# Laser induced autofluorescence of bones and teeth in newborn rats after maternal administration of indinavir

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*Abstract*—**This paper presents autofluorescence of bones and teeth for 14-day and 28-day old newborn rats. The measurements were carried out by means of the fibre optical spectrometer with laser excitation source (407 nm). Differences in emission spectra of incisor, mandible and femur of rats with age were observed. It was also found that maternal administration of antiviral drug – *indinavir* (500 mg/kg po.) caused changes in autofluorescence of the studied tissues.**

## I. Introduction

OPTICAL spectroscopy is one of the most widely used spectroscopic techniques in the fields of biochemistry and molecular biophysics today [1]-[2]. Among instrumental techniques, fluorescence spectroscopy measuring the emission intensity of the sample, is recognized as one of the more sensitive. The reason for the high sensitivity of fluorescence techniques is that the emission signal is measured above a low background level which is essential in life science research. Additional advantage of optical fluorescence spectroscopy is that this technology can be utilized without damaging specimens.

Fluorescence spectroscopy requires fluorophores which can be of three types i.e. the exogenous, the exogenously induced (synthesized in patient body from exogenous precursor such ALA) or endogenous ones. Most works in this field deal with exogenous fluorophores currently being assessed as contrast agents for cancer detection (PDD - photodynamic diagnosis) and therapy (PDT) [3]-[5].

This paper focuses on endogenous fluorophores whose natural emission is often called autofluorescence unlike fluorescence of exogenous ones. The most frequently occurring endogenous fluorophores in biological tissues are amino acids (tryptophan, tyrosine, phenylalanine), structural proteins (collagen, elastin), metabolic coenzymes (flavins, NADH, NADPH), lipids and porphyrins [6]-[7]. Their fluorescence is sensitive to the environment and the metabolic status of the tissue which change with progresses of the tissue from normal to the pathologic state. This makes autofluorescence spectroscopy a valuable, noninvasive tool to study the health of biological tissues.

Recently autofluorescence spectra of human bronchial tissue, human skin or teeth for in *vivo* detection of various diseases and pathologies have been reported [8]-[14]. However, according to our knowledge no systematic study of bone autofluorescence has been conducted so far. Thus an animal model was used to study the changes in autofluorescence of bones in newborn rats with age and due to drugs administered.

The paper describes preliminary spectroscopic studies *in vitro* of flat and long bones as well as teeth of rats during the first month of life.

It is known that anti- HIV therapy can have a toxic effect on different internal organs [15]. Therefore it is of interest to find out whether maternal administration of anti-viral drug *indinavir* affects bone ontogenesis in newborn rats. The examination of autofluorescence from various bone tissues seems to be beneficial both from the scientific as well as the application point of view.

## II. Materials and Methods

The study was performed in female Wistar rats from the Centre of Experimental Medicine of Medical University of Silesia. After fertilization rats were administered intragastrically with antiviral drug – *indinavir* in a dose of 500 mg/kg po from $6^{th}$ to $15^{th}$ day of pregnancy. Rats of control group were administrated distilled water in volume of 5ml/kg using intragastric tube. The bone tissues were sampled from 14- and 28-day-old rats. Each group consisted of 6-8 newborns. The bones were cleaned from soft tissues, brushed in purified water a number of times and then air-dried. After preparation the bones were separated according to kind of bone, age and maternal drug administered. The autofluorescence studies were performed for mandible (flat bone), femur (long bone) and incisor (example of tooth). Two (A, B) and three areas (A, B, C) on the bone surface were chosen for examination of mandible and femur, respectively. Regions of interest of the studied rat bones are illustrated in Fig. 1.

Manuscript received June 3, 2008. This work was supported in part by Polish Ministry of Education under project No. 2P05D085.

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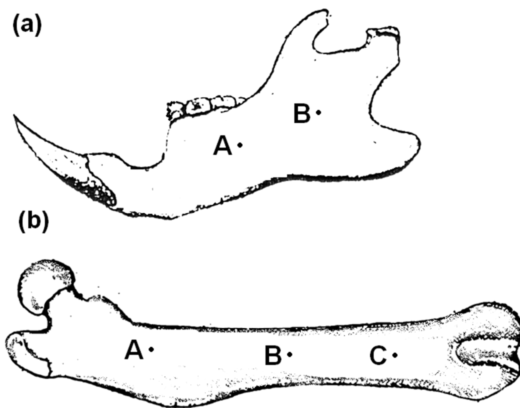



Fig.1. Regions of interest of rat mandible and femur

Fluorescence spectroscopy basing on fiber-optical techniques is especially usefull in measuring the fluorescence emission from biological tissues. The optical apparatus used in this work is presented in Fig. 2.

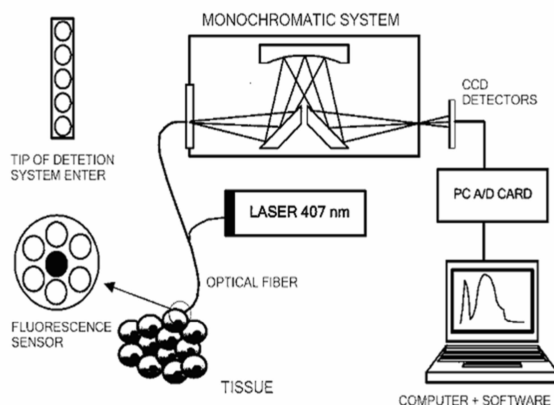



Fig. 2. The optical scheme of laser (407 nm) induced fluorescence (LIF).

Fiberoptical fluorescence analyzer was constructed using BIOSPEC technology and LESA 6 software [16]. The excitation wavelength (407 nm) was generated by the semi conductive GaN laser (initial energy 10 μW). The emission spectra were collected at a given site by means of a fiber bundle of 1.9 mm diameter that was placed at a fixed distance in the vicinity of the site to be measured. The raw spectra were recorded in the range up to 814 nm. Each data collection set of 10 raw spectra was considered to be an independent measurement. The spectra were normalized to the excitation peak at 407 nm take as the reference signal. A linear background was defined by the signal 407 and 814 nm and subtracted from each measurement. Then the spectra were averaged to obtain the typical spectrum of bone tissue, provided there were no statistical differences between the regions of interest.

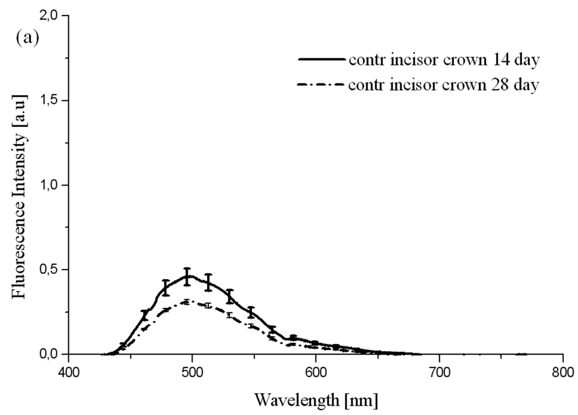
The obtained results were formulated using STATISTICA and ORIGIN software. Shapiro-Wilk's test to check the normality of the distributions together with ANOVA and Duncan's test to obtain significance level coefficient were used.

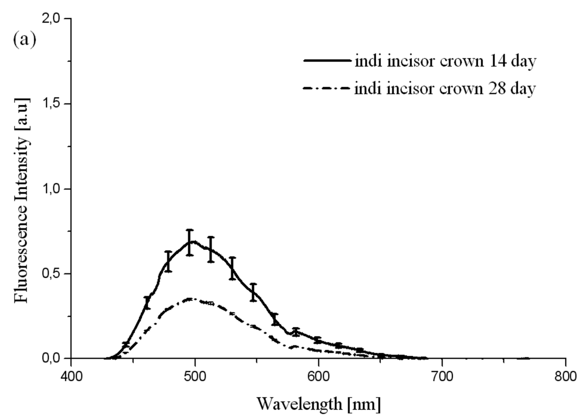
## III. Results and Discussion

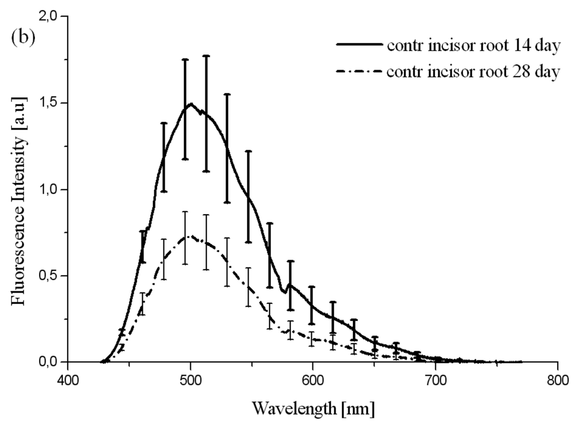
Figure 3 - 6 present typical emission spectra of the studied bone tissues for 407 nm as the excitation wavelength. Autofluorescence from an anatomic crown (a) and an anatomic root (b) of incisor for healthy 14- and 28-day old rats are presented in Fig. 3. Analogous spectral characteristics for mandible (a) and femur (b) are presented in Fig. 5. Similar set of the results for incisors, and flat and long bones of newborns whose mothers were treated with *indinavir* during pregnancy are illustrated in Fig. 4(a), 4(b) and 6(a), 6(b). Emission spectra with the broad band located near 500 nm maximum together with two apparent side bands on either side (blue and red) are observed. These spectra are substantially similar to those reported earlier [12]-[14]. Contributions from endogenous fluorophores such as porphyrins, flavins and vitamins seem to be present. Porphyrins related to heme biosynthesis yield a red fluorescence with peaks 630 nm and 670 nm. Flavin components essential in cellular metabolism exhibit emission maximum at 535 nm. However 1.25-dihydroxyvitamin D regulating transcellular transport in bone can give fluorescence contribution at about 480 nm.

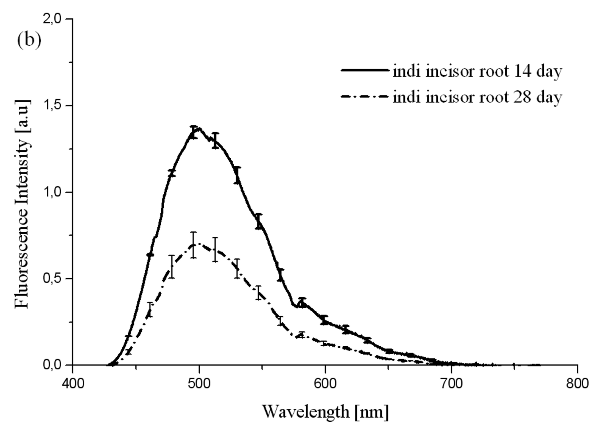
Emission spectra seen in Fig. 3-6 disclose that intensity of fluorescence depends markedly on the kind of bone tissue as well as on the age of newborn rats. The highest emission intensity from femur while the lowest emission from an anatomic crown are observed. This behavior of autofluorescence seems to be justified by the composition of the bone tissue. An anatomic crown consists of three tissues: enamel, dentin and pump. It is known that approximately 96% of enamel is mineralized, containing the most dense hydroxyapatite in the body. The organic material constitutes only 1% of the enamel mass. Thus the low emission from the surface of incisor crown seems to be accepted. Our measurements show that an increase of organic matrix up to 20% in the other forms of bone tissue leads to enhancement of fluorescence intensity.

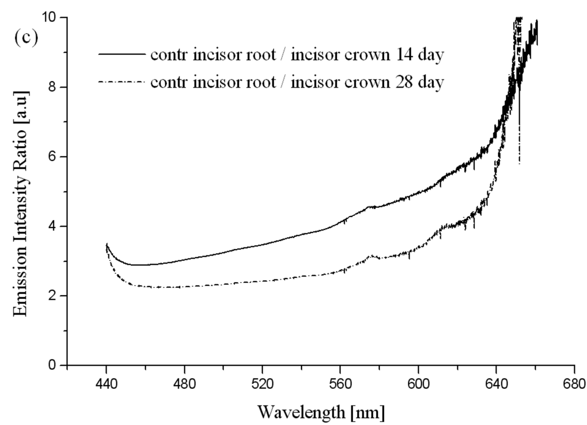
There are some differences in fluorescence originating from tooth, flat and long bones. To better delineate the spectral differences, emission spectrum ratios were calculated and shown in Fig. 3(c) and 5(c) for control tissues. The calculated spectra ratios for 14-day and 28-day old newborns show similar plots against wavelength (Fig. 3(a).). These spectral ratios change monotonically at shorter wavelengths and rapidly increase at longer wavelengths (above 600 nm). Analogical plots of mandible/incisor and femur/incisor ratio are obtained but with higher emission intensity ratio values than the incisor root /incisor crown ratios. It should be noted that the femur is the most emissive bone.



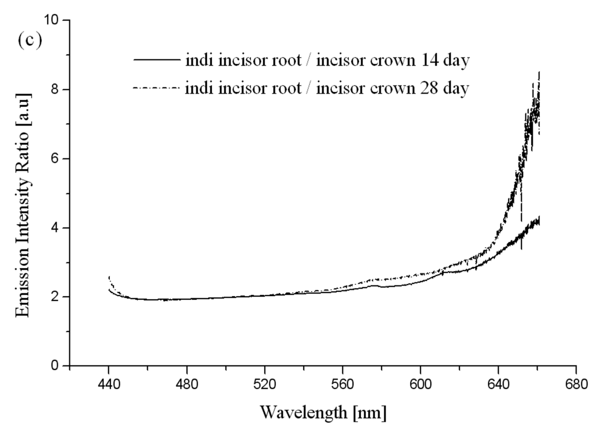
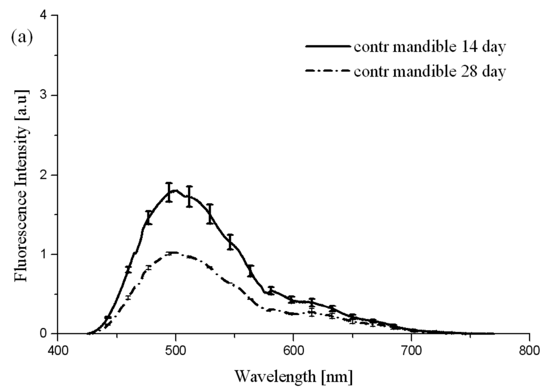
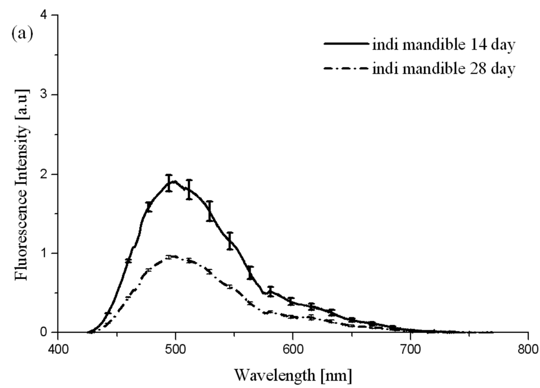
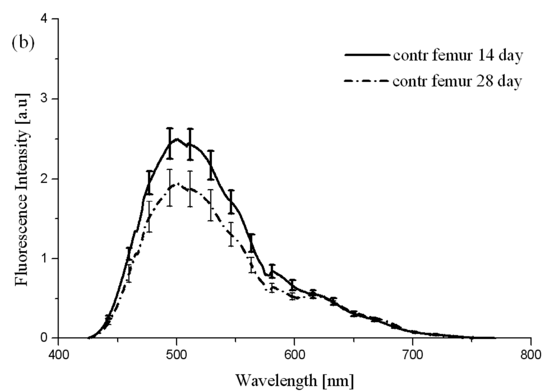



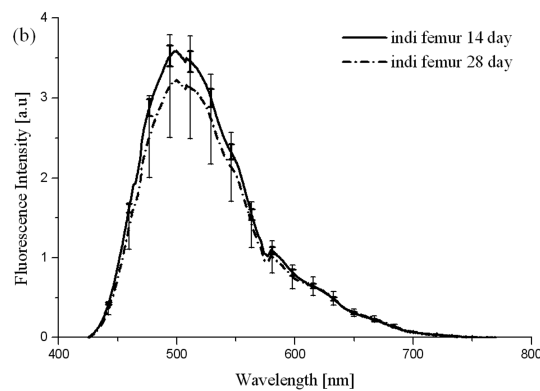

Fig. 3. Typical spectra of an anatomic crown (a) and an anatomic root (b) of incisor for 14-day and 28-day old newborns. Emission intensity ratio (c). An excitation wavelength is 405 nm.

Fig. 4. Typical spectra of an anatomic crown (a) and an anatomic root (b) of incisor for 14-day and 28-day old newborns after maternal administration of *indinavir*. Emission intensity ratio (c). An excitation wavelength is 405 nm.



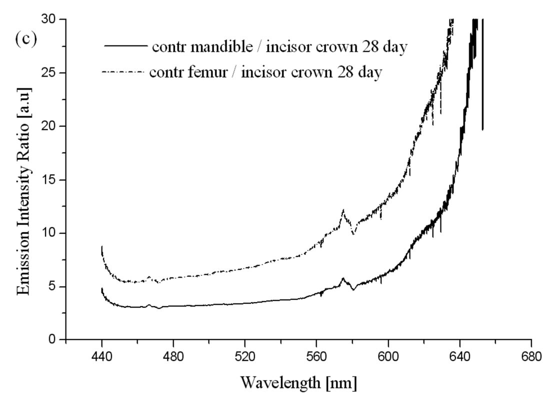




Fig. 5. Typical spectra of mandible (a) and femur (b) for 14-day and 28-day old newborns. Emission intensity ratio (c). An excitation wavelength is 405 nm.

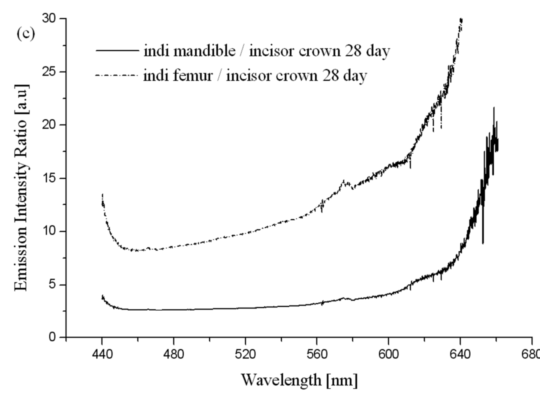



Fig. 6. Typical spectra of mandible (a) and femur (b) for 14-day and 28-day old newborns after maternal administration of *indinavir*. Emission intensity ratio (c). An excitation wavelength is 405 nm.

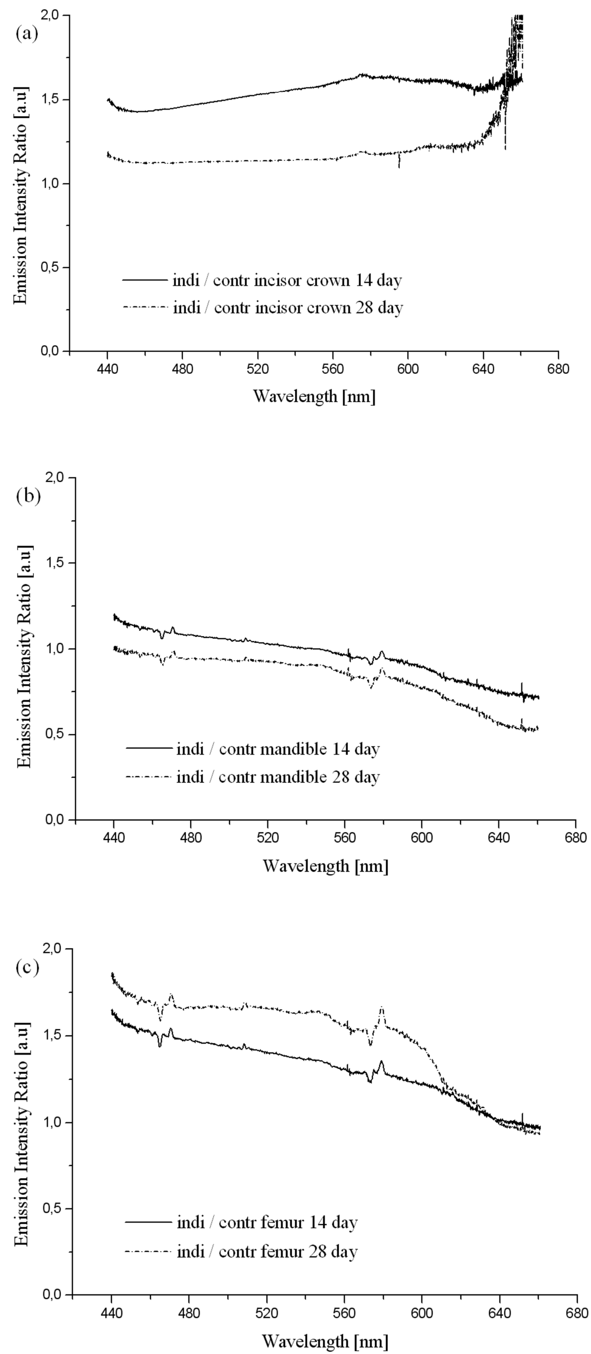



Fig. 7. Spectrum ratios: incisor crown after maternal administration of *indinavir*/control incisor crown (a) mandible after maternal administration of *indinavir*/control mandible (b) femur after maternal administration of *indinavir*/control mandible (c).

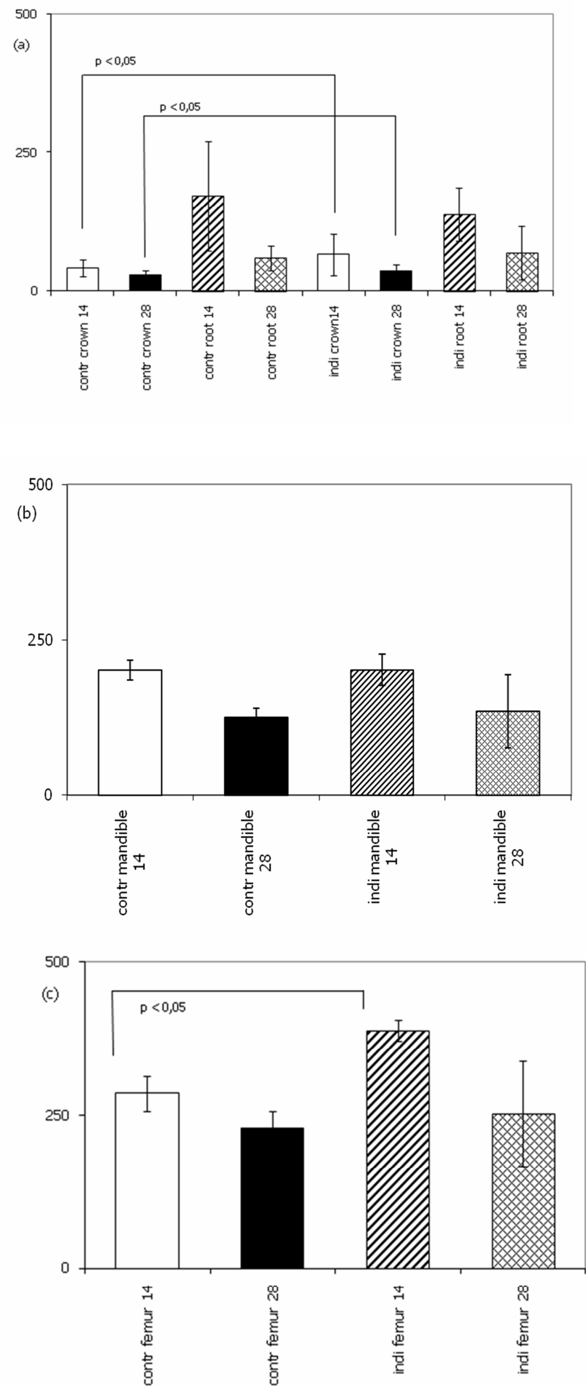



Fig. 8. Areas under fluorescence bands for incisor (a), mandible (b) and femur (c) of 14-day and 28-day newborns that mother were treated with *indinavir* and control group

Moreover it is noteworthy that the magnitude of autofluorescence decreases with the age of newborns for each studied group (Fig. 3-8). It seems to be caused by the increase of mineralization in bones with the age of newborns. However the degree of bone mineralization is due to the health state of teeth and bones. Intensity and shape of autofluorecence spectra of the studied tissues sampled from newborns after maternal administration of *indinavir* (Fig. 4 and 6) differ in comparison with the control tissues.

Overall autofluorescence is the mixture of emission from molecular fluorophores as well as of the diffusion and scattering properties of the biological tissues [2], [17]-[18]. Moreover an interaction of light with tissue depends on its wavelength. As the excitation wavelength changes towards longer, deeper layers of tissue are probed. Spectral characteristic depends on the excitation wavelength. This makes it extremely difficult to extract valuable biochemical information about the tissue which is present in the shape and intensity of the fluorescence spectrum. If appropriate wavelength bands are selected and their intensities are compared with one another then in-depth information can be obtained. The calculated spectral emission spectrum ratios of intrinsic fluorescence after maternal administration of antiviral drug to proper control group are shown in Fig. 7. The results indicate markedly changes for incisor crown and femur tissue. It should be noted that the femur from *indinavir* group was more emissive at shorter wavelengths (below 620 nm). In turn the enhanced emissity of the incisor crown increases even more in the red range. The changes in autofluorescence seems to be compatible with the morphological estimations which displayed some disturbance of cell architecture for the incisor of 7-day old rat in the case of *indinavir* group (non shown in this paper).

Integral fluorescence is often considered to be the most appropriate criterion for estimating the state of tissue therefore areas under emission spectra have been calculated and presented in Fig. 8. One can see that emission from tooth and bone depends on its form and decreases with age of newborns. Moreover maternal administration of antiviral drug modifies the magnitude of fluorescence intensity. The results with significance level $p<0.05$ linked to control group for femur and incisor crown are obtained. A tendency for regeneration of the studied tissues with age of newborns should be also noted (Fig.8.).

## IV. CONCLUSION

The laser-induced (407 nm) fluorescence can be used in examination of teeth and bones in newborn rats with regard to their form and age. Autofluorescence revealed some differences in the development of the studied biological tissues after maternal administration of i*ndinavi*r in comparison with the control groups. However further analysis/quantification of fluorescence spectra are required to confirm the effect of other drugs used in pregnancy on autofluorescence of animal bones and teeth.

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