Preparation of unfixed undemineralized bone sections: the Bright bone cryostat

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SYNOPSIS A bone cryostat is described with which cold sections of unfixed, undemineralized calcified tissue can be prepared.

The bone cryostat is suitable for the preparation of the sections required for the histochemical and immunofluorescent study of bone, for electron microprobe analysis, and for bone and joint autoradiography with soluble compounds.

The histochemical and immunofluorescent investigation of bone necessitates the use of cold microtome sections. Many important diagnostic and research techniques cannot be applied with certainty to fixed and demineralized tissue. Conventional cryostats can be employed only with difficulty to cut immature or embryonic bone and are generally incapable of preparing sections of mature bone. This is a serious disadvantage in the study, not only of bone, but also of the soft tissues of small joints such as those of the ear and larynx from which cartilage and synovia cannot be detached without destroying histological integrity.

It was therefore decided to design a refrigerated microtome with which rapidly chilled blocks of unfixed, undemineralized bone could be cut at low temperature.

Description and Methods

The Bright Instrument Company manufactured a prototype instrument¹, based on their cryostat made for whole animal autoradiography. The external dimensions of the cabinet are 167 cm (length) × 76 cm (breadth) × 91 cm (height). A Jung K microtome was selected for microtomy and was incorporated into the horizontal cryostat cabinet.

The microtome is driven at constant speed allowing sections to be cut slowly with a minimum of vibration. A direct drive mechanism is used. The drive speed can be varied for different material by means of a variable speed gearbox. The speed per

stroke of the microtome can be adjusted from 0.01 to 3 seconds. The drive shaft of the microtome is extended through a bearing to the outside of the cabinet. A toothed flywheel is connected by a belt to a variable speed gearbox situated in the motor compartment and driven through a flexible coupling by a 1/3 hp constant speed electric motor fitted with a thermal overload. The gearbox is protected by a torque clutch.

The chilled tissue is mounted on a brass block holder (6.5 cm × 4.0 cm × 2.5 cm) chilled in crushed cardice. Tissue-Tek O.C.T. Compound² is then placed on the holder. The chilled specimen is then manipulated into the required position and the O.C.T. Compound rapidly frozen with Polar Spray¹.

The technique employed in obtaining sections is similar to that used with the conventional cryostat. The optimum cutting temperature of the cabinet varies with the nature of individual specimens, but -20°C to -30°C is a normal working range. Cutting speeds have to be extremely slow and the angle of the guide or anti-roll plate adjusted accurately. The arrangement of knife, guide plate, and specimen can be seen in Figure 1. The knives supplied with the Jung K microtome vary in cutting profile from K1 to K3, K3 having the more obtuse angle: this angle is recommended for cutting harder tissue. The angle of the knife to the specimen is fixed and can only be altered by exchanging the knife for one of a different number, and thus of different profile. However, the horizontal angle of the knife to the specimen track should remain as acute as possible. Sections of 6 to

¹Further information is available on request from Bright Instrument Company Limited, St Peter's Road, Huntingdon.

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²O.C.T. Compound is a water-soluble supporting medium that cuts well at low temperature. It is supplied by Ames & Company, Stoke Court, Stoke Poges, Slough, Buckinghamshire.

Fig. 1 Guide plate (G), being lowered into position against knife (K), in preparation for sectioning specimen (S).

10 μ m are adhered to warm slides by rapid thawing. Difficulty may be experienced at first with the large bevel of the knives³. It is not possible to obtain serial sections, but a short series of sections has been cut.

Results

Morphological investigations of tissue prepared and cut in the above manner have shown that tissue is extremely well preserved. There is excellent retention and accurate localization of enzyme activity. Figures 2 and 3 show sections of rat bone and articular cartilage prepared by the present technique and stained by an azo dye method for alkaline phosphatase (Pearse, 1960). No artefactual spaces surround chondrocytes or osteocytes, and the cells lie in close contact with the surrounding matrix.

A systematic programme of enzyme studies has begun. It has already been shown, for example, that the superficial chondrocytes of rat articular cartilage display no alkaline phosphatase activity but that there is high activity in relation to the cells of the deeper cartilage layers and of the osteocytes of the bone end plate.

Discussion

Sections of rapidly chilled tissue have been shown to be ideal for the study of tissue enzyme activities.

³Glen Creston, The Red House, 37 The Broadway, Stanmore, Middlesex, offer a knife exchange service for K knives.

However, it is not possible to cut adequate sections of fully mineralized bone on any cryostat microtome available in the United Kingdom. In the analysis of bone histochemistry, investigators have used other methods to prepare sections with varying degrees of success: many have fixed and demineralized the bone before sectioning (Schajowicz and Cabrini, 1959; Papoušek, Horn, Urbanová, and Gregorová 1967; Radden and Fullmer, 1969; Fullmer and Link, 1964; Vainio, 1970). Balogh (1962) stated that although sufficient oxidative enzyme activity can be preserved after decalcification with ethylenediamine tetraacetic acid to permit effective histochemical localization, tissues which have been decalcified before sectioning are less suitable for oxidative enzyme study than undecalcified sections. Many investigators have been restricted to the study of young animals in the tissues of which the calcium hydroxyapatite crystals are dispersed at low density (Schajowicz; Cabrini, 1960; Balogh, Dudley, and Cohen, 1961; Fullmer, 1964; Handelsman, Morse, and Irving, 1964; Fullmer, Link, and Baer, 1964). Wergedal and Baylink (1969) obtained thin, undecalcified slices of bone for histochemical analysis by grinding sawn sections on a ground glass plate, but it was admitted that a proportion of activity was lost during grinding.

Each of these methods has inherent disadvantages which can be eliminated or greatly reduced by the use of the cryostat microtome described in this paper. With the present technique it is possible to study

Fig. 2 Rat ankle cartilage and bone; alkaline phosphatase activity. Azo dye method—incubation time—10 min at pH 9.4 and 20°C. The arrow indicates cartilage surface. $\times 120$

Fig. 3 Rat ankle cartilage and bone; alkaline phosphatase activity. Azo dye method—incubation time—10 min at pH 9.4 and 20°C. $\times 40$

enzymes in the cartilage and connective tissue immediately surrounding bone, or in soft tissues, such as synovium, within the diarthrodial joint. In small laboratory animals, it is normally very difficult to dissect these soft tissues free from bone without causing damage to tissue and disruption of enzyme localization.

The refrigerated bone microtome may have applications outside the research laboratory. It may, for example, be used in hospital laboratories to define the extent of spread of benign or malignant bone tumours and can be employed to prepare fresh bone sections for the study of metabolic bone disease by microradiography, for electron microprobe analysis (Gardner and Hall, 1969), or for autoradiography with soluble labelled compounds. The advantages of the bone cryostat for immunofluorescent techniques appear considerable.

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