Ion probe analysis of discolored areas in the enamel of deciduous teeth

A pilot study

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Discolored and normal areas in the enamel of eleven primary teeth from children born to diabetic mothers were analysed with ion probe technique. These teeth were compared with four teeth from children born to healthy mothers. Fourteen different mass numbers were recorded. The affected areas showed a proportionally higher content of organic material, but the differences in recorded values reached statistical significance only in the postnatal enamel. The study revealed a considerable biological variation in the chemical composition of deciduous tooth enamel. The brown areas may be partly related to variation in physical properties.

Key-words: Mineralization disturbances; human teeth; diabetes

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In ground sections from human deciduous teeth brownish colored areas are often observed in the enamel (Fig. 1). These «discolorations» vary in shade from light yellowish brown to dark brown in transmitted light. Studies in incident light show that they do not represent a pigmentation (7). Under higher magnification it becomes obvious that these areas usually contain prisms with an undulating and irregular configuration. An early investigation showed that in 70–80 per cent of discolored areas the mineralization was less than in adjacent enamel (10). However, light microscopy is unreliable for determining the degree of mineralization. More reliable observations may be obtained with microradiography and examination under polarised light (2, 8).

Neonatal distress has been reported to interfere with normal mineralization of enamel (4). For example, it has been observed that children of diabetic mothers have an increased frequency of mineralization disturbances and brown areas in the enamel of their primary teeth (6). These children develop a functional hypoparathyroidism causing postnatal hypocalcemia. Children of diabetic mothers might therefore be expected to exhibit variation in chemical compositon of the enamel of their primary teeth.

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In recent years ion probe analysis has proved valuable for detailed studies of element distribution in predeterminded areas of dental hard tissues (1). The purpose of this pilot study was to analyse, by ion probe technique, brown and normal areas of enamel in primary teeth from children born to diabetic mothers.

MATERIAL

The analyses were carried out on ground sections of upper central primary incisors selected from children of diabetic mothers (for details, see Norén, Grahnén & Magnusson 1978). After histological and microradiographic examination, 11 teeth showing distinct discolorations in the pre- and/or postnatal enamel were analysed. Four teeth from children born to healthy mothers and without noticeable morphological changes in the enamel served as controls. Only the buccal side of the tooth was analysed.

METHODS

A Cameca IMS 300 ion analyser was used (1, 5). The enamel surface was bombarded with O^- ions with a total current of about 0.8 μA and with an energy of 14.5 keV. The primary ion beam had a diameter of 75 μm. Positive secondary ions were collected from the center of the sputtered area, which had a diameter of about 40 μm. The total secondary ion current sampled for the matrix element calcium was in the range of $1-2 \times 10^{-13}$ A.

The ground specimens were additionally polished with diamond paste, cleansed in an ultrasonic bath and mounted in a sampleholder for the ion probe. The surface was coated with an approximately 0.1 μm thick layer of evaporated gold.

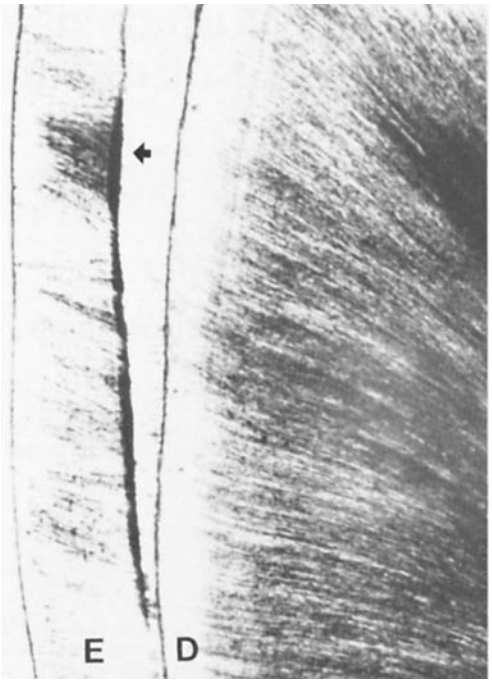

Fig. 1. Discolored area in prenatal enamel (arrow). E denotes enamel, D dentin. Ground section, x 100.

The sections were analysed at 5 points, two in the prenatal and two in the postnatal enamel, and one in the dentin for technical reference (4). The recorded mass numbers, chosen for reliability in ion probe detections, are listed in Table 1. Measured secondary ion currents from the different mass numbers were normalized to the recorded value of calcium 44 (3, 4).

Student's t-test was used for the statistical analysis.

RESULTS

The differences in chemical composition between normal and brown areas were small (Table 1). The values from prenatal enamel showed no stat-

Table 1. *Recorded mass numbers and corresponding ion species (4). Normalized ion currents in pre- and postnatal enamel. Mean values and standard deviations (in brackets). The figures represent 8 measurements in normal and 22 in disturbed enamel*

Massnumber	Positive ion species	PRENATAL normal	PRENATAL disturbed	POSTNATAL normal	POSTNATAL disturbed
12	C	0.00174 (±0.00072)	0.00139 (±0.00027)	0.00112 (±0.00021)	0.00142 (±0.00050)
20	Ca^{++}	0.228 (±0.202)	0.228 (±0.022)	0.212 (±0.008)	0.247 (±0.040)
23	Na	1.61 (±0.32)	1.70 (±0.45)	1.36 (±0.25)	1.82 (±0.36)*
24	Mg	0.140 (±0.0082)	0.128 (±0.0201)	0.139 (±0.0896)	0.117 (±0.0426)
31	P	0.109 (±0.0167)	0.120 (±0.0198)	0.0913 (±0.0137)	0.117 (±0.0426)
39	K	0.101 (±0.0231)	0.101 (±0.0255)	0.111 (±0.0471)	0.111 (±0.0204)
52	CaC	0.00158 (±0.00101)	0.00247 (±0.00094)	0.00123 (±0.00058)	0.00190 (±0.00035)
57	CaOH	1.35 (±0.30)	2.47 (±0.84)	1.17 (±0.18)	2.32 (±0.97)*
59	CaF, CaO, CaOH	0.0196 (±0.0196)	0.0288 (±0.0089)	0.0196 (±0.0018)	0.0297 (±0.0116)
60	CaO	0.0925 (±0.0105)	0.0876 (±0.0876)	0.0863 (±0.0349)	0.0892 (±0.0147)
61	CaOH	0.0344 (±0.0093)	0.0573 (±0.0225)	0.0289 (±0.00583)	0.0289 (±0.00583)
71	CaP	0.0187 (±0.0022)	0.0161 (±0.0008)	0.0193 (±0.0018)	0.0160 (±0.0014)**
88	Sr	0.00233 (±0.00022)	0.00233 (±0.00036)	0.00233 (±0.00044)	0.00256 (±0.00062)

* $p < 0.05$
** $p < 0.01$

istically significant differences. However, the brown areas seemed to contain more sodium and carbon than normal enamel and the content of potassium was decreased. Greater deviations were seen in the postnatal enamel and they rached significant levels for sodium, certain hydroxide complexes and CaP (mass numbers 23, 59, 71). Irrespective of origin from controls or disturbed teeth, the normal and discolored enamel areas showed large biological variations in chemical composition.

DISCUSSION

Great care was taken to analyse identically situated areas in enamel from different teeth. In all probability, the obvious variation, with standard deviations as large as ± 10 % of the mean values, reflected a biological variation between individual points. With the Cameca ion probe the measured area is about 2.5 x 10^3 μm^2 and the error of measurement is small (4). Thus the study confirmed large variations between individuals and within different parts of the normal enamel (11, 12).

The small differences between normal and discolored areas in the prenatal enamel and the comparatively smaller standard deviations may be related to the fact that the foetus is protected during intrauterine life. The newborn infant appears to be more sensitive to

factors influencing mineralization. The high values for sodium and calcium hydroxide mass peaks, the decrease in CaP and the tendency to increase in carbon suggest a relative increase in non-prismatic substance in the discolored areas. This is in accordance with concepts derived from histological studies (2, 5, 6, 8).

This pilot study clearly shows a need for further and extended studies to establish the range of normalcy of the mineral content and its distribution in human enamel. The observations suggest that the phenomenon of brown areas in deciduous tooth enamel is also related to variation in physical properties and not only to chemical changes.

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