



**MORPHOLOGICAL AND CYTOCHEMICAL ASPECTS OF THE DEVELOPING  
MOUSE MOLAR, WITH REFERENCE TO NEURAL CREST INVOLVEMENT.**

**A light and transmission  
electron microscopic study**

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## SUMMARY

It is still uncertain if neural crest cells initiate tooth development in mammals since current information on the subject is extrapolated from studies on amphibian embryos. This investigation was undertaken to identify and examine the role of neural crest-derived mesenchyme (ectomesenchyme) in mammalian tooth formation using glycogen, alkaline phosphatase and ribonucleic acid (RNA) content as the criteria for its identification.

Mouse embryos at 12.5, 13 and 14 days of gestation were killed and the heads serially sectioned to determine the early stages of molar tooth development. The tooth bearing areas of the maxillary first molar were then examined histochemically by light and electron microscopy for the distribution of glycogen, alkaline phosphatase and RNA activity. The following histochemical reactions were used.

- (a) Periodic-acid Schiff reagent (PAS) for demonstrating glycogen. At the ultra-structural level, glycogen was easily identified as electron-dense particles 15-40 nm in diameter which appeared either as single particles or in rosettes.
- (b) A metal salt technique, using sodium  $\beta$ -glycerophosphate as substrate and lead citrate as a coupler, for demonstrating alkaline phosphatase activity both at light and electron microscopic level.
- (c) Methyl-green pyronin stain and the ultracytochemical acridine orange method to demonstrate RNA.

Control experiments for each of these substances were also performed.

The first sign of tooth development was seen in 12.5 day old embryos in the form of a thickened oral epithelium. This developed into a dental lamina on day 13 followed by a bud structure in 14 day old embryos. Throughout these early stages of molar tooth development a condensation of the underlying mesenchyme was observed.

The thickened oral epithelium and the central cells of the dental lamina and tooth buds were rich in alkaline phosphatase while the underlying mesenchyme showed weak enzyme reaction. High RNA content was seen in both the epithelial and mesenchymal components of the developing tooth germ throughout the stages examined. Glycogen

was present in minimal amounts in the surface cells of the thickened oral epithelium and in the central cells of the dental lamina and tooth bud. The mesenchyme immediately adjacent to the tooth germ was devoid of glycogen throughout the stages examined. Glycogen was however, present in large amounts in the mesenchyme surrounding this region from the 13th day onwards.

Glycogen-rich cells which also showed high RNA content and some alkaline phosphatase activity on their cell membrane were located in the dental mesenchyme from the 13th day onwards. These cells possessed features suggestive of migratory activity and were closely associated with the matrix fibrils and amorphous material in the extracellular matrix. It is suggested that these particular mesenchymal cells are of neural crest origin.

In this study, the morphological changes that occur in the maxillary molar tooth germ during the stages up to the bud stage have also been investigated and described.

## STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except when due reference is made in the text of the thesis.

Jagjit Singh

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