Area of the organic-inorganic interface of dental enamel

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The area of the organic-inorganic interface of several kinds of dental enamel was determined by examining on a computer images based on electron micrographs of decalcified sections. The mean values varied between 0.1363 m^2/mm^3 and 0.2229 m^2/mm^3. Assuming a specific gravity for dental enamel of 3.15 the corresponding values were 43 m^2/g and 71 m^2/g, respectively. The possibility that fluoride may influence the area of the organic-inorganic interface was discussed.

Key-words: Dental enamel; species specificity; histology, comparative; microscopy, electron

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Since chemical reactions take place on the surfaces of the mineral crystals of dental enamel, attempts have been made to determine the internal accessible surface area of fully mineralized enamel tissue. Most frequently gas absorption measurements of powdered enamel have been taken to assess the specific surface area (*Wood*, 1947; *Hendricks & Hill*, 1950; *Likins, Cowie & Mc Clure*, 1951; *Mc Cann & Bullock*, 1955; *Little*, 1961; *Brudevold, McCann & Grön*, 1965). As could be expected the studies showed that the specific surface area is dependent on particle size in such a way that the surface area increases as the particle size decreases. However, the estimates of the specific surface area arrived at by the gas absorption methods showed considerable variations with values ranging between 1 m^2/g and 28 m^2/g. *Poole* (1965) found that the void volume of enamel accessible to water was 4.7 per cent and assessed the internal surface area to be approximately 10 m^2/g. *Sognnæs* (1965) calculated that enamel containing crystals about 300 Å wide would have a surface area of about 50 m^2/g. *Selvig & Halse* (1972a) took measurements of rat crystal thickness and width and calculated averages of 250 Å and 450 Å respectively. Assuming a density of 900 crystals per μm^2 enamel and a specific gravity of 3.15 these authors found that the specific surface area of rat enamel may be 34 m^2/g.

Previous electron microscopic work has shown that dental enamel crystals are surrounded by an organic sheath (*Syrrist*, 1949; *Nylén & Omnell*, 1962; *Scott & Omnell*, 1962; *Rönnholm*, 1962; *Travis & Glimcher*, 1964; *Sundström & Zelander*, 1968; *Silness & Gustavsen*, 1969; *Gustavsen & Silness*, 1969; *Silness & Gustavsen*, 1970; *Gustavsen*, 1972). It has also been demonstrated that the structure of the organic matrices may be retained through careful decalcifying procedures, so that a detailed study of the morphology and

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dimensions of the tubular organic sheaths surrounding the enamel crystals is made possible (*Travis & Glimcher*, 1964; *Sundström & Zelander*, 1968; *Silness & Gustavsen*, 1969; *Gustavsen & Silness*, 1969; *Silness & Gustavsen*, 1970; *Gustavsen*, 1972). It has been suggested that the organic layer in intimate contact with the crystal surfaces may serve to prevent fusion between neighbouring crystals (*Nylén, Eanes & Omnell*, 1963). In fact, it has been shown that in fully mineralized enamel the width of the intercrystalline spaces may correspond to the thickness of the organic sheaths recovered after decalcification (*Gustavsen*, 1972). Now, if in a given volume of tissue the surface area of the organic sheaths could be determined, the area of the organic-inorganic interface on either side of the sheaths could be known and thus, the total area of the organic-inorganic interface. The purpose of the present study was to determine the total surface area of the organic sheaths per unit volume of dental enamel tissue in order to get an estimate of the area of the organic-inorganic interface.

MATERIAL AND METHODS

The material consisted of dental enamel from a varying number of teeth of shark, alligator, hedgehog, sound human permanent and deciduous teeth as well as unerupted fluorosed human teeth with fully developed roots. The degree of fluorosis of the last-mentioned teeth was evaluated as mild, according to the system proposed by *Dean* (1938). Only the mottled areas and the tissue beneath such areas were examined. The teeth were either stored in neutral formalin or in small, stoppered test tubes containing moist cotton and thymol.

Bucco-lingual sections of the teeth were cut with a »Gillings Hamco Thin Sectioning Machine» under waterspray. These sections were ground and polished to thicknesses from 15 to 50 μm.

The decalcifying fluid was prepared as a 0.5 per cent aqueous solution of Chromic Sulphate Basic (approx. $Cr_2(SO_4)_3 \cdot 6H_20$), The British Drug Houses Ltd., England. By adding alkali (NaOH) the chromium solution was brought to a pH slightly above 3 (3.1—3.3) (see *Sundström*, 1966; *Sundström & Zelander*, 1968).

The ground sections were placed on the bottom of Petri dishes filled with the decalcifying solution which was changed daily. The decalcification process, assessed as explained by *Sundström* (1966), was completed after 4—9 days. The decalcified dehydrated matrices were placed in aluminium foil containers on top of a polymerized layer of Araldite and covered with Araldite syrup. After polymerization small, matrix containing blocks were placed on the bottom of gelatin capsules, which were subsequently filled with Araldite syrup. Within the capsules the specimens were oriented so as to permit sectioning in known relation to tissue areas. Sectioning was done with glass knives on an LKB Ultrotome. For the present study sections from the mid-buccal area about half way through the tissue were used. From the human fluorosed teeth sections from slightly beneath the surface (area 1), sections from the subsurface zone (area 2) and sections from the zone (area 3) on the dentinal side of the subsurface were examined. All sectioning was carried out so as to cut the organic tubes more or less at right angles to their long dimension.

The electron microscope (Siemens Elmiskop I) was operated at 80 kv. Micrographs were obtained at a plate magnifica-

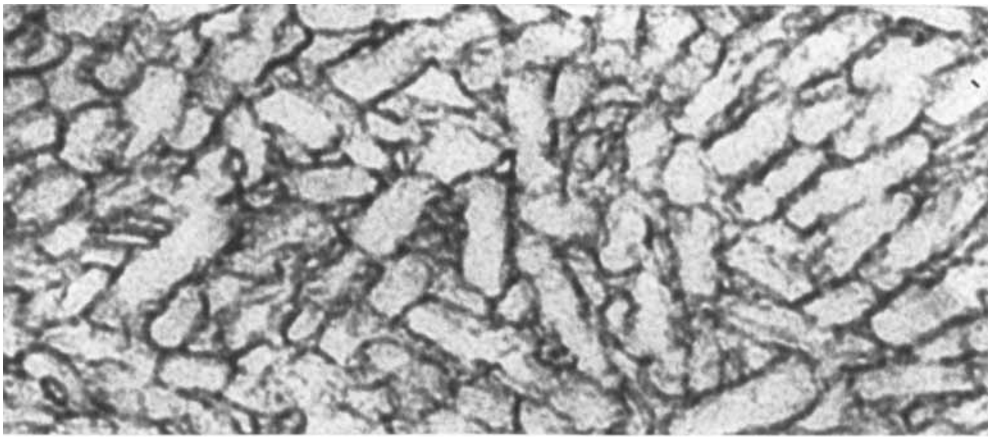

Fig. 1. Electron micrograph on which the tracing shown in Fig. 2 was based.

tion of 40.000 diameters, and prints, enlarged six times, were prepared. To achieve good detection images based on the electron micrographs showing representation of the organic phase were prepared by tracing the linear structures on transparent tracing paper by means of a fine line ink marker (Penol 1300). These tracings were subsequently photographed and prints made on high contrast photographic paper (Figs. 1 and 2).

The analyses were carried out by examining the images on a Quantimet 720 using an Epidiascope (Imanco, Image Analysing Computers Limited, England). Area and intercept measurements were taken. From the intercept measurements it is possible to derive the total length of the linear structures within a given area (mm/mm^2). When this ratio is known, the surface area of the same structures (the organic sheaths) per unit volume (mm^2/mm^3) can be calculated. All data recorded were measured in picture points, the absolute value of which depends on the magnification of the system. For the

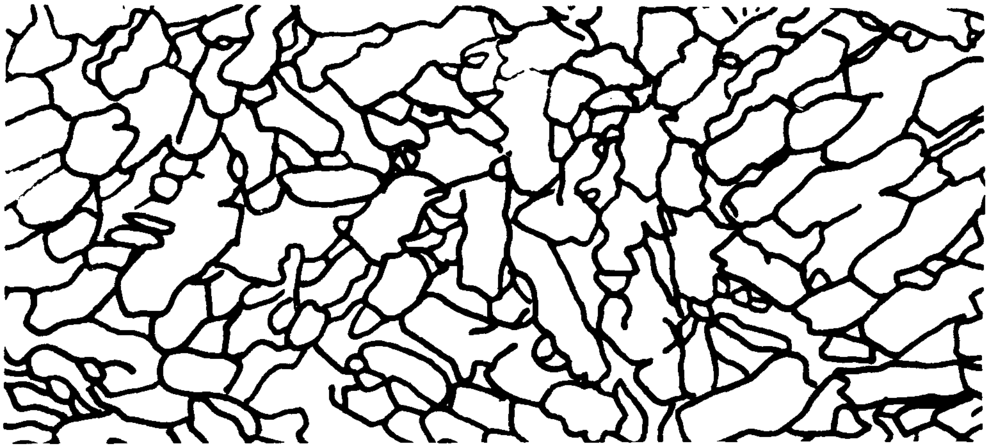

Fig. 2. Image of tracing based on the electron micrograph shown in Fig. 1.

present examination 1 linear picture point equalled 0.0916 mm and 1 area picture point 0.00839 mm^2. The method error of the tracing procedure was assessed by analysing statistically the difference between duplicate tracings of three electron micrographs. The method error was calculated from the following equation:

$$e(met) = \sqrt{\frac{D^2}{2N}}$$

where D is the difference between the duplicate measurements and N is the number of double measurements. The method error was 3.8 per cent of the mean length.

Values for the specific surface area of dental enamel are usually given as m^2/g whereas in the present report the values emerge as m^2/mm^3. To facilitate comparison with previous work calculations have been made to present comparable values (m^2/g) assuming specific gravities of dental enamel of 3.15, 3.00, 2.85, and 2.70.

RESULTS

The mean values and ranges for the total surface area of the organic sheaths from three zones of each of the different dental enamels are listed in Table I. On the assumptions made the listed values correspond to the values for the area of the organic-inorganic interface. As can be seen from Table I the mean values varied between 0.1363 m^2/mm^3 and 0.2229 m^2/mm^3. Assuming a specific gravity for dental enamel of 3.15 the corresponding values were 43 m^2/g and 71 m^2/g respectively. As could be expected the calculations based on lower specific gravities showed higher values for the specific surface area of dental enamel.

DISCUSSION

In the present work the organic sheath recovered in the decalcified ground sections have been thought of as having been

Table I. *Area of the organic-inorganic interface of dental enamels*

		Area of the organic-inorganic interface					
Material	No of areas	m^2/mm^3 $\bar{x}$	m^2/mm^3 range	m^2/g sp.gr. 3.15 $\bar{x}$	m^2/g sp.gr. 3.00 $\bar{x}$	m^2/g sp.gr. 2.85 $\bar{x}$	m^2/g sp.gr. 2.70 $\bar{x}$
Deciduous human enamel	3	0.2229	0.2177—0.2290	71	74	78	83
Permanent human enamel	3	0.1922	0.1834—0.2005	61	64	67	71
Fluorosed enamel area 3	3	0.1901	0.1889—0.1908	60	63	67	70
Fluorosed enamel area 2	3	0.1630	0.1533—0.1751	52	54	57	60
Fluorosed enamel area 1	3	0.1527	0.1430—0.1586	48	51	54	57
Shark enamel	3	0.1363	0.1336—0.1409	43	45	48	50
Alligator enamel	3	0.1951	0.1912—0.1973	63	65	68	72
Hedgehog enamel	3	0.1985	0.1853—0.2063	63	66	70	74

contained within the comparatively narrow capillary or intercrystalline spaces in dental enamel. With the methods used an estimate of the total surface area of sheath per unit volume of tissue can be obtained and thereby an estimate of the area of the organic-inorganic interface of dental enamel. The area of this interface is, however, also dependent on the thickness of the sheaths, i.e. the width of the intercrystalline spaces in such a way that an increase in width would imply a decrease in the interface area and *vice versa*. The analytical data revealed that in the images traced on basis of the electron micrographs the thickness of the lines showed average values between 30—35 Å. These values are in good harmony with the thickness values (30—50 Å) of the organic sheaths reported in earlier electron microscopic work (*Travis & Glimcher*, 1964; *Sundström & Zelander*, 1968; *Silness & Gustavsen*, 1969; *Gustavsen & Silness*, 1969, *Silness & Gustavsen*, 1970; *Gustavsen*, 1972). The values also compare favourably with the width dimensions (10—70 Å) of the intercrystalline spaces given by earlier authors (*Rönnholm*, 1962; *Frank*, 1965; *Selvig & Halse*, 1972b; *Gustavsen*, 1972).

In addition to the errors due to the tracing technique, the photographic procedures including shrinkage of the emulsion and shrinkage of prints should be considered. Since the work was carried out with standardized routine techniques we have no reason to believe that the error due to these techniques had an order of magnitude that would invalidate the estimates presented.

Errors may also be introduced by the preparatory procedures of decalcification, fixation, embedding and sectioning. In this connection shrinkage of the organic matrices is a critical point since shrinkage would tend to result in an overestimation of the area of the organic-inorganic interface. Thus, it can be shown that a shrinkage of ten per cent would necessitate a correction of the estimates by the same percentage. We have reason to believe that the shrinkage is less than ten per cent.

With the restrictions inherent in the methods the results of the present study indicate average organic-inorganic interface areas of dental enamel varying between 0.1363—0.2229 m^2/mm^3. With a specific gravity of dental enamel of 3.15 the corresponding values expressed as m^2/g are 43 and 71, respectively. These values compare fairly well with the specific surface area (50 m^2/g) calculated by *Sognnæs* (1965) for human subsurface enamel and the specific surface area for rat incisor enamel (34 m^2/g) reported by *Selvig & Halse* (1972a).

When comparing the different dental enamels, it must be kept in mind that the average values for the area of the organic-inorganic interface were based on few analyses. Additional investigations are, therefore, necessary to elucidate the significance of the results. None the less, we would like to direct attention to the results obtained for shark enamel and human fluorosed enamel as compared to the others. Shark enamel crystals show regular polygonal habits when viewed in cross-sections (Fig. 3), and are possibly fluorapatite crystals (*Glas*, 1962). Observations on decalcified human fluorosed enamel from the surface layer (area 1) also indicate regular polygonal cross-sectional shapes of the crystals (Fig. 4). This may be the reason why the values for the area of the organic-inorganic interface of shark enamel and fluorosed enamel (area 1) were low and agreed well. X-ray diffraction studies by *Posner et al.* (1963) showed that fluoride, when incorporated in bone

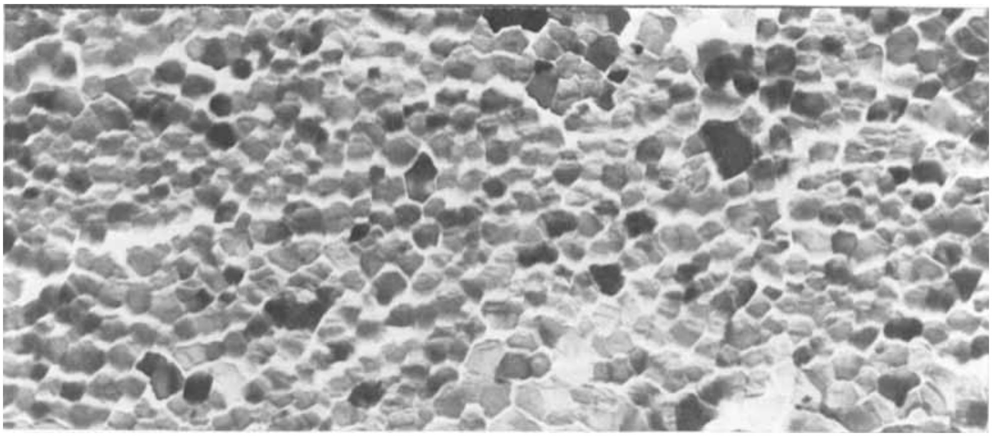

Fig. 3. Shark enamel (Lamna carnubica). Transversely sectioned enamel crystals from functional tooth (approx. × 69 000).

apatite, produced an improvement in crystallinity, where the more perfect and/or larger crystals are said to be more crystalline. It would not be surprising, therefore, that the well-formed crystals in shark enamel and human enamel with mild fluorosis and the apparent decrease in the area of the organic-inorganic interface of these enamels result from the effects of fluoride. The findings of higher values for the area of the organic-inorganic interface in the depth of the fluorosed enamel need not be inconsistent with such an assumption since it has been shown that in fluorosed enamel of unerupted teeth there is a gradual decrease in the fluoride content from the surface towards the dentine (*Isaac et al.*, 1958).

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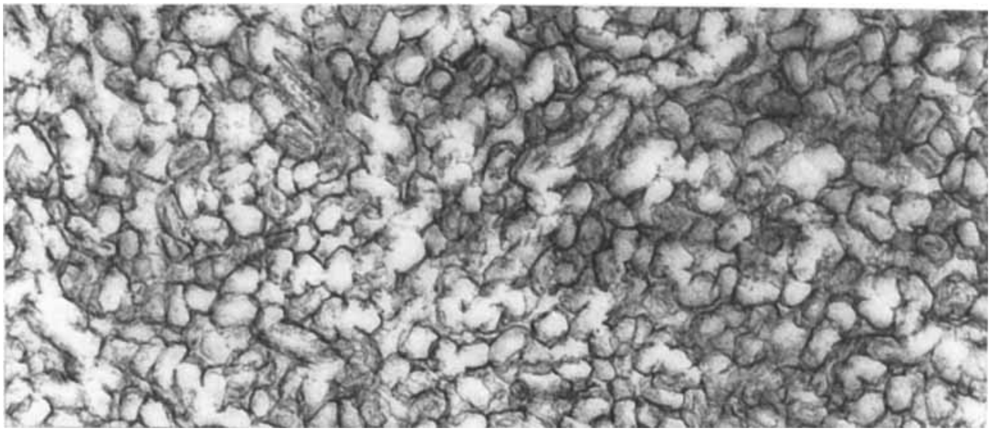

Fig. 4. Fluorosed human enamel. Decalcified section showing transversely sectioned organic tubes (approx. × 69 000).

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