# Embedding of the isolated enamel organ of the rat incisor for electron microscopy

KAJ JOSEPHSEN

Departments of Anatomy and Electron Microscopy, Royal Dental College, Aarhus, Denmark

Josephsen, K. Embedding of the isolated enamel organ of the rat incisor for electron microscopy. *Acta Odont. Scand.* 32, 379—382, 1974.

A detailed histologic and cytologic investigation of the enamel organ of the rat incisor requires that it be divided into small pieces before embedding. This report describes a method for easy identification, embedding and orientation of the individual tissue pieces. After initial fixation isolated enamel organs from 3 month-old rats are divided so that the form of each specimen indicates its original position in the enamel organ. All specimens from an individual enamel organ are subsequently postfixed and embedded in a special tray. Small blocks containing the specimens are mounted with the desired orientation at the end of a cylindrical acrylic stub. This procedure or parts thereof may be adopted to many other types of organized tissues.

*Key-words:* Amelogenesis; incisor; rats; histological technics

*Kaj Josephsen, Department of Anatomy, Royal Dental College, Vennelyst Boulevard, DK-8000 Aarhus C, Denmark*

The continuously growing incisor of the rat is suitable for the study of amelogenesis, as it is possible to follow the development, function and regression of the enamel organ in the same tooth. In 3 month-old rats the average length of the enamel organ associated with the maxillary incisor is 18.4 mm and that associated with the mandibular incisor is 21.0 mm (*Josephsen*, 1974). A detailed investigation of the multiple zones along the isolated enamel organ requires that it be divided into small specimens before embedding. This article describes a method whereby each piece of tissue can be oriented as desired in the final embedment, with complete assurance as to its original position in the enamel organ. This method or part of it can be easily applied to other types of tissues.

## MATERIALS AND METHODS

Three month-old rats were fixed by perfusion with 2.5 % glutaraldehyde in cacodylate buffer at pH 7.4. The jaws were removed and the enamel organs isolated using a method described elsewhere (*Josephsen*, 1974). The isolated enamel organ consisted of three parts (Fig. 1): (A) the apical cone which represents 3—5 mm of the growing end of the tooth, (B) the intermediate portion, and (C) the part adjacent to the mature enamel. The

Received for publication, March 18, 1974.

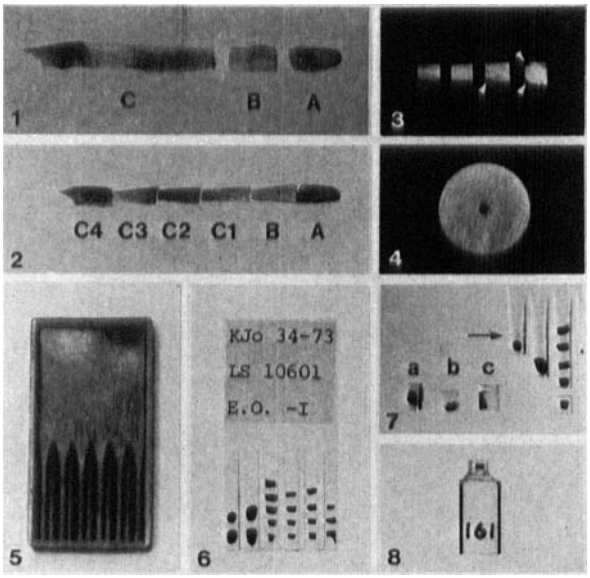


Fig. 1. Enamel organ isolated from maxillary incisor and consisting of (A) the apical cone, (B) the intermediate portion, and (C) the part adjacent to mature enamel. × 2.
Fig. 2. Incisal part of enamel organ (C) is subdivided into four pieces (C 1—4). All elongated specimens are tapered in the incisal direction.
Fig. 3. Each of tapered specimens is cross-sectioned into smaller pieces, which are processed in the same vial during the subsequent procedures. The two apical specimens are further marked by cutting off one or more of the corners (arrow heads). Shape and relative size of the tiny specimens secure the registration of the original sequence of the tissue pieces in the enamel organ.
Fig. 4. Serrated steel disk used for separation of the individual tissue specimens.
Fig. 5. Tray (40 × 20 × 3 mm) for embedding of the enamel organ specimens.
Fig. 6. Casting of Spurr's epoxy mixture containing most of an enamel organ from a mandibular incisor. Label with registration numbers is embedded in the casting.
Fig. 7. Specimen blocks are separated (arrow) and may be orientated in anyone of the three planes (a, b and c).
Fig. 8. An embedded specimen mounted on a stub of acrylic resin with »Tensol» cement. Registration number is engraved with a little bur into the side of the stub (the engraving is here colored for visualization).

latter part was 11—12 mm long and was subdivided into four pieces of nearly equal size. The most incisally of these represented the supraalveolar part of the enamel organ (Fig. 2, C4).

For electron microscopy, each part of the enamel organ was partitioned with a razor blade under a stereo microscope. To assure the registration of the original sequence of the specimens, each of the elongated parts was lightly tapered in the incisal direction (Fig 2), and finally was cross sectioned into smaller pieces, which were processed in the same vial during subsequent procedures. The most apical tissue pieces in a series were further marked by cutting off one or more of the corners (Fig. 3). The apical cone and the supraalveolar part were immediately cut into 1 mm thick slices which were processed together in one vial, since identification of the individual slices was easy because of their shape and relative size.

The specimens were postfixed in osmium tetroxide, dehydrated in alcohol and infiltrated with Epon 812 (*Luft*, 1961), Spurr's epoxy mixture (*Spurr*, 1969) or Vestopal W (*Ryter & Kellenberger*, 1958).

The tissue pieces from a single enamel organ were embedded in a special tray in which root files for use in dental practice[1]) are packed for sale (Fig. 5). The tray is made of soft plastic. Partitions running half the length of the tray divide it into six contiguous compartments. Registration numbers were recorded on a label, which was placed in the non-partitioned end of the tray. Subsequently

[1]) Stainless Hedström Files, Svenska Dental Instrument, Stockholm, Sweden.

the tray was filled with resin almost to the top. All tissue pieces from a single vial were temporarily placed in the undivided end of the tray. Under a stereo microscope the individual specimens were then brought to their final position in one of the compartments in correct sequence as evident from their shape and relative size. Each tissue piece was orientated with its convex labial surface against the bottom of the tray. The disk-shaped specimens from the apical cone were placed on their flat surface. The orientation of the individual tissue pieces was greatly facilitated by the presence of the partitions, which prevented already positioned specimens from being displaced by streaming resin. To secure a flat upper surface of the entire embedding, the tray was filled completely with resin, so that the partitions were covered. After polymerization and cooling to room temperature, the casting was easily separated from the tray. The casting had the form of a comb whose teeth contained the tissue pieces (Fig. 6). Before sectioning, the individual specimens were separated with a rotating steel disk[1]), which gave a narrow kerf (Fig. 4).

The embeddings were individually mounted in the desired orientation on a 1 cm high stub of transparent acrylic resin with »Tensol®« cement no. 7, a self-curing acrylic cement[2]) (Fig. 8). The stubs were premanufactured from long acrylic resin rods (6 mm in diameter). For identification, consecutive registration numbers were engraved with a rotating round bur into the sides of the stubs. Surplus mounting cement should be avoided as it partially dissolves the embedding resin. After curing for 20 to 30 minutes at 60°C or one hour at room-temperature, the blocks were ready for trimming and ultramicrotomy.

[1]) ½ or ¾ inches saw-blades (Kressäger) from Dreve-Dentamid, D-4750 Unna, W-Germany.

[2]) Manufactured by Imperial Chemical Industries (ICI), Welwyn Garden City, Great Britain.

## RESULTS AND DISCUSSION

The described method is designed to facilitate a number of steps which may otherwise introduce varying degrees of unnecessary and tedious work, as well as uncertainty and inaccuracy concerning the identification of the sliced tissue pieces and their final orientation in the ultramicrotome. While the method may be used in combination with most types of embedding media, Epon 812 has been found most suitable because its viscosity is such that the tray may be moved about without displacement of the specimens. In contrast, it was always necessary to readjust the orientation of the specimens when Spurr's epoxy mixture was used. The design of the tray for embedding makes it possible, if correctly filled with resin, to achieve a flat surface on the cast, because the partitions between the individual compartments are lower than the sides of the tray so that they do not interfere with the formation of a smooth horizontal surface. When the two types of epoxy were employed, the polymerization produced a flat, even, and bright surface, whereas Vestopal W left a ragged and uneven surface. Furthermore, the latter resin expanded during the curing process, resulting in breakage of the tray in one or more corners.

The procedure is economical as the volume of required chemicals is held to a minimum because many tissue pieces from one specimen may be processed together, since they are unequivocally identifiable due to the dissection method. Thus only

3 ml of resin required to embed a full tray that often contains 20 or more specimens.

Finally, this embedding procedure may be adopted for many types of specimens where identification of the individual tissue pieces and their orientation during ultramicrotomy is essential for subsequent electron microscopical examination.

*Acknowledgments.* I wish to thank Mrs. M. Andersen for skillful technical assistance, Mrs. A. Dalmose for preparing the illustrations and Mrs. M. Arvad for secretarial assistance. The valuable criticism and suggestions offered during the preparation of the final manuscript by Drs. O. Fejerskov, B. Tandler and J. Theilade are gratefully acknowledged.

REFERENCES

*Josephsen, K.* 1974. A technique for isolating the enamel organ of the rat incisor for histologic studies. Scand. J. Dent. Res. 82, 229—238

*Luft, J. H.* 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9, 409—414

*Ryter, A. & Kellenberger, E.* 1958. L'inclusion au polyester pour l'ultramicrotomie. J. Ultrastruct. Res. 2, 200—214

*Spurr, A. R.* 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26, 31—43