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# The Total Luminescence of Bovine and Human Dental Enamel

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The corrected emission and excitation spectra of human and bovine dental enamel and its separated organic material were determined at room temperature and liquid nitrogen temperature. In both materials three distinct *luminescence* peaks were found in the region 350-360nm, 405-410nm, 440-450nm. The intensity ratios of the peaks of the enamel do not differ substantially upon removal of the inorganic component. Hence a conclusion is confirmed that the organic component is the most responsible material for the total luminescence of the enamel. Several hypothesis are available for determination of the nature of luminescence of the organic components. Differences in the excitation spectra are discussed. Phosphorescence emission and phosphorescence lifetimes were determined.

*Key words:* Luminescence — Enamel — Protein

## Introduction

The fluorescence and phosphorescence of the hard calcified tissues are known phenomena, observed and described by several authors. Reviews have been given e.g. by Scharf (1971) or by Hefferren *et al*, (1971). Emission bands were observed at about 410nm [1, 2, 12, 13] and at about 450nm [3, 5, 14].

The origin of the luminescence is presumed to be in the organic components of the tissues [6].

We have measured the total luminescence of human and bovine dental enamel at room and liquid nitrogen temperatures in the range 240—600nm. All the spectra were corrected for the spectral dependence of the apparatus. Three significant emission peaks located at 350, 405 and 450nm were found together with a smaller peak at 520nm. The intensity ratios of the different peaks in the investigated materials were determined. Phosphorescence spectra and phosphorescence lifetimes were measured.

Also, such corrected spectra were determined of the organic component of the enamel, obtained in suspension by dissolving the inorganic material.

## Materials and Methods

The samples were prepared from the sound human and bovine enamel of mature, freshly-extracted incisors. Special care was taken to ensure that no dentine was present in the enamel samples. A labial enamel layer was cut off the tooth and ground from the dentino-enamel junction side until no dentine was detectable by visual and microscopical inspection and under UV light. The presence of organic materials other than the enamel matrix (e.g. collagen) cannot be completely excluded on the basis of this preparative procedure. However, it then could be a matter of discussion if this material should not be regarded as a component of

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enamel. Also control measurements were made on several slabs ground from both sides and suspensions obtained therefrom. Of all samples, the flat side (or sides) was exposed to the incident light and therefore it was optically polished to diminish surface reflections. The area of the samples was about 50 $mm^2$, the thickness about 0.5 mm.

For the organic *material* luminescence measurements, the dentinefree enamel was ground and the powder (1 g) suspended into 0.01 M HCl (150 ml). During the dissolution process the pH was kept constant by the addition of acid. The resulting suspension was then dialysed for several days against daily refreshed water; subsequently the content of the bag was freeze-dried. An aqueous suspension of this material was used for the luminescence measurements.

The luminescence spectra were measured by means of a MPF 3 Perkin-Elmer Fluorescence Spectrophotometer with a corrected spectra accessory. This unit continually corrects the emission spectra for detector and monochromator transmission from 300 nm till 600 nm and excitation spectra for lamp output and monochromator transmission from 250 nm till 600 nm.

The solid samples were mounted in the Perkin-Elmer Solid Sample Holder Accessory 018-0051. For the liquid samples conventional fluorescent-free quartz 10 mm × 10 mm cells were used. The sensitivity settings varied between 0.3 and 10. The slit widths used for the slab were:—excitation 10 nm, emission 5 nm. The slit widths used for the suspension: excitation 20 nm, emission 10 nm.

A band filter D25 on the excitation side and different cut-off filters on the emission side were always used in order to eliminate the harmonics and stray light respectively. The band filter was omitted when recording the excitation spectra.

The measurements at liquid nitrogen temperature were performed in a metal cryostat (Cryoson) with temperature control and by means of the Perkin-Elmer Phosphorescence Accessory 018-0073. The same slit widths and sensitivity settings were used as during the measurements at room temperature. The phosphorescence lifetime was measured by means of a shutter in the excitation beam and replotting the regular decay curve on semi-logarithmic paper.

## Results

No substantial differences were found between the luminescence of human and bovine enamel. In spite of their different absorption spectra [15] no important differences in the sensitivity ratios of differents emission peaks in human and bovine enamel could be observed. Three emission peaks together with their excitation maxima for the enamel and the aqueous suspension of its organic component are shown in Figs. 1, 2, 3.

These spectra are not corrected for reabsorption, but from the data on absorption and scattering [15] it can be estimated that reabsorption of radiation emitted at 350 nm is around 10 %, this value decreasing with increasing wavelength.

Table 1 summarizes the maxima of emission bands together with their excitation maxima for both investigated materials (solid and liquid) at room temperature. A minor shoulder in the *luminescence* emission was present at 520 nm. There is a remarkable difference between the excitation spectra of solid and liquid material when the emission wavelength is set at 440—450 nm.

No substantial difference in the positions of the *luminescence* maxima was found when measured at the low temperature. Fig. 4 shows emission and excitation of total emission and phosphorescence spectra for the enamel and the suspension of its organic component. In Table 2 the experimental maxima are shown. Phosphorescence decay times were found to be $4 \pm 1$ s for the solid enamel as well as for the organic component in the ethyleneglycol-water glass.

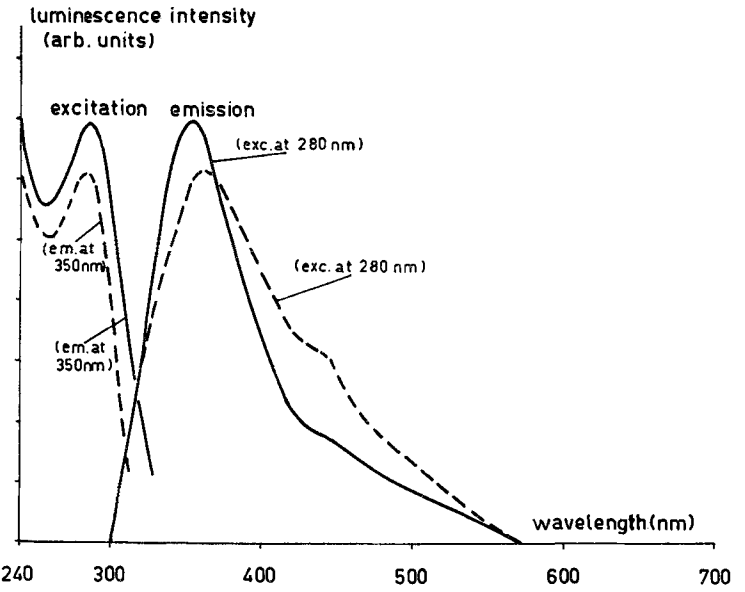


Fig. 1. The corrected emission and corrected excitation of the bovine dental enamel slab (full line) and of the aqueous suspension of its organic component (dashed line). The units of the luminescence intensity of the solid enamel are not the same as the luminescence intensity units of the organic component of the enamel; the units of luminescence intensity for one and the same material in Figs. 1, 2, 3 are the same. Cut-off filter UV-31 was used

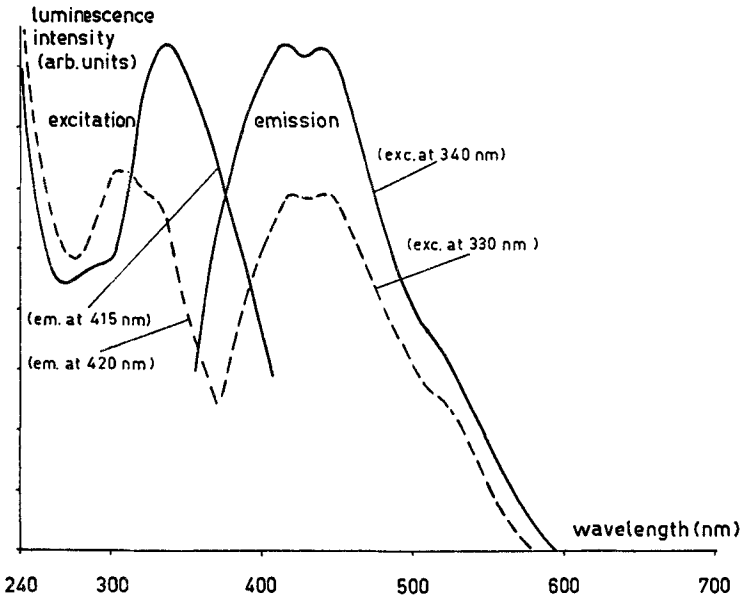


Fig. 2. Same as Fig. 1, but other wavelengths as indicated. Cut-off filter UV-35 was used

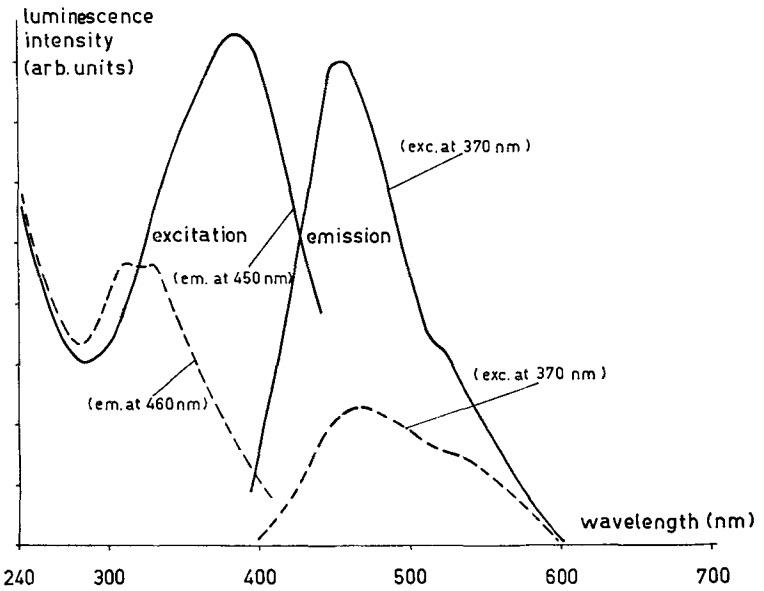


Fig. 3. Same as Fig. 1, but other wavelengths as indicated. Cut-off filter UV-39 was used

Table 1. Luminescence of enamel at room-temperature. Emission and excitation maxima of bovine enamel and its organic component

| Material | This work | | Previous work | | Ref. |
|---|---|---|---|---|---|
| | Exc. wavelength (nm) | em. wavelength (nm) | Exc. wavelength (nm) | em. wavelength (nm) | |
| Solid Enamel | 285 ± 5 | 350 ± 3 | | | |
| | 335 ± 5 | 405 ± 3 | | | |
| | 375 ± 5 | 450 ± 3 | 370 | 450 | [4] |
| | | | 370 | 460 | [14] |
| | | 520 (shoulder) | | 530 (shoulder) | [14] |
| Organic Component | 285 ± 7 | 360 + 5 | | | |
| | 330 ± 7 | 410 ± 5 | 325 | 402 | [12] |
| | | | 292 | 408 | [12] |
| | 330 ± 7 | 455 ± 5 | | | |

## Discussion

In the emission spectra of enamel and the suspension of its organic material (Figs. 1-3) at least three distinct emission bands are seen. The visible emissions each have been observed in these materials by others (c.f. Table 1).

A spectrum of the 450 nm emission band in enamel has been reported by Hall *et al.* (1970), a corrected emission spectrum including the shoulder around 530 nm and also a corrected excitation spectrum of the emission peak have been shown by Scharf (1971). These results are in agreement with ours.

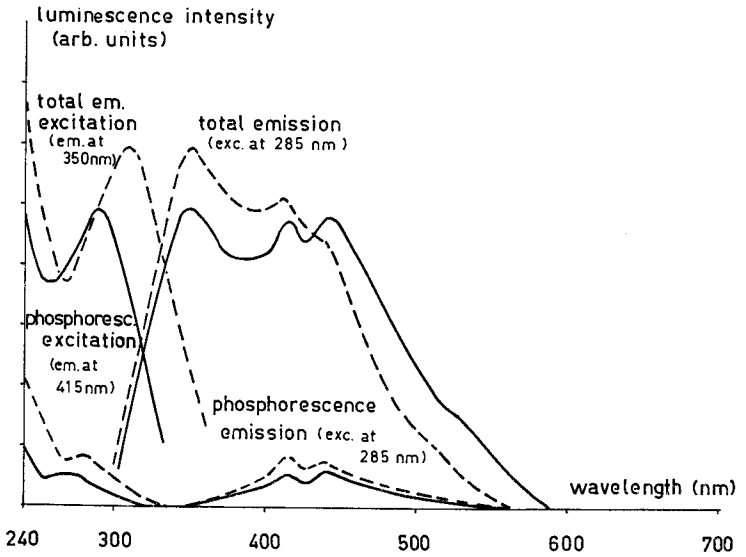


Fig. 4. The corrected emission and corrected excitation spectra of the dental enamel (full line) and its organic component suspension in ethyleneglycol-water (1 : 1) glass (dashed line) at 77 K. The units of the luminescence intensity of the solid enamel are not the same as the luminescence intensity units of its organic component

Table 2. Luminescence of enamel at 77 K

| Material | This work | | | Previous work | | | Ref. |
|---|---|---|---|---|---|---|---|
| | Exc. wavelength (nm) | Em. wavelength (nm) | decay times (s) | Exc. wavelength (nm) | Em. wavelength (nm) | decay times (s) | |
| Solid Enamel | Total Emission | | | | | | |
| | 285 ± 5 | 350 ± 5 | | | | | |
| | 335 ± 5 | 410 ± 5 | | | | | |
| | 375 ± 5 | 440 ± 5 | | | | | |
| | Phosphorescence | | | | | | |
| | 285 ± 5 | 405 ± 3 | 4 ± 1 | | | | |
| | 285 ± 5 | 440 ± 3 | 4 ± 1 | 310 | 455 | | [5] |
| | 340 | no detectable | | 311 | | 4.25 | [6] |
| | 370 | phosphorescence | | | | | |
| Organic Component | Total emission | | | | | | |
| | 305 ± 5 | 350 ± 5 | | | | | |
| | not exactly | 410 ± 5 | | | | | |
| | determinable | 440 ± 5 | | | | | |
| | Phosphorescence | | | | | | |
| | 285 ± 7 | 410 ± 5 | 4 ± 1 | | | | |
| | 285 ± 7 | 445 ± 5 | 4 ± 1 | | | | |

The 405nm-band has been observed in enamel parent gelatin by Mancewicz and Hoerman (1964). These authors used two preparations, one prepared with EDTA at neutral pH, and a second prepared from the first by hydrolysis in 3.6N HCl for 7 days. Their acid treatment shifted the excitation from 325nm to 295nm which is in agreement with our observation of two unresolved excitation peaks (Fig. 2) in material obtained after the use of a weaker acid treatment (0.01M HCl for several hours).

Our spectra indicate that the radiation emitted in these two visible bands (405nm and 450nm), when excited at optimum wavelength, is of the same order of magnitude as the amount of radiation emitted by tryptophan residues, when excited at optimum wavelength. However, the absorption in the range 330 to 500nm is much smaller than the absorption at 285nm [15], the tryptophan absorption. We therefore expect that the long-wavelength chromophores have a much higher quantum yield (of the order of a factor of 5) than the tryptophan residues. The last mentioned value differs from protein to protein but most values quoted are of the order of 10 % [8].

The shift in the excitation spectrum of the 450nm emission band which occurs upon decalcification of the enamel (Figs. 2, 3) may perhaps be due to differences in scattering coefficient and wavelength dependence thereon [15]. Differences in true optical path length and thus in absorption could cause relatively large spectral changes between both samples. More likely seems to be an excited state effect. Such could be the existence of an excimer [17] in the suspension but not in the enamel, or energy transfer in suspension but not in enamel. Quantative spectra, corrected also for scattering effects, are needed before definite conclusions can be drawn. Such experiments are in progress. Anyway, it seems very likely that both the 405nm and 450nm bands arise from related chromophores in different degree of relation in enamel and supension, or from one chromophore in different states.

The positions of the peaks of the low-temperature luminescence (Fig. 4) can be compared to the phosphorescence maxima of enamel as reported by Hoerman and Mancewicz (1964). The data relevant to this work are included in Table 2.

With regard to the origin of all these emissions several conclusions are possible. In enamel at room temperature three different bands with different excitation spectra lead to the conclusion that three different luminescing entities are present. In the suspension both the 410nm and the 450nm emissions have about the same excitation spectrum. In this case at least two luminescent entities are present. A comparison of Figs. 1-3 shows that all peaks of the enamel are also present in the suspension spectra with comparable intensity ratios, this confirming previously reached [6] conclusions that hydroxyapatite does not play an important, if any, role in enamel luminescence.

The fine structure of the phosphorescence emission of enamel and the suspension (Fig. 4) agrees well with the phosphorescence spectrum of tryptophan and tryptophan containing (Class B) proteins. Our decay times agree well to tryptophanic residues ($\tau_{tryp} = 6.65$s, $\tau_{prot} \cong 5$s [17]) but are too long for tyrosine residues ($\tau_{tyr} = 2.90$s [17]). We conclude therefore that the phosphorescence is due to tryptophan residues in the enamel protein.

The fluorescence emission at room-temperature at 350nm, excited at 285nm, agrees with known tryptophan emission data for various circumstances and will also be of tryptophanic origin. However the position of the short-wavelength emission of enamel and suspension at 77K is not in agreement with the low-temperature emission of tryptophan, its derivatives and globular Class B proteins in the same solvent, which are all located around 325nm [17]. Apparently, in enamel protein the microenvironment of tryptophan does not change when the temperature is decreased. Perhaps the chromophore is not in contact with liquid at all. In this respect it might be of interest that Steen (1968) found that fluorescence of dry tryptophan at 77K emitted 25nm to the red from a solution in a ethyleneglycol-water glass. However, dry tryptophan may be a more complicated system due to chromophore interaction, for instance no phosphorescence is observed.

The 405nm and 450nm emission bands in enamel and suspension clearly arise from a chromophore that is attached to either a water-insoluble, or to a nondialysable, i.e. large molecule. Similar or the same bands have been observed in many connective tissues. Reviews have been given by Hoerman (1971) and Scharf (1971).

La Bella and co-workers [9, 10] suggest that these emissions are due to bityrosine crosslinks as studied by Lehrer and Fasman (1967), but Hoerman in his review article [7] reaches another conclusion. More recent evidence by Armstrong and Horsley (1972) indicates that the fluorescent material was not intrinsic to calcified tissue collagen, but rather another substance bound to the matrix.

In order to conclude whether or not these emissions are of the same origin as the 405nm and 450nm bands in enamel, complete emission spectra with excitation spectra for several proteins have to be available.

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