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SERIAL UNDECALCIFIED SECTIONS OF RAT BONES*

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Introduction

Effort has been devoted to determination of the effects of hormonal agents on rates of lamellar bone formation and resorption. This work created need for a method of undecalcified serial sectioning of standard bones, such as tibias, in such fashion that absolute bone volume could be measured as well as variations in resorptive activity, in new lamellar bone formation and in other modalities in the entire diaphysis. Bone resorption is usually measured by a method of Frost, Villanueva, and Roth,[4] while bone formation has been labelled with tetracycline antibiotics in vivo according to the method of Frost, Roth, Villanueva, and Stanisavljevic.[3]

We report here a simple method of preparation of serial undecalcified sections of bones for such measurements.

Materials

A scroll-cutting saw having a blade containing over 70 teeth to the inch is used for cutting the initial sections. Coarse-toothed saws fragment rodent bone which is unsually brittle. A wood V-block holds the bones to be cut. Carborundum surfaced waterproof sandpaper, #360 or #420 grit, plus a flat surface and a source of gently running water are needed for grinding the sections.[1]

A dozen small glass containers with identfying numbers are needed to hold individual sections and prevent loss of serial order. A dilute solution of household detergent, such as "Joy", "Whisk", or "Lux" is needed for washing finished sections prior to mounting.

Methods

Bone sections are cut transversely to the axis, one section per millimeter usually being sufficient. The levels at which saw cuts are made are marked with India Ink beforehand.

Each section is placed in its corresponding glass container after cutting with tap water to prevent drying. Systematically numbering sections with low numbers at the proximal and high numbers at the distal diaphysis helps avoid mistakes in identification.

Sections are ground by Frost's method.[1] If the sections are to be stained they should be left 100 or 200 microns thick for staining, being reduced by additional grinding after staining to the desired 50 microns. Staining should be with a basic fuchsin concentration of about 0.01 percent.[2]

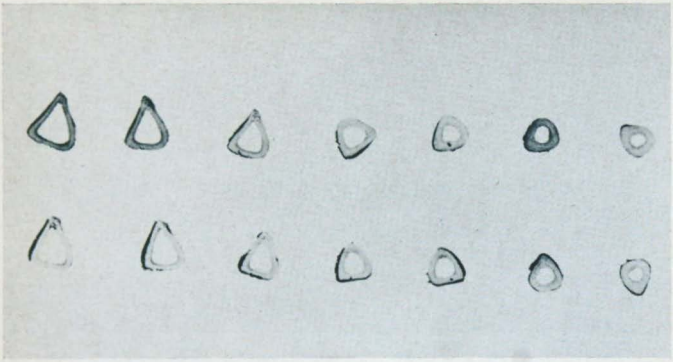

Figure 1

Shows a finished mount of right and left tibial serial sections from the same animal, the left having been subjected to an in vivo experimental procedure while the right one was used as control.

Completed sections are dried in air. On a clean microscope slide a number of small drops of mounting resin are placed equidistantly and equal in number to the number of sections to be mounted. The dried sections are pressed down in serial order on the drops of resin and allowed to set overnight. The following day the mountant is hard enough to permit mounting under standard cover slips with additional mountant.

Microradiographic or other studies may be done prior to mounting in the usual way.

Discussion

Mounts prepared as recommended permit measurement of absolute bone volume of the diaphysis, of Howship's specific surface for the entire diaphysis,[4] of osteoid seams per unit volume averaged for the entire diaphysis,[5] of total new bone formed per total absolute volume of original diaphysis (done with the multiband tetracycline labelling method)[3]. By using the rapid hematoxylin and eosin method[6] counts of the absolute numbers of osteoblasts, osteoclasts, or other cell types in the entire diaphysis may be made.

While these studies are often laborious and will be applicable only in research areas, they provide valuable checks on less direct methods depending upon use of isotopes or spectrophotometry.

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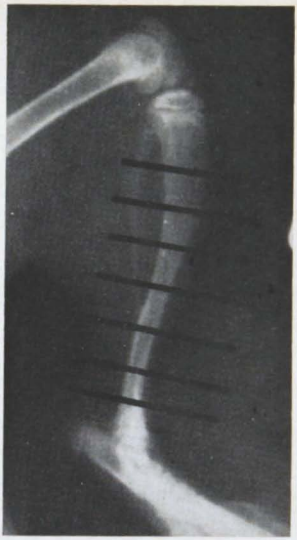

Figure 2

Diagrams the serial sequence of the sections on a rat tibia.

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