Clinical and histologic appearance in enamel of primary teeth from children with neonatal hypocalcemia induced by blood exchange transfusion

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This investigation was undertaken to study the clinical and histologic appearance of deciduous enamel from 11 infants who were subjected to blood exchange transfusions (ET) during the first days of life. As a result of the treatment they had a mean of 3 consecutive hypocalcemic days. At the age of 5 years a dental examination of their primary teeth was performed. Four of the children had clinically recorded enamel aberrations correlated with the neonatal period. Exfoliated teeth were then examined histologically. All had a normal major enamel morphology, and the neonatal line was present in all teeth. Histologically investigated teeth with enamel hypoplasia had the aberrations located close to the neonatal line. The conclusions drawn from this study are that hypocalcemia caused by ET in the newborn period did not affect the width of the neonatal line or the major enamel morphology to any extent. Enamel aberrations occurred only when four or more ETs were performed. □ *Dental enamel; enamel hypomineralization; enamel hypoplasia; ionized calcium; neonatal line*

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Each tooth accompanies a child for a long time, and the mineralized enamel contains a history of the child's early life and events (1–3). Factors such as trauma and various medical conditions are known to disturb tooth formation, and as a result enamel aberrations develop (3–5). Among medical conditions, hypocalcemia has been proposed by several investigators to be one of the main etiologic factors underlying enamel aberrations (3, 5–9) and the appearance of the neonatal line (9, 10).

However, a recently performed clinical and histologic investigation of deciduous enamel from healthy infants with low values of blood ionized calcium on the first days after birth showed no relationship between calcium values and the morphology of deciduous enamel (11). Nevertheless, it is of interest to further evaluate the effect of very low values of blood ionized calcium during the first days of life on the enamel morphogenesis.

The purpose of this investigation was to study the deciduous enamel, clinically and histologically, in a group of children who had very low values of blood ionized calcium on the first days of life, owing to their need for blood exchange transfusions.

Materials and methods

Thirty-five infants took part in a study, in which blood ionized calcium was measured before, during, and after blood exchange transfusion, performed on their first days postpartum (12). At the time of this investigation, 11 children were available for a detailed dental examination of their primary teeth.

Medical data

All children had the diagnosis of hemolytic disease of the newborn and were treated with one or more blood exchange transfusions (ET) during the first days of life. Repeated blood samples were collected from each child, and measurements of blood ionized calcium were performed on an ICA-1 (Radiometer A/S, Copenhagen, Denmark) (12).

The following medical data concerning each child were compiled: gestational age at birth, mode of delivery, birth weight, complications at birth, medical treatment during the first weeks postpartum, number of ETs, day postpartum when the first ET was performed, initial value of blood ionized calcium before ET, number of days postpartum when low values of blood ionized calcium were recorded, and any calcium supplement during or after ET.

Clinical examination

A detailed case history concerning the first 2 years was taken, including facts concerning medical health, time of tooth eruption, trauma, and fluoride exposure. After parental consent, data were also collected from the medical files with regard to postnatal growth, development, and general health. Two of the authors (L.

Table 1. Classification of enamel defects

Types of defects
Hypoplasia (P)
Hypomineralization (M)
M with substance loss (MSL)
Localization of defects
Gingival half
Incisal half
Occlusal
Cuspal
No. of defects per located surface
1
2
≥3
Extent of defects on located surface
<1/4
>1/4 <1/2
>1/2
Appearance
Circle
Grooves
Diffuse, patchy
Diffuse, fine white lines
Pits
Colour
White/cream
Yellow
Brown

Ranggård and J. Östlund) examined the primary teeth, which included registration of number of teeth, number of decayed, extracted, and filled surfaces (def-s), and a characterization of enamel defects (Table 1).

Enamel hypoplasia (P) was defined as a quantitative defect of the enamel involving the surface, associated with reduced enamel thickness. The borders of the defect had to be rounded and smooth. Enamel hypomineralization (M) was defined as a qualitative defect of enamel, visually identified as a change in its translucency, seen as a discoloration on the surface.

The fluoride content in the children's drinking water was assessed by the Public Dental Health Clinics.

Histologic examination

One exfoliated deciduous tooth was obtained from each child. The teeth were coded, and the histologic examination was thus made blind. The teeth were placed in absolute alcohol for 24 h and thereafter embedded in Epofix (Struers, Copenhagen, Denmark), a cold-mounting epoxy resin.

Sagittal buccolingual 100-μm-thick sections were prepared with a Leitz 1600 low-speed saw microtome (13). After being polished, the sections were examined in a polarized light microscope (Olympus BH), using strain-free objectives, both dry in air and after water absorption (24 h).

The width of the neonatal line was measured with a Leitz Microvid equipment connected to a computer.

Table 2. The range and mean blood ionized calcium values (in mmol/l), measured at first ($n = 11$) blood exchange transfusion (ET) and at additional analyzed blood exchange transfusions ($n = 13$ of 23). The initial value was measured at the start of ET, and the lowest value was measured during or immediately after ET

ET	Mean	Range	No.
First			11
Initial	1.12	0.87–1.23	11
Lowest	0.44	0.24–0.69	11
Additional			23
Initial	0.86	0.54–1.13	13
Lowest	0.43	0.17–0.76	13

The measurements, which had an accuracy of 0.1 μm, were performed directly in the microscope (14). The neonatal line was identified and then measured in an area in the middle part of the enamel between its inner and outer limits. A magnification of 40× was used, and five measurements of the neonatal line were performed on a length of 220 μm.

Analysis of data

For the statistical analysis Student's t test and linear regression analysis were used.

Results

Medical data

The 11 newborn infants had a mean gestational age of 35 (range, 31–41) completed weeks. The mean birth weight was 2524 g (range, 1230–3640 g).

Three children were delivered with a normal vertex presentation, five by planned caesarean section, and the last two (identical twins, Patients 7 and 8 in Table 3) were delivered by acute caesarean section. Postnatal asphyxia (Apgar score <7 at 5 min postpartum) was recorded for one of the infants (Patient 4 in Table 3).

Blood exchange transfusions

Altogether, 34 blood exchange transfusions (ET) were performed on the 11 infants. The number of ET needed for each child varied within the group from one to eight, with a mean of three. Nine infants had their first ET during their 1st day of life, one on the 2nd day, and one on the 3rd day of life.

Before the first ET the levels of blood ionized calcium were analyzed for each child ($n = 11$). Two infants were hypocalcemic with values below 1.10 mmol/l (15, 16) already before first ET; six infants had ≥ 1.10 mmol/l but less than 1.20 mmol/l, and the last three infants had ≥ 1.20 mmol/l. During the first ET ($n = 11$) the values of blood ionized calcium decreased (Table 2) to very low values in all infants ($p < 0.001$).

Table 3. For each patient the number of teeth with enamel hypoplasias (P) and/or enamel hypomineralizations (M) is presented. The calculated mean width (μm) of the neonatal line is presented in the second column

Patient	Hypoplasias/ hypomineral.	Neonatal line width
1	1M	19.4
2	6P	4.7
3	1M, 1P + M	8.3
4	1P	16.1
5	4P, 1M	14.2
6	3M	2.7
7	2P	8.0
8	9P, 1M	5.7
9	None	7.2
10	None	6.8
11	None	5.6

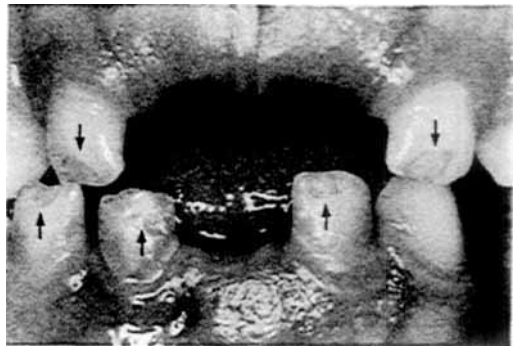

Fig. 1. A child with multiple enamel hypoplasias (arrows). Missing teeth were also affected, but at the time of this photo the upper ones were extracted owing to trauma, and the lower one was exfoliated.

Eight of the infants needed more than 1 ET, and at 13 of totally 23 additional ETs, the blood ionized calcium was analyzed. The initial values of blood ionized calcium at additional ETs was significantly lower ($p < 0.001$) than the initial values of blood ionized calcium at the first ET (Table 2). The additional ETs had the same result as the first ET (Table 2): very low blood ionized calcium values ($p < 0.001$).

On the basis of the number of days with ET treatment and, further, the blood analysis demonstrating hypocalcemic values (15, 16) of blood ionized calcium, the number of hypocalcemic days was calculated for each child. The children had a mean of 3 hypocalcemic days, with a range from 1 to 4 days.

Calcium supplementation can be given during or after a blood exchange transfusion to increase the very low blood calcium values that develop during the ET (12). Seven of the infants received calcium infusions during blood exchange transfusion, and within 1 min after the first calcium injection the mean blood ionized calcium value was 2.11 mmol/l (range, 1.45–2.65 mmol/l). However, already after 5 min the mean value of blood ionized calcium was 0.53 mmol/l (range, 0.34–0.90 mmol/l). One child also received calcium orally after blood exchange transfusion, owing to prolonged hypocalcemic values (4 days).

Clinical findings of enamel aberrations

The frequency of enamel hypomineralization was 45% and of enamel hypoplasias 55%. Both types were present in 27% of the cases.

Eight of the 11 children had one or more teeth with enamel aberrations (Table 3 and Fig. 1). Two of the children had one enamel aberration each, two had two aberrations each, and four children had 3, 5, 6, and 10 affected teeth, respectively.

Patients 2, 5, 7, and 8 (Table 3) had enamel hypoplasia, located symmetrically and at levels on the teeth corresponding to the gestational age of the infant at birth. These four infants had in common that they had all received more than four ETs, that they had at least 2 consecutive days when ET was performed, and that they had at least 3 days with hypocalcemia. Further, they all had a gestational age less than 37 completed weeks, and none of them had a birth weight of more than 2800 g. In Table 4 these factors are presented for all 11 infants. The main difference between infants with multiple and symmetric neonatal enamel aberrations ($n = 4$) and the other infants ($n = 7$) was the large number of ETs and the number of consecutive days on which additional ETs were performed.

The fluoride exposure was low in all children ($n = 11$); six of them were caries-free at the examination, and the other children had caries in relation to the enamel

Table 4. The number of blood exchange transfusions (≥ 4 ETs), the consecutive number of days with ET treatment (≥ 2), the number of hypocalcemic days (≥ 3), the gestational age (≤ 36 weeks), and the birth weight (≤ 2800 g) were the common factors for the four infants (Patients 2, 5, 7, and 8) with symmetric and multiple enamel aberrations. Fulfilled criterion (X) for each factor is presented for all infants

Patient	ETs ≥ 4	ET days ≥ 2	Hypocal. ≥ 3 days	Gest. age ≤ 36 weeks	≤ 2800 g
2	X	X	X	X	X
5	X	X	X	X	X
7	X	X	X	X	X
8	X	X	X	X	X
1	—	—	—	—	—
3	—	—	—	X	—
4	—	—	—	X	X
6	—	—	—	—	—
9	—	—	—	—	—
10	—	—	X	X	X
11	—	—	X	X	X

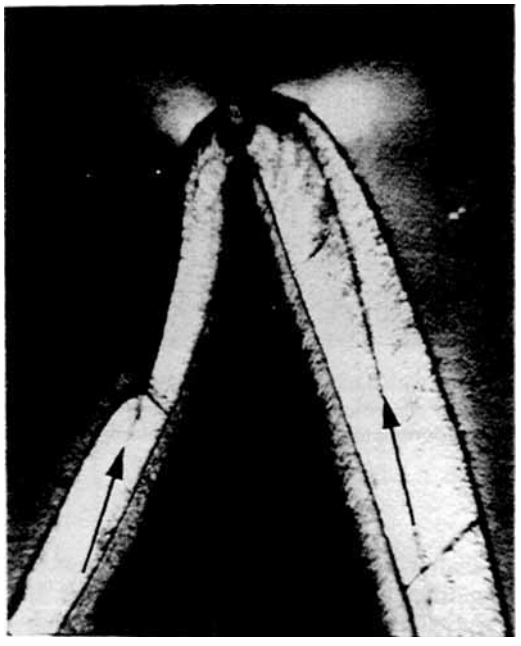

Fig. 2. A ground section of a deciduous lower incisor from a preterm infant. The neonatal line (arrows) is located incisally. Lingually, enamel hypoplasia is seen, with the neonatal line at its bottom.

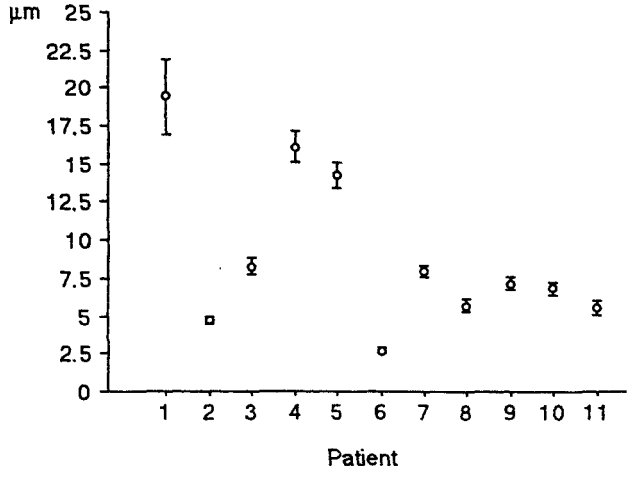

Fig. 3. The mean ± SEM width after five measurements of each measured neonatal line.

aberrations seen. None of the infants were exposed to any known dental trauma before the age of 2 years.

Histologic observations

One exfoliated primary tooth was obtained from each of the 11 children. Four of them were upper incisors (three medial, one lateral), and the other seven were lower incisors (six medial, one lateral).

The neonatal line was found in all investigated incisors. The location of the line varied on the basis of gestational age. Children with low gestational ages thus had neonatal lines located more incisally than those who were full-term (Fig. 2).

The neonatal line width varied within each specimen; thus a mean value of the neonatal line width was calculated for each tooth (Fig. 3). The mean width ranged from 2.7 µm to 19.4 µm (Table 3), with a median value of 7.2 µm. There was no statistically significant correlation between the number of hypocalcemic days or the number of ETs performed and the neonatal line width.

The presence of the neonatal line made it possible to distinguish the prenatal enamel from the postnatal enamel. The enamel generally had a high degree of mineralization, seen as a negative birefringence both in air and after water absorption, indicating a pore volume distribution of less than 1% (9). Incisally, mostly on the buccal side, the enamel appeared to have an area of less mineralized enamel, positively birefringent in air and negatively after water absorption, indicating a poor volume distribution between 1% and 5% (9). In full-term infants this area was located in the prenatally formed enamel, but in preterm infants both pre- and post-natal enamel was involved, since the neonatal line passed through this area.

In three of the teeth (27%) small areas of hypomineralized enamel with a pore volume distribution of less than 5% but more than 1% were seen beneath a well-mineralized surface in the cervical area of the postnatal enamel. These lesions were not associated with the neonatal line, which was located in earlier formed enamel.

Four children had clinically recorded enamel hypoplasias, located symmetrically and at levels on the teeth corresponding to the gestational age of the infant at birth. One tooth from each of those children was among the teeth that were analyzed histologically. One of them had buccal enamel hypoplasia, two had lingual enamel hypoplasias, and one had enamel hypoplasias located both buccally and lingually. All hypoplasias were located on the incisal part of the crowns, in relation to the neonatal line, with the neonatal line in the bottom part of the enamel aberration (Figs. 2, 4).

Discussion

In newborn infants blood ionized calcium is not measured unless a hypocalcemic state is suspected. It is therefore difficult to determine whether there is a connection between neonatal hypocalcemia and enamel aberrations detected many years later at a dental examination. Thus this investigation had two main advantages despite the limited number of patients: all infants developed hypocalcemia owing to blood exchange transfusion, and, further, measured values of blood ionized

Fig. 4. A ground section of a deciduous lower incisor with enamel hypoplasia in relation to the neonatal line (arrows) (E = enamel; D = dentin).

calcium before, during, and after blood exchange transfusion enabled acquisition of detailed information about the hypocalcemic state. These facts in combination with medical and odontologic case histories gave us new possibilities for evaluating clinical and histologic findings of the enamel during a hypocalcemic state.

During a blood exchange transfusion a great deal of ionized calcium disappears from the blood. Thereafter, the blood ionized calcium usually normalizes spontaneously within the following 24 h (12). Repeated blood exchange transfusions aggravate the hypocalcemic state, explaining why some of the infants received a calcium supplement during the next blood exchange transfusion, with the aim of preventing extremely low values of blood ionized calcium. However, it has been found that after 5 min the effect of the calcium injection disappears (12). Consequently, calcium supplementation during blood exchange transfusion is no longer routine in many neonatal intensive care units.

Healthy, newborn infants normally have low, but not by definition hypocalcemic, values of blood ionized calcium during the first days of life (17). With two exceptions the initial values of ionized calcium before the first blood exchange transfusion were typical of newborn, healthy infants. The hypocalcemia measured during and after blood exchange transfusion should thus mainly be attributable to the ET.

Of special interest are enamel hypoplasias, located on several teeth with symmetric and chronologic distribution, as they are probably caused by the same factor and during the same period (18–20). This was seen in four cases. The histologic examination showed that these enamel aberrations had occurred during the neonatal period, as the enamel hypoplasias were located in immediate relation to the neonatal line (9). Common to all the newborn infants in the present study was their need for ET. Despite severe hypocalcemia caused by the ET, most of the infants had no enamel aberrations that had occurred neonatally. Only infants subjected to more than four ETs had affected teeth. The enamel aberrations seen in those infants might be caused by the extreme hypocalcemia but may as well have been caused by other unknown factors.

Histologically, the enamel morphology was typical for primary teeth (9, 11). Incisally, a less mineralized area within the prenatal enamel, mainly buccally, of full-term infants is common. However, in preterm infants this microporous zone extended into the postnatal enamel. This can be explained by the more incisally located neonatal line in preterm infants, which thus divided the hypomineralized area into a prenatal and a postnatal portion.

The neonatal line width was measured, as hypocalcemia is mentioned as one factor contributing to the neonatal line width (9, 10). In a recent investigation of optimally selected infants there was no correlation between low but normal values of ionized blood calcium during the first days postnatally and the neonatal line width (11). The present investigation of newborn infants with 1–4 consecutive days of hypocalcemia showed no significant correlations between clinical data and neonatal line width. The neonatal line does not seem to be affected by the low values of blood ionized calcium occurring during the first days of life.

Among other theories as to why the neonatal line occurs, the trauma of birth itself has been proposed to be of central importance (5, 21, 22). Thus, the neonatal line width in infants delivered by caesarean section is of special interest. It has been proposed that infants have thinner neonatal lines when delivered by caesarean section, as they are not exposed to the normal birth trauma (21). This was not seen in this study, as the children delivered by caesarean section had both thin and wide lines. Thus, the cause of the neonatal line is still unclear, and further investigations are needed to explain how and why it occurs.

The conclusions drawn in this study are that all infants selected for analysis of their primary teeth were hypocalcemic for at least 1 day. Enamel aberrations correlated to the neonatal period were seen in infants who received four or more ETs, but otherwise not. Thus the hypocalcemic state is probably not responsible for enamel aberrations to any great extent, as earlier

believed. This is further supported by the fact that the neonatal line width was mainly thin and was not correlated with the duration or degree of the hypocalcemic state.

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References

1. Lunt RC, Law DB. A review of the chronology of calcification of the deciduous teeth. J Am Dent Assoc 1974;89:599–606.
2. Fejerskov O. Odontogenesis. In: Mjör IA, Fejerskov O, editors. Histology of the human tooth. Copenhagen: Munksgaard, 1979: 21–32.
3. Magnusson BO, Persliden B. Development and its aberrations. In Magnusson BO, editor. Pedodontics. A systematic approach. Copenhagen: Munksgaard, 1981:79–128.
4. Pindborg JJ. Aetiology of developmental enamel defects not related to fluorosis. Int Dent J 1982;32:123–34.
5. Seow WK. Enamel hypoplasia in the primary dentition: a review. J Dent Child 1991;58:441–52.
6. Stimmler L, Snodgrass GJA. Jaffe E. Dental defects associated with neonatal symptomatic hypocalcemia. Arch Dis Child 1973; 48:217–20.
7. Levine RS, Keen JH. Neonatal enamel hypoplasia in association with symptomatic neonatal hypocalcemia. Br Dent J 1974;137: 429–33.
8. Nikiforuk G, Fraser D. Etiology of enamel hypoplasia and interglobular dentin. The roles of hypocalcemia and hypophosphatemia. Metab Bone Dis Rel Res 1979;2:17–23.
9. Norén JG. Human deciduous enamel in perinatal disorders. Morphological and chemical aspects [thesis]. Göteborg: Göteborg University, 1983.
10. Norén JG, Koch G, Rasmussen P. Disturbances in tooth development and eruption. In: Koch G, Modéer T, Poulsen S, editors. Pedodontics—a clinical approach. Copenhagen: Munksgaard, 1991:250–74.
11. Ranggård L, Norén JG, Nelson N. Clinical and histologic appearance in enamel of primary teeth in relation to neonatal blood ionized calcium values. Scand J Dent Res 1994;102:254–9.
12. Nelson N, Finnström O. Blood exchange transfusions in newborns, the effect on serum ionized calcium. Early Hum Dev 1988;18:157–64.
13. Norén JG, Engström C. Das Schneiden mineralisierter, harter Gewebe mit dem Leitz-Sägemikrotom 1600. Leitz Mitt Tech 1987;9:49–52.
14. Johansson CB. On tissue reactions to metal implants [thesis]. Göteborg: Göteborg University, 1991.
15. Cruz ML, Tsang RC. Disorders of calcium and magnesium homeostasis. In: Yeh TF, editor. Neonatal therapeutics. 2nd ed. St Louis (MO): Mosby Year Book, 1991:253–71.
16. Loughead JL, Mimouni F, Tsang RC. Serum ionized calcium concentrations in normal neonates. Am J Dis Child 1988;142:516–8.
17. Nelson N, Finnström O, Larsson L. Neonatal reference values for ionized calcium, phosphate and magnesium. Selection of reference population by optimality criteria. Scand J Clin Lab Invest 1987; 47:111–7.
18. Sarnat BG, Schour I. Enamel hypoplasia (chronologic enamel aplasia) in relation to systemic disease: a chronologic, morphologic and etiologic classification. I. J Am Dent Assoc 1941;28:1989–2000.
19. Sarnat BG, Schour I. Enamel hypoplasia (chronologic enamel aplasia) in relation to systemic disease: a chronologic, morphologic and etiologic classification. II. J Am Dent Assoc 1942;29:67–75.
20. Pindborg JJ. Disturbances in tooth formation: types. In: Pindborg JJ, editor. Pathology of the dental hard tissues. Copenhagen: Munksgaard, 1970:75–107.
21. Eli I, Sarnat H, Talmi E. Effect of the birth process on the neonatal line in primary tooth enamel. Pediatr Dent 1989;11:220–3.
22. Schour I. The neonatal line in the enamel and dentin of the human deciduous teeth and first permanent molar. J Am Dent Assoc 1936;23:1946–55.

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