

A METHOD FOR PREPARING LONGITUDINAL SEMI-THIN EPON SECTIONS OF ENTIRE RAT INCISORS

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Summary—This method permits the preparation of Epon sections of rat incisors at 0.5 or 1 μm thickness and up to 1 cm in length. The upper and lower incisors were divided into three or four cross-sectional segments and these segments were split in half, the cut surface becoming the block face. The blocks were trimmed in a specific fashion and cut with routine glass knives on either LKB or Porter-Blum MT1 ultramicrotomes. The entire length of the incisor could then be studied by combining sections from consecutive segments.

While planning an investigation of cell proliferation and cell migration in the rat incisor, it became apparent that the better resolution provided by semi-thin Epon sections was required. However, conventional methods which routinely employ small blocks would be impractical since it was important to follow precisely the movement of cell populations over long distances as they advance from the apical towards the incisal end of this tooth. The technical problems involved in cutting good Epon sections become more acute with a larger block face. For example, the width of the block face is limited by the amount of usable knife edge available which, in the case of glass, is normally no more than the left half of very carefully prepared knives. The length of the block face can be increased considerably but this leads to a greater chance of chattering particularly with manual ultramicrotomes where the maintenance of a steady, even cutting motion becomes a limiting factor. Inconsistencies within the embedded material itself, either in the form of irregular polymerization of the Epon or by dense areas alternating with areas of less density, become magnified in larger blocks and thus are more likely to promote vibration of either the knife or block with resultant chatter. Also, the cutting of large sections causes a glass knife to dull very quickly thus creating an additional source of chatter. Further complications arise if many serial sections are being prepared since knives must be frequently changed. Despite such inherent difficulties, a reproducible method was developed which permitted the preparation of Epon sections of rat incisors at 0.5 or 1 μm thickness and up to 1 cm in length. It was felt that a description of this method would benefit those workers using rat incisors who need the improved resolution of semi-thin Epon sections and the ability to include either a long portion of the tooth in one section or a means to cover the entire length of the incisor.

The method of Warshawsky and Moore (1967) was used for fixation and decalcification of the rat incisors.

After decalcification the upper and lower jaws were handled as follows. In upper jaws, a razor blade cut was made transversely across the skull perpendicular to the cranium and the palate, and at the anterior border of the upper first molar (Fig. 1, cut A-a). The posterior portion was discarded and the anterior portion, containing the whole upper incisor, was then divided into three or four cross-sectional segments using several landmarks. The limit of the first segment was established by a cut perpendicular to the labial surface of the tooth and made at the level of the anterior border of the root of the zygoma (Fig. 1, cut B-b). The remaining segments were prepared by moving incisally any desired distance and making cuts kept perpendicular to the labial surface of the tooth at each point (Fig. 1, cuts C-c and D-d). The segments were thus similar to the wedges of a pie, the circumference of which was the curvature of the labial surface of the incisor. For 300-g rats, usually four segments were prepared which covered all the morphological regions of the ameloblast layer including the gingival margin (Warshawsky and Smith, 1974). Each segment was placed in a separate specimen bottle containing phosphate buffer (Warshawsky and Moore, 1967).

Cross-sectional segments from the lower incisor were prepared after making a preliminary cut from the medial to the lateral surface of the jaw at the level of the mandibular foramen and perpendicular to the labial surface of the tooth (Fig. 1, cut E-e). The small posterior portion of the mandible was discarded. The incisal limit of the first segment was determined by a cut made perpendicular to the labial surface of the incisor and aligned with the point of junction of the body and ramus of the mandible. This cut was made from the medial to the lateral surface of the jaw (Fig. 1, cut F-f). The remaining segments were prepared by making cuts perpendicular to the labial surface of the tooth at any desired points (Fig. 1, cuts G-g and H-h). For 300-g rats four segments of equal length included all of the embedded portion of the incisor and the soft tis-

sue at the gingival margin. As in the upper preparation, each lower segment was placed in a separate bottle of buffer wash.

Upper and lower cross-sectional segments were then split into medial and lateral halves. The first segment from the upper or lower incisor was held such that the cut incisal surface pointed directly at the investigator while the concealed apical end pointed away. A thin razor-blade was positioned on the incisal surface between the midline of the enamel organ at the labial side and the midline at the lingual side of the tooth. Taking care to keep the razor-blade perpendicular on these midlines, a clean, single-stroke slice was made along the length of the segment. This was easy in the straight first segment of the upper incisor, but difficult for the same segment from the lower incisor because there is a lateral displacement and a slight medial bend near the apical end. Halving of the remaining segments was less blindly accomplished since the tooth could be seen on the cut surface at both ends of the segment. Thus, it was possible to align the razor blade on the midlines at one end of the segment, to start the halving slice, then to turn the whole segment around and watch the progress of the cut as it was carried towards the investigator. However, for such segments from the lower incisor this procedure was modified to compensate for the lateral pitch and curvature in this tooth. This made it necessary to direct the cut along the straightest possible line which would connect the curving midlines. Here, the slice was started at one end of the segment, the segment was turned around and the razor blade was tipped so that the entire length of the enamel organ at the labial side was cut at the midline first, and then the slice was completed by passing the blade towards the lingual side.

Osmication time was prolonged from 2 to 4 hr to accommodate for the larger mass of tissue. Infiltration in dilute acetone-Epon (3:1, 2:1) was prolonged up to 3 days to allow thorough penetration. The specimens were left in pure Epon for 8 hr before flat embedding in Beem capsules (Condick, 1970). The longitudinally cut surface of each half-segment formed the block face. After polymerization at 60°C, the blocks were trimmed to the conventional tapering, pyramid shape, but with the following modifications. The bottom of the pyramid was prepared to form a right angle with the surface of the block face. The enamel organ side of the pyramid was trimmed following the curvature of this surface. The other side of the pyramid was cut straight and was made so that the top of the block would be

narrower than the bottom. Furthermore, the width of the entire block face was adjusted with this cut to occupy the usable knife edge available. Where consecutive blocks from the same tooth were to be used, no tissue was trimmed away from the top or bottom of the pyramid. Sections were cut on either a Porter-Blum MT1 or LKB ultramicrotome at 0.5 or 1 μm using glass knives. Care was necessary to make knives of the highest quality (using an LKB Knifemaker), and knife changes were usually necessary after the cutting of five sections. For serial sections, the procedure of Merzel (1971) was followed. Despite their enormous size, sections could easily be picked up with a hair or glass needle, at the midpoint of their length, and transferred without wrinkling to glass slides. The sections were heated on a hot plate for at least 1 hr. Shorter heating seemed to allow the sections to separate from the slides.

Segment one in the upper incisor included the apical end of the tooth up to the zone of maturation (Warshawsky and Smith, 1974), while for the lower incisor, segment one terminated within the zone of enamel secretion (region of inner enamel secretion). The contents of the other segments varied with their lengths. Figure 2 is a montage of segments from the upper and lower incisor in a 300-g rat. It illustrates the usefulness of this method when the entire length of the tooth is under consideration (Smith and Warshawsky, 1973).

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Résumé—Cette méthode permet la préparation des sections Epon d'incisives de rats d'une épaisseur de 0,5 ou 1 μm et d'une longueur jusqu'à 1 cm. Les incisives supérieures et inférieures ont été divisées en trois ou quatre segments sectionnés transversalement et ces segments furent divisés en deux, la surface coupée devenant la face du bloc. Les blocs ont été coupés d'une manière spécifique et tranchés avec des couteaux usuels pour le verre sur un ultramicrotome MT1 de type LKB ou Porter-Blum. Puis, l'entière longueur de l'incisive put être étudiée en combinant des sections de segments consécutifs.

Zusammenfassung—Dieses Verfahren erlaubt die Zubereitung von Epon-Abschnitten von Ratenschneidezähnen mit einer Stärke von 0,5 oder 1 μm und einer Länge bis zu 1 cm. Die oberen und unteren Schneidezähne wurden in drei oder vier lange Querschnittsegmente geteilt und diese Segmente wurden in die Hälfte gespalten, wobei die geschnittene Fläche die Blockstirnfläche wurde. Die Blöcke wurden auf bestimmte Art zugerichtet und mit üblichen Glasschneidern entweder auf einem LKB oder Porter-Blum MT1 Ultramikrotom geschnitten. Die gesamte Länge des Schneidezahns konnte dann durch Kombination von Abschnitten aus aufeinanderfolgenden Segmenten untersucht werden.

Fig. 1. Mesial surface of the left half of the skull and left mandible showing the location of cuts which isolate the incisors (A-a, E-e) and delineate cross sectional segments (labelled 1-4). The short unlabelled lines indicate the erupted portions of the incisors which were routinely discarded. x 2

Fig. 2. Longitudinal $1\ \mu\text{m}$ sections from consecutive segments similar to those illustrated in Fig. 1. The spaces between the sections have been intentionally included in the montage. Points marking the start of enamel secretion (ES), the maturation of enamel (M), and the gingival margin (G) on the upper and lower incisor are indicated with arrows. Toluidine blue. x 6.5

