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LASER-INDUCED AUTOFLUORESCENCE SPECTROSCOPY OF DENTAL CARIES

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Abstract - We studied spectral autofluorescence characteristics of dental caries. A wide range of carious lesions revealed characteristic emission of endogenous fluorophores with strong fluorescence bands in the red spectral region when excited with 407 nm line radiation of a krypton ion laser. Healthy hard dental tissue exhibited no emission bands in the red. The fluorescence spectra, fluorescence excitation spectra as well as the reflectance spectra of carious lesions were found to be typical for fluorescent porphyrins, mainly protoporphyrin IX. A possible source of these porphyrins within carious tissues is bacterial biosynthesis. Non-invasive sensitive *in vivo* caries detection by means of appropriate excitation sources and porphyrin fluorescence detectors should be possible.

Key words. Dental caries, fluorescence diagnosis, autofluorescence, porphyrins

INTRODUCTION

Conventional methods of caries detection include clinical, chemical and radiographic techniques. Clinical diagnosis involves visual recognition of color changes and tactile exploration with hard instruments. These methods are not well suited for detection of early lesion development. Use of chemical systems for caries diagnosis is moderately effective, but time-consuming and difficult to utilize where access is restricted (e.g. Franco and Kelsey, 1981; Fusayama, 1979; Zinck *et al.*, 1988). Concerns regarding radiographic techniques include undesirability of repeated exposure to ionizing radiation and difficulties associated with conversion of 3D- into 2D-images. Another approach of early caries diagnosis is the application of novel optical methods, such as reflectance spectroscopy and laser-induced autofluorescence (e.g. reviews: Angmar-Mansson and ten Bosch, 1987, 1993).

Light exposure of thick biological tissue results in reflection, scattering and absorption of incident photons. After launching into tissue, some backscattered photons will leave the tissue again. Together with direct reflected photons (Fresnel reflection at the boundary air-tissue), these scattered photons are the origin for reflectance light. For example, the remittance of human Caucasian skin at 630 nm is about 0.6. That means 60% of incident photons will leave the tissue (König *et al.,* 1994). The remittance depends on the refraction index, the scattering and absorption behavior of the tissue. The remaining photons within the tissue are absorbed by biomolecules. This results in heat formation, photochemical reactions and in autofluorescence. Autofluorescence is based on the presence of endogenous fluorophores. Important fluorophores are the aminoacids tryptophan and tyrosine, the coenzymes NADH, NADPH and flavins, as well as endogenous metal-free porphyrins. Fluorescence spectrum and fluo-

rescence lifetime are characteristic for a specific fluorophore. Extremely sensitive fluorescence detectors allow counting of single fluorescence photons.

Autofluorescence detection is an interesting tool for the differentiation between various tissues and for gathering of information about cell metabolism (see review: König and Schneckenburger, 1994). The method was also applied to study autofluorescence of dental tissues. Using a Wood lamp, Bommer was likely the first who detected in 1927 an orange and red fluorescence of the "tooth film" in patients. Benedict (1928) and Hartles and Leaver (1953) reported on tooth fluorescence under UV irradiation. Armstrong (1963) studied the fluorescence of healthy and carious human dentine samples. Since the eighties, different groups have attempted to localize carious regions by means of laser-induced autofluorescence (e.g. review Angmar-Mansson and ten Bosch, 1993). Alfano *et al.* (1984) investigated autofluorescence of dental tissue during 488 nm excitation. A fluorescence maximum around 550 nm was found for both carious and non-carious regions. No significant differences in the spectral behavior were measured. Albin *et al.* (1988) used the same excitation wavelength but measured a single maximum for carious regions at 590 nm compared with the fluorescence of surrounding sound tissue with a main peak at 553 nm. Hafström-Björkman *et al.* (1991) reported on differences in the fluorescence intensity at 540 nm during 488 nm excitation. Also Bjelkhagen *et al.* (1982) used the argon ion laser at 488 nm, although most of endogenous fluorophores absorb mainly in the UV and in the short-wavelength region of the visible spectrum. Alfano *et al.* (1981) varied the excitation wavelength (350 nm, 410 nm, 530 nm) and found a higher fluorescence intensity in the red spectral region (unstructured spectrum) for carious than for healthy dentin and enamel, especially for UV and 530 nm excitation. They determined the same emission lifetime for carious and non carious regions of about 2.3 ns.

Autofluorescence in the red spectral region is often based on endogenous fluorescent porphyrins. Different microorganisms, such as yeast cells (König and Schneckenburger, 1994), *Propionibacterium acnes* (Kjeldstad *et al.*, 1984), *Bacteroides melaninogenicus* (Shah *et al.*, 1979), *Pseudomonas areuginosa* (König *et al.*, 1992) are able to synthesize proto- and coproporphyrin. These fluorophores absorb mainly in the violet spectral region around 405 nm. It seems therefore possible that oral bacteria present in carious lesions may cause a typical autofluorescence in the red spectral region during appropriate light excitation.

It was the aim of this study to investigate autofluorescence in the red spectral region of healthy and carious hard dental tissue using the 407 nm line of a krypton ion laser as optimal excitation radiation for porphyrin fluorescence.

MATERIALS AND METHODS

The experimental set-up is shown in fig. 1. The 407 nm radiation of a krypton ion laser, transmitted via quartz prisms to separate the background radiation of the laser, was coupled into a single 0.6 mm quartz fiber. The sample was irradiated with an excitation power of 3 mW, the irradiated area was about 5 mm^2. Eight peripheral 0.4 mm fibers were used as fluorescence detection fibers. The dichroic long-pass filter (cut-off wavelength: 460 nm, Zeiss/Germany) in front of the slit of the polychromator prevented the entrance of backscattered excitation light (rejection at 407 nm: 0.99). Spectra were measured within 1 s using an optical multichannel analyzer (OMA, ZWG, Academy of Sciences, Berlin/Germany).

Reflectance spectra were measured with the same fiber optical sensor and detection system, but without the long-pass filter at the entrance slit. A stabilized high pressure xenon lamp (polychromatic light source) was used as irradiation source. Spectralon (remittance R= 0.99) served as white standard. This special experimental set-up and the exact correction procedure have been described earlier (König *et al.*, 1994).

Fluorescence excitation spectra were obtained using the fluorescence spectrometer SFM 25 (Kontron Instruments, Germany) with a modified sample holder.

One hundred permanent human teeth extracted for prosthodontic or orthodontic purposes were examined. All teeth had at least one carious lesion, as prediagnosed by clinical and radiographic determination. The time interval between extraction and fluorescence investigations usually averaged 3-5

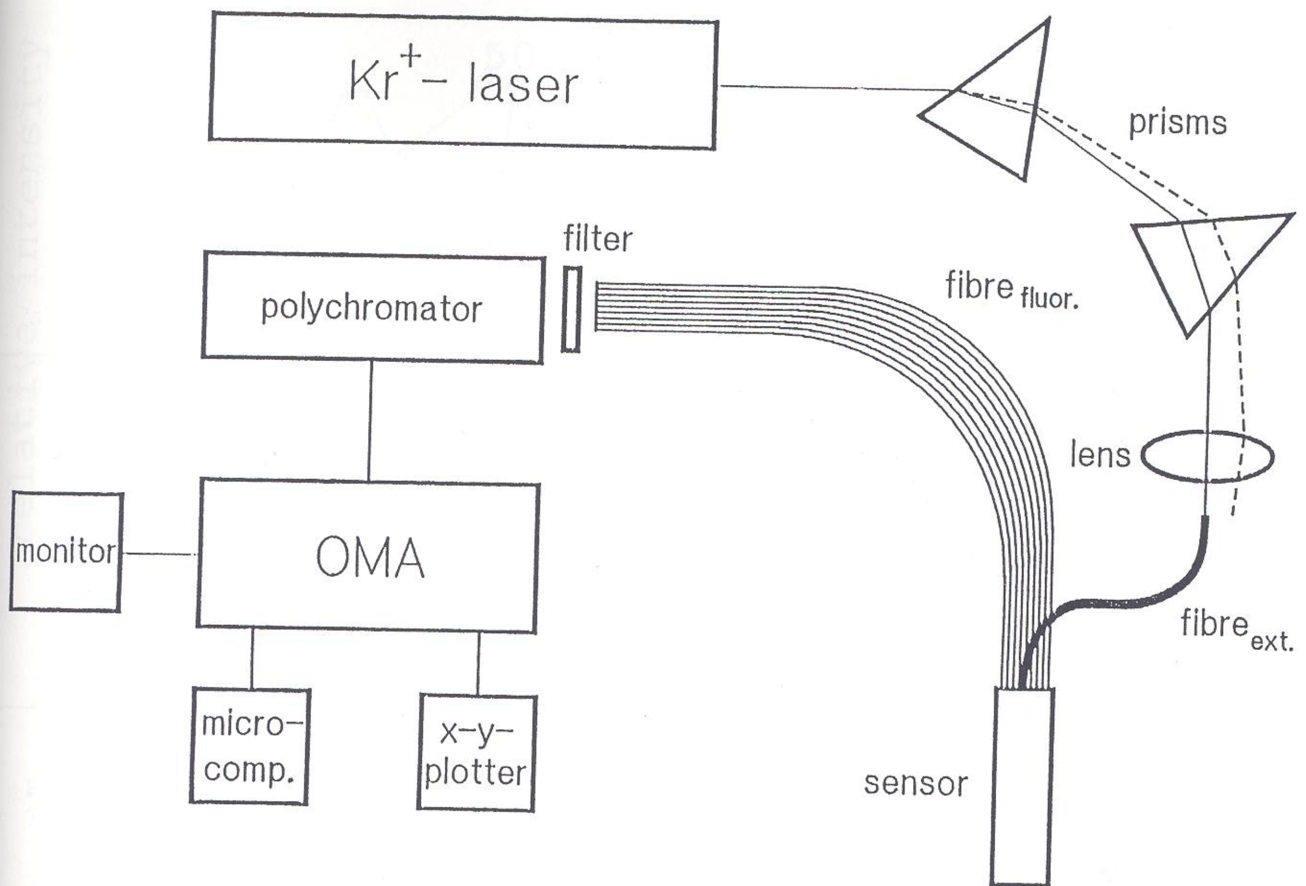

Fig. 1 *Experimental set-up for sensitive fluorescence spectroscopy*

hrs. In the meantime specimens were stored in isotonic NaCl, in an attempt to maintain the existing tooth flora as accurately as possible. During fluorescence measurements, teeth were removed from the NaCl solution, but not dried in any way.

RESULTS

Healthy dental hard tissue as well as carious lesions exhibited autofluorescence in the red spectral region when excited with 407 nm radiation. However, major differences were found in spectral characteristics. Healthy hard tissue (dentin, enamel) showed no fluorescence maxima in the red spectral region. In contrast, all carious lesions exhibited characteristic autofluorescence peaks in the red. Most of the carious regions had a strong fluorescence maximum at 635 nm and a shoulder around 700 nm.

Fig. 2 demonstrates autofluorescence spectra from a tooth with various carious regions and a filling material. The first spectrum exhibited autofluorescence of caries-free regions showing a continuous decrease of fluorescence intensity with increasing wavelength. The next spectrum of a small superficial carious lesion (caries I) revealed a peak at 635 nm. This characteristic red fluorescence was superposed by the structureless autofluorescence of surrounding healthy tissue. The following spectrum II of a larger carious lesion indicated an additional band around 590 nm. Spectrum III consisted of a superposition of two main bands with maxima at 625 nm and 635 nm. The spectrum of carious lesion IV was found to be similar to that of caries II, but with a relative higher fluorescence around 670 nm. Whereas all extracted teeth exhibited the 635 nm fluorescence band in carious tissues, the 590 nm emission was found in four teeth and the 625 nm

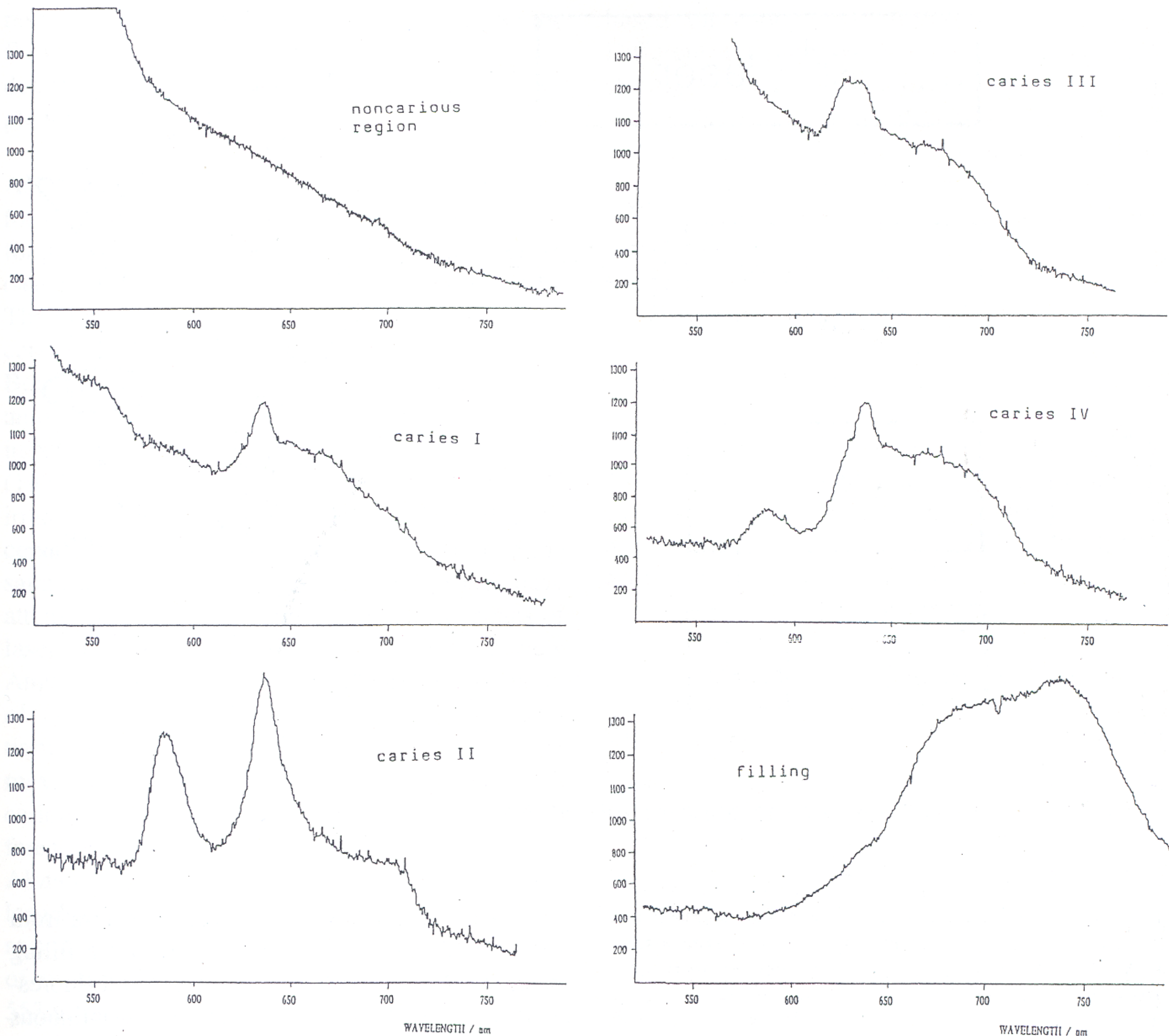

Fig. 2 *Autofluorescence spectra of one freshly extracted human tooth with various carious lesions and a filling material.*

band in two teeth only. The autofluorescence intensity decreased with increased excitation light dose. The photobleaching rate for the 590 nm fluorescence was found to be higher than for the 635 nm autofluorescence. The spectrum of a filling material showed a broad fluorescence band in the far red spectral region with a bandwidth of more than 100 nm.

In order to gain more information on the fluorophores responsible for the characteristic caries fluorescence, fluorescence excitation spectra were taken. A fluorescence excitation spectrum of a typical large carious lesion is shown in fig. 3. A main excitation band occurred in the violet spectral region, followed by smaller bands at 466 nm, 505 nm, 540 nm, 582 nm and 632 nm. This excitation spectrum indicates that the most efficient fluorescence excitation wavelength to induce the structured red fluorescence in carious lesions is around 400 nm.

Additional information was obtained by backscattered light measurement during high pressure

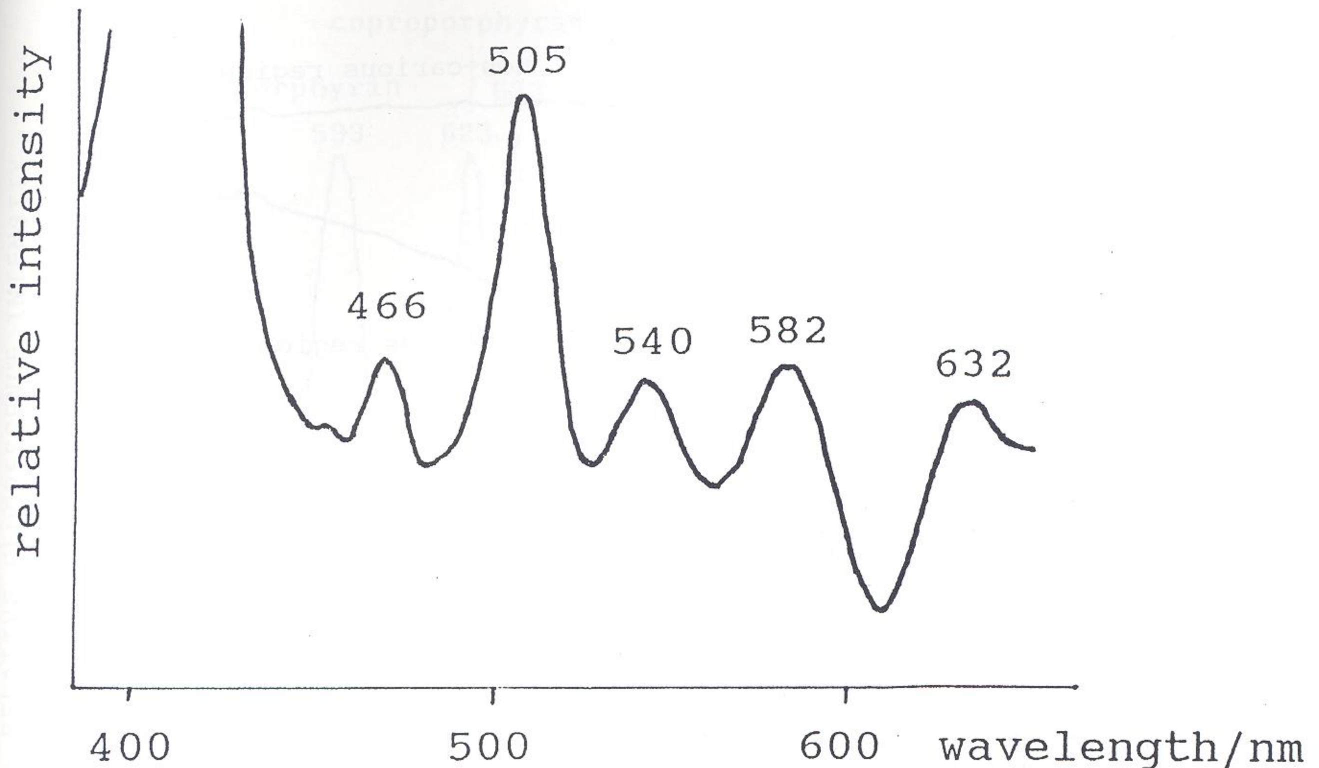

Fig. 3 *Fluorescence excitation spectrum of a strong fluorescent carious region.* Fluorescence was detected at 700 nm in order to get information on the absorption behavior in the red spectral region.

xenon lamp irradiation (Fig. 4). Reflectance of the carious region was around 10% in the visible spectrum up to 600 nm, in contrast to 50% remittance of surrounding healthy hard tissue. Of course, this value is even higher in the case of sound white teeth. The reflectance minima at 510 nm, 548 nm and 584 nm measured in carious lesions correspond nearly to the fluorescence excitation bands. No such bands were found in healthy tissues.

The autofluorescence spectra document the possibility to differentiate between healthy and carious regions by sensitive fluorescence spectroscopy. The spectral characteristics of fluorescence and absorption in carious regions are typical for fluorescent porphyrins. Compared to fluorescence spectra of different porphyrins in solution (Fig. 5), the 635 nm autofluorescence maximum of caries can be attributed to endogenous protoporphyrin IX, the 625 nm band to coproporphyrin and the 590 nm emission to fluorescent metallo-porphyrins, such as Zn-proto-porphyrin.

DISCUSSION

The sensitive laser-induced fluorescence spectroscopy indicates that carious regions emit differently from adjacent healthy dental tissue and filling material in the red spectral region. Initial caries detection by fluorescence spectroscopy appears therefore possible. The endogenous fluorophores responsible for the caries fluorescence seems to be mainly protoporphyrin IX as shown by emission spectra, fluorescence excitation spectra, and reflectance spectra. Additional time-resolved and time-gated fluorescence measurements indicate the presence of metal-free porphyrins (König *et al.*, 1999). The fluorophores are photolabile. Irradiation of protoporphyrin leads to oxygen-dependent photodestruction due to photobleaching and photoproduct formation as shown in former experiments (König *et al.*, 1993). Therefore, low excitation light doses and sensitive detectors are required for caries detection by autofluorescence.

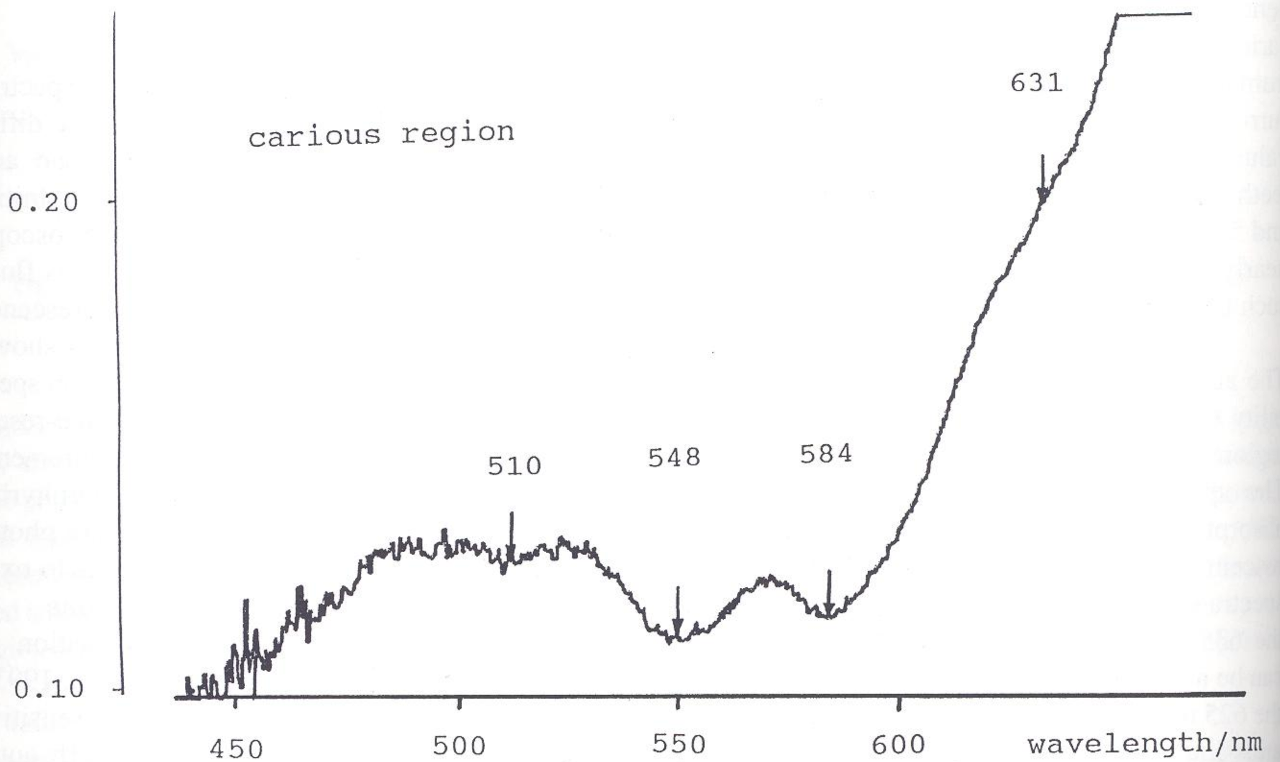

Fig. 4 *Reflectance spectrum of the same carious lesion as in fig. 3.*

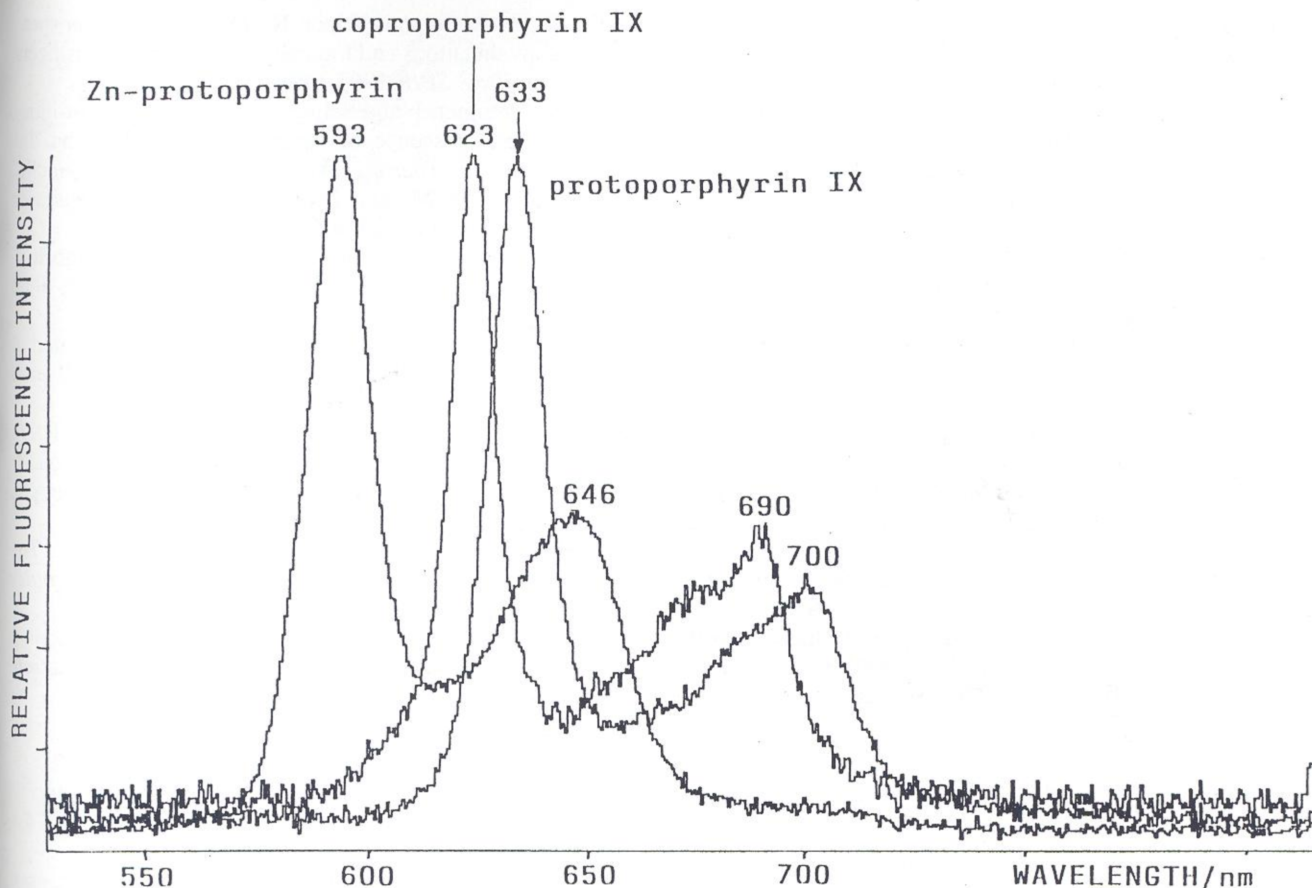

Fig. 5 *Emission spectra of different porphyrin solutions* (solvent: dimethylsulfoxide).

Various microorganisms are able to synthesize fluorescent porphyrins. Further investigations are necessary to determine the exact origin of the endogenous porphyrins and to clarify which caries-involved bacteria are responsible for the porphyrin fluorescence.

In order to build an *in situ* caries detector for clinical use excitation sources with emission in the violet/blue spectral range are recommended, such as mercury lamps, krypton ion lasers and blue-emitting (laser) diodes. The fluorescence excitation radiation should be transmitted via a light fiber system to allow examination of dental cavities. Appropriate sensitive low-cost detectors are photomultipliers equipped with special spectral filters.

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