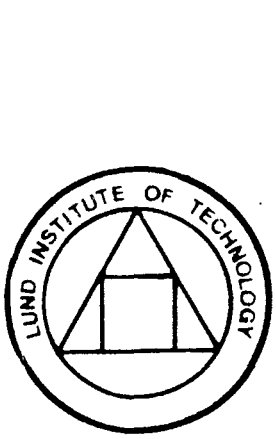
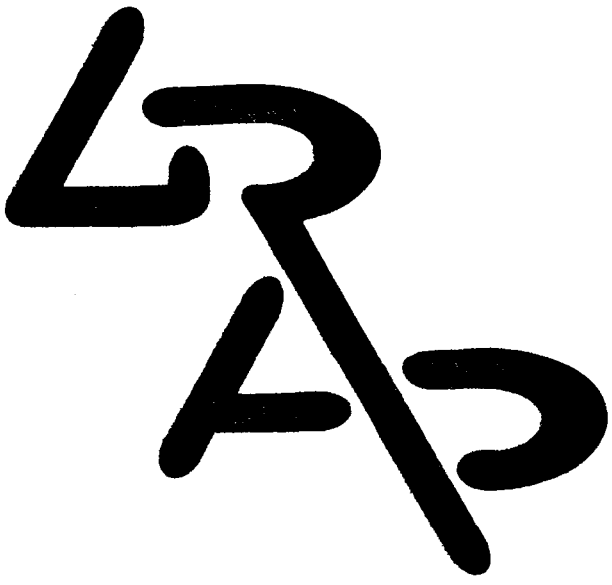
Lund Reports on Atomic Physics

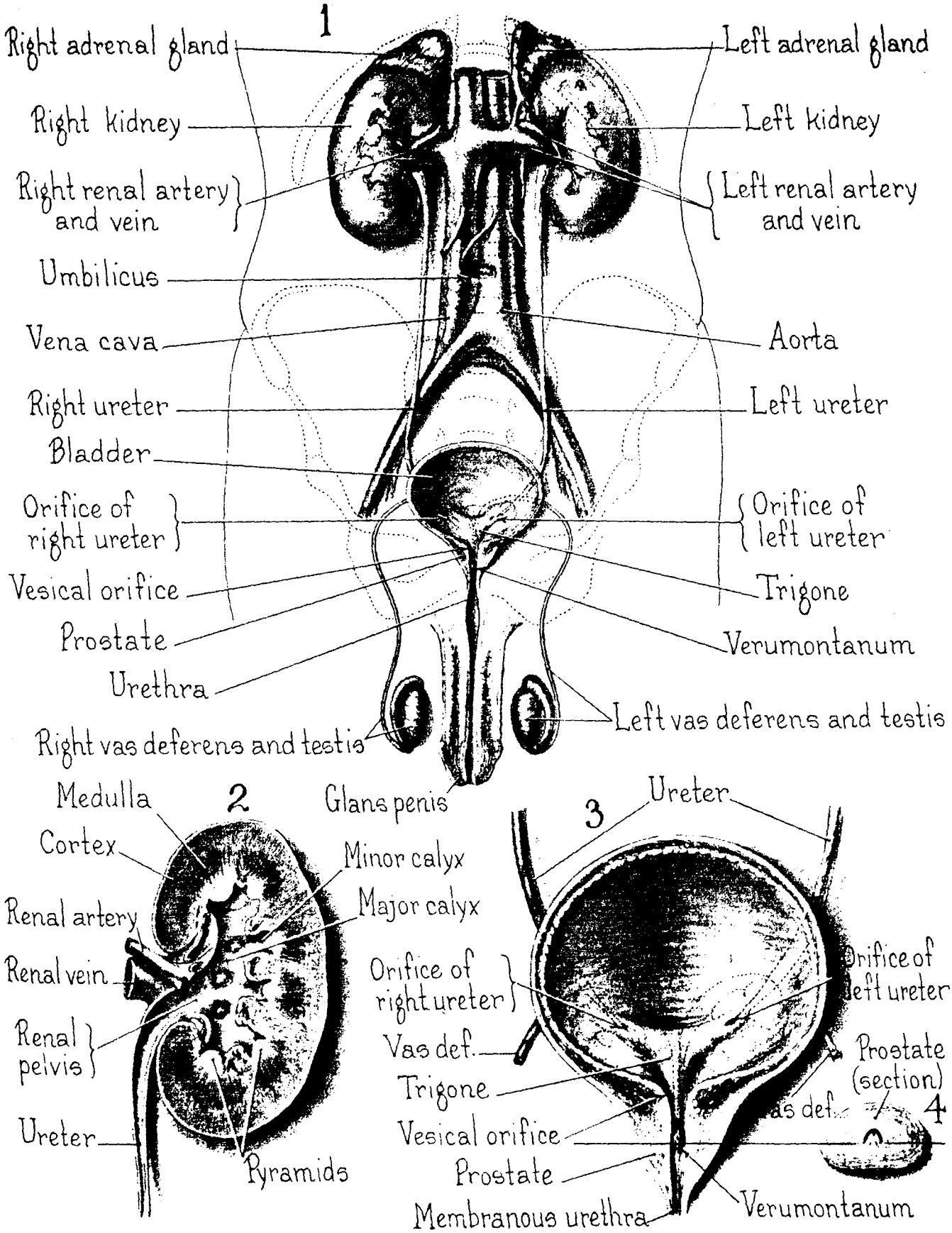
INVESTIGATIONS OF LASER-INDUCED FLUORESCENCE IN ORGANIC TISSUE.

SUNE MONTÁN

LRAP-17






1
Right adrenal gland
Left adrenal gland
Right kidney
Left kidney
Right renal artery and vein
Left renal artery and vein
Umbilicus
Vena cava
Aorta
Right ureter
Left ureter
Bladder
Orifice of right ureter
Orifice of left ureter
Vesical orifice
Trigone
Prostate
Verumontanum
Urethra
Left vas deferens and testis
Right vas deferens and testis
Glans penis
2
Medulla
Cortex
Minor calyx
Major calyx
Renal artery
Renal vein
Renal pelvis
Ureter
Pyramids
3
Ureter
Orifice of right ureter
Orifice of left ureter
Vas def.
Prostate (section)
Trigone
Vesical orifice
Prostate
Membranous urethra
Verumontanum
4

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I föreliggande arbete har bl a möjligheterna att detektera förstadier till blåscancer med hjälp av laserinducerad fluorescens undersökts. Blåscancer är bland de vanligaste tumörerna. I sina enklare stadier kan dylika tumörer ofta behandlas via urinröret. I svårare fall är den effektivaste behandlingen att avlägsna urinblåsan och att ge strålbehandling. Detta är en påfrestande och delvis invalidiserande behandling. De tumörer, som fordrar denna behandling har sannolikt oftast funnits i form av ett förstadium. Tidig upptäckt och lokalisering av dessa tidiga tumörstadier kan möjliggöra en lindrigare behandling, t ex genom Nd-YAG-laser-bestrålning. Under 1960-talet undersöktes fluorescens hos tumörer efter tillförsel av ett antibiotikum, tetracyklin. Man fann då att tidiga tumörstadier, s k cancer in situ, gav gul fluorescens vid belysning med ultraviolett ljus. Detta användes under en följd av år för att lokalisera tumörer, som ännu var osynliga för blotta ögat. Lämplig behandling saknades dock vid den tiden. Av oklara skäl slutade metoden att användas i början av 1970-talet. För att pröva metodens användbarhet, har vi undersökt fluorescens inducerad med en kvävelaser, hos prover från olika typer av blåstumörer. Spektra upptogs dels med, dels utan föregående tillförsel av tetracyklin. Vi kunde inte finna någon säker karakteristisk fluorescens hos dessa tumörer. Vidare undersökningar bör dock utföras, innan metoden definitivt säges sakna värde.

Även laserinducerad fluorescens från andra organiska material har undersökts. Det visade sig, att denna fluorescens är tämligen lika för olika material. Vissa undantag finns dock,

bl a från försök på huden. En del födelsemärken gav avvikande spektra. Dessa märken opererades bort och analyserades, men kunde ej histologiskt skiljas från vanlig hud.

INTRODUCTION

In this work the possibilities to detect early stages of cancers of the urinary bladder by means of laser-induced fluorescence have been investigated. Cancers of the urinary bladder have different tendencies to grow deeply into the bladder wall (Fig. 1). Generally they remain superficial, and are then possible to treat transurethrally. Their aggressivity or tendency to grow deeply into the bladder wall is correlated to their microscopical appearance, the malignancy grade. Aggressive cancers usually shed a large number of cells into the urine, where they can be demonstrated by cytological examination.

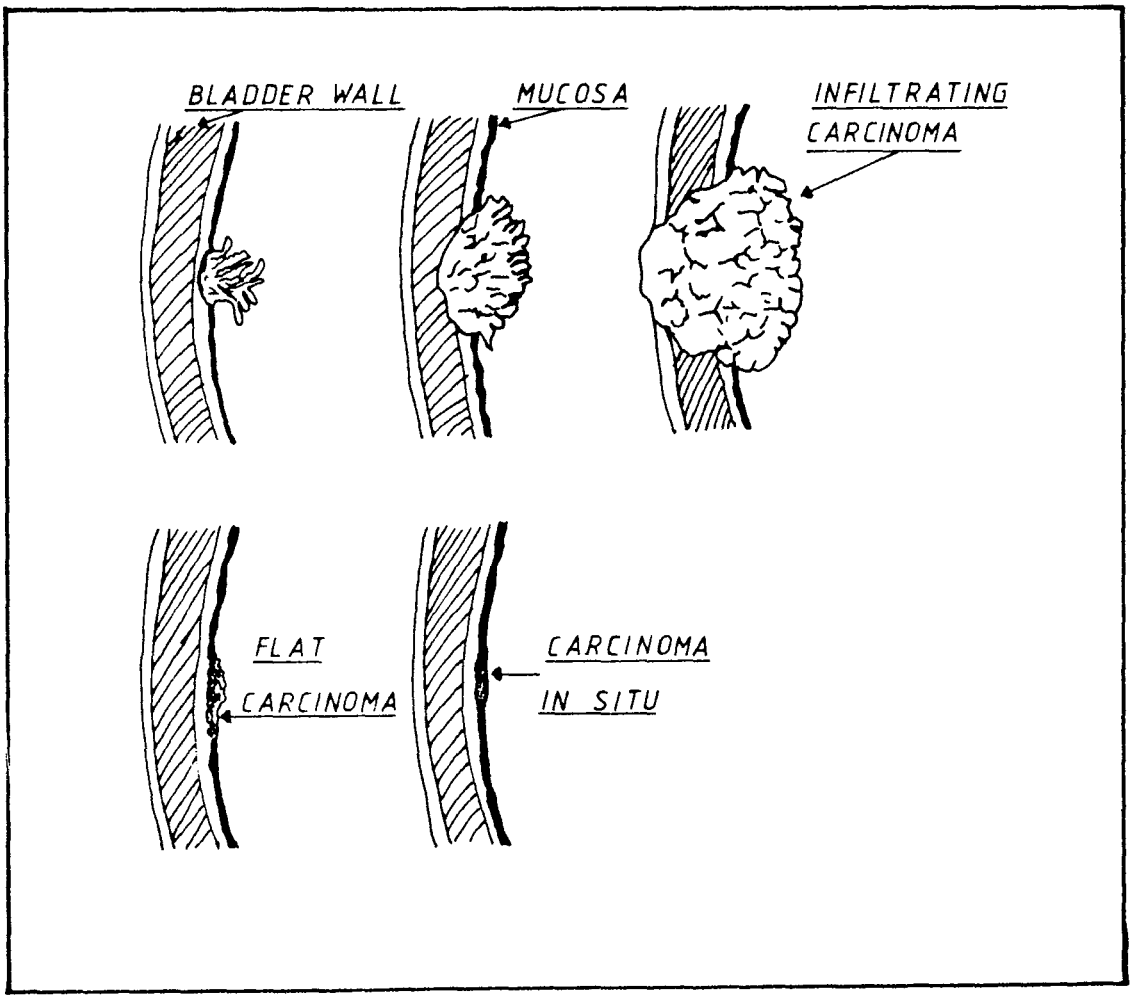


Fig. 1. Different kinds and development stages of human urinary bladder tumors.

The tumors also have different tendencies to grow into the bladder lumen (Fig. 1). Tumors that do so are easily recognized by cystoscopic examination (Figs. 2a, 2b). In very early stages of aggressive cancer, the tumor may not be demonstrable at cystoscopy, although urinary cytology shows malignant cells. In this situation, the urologic surgeon is facing a difficult problem. By taking multiple biopsies from the urinary bladder the diagnosis may be confirmed, but the exact localization of the tumor remains unknown. This situation is termed carcinoma in situ, and represents a premalignant disease, which may develop into an infiltrating bladder cancer. If the exact carcinoma in situ localization were known, it could be treated by e g Nd-YAG laser irradiation. Other used therapeutic modalities have not proved to be generally efficient.

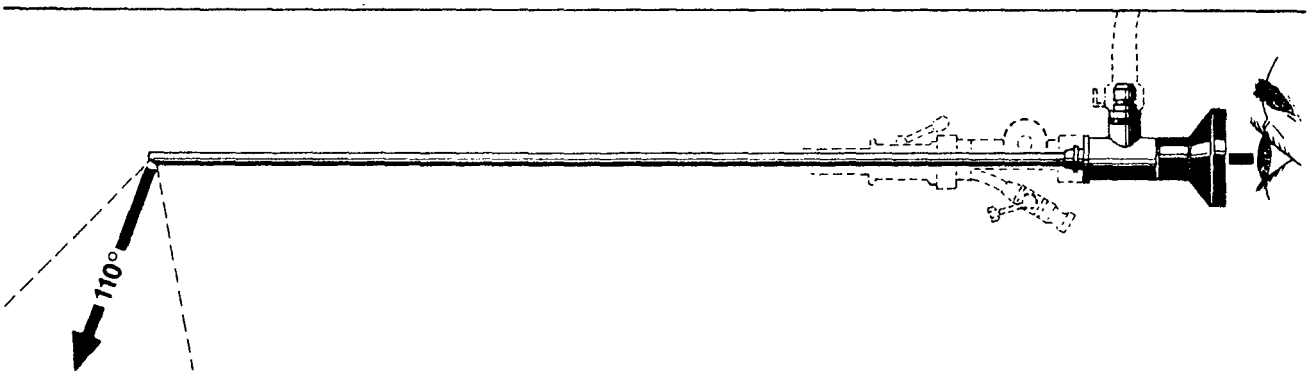


Fig. 2a). Cystoscope.

Also laser-induced fluorescence in other organic materials has been investigated. This fluorescence proved to be quite similar for different materials. There are exceptions though. Certain naevi showed different spectra. These naevi were removed and analyzed histologically. No difference between them and normal, pigmented naevi could be found, however.

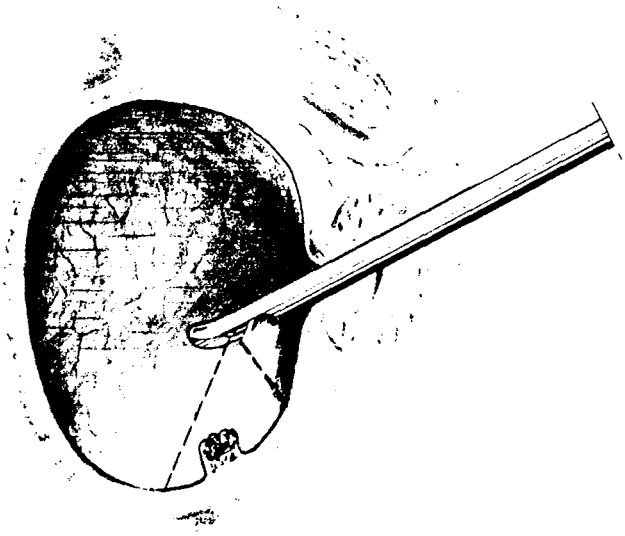


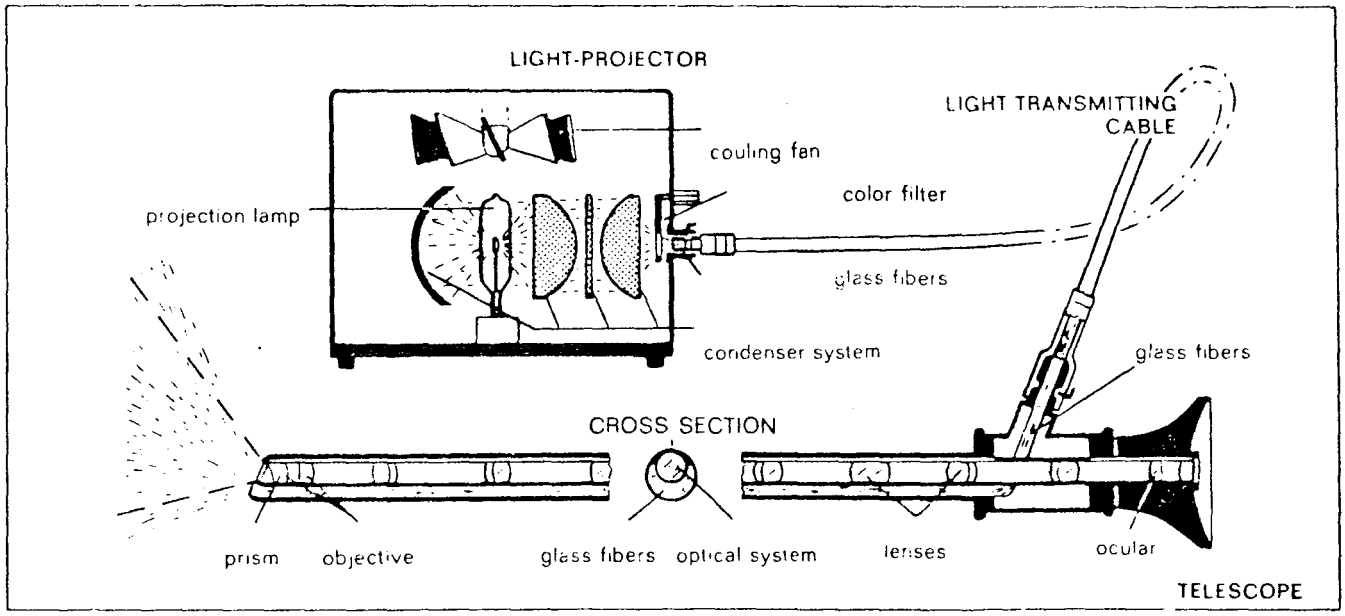
Fig. 2b). Cystoscopic examination of the urinary bladder.

PREVIOUSLY INVESTIGATED METHODS FOR DIAGNOSIS AND LOCALIZATION OF CARCINOMA IN SITU

About 1955 it was known that an antibiotic substance, tetracycline, produced yellow fluorescence when irradiated with ultra-violet light. It was also noted, that this substance

was enriched in tumorous tissue, where it also remained after cessation of supply of the antibiotic [1]. Specifically to the urinary bladder this technique was applied in 1964 [2]. The ordinary cystoscope had to be rebuilt to transmit ultra-violet light. The fibre light bundle and light guide (Fig. 3) were replaced by quartz glass fibres. The UV--source was a high-pressure mercury-arc lamp, from which the 366, 403 and 430 nm lines were utilized. The standard procedure was to supply tetracycline by mouth for 4 days preoperatively, with a free interval of 36 hours before the examination to prevent that all tissue structures were saturated with tetracycline, producing a general yellow fluorescence.

Frank tumors only rarely exhibited yellow fluorescence, while carcinoma in situ generally did so.



In this illumination system the light of a separate light source (projector) is transmitted via a detachable flexible light cable to the instrument and via a conductor of glass fibers inside the instrument to the distal end. That means that the illumination is not accomplished by a small tungsten lamp as formerly. The heat produced by the lamp is mainly eliminated by a filter, the rest is absorbed by the fibers so that actually cold light leaves the distal end of the instrument. Excessive heating of the condenser system and the lamp is avoided by means of a fan.

Fig. 3. Fiber light illumination.

THE MECHANISM OF TETRACYCLINE BINDING TO TUMOR TISSUE

Initially it was generally considered, that tetracycline was enriched in neoplastic tissue by unknown mechanisms. It has however been shown, that tetracycline has a strong affinity to calcium ions. These in turn have a pronounced affinity to necrotic cells [3]. Since tumors often contain a large number of dead cells, this seems to be the probable explanation of tetracycline affinity to cancer cells. This is reinforced by the fact, that urinary cells in the urine generally do not contain tetracycline. These cells are viable in about 80%. Neither do frank tumors generally carry necrotic cells on their surface, which may explain their abscence of yellow fluorescence.

It seems fair to assume that urinary tetracycline is chelated to calcium ions in necrotic carcinoma in situ cells. The rationale for supplying tetracycline for so long time before the examination thus seems doubtful. The free interval prior to examination however seems justified.

We made the choice to use a single dose of a tetracycline derivative, doxycycline, which gives a high urinary concentration for about 24 hours after administration. The free interval was chosen to 48 hours.

THEORY OF THE FLUORESCENCE PHENOMENA

The method used in our investigations has been laser-induced fluorescence. The theory behind it can briefly be explained as follows:

The electronic energy levels of a molecule are split into vibrational and rotational energy sublevels. While typical electronic energy-level splittings are of the magnitude 1 eV, vibrational and rotational energy level splittings are of the magnitude 10^{-1} eV and 10^{-3} eV, respectively. In complex organic molecules, these sublevels are broadened and an energy band is obtained rather than energy levels. Therefore, when a light quantum hits a molecule, the probability is high, that the quantum will be absorbed and the molecule transferred to an excited state, provided that the energy of the incident light quantum is greater than the gap between the two lowest electronic energy levels. This gap generally corresponds to a light quantum in the visible region. From the excited state, the molecule will decay back to lower energy levels. It may do this either by emitting a photon or by radiationless processes. The light emitted at the radiation transfer process is termed fluorescence light. This light contains longer wavelengths than the exciting laser light, since the molecule always first relaxes radiationless to the lowest energy level in the first excited electronic energy state, (Fig. 4). The spectral distribution is hence determined by the vibrational and rotational energy levels of the electronic ground state, and the energy gap between the

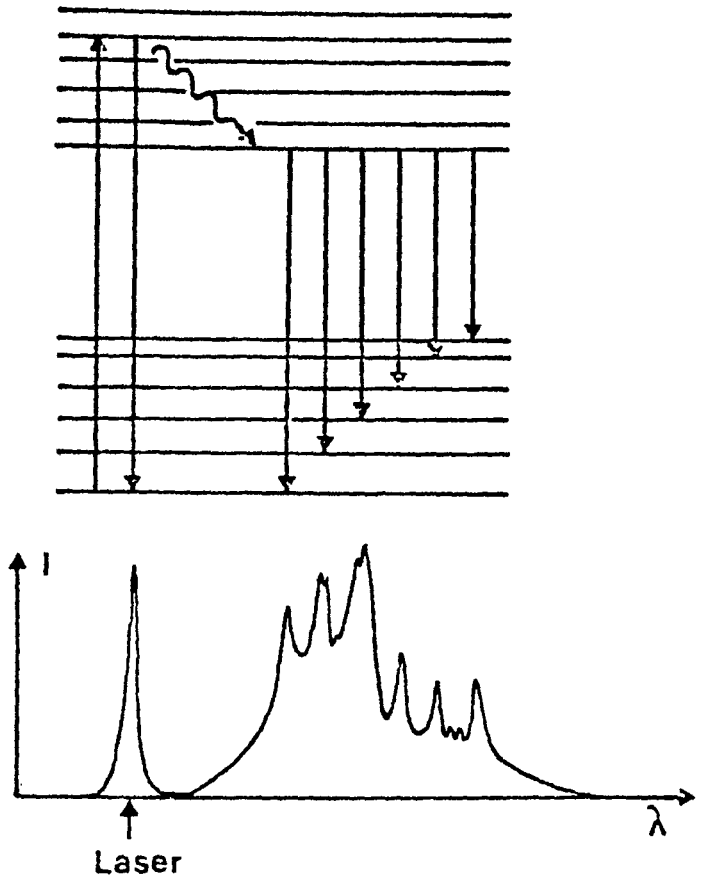


Fig. 4. General energy-level diagram and fluorescence spectrum.

first excited electronic state and the ground state of the molecule. The distribution is largely independent of the wavelength of the exciting light. Thus, the fluorescence is characteristic of the molecule in question. The intensity of the fluorescence light depends partly on the probability for radiation decay versus radiationless decay (Refs. 4,5).

LABORATORY SET-UPS

Essentially two different set-ups were used for the experiments (Figs. 5,6). The light source was a nitrogen laser, which emits ultra-violet light with a wavelength of 337 nm. The used laser emitted pulses of 0.75 mJ maximum energy and

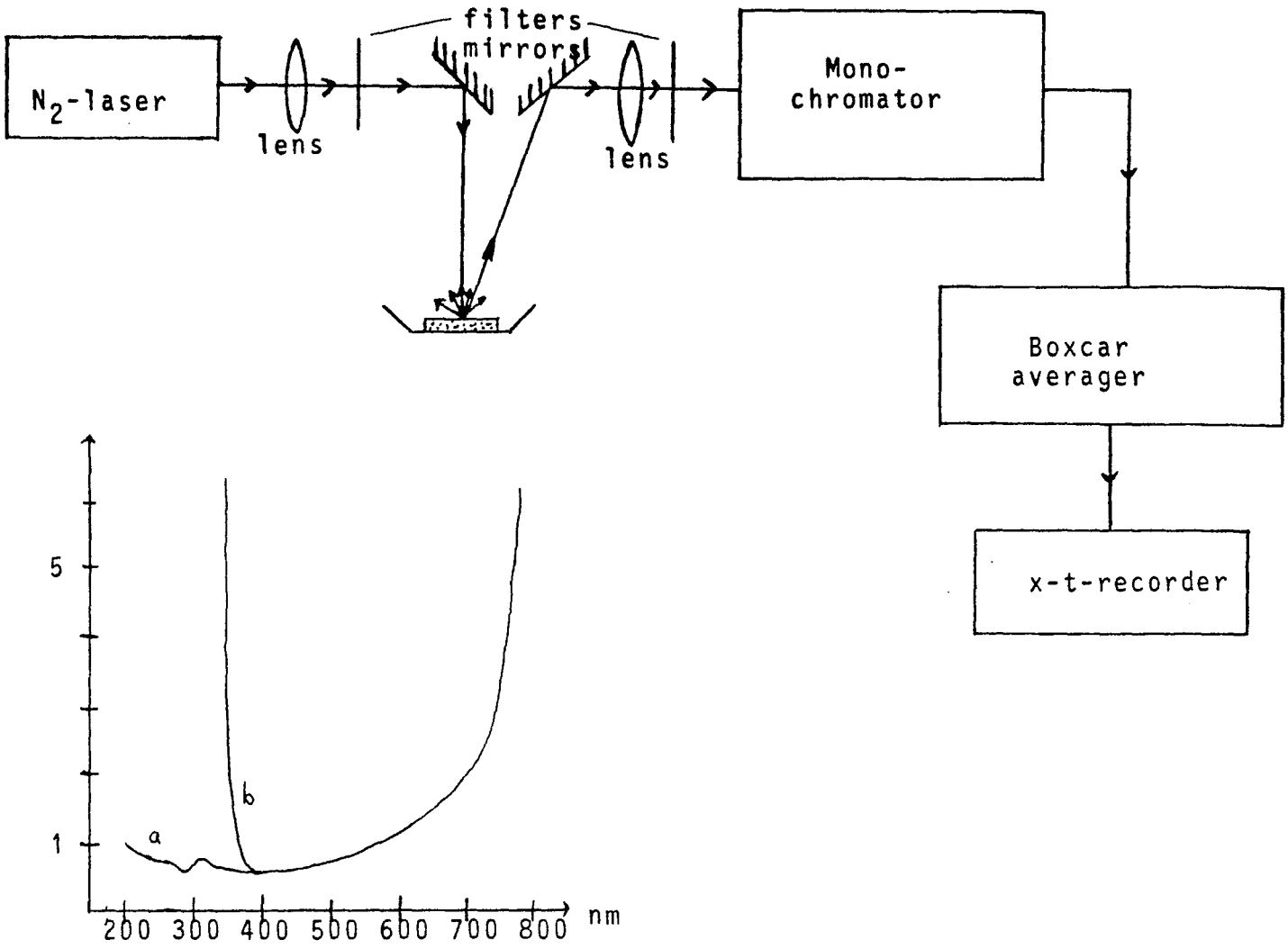


Fig. 5. Set-up for fluorescence measurements. The filters used were an interference filter for the laser light and a cut-off filter in front of the monochromator to suppress elastically scattered laser light. Down left, the multiplicative correction function for this set-up a) without cut-off filter and b) with cut-off filter.

10 ns length. The size of the laser beam was about 7 x 35 mm and the divergence about 3 x 15 mrad. The laser light was focused on to the sample under investigation and passed through a 337 nm interference filter to suppress other spontaneously emitted lines from the N_2 discharge. The filter had a transmission of about 35 per cent for the laser light. The mirrors were high-reflectance front-surface mirrors. The fluorescence light was detected by either

For both the described set-ups, the obtained spectra differ from the real ones. The detector efficiency of the DARSS, the transmission of the monochromator, the response of the photo-multiplier tube, the reflectivity of the mirrors and the transmission of the lenses vary with wavelength. The DARSS spectra can be corrected in the computer. This has not been done in this work. The multiplicative correction function, which includes the cut-off filter and the spectral response of the DARSS system and the photomultiplier tube, respectively, is roughly drawn in Figs. 5 and 6.

To produce a strong fluorescence-light signal, a broad entrance slit of the DARSS detector was used. This meant that the resolution was reduced to 33 nm.

The samples investigated were placed in aluminum cups that were discarded after each measurement to avoid pollution of new samples. These cups do not produce any fluorescence. As intensity reference, a solution of 100 mg Rhodamine 610 in 20 ml methanol and 6000 ml ordinary tap water was used in a 1 cm thick layer. The peak of this spectrum has been given the intensity 1.0 in the different set-ups.

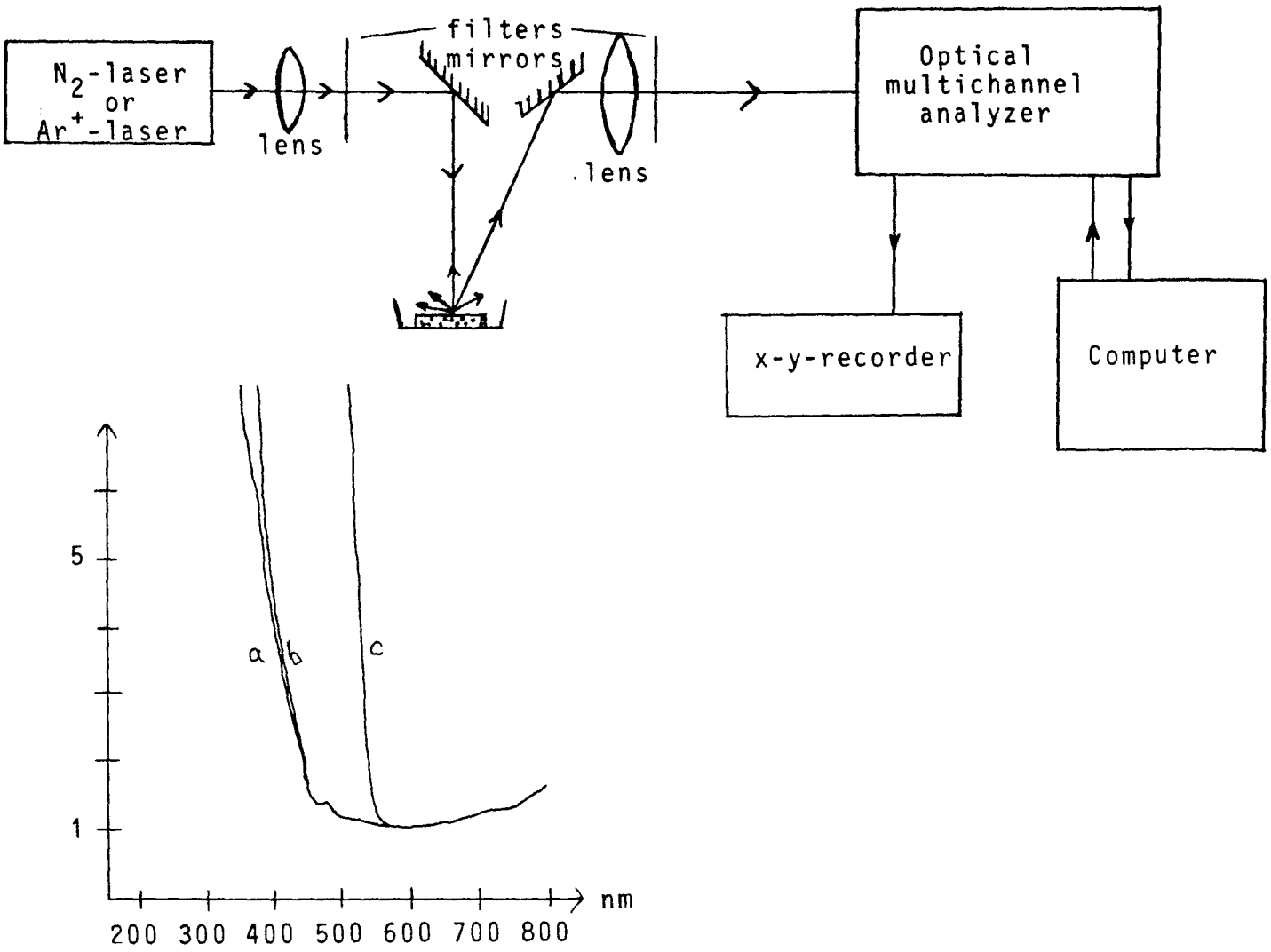


Fig. 6. Set-up for fluorescence measurements. Together with the Ar^+-laser no interference filter was used. Down left, the multiplicative correction function for this set-up: a) without cut-off filter, b) with the cut-off filter used together with the N_2-laser and c) with the cut-off filter used together with the Ar^+-laser.

a Diode Array Rapid Scan Spectrometer (DARSS) from Tracor Northern or a scanning monochromator, Jobin Yvon HR 1000, in connection with a photo-multiplier tube, EMI 9558B, and a boxcar integrator, Princeton Applied Research, Model 162. In both cases the fluorescence light was focused on the entrance slit of the detection system to enhance the fluorescence-light signal. A cut-off filter (GG 375) was used to suppress elastically scattered laser light.

The boxcar integrator averages the pulses from the photo-multiplier tube. The boxcar integrator was gated, which means, that it accepted an input signal only during a certain time interval after trigging. The integrator was triggered by the signal from a photo diode, placed in the laser beam. In this way, the background light was very efficiently suppressed. In fact, it was possible to perform the measurements in full day light. As the wavelength of the monochromator was scanned, the laser-induced fluorescence spectrum of the sample was obtained on a strip-chart recorder.

The DARSS system was used as an alternative. In this unit, the light is spread out over a photo-diode array (TN-1223-4GI) by means of a grating. The array contains 1024 diodes. Under light exposure, charges are produced, and a number of counts, proportional to the accumulated charge, is read out at given time intervals. The dark current of the DARSS is much larger than that of the photomultiplier tube. In the DARSS, however, there are possibilities to add and subtract spectra, and thus it is possible to subtract background light to improve the signal-to-noise ratio. It is also possible to store the obtained spectra on a floppy-disc via a computer and later reproduce them or plot the spectra with an x-y-recorder. The most important difference, though, is that while the monochromator unit detects the intensity of a very small wavelength interval and thus has to be scanned to obtain a spectrum, the DARSS system captures a whole spectrum for each individual laser pulse.

PRELIMINARY LABORATORY INVESTIGATIONS

Methods and Material

In the first preliminary investigations, a rat was dissected and different tissues were examined. Different pieces of pork and beef were also examined. These measurements were done with the set-up shown in Fig. 6. In order to find a system that could be used with a cystoscope we tried to transmit laser light, Fig. 7a, and fluorescence light, Fig. 7b, through three different optical fibres: the Pilkington Hytran (designed for infra-red light from the Nd:YAG laser, 1.06 μm), the Storz liquid light guide, and a conventional light guide made of glass. We also tried to excite the sample through two of the fibres, Fig. 7c. A set-up used in experiments further on,

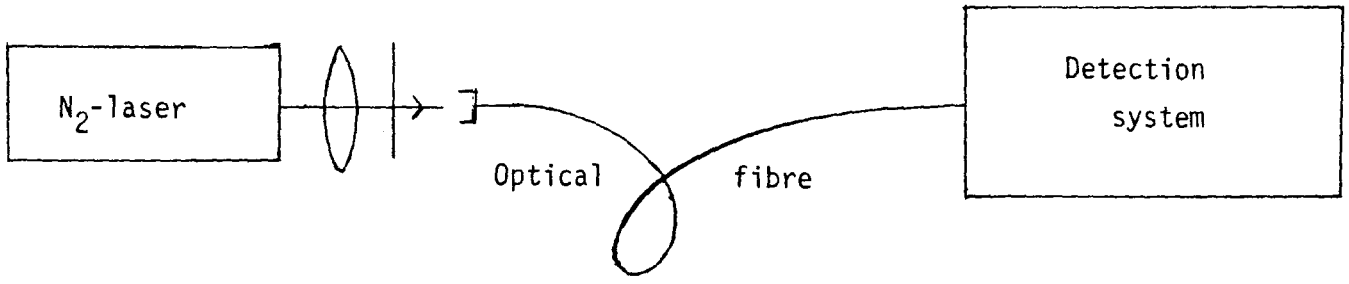


Fig 7a). Set-up for examination of the transmission of the laser light of the different optical fibres.

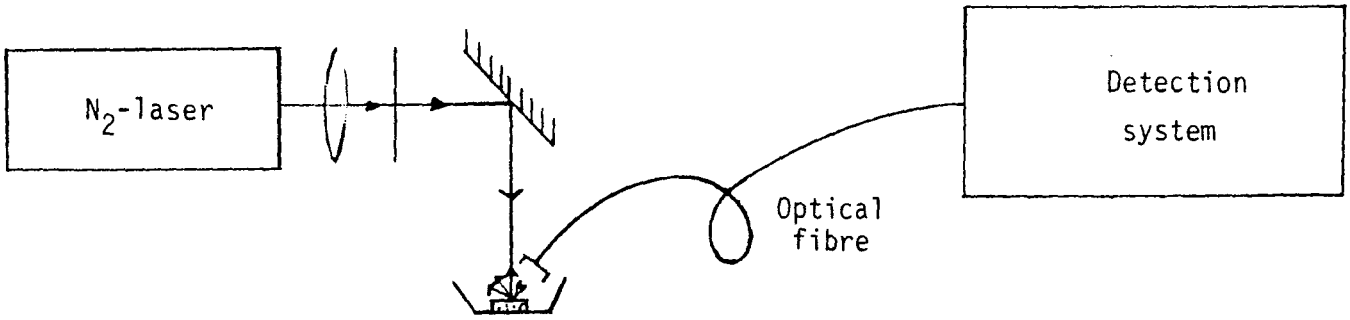


Fig 7b). Set-up for examination of the transmission of the fluorescence light of the different optical fibres.

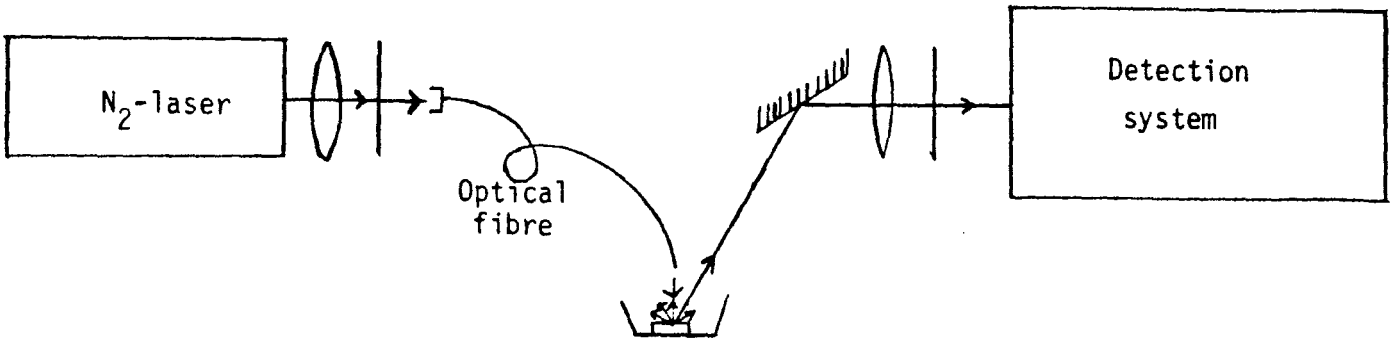


Fig. 7c). Set-up used at an attempt to excite the sample with laser light led through an optical fibre.

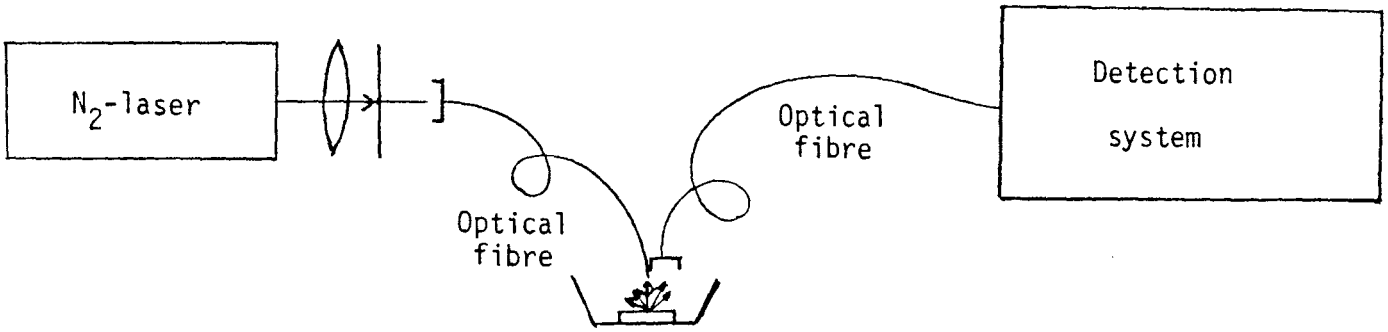


Fig. 7d). Set-up where both the laser light and the fluorescence light are led through optical fibres.

where both the laser light and the fluorescence light were taken through optical fibres, Fig. 7d, was examined. The laser light was transmitted through the Pilkington Hytran fibre and the fluorescence light through the conventional glass fibre light guide. Measurements were done on some drug substances: Pivmecillinam (Selexidin^R, Lövens, Denmark), Cefuroxim (Zinacef^R, Glaxo, UK), Doxycyklin (Vibramycin^R, Pfizer, USA), Estramustin (Estracyt^R, LEO, Sweden), Fig. 8.

Pivaloyloximetylester av (2*S*,5*R*,6*R*)-6-[[(hexahydro-1*H*-azepin-1-yl)metylen]amino]-3,3-dimetyl-7-oxo-4-tia-1-azabicyklo[3,2,0]heptan-2-karboxylsyra

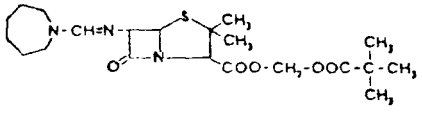


Fig. 8a). Pivmecilliamum, the active substance of Selexidin.

(6*R*,7*R*)-7-[(*Z*)-2-(2-Furyl)-2-(metoxiimino)acetamido]-3-karbamoyloximetyl-8-oxo-5-tia-1-azabicyklo[4.2.0]okt-2-en-2-karboxylsyra

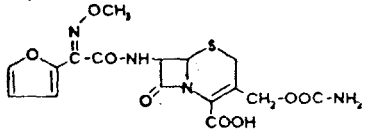


Fig. 8b). Cefuroximum, the active substance of Zinacef.

(-)-4-Dimetylamino-3,6,10,12,12a-pentahydroxi-6-metyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-oktahydronaftacen-2-karboxamid

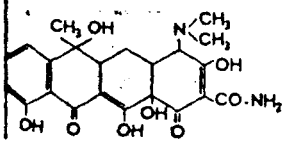


Fig. 8c). Tetracyclinum.

6-Deoxi-5-hydroxitetracyklin

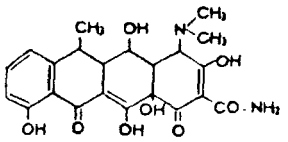


Fig. 8d). Doxycyclinum, the active substance of Vibramycin.

The three first ones are antibiotics. The fourth one is a cytotoxic drug. We also tried to induce fluorescence with an Ar^+-laser, as this laser has very attractive surgical qualities. The same set-up was used here as in Fig. 6, except for the interference filter, that was removed, and the cut-off filter,which was exchanged for an OG 515. The laser was operated in the green region on the lines 514, 502, 496 and 488 nm. No intensity reference was acquired here. To see if it would be possible to realize any fluorescence measurements in the bladder, some fluids of current interest were examined, viz. urine, taken in the morning and in the afternoon, normal saline solution and sterile water. This was done with the set-up in Fig. 5.

Results

The "rat spectra" are shown in Figs. 9-11. They all have their peaks at the same wavelength, only the intensity differs. They seem to have different properties in the red and infra-red region, though. To the human eye they all seemed blue, with a difference in intensity only.

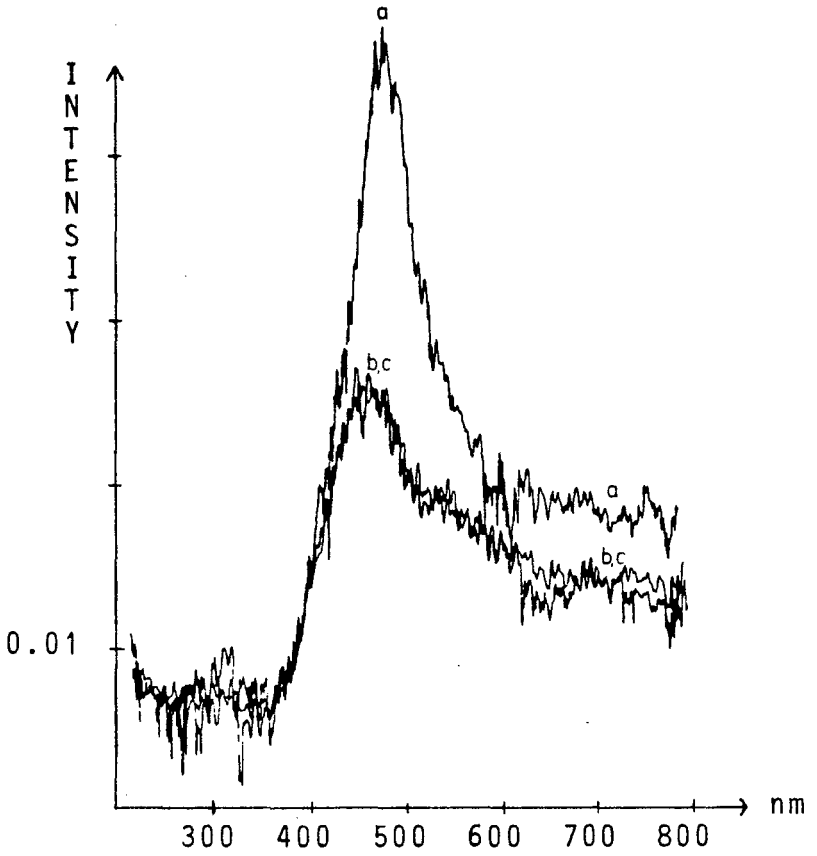


Fig. 9. Fluorescence spectra of different parts of a rat: a) muscle, b) lung and c) blood.

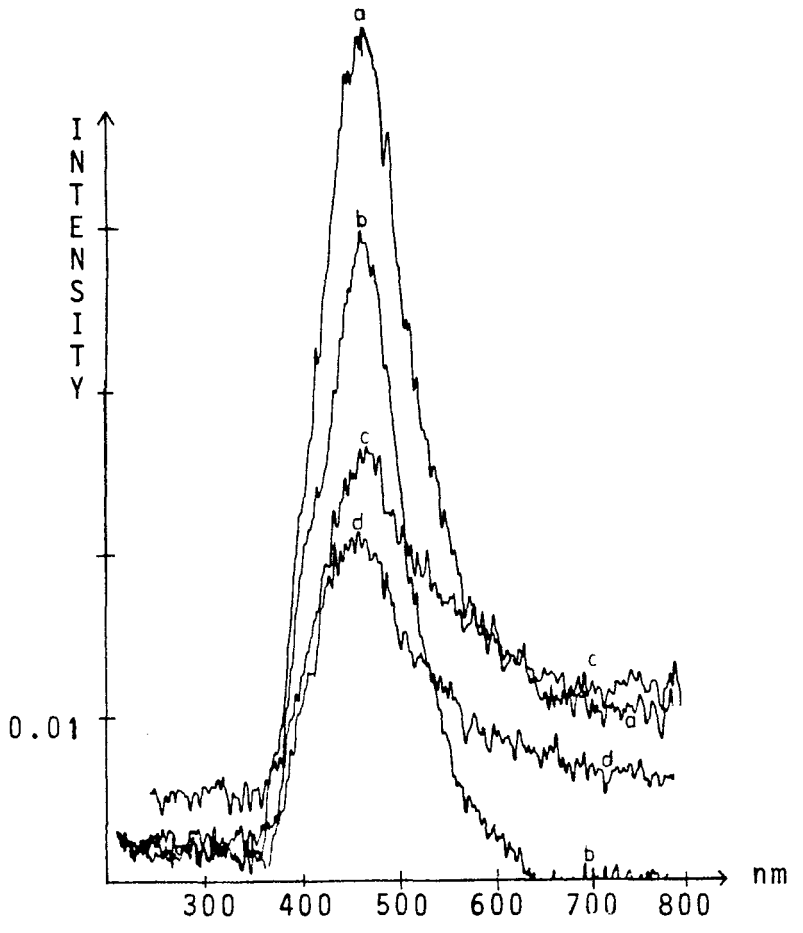


Fig. 10. Fluorescence spectra of parts of a rat: a) stomach mucosa, b) urinary bladder, c) liver and d) spleen.

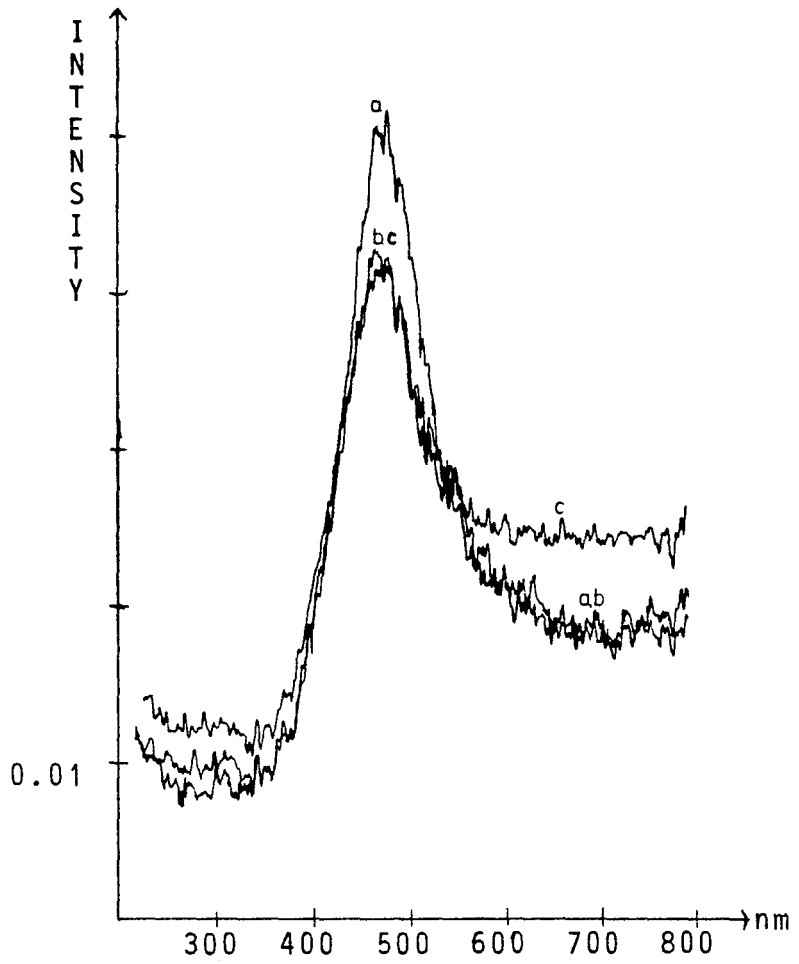


Fig. 11. Fluorescence spectra of parts of a rat: a) lard, b) testicle and c) prostate.

The spectra from pork and beef can be seen in Figs. 12 - 16. These spectra have their peaks at essentially the same wavelength as the rat. It is possible though to find other peaks, and especially from the porcine heart there are two curves that differ very much from each other, Fig. 13. The lower one, c), appeared yellowish to the eye, while the other one, b), appeared blue. No difference could be discovered in the tissue surface at ocular inspection. Beef compared to corresponding pieces of pork are shown in Figs. 14 - 15.

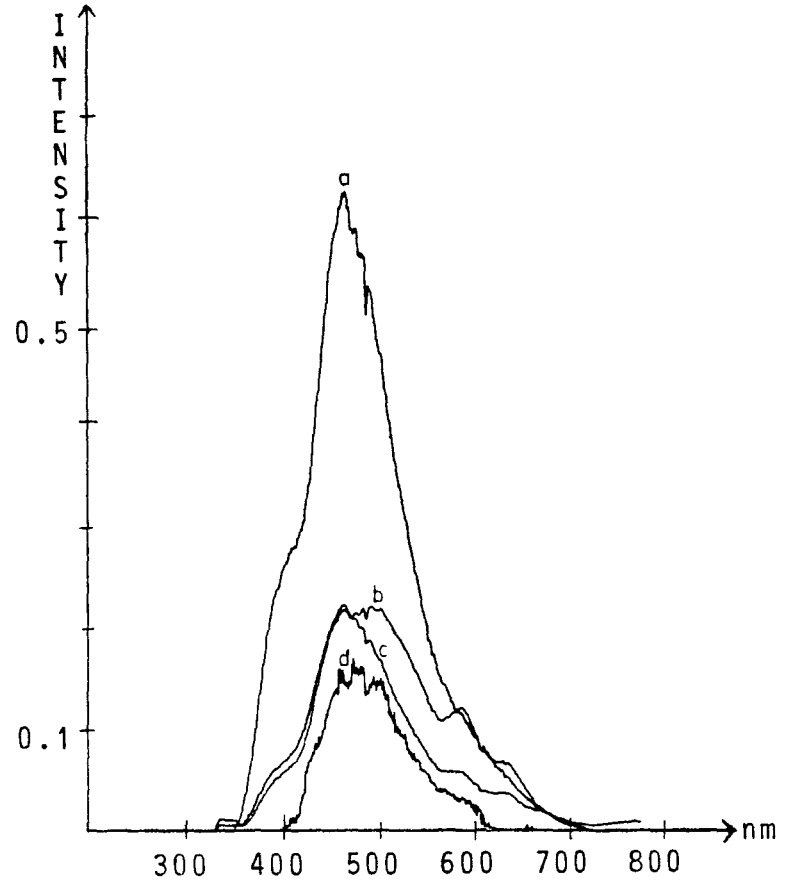


Fig. 12. Fluorescnece spectra of different pieces of pork: a) lard, b) muscle c) tounge and d) liver.

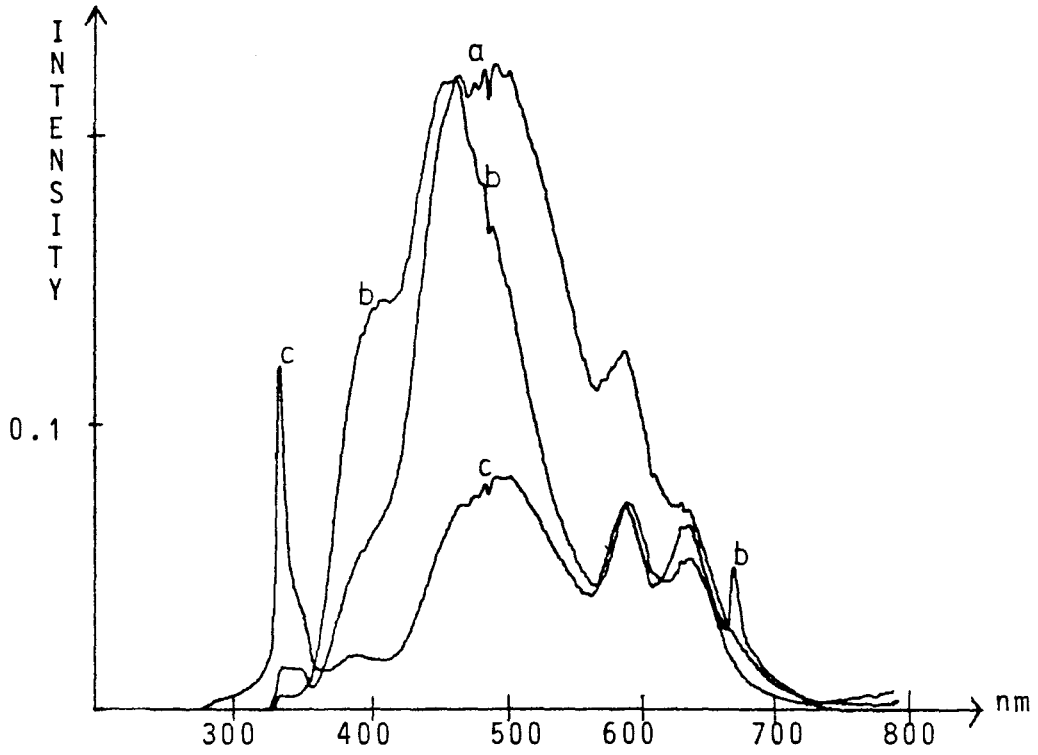


Fig. 13. Fluorescence spectra of a) porcine muscle, b) porcine heart fluorescing blue and c) porcine heart fluorescing yellow. No difference in the tissue surface could be discovered at an ocular inspection.

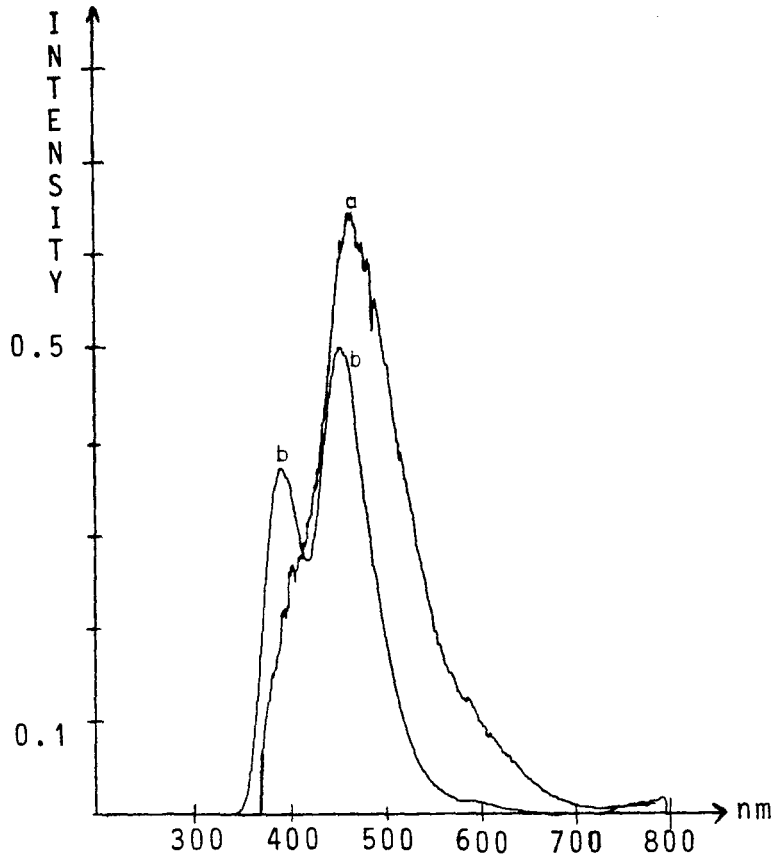


Fig. 14. Fluorescence spectra of a) porcine lard and b) beef lard.

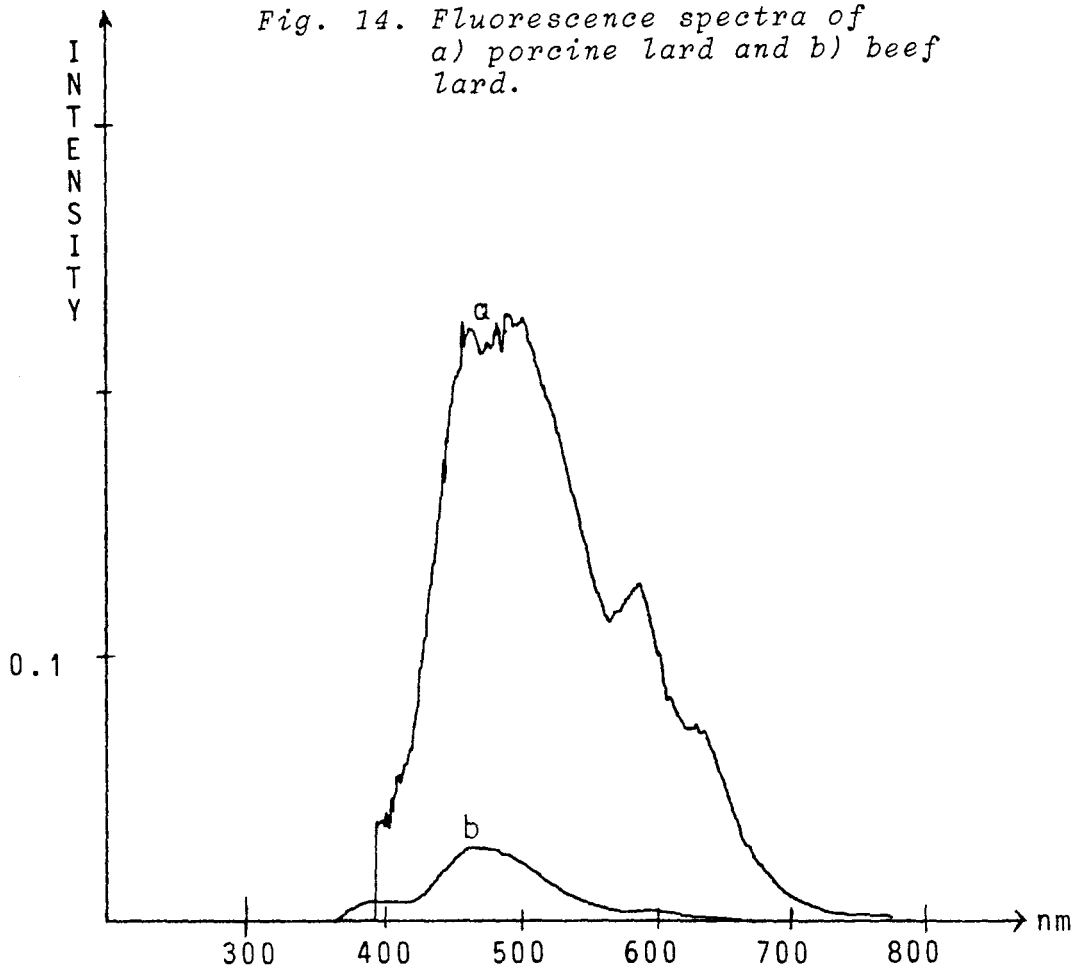


Fig. 15. Fluorescence spectra of a) porcine muscle and b) beef muscle.

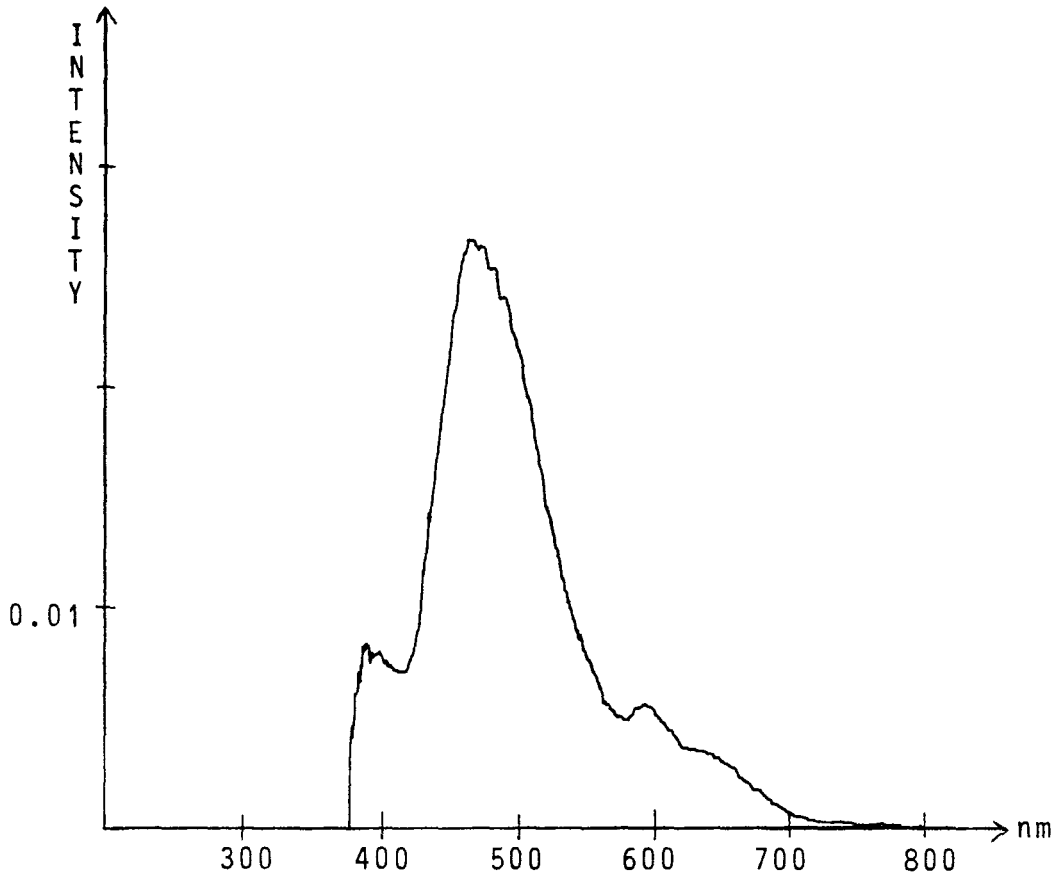


Fig. 16. Fluorescence spectrum of beef muscle (Fig. 15 b)) enlarged.

The transmission of the optical fibres was investigated with the set-up in Fig. 7. Storz transmits the laser light best, Fig. 17, but produces fluorescence light of its own as well. The Pilkington Hytran has very good properties. It transmits the laser light well and gives no fluorescence. The conventional light guide, finally, is completely opaque to light of the wavelength 337 nm, and gives fluorescence light only. The peaks to the right in the spectra originate from the second-order diffraction of the laser light in the grating monochromator detector. The peaks are broad because

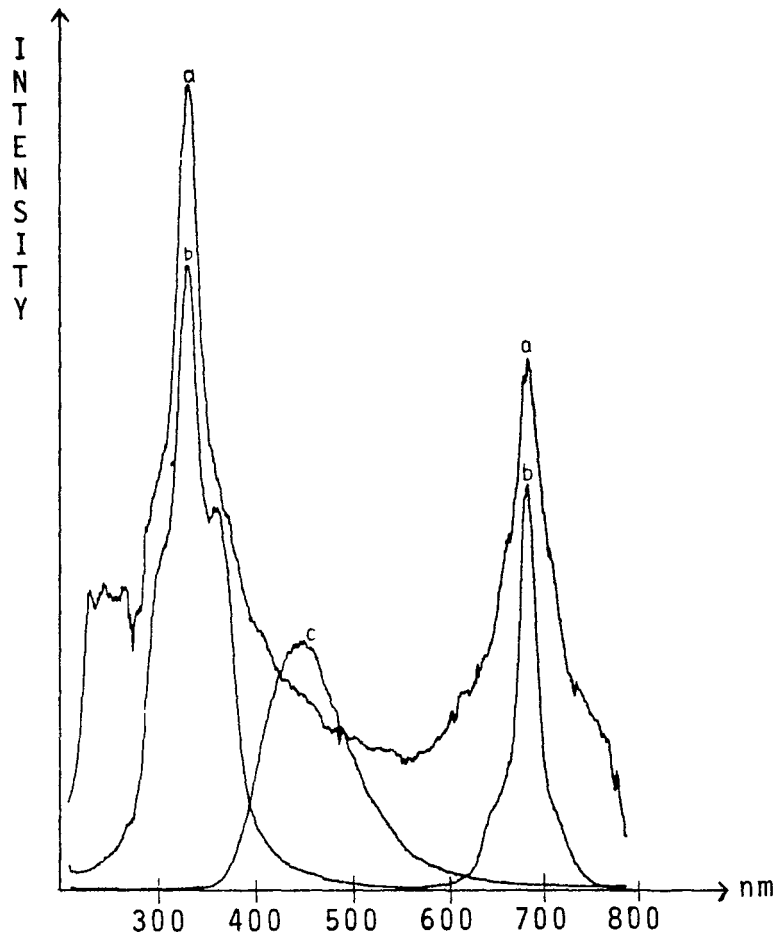


Fig. 17. Laser light transmitted through a) Storz, b) Pilkington Hytran and c) a convetional light guide. The peaks to the right of the spectra originate from the second order diffraction of the laser light.

of the low resolution. The first two fibres were used to excite normal paper. Spectra are shown in Fig. 18, where also the spectrum from paper excited and detected with the set-up in Fig. 6 is plotted for comparison. Detection through the different fibres, Fig. 19, was tried as well. The laser light transmitted through the Storz fiber can easily be suppressed by means of a filter. The set-up in Fig. 7d gave a correct spectral result, but the intensity was reduced by about a factor 25.

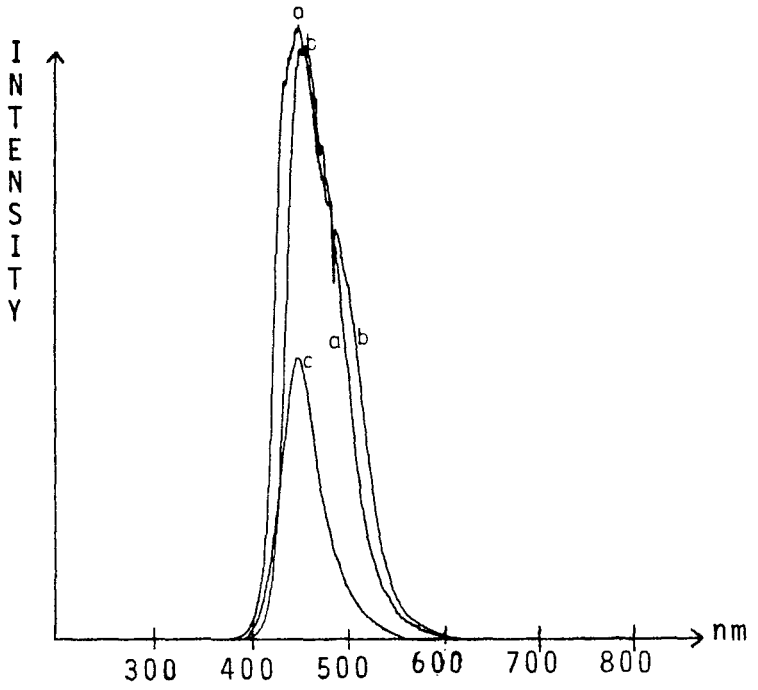


Fig. 18. Sample of normal paper excited with laser light led a) normally, as in Fig. 6., b) through Storz liquid light guide and c) through Pilkington Hytran.

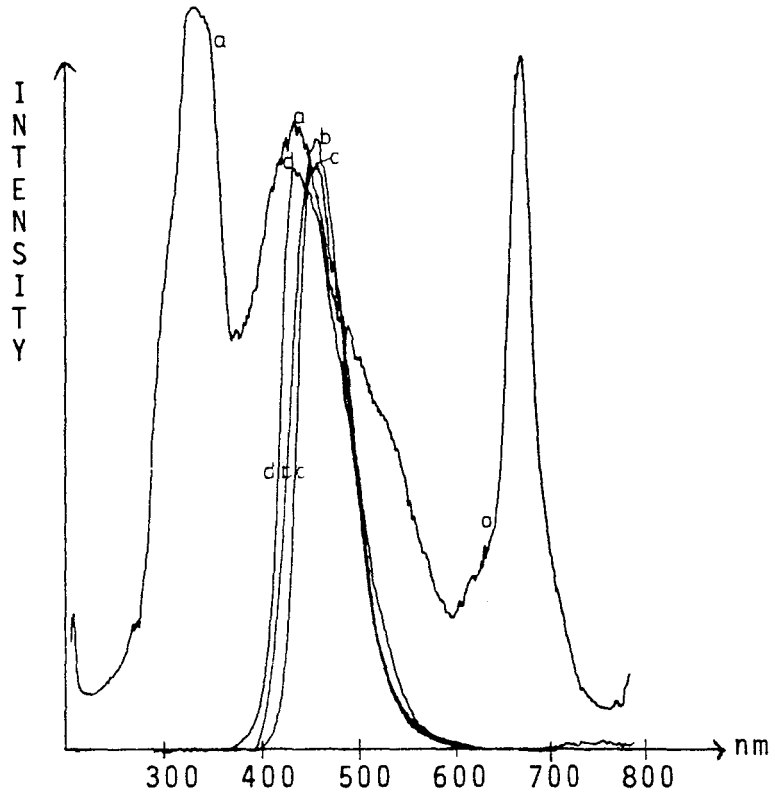


Fig. 19. Sample of normal paper excited with the set-up in Fig. 6. and detected a) through Storz liquid light guide, b) through the conventional light guide (spectrum enlarged 64 times), c) in the way shown in Fig. 6. and d) through Pilkington Hytran.

The drugs turned out to produce very different fluorescence spectra, Figs. 20 - 23. Estramustin (measured with N_2-laser only) showed very strong fluorescence (Fig. 21), and so did Cefuroxim. Doxycycline produced less intense fluorescence, Fig. 20, but still more than normal tissue. Pivmecillinam gave no fluorescence at all. The same applies to the spectra where an Ar^+-laser was used as exciting light source. An interesting observation is that the fluorescence yield from the calcium solution mixed with doxycycline is more intense than when the two are excited separately and their respective spectra are added, Fig. 23.

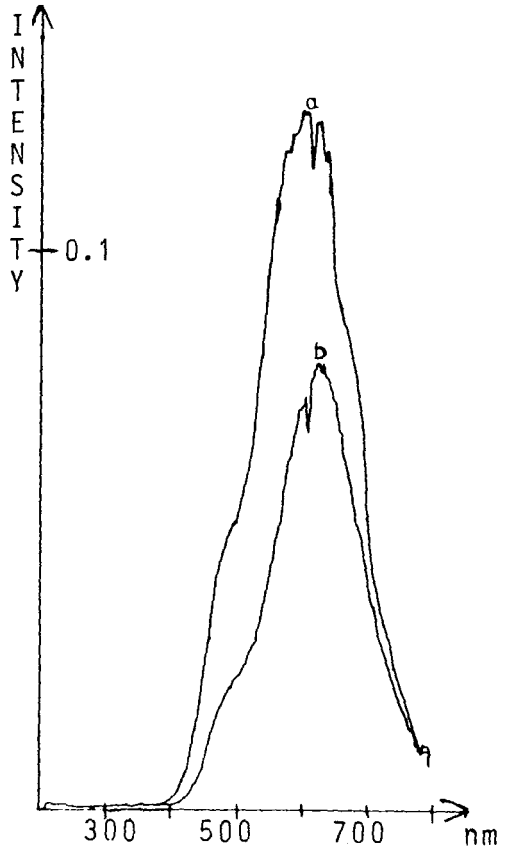


Fig. 20. Fluorescence spectra of a) doxycycline mixed with calcium b) doxycycline alone, excited with the N_2-laser.

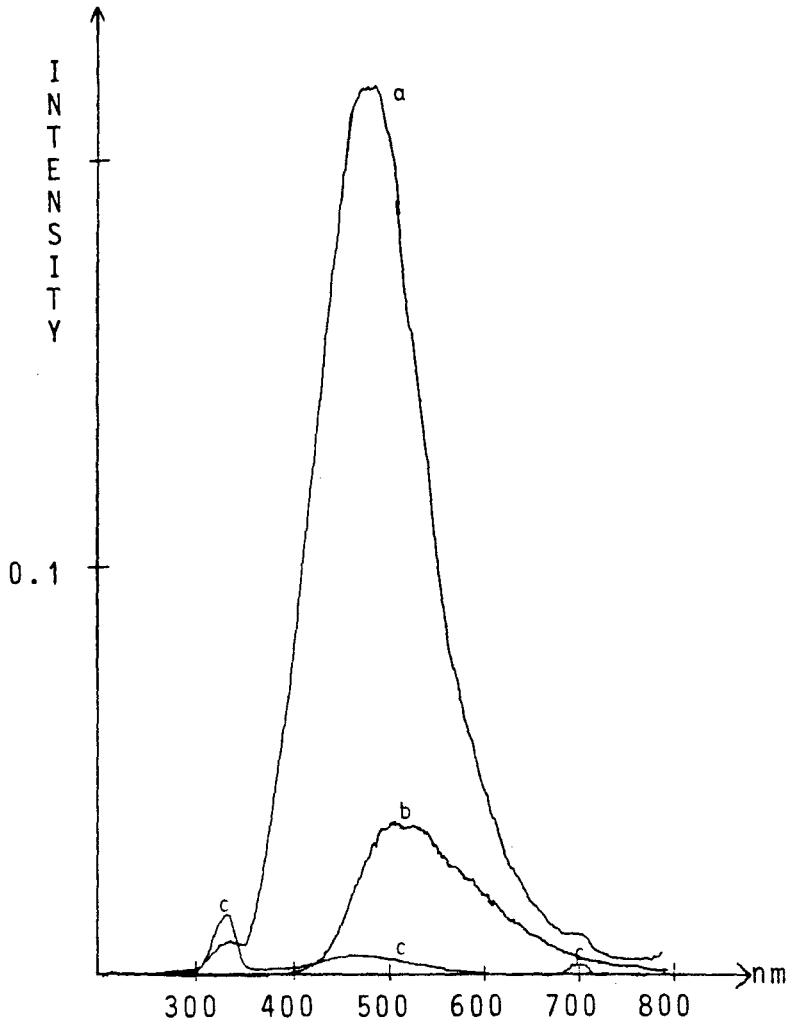


Fig.21. Fluorescence spectra of a) estramustin, b) cefuroxim and c) pivmecillinam, excited with the N_2-laser.

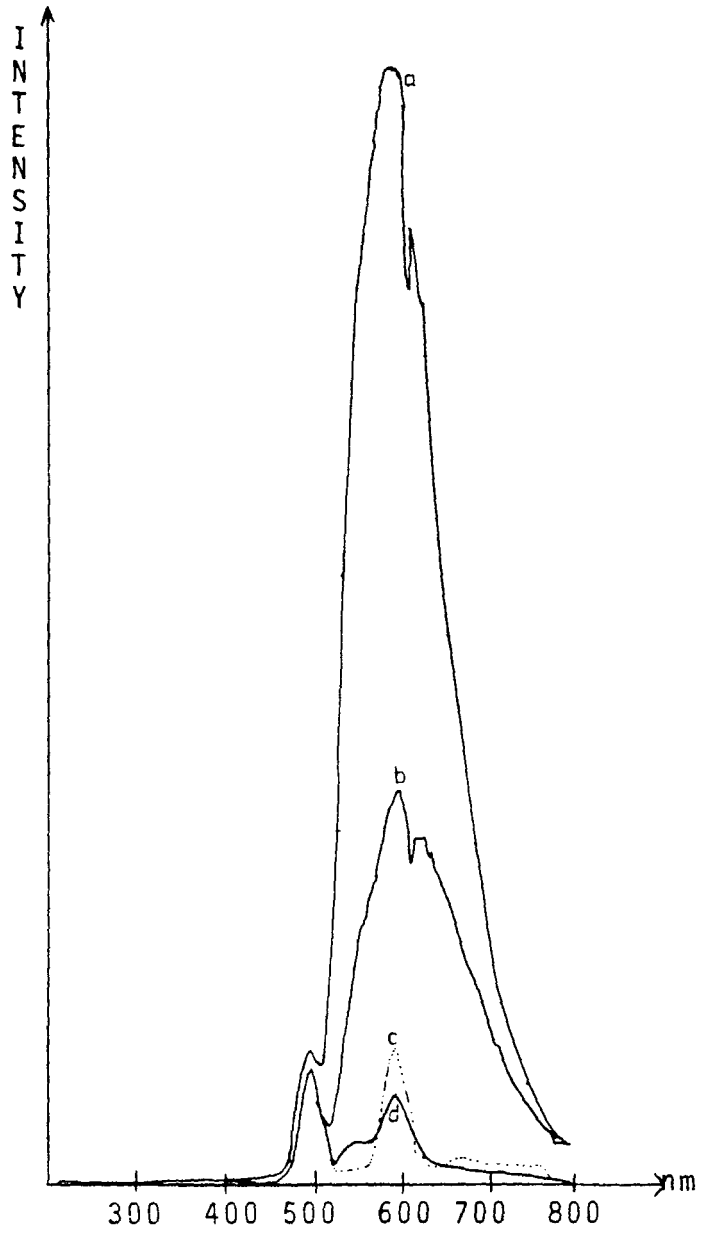


Fig. 22. Fluorescence spectra of a) cefuroxim, b) doxycycline, c) saline solution and d) pivmecillinam, excited with an Ar^+-laser.

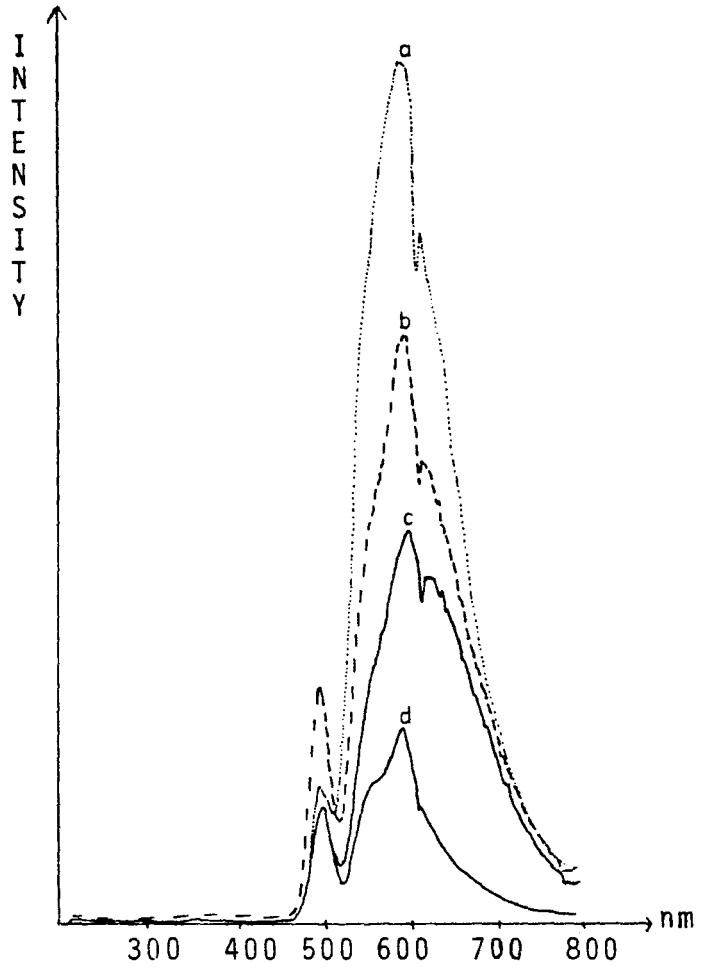


Fig. 23. Fluorescence spectrum of a) doxycycline mixed with calcium, c) doxycycline alone and d) calcium solution alone. b) is spectra c) and d) added. All samples were excited with an Ar^+-laser.

The urine has quite a strong fluorescence, Fig. 24, which seems to be independent of the difference of contents between morning and afternoon. Neither the NaCl-solution, nor the sterile water produce any fluorescence. The peak at 384 nm seen in the spectra originate from the OH stretch Raman-shift of the water molecule. The Raman scattering is a generally much weaker process than fluorescence.

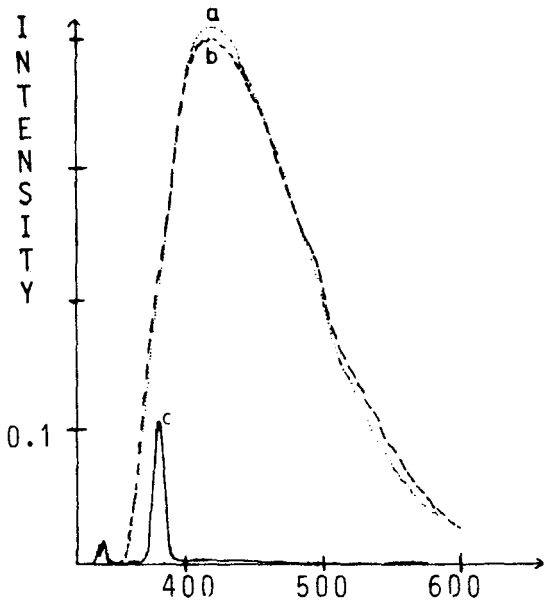


Fig. 24. Fluorescence spectra of a) urine, taken in the morning, b) urine, taken in the afternoon and c) sterile water and normal saline solution (identical spectra).

Conclusions

The "rat spectra" are very similar. The fluorescence light can not be used to distinguish different kinds of tissue if only the human eye is used as a detector. Beef and pork are different, and some different tissue structures seem to be revealed in the pork heart. The reason for this finding is unexplained. Of the fibres, the most suitable of the investigated ones for transmitting the laser light is the Pilkington Hytran, since it does not produce any fluorescence light itself. For detection, the conventional light guide seems suitable. It transmits the visible wavelengths well, and works as a cut-off filter since no laser light is trans-

mitted. Thus, for the set-up in Fig. 7d, it is natural to choose the Pilkington Hytran as transmitter for the laser light and the conventional light guide for the fluorescence light.

The strong fluorescence light from estramustin and cefuroxim could be utilized to trace the substances in the body. As earlier mentioned, doxycycline has a great affinity for calcium. As this compound fluoresces intensely, it should be possible to find calcium in the body, and thus to discover carcinoma in situ in the urinary bladder due to the earlier described mechanism.

It seems like the Ar^+-laser was as well suited for these investigations as the N_2-laser. The wavelength is evidently short enough to induce fluorescence, and the light produced from the samples lies in the visible region. It will be necessary to observe through a filter, though, since otherwise nothing but the elastically scattered laser light will be seen. We did not measure any spectra from tissue with this laser. We consider this as an interesting approach for future investigations.

Urine gives rise to strong fluorescence. This is no problem, however, since the bladder can be continuously washed with saline solution or sterile water, and these liquids do not fluoresce at all. They are also sufficiently transparent to the UV laser light.

CLINICAL IN VITRO INVESTIGATIONS

Material

Biopsy specimens were obtained at cystoscopy in 4 patients, ennumerated I, II, III and IV.

I) This patient suffered from recurrent superficial grade I tumors. 4 specimens were randomly taken from tumor and normal mucosa.

II) This patient had a history similar to the patient described in I).

III) This patient had previously undergone partial resection and coagulation of a grade I tumor. After that he had developed what seemed to be a calcified necrosis. A part of the calcification was removed for examination. Biopsy specimens from the underlying fibrous tissue, the boarder of the lesion, and from a macroscopically normal portion of the mucosa were obtained.

IV) This patient suffered from a well-known carcinoma in situ of the urinary bladder. 6 biopsies were taken from randomly chosen parts of the bladder.

The collected biopsy specimens were placed in normal saline solution. Their identity were unknown to the fluorescence investigator. The patients II), III), and IV) had received 200 mg of doxycycline (Vibramycin, Pfizer) 48 h prior to the examination. After fluorescence examination, the specimens were examined histologically.

Methods

For the investigation of the specimens from the patient I), the set-up in Fig. 6 was used. It was difficult to hit the small specimen with the laser beam, and therefore the set-up in Fig. 7d was used for the other measurements. The specimens were placed in aluminum cups. The specimens from I) were immersed in NaCl-solution (9mg/ml) in the cups. One of them was irradiated both with and without NaCl-solution. The other ones, from II), III), and IV) were irradiated without fluid.

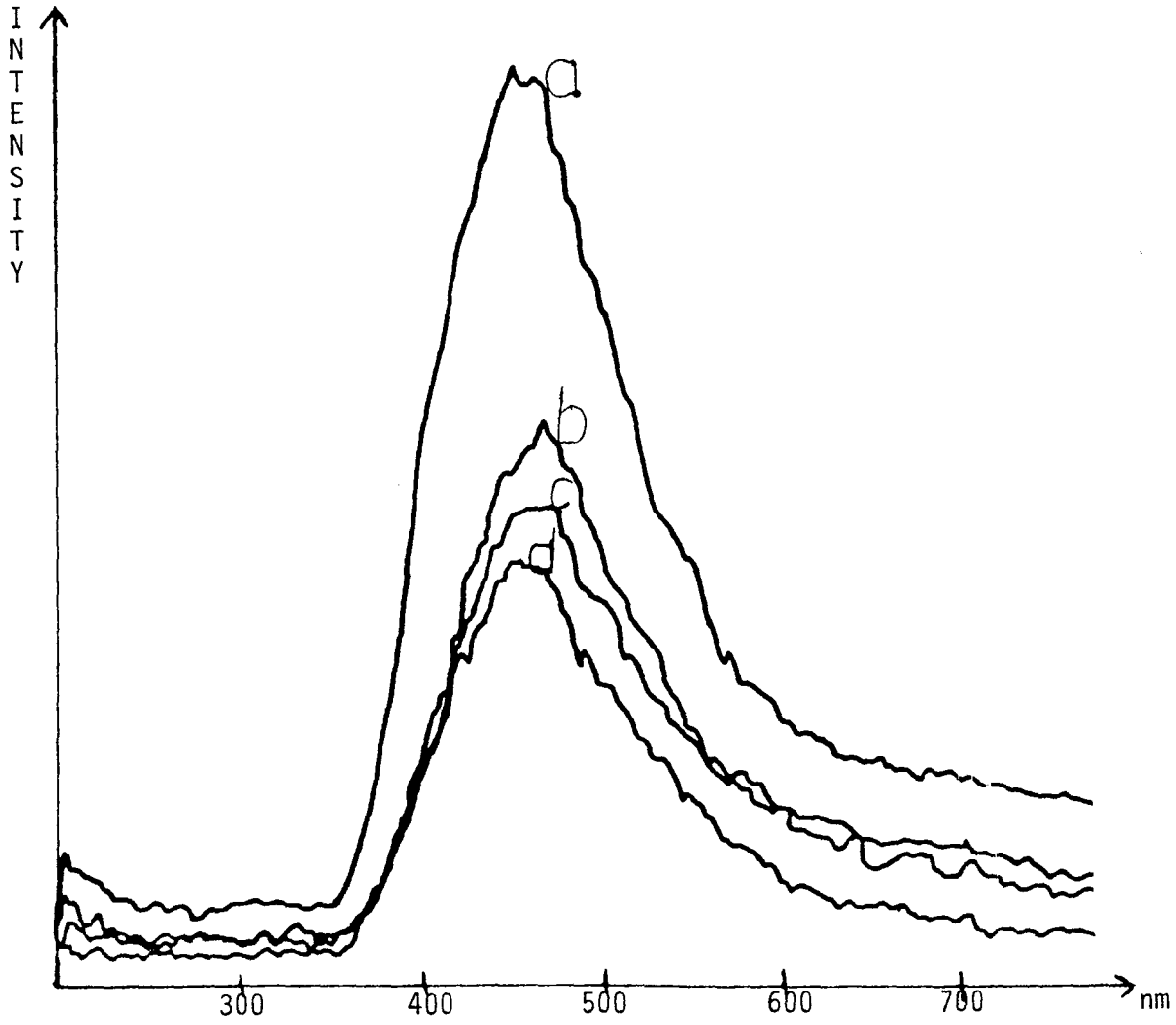


Fig. 25. Fluorescence spectra of biopsy specimens from the urinary bladder. a) proved to contain no tumor cells but calcifications and fibrous tissue, whereas b), c) and d) were fragments of grade I carcinoma.

Results

The specimen from I) irradiated both without and with the NaCl-solution produced identical spectra in the two cases. The spectra from I) can be seen in Fig. 25. There is no spectral difference between the specimens. The a)-curve has a higher peak. Histology revealed tissue without tumor cells, but with calcification and fibrous tissue. The other specimens were fragments of grade I carcinoma.

Neither can any significant difference be found between the specimens from patient II), Fig. 26. Microscopy showed no tumor cells at all, but ordinary mucosa.

Among the III) specimens, Fig. 27, a small shift towards longer wavelengths can be seen in the b). The other ones seem to have approximately the same spectral distribution as the

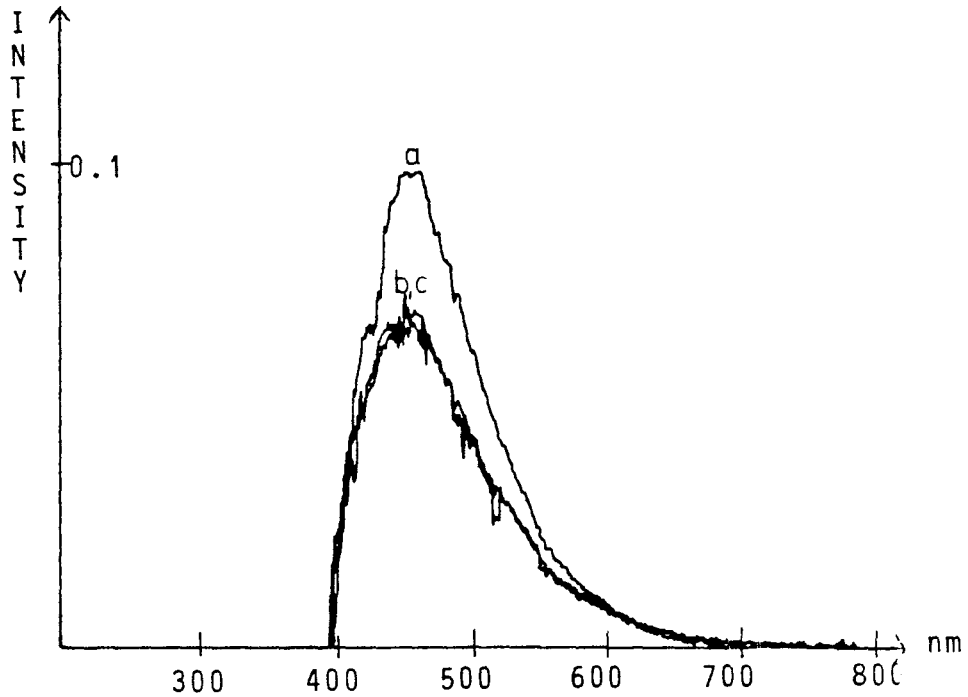


Fig. 26. Fluorescence spectra of biopsy specimens that turned out to be ordinary mucosa. This patient had been given doxycycline prior to examination.

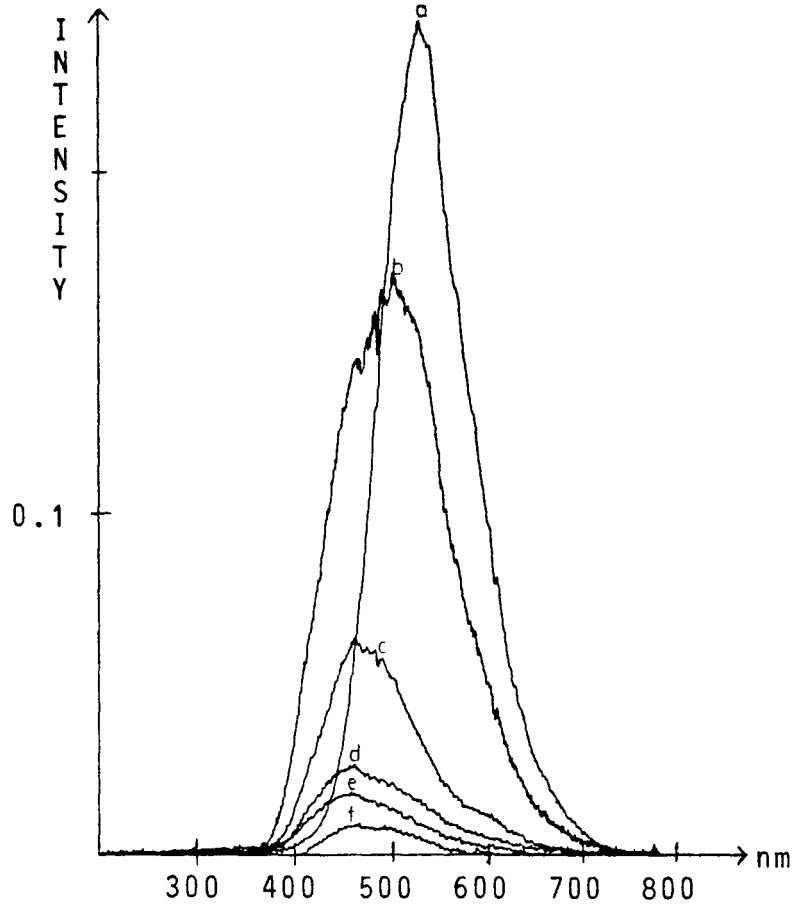


Fig. 27. Fluorescence spectra of a) calcification, b), c), d), e) inflamed tissue adn f) calcified scarry tissue. This patient had been given doxycycline prior to examination.

specimens from I) and II). The calcification, a), clearly has its peak at a longer wavelength. It appeared bright yellow to the eye whereas the low intensity specimen appeared blue. The b) appeared yellow. It has its peak at about 520 nm while the Vibramycin - calcium compound has its peak at 580 nm. This might be explained by the fact that in this case the fluorescence from normal tissue is involved as well and added in some way which may produce this shift towards shorter wavelength. The pathological examination here however, showed that b), c), d) and e) contained inflamed tissue while f) contained almost exclusively calcified scarry tissue.

The spectra of patient IV), Fig. 28a) and b), also show almost identical distributions. They also show similarity to the spectra of case I, where no Vibramycin was given. The specimens all turned out to be cancer in situ.

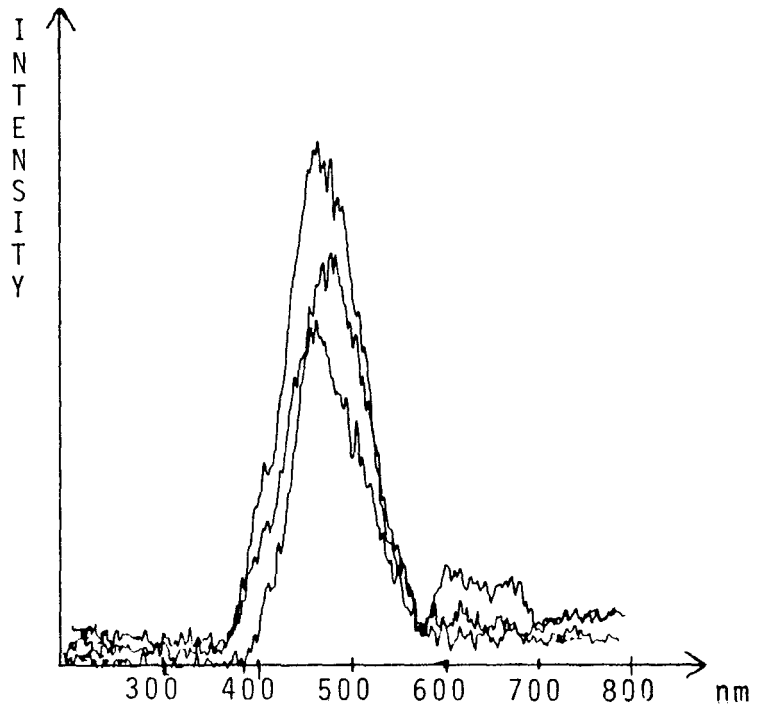


Fig. 28a). Fluorescence spectra of specimens that proved to be carcinoma in situ. This patient had been given doxycycline prior to examination.

Conclusions

No difference in fluorescence light could be detected between ordinary mucosa, inflamed tissue, frank tumors and carcinoma in situ. This confirms the doubts in the earlier mentioned results from 1964, where some of the specimens that produced fluorescence light proved to be normal tissue. Se-

veral explanations may be possible. Doxycycline maybe does not enrich in cells in the same way that tetracycline does. Our free interval might have been too long. In 1964 tetracycline was given for a longer time, which might have caused all tissue to be saturated with tetracycline. Further investigations ought to be made, however, before the method is finally rejected.

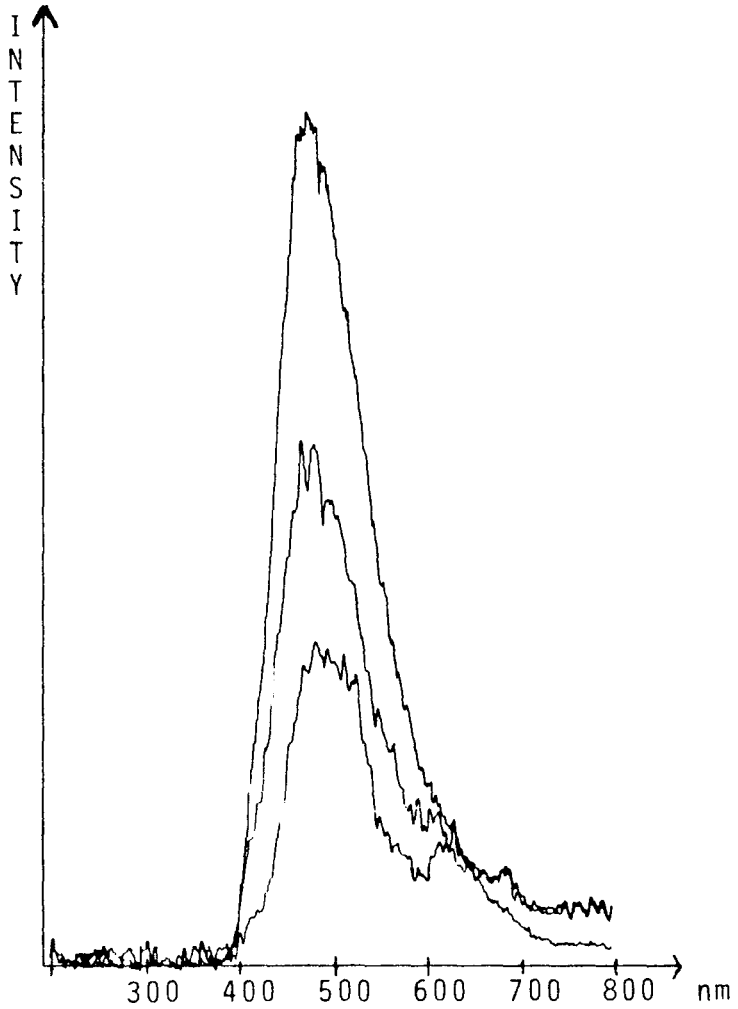


Fig. 28b). Fluorescence spectra of specimens that proved to be carcinoma in situ. This patient was given doxycycline prior to examination.

Discussion

Our experiments have made it less probable that the method, to mark tumors with tetracycline is reliable. This unreliability might be the reason for the cessation of its use in the beginning of the seventies. Still, we do not consider the method proved to be worthless. Reports show, however, that other substances seem to be of more interest, especially HPD, hemato-porphyrin derivative. HPD is a known tumorseeking fluorescent dye and has proved to mark extremely small tumors in the human bronchus (Ref. 6) and in the bladder of rabbits (Ref. 7) very efficiently. Also acridin orange seems to be of interest.

All tissues seem to produce very similar fluorescence spectra when irradiated with UV-light. We consider however the clearly distinguishable yellow fluorescence of the porcine heart an interesting object for further investigations.

INVESTIGATION OF NORMAL SKIN, NAEVI AND MELANOMA METASTASES

Material

Normal skin and naevi of the authors were investigated. One patient with cutaneous metastases of malignant melanoma was also examined.

Method

The set-up in Fig. 7d) was used as a probe and the laser--induced fluorescence from different areas of the skin of the authors was recorded. Some of the naevi were later removed for histological examination. The patient with melanoma metastases was examined in the same way.

Results

Two of the naevi had a separate peak around 660 nm, Fig. 29. This is very close to the second-order diffraction of the laser light. It is not likely, however, that it is the laser light that has given rise to this peak, since there is no first-order diffraction. The other naevi showed the same spectra as normal skin, with less intensity only. The same was true for the finger nail, but there more intensity was obtained, Fig. 30a. The skin of different individuals showed different spectra, Figs. 30a and 30b. Scarry tissue and areas which had been irradiated with a Nd:YAG laser showed spectra differing from the spectrum of normal skin of the same person.

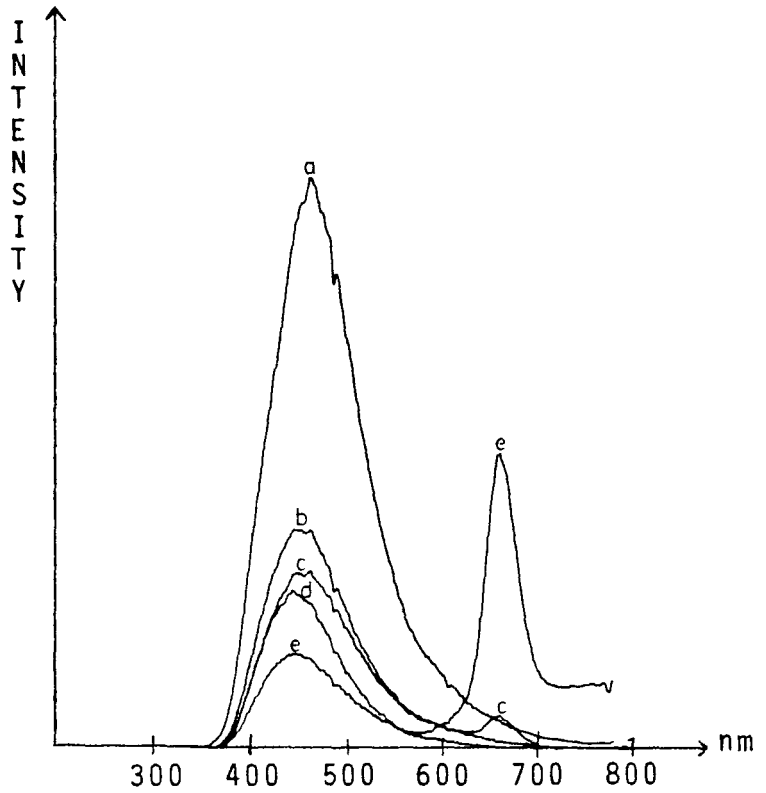


Fig. 29. Fluorescence spectra of a) normal skin, b), c), d), and e) different naevi. c) and e) were later removed and examined histologically.

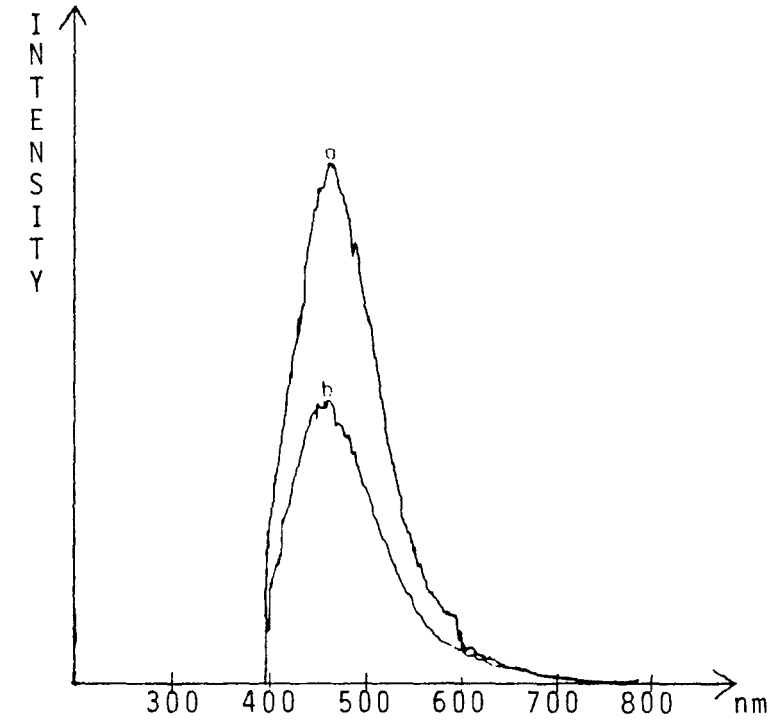


Fig. 30a). Fluorescence spectra of a) normal skin and b) thumb nail.

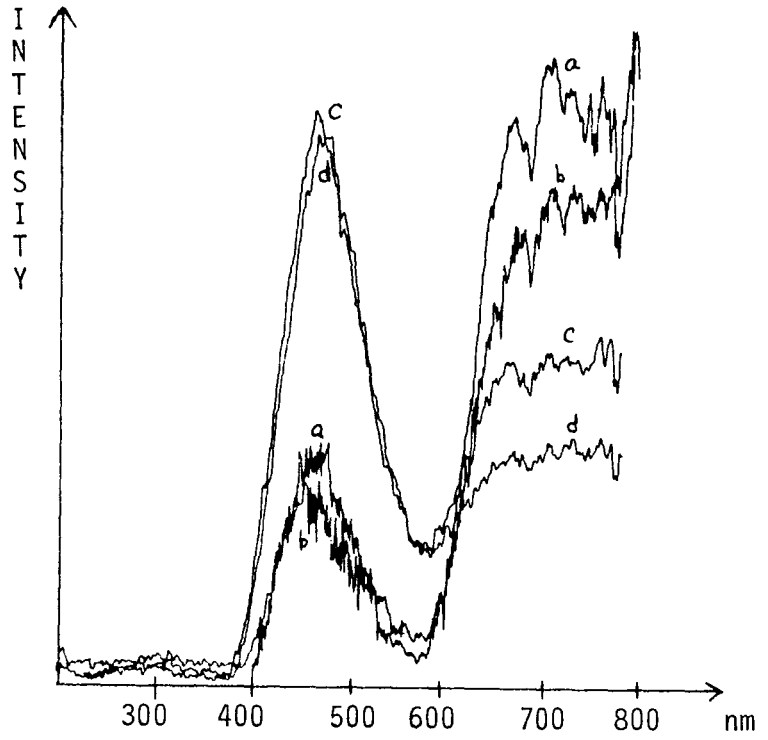


Fig. 30b). Fluorescence spectra of a) and b) areas from which naevi had been removed by means of Nd:YAG laser irradiation two months earlier, c) one year old scarry tissue of the skin and d) normal skin.

The spectra from the cutaneous metastases, Figs. 31 - 34, showed the same distribution as the normal skin of the patient, Fig. 31, except for one, which gave much more infra-red light, Fig. 34. The earlier mentioned red peak can be seen in the spectra in Figs. 31 - 34, although it is weak. Histological examination of the naevi in Fig. 29 revealed ordinary pigmental naevi.

Conclusions

Cells, that at a histological examination seem to be identical, show different spectra. This prove that it is possible to trace differences with laser-induced fluorescence that are impossible to detect histologically. It has not been investigated from where the differences origin.

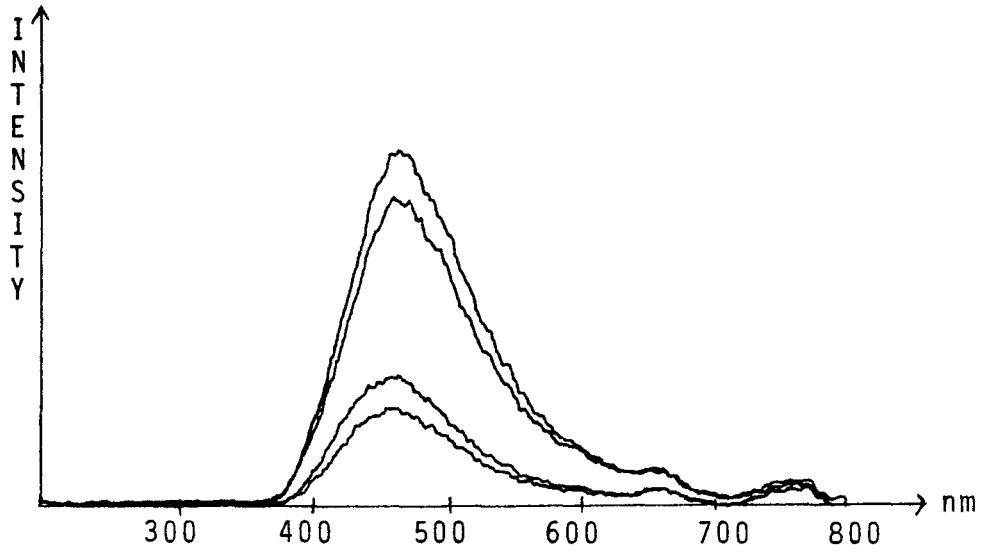


Fig. 31. Fluorescence spectra of normal skin of a patient with cutaneous metastases. a) and b) light skin, c) and d) sunburnt skin. The intensity of these spectra is reduced with a factor 4 compared to Figs. 32 - 34.

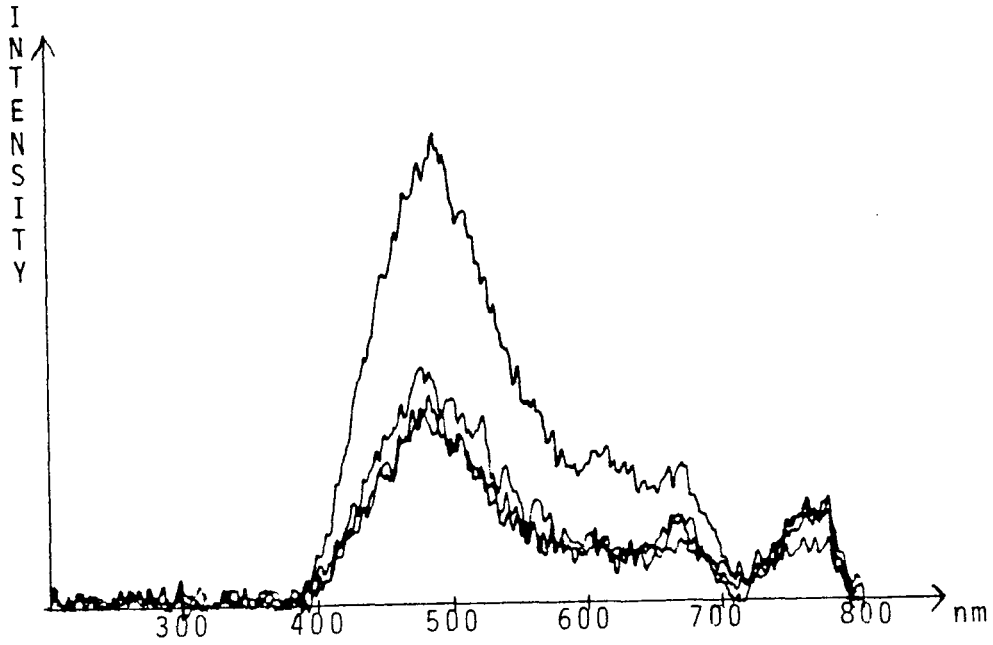


Fig. 32. Flucrescence spectra of naevi of a patient with cutaneous metastases of malignant melanoma.

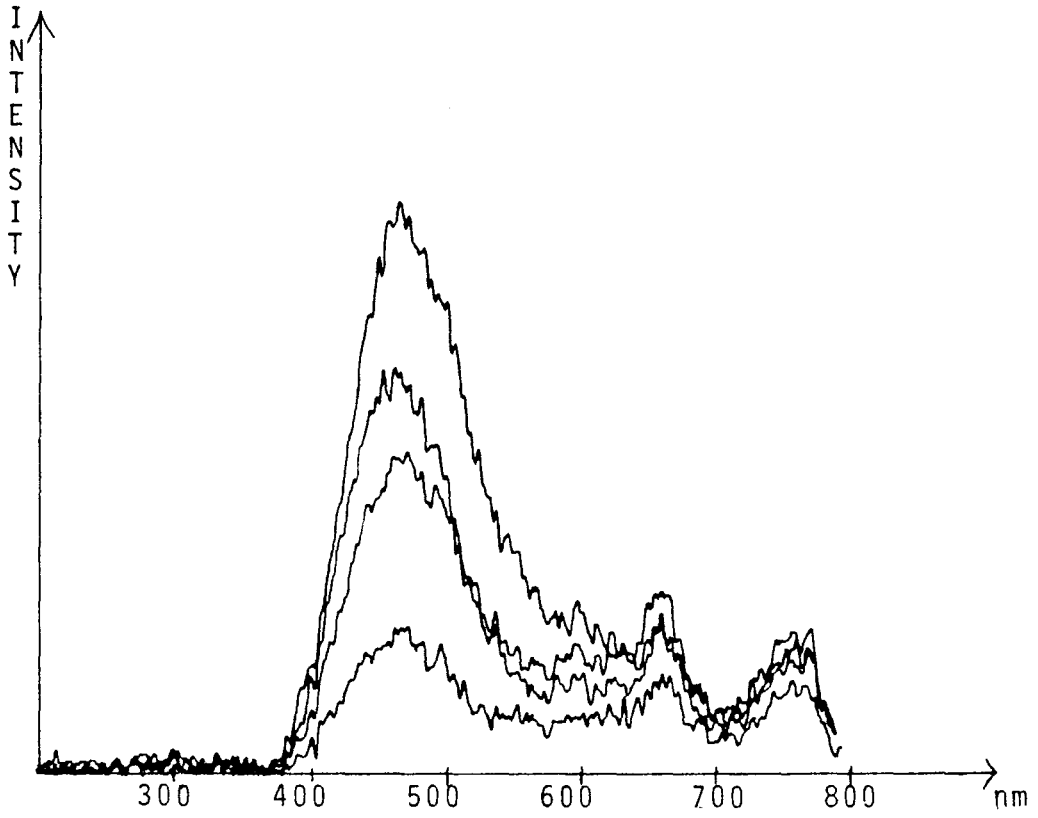


Fig. 33. Fluorescence spectra of cutaneous metastases of malignant melanoma.

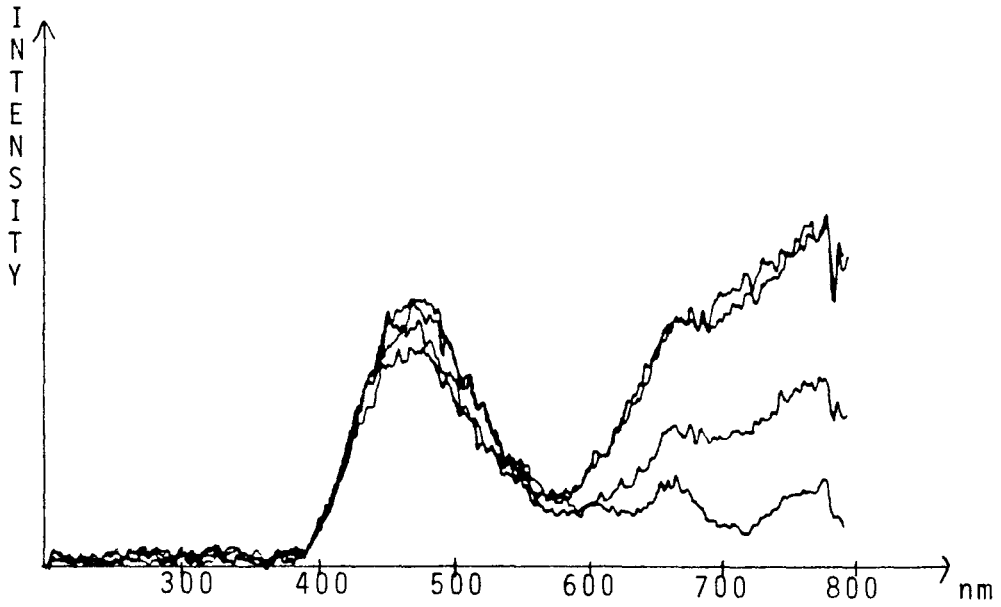


Fig. 34. Fluorescence spectra of different parts of one metastase of malignant melanoma.

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ORDLISTA

antibiotic =	medel mot bakterieinfektion
biopsy =	"provbit", tagen t ex från urinblåsans slemhinna, för mikroskopisk undersökning
calcification =	förkalkning
calcified =	förkalkad
carcinoma =	cancer
clinical investigation =	undersökning rörande patient
cutaneous metastases =	dottersvulster i huden
cystoscopy =	att titta in i urinblåsan genom ett via urinröret infört instrument, cystoskop
cytology =	mikroskopisk undersökning av lösa celler, till skillnad från hela vävnadsstycken
fibrous tissue =	bindväv
Frank tumor =	tumör uppenbart synlig vid cystoskopi
history =	i detta sammanhang: sjukhistoria
infiltrating =	djupväxande
in vitio =	"i provröret", t ex lab-undersökning
in vivo =	"i levande livet"
lard =	fett
lesion =	förändring
malignancy =	"elakartadhet"
malignant =	elakartad
melanoma =	pigmenterad hudcancer
mucosa =	slemhinna
naevi =	"födelsemärken" med oftast brun färg

necrotic cell =	död cell
neoplastic tissue =	cancervävnad
normal saline =	fysiologisk koksaltlösning
premalignant =	förstadium till cancer
recurrent =	återkommande
resection =	skära bort (t ex en tumör)
scarry tissue =	ärrvävnad
specimen =	ett prov från t ex vävnad
spleen =	mjälte
surgeon =	kirurg
transurethral =	via urinröret
tumourous tissue =	tumörvävnad
urinary bladder =	urinblåsan
viable =	levande