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# Incremental lines in mouse molar enamel

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ABSTRACT

*Objective:* The purpose of the present study was to investigate the occurrence and periodicity of enamel incremental lines in mouse molars in an attempt to draw attention to some key questions about the rhythm in the activity of the secreting ameloblasts during formation of mouse molar enamel.

*Methods:* The mouse molars were ground, etched, and studied using scanning electron microscopy.

*Results:* Lines interpreted as incremental lines generally appeared as grooves of variable distinctness, and were only observed cervically, in the region about 50–250 μm from the enamel–cementum junction. The lines were most readily observable in the outer enamel and in the superficial prism-free layer, and were difficult to identify in the deeper parts of enamel, i.e. in the inner enamel with prism decussation. However, in areas where the enamel tended to be hypomineralized the incremental lines were observed as clearly continuous from outer into inner enamel. The incremental lines in mouse molar enamel exhibited an average periodicity of about 4 μm, and the distance between the lines decreased towards the enamel surface.

*Conclusions:* We conclude that incremental lines are to some extent visible in mouse molar enamel. Together with data from the literature and theoretical considerations, we suggest that they probably represent a daily rhythm in enamel formation. This study witnesses the layered apposition of mouse molar enamel and supports the theory that circadian clock probably regulates enamel development.



## 1. Introduction

Circadian clock genes are expressed in mouse molars, suggesting that these genes may be involved in the regulation of ameloblast and odontoblast functions, such as enamel and dentine matrix secretion and mineralization.[1] Using an ameloblast cell line, other researchers have explored the potential links between circadian control and stage-specific regulation of ameloblast genes.[2] Another recent study has demonstrated that circadian clock genes in an ameloblast cell line and amelogenin gene (*Amelx*) in 2-day postnatal mouse molars oscillate in a circadian pattern.[3] These studies suggest that circadian clock genes modulate enamel development, and that amelogenesis is subject to diurnal rhythms in gene-expression levels and cell activity during development of mouse molar enamel.

Enamel formation starts at the enamel–dentine junction and proceeds outward by layered apposition of the matrix produced and secreted by the retreating ameloblasts. This movement of ameloblasts brings them from the enamel–dentine junction to the surface of the enamel. The path pursued by each individual ameloblast is traced out by the prisms, while the movement of the ameloblast layer as a whole is mirrored by the incremental lines of enamel, the Retzius lines.[4] These lines, therefore, indicate the position of

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the ameloblast layer and of the developing enamel surface at different points of time and may evidence physiological or pathological events affecting enamel formation. A system of evenly spaced incremental lines, especially evident in the outer and cervical enamel of humans and other primates has been thoroughly described, and is highly indicative of a physiological rhythm in enamel formation.[5–9] Shorter increments, the prism cross-striations, probably represent a diurnal rhythm in enamel formation.[10–12] The number of prism cross-striations between any two regularly spaced Retzius lines is reported to vary between 6 and 11.[9] These incremental growth tracks, Retzius lines and prism cross-striations, represent an internal record of time which, linked to a time scale, may serve as a valuable tool in determining the rate of enamel formation[11] and the crown formation time.[13]

Rodent and human enamel exhibits the same basic structural elements, prisms and interprism. However, the spatial arrangement of the prisms, i.e. the prism pattern, is considerably different. In rat and mouse incisors the enamel exhibits two main layers, an inner layer with extreme prism decussation and an outer layer with parallel and incisally inclined prisms.[14,15] Rat and mouse molar enamel resembles incisor enamel, but prism decussation is absent in some areas.[16,17] Incremental lines (Retzius lines and prism cross-striations) are not conspicuous in rat and mouse enamel,[14,15,18] possibly because enamel apposition occurs at a faster rate than in human enamel.[15,19,20] Lines resembling incremental lines, with a periodicity of about 1 μm, have been observed in the aprismatic enamel in mouse molars.[17] Korvenkontio reported the presence of incremental lines in certain rodent species, but with no conclusive evidence for rat and mouse enamel.[21] Also, lines which are parallel with the surface of developing rat enamel have been induced experimentally by injections of sodium fluoride[22] and tetracycline.[23]

The occurrence and periodicity of incremental markings in mouse molars have not been thoroughly investigated. We have occasionally observed lines resembling incremental lines in mouse molar enamel. In view of the renewed interest in clock genes and circadian rhythms in amelogenesis we wanted to do a systematic study on the occurrence, conspicuousness and periodicity of incremental lines in mouse molar enamel.

## 2. Materials and methods

### 2.1. Experimental animals

Ten phenotypical adult mice (CD-1 strain) were randomly selected for the study. All animals were kept at a 12 h light:dark cycle at 21 °C with a relative humidity of 65%. Prior to experimental use, animals were given standard laboratory fodder and water ad libitum. The animals were kept according to the regulations of the Norwegian Gene Technology Act of 1994.

### 2.2. Scanning electron microscopy

After cervical dislocation, the right upper and lower jaws containing all three molar teeth were dissected out and fixed in 70% ethanol. After fixation, all soft tissue was carefully removed by dissection under a stereomicroscope and by light brushing under running tap water. The specimens were air-dried, embedded in Epon, and ground longitudinally in a mesiodistal direction under a stereo-microscope using grits 800 and 1200 3M (3M, St. Paul, MN, USA) waterproof silicone carbide paper in a specially designed apparatus.[24] The ground surface was then polished against the backside of the 3M waterproof silicone carbide paper with 0.05 μm particle size alumina powder (Buehler Micropolish, Buehler, Lake Bluff, IL, USA) in water. After careful brushing under running tap water, teeth were etched for 45 s in 0.1% nitric acid, air-dried overnight, sputter-coated with 30 nm gold–palladium and observed in a Philips XL30 ESEM (Philips, FEI, Netherlands) operated at 10 kV.

### 2.3. Measurements and statistical analysis

SEM micrographs of ground and etched molars were used to investigate the occurrence of incremental lines and also their periodicity, i.e. the distance between the lines. Mean values and standard deviations were calculated using Microsoft Excel Worksheet (Microsoft Office Excel, 2010).

## 3. Results

### 3.1. Occurrence of incremental lines in mouse molar enamel

The enamel exhibited the characteristic division into two main layers, i.e. outer enamel and inner enamel (Fig. 1a). Incremental lines generally appeared as grooves of variable distinctness (Figs. 1–3). The lines were only observed cervically, in the region about 50–250 μm from the enamel–cementum junction. They were visible in 67–74% and 61–78% of maxillary and mandibular molars, respectively (Table 1). The lines were most readily observable in the outer enamel and in the superficial prism-free layer, and were difficult to identify in the deeper parts of enamel, i.e. in the inner enamel with prism decussation (Fig. 1). However, in areas where the enamel tended to be hypomineralized (darker with less distinct crystals and prisms/interprism in SEM) the incremental lines were observed as clearly continuous from outer into inner enamel, where their visibility faded more or less abruptly or gradually (Figs. 2 and 3). Where the lines were not unduly blurred by the hypomineralized state, it appeared that crystal discontinuity and/or crystal deficiency may contribute to their visibility (Figs. 1b and 2c, d). Closest to the enamel–cementum junction the incremental lines tended to be parallel with the enamel surface (Figs. 1a, b, 2a, b and 3a, b), further occlusally they reached the surface at an angle of about 10–15° (Figs. 2c, d and 3c, d).

### 3.2. Periodicity of incremental lines in mouse molar enamel

There was no significant difference in periodicity between distal and mesial aspect of the molars. Therefore, the results are presented collectively for both aspects of the teeth