Maternal diabetes and changes in the hard tissues of primary teeth

III. A histologic and microradiographic study

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According to clinical studies, infants of diabetic mothers (IDM) seem to run a higher risk of mineralization disturbances in the enamel than normal healthy infants. In order to evaluate this observation at the histologic level, exfoliated primary incisors were collected from 74 IDM, representing two types of care during gestation and the perinatal period, and from 52 healthy children.

The width of the neonatal line was evaluated in the enamel and the dentin by study of bucco-lingual undemineralized sections. Pre- and postnatal enamel was classified according to discolorations, deviations of prisms and hypoplasia. Microradiographic studies included measurements of the neonatal lines and classification of the degree of mineralization in the pre- and postnatal dental hard tissues.

Irrespective of treatment, the IDM showed a significantly higher incidence of widened neonatal lines and also an increased frequency of postnatal disturbances compared to the controls. The observations are discussed against the background of the neonatal hypocalcemia reported in IDM.

Key-words: Mineralization disturbances; neonatal line; enamel; dentin

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Infants of diabetic mothers (IDM) treated by older methods and delivered in the 36th–37th week of pregnancy show a higher frequency of enamel hypoplasia than full-term healthy infants without complications during delivery and the neonatal period (8). A higher frequency of mineralization defects was also noted in this IDM group as compared to a group of IDM treated according to Möller & al. (13) with delivery postponed to almost full-term and free from complications. The higher frequency of hypoplasia in the first group may to some extent be ascribed to pre-term delivery with coexisting complications, such as asphyxia and hyperbilirubinaemia during the neonatal period. On the other hand, some of these IDM also had prenatal hypoplasias in their teeth.

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Consequently, the maternal diabetes as such cannot be excluded as a contributory factor.

As clinical studies of the tooth surface only give limited information, it was decided to collect exfoliated teeth from the investigated children for histologic and microradiographic examination. The purpose of the present study was to analyse further the influence of maternal diabetes on enamel and dentin at a micro-level.

MATERIAL

The clinical material consisted of three groups (for details, see 8, 9, 11), namely:

IDM 1. Thirty-nine children of diabetic mothers delivered in the 32nd to 40th week (mean 36 weeks). The mothers had been treated in the usual, conventional way during pregnancy. During the neonatal period the infants had been under observation by the Pediatric Departments in Malmö, Lund or Umeå, Sweden.

IDM 2. Fifty-eight children of diabetic mothers delivered in the 35th to 40th week (mean 38.5 weeks) at the Department of Obstetrics in Borås, Sweden. During pregnancy the mothers had been treated according to the Pederson method as modified by Möller & al (13).

Controls. Eighty-nine full-term healthy children born at the Departments of Obstetrics in Malmö or Umeå.

The histologic study was carried out on primary incisors collected from the three groups. One tooth or more was obtained from 29 children in the first group, 45 in the second group, and 52 children in the control group. The proportion of girls to boys was fairly equal in the three groups, 40 to 50 per cent. The majority of the teeth investigated were upper central primary incisors (54 %) followed by upper lateral incisors (32 %), lower lateral incisors (7 %) and lower central incisors (7 %).

METHODS

The clinical examination was performed when the children were 2–3 1/2 years of age. The parents were provided with a test tube containing 10 per cent neutral buffered formalin and requested to return any exfoliated teeth in the tube.

The collected teeth were dehydrated in rising concentrations of ethanol and subsequently in methyl methacrylate. Slices, approximately 150 μ thick, were sawn out of the embedded tooth with an Isomet low-speed saw (Buehler Ltd., USA). The sections were ground and polished by hand to an approximate thickness of 70–90 μ.

Pilot studies revealed that the most precise picture of the neonatal line was achieved in central bucco-lingual sections. This structure was also recognized more readily in incisors than in molars. One incisor was chosen from each patient and oriented during embedding to ensure buccolingual sections. Two sections were prepared from each tooth.

After preliminary visual examination under a light microscope, microradiograms were produced of each section (25 kV, 20 mA, focus-film distance 25 cm, exposure time 1 hour on Kodak High Resolution plate). An aluminium reference scale was utilized as a gross control of variations in the thickness of the sections. Finally, the sections were mounted on glass slides in Diatex and subjected to histologic examination.

Criteria of examination

The observations from the sections and the microradiograms were registered according to criteria listed in Table 1. In the histologic evaluation of the neonatal line and extra lines, the influence of oblique sectioning was estimated by focus variation. Discolored areas were also observed under incident light to rule out the possibility that they represented pigmentations. In the dentin an abundance of dentinal tubules filled with air

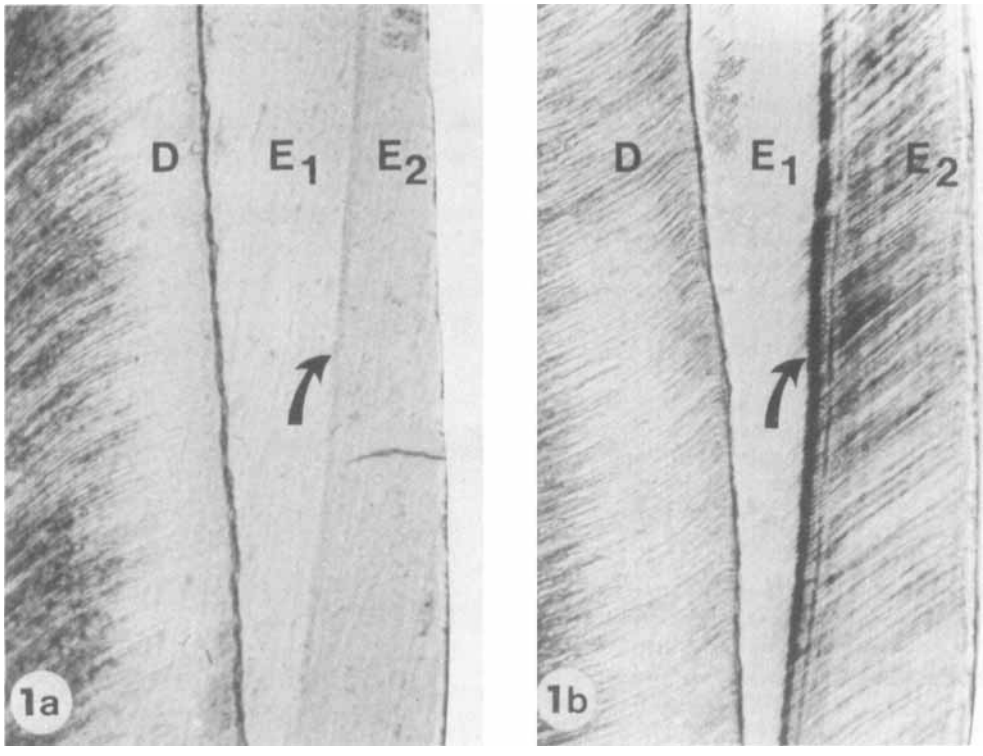

Fig. 1. *a* Normal neonatal line (arrow) and normal mineralization of enamel. Control group. *b* Widened neonatal line in enamel (arrow), postnatal extra lines and mineralization disturbances. Infant born to diabetic mother. D denotes dentin, E_1 prenatal enamel and E_2 postnatal enamel. Ground sections. X 75.

obscured all histological observations other than the existence of a neonatal line and any extra lines.

In evaluation of pre- and postnatal enamel in the microradiograms, reference was made to the aluminium scale which was always included. Caries was readily discriminated from other variations in mineralization. All observations were carried out with the sections and the microradiograms coded, *i.e.* without knowing to which group the specimens belonged.

In the statistical analysis chi-square distribution with Yate's correction was used (16).

OBSERVATIONS

Since none of the variables studied was found to vary with sex, the results of the analyses are pooled for both sexes.

In the histologic observations of the enamel the neonatal line was detected in all but seven teeth, coming from all three groups (Fig. 1). A widened line was observed about three times as frequently in IDM than in controls (Table 2). Statistical analyses showed significant differences between the IDM and the controls. When compared to each other, the two IDM groups exhibited fairly equal conditions. Generally, a widened neonatal line was more often observed on the buccal than on the lingual side of the tooth (Table 2).

The prenatal as well as the postnatal enamel exhibited a higher prevalence of moderate to severe disturbances – mostly deviations of prisms and discolorations – in the two IDM groups (Fig. 1–2). As the number of severe disturbances was low, they are reported together with the moderate deviations in Table 2. Only as regards the

Table 1. *Criteria in histologic and microradiographic examinations*

Histology			
Enamel		Dentin	
Neonatal	Pre & postnatal	Neonatal	Pre & postnatal
I. No visible neonatal line	*I.* Free from disturbances or only minor disturbances *i.e.* even arrangement of prisms, no or minor areas of light discoloration, no hypoplasia	*I.* No neonatal line	Existence of extra lines
II. Normal width of neonatal line	*II.* Moderate disturbances. Minor deviations in course of prisms or light brown areas occupying more than 25 % of the enamel. No hypoplasia	*II.* Existence of neonatal line	
III. Widened neonatal line	*III.* Severe disturbances. One or several of the following criteria: Marked deviations in arrangement of prisms; undulating or whirling configuration; strong discoloration or light brown areas occupying major part of the enamel, and/or hypoplasia		

postnatal enamel did the group IDM 2 exhibit a difference from the controls at a significant level, but the number of postnatal changes in the group IDM 1 was also almost significant.

The microradiograms partly confirmed the histologic observations, as a widened neonatal line (Fig. 3) was observed significantly more often in the IDM groups. The number of moderate to severe disturbances was less in comparison to the histologic findings. Significant differences from the controls were noted as regards the postnatal enamel in teeth from children in the IDM groups. (Table 2.)

In the microradiograms there was a higher percentage of teeth from IDM than in the control group, where the postnatal enamel was considered less well mineralized than the prenatal enamel.

When histologic and microradiographic observations were pooled, the noted differences persisted. Above all, the two IDM groups did not differ from each other. Both had a significantly higher incidence of widened neonatal lines and postnatal disturbances in mineralization compared to the control group.

In all three groups, a widened neonatal line observed histologically was frequently linked to disturbances in postnatal enamel (72–95 %). The association with prenatal disturbances was less consistent (37–54 %). Although weaker, the same tendency was

Microradiography			
Enamel		Dentin	
Neonatal	Pre & postnatal	Neonatal	Pre & postnatal
I. No visible neonatal line	*I.* Normal appearance, no visible deviations in enamel	*I.* No visible neonatal line	*I.* Low degree of mineralization, roughly corresponding to that of the mantle dentin. No or only minute areas of globular dentin. Areas of interglobulary dentin
II. Neonatal line narrower or equal to 10–12 μ	*II.* Moderate disturbances, areas with lower degree of mineralization, corresponding to 1/2–1 step on the reference scale	*II.* Neonatal line narrower or equal to 25 μ	*II.* Moderate degree of mineralization, 1/2–1 step higher than the mantle dentin. The greater part of the prenatal dentin had a globular character. In the postnatal dentin the globulary structure occupied 1/4 of the distance to the pulp
III. Widened neonatal line, widht exceeding 10–12 μ	*III.* Severe disturbances, areas with low mineralization corresponding to 1 step or more on the reference scale, and/or hypoplasia	*III.* Widened neonatal line, width exceeding 25 μ	*III.* Heavy mineralization more than 1 step higher than the mantle dentin. Globular structures in the entire prenatal and in the outer half of the postnatal dentin

noted in the observations from the microradiograms.

In the histologic observations of dentin, the neonatal line was visible in about 90 % of the teeth from children in group IDM 1 and in about 75 % from group IDM 2, but only in about 58 % of the controls. This probably reflected a higher incidence of widened neonatal lines in the children of diabetic mothers.

In the microradiograms the neonatal line was more readily discerned in the dentin, but was not visible in 15 % of the teeth from the controls. It was considered widened in a higher percentage of teeth from one of the IDM groups (59 % and 44 % respectively) than in controls (44 %). However, this difference was not statistically significant (Table 3). Nor did the observations regarding the general degree of mineralization reveal any differences, as the distribution of low or high levels was fairly equal in the three groups (Table 3).

Extra lines resembling the neonatal line were seen in rather few cases. Most of them were observed in the group IDM 2, with postnatal location.

DISCUSSION

Teeth are unique among mineralized tissues as they remain stable after their initial development, at least at the macroscopic

Table 2. *Prevalence of disturbances in the enamel. IDM denotes infants born to diabetic mothers and C controls*

	No. in group			Differences	
	IDM 1	IDM 2	C	IDM1-C	IDM2-C
Total no. incisors investigated	29	45	52		
Histologic observations:					
Neonatal line widened:					
buccally	20	32	18	**	***
lingually	11	17	8	*	•
buccally and/or lingually	21	32	18	**	***
Prenatal moderate/severe distubances					
buccally	13	19	15	ns	ns
lingually	6	5	6	ns	ns
buccally and/or lingually	14	19	16	ns	ns
Postnatal moderate/severe disturbances					
buccally	15	31	18	ns	**
lingually	12	20	12	ns	•
buccally and/or lingually	17	31	20	ns	**
Microradiographic observations:					
Neonatal line widened:					
buccally	17	21	10	***	**
lingually	2	2	0	ns	ns
buccally and/or lingually	17	21	10	***	**
Prenatal disturbances					
buccally	10	9	14	ns	ns
lingually	9	6	6	ns	ns
buccally and/or lingually	10	9	14	ns	ns
Postnatal disturbances					
buccally	20	22	12	***	*
lingually	20	22	8	***	***
buccally and/or lingually	22	22	13	***	•

• $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ ns = non significant

and light microscopic level. Consequently, defects in the structure of enamel and dentin will remain throughout the lifespan of the tooth, witnessing disturbances that have occurred during its mineralization. As the crowns of primary teeth are easily accessible after exfoliation, they present a possibility of evaluating mineralization disturbances that have occurred from about the 14th week in utero to the first postnatal months. A study of this type was carried out by Bergman, Bille & Lyttkens (1) in children with minimal brain dysfunction.

However, the character of the dental hard tissues also creates technical problems. The preparation of thin planoparallel undemineralized sections is difficult and time-consuming. Consequently, there are limitations to the information that can be obtained with conventional histologic and microradiographic techniques. The pilot studies confirmed the observation of Schour

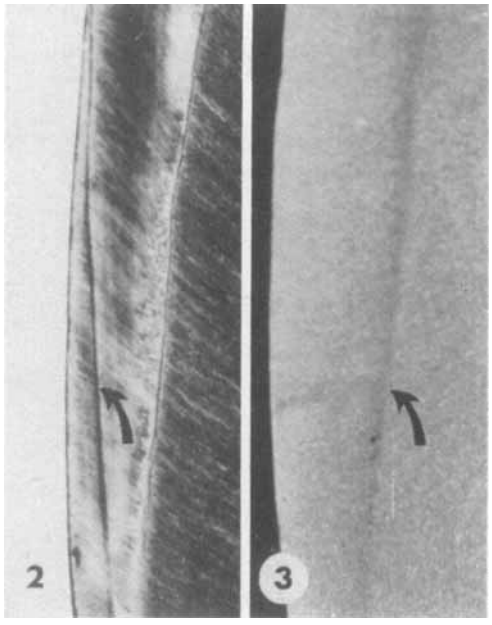

Fig. 2. Widened neonatal line in enamel (arrow). Prenatal and postnatal disturbances of mineralization. Infant born to diabetic mother. Enamel left, dentin right. Ground section. X 40.

Fig. 3. Widened neonatal line in enamel (arrow). From the IDM groups. Microradiogram. X 100.

(15) that bucco-lingual sections of incisors yield a comparatively large amount of information. The study of exfoliated teeth limits the observations on dentin as the dentinal tubules become filled with air and appear black under transmitted light.

The most frequent changes noted in the enamel were brown areas appearing pre- and postnatally. They were not related to pigmentation, but in all probability represent variations in mineral content and/or minor changes in the orientation of the enamel prisms. It is noteworthy that where changes in the orientation of the prisms were obvious in the sections, they were accompanied by brown discolorations in the same area. Where more than one line was visible in the enamel or dentin, the position of the neonatal line was calculated on the basis of the length of the gestation. If several lines were observed close to each other, the most distinct one was considered to be the neonatal line.

The relationship between mineralization defects of teeth and variations in serum calcium levels has been proven by clinical evidence (10) and by experimental studies (6, 12). In recent years pediatricians have focused interest on neonatal hypocalcemia. Normal infants exhibit a decrease in plasma Ca during the first 48 hours of life (5), a fact which may be related to the appearance of a neonatal line in teeth. Furthermore, marked or prolonged hypocalcemia has been observed in connection with perinatal disorders, such as prematurity, asphyxia and general perinatal complications (4, 7, 19). The IDM show more immature features than their gestational age would indicate (20) and their regulation of calcium-phosphate homeostasis is inadequate as in premature. They develop hypocalcemia and hyperphosphatemia, seemingly as a result of functional hypoparathyroidism (2, 19). This condition is thought to exist already in the fetal period, but its effect will be accentuated during the first days of life.

The present study confirmed earlier clinical observations that children born to diabetic mothers suffer a greater risk of mineralization disturbances than children born to healthy mothers. The findings at

Table 3. *Microradiographic observations in the dentin. IDM denotes infants born to diabetic mothers, C controls*

	No. in group IDM 1	IDM 2	C
Total no. incisors investigated	29	45	52
Neonatal line widened			
buccally	17	19	22
lingually	10	10	13
buccally and/or lingually	17	20	23
Degree of mineralization			
prenatal low	8	4	4
prenatal moderate	11	17	25
prenatal high	10	24	23
postnatal low	13	24	22
postnatal moderate	10	14	20
postnatal high	6	7	10

Statistical analysis revealed no differences at significant level.

the micro-level are most compatible with an occurence of hypocalcemia in IDM, and this is especially true as regards the widened neonatal lines.

It must be kept in mind that this study does not allow direct comparisons with the earlier clinical observations of the enamel surface. No attempt was made to orientate the sections so as to cover areas with macroscopic defects. Thus, the difference noted earlier in the frequency of hypoplasia between the two IDM groups may reflect variations in the care of the pregnant mother and the neonate. The present study corroborates a link between mineralization disturbances of teeth and the systemic disease of the diabetic mother.

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