TIME-RESOLVED LASER FLUORESCENCE AND PHOTobleaching OF SINGLE CELLS
AFTER PHOTOSENSITIZATION WITH HEMATOPORPHYRIN DERIVATIVE (HpD)

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INTRODUCTION

Because of its preferential retention in tumor cells hematopor­
phyrin derivative (HpD) is used for diagnosis1,2 and phototherapy 3
of tumors. The cytotoxic effect has been attributed to the formation
of singlet oxygen after intermolecular energy transfer HpD→O2 4 or
to the production of HpD radicals 5.

Quantitative fluorometric detection of HpD is limited by cell
autofluorescence which cannot be completely suppressed by spectral
filtering. However previous results obtained using mouse tissue cells
and human lymphocytes 6 indicate that HpD fluorescence and autofluo­
rescence can probably be separated according to their different de­
cay times. These different decay patterns have been investigated fur­
ther in this paper using human bladder cells and rabbit Brown-Pearce
tumor cells.

EXPERIMENTAL

Cultures of human cells (normal urothelial and bladder cancer
cells) or Brown-Pearce carcinoma cells 7, were incubated with an
aqueous HpD solution (5 μg/ml) for 12 or 36 hours, respectively.

Single cells were irradiated in a fluorescence microscope
(Fig. 1) with UV pulses of 364 nm and 100 psec duration from a mode­
locked argon ion (Ar+) laser. Laser pulses with a repetition rate

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of 250 kHz were selected from the 82 MHz pulse train by means of an electro-optic shutter.

The laser beam, focused to a diameter of 100 μm, illuminated the cells homogeneously with a pulse energy of $2 \cdot 10^{-11}$ Joule. The fluorescence decay curve was obtained using a single photon counting system, as well as optical long pass and interference filters appropriate for HpD emission (600-700 nm). The time-resolution of the entire detecting system was about 0.5 nsec.

RESULTS AND DISCUSSION

Fig. 2 shows the fluorescence decay curves for a single human bladder cell incubated with HpD and for a reference cell not exposed to HpD. Both curves show fast decay times of about 1 nsec which are attributed to autofluorescence. The cell treated with HpD also shows a long-lived fluorescence component which decays with a time constant of $(6.5 \pm 2)$ nsec. The same decay time was measured for all normal and tumor cells incubated with HpD, in agreement with results recently obtained by Docchio et al. using mouse tissue cells.

A decrease of fluorescence intensity due to photobleaching was observed within a few minutes of irradiation. Fig. 3 shows the bleaching behaviour of an HpD treated Brown-Pearce tumor cell at an average irradiance of 300 mW/mm². The fading of short-lived autofluorescence is relatively weak, but the fluorescence photon rate of HpD is reduced by more than a factor of 3. Even at a power density of only 3 mW/mm², the value commonly used for time-resolved fluorescence