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Light-Stimulated Ultraweak Photon Reemission of Human Amnion Cells and Wish Cells

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ABSTRACT

Photon reemission in the ultraweak intensity range that is observed after irradiation of cell suspensions with light, reveals characteristic differences between normal human amnion cells and transformed Wish cells from the same parental tissue. The reemission kinetics, approximated best by a hyperbolical process, were studied as a function of cell density, showing that: malignant Wish cells have a photon storage capacity that is not improved by increasing the cell density; and that normal amnion cells exhibit a photon storage capacity that strongly increases with increasing cell density. The interpretation of this effect and the nature of the emitter are discussed.

Index Entries: Photon reemission, kinetics of, and differences in; photon storage capacity, of cells; human amnion cells; wish cells.

INTRODUCTION

Spontaneous ultraweak photon emission (PE) in the visible region has been well documented for living cells and tissues of plant- as well as animal origin (*1–3*).

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The mechanisms of this phenomenon and its biological significance are discussed quite controversially. The "chemiluminescence concept" is based on the occurrence of oxidative reactions, which usually are associated with lipid peroxidation (*4–6*).

In the "genetic information concept," the nucleus and especially the DNA are considered to be the essential source of the PE, a view which is supported by experiments with ethidium bromide, which intercalates into DNA and modulates emission intensity (*7,8*). Taking theoretical considerations on energy storage and long-range coherence in biological systems (*9*) as a base, some authors have come to the conclusion that this emission plays a key role in inter- and intracellular regulation (*10–12*).

This model predicts a decay behavior for the light-induced reemission of cell systems. Under the assumption of an energy quantum storage mechanism within and a coherent feed-back coupling between cells, the decaying kinetics after excitation should follow a hyperbolic rather than an exponential law (*13*). This indeed has been observed with plant material (*11*) and also with mammalian cell cultures (*14*), both after irradiation with light.

Furthermore, the decaying kinetics seem to depend on the cell status. The comparison between normal tissue and tumor tissue of a plant (*Bryophyllum*) revealed a difference in the reemission kinetics after irradiation with light, whereas no difference was seen in the ultraweak, but well observable spontaneous PE (*11*).

A dependence on the cell cycle has also been found with fractions of microsporocytes and cones of Larix (*15*), where the spontaneous PE and also the light-induced reemission intensity show a pronounced minimum at the stages of the haplophase.

Rat hepatocytes and hepatoma cell lines investigated most recently by Schamhart et al. (*14*) did not exhibit any significant spontaneous PE. After irradiation with white light, the reemission intensity within the first five s was found to be different at high cell densities for the different cell types studied, yielding the lowest value for normal hepatocytes and the highest value for the least differentiated tumor cell line.

In this report we compare the reemission characteristics of normal human amnion cells with those of the transformed Wish cell line (ATCC CLL 25), which was derived from human amnion tissue (*16*) and exhibits ultrastructural features of a malignant cell line (*17*).

MATERIALS AND METHODS

Registration of Photon Emission

For detection and registration of spontaneous and stimulated PE we used a single photon counting device (as described in *1,11*), equipped with a cooled EMI 9558 QB photomultiplier tube with high sensitivity in the range between 250 and 800 nm. The integral intensity values within

given time intervals (.1 s) were stored and processed by an interfaced computer.

A quartz sample cuvette containing 10 mL of cell suspension or medium was kept in a dark chamber in front of the multiplier. Irradiation of the cuvet was performed perpendicular to the detecting direction with focused white light from a 150 watt tungsten lamp, passing a heat reflection filter that cuts off wavelengths above 720 and below 310 nm.

Each measuring cycle started by irradiating the sample for 3 min; the following stimulated emission was recorded and evaluated from .7 to 55 s after the end of excitation. Every sample was irradiated and measured three times consecutively and was continuously stirred.

Cell Cultures

Human amnion cell cultures were received as a gift from the Institute for Anthropology and Human Genetics, University of Heidelberg. The cells had been collected by amniocentesis and cultured for 4–6 wk in HAM F-10 medium supplemented with 20% FCS. For measurement of one series of cell densities, the cells from about 30 culture flasks were trypsinized, washed once, and resuspended in 10 mL MEM Dulbecco "for chemiluminescence" (Boehringer, Mannheim). Viability of the final cell suspension was about 80%.

Wish cells were obtained from Flow Laboratories and cultured in MEM H medium containing 20% FCS. Starting with $3 \cdot 10^6$ cells/15 mL, confluency was reached within approx 10 d. For measurement of one series of cell densities, 5–6 flasks (75 cm^2 each) containing confluently grown cultures were used and prepared as above. Viability of the final cell suspension was about 85%.

RESULTS

Human amnion cells and Wish cells in suspension at the maximum cell density, that is, $3 \cdot 10^6$/mL and $33 \cdot 10^6$/mL, respectively, did not exhibit any significant spontaneous photon emission, as compared with the dark count rate of the photomultiplier.

For the photon reemission kinetics after irradiation, the cell densities of the suspensions ranged from .15 to $3 \cdot 10^6$ cells/mL (amnion) and from .6 to $33 \cdot 10^6$ cells/mL (Wish).

The decay behavior of the stimulated PE within the time interval from .7 to 10 s is plotted exponentially (log I vs t) for two representative records with amnion and Wish cells in Fig. 1a and 1b, respectively, whereas in Fig. 1c, the same data are presented as a hyperbolic plot (log I vs log t).

For both decay functions approximation curves were computed according to the least square fit method. In the exponential case ($y = \alpha_1 \cdot \exp[-k_1 \cdot t]$), correlation coefficients of .946 (amnion) and .979 (Wish)

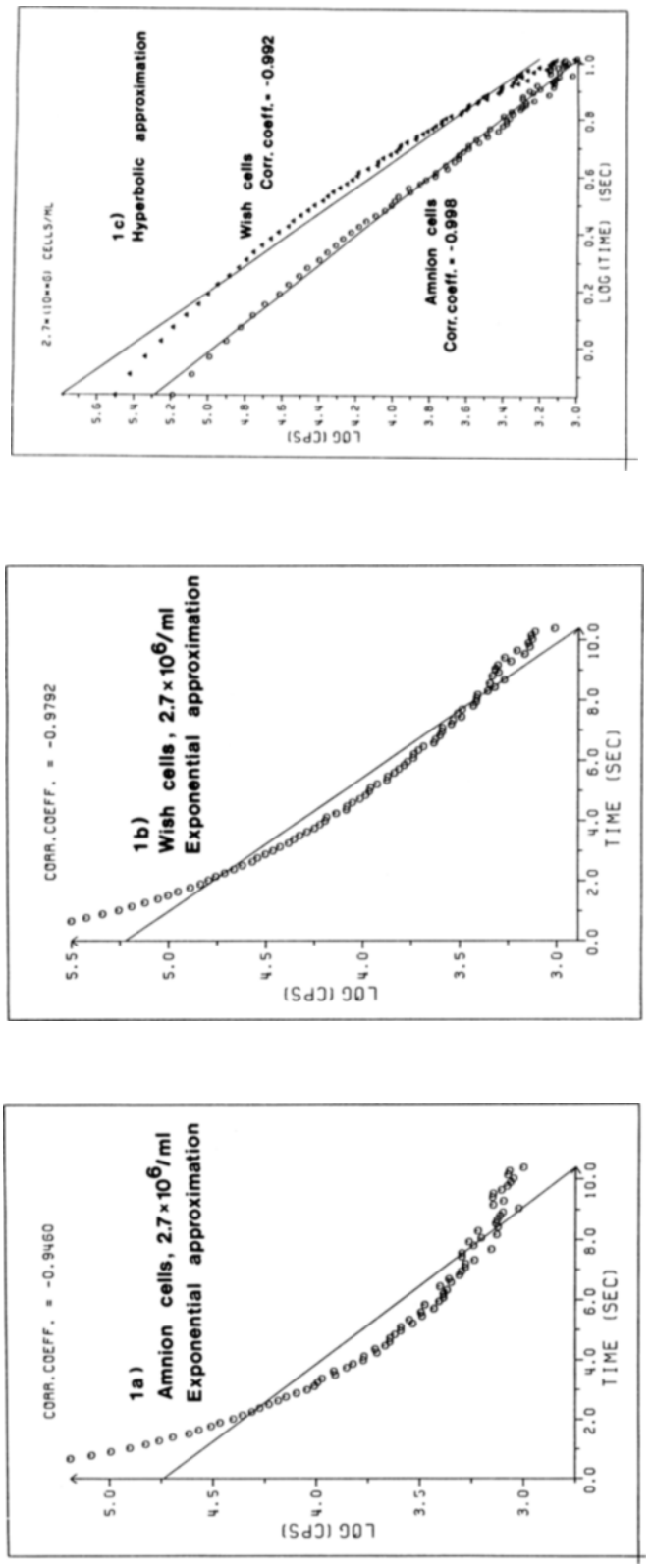

Fig. 1. Decay kinetics of ultraweak photon emission of cell suspensions following irradiation with white light, approximated by exponential (1a and b) or hyperbolic (1c) decay law. Points, experimental data; drawn line, calculated approximation. Cells were suspended in 10 mL MEM medium without phenol red and continuously stirred at 34°C. Measuring cycle .123 s, first evaluated value at .7 s.

are obtained, and a systematical deviation is apparent from the plot. The hyperbolic approximation ($y = \alpha_2 \cdot t^{-k_2}$), on the contrary, gives an excellent fit with correlation coefficients of .998 (amnion) and .992 (Wish).

This result is in agreement with previous observations on the hyperbolic decaying kinetics of cucumber seedlings after irradiation (*11*), and of rat hepatocytes and hepatoma cell lines (*14*), and also with theoretical considerations on the radiation decay behavior of a system with coherent feedback (*13*).

Based on these results, all recorded kinetics with amnion and Wish cells were approximated hyperbolically on a personal computer over the time interval from .7–56 s after irradiation, yielding a decay constant $-k$ and an amplitude factor α. The variation of α with cell density is relatively small and does not exceed the deviation of the control values; therefore α was not taken into account for evaluation. The decay constant $-k$, on the other hand, is a rather characteristic parameter, especially when studied at different cell densities: Fig. 2 shows the decay constant of amnion and Wish cells after irradiation as a function of the cell

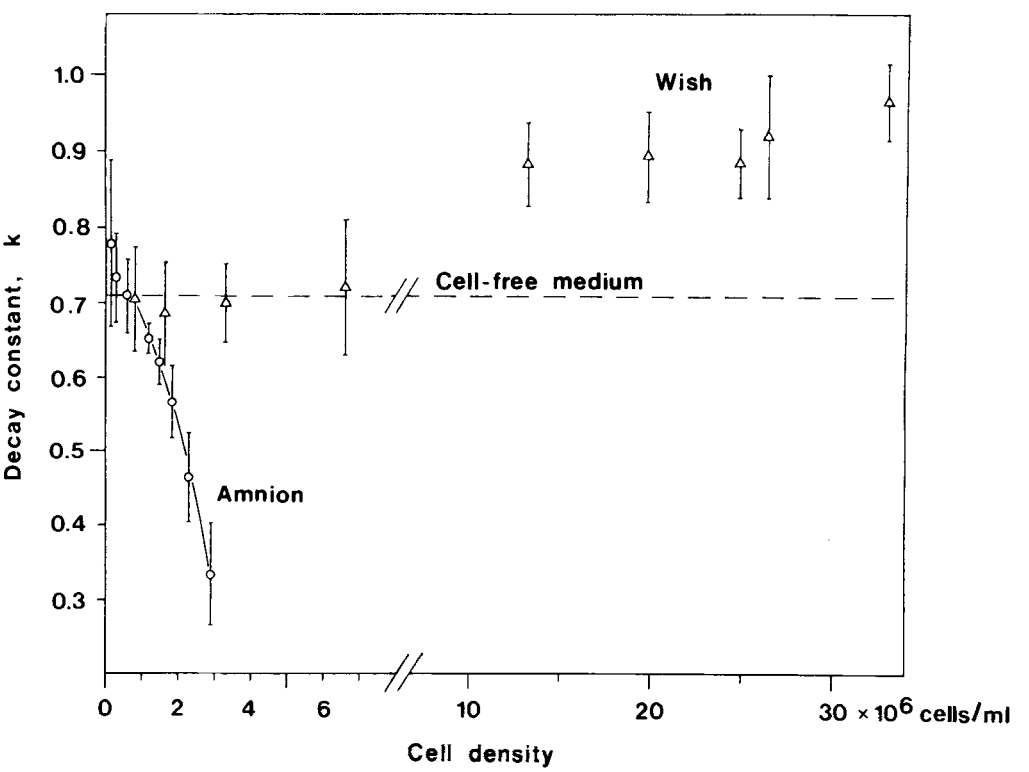

Fig. 2. Dependence of the reemission decay constant $-k$ on cell density for amnion and Wish cells. The recorded decay curve from .7 to 56 s has been approximated to give $-k$. Each point represents the mean value of three determinations with standard deviations as indicated. The control value for MEM medium without cells was determined from seven experiments to be .71 ± .04. Circles, amnion cells; triangles: Wish cells. Other conditions as in Fig. 1.

density. The plot reveals strikingly different behavior for the two cell types; Wish cells result in a roughly constant value for $-k$ at moderate cell densities, which tends to increase at very high densities, whereas amnion cells exhibit a drastic decrease of $-k$ with increasing cell density.

In the experiment presented in Fig. 2, the dilutions were prepared linearly from high to low cell densities. In a second experiment, the dilution series was permuted to check the possible influence of cell aging; the result was similar and no time dependence was observable (data not shown).

The decrease of $-k$ with increasing amnion cell density is equivalent to a prolonged storage of the excitation energy. This becomes obvious by examining the remaining reemission intensity after a long time; Fig. 3 presents the mean intensity values between 49.3 and 55.4 s after irradiation.

In Wish cell suspensions, the excitation energy after this time is decayed almost completely (Fig. 3a); solely at cell densities of about $3–10 \cdot 10^6$/mL is the remaining intensity elevated to 130% above the base level.

On the contrary, the remaining intensity in amnion cell suspensions continuously increases with increasing cell density (Fig. 3b), reaching a peak value of about 300% above the base level at the highest cell density ($3 \cdot 10^6$/mL).

DISCUSSION

The presented reemission kinetics of amnion and Wish cell suspensions show that irradiation with white light induces an energy storage process with a different decay behavior in the two cell types studied.

The decay has been recorded from .7 to 56 s after the end of irradiation; the kinetic is approximated well by a hyperbolic decay function, whereas the exponential approximation deviates systematically.

This is in accordance with previous observations of Popp et al. (*11*) with cucumber seedlings and of Schamhardt et al. (*14*) with rat hepatocytes and hepatoma cell lines and also with theoretical considerations concerning the decay behavior of a system with a coherent feedback coupling (*13*).

Popp et al. found for the decay constant k with plant material $1 < k < 3$ in most cases; from Schamhardt's kinetic data, decay constants of 1.0 to 1.7 can be calculated for a cell density of $4 \cdot 10^6$ cells/mL.

In our system, we observed a dependency of the rate constant upon the cell density; for Wish cells values we arrived at increased from .7 to 1.0 with increasing cell density, whereas the values for amnion cells decreased from .7 to .3 with increasing cell density. This points to a very high storage capacity for excitation energy in the case of healthy human cells, giving rise to a reemission level that after about 50 s, is still about 300% above the control level at the amnion cell density of $3 \cdot 10^6$ cells/mL.

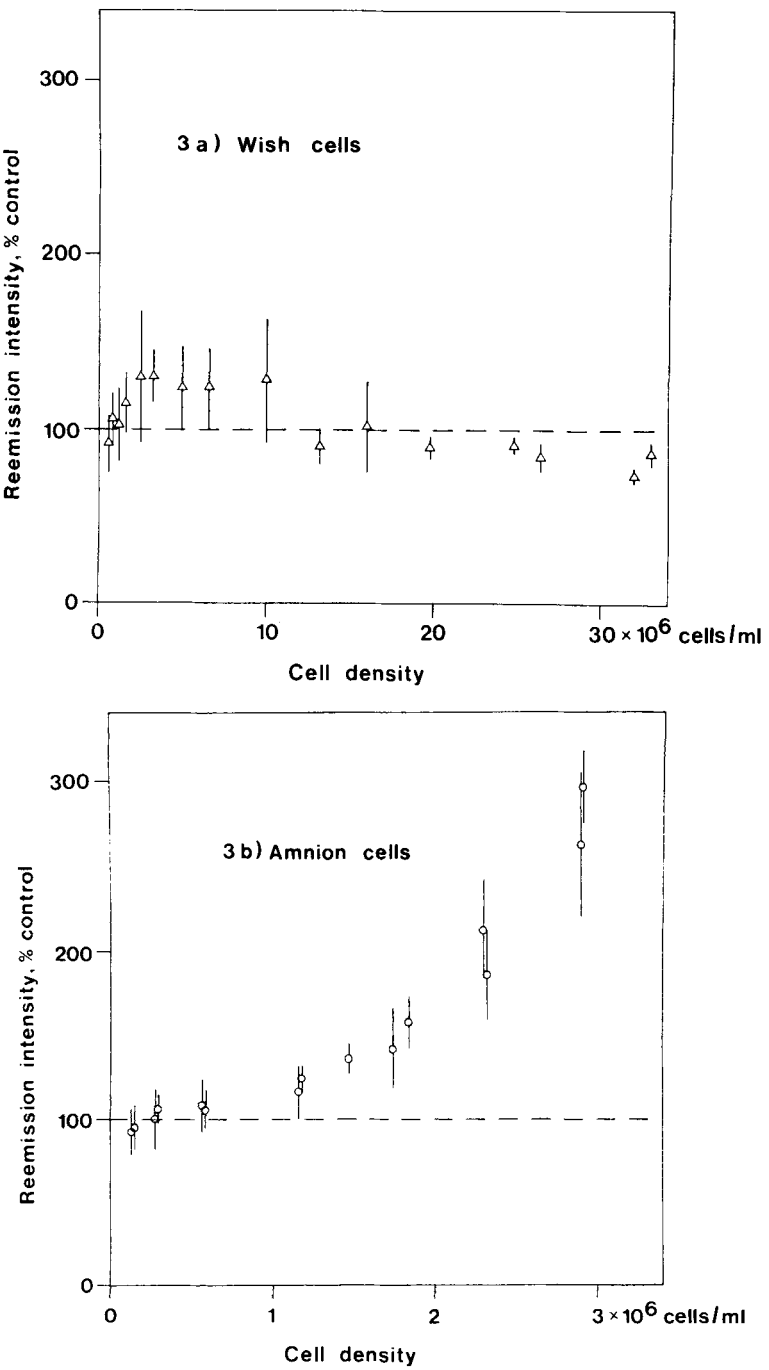

Fig. 3. Relative Reemission Intensity 49–55 s after irradiation as a function of cell density for Wish cells (3a) and amnion cells (3b). The plot gives the mean count rate with cells, registered within the time interval from 49–55 s and related to the control count rate with medium, which is set to 100%. The absolute control value for amnion (7 controls) is 231 ± 23 registered counts per second (cps), for Wish (6 controls) 261 ± 49 cps. Each plot is based on two independent cell dilution series, each point representing the mean of three determinations with standard deviation. Other conditions as in Fig. 1.

Plotting the decay constant vs cell density results in a steep decrease for amnion cells with increasing density.

Wish cells exhibit strikingly different behavior; they show a much less pronounced density dependence, whereby the k values tend to increase at higher densities. Correspondingly, the reemission intensity after about 50 s is equal to, or only slightly increased over, the control level.

What is the Mechanism of this Slow Photon Reemission?

One possibility could be the generation of a chemically reactive, short-lived intermediate, such as activated oxygen. It is known that flavins and flavoproteins are able, upon irradiation, to produce $O_2^{\bullet -}$ (*18*), which yields singlet oxygen ($^1O_2$) when dismutating spontaneously (*19*), whereas the superoxide dismutase (SOD, E.C. 1.15.1.1)-catalyzed dismutation reaction results in non-excited $^3O_2$.

Therefore, one would expect the intensity of the widely discussed singlet oxygen dimol emission (*4–6*) to be inversely related to the SOD-content of the cells studied.

This seems not to be the case here; for amnion cells, the integrated intensity that depends exponentially on k, exceeds that of Wish cells at all densities, despite the fact that tumor cells in general contain much lower SOD-levels than normal cells (*20*).

Besides that, the observed drastic increase of k with increasing amnion cell density is controversial to the constant SOD-content per cell, pointing to a different mechanism of emission, which seems to be sensitive to the intercellular distance.

An alternative interpretation of the results could be the generation of an electronically excited state with a long lifetime within the cellular structure, either as a triplet state (as discussed by Campa and Cilento, *21*) for photo-induced luminescence, or as exciplex formation within the homogeneous periodic structure of the DNA, as it has been postulated and discussed by Popp (*8,22,23*). Besides the above-mentioned apparent accordance of the observed hyperbolic kinetics with the theoretically deduced decay law, this model also predicts a higher photon storage capacity for normal than for tumor cells, in agreement with the results presented here.

For an unequivocal interpretation of the present phenomenon, further work has to clarify the mechanism of the reemission, particularly the nature and localization of the emitter and the role of oxygen.

If this phenomenon holds true for other cell systems, it offers a criterion for the distinction between normal and malignant cells; normal cells showing an increasingly slower decay with increasing cell density, whereas malignant cells (having eventually lost their capacity to store excitation energy in a cooperative way) exhibit a constant or even accelerated decay at higher cell densities.

Such a biophysical, non-invasive method would prove very useful in testing the effect of drugs on inducing or reducing malignant aberrations of cells.

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