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STRUCTURAL AND CALCIFICATION PATTERNS OF THE NEONATAL LINE IN THE ENAMEL OF HUMAN DECIDUOUS TEETH

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Abstract

The neonatal line of the enamel in human deciduous teeth was observed by scanning electron microscopy using secondary and backscattered electron signals. The neonatal line containing irregular structures of enamel prisms with disordered crystal arrangements was basically formed by the abrupt bending of the prisms towards the root. Usually, the prisms gradually bent back again to regain their previous orientation, but the prisms in the inner and the surface layer sometimes ran straight ahead after bending. The prism sheath regions showing hypocalcification contained a relatively large amount of organic material as shown by treatment with chromium sulfate. When etched with ethylenediaminetetraacetic acid (EDTA), such prism sheath regions were extensively eroded due to the lower density of crystals caused by the abrupt bending of the prisms. Abnormally shaped prisms and small prismless areas were occasionally present. The neonatal line formed by the double bending of the prisms is likely to represent a particular type of rhythmic Retzius line rather than a pathologic Retzius line, whereas the hypocalcified sheath regions may be similar to those of some pathologic lines of Retzius.

Key Words: Tooth enamel, neonatal line, prism bending, prism sheath, prismless enamel, Retzius line, fine structure, calcification, scanning electron microscopy, backscattered electron imaging.

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Introduction

The incremental lines of human enamel running concentrically around the long axis of a tooth are generally divided into the regular or rhythmic and the irregular or pathologic lines of Retzius [5, 6, 14]. The rhythmic Retzius lines clearly seen in the surface enamel are arranged in about equal intervals, whereas the pathologic Retzius lines occasionally seen in the enamel may be formed by some abnormal factors [5, 6, 19]. Most Retzius lines show hypocalcification [6, 19], while some pathologic lines of Retzius may show hypercalcification [6].

The neonatal line of the enamel, which divides the enamel into pre- and the postnatal enamel in deciduous teeth and first permanent molars [12, 23, 30], may be a kind of pathologic Retzius line [6] or it may be similar to the rhythmic Retzius line [28]. On the other hand, the neonatal line may be somewhat different from these Retzius lines [30].

It has been reported that the neonatal line, showing hypocalcification [3, 28], is formed by the dislocation of enamel prisms [28, 30] and by irregular prism structures with a disordered crystal arrangement [28]. However, three-dimensional (3-D) computer graphics could not reveal the prism dislocation except for a disorder in the prism arrangement [1]. It has been also reported that the neonatal line contains a relatively large amount of organic material as seen in decalcified sections [24]. No reliable morphological studies of the neonatal line have been reported since 1978 [24, 28, 30]. However, the fine structural and calcification patterns of the neonatal line have not yet been sufficiently elucidated.

In this study, we observed the neonatal line of the enamel by scanning electron microscopy (SEM) after mild etching with several chemical agents and by backscattered electron (BSE) imaging in the scanning electron microscope without chemical treatment. The neonatal line was compared with the Retzius lines previously reported [2, 4, 5, 6, 8, 10, 14, 19, 22, 27, 29].

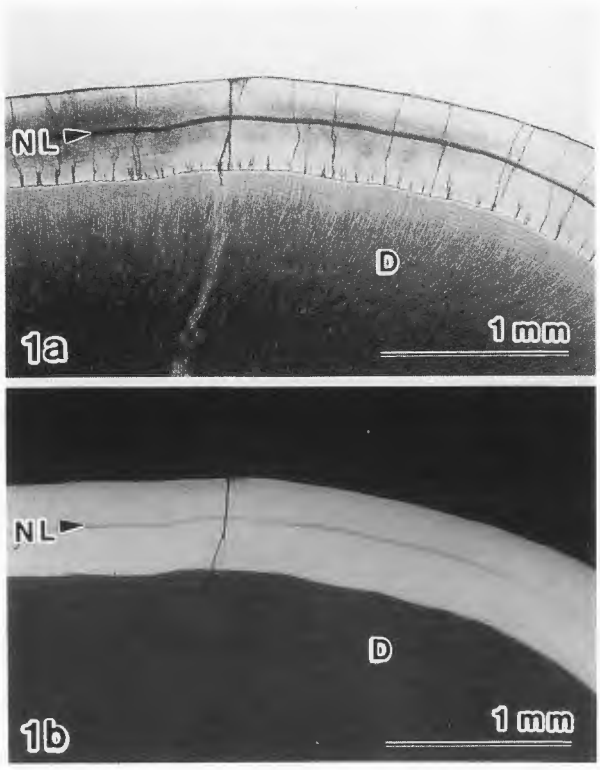


Figure 1. (a) Transmitted light micrograph of the transverse ground section of a deciduous incisor. (b) Microradiograph of (a). NL: neonatal line of the enamel; D: dentin.

Materials and Methods

A large number of exfoliated human deciduous incisor teeth were fixed in 10% neutral formaldehyde and then preserved in 70% ethanol. Twenty caries-free teeth, aged 6 to 7 years, were selected and their mid-crowns were cut transversely into 2 or 3 slices with a diamond wheel. All specimens were ground with grinding stones to obtain about 100-μm-thick sections. They were observed under transmitted light microscopy and microradiographs of the sections were prepared. After the incisal-side ground surfaces were polished with 5- and 0.3-μm alumina on polishing cloths, the polished surfaces of most samples were etched with 0.01 M lactic acid for 5 minutes, 1% ethylenediaminetetraacetic acid (EDTA) at pH 7.2 for 15 minutes [15, 16], or 0.5% chromium sulfate for 17 hours. Lactic acid treatment reveals the crystal arrangement of enamel prisms [14, 18]. EDTA selectively erodes prism sheath regions of the enamel [11, 13], while chromium sulfate retains organic materials in the enamel [7] and the hypercalcified peritubular dentin [25]. This etching was followed by rinsing in running water, dehydration with ethanol and critical point drying from CO_2. The etched surfaces were observed with a S-430 Hitachi (Tokyo, Japan) SEM operated at an accelerating voltage of 15 kV after coating with a 10 to 15 nm gold layer.

The other samples were not chemically treated and were cleaned ultrasonically and air-dried. To determine the extent of calcification, BSE images of the polished surfaces were obtained with a S-2500CX Hitachi SEM at 20 kV after carbon coating.

Results

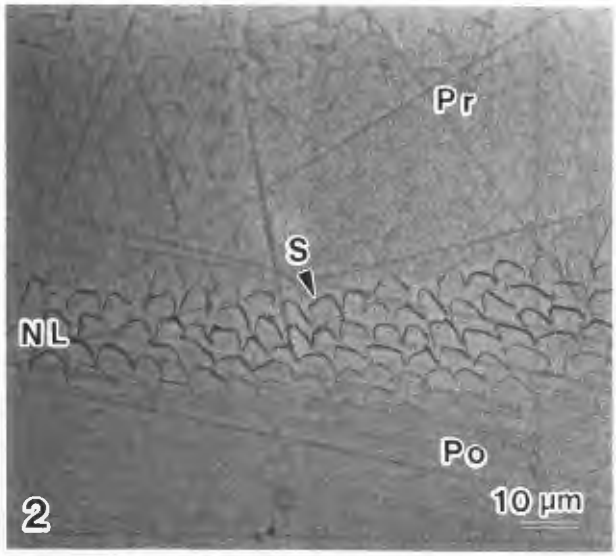
Under transmitted light microscopy of the transverse ground sections of the deciduous teeth, the neonatal line of the enamel appeared as a dark zone (Fig. 1a). In the same section as Figure 1a, the neonatal line showed a lower radiopacity by microradiography than pre- and postnatal enamel (Fig. 1b), although the opacity and thickness varied in the different regions of the enamel as well as in individual teeth.

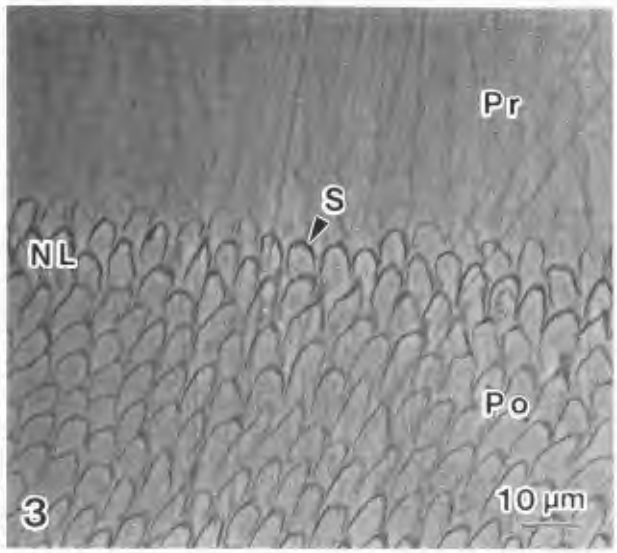
When the polished surfaces of the transverse ground sections without chemical treatment were observed by BSE, the neonatal line had more evident (Fig. 2) or thicker prism sheath regions with a low BSE signal (Figs. 3 and 4), which included prism boundaries and peripheral regions, compared to pre- and postnatal enamel. In addition, the prisms and the interprismatic or prism-tail regions did not show a low BSE signal anywhere in the neonatal line. When EDTA-etched sections were observed by SEM (Fig. 5), the prism sheath regions in the neonatal line were more strongly dissolved than those in pre- and postnatal enamel, and the prisms showed a more or less irregular structure. The thickness of their neonatal lines (Figs. 2 to 5) ranged from about 10 to 30 μm.

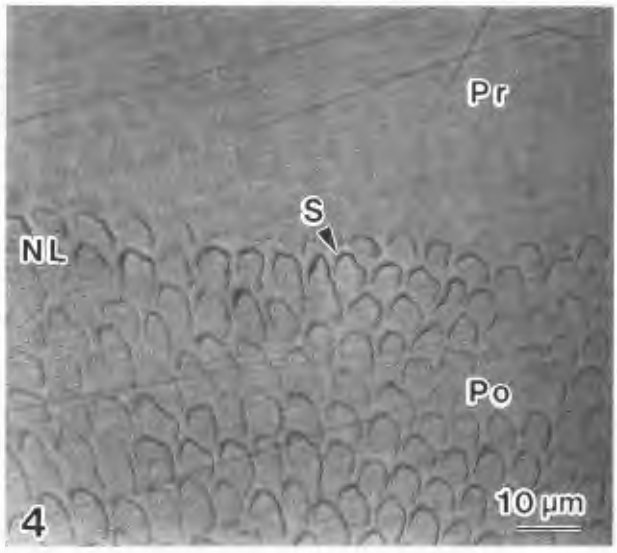
In SEM of samples etched with lactic acid, the zone of the neonatal line was unclear. However, the neonatal line contained areas with a remarkably disordered crystal arrangement (Fig. 6). The arrangement seemed to be formed by minute prism-like structures and wider interprismatic or prism-tail regions. When the sections were treated with chromium sulfate and the incisal-side surfaces were observed by SEM (Figs. 7 and 8), the neonatal line contained a relatively large amount of chromium sulfate-insoluble material in the prism sheath regions with the prism peripheries abruptly rising from the plane of the prenatal enamel. The chromium sulfate-insoluble material gradually decreased towards the postnatal enamel. Some prisms ran longitudinally within the neonatal line surrounded by the cross-sectioned prisms in pre- and postnatal enamel (Fig. 8). The neonatal line (Figs. 7 and 8) measured about 15 to 20 μm in thickness.

The transverse ground surfaces of teeth were etched with EDTA and observed by SEM at higher magnification (Figs. 9 to 12). In Figures 9 and 10, the

Figures 2-4 (at right). Backscattered electron images of the middle enamel layer in deciduous incisors. The polished surfaces of the transverse ground sections were obtained without chemical treatment. NL: neonatal line; S: prism sheath region; Pr and Po: pre- and postnatal enamel.







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prism sheath regions with their surroundings in the neonatal lines adjacent to the prenatal enamel showed a stronger erosion, and the EDTA-soluble regions were gradually weakened towards the postnatal enamel. The neonatal lines were about 15 to 20 μm thick.

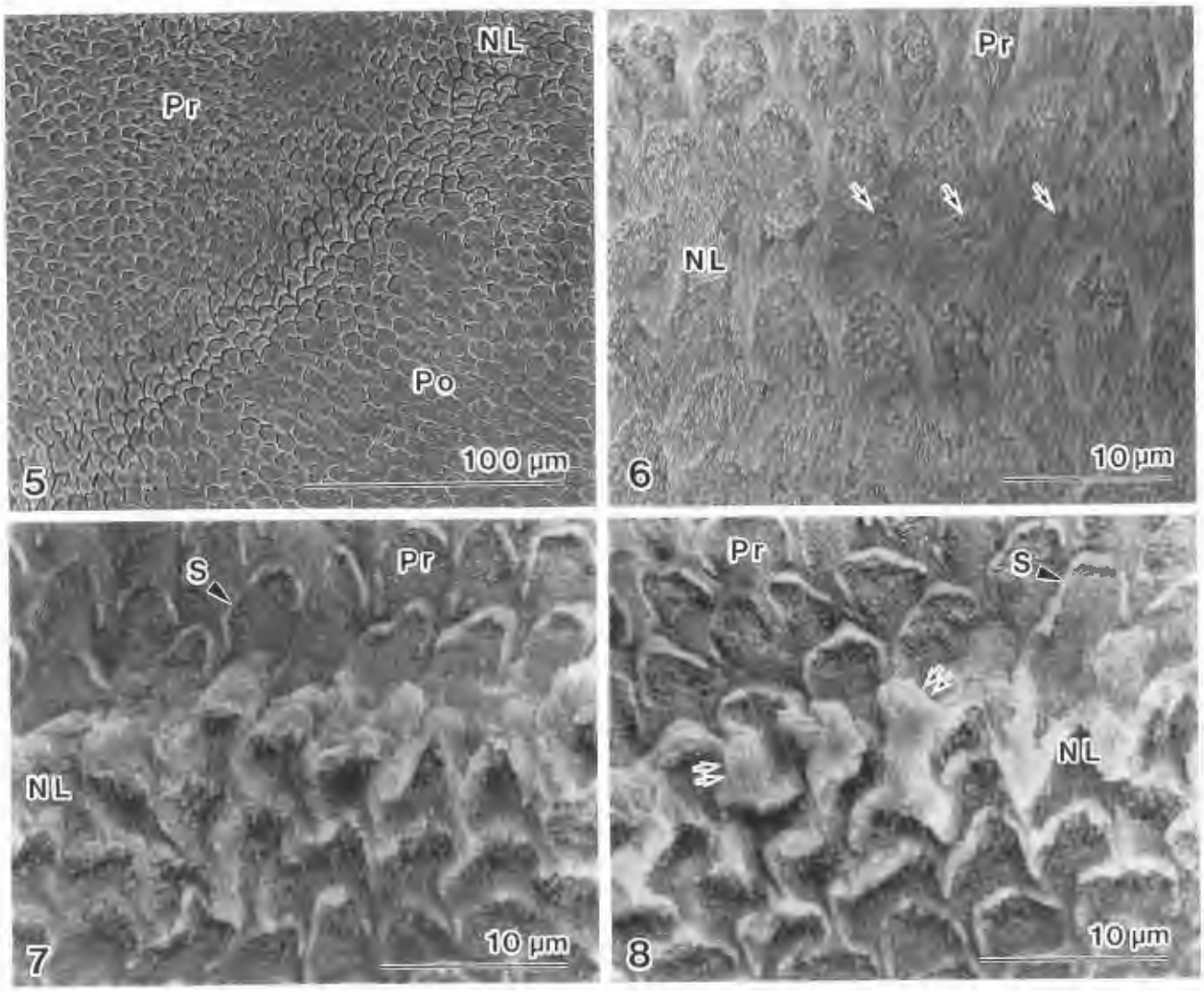
In some cases, when the neonatal line ran through the cervical-side inner surface and the incisal-side surface layer, the transversely to obliquely cut prisms in the prenatal enamel had changed into obliquely to longitudinally cut prisms with clearly etched sheath regions in the postnatal enamel (Fig. 11). That means the prism orientation did not return, and the prisms went straight ahead towards the root after prism bending. Such a neonatal line was recognized as a boundary line between pre- and postnatal enamel. As a result, in this case, the neonatal line had no thickness.

The neonatal line occasionally contained abnormally shaped prisms including stunted (Figs. 9, 10 and 12), circular (Fig. 9), and spiral (Fig. 10) prisms. The fusion of several prisms was also occasionally observed in the neonatal line of some teeth (Fig. 9). Such fused structures are recognized as prismless enamel, although the areas were very small. In the neonatal line of one of the teeth, scattered prismless areas with a blurry outline with indistinct prism structures were observed (Fig. 12). The neonatal line was about 30 μm in thickness.

Discussion

The neonatal line of human tooth enamel, as previously reported [3, 28], showed a somewhat lower degree of calcification than the pre- and the postnatal enamel by microradiography (Fig. 1b). The BSE images of the cross-sectioned prisms in the neonatal line (Figs. 2 to 4) revealed that the hypocalcification appeared in the prism sheath regions or the prism boundaries with the peripheral regions rather than in the prisms and the interprismatic or prism-tail regions, some of which might contain cross-bands or striations [28, 30]. This indicates that the expanded sheath regions as noted by Weber and Eisenmann [28] show a lower density of crystals than the cross-striations. Thus, the prism sheath regions will selectively be dissolved with HCl [30] and EDTA as shown in Figures 5, 9, and 10 [11, 13, 15, 20].

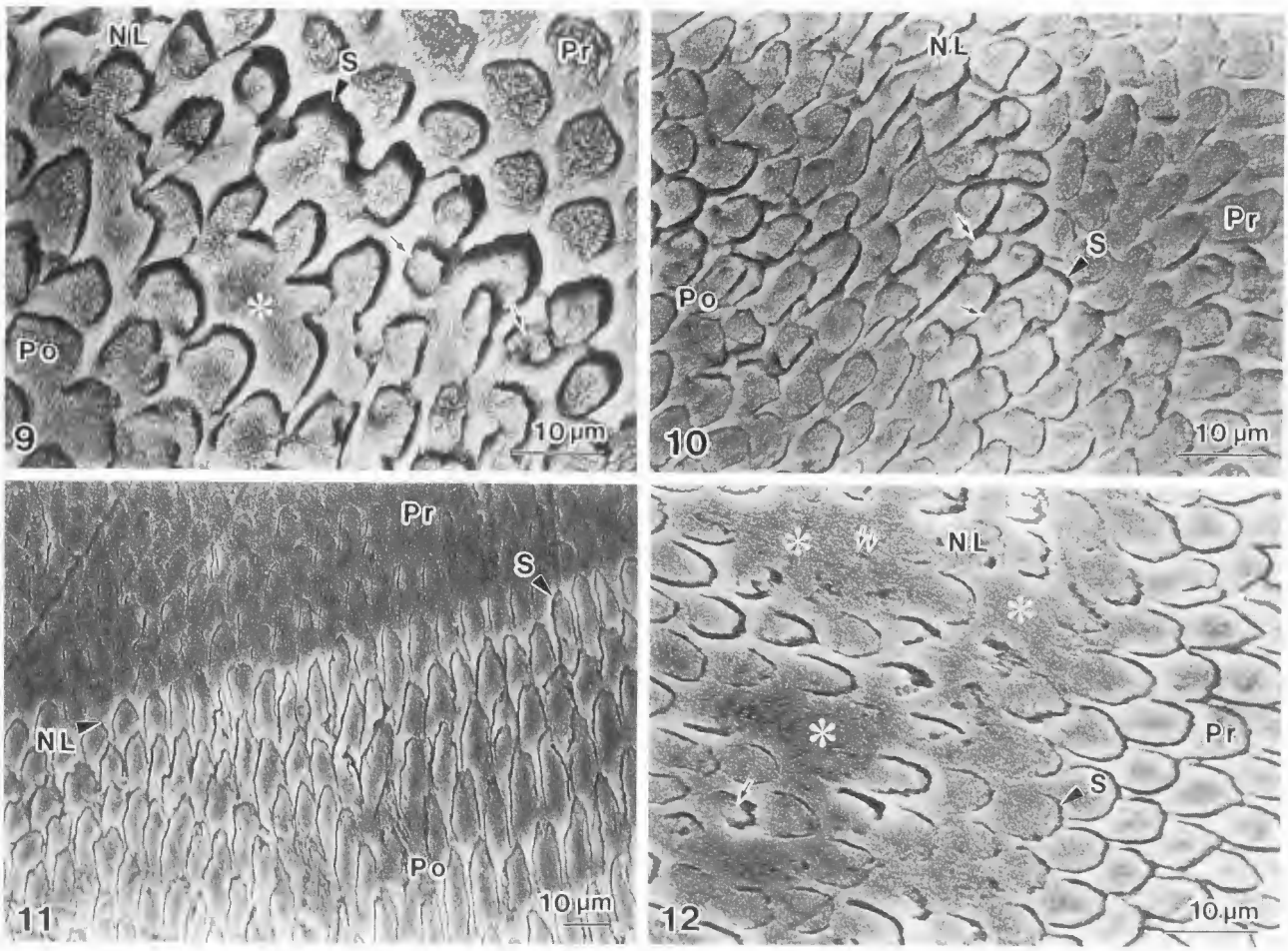
Sognnaes [24] reported that the prism sheath regions in the neonatal line contained a relatively large



Figures 5-8. Scanning electron micrographs of the middle enamel layer in transverse ground sections of deciduous incisors. The polished surfaces were etched with EDTA (**Fig. 5**), lactic acid (**Fig. 6**), and chromium sulfate (**Figs. 7 and 8**). NL: neonatal line; S: prism sheath region; arrow: minute prism-like structure; double arrow: prism running longitudinally; Pr and Po: pre- and postnatal enamel.

amount of trichloroacetic acid-insoluble material. In this study, the sheath regions with their surroundings contained a larger amount of chromium sulfate-insoluble material instead of low density enamel crystals than those of the pre- and the postnatal enamel (Figs. 7 and 8). Gustavsen and Silness [7] reported that the compartments of prism sheath regions containing the organic subcompartments in the crystals differed from those of the prisms, when mature enamel of human teeth was decalcified with chromium sulfate and examined by transmission electron microscopy. The organic material in the neonatal line may be strongly absorbed by crystal surfaces but fully present within crystal cores.

It is known that the neonatal line shows a dislocation of the prisms [28, 30]. Whittaker and Richards [30] suggested that the prism orientation of the prenatal enamel was almost the same as that of the postnatal enamel. In this study, we confirmed that such a neonatal line was formed by the double bending of the prisms; at first, the prisms abruptly bent towards the root and gradually bent back again to regain their previous orientation as seen after chromium sulfate treatment (Figs. 7 and 8) and EDTA etching (Figs. 9 and 10). When etched with lactic acid, the minute prism-like structures and the wider interprismatic or prism-tail regions, which showed a disordered crystal arrangement as reported by Weber and Eisenmann [28], were observed in the neonatal line by SEM (Fig. 6).



Figures 9-12. Scanning electron micrographs of the EDTA-etched enamel surfaces in transverse ground sections of deciduous incisors. **Figures 9, 10, and 12** show the middle layer and **Figure 11** shows the cervical-side inner layer. NL: neonatal line. S: prism sheath region; *: prismless area; arrow: abnormal prism; Double arrows: indistinct prism structures; Pr and Po: pre- and postnatal enamel.

The scanning electron micrograph should be interpreted as showing that the prisms bend abruptly. The double bending of the prisms may be caused by reversible morphological changes in Tomes' processes [2, 9, 16, 22, 26, 27], such as shrinkage or shift of position and then regaining of their original orientation, while ameloblasts, which might temporarily change their orientation [2, 9, 22, 27], will gradually leave the first-formed surface of the neonatal line. In the simple bending (Fig. 11), on the other hand, the shapes of Tomes' processes may remain the same through the postnatal prismatic enamel after shifting of position.

The thickness of the neonatal line ranged from about 10 to 30 μm thick to zero thickness of the simple bending in this study. Whittaker and Richards [30] reported that the thickness exceeded 40 μm in the majority of the specimens, although zero thickness was also present. The heterogeneous thickness of the neonatal lines may be caused by variable duration and intensity of stress against ameloblasts in the different regions of the enamel as well as in individual teeth.

The "prismless" enamel, which occasionally contains abnormal and indistinct prism structures, is commonly distributed in the surface layer of the enamel [15, 16, 18] but relatively rarely in the inner layer [17]. In some teeth with enamel hypoplasia, however, the prismless enamel is scattered from the surface to the inner layer [19] or in the middle layer surrounded by the pathologic Retzius lines [21]. In this study, small prismless areas were found in some neonatal lines (Fig. 9). The neonatal line of one of the teeth had scattered prismless areas with a blurred outline with indistinct prism structures (Fig. 12). Previously, we have shown that the crystal orientation of the surface prismless enamel is continuous with that of the interprismatic or prism-tail regions [17]. This means that

the crystal orientation in the neonatal line containing prismless structures differed from that of the prisms in the pre- and the postnatal enamel as seen in the prismatic neonatal line. In such a neonatal line, some or many Tomes' processes may have temporarily disappeared or remarkably shrunk [16, 18, 19, 22].

In enamel showing prism orientation disorders, for example in the Hunter-Schreger bands [26], gnarled enamel [20], and inner enamel adjacent to the innermost enamel of an about 10 to 15 μm layer [1, 17, 22], abnormal shaped prisms have been reported, although there are no reports on the Retzius lines except for constricted prisms or the remarkable cross-striations in the rhythmic Retzius lines [14], as shown in Figure 6. We found that the neonatal line occasionally contained such abnormal-shaped prisms as stunted, circular, and spiral. These prisms are probably caused by the temporary changes in the Tomes' process shapes.

According to previous studies, the rhythmic Retzius lines are basically formed by the double bending of the prisms in a narrow zone [4, 6, 10, 14, 27, 29] with the thin cross-bands or striations [2, 5, 8, 14, 22, 29], whereas the pathologic Retzius lines are known as the wider zone of the successive clear cross-striations [5, 6, 14] or the zone showing a disorder of prism orientation [6, 8, 14]. The hypocalcified regions of the rhythmic Retzius lines are probably present in the prism-bending zone containing the cross-striations, whereas the hypocalcified regions of the pathologic Retzius lines may be seen in the successive clear cross-striations [5, 6, 14], the prism sheath regions [6, 19], or the prisms themselves for a relatively long distance [14]. Therefore, the neonatal line formed by the double bending of the prisms, as already suggested [28], is likely to show a particular type of the rhythmic Retzius rather than the pathologic Retzius lines, except for the simple bending of the prisms and the occasional appearance of prismless areas. On the other hand, the lower calcified and expanded prism sheath regions may be similar to those of some pathologic lines of Retzius [6, 19].

In summary, we obtained several findings on the neonatal line of human tooth enamel by BSE and SEM, which add to those in previous studies [24, 28, 30].

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Discussion with Reviewers

Reviewer I: If all ameloblasts on the developing enamel drifted 5 μm cervically, the surface of the ameloblast layer facing the developing enamel would become, at least two-dimensionally, 10 μm longer than the surface of the developing enamel. However, the number of cells composing the ameloblast layer is fixed. How are those disagreements in length between enamel surface and ameloblast surface compensated?
Authors: Probably, ameloblasts showing reversible morphological changes of Tomes' processes [2, 9, 17, 22, 26, 27] gradually leave the first-formed surface of the neonatal line during the period of forming the neonatal line. The basic movement of the ameloblast might be similar to that of the entire enamel formation [2, 22, 26], although the prism bending may occur in the neonatal line. When ameloblasts are under some stress for a long time after birth, for example, jaundice of the newborn, the distance between the layer of enamel ameloblasts, which have finished forming the neonatal line, and the first-formed surface of the neonatal line will be more than 10 μm.

Reviewer III: The double bending should be more apparent in longitudinal rather than in transverse sections. However, all figures are from sections cut transversely.
Authors: Indeed, only transverse sections of the teeth were used in this study. Unfortunately, we could not observe the prism through the neonatal line running from pre- and postnatal enamel in the longitudinal sections anywhere, although we previously have reported the prism crossing over the rhythmic Retzius line [14]. In the transverse sections, when the single bending of prisms is performed, the prism orientation will take on the appearance shown in Figure 11. On the other hand, when the oblique to longitudinally cut prisms in the neonatal line and the cross-sectioned prisms in the pre- and the postnatal enamel are observed, the prisms should bend towards the root at first and then bend back again to regain their previous orientation in the neonatal line. We believe that Figures 7 and 8 clearly illustrate the double bending of the prisms.

Reviewer III: Can you comment further on the neonatal line: the moment when it is formed, why and how?
Reviewer IV: What is the original information in the present paper?
Authors: In 1936, Schour [23] found one set of remarkable incremental lines formed at the same time in the enamel and the dentin of human deciduous teeth and first permanent molars. He introduced the term **neonatal lines** of the enamel and the dentin based on the periods of the formation of the teeth. Since 1936, this remarkable incremental line in the enamel, as well as in the dentin, has been recognized as the neonatal line between the pre- and the postnatal enamel [2, 3, 5, 6, 12, 24, 28, 30]. The neonatal line may be due to the fact that ameloblasts are subjected to stress at birth or as a result of jaundice of the newborn; however, there is as yet insufficient evidence for this theory.

The neonatal line has previously been described by light microscopy (LM) [24], transmission electron microscopy (TEM) [28], and SEM [30]. However, the structure and calcification patterns have not been sufficiently elucidated in the published figures, especially the 3-D structure of the prism bending [28, 30]. We now clearly illustrated these structures by BSE imaging without etching and by SEM after mild etching. Moreover, we found that the neonatal line occasionally con-

tained abnormally shaped prisms and prismless areas. In a comparison between the rhythmic and pathologic lines of Retzius, we obtained results which somewhat differed from previous reports [6, 28, 30].

Reviewer IV: Please elaborate on the biological developmental mechanisms in enamel.
Authors: There have been no reports on the structure of the neonatal line during its formation or immediately after, because it has not been possible to collect human teeth from new-borns. Therefore, it is not possible to discuss any mechanisms except for the temporal morphological changes of the Tomes' processes [2, 9, 20, 22, 26, 27]. We think that the data are too limited to warrant further discussion.

Reviewer IV: Can the authors really distinguish between original structure and artefactual structure which develops as a result of the preparation procedure for the specimens? What the authors and others call etching in respect of enamel is the combined result of dissolution at crystal surfaces in depth within the tissue (which is strongly influenced by the accessibility of the microscopic location to the etching fluid), reprecipitation phenomena occurring simultaneously and at the moment of cessation of the etching process, and shrinkage distortion and cracking developing in the partially demineralized three-dimensional layer in the surface which has been attacked. It is not possible to interpret these effects in a superficial way.
Authors: If SEM using etching agents is considered to be artificial, TEM of decalcified sections of enamel must also be considered to be artificial. However, even when undecalcified enamel is sectioned, the prism boundaries with lower crystal density may undergo some fracturing by the physical stress. According to this view, the ground enamel surfaces in BSE and the fractured surfaces in SEM would also be artificial. We do not regard the images of the etched enamel in SEM to be entirely artificial, because the images of the etched specimens must be derived from the original structures of the enamel, even though some features in the images may be exaggerated.

Reviewer IV: A prism sheath is not a structure which occurs in normal enamel, but one which develops as a result of acid etching of enamel. The term is mistakenly used in the literature to refer to the tissue lying at prism boundaries or junctions (prism boundary discontinuities) but proper scholastic treatment of the literature will make it quite clear that the sheath is the artifact of etching.
Authors: Enamel principally consists of apatite crystals. However, the terms "prism sheath" in LM and "prism boundary" in TEM are commonly used in a number of references and text books. In this study, we used the term "prism sheath region" according to the study of Gustavsen and Silness [7]. When BSE or SEM is carried out on unetched or etched specimens, this term means a prism boundary with the peripheral region. Certainly, the prism boundaries are usually discontinuous in the greater part of the enamel. However, the prism sheath regions are sometimes connected with each other as seen in our SEM observations of etched specimens [14, 15, 17], and this has also been published elsewhere [7, 8, 10]. In addition, the continuous sheath regions are frequently seen in the outer enamel layer in SEM of etched specimens [31]. On the other hand, the Tomes' processes will be more or less temporally shortened at the beginning of the neonatal line with and without prismless enamel, due to the abrupt bending of prisms in the neonatal line (Figs. 7-10) and the prismless enamel formation (Fig. 9) [17, 19, 20]. In such regions, we consider that the prism sheath regions (prism boundaries with the peripheral regions) are likely to become connected.

Reviewer IV: Enamel is a continuous structure only partly sub-divided by its discontinuities. It is as much nonsense to talk about prism and interprismatic regions as it is to talk about heads and tails. It is equally nonsensical to say that several prisms are continuous. It is the normality for all sub-regions in enamel to be connected with all other sub-regions.
Authors: As mentioned above, enamel consists principally of apatite crystals. According to this view, the enamel is certainly continuous. However, prisms and interprismatic regions or prism heads and tails are commonly used terms as well as prism sheaths or boundaries. Regarding your comment that several prisms are continuous, we indeed illustrated the fusion of several prisms. As mentioned previously, in the neonatal line as one of the abnormal zones, we regard the fusion of several prisms to be temporally formed by the degeneration of Tomes' processes. Basically, the prismless enamel contains no prism sheath regions because of the parallel crystal arrangement (see previous answer). In general, however, the prismless enamel occasionally contains several abnormal-shaped prisms [17-20]. The fused areas of several prisms can be identified as prismless enamel which is locally formed.

Additional Reference

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