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Preparation Methods and LAMMA Analysis of Dental Hard Tissue with Special Respect to Fluorine*

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Präparationsmethoden und LAMMA-Analyse von Dentalhartgewebe unter besonderer Berücksichtigung von Fluor

Key words: Best. von Fluor in Zahngewebe; Massenspektrometrie, Lasermikrosonde

Microprobe analysis of dental hard tissue is commonly performed by electron- and ion-microprobe instruments. Unfortunately, energy dispersive X-ray microprobe fails to detect trace elements particularly of low mass numbers such as for instance fluorine as an important trace constituent of dental materials. Such restrictions do not exist for Laser Microprobe Mass Analysis (LAMMA-method). However, LAMMA analysis cannot be performed on bulk specimens so far. On the other hand the preparation of very thin sections in the micrometer range is possible with decalcified hard substance only. Cutting enamel specimen by means of a hard tissue microtome results in crumbling fragmentation. The resulting fragments cannot be correlated any more to defined tooth areas, e.g. distinct growth layers. Many investigators have tried to grind larger areas down to thin sections in order to applicate the microradiographic technique, but in most cases the results are not below 10 or some 100 µm (references see [1]). For our first applications of the LAMMA method we had used this large area grinding technique [2], but the efforts for gaining defined and unbrittled specimen were often subjected to fortune. Therefore, our aim was to develop a preparation method which could avoid the preparation difficulties and which would offer the possibility for the preparation of small specimen areas down to a few µm thickness in any selected area of a whole tooth section [3, 4].

Therefore we developed the following preparation technique: The tooth is embedded in a cold curing synthetic resin (Fig. 1a). A first cut is made by a modified commercially available annular saw cutting machine (Fig. 1b). The resulting area on the bulk is polished. A thin glass slide mounted in a Plexiglas-ring is sticked on the surface (Fig. 1c) and a second cut is done (Fig. 1d). Now this thin section of about 150–200 µm thickness is ground down and polished on the resulting specimen surface (Fig. 1e). Then a groove is milled with a fine grinding wheel through the glass slide into the sample, until an area of only a few µm thickness remains in the tooth area of interest (Fig. 1f). This procedure is done under visual control. The specimen is fixed in a special sample holder and mounted on the stage of the LAMMA microscope (Fig. 1g).

Now some examples for the application of the method are given. Figure 2 shows two grooves in the transversal cut section of a rat incisor. In Fig. 3 the indicated area of Fig. 2 is shown with higher magnification. The thickness of this area can be estimated by the fact that the direction of enamel prisms indicated by arrows and the scratches from the rotating grinding wheel can be seen very clearly (the diameter of enamel prisms is in the region between 3 and 8 µm). In Fig. 4a another groove across a rat incisor section is shown. The arrows indicate holes from LAMMA analysis in a rat incisor dentine specimen. This tooth was taken from an experiment performed by Kato and Gabriel [5] to investigate the interference between lead ions and fluorine ions during hard tissue formation. (The use of lead ions as a time marking agent for the investigation of hard tissue growth velocity is recommended and has been investigated by Kato and coworkers, see e.g. [6, 7]). Figure 4c shows a decalcified microtome section of the same tooth. The lead resulting from the subcutaneous

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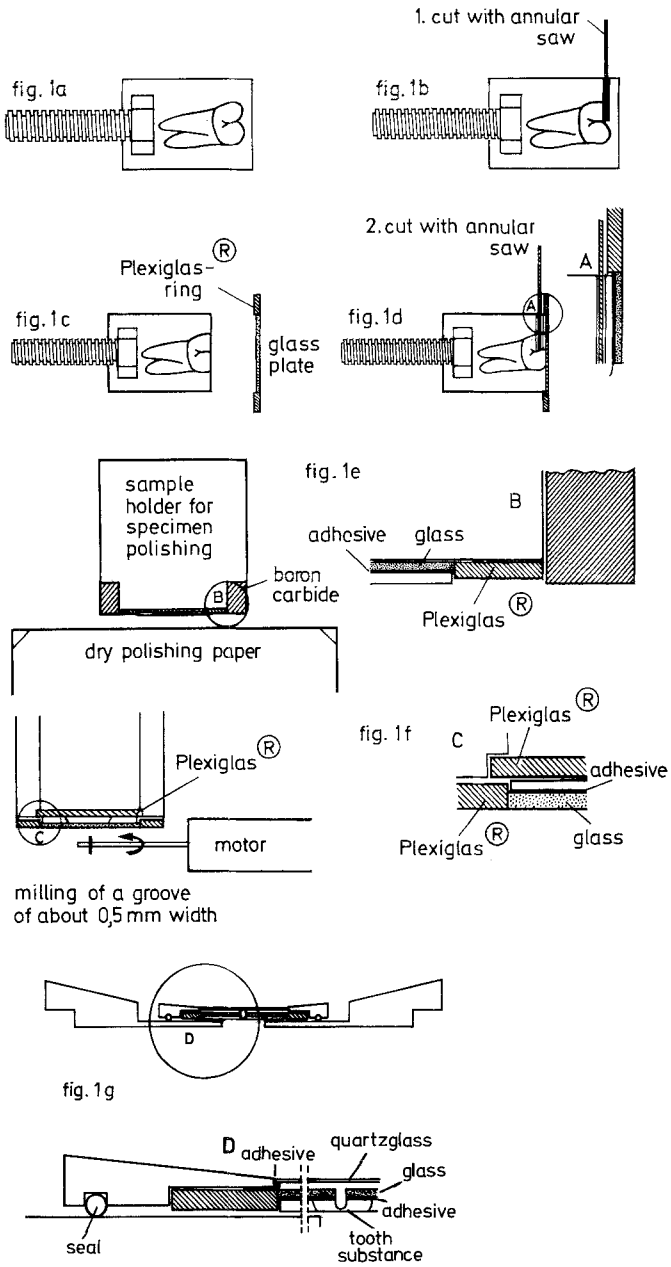

Fig. 1a–g. Groove milling technique for the preparation of thin areas in biological hard tissue and other hard substances

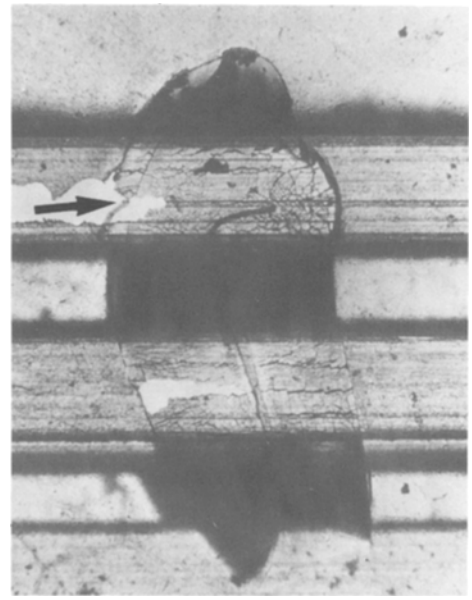

Fig. 2. Grooves in a transversal cut section of a rat incisor

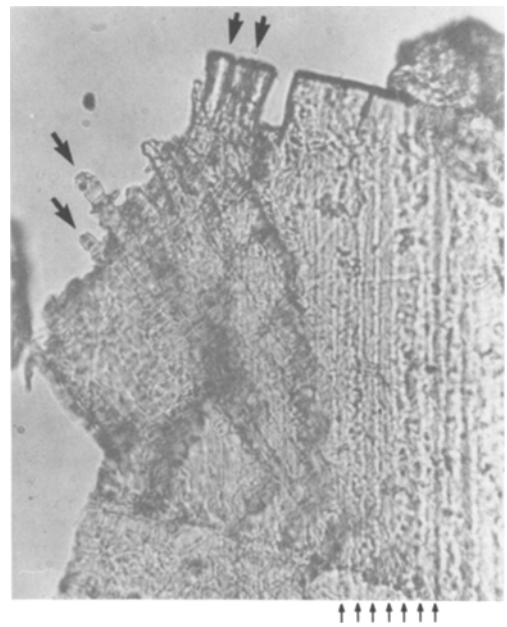

Fig. 3. Scratch direction resulting from the milling process → and single enamel prisms ➡

and intravenous injection has deposited in dentine and has been made visuable by H_2S-precipitation. Two different injections of NaF-solution have been made at the 4th and the 5th day after the lead injections. The distance of the two F-injection lines is clearly seen as well as in the groove of the hard tissue section (Fig. 4b). From the laser holes it is evident that with this technique the analysis of growth layers in dentine is possible, which have a distance of about 15 µm equivalent to a difference of growth time of 1 day.

It should be possible to improve these resolution values. In very thin specimen regions of hard tissue, it is possible to reduce the diameter of the laser holes below 2 µm. This is shown by Fig. 5. In addition, line scanning analysis across a specimen is possible. This is shown by Fig. 6. On the other hand, it is known from literature [8] that the maximum growth velocity of rat incisors is in the order of about 150 µm/day. This leads to the conclusion that it should be possible to analyze growth layers which differ in a growth time of below 1 h.

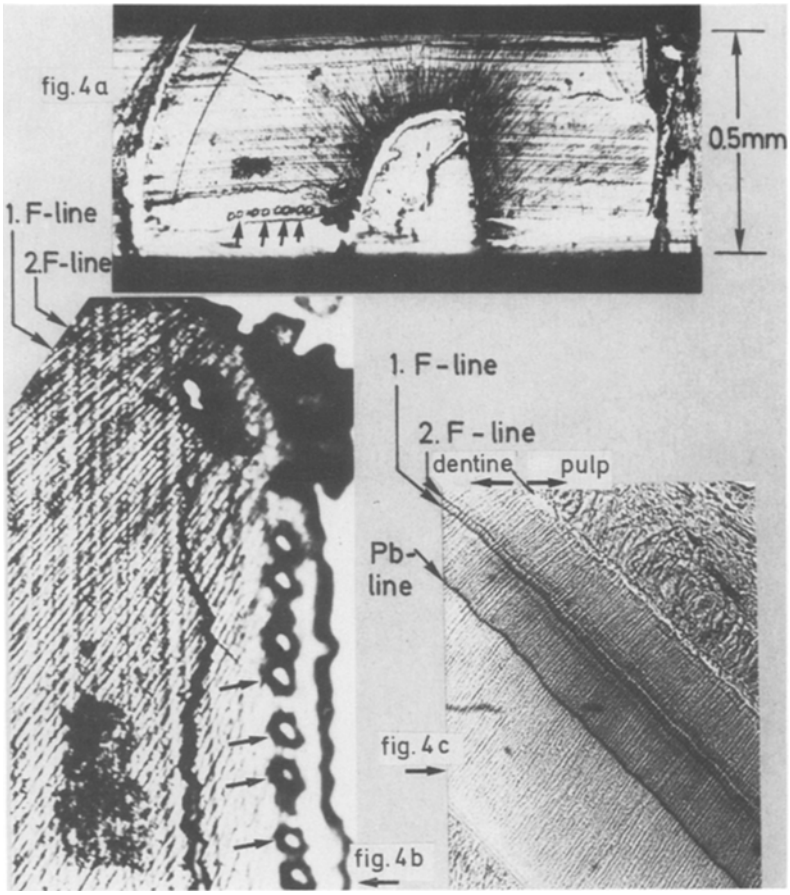

Fig. 4a—c
Influence of Pb- and F-ions on dentine formation in rat incisors. Pb-deposition in rat incisor dentine after i.v. injection of Pb-acetate solution (2 mg/kg Pb-Ac) at the 1. day + 500 mg/kg $PbCO_3$-powder in 2% CMC sol. s.c.
a, b undecalcified annular cutting machine section, milled down along a slit of 0.5 mm width to a few µm thickness, diameter of the holes: between 5 and 12 µm. **c** decalcified microtome section, lead-precipitation by H_2S. 1. F-line: influence of NaF-injection at the 4th day (30 mg/kg i.p. after Pb-injection), 2. F-line: influence of NaF-injection at the 5th day (30 mg/kg i.p. after Pb-injection), distance of the F-lines: about 15 µm

Figure 7 shows some LAMMA spectra taken from human enamel and rat incisor dentine. The determination of fluorine is very easy because no elements or molecules with the mass number 19 (fluorine) occur. At higher mass numbers the coordination between mass numbers and elements or molecules becomes more difficult. This is especially valid for dentine in the negative spectrum because there are a lot of organic molecule fractures. For further details see explanations within the figures. To achieve further interpretation and quantification of the spectra, special reference standards have to be developed. In addition, recent results and developments of the LAMMA method presented by Kaufmann, Hillenkamp, Wechsung, Heinen and Vogt (e.g. [9, 10] and during this symposium) must be applied.

It is obvious that this combination of the LAMMA method with our preparation technique offers new possibilities for the investigation of the effects of trace elements (or toxic agents) on the growth of hard tissue as well as of possible interference effects of different agents. This might be of great importance for the investigation of toxic agent effects on animals, e.g. as a kind of "biological environmental pollution monitoring". The continuously growing hard tissue, e.g. the incisor, acts as a "registering strip" and by means of the LAMMA method we should be able to learn how to interprete the information, which had been imprinted in the different growth layers, in terms of a "biological

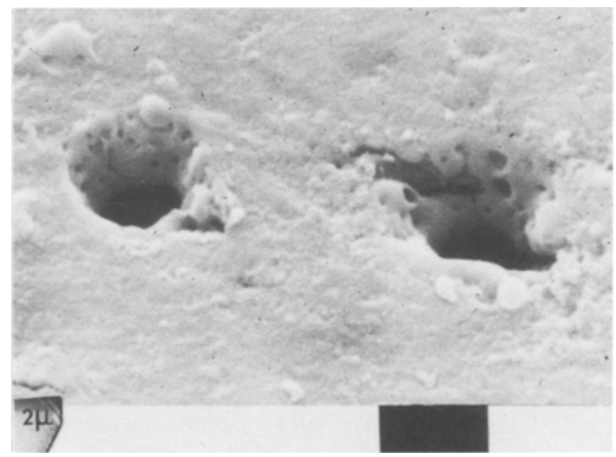

Fig. 5. Scanning electron micrographs of laser holes in human dental enamel specimen

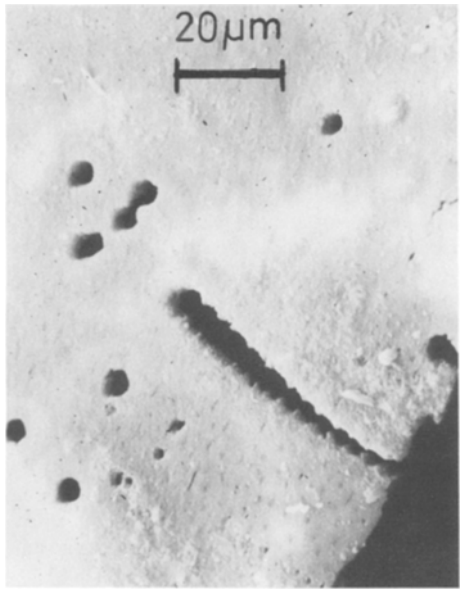

Fig. 6. Line scanning analysis across a human dental enamel specimen

curriculum vitae". We think that no other analytical technique is suitable to solve such problems.

In order to get a survey about the possibilities of different analytical methods which are commonly used for the determination of fluorine in dental hard tissue we have tried a comparison by compiling a nomogram. The results are shown in Fig. 8. (The first version of this nomogram was presented in [2]): Each method for the analytical determination of a trace element needs a certain minimum quantity (weight) of matrix substance in order to determine the quantity of the trace element of interest (here: fluorine) in this matrix. This magnitude of needed substance is given on the abscissa together with some really occurring mass values corresponding to teeth and tooth structures. On the ordinate the amount of trace element (weight) contained in the matrix is plotted. In dental hard tissue of apatitic structure fluorine occurs within a concentration range between about 1 and 1000 ppm. That means that the absolute amount of fluorine which is to

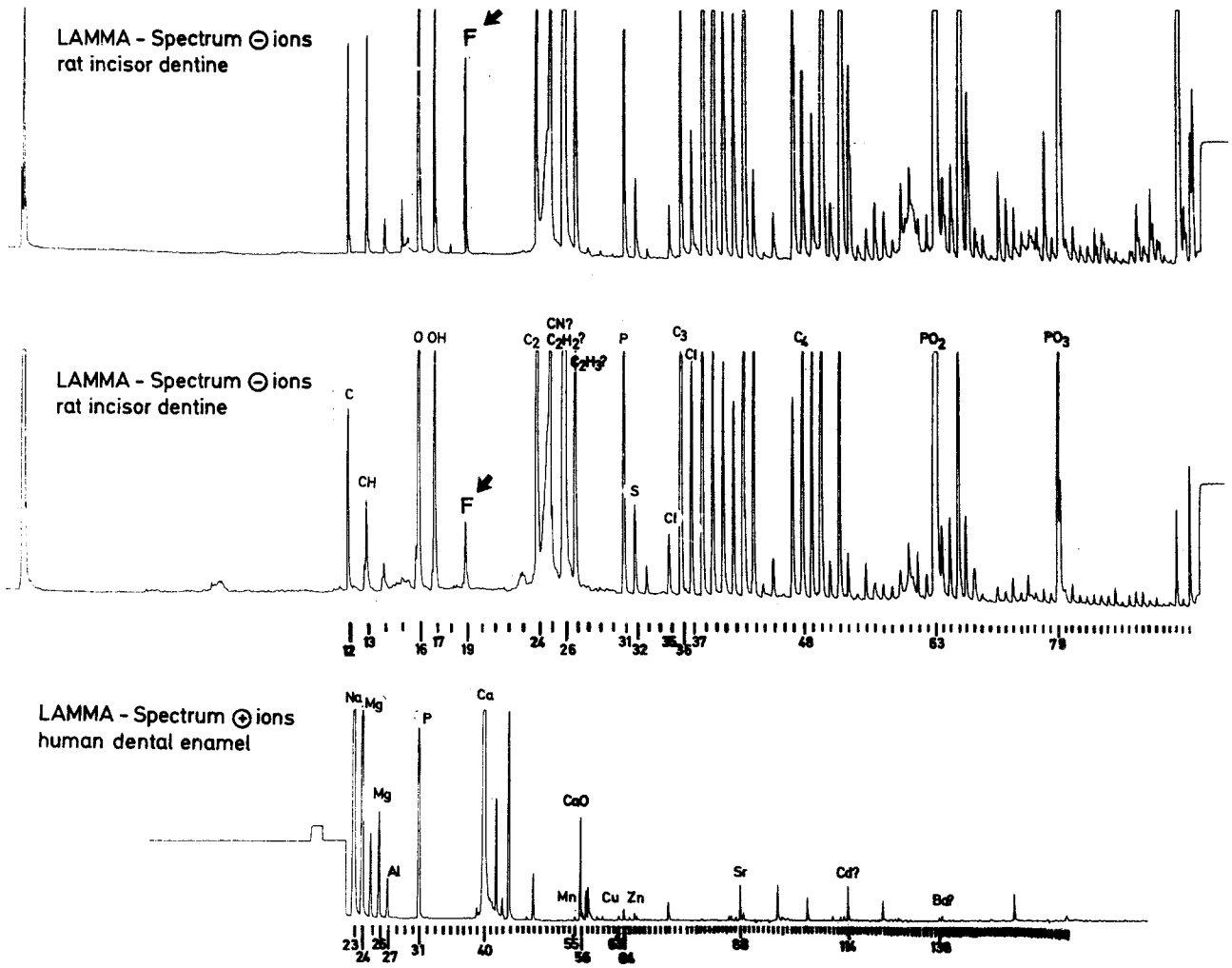

Fig. 7. LAMMA spectra of dental hard tissue

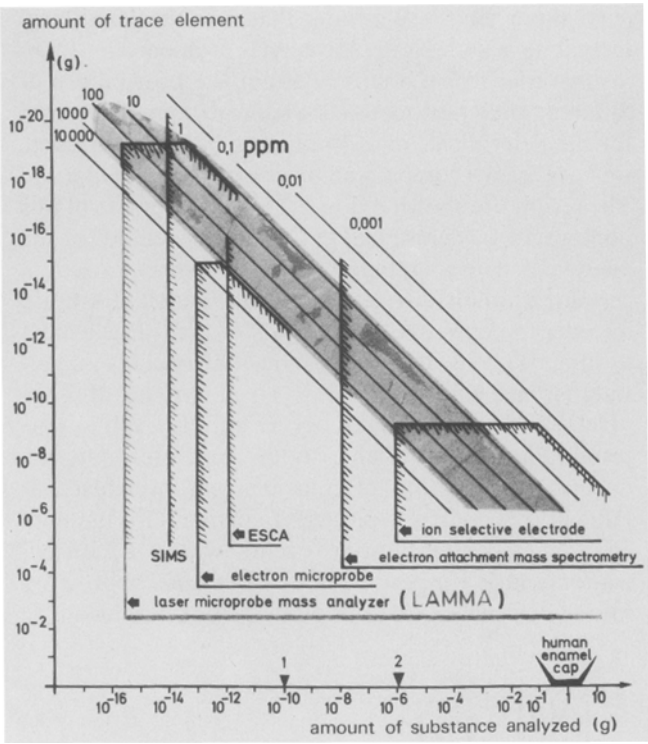

Fig. 8. Detection limits and sensitivity of different microanalytic methods for fluorine analysis. ad 1▼: The growth velocity of the continuously growing enamel matrix of the rat incisor is about 1 µm/8 min. If a minimum distance of 4 µm for two separate laser holes (see Figs. 5 and 6) within a scanning trace is assumed, growth layers can be analysed which differ by a generation time of about half an hour. – The substance which is needed for each analysis is about 10^{-10} g. ad 2▼: The total mass of a human enamel prism is about 10^{-6} g (mean prism diameter 4–7 µm)

be determined by the analytical method is determined by the abscissa, the ordinate and the area between the 1 and the 1000 ppm lines diagonally from left above to right down (grey area). Fluorine concentrations of that quantity are detectable with the ion-selective electrode if big substance quantities are available, e.g. a whole tooth or a big part of it. If the quantity of substance which should be analyzed becomes smaller and smaller one has to apply the electron attachment mass spectrography, the ESCA or the SIMS method. Extremely low quantities e.g. within single enamel prisms are only detectable with the aid of the LAMMA method.

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