are fixed. The most widely used fixatives are 70% ethanol or 10% formalin (at pH = 7.0). After dehydration, bone samples are filled with monomeric plastics, usually methyl methacrylate, which gives the resulting blocks a strength similar to that of bone. At the next stage, using special microtomes equipped with a diamond knife, sections 1-2 μm thick are prepared. In some laboratories, the plastic is dissolved before staining the sections, while in others this is not done, wishing to preserve the section architecture. Subsequently, the sections are stained. When choosing a staining procedure, it is imperative to use a method that ensures unambiguous identification of osteoid and bone tissue cells [2].

RESULTS AND DISCUSSION

It should be noted that the above-mentioned methods of filling, obtaining sections and their staining require the presence of special expensive equipment and reagents, and are also characterized by the complexity and duration of the procedure. In this connection, this method is practically not used in Ukraine. We have developed a method for obtaining non-decalcified sections of bone tissue of Wistar rats and their subsequent staining for the study of static histomorphometric parameters, which allows us to avoid these inconveniences.

The obtained semi-thin sections are stained using the Koss method with subsequent contrast staining. Unlike staining with Solochrome cyanine R and Goldner trichrome, this method is simple to perform and has readily available reagents. The Koss method is a classic reference method for detecting calcium in tissues. In this case, the bone mineral is stained black due to silver deposition, while the osteoid remains susceptible to contrast staining [3].

Staining technique

1. The sections are placed in a 1% aqueous solution of silver nitrate and illuminated with a strong light source (sunlight is best) for 2-15 minutes (the duration may vary depending on the intensity of the light and the freshness of the solution).
2. Rinse in three changes of distilled water.
3. Treat with 2.5% sodium thiosulfate

2-3 minutes.

4. Rinse well in distilled water.

In subsequent stages, cells and osteoid were stained.

5. Stain with 1% methylene blue solution for 30-60 seconds. Method for preparing 1% methylene blue solution: first, prepare a saturated aqueous solution, which is mixed with 90% ethanol in a 1:1 ratio. Then, prepare a 1% aqueous solution of methylene blue from this mother liquor.
6. Rinse in running water.
7. Stain with 0.05% basic fuchsin on 2.5% ethanol for 30 seconds.
8. Rinse in running water.
Initially, pieces of bone tissue are fixed in 70% ethyl alcohol for 24 hours. Considering the fact that using the same technique it is possible to study dynamic histomorphometric parameters (with preliminary double administration of tetracycline to animals), formalin is less acceptable, since in comparison with alcohol, it leads to greater washing out of tetracycline labels.
The sections obtained in this way allow the determination of the following static parameters of bone formation and resorption recommended by the Nomenclature Committee on Histomorphometry of the American Society of Bone and Mineral Research [4]:
1) parameters of bone formation:
- OV/BV – osteoid volume – the volume of osteoid (%) of spongy bone tissue that has not undergone calcification;
- Os/Bs – osteoid surface – the surface of osteoid (%) of the total perimeter of spongy bone tissue that is covered by osteoid;
- O.Th – osteoid thickness – the average thickness (μm) of osteoid layers;
- Ob.s/Bs – osteoblast surface – the surface of osteoblasts – the surface (%) of the total perimeter of spongy bone tissue covered by active osteoblasts;
2) resorption parameters:
- Es/Bs – eroded surface – the part (%) of the surface of spongy bone covered with resorption lacunae.
- Oc.s/Bs – osteoclast surface – osteoclast surface – part (%) of the total perimeter of spongy bone tissue covered with osteoclasts.

CONCLUSION

Instead of the last indicator, it is possible to determine the number of osteoclasts N.Oc (number of osteoclasts) per square millimeter of bone section or N.Oc/Bs the number of osteoclasts per millimeter of spongy bone surface.
A similar technique can be used to determine the dynamic parameters of bone remodeling, which we will do in the future.

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