Photon emission in tumor biology

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Abstract. Photon emission from mammalian cells has been subject of study for many years. Growing research activity is directed on the photon emission within the field of tumor biology. These studies, applying high-sensitivity photon counting methods, have paid attention to several aspects, including photon emission from serum of tumor-bearing animals, photon emission of tumors and of isolated tumor cells. In addition, research activity is increased with respect to the photon emission induced by white light from cultured tumor cells. In this review we report on the different aspects of spontaneous and induced photon emission of tumor cells as compared to normal cells. Throughout these studies the question of a functional biological role of this spontaneous and light-induced photon emission has been raised and some different points of view will be discussed.
Key words. Biophoton review; photobiology; spontaneous chemiluminescence; photon reemission; mammalian cells; tumor cells; tumor tissue.

Introduction

In the studies of the mechanisms underlying tumor development, a variety of biochemical and biophysical techniques have been applied in the past. More recently, growing research activity in the field of photon emission is directed on carcinogenesis. This increased activity reflects the recognition that luminescence is emitted by all biological systems and associated with fundamental physiological processes. The modern technology of the super-high sensitivity photon counting methods allows the detection and spectral analysis of extra-weak bioluminescence and chemiluminescence from living cells and tissues. A number of studies, applying this modern technology, have paid attention to the photon emission characteristics of tumor-bearing animals, of tumors and of isolated tumor cells. In addition, research activity is increased with respect to the development of biochemical and biophysical models in order to describe the creation by organisms of electronically excited states and photons, the link between them and physiological processes, and the evaluation of their possible informative role.

In this paper we make an attempt to present the results of studies of extremely weak bioluminescence and chemiluminescence in the field of oncology. We will consider primarily some studies on the photon emission from blood samples of animals which develop tumors, and then the studies including malignant tumors and isolated tumor cells. Furthermore, we will consider studies on the light-induced photon emission of isolated tumor cells.

Photon emission (PE) from blood serum during carcinogenesis

Luminescence, originating from living systems was proposed at the beginning of this century by G. Gurwitsch to explain some experimental observations with respect to growth regulation [19]. Research on weak spontaneous photon emission from biological systems has been continued in the countries of the GUS and in Eastern European countries. In several recent meetings a number of contributions have given an overview of this work [50, 67]. A general hypothesis underlying most of this recent work has connected the emitted radiation to biochemical processes as well as to the physiological state. According to this hypothesis the intensity of the biochemiluminescence correlates to the severity of a number of pathologies.

The origin for the chemiluminescence of tissues and body liquids is the non-enzymatic oxidation of complex organic compounds, primarily lipids, by free radicals. Thus, peroxide oxidation of lipids leads to peroxide radicals. The latter compounds, by recombining, release an energy sufficient to emit a quantum in the visible part of the spectrum. This spontaneous chemiluminescence of liquids is a continuous and very weak emission in the near-ultraviolet, visible and near-infrared parts of the spectrum. It has, according to the current hypothesis, a metabolic origin.

The relation between chemiluminescence, stress and pathological conditions can, in a simple form, be presented in four steps: Under conditions of stress, blood is saturated with oxygen and non-saturated fatty acids in order to provide energy to cope with stress conditions. Depending on the coping strategy, energetic substrates are not used for some time and remain present at increased levels. This increases the probability of direct contact between oxygen and substrates that leads to increased activation of free-radical oxidation. Free radicals, aldehydes and other compounds could be formed by toxins affecting membranes, creating a prepathological condition.

In order to survive an organism has a complex of devices, including a whole set of antioxidant systems, to protect against oxygen toxicity. There is no doubt that the state of pro- and antioxidant balance is of vital importance. This state has been the subject of research that was aimed at judging the pro- and antioxidant equilibrium in the host, its liquids and cells, in different pathologies. For this reason it was suggested that a technique which allows an integral, simple and rapid evaluation of the equilibri-

um state can be based on the analysis of chemiluminescence intensity in biologic liquids, like blood serum.

The state of chemiluminescence research in the field of oncology has been previously discussed by Baraboy [2] and we consider some findings with respect to animals in the early stages of carcinogenesis, animals with growing transplantable tumors, and human clinical trials.

a) *Early stages of carcinogenesis.* Following injection of nitrosodiethylamine to rats, a persistent enhancement in photon emission of blood serum was observed. But different carcinogens exert a contrary effect. Injection of 3,4-a-benzpyrene and of DMBA resulted in decreased serum photon emission.

b) *Animals with transplanted tumors.* In the initial 1–2 days of growth after transplantation of a tumor increased serum photon emission values were usually observed. The magnitude of the increase depended on the duration of tumor growth and type of tumor. Serum photon emission from animals with experimentally transferred tumors revealed that in Walker carcinosarcoma, Crocker sarcoma, sarcoma 45 and some other tumors, the intensity of photon emission drops below the threshold level. In contrast, transplanted Guerin's carcinoma, Pliss' lymphosarcoma, and Shwets' erythromyelosis during growth cause increased intensity of serum photon emission.

c) *Clinical trials.* Clinical trials of serum photon emission of cancer patients revealed increased values in the majority of examined cases. This is characteristic of patients with carcinoma of the oesophagus, stomach, colon, squamous cell carcinoma of the lung, breast and cervix. However, the clinical observations did not permit an unequivocal conclusion about increased serum photon emission values as typical for neoplasia. It had been shown that the presence of pulmonary adenocarcinoma in patients is normally associated with a reduction of serum emission values.

The aim of this research has been to devise a laboratory test, in which serum photon emission intensities would give an indication of the conditions of the patient suffering from specific malignancies. As the data seem contradictory, further investigation in this field is needed. It must also be taken into account that an increase of PE intensity occurs in inflammatory diseases, intoxication, radiation lesions and cardiovascular pathology [48, 64, 67].

Photon emission from normal and cancerous human tissues

The question can be raised whether tumor tissues are characterized, like blood serum, by a different PE intensity as compared to the neighboring tissue. Recently, photon emission in the visible and near ultraviolet range by samples of human tissue have been recorded [18]. Samples were human tissues coming from surgical operations. As soon as they had been taken from the living body, the samples were put into a dark container and maintained at 37 °C, immersed in Ringer's solution to allow metabolic processes to be maintained. They were measured 30–100 min after their removal. The authors concluded that all the normal, non-tumor, samples emitted light with negligible intensity, whereas most of the tumor samples emitted light with a much higher intensity. The samples belonging to the sub-set of tumor tissues have a much wider distribution of PE values (300 ± 90 photons/cm^2 min) than the normal tissues (22 ± 6 photons/cm^2 min). Their data further show that some tumor samples have a low PE value which cannot be distinguished, within the limits of statistical error, from that of normal tissues. In 3 cases malignant tissue could be compared with normal tissue. One of these cases showed large differences between normal and malignant tissue.

It is interesting that studies have reported the wide distribution of photon emission values of tumors [18] and that of blood serum [2]. In this respect a wider experimental basis is required, including parallel measurements of photon emission of tumors and serum of the same patient to validate photon emission as a simple, non-destructive analytical tool in tumor diagnosis. The question of whether photon emission from tumors is caused by tumor cells or by other cells present in the tumor has not been directly answered. Some evidence, however, indicates that non-tumor cells may be involved in tumor photon emission. In tumors a diversity of cells is present and with respect to photon emission special attention must be paid to the population of phagocytes. Once they leave the bloodstream, monocytes mature into macrophages, which together with neutrophils, also called polymorphonuclear leucocytes, are the main professional phagocytes in the body. Activated phagocytes reduce molecular oxygen to superoxide via NADPH-oxidase, a process called respiratory burst. Superoxide radicals form hydrogen peroxide which then serves as a substrate for myeloperoxidase reaction generating a variety of highly toxic metabolites. These processes produce electronically excited states which by relaxing to the ground state emit photons.

The data from several experimental tumor models have demonstrated that high-growth and low-growth fractions exist in tumors depending on the site. Moreover, the continuous increase of tumor size causes local conditions of decreased vascularization, nutrient deprivation, tumor cell death and necrosis. From these points of view it is likely to suggest that phagocytes, which can populate tumors to a various extent depending on the (local) conditions of the tumor are, at least in part, responsible for the photon emission of solid tumors. However, experimental data are not available, although in a few studies it has been shown that monocytes showed increased photon emission activity in solid tumor patients [8, 9].

It must be considered that not (only) the number of phagocytes but their structural environment is responsible for the photon emission intensity from the tumors. The structure of the extracellular matrix is mainly deter-

Table 1. Effect of collagen on luminescence of human PMN leucocytes[a]

Collagen concentration (µg/ml)	Lagtime (min)	Increase per 10 min	Time of maximal luminescence (min)	Maximum luminescence
0	14	20×10^5	35	27×10^5
50	12	34×10^5	25	37×10^5
100	9	23×10^5	25	27×10^5
200	3	4.5×10^5	40	10×10^4

[a] PMN leucocytes (5×10^5) were incubated in a liquid medium or in a collagen gel. At the start of incubation at 37 °C, PMA was added. The increase in the rate of luminescence was determined during a 10-min period after the lag period. At high (200 µg/ml) collagen concentration the time of maximal luminescence cannot be determined since maximal luminescence occurs during a long period of time.

mined by fibroblasts. This cell type is a producer of collagen[16,35] and glycosaminoglycan[20,42] and is able to restructure the cellular environment by contracting collagen[1,3,4,51,52]. We demonstrated earlier that when polymorphonuclear leucocytes were incubated in a matrix of collagen, the photon luminescence was strongly influenced by the collagen concentration[57]. Photon emission of leucocytes and macrophages has been measured both by directly applying photomultipliers and by the use of various amplifiers, for instance luminol[26]. In order to demonstrate the influence of collagen packing on phagocytic activity of polymorphonuclear leucocytes, the luminescence of these cells, incubated in nutrient-rich medium plus luminol, was investigated in relation to collagen concentration[61]. For the preparation of collagen matrices mixtures for gels containing up to 0.2 mg collagen per ml and leucocytes were prepared and polymerized. The cells were activated by phorbol-myristate-acetate. As shown in table 1 an increasing collagen concentration causes the onset of the response to advance. The lowest collagen concentration causes an acceleration and amplification of the peak response. Higher concentrations retard and diminish this phase. These type of data show that leucocyte activation is strongly activated by collagen packing. The packing of collagen is mainly regulated by fibroblasts. The contracting forces exerted by fibroblasts depend on the fibroblast density and the collagen concentration[51,52,61]. In tumor tissue the structure of the extracellular matrix shows a dynamic behavior. Various enzymes, among them collagenases and enzymes which metabolize glycosaminoglycans, are produced by tumor cells and modulate the matrix continuously[27,33].

Photon emission from isolated normal and tumor cells

A second approach to study the origin of photon emission from tumors is the analysis of photon emission of isolated tumor cells. The liver has been subject of extensive research with respect to photon emission. Emission of weak radiation in the visible range by the liver has been described by several authors[6,7,49]. This emission has been registered both in situ and from isolated livers. The phenomenon of photon emission was still present after isolation of individual hepatocytes, amounting to approximately 30 photons/s per 10^6 cells[10]. In the past decades increasing emphasis has been placed on comparative biochemical and biophysical studies of liver and hepatoma cells[44,54,55,62,63,65,66]. In recent years, we have investigated spontaneous photon emission from suspensions of rat hepatocytes and of cells of the hepatoma cell lines Reuber H35 and HTC. Rat hepatocytes exhibit photon emission but that of hepatoma cell lines was not detectable[59,60]. Several attempts have been made to detect emission from suspensions of the two cell lines. These attempts were unsuccessful, although a) the absorbance studies have shown that self-absorbance of light by the HTC and Reuber H35 cells was less than that by hepatocytes, and b) the cell densities of the tested suspensions ranged from 10^5 to 10^7 per ml. The altered photon emission may be part of the general shift in the production of superoxide radicals with increasing malignancy[17]. From these data it is not unlikely to propose that tumor cells can be considered as a protective-adaptive cell state protecting the body at that site against activation of free radical oxidation and injury.

Light-induced photon emission (IPE) studies: different concepts

The long decay time of the light-induced photon emission from living systems was first described by Popp and co-workers for plant material, including cucumber and *Bryophyllum*[37]. In these studies decay of the light emission after illumination lasted up to 1 h. Similar decay times of the IPE were observed for the microsporocytes and for the intact male inflorescences of larch[12,13]. In the latter studies the authors paid considerable attention to variations of IPE from cells in different stages of development. They observed that IPE from cells undergoing meiosis were considerably different from those observed for the whole inflorescences. The investigations of the activity level of DNase and RNase and of the intensity of IPE from microsporocytes of larch during development[14] revealed the close positive correlation between changes in the DNase activity and of the intensity of the IPE. These results have been considered as support for the concept that DNA, possibly in the form of chromatin, is one of the sources of biophotons. This concept is strongly promoted by Popp and co-workers. They offered a biophysical model as a basis for understanding the photon response from light (stress)-perturbed biosystems. This model has been called the 'photon storage hypothesis'. In their model a regulatory system is illustrated by a resonator cavity. The response of the resonator to a stressor depends on several factors and may be quantified by the value of the 'quality factor' (Q). A stable resonant system has a high Q-value. Such system loses only a very small amount of its energy E. This

feature agrees well with a low intensity luminescence from intact (non-perturbed) organisms. When a bioregulatory system is stressed it will give a larger response to the stimulus, reflecting the partially disintegrated system with a diminished Q-value due to the malfunction of the negative feedback [32, 37]. The level of the IPE intensity can be considered an indicator of the sensitivity of a cell to the regulating action of biophotons. According to these authors the source of biophotons is the genetic apparatus of the cell. For this reason this model has been also indicated as the 'genetic information concept'. Rattemeyer et al.[41] observed a close correlation between changes in the degree of condensation of the cellular DNA and the spontaneous photon emission activity of cucumber seedlings treated with ethidium bromide. In other experiments using suspension cultures of soya cells photon emission was also changed by ethidium bromide: photon emission increased after exposure to ethidium bromide at concentrations up to 30 µg per ml, and decreased again when the cells were exposed to higher concentrations up to 3 mg per ml [38]. However, in the latter experiment data are not available with respect to the effect of these concentrations of ethidium bromide on DNA structure. No biochemical fractionation of these cells could prove that DNA is the sole source of binding of ethidium bromide or to what extent other molecular principles might be responsible for the observed differences. Another argument which has sometimes been used in favor of the 'genetic information concept' was the observation that photon emission could not be detected in measurements using mature red cells of blood, in which the nuclear apparatus is missing. The validity of this argument, however, is doubtful because several other studies including mammalian cells with intact nucleus report that photon emission could not be detected (this review). In this respect it is more likely to repeat these experiments using enucleated plant cells.

In addition to the experimental data in favor of the 'genetic information concept' this group of researchers added theoretical indications [24, 25, 39] that point to biopolymers, in particular to the exciplexes of DNA, as essential source of a coherent electromagnetic field within living tissues. They compared a number of expected properties of a chaotic (spontaneous) luminescence field versus an ideal coherent (regulatory) field [40]. A number of experimental results are in favor of the coherent field hypothesis.

Besides the previously mentioned experimental results the decay behavior as well as the transparency of the tissue for photons was considered to be of decisive importance.

Under ergodic conditions hyperbolic decay is a necessary and sufficient condition for coherent scattering. And instead of an exponential decay, living cell populations exhibit a hyperbolic relaxation of photon intensity after exposure to white-light illumination [13, 37, 40]. The investigations of transparency of the tissue for photons, originating from the interior part of the system became also an important step in searching for the chaotic or coherent character of luminescence. Measurements of transparency of disperse media as well as of cell layers have yielded evidence that photon emission itself induces an extinction coefficient that is at least one order of magnitude lower than that for comparable light [38].

The mechanism behind the phenomenon of IPE and its biological significance are discussed quite controversially. In principle the 'chemiluminescence concept' which is based on the biochemical model including oxidative radical reactions in biomembranes, usually associated with lipid peroxidation might offer an explanation for IPE too. According to this model, in a perturbed regulatory system a small stress, as white light irradiation, can initiate a large perturbation. This can cause an increase in endogenous free radical production, possibly overwhelming the various anti-oxidant defences.

Although for photon emission of plant tissue many experimental data confirm the chemiluminescence concept, it is not excluded that part of photon emission can be ascribed to other sources and is linked to IPE. In order to discriminate between the different concepts several characteristics of IPE, including spectral analysis, decay characteristics, transparency and cellular origin need to be studied. It is not the purpose of this paper to evaluate these data from plants. In this period of time the first measurement of IPE of mammalian tumor cells were found. They will be the subject of this paper.

Induced photon emission (IPE) from mammalian cells

Several studies have demonstrated that mammalian cells illuminated with white light exhibit a short lived photo-induced luminescence. The first mammalian system was studied by Schamhart and van Wijk [45, 56] and consisted of normal, isolated rat hepatocytes and the hepatoma-derived cell lines, Reuber H35 and HTC, with different degree of differentiation. The second system was studied by Scholz et al.[47] who compared normal human amnion cells and transformed Wish cells derived from the same parental tissue. The third mammalian cell type was the Cloudman S91 melanoma cells, studied by Niggli [34].

As proposed previously one of the hypothetical mechanisms to explain this short lived photo-induced luminescence, is the connection with photooxidation/reduction processes of endogenous cell components such as e.g. flavins. Some studies with mammalian cells have presented evidence that mitochondria are targets for light irradiation [43]. For rat liver mitochondria irradiation was found to cause both an increase in the electrochemical proton gradient and ATP extra-synthesis [36]. Further along this line of thinking it can be suggested that under photo-oxygenation conditions, photoreduced flavin undergoes a series of reactions in which oxygen free radicals such as singlet oxygen, hydroxyl, perhydroxyl and superoxide-anion may be formed, or alternatively, singlet oxy-

gen may be formed from the reaction of superoxide-anion and hydrogen peroxide[15,28,31]. Unfortunately further knowledge on light-harvesting principles and coupled acceptor molecules in mammalian cells is limited.

The second hypothetical mechanism, in analogy to the plant studies, is that IPE arises from DNA or the chromatin according to the photon storage model.

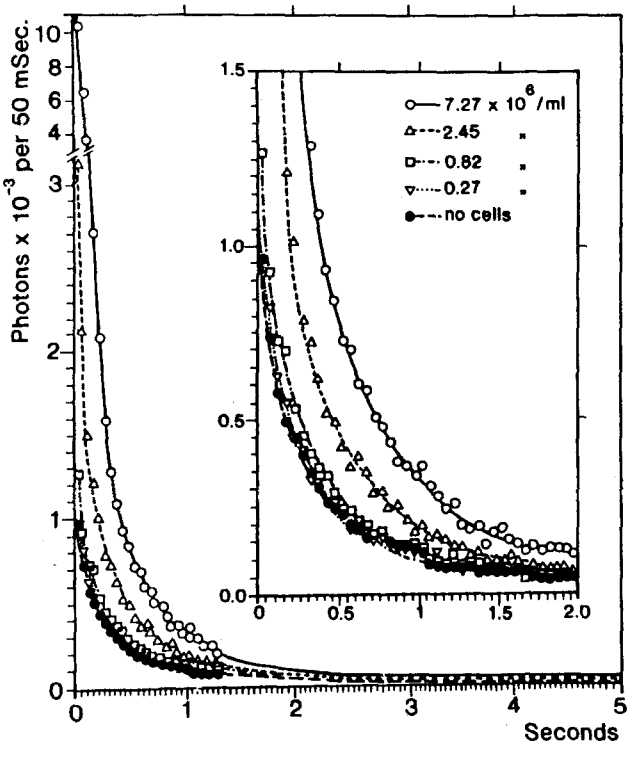
The results of the studies on mammalian IPE are comparable to some extent and will be discussed in order to throw light on the validity of the two models for mammalian IPE. The results of these studies include the duration of IPE, the re-emission relaxation dynamics, the light-transmission characteristics, cell fractionation studies with respect to possible source(s) of IPE, and spectral analysis studies.

a) *Duration of IPE*

The decay of the light emission after illumination was of the order of a few seconds (fig. 1 A). A complicating factor is the observation that a low level of induced photon emission was also detected after illumination of an empty cuvette or a cuvette with medium but without cells. It is suggested that quartz glass is responsible for a background IPE and this must be taken into consideration in order to calculate cell specific IPE. However, when very careful measurements were performed, including at least 10-fold measurements the decay can be detected for about 30 s and thereafter it was not distinguished from the initial value without pre-illumination[58].

b) *Reemission relaxation kinetics*

Following the procedure of a 10-fold measurement which eliminates disturbances caused by irregularities in the dark count rate, the cell-specific IPE could be followed into the last part ('tail') of the decay curve up to 30 s. For HTC cell populations the decay kinetics were determined by determining the maximal amount of photons emitted after illumination (from the start of the measurement) as well as the fraction that has been produced at each time or the fraction that was still not emitted yet[58]. The analysis of the decrease of the latter fraction in time shows that the tail part of the curve is a straight line in the logarithmic plot of fraction versus time (fig. 2). The decay occurs with a half time of approximately 3–4 s in the period of 5–25 s after the start of the measurement (the example is shown for 4–15 s). To study earlier kinetics the contribution of the 3–4 s decay half time has been eliminated from the earlier data points. An example of the cumulative data curves for a period of about 1.5 s for HTC cell populations with various cell densities is shown



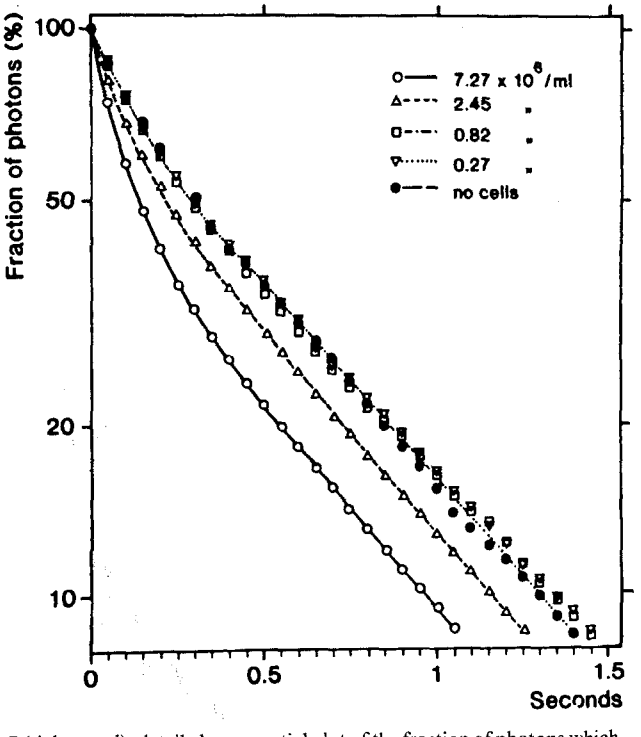


Figure 1. Effect of cell concentration of light-induced photon emission of HTC cells. HTC cell populations were incubated at densities (cells/ml) of (○) 7.27 × 10^6; (Δ) 2.45 × 10^6; (□) 0.82 × 10^6; (▽) 0.27 × 10^6; (●) no cells. *A* (left panel): photons per 50 ms were determined at various times after the start of the measurement. *B* (right panel): detailed exponential plot of the fraction of photons which were not emitted yet at the time of measurement as specified on the X-axis.

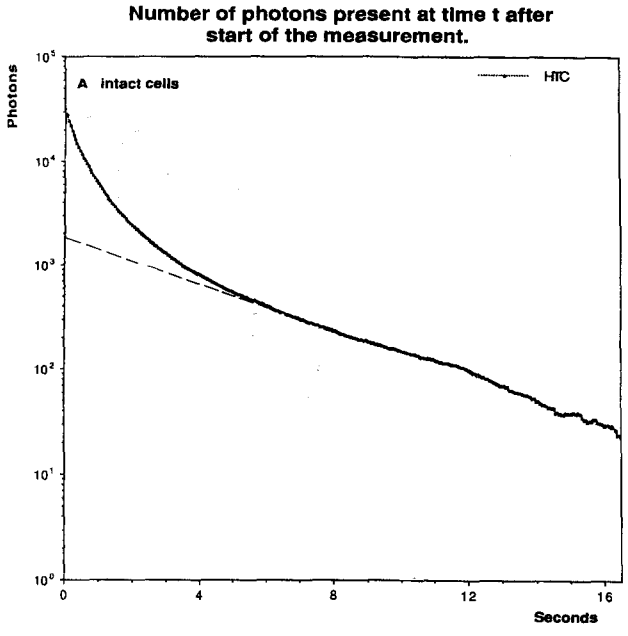


Figure 2. Exponential plot of the number of photons which were not yet emitted from a HTC cell population at the times of measurement as specified on the X-axis.

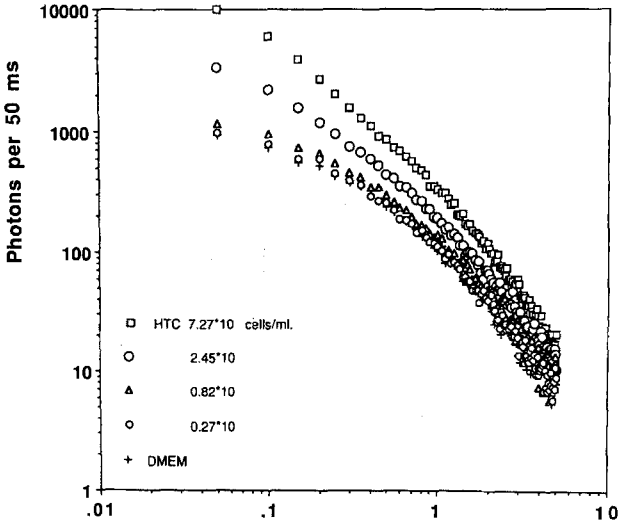


Figure 3. Decay kinetics of light-induced photon emission from HTC cells approximated by hyperbolic decay law. The early part (0.5 s) is cell density dependent. Cell densities were: (□) 7.27×10^6; (○) 2.45×10^6; (△) 0.82×10^6; (●) 0.27×10^6; (+) no cells.

in figure 1 B. At all densities, including the conditions that the cuvette with medium but without cells was irradiated, the second part of the curves showed a straight line, indicating a half life time of about 300 ms in the period of 500–2000 ms after the start of the measurements.

Before that time, i.e. in a period of about 500 ms after the start of the measurement, the decay is even more rapid and it is dependent on the cell density [58].

In a few studies the differences in decay kinetics were expressed in terms of the initial rate [34, 56]. The initial rate of decay of photon emission as measured during an early interval directly after the start of the measurement can be of some value, but a correction for non-cell-specific IPE had to be made.

In order to develop a better measure attention was paid to the observation that for a given cell density the rate of decay is not constant. It corresponds to the observation that the early part of the decay curve, the part which shows cell density dependence, cannot be described by a single exponential decay law (fig. 3). A better description of the time course is given by a hyperbolic decay law [47, 59]. Again this part can be obtained from the original data only by subtracting the data obtained with cuvette and medium but without cells. It is evident that when photon emission is small as compared to the cuvette value the interpretation of the data – without any correction – is dominated by the two constant decay half times which are cell density independent.

Following correction for cell independent decay, the best fitting of the hyperbolic decay according to

$$i(0,t) = \alpha \cdot t^{-k}$$

is determined and the values α (amplitude factor) and $-k$ (decay constant) are then determined. This procedure has been applied – without correction however – by Scholz et al. [47].

c) *Cell type-dependence of IPE*

In the few comparative studies on IPE of a cultured tumor cell type and its normal counterpart, characteristic differences were observed between the normal and the transformed cell [45, 47, 56]. The lowest value was found for the normal cell and the highest value for the tumor cell. However, the quantitative data of these studies cannot be compared, since conditions for measurements were different in these investigations. In this context, in a preliminary study of van Wijk, van Aken, Wei-Ping and Popp it was decided to draw up an inventory of mammalian cell types present in the laboratories with respect to their IPE activity. The IPE was detected in all mammalian cells tested including cat, Chinese hamster, cow, dog, human, monkey, mouse and rat cells.

The intensity of this emission is dependent on cell type. In general lowest IPE values ranging between 4 and 8 photons per 10^4 cells were found for normal, non-mesodermal, cells. The IPE values for tumor cells ranged between 7 and 36 photons per 10^4 cells. Relative high IPE values, between 30 and 100 photons per 10^4 cells were found in suspensions of fibroblasts.

d) *Light transmission characteristics*

Total photon emission by a cell population of different densities has been used to study the relation of measured photon emission, cell number, and absorbance. This has been studied for HTC cells (fig. 4). By expressing the total number of photon counts emitted in 5 s as a function of cell density (ranging from $0.05 - 8 \times 10^6$ cells/ml) HTC cells displayed an increasing emission with increas-

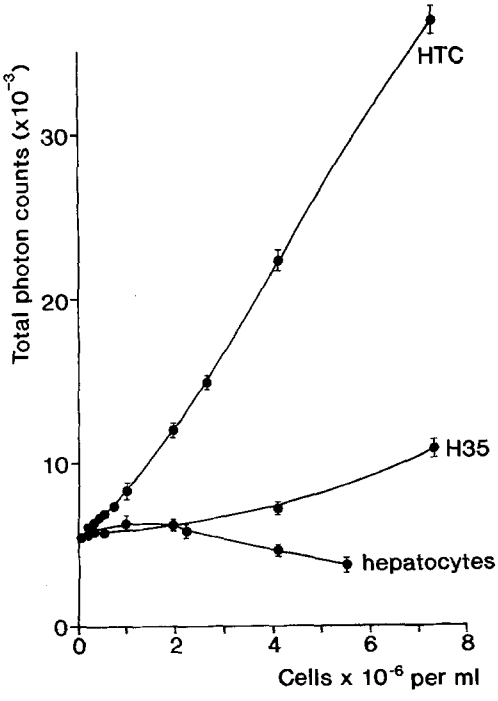


Figure 4. Cell density dependence of total emitted photons of suspensions of HTC cells, Reuber H35 cells and isolated hepatocytes.

ing cell density[56, 59]. The transmittance at cell densities of $2 - 3 \times 10^6$ cells/ml is reduced to 10% and less[59]. Any correction of cell-dependent IPE for absorbance by the suspension will cause a dramatic non-linear increase at the higher cell densities.

A similar conclusion can be derived from the biophoton reemission of Cloudman S91 mouse melanoma cells after pre-illumination with white light at concentration of 1 and 2×10^8 [34].

A completely different example of the relation between cell density and IPE is that of a population of normal rat liver cells (fig. 4). In this case at higher cell densities the IPE was lower than that of the cuvette and reached about 70% of that value[60]. These data demonstrate that a simple correction as with HTC cells cannot be made since cells can interfere with cuvette-dependent emission, too, in a way that is cell density dependent. Especially this will influence the interpretation of data from cells with low IPE value compared to the cuvette IPE.

These data show that in the case of IPE in suspensions of hepatocytes and hepatoma cells a consistent explanation in terms of delayed luminescence of these cells meets several difficulties. Especially with respect to the application of the average extinction coefficient of hepatoma cells the self-absorbance characteristics of the delayed luminescence could not be explained. The photon emission of HTC cells increases although transmission is reduced to less than 1%.

Comparing the two models, the phenomenon of decreased self-absorbance can be explained, according to Li and Popp[24, 25, 38–40] by assuming a coherent character of IPE. As outlined above, the hyperbolic instead of the exponential decay kinetics are also in agreement with the 'photon storage model'. On the other hand Kochel[23] presented evidence that the hyperbolic decay kinetics is not in contrast to the biochemical model with oxidative radical reactions.

For further discrimination between the models the origin of mammalian cell IPE is important.

e) *Source of mammalian IPE*

With respect to the nature of the molecular principle of IPE, several approaches have been used, including extracellular changes and cell fractionation studies.

A very impressive set of data has been presented by Niggli on the influence of cholera-toxin and of forskolin on Cloudman S91 mouse melanoma cells[34]. Both substances induce melanin synthesis via the cyclic AMP pathway. When these cells were light irradiated the reemission relaxation dynamics is no longer observed. In such treated melanoma cells biophoton reemission was not different from that of the cuvette without cells.

At first sight these results suggest that IPE is related to the altered membrane characteristics, but other data indicate that not the plasma membrane is involved. These data came from studies on disrupted and fractionated HTC cells[58–60] and Cloudman S91 cells[34]. Several methods for cell disruption were applied.

When HTC cells were disrupted by the addition of sodium dodecylsulphate to a final concentration of 0.5% to the medium it caused a decreased initial value of IPE decay curve[58]. In fact, this treatment leads to a decreased total amount of photon emission by the elimination of the fraction with the fast and variable half life value (fig. 5). When HTC cells were disrupted by a Dounce homogenizer we found remarkably similar IPE intensities of broken cells as compared to intact cells, indicating that the principle or process leading to IPE requires no intact cells. The IPE of cell fractions were tested, showing that the cytoplasmic fraction, or the fractions containing mitochondria and subribosomal particles have a very low activity, less than 5% of that of the nuclear fraction. In contrast a very efficient reemission of biophotons was observed of the nuclei of mouse melanoma cells[34] and of hepatoma cells[35, 36]. The further fractionation of the nuclei shows that intactness of nuclei is not required for IPE (table 2). The isolated chromatin-containing fraction has IPE values that are even higher than IPE values of nuclei. However, phenol-extracted DNA did not produce any IPE[59, 60].

The observation that pure DNA has no reemission capacity points to an essential importance of whole chromatin – possibly for conformational reasons. In the two models this set of data is in favor of the biophysical model which proposes DNA or chromatin as a source of biophotons. In addition, several other data with respect to the source of IPE are not in favor of oxidative radical reactions.

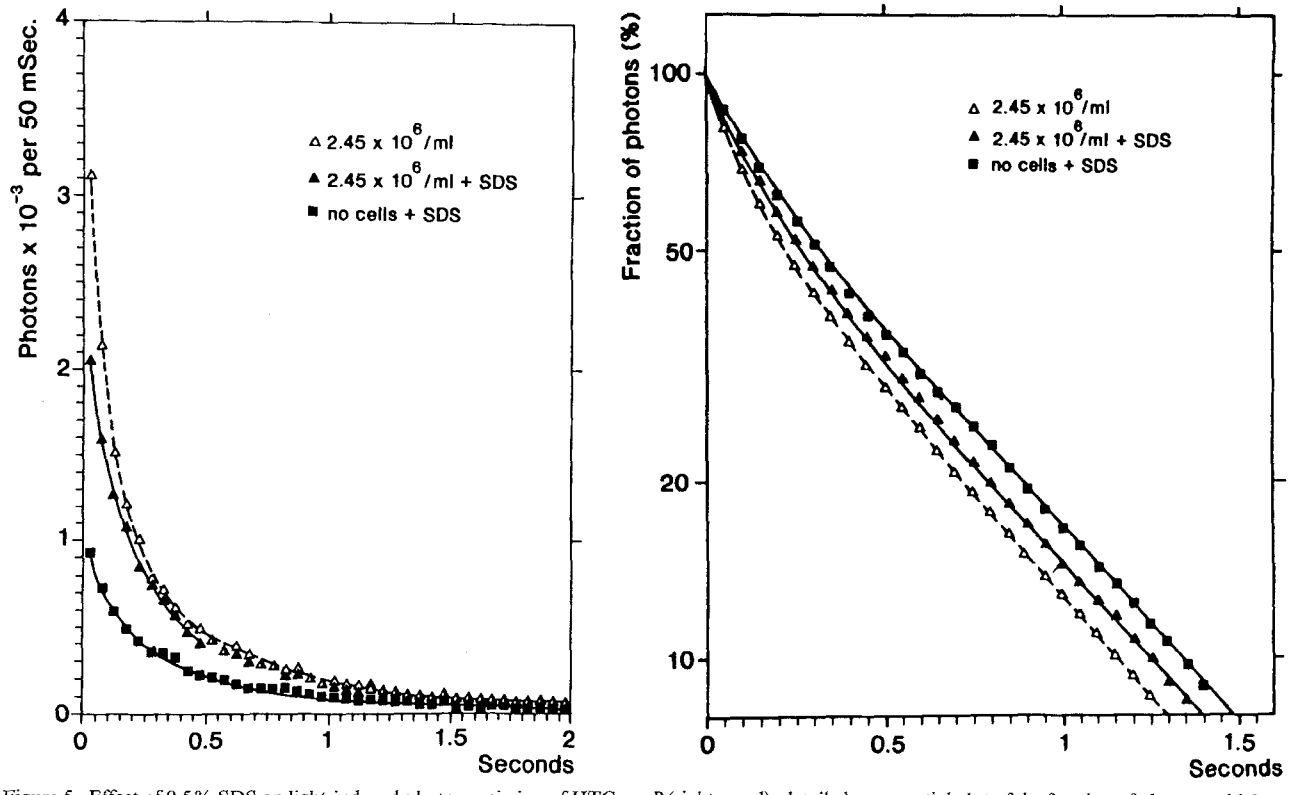


Figure 5. Effect of 0.5% SDS on light-induced photon emission of HTC cells (cell density 2.45 × 10^6 cells/ml). (Δ) cells; (▲) cells plus SDS; (■) medium with and without SDS.
A (left panel): photons per 50 ms were determined at various times after the start of the measurement.
B (right panel): detailed exponential plot of the fraction of photons which were not emitted yet at the time of measurement as specified on the X-axis.

Table 2. Light-induced photon emission of HTC cells and cellular fractions[a]

	Total photon counts in 0.5 s corresponding with 10^6 cells	Transmission at 500 nm, %
Whole cells	4053 ± 770	18
Cytoplasmic fraction	1736 ± 581	87
Nuclear fraction	5670 ± 1463	36
Chromatin fraction	13230 ± 3206	44
DNA (phenol-extracted)	388 ± 72	96

[a] Cell fractions were measured at amounts that correspond with the original cell number. Values (total photon counts in 0.5 s per 10^6 cells) were obtained in 5 different experiments and are given for the mean ± SD after subtracting the background values of cuvette with buffer. Measured IPE was not corrected for absorption of light by the cell suspension. Transmission at 500 nm was determined spectrophotometrically.

In studies with HTC cells the oxygen dependency of IPE was measured. Although the cuvette containing the cell suspension was flushed with argon for at least 15 min prior to the start of the measurements and the culture was kept under these conditions for 1 h the IPE values were not decreased. Furthermore, reoxygenation of the hypoxic cells by flushing these cultures with 95% oxygen/5% CO_2 did not increase the IPE values, indicating that IPE does not require any oxygen-dependent metabolism.

The characteristics of IPE of HTC hepatoma cells and Cloudman S91 melanoma cells can be explained by the 'photon storage model' including chromatin-dependent emission of light with a coherent character. As we have discussed before, the hyperbolic instead of the exponential decay kinetics is in favor of coherence. It might also (at least in part) explain the increased transparency for IPE as compared to artificial light.

The data are not in favor of the flavin concept. This model cannot explain the increase of transmittance of light, neither does it fit with respect to the lack of IPE in the mitochondrial fraction and its occurrence in the nuclear fraction. The independence of oxygen is also not an argument for this explanation.

Although the data are in favor of the 'photon storage model' the question can be raised whether other possible explanations for mammalian IPE can be presented. At present we found one other type of explanation, supposing that the cell-dependent IPE of mammalian cell populations is in fact emitted by the quartz cuvette. Thus, emission of the quartz cuvette might be increased in suspensions of HTC and H35 cells under conditions in which transparency for white light approaches zero. Furthermore in hepatocytes, it can be assumed that these cells show less stimulation of cuvette IPE, leading to a small cell density dependent increase of IPE of the cu-

vette quartz emission values, but at higher densities the absorbance increased and can repress the IPE derived from the quartz parts of the backside of the cuvette. This explanation focusses the attention to the quartz dependent photon reemission. Recently Black and Rogers[5] drew attention to the luminescence of cells in quartz glass and plastic containers. They warned that this effect could have profound influence upon both photochemical and photobiological reactions, perhaps resulting in specious interpretations. The observation of luminescence requires the stimulation of trapped charge carriers. The charge carrier stimulation can be achieved either via thermal energy (thermoluminescence) or via the absorption of optical energy. Often the illumination is observed to induce glow peaks[29,30]. This can be interpreted as the release of charge from the traps. Photonically-ejected charge carriers (electrons or holes) from traps are redistributed and captured in other traps. The transfer efficiency is very much dependent on the temperature of the sample during illumination. The nature of this latter dependence indicates that the charge release from the residual traps occurs as a result of the combined effect of excitation by photons and thermal stimulation. The observation of phototransfer in quartz has been done by Schlesinger[46] and a great effort has been made to determine the precise nature of the phototransfer mechanism[11,21,22,53].

A spectral analysis of the IPE of the cuvette (plus medium) and that including the cell suspension should elucidate the spectral characteristics of the cell-specific IPE, and indicate its difference with the quartz IPE spectrum. Although in all papers on mammalian IPE the quartz IPE has been mentioned and a few data have also been published on spectral analysis of HTC cells[59,60], no comparison has yet been made between cell-specific and cuvette values.

The spectral analysis of induced photon emission was analyzed by interposing glass absorption filters between the cuvette and the photocathode. The filters were characterized by a maximal transmission at 360 nm (BG3), 500 nm (BG39) or a cut-off at 610 (RG610). IPE of the cuvette as well as that of the cuvette plus cells was maximal using the filter with maximal transmission at 500 nm. In the three parts of the spectrum the cell suspension caused an emission increase of a factor 3.4 as compared to the emission of the cuvette.

In fact these data on spectral analysis did not reveal any characteristic differences between cell-specific IPE and cuvette IPE. However, they are no evidence for the 'cuvette hypotheses' as long as no detailed analysis has been performed.

Conclusion

The main purpose of this review was to present the data on photon emission with respect to malignancy. As we have seen, the general picture is fragmentary. Nevertheless, the data point to a large variability of photon emission values between tumors as well as between the serum values of the tumor-bearing hosts. The limited data suggest that the host-response to the tumor is responsible for the photon emission of tumors. No evidence was found for an increased photon emission of the tumor cells themselves. The spontaneous photon emission is explained by multiple enzymatic and non-enzymatic reactions. These data led to the speculation that tumor cells are a protective-adaptive state protecting the body at that site against activation of free radical oxidation and injury. Such an inhibition might lead to a decrease of the cytotoxic products inhibitory for cell growth, which creates conditions for rapid growth of tumor cells. On the other hand the uncontrolled growth causes a number of reactions of the tumor-bearing host as seen by the increased photon emission of the host' serum and that of monocytes.

Photo-induction of photon emission was evident from tumor cells. The studies on IPE of tumor cells have demonstrated that this type of photon emission is not easily explained by the 'oxygen radical concept'. Another concept, that of 'genetic photon storage', is able to explain more of the characteristics of IPE. From the data presented in this review it can be stated that for an unequivocal interpretation of IPE further clarification of the mechanism is necessary. The IPE shows a distinction between normal and malignant cells. For this reason this phenomenon deserves further attention and connection with the biochemical data of normal and malignant cells.

1 Allen, T. D., and Schor, S. L., The contraction of collagen matrices by dermal fibroblasts. J. Ultrastructure Res. *83* (1983) 205–219.

2 Baraboy, V. A., Chemiluminescence in oncology, in: Biological Luminescence, pp. 470–491. Ed. B. Jezowska-Trzebiatowska. World Scientific Publishing, Singapore/New Jersey/London/Hong Kong 1990.

3 Bell, E., Ivarsson, B., and Merrill, C., Production of a tissue-like structure by contraction of collagen lattice by human fibroblasts of different proliferative potential in vitro. Proc. natl Acad. Sci. USA *76* (1979) 1274–1278.

4 Bellows, C. G., Melcher, A. H., and Aubin, J. E., Contraction and organization of collagen gels by cells cultured from peridontal ligament, gingiva and bone suggest functional differences between cell types. J. Cell Sci. *50* (1981) 299–314.

5 Black, H. S., and Rodgers, M. A. J., Glass and plastic photoluminescence: interaction with aqueous media and cultured mammalian cells. Photochem. Photobiol. *51* (1990) 383–386.

6 Boveris, A., Cadenas, E., Reiter, R., Filipkowski, M., Nakase, Y., and Chance, B., Organ chemiluminescence: non-invasive assay for oxidative radical reactions. Proc. natl Acad. Sci. USA *77* (1980) 347–351.

7 Boveris, A., Cadenas, E., and Chance, B., Ultraweak chemiluminescence: a sensitive assay for oxidative radical reactions. Fedn Proc. *40* (1981) 195–198.

8 Braun, D. P., Harris, J. E., Maximovich, S., Marder, R., and Lint, T. F., Chemiluminescence in peripheral blood mononuclear cells from solid tumor cancer patients. Cancer Immun. Immunother. *12* (1981) 31–37.

9 Braun, D. P., DeBoer, K. P., and Harris, J. E., Chemiluminescence, suppression and cytotoxicity in peripheral blood mononuclear cells from solid tumor cancer patients. Cancer Immun. Immunother. *14* (1982) 86–91.

10 Cadenas, E., Wefers, H., and Sies, H., Low-level luminescence of isolated hepatocytes. Eur. J. Biochem. *119* (1981) 531–536.

11 Chandra, B., Lakshmanam, A. R., and Bhatt, R. C., Effect of deep traps on sensitization in LiF (TLD-100 phosphor). Phys. Stat. Sol. (A) *66* (1981) 335–340.

12 Chwirot, W. B., Dygdala, R. S., and Chwirot, S., Ultraweak photon emission during microsporogenesis in *Larix europaea*. Cytobios *44* (1985) 95–102.
13 Chwirot, W. B., Dygdala, R. S., and Chwirot, S., Optical coherence of white-light-induced photon emission from microsporocytes of *Larix europaea*. Cytobios *44* (1985) 239–249.
14 Chwirot, W. B., New indication of possible role of DNA in ultraweak photon emission from biological systems. J. Plant Physiol. *122* (1986) 81–86.
15 Fontes, A. G., DelaRosa, F. F., and Gomez-Moreno, C., Continuous photochemical production of hydrogen peroxide catalyzed by flavins. Photochem. Photobiophys. *2* (1981) 355–364.
16 Fleischmajer, R., Olson, B. K., and Kuhn, K. (Eds), Biology, Chemistry and Pathology of Collagen. Ann. N.Y. Acad. Sci., vol. 460. New York 1985.
17 Galeotto, T., Bartoli, G. M., Bartoli, S., and Bertoli, E., Superoxide radicals and lipid peroxidation in tumor microsomal membranes, in: Development in Biochemistry, vol. 11B, pp. 106–117. Eds W. H. Bannister and J. V. Bannister. Elsevier, North Holland/New York 1980.
18 Grasso, F., Grillo, C., Musumeci, F., Triglia, A., Rodolico, G., Cammisuli, F., Rinzivillo, C., Fragati, G., Santuccio, A., and Rodolico, M., Photon emission from normal and tumor human tissues. Experientia *48* (1992) 10–13.
19 Gurwitsch, A. A., A historical review of the problem of mitogenic radiation. Experientia *44* (1988) 545–550.
20 Hascall, V. C., and Hascall, G. K., Proteoglycans, in: Cell Biology of Extracellular Matrix, pp. 39–63. Ed. E. D. Hay. Plenum Press, New York 1981.
21 Jain, V. K., Radiation-induced sensitization and UV effects in lithium fluoride TLD phosphor. Phys. Stat. Sol. (A) *60* (1980) 351–356.
22 Jain, V. K., Phosphotransfer, sensitization and re-estimation of dose in lithium fluoride TLD. Health Phys. *41* (1981) 363–370.
23 Kochel, B., Time-resolved luminescence of perturbed biosystems: stochastic models and perturbation measures. Experientia *48* (1992) 1059–1069, this issue.
24 Li, K. H., Bioluminescence and stimulated coherent radiation. Laser Elektro-Optik *3* (1981) 32–35.
25 Li, K. H., Popp, F. A., Nagl, W., and Klima, H., Indications of optical coherence in biological systems and its possible significance, in: Coherent Excitations in Biological Systems. Eds H. Fröhlich and F. Kremer. Springer Verlag, Berlin/Heidelberg 1983.
26 Lilius, E.-M., Leukocytes as immunosensors, in: Biological Luminescence, pp. 449–459. Ed. B. Jezowska-Trzebiatowska. World Scientific Publishing, Singapore/New Jersey/London/Hong Kong 1990.
27 Liotta, L. A., and Rao, C. N., Role of the extracellular matrix in cancer. Ann. N.Y. Acad. Sci. *460* (1985) 333–344.
28 Massey, V., Strickland, S., Maykew, S. G., Howell, L. G., Engel, P. C., Matthews, R. G., Schumann, M., and Sullivan, P. A., The production of superoxide anion radicals in the reaction of reduced flavins and flavoproteins with molecular oxygen. Biochem. biophys. Res. Commun. *36* (1969) 891–897.
29 Mayhugh, M. R., Christy, R. W., and Johnson, N. M., Thermoluminescence and color center correlations in dosimetry LiF. J. appl. Phys. *41* (1970) 2968–2976.
30 Miller, L. D., and Bube, R. H., Luminescence, trapping and F centers in Lithium fluoride crystals. J. appl. Phys. *41* (1970) 3687–3697.
31 Murray, R. W., Chemical sources of singlet oxygen, in: Singlet Oxygen, pp. 59–114. Eds H. H. Wasserman and R. W. Murray. Academic Press, New York 1979.
32 Nagl, W., and Popp, F. A., A physical (electromagnetic) model of differentiation. I Basic considerations. Cytobios *37* (1983) 45–62.
33 Nakajima, M., Irimura, T., DiFerrante, N., and Nicolson, G. L., Metastatic melanoma cell heparanase. J. biol. Chem. *259* (1984) 2283–2290.
34 Niggli, H. J., Biophoton re-emission studies in carcinogenic mouse melanoma cells, in: Recent Advances in Biophoton Research and its Application, pp. 231–242. Eds F. A. Popp, K. H. Li and Q. Gu. World Scientific Publishing, Singapore/New Jersey/London/Hong Kong 1992.
35 Olson, B. R., Collagen biosynthesis, in: Cell Biology of Extracellular Matrix, pp. 139–177. Ed. E. D. Hay. Plenum Press, New York 1981.
36 Passarella, S., Casamassina, E., Molinari, S., Pastore, D., Quagliariello, E., Catalano, I. M., and Cingolani, A., Increase of proton electrochemical potential and ATP synthesis in rat liver mitochondria irradiated in vitro by helium-neon laser. FEBS Lett. *175* (1984) 95–99.
37 Popp, F. A., Ruth, B., Bahr, W., Bohm, J., Grass, P., Grollig, G., Rattemeyer, M., Schmidt, H. G., and Wulle, P., Emission of visible and ultraviolet radiation by active biological systems. Coll. Phenomena *3* (1981) 187–214.
38 Popp, F. A., Nagl, W., Li, K. H., Scholz, W., Weingärtner, O., and Wolf, R., Biophoton emission: New evidence for coherence and DNA as source. Cell Biophys. *6* (1984) 33–52.
39 Popp, F. A., and Nagl, W., Towards an understanding of stacked base interactions: non-equilibrial phase transitions as a probable model. Polymer Bull. *15* (1986) 89–91.
40 Popp, F. A., Li, K. H., Mei, W. P., Galle, M., and Neurohr, R., Physical aspects of biophotons. Experientia *44* (1988) 576–585.
41 Rattemeyer, J., Popp, F. A., and Nagl, W., Evidence of photon emission from DNA in living systems. Naturwissenschaften *68* (1981) 572–573.
42 Ruoslahti, E., Structure and biology of proteoglycans. A. Rev. Cell Biol. *4* (1988) 229–255.
43 Salet, C., and Moreno, G., Photosensitization of mitochondria. Molecular and cellular aspects. J. Photochem. Photobiol. *5* (1990) 133–150.
44 Schamhart, D. H. J., van der Poll, K. W., and van Wijk, R., Comparative studies of glucose metabolism in HTC, RLC, MHC and Reuber H35 hepatoma cells. *39* (1979) 1051–1055.
45 Schamhart, D. H. J., and van Wijk, R., Photon emission and the degree of differentiation, in: Photon Emission from Biological Systems, pp. 137–152. Ed. B. Jezowska-Trzebiatowska. World Scientific Publishing, Singapore/New Jersey 1987.
46 Schlesinger, M., Optical studies of electron and hole trapping levels in quartz. J. Phys. Chem. Sol. *26* (1965) 1761–1766.
47 Scholz, W., Staszkiewics, U., Popp, F. A., and Nagl, W., Light-stimulated ultraweak photon re-emission of human amnion cells and wish cells. Cell Biophys. *13* (1988) 55–63.
48 Sherstnev, M., Chemiluminescent express-methods in clinical medicine, in: Biological Luminescence, pp. 504–531. Ed. B. Jezowska-Trzebiatowska. World Scientific Publishing, Singapore/New Jersey/London/Hong Kong 1990.
49 Shlyakhtina, L. L., and Gurwitsch, A. A., Radiations from the mouse liver at normal temperatures and on cooling. Biophysics *17* (1972) 1146–1150.
50 Slawinski, J., Present status and prospects of PEBS' investigations, in: Photon Emission from Biological Systems, pp. 7–21. Ed. B. Jezowska-Trzebiatowska. World Scientific Publishing, Singapore/New Jersey 1987.
51 Souren, J. E. M., Ponec, M., and van Wijk, R., Contraction of collagen by human fibroblasts and keratinocytes. In Vitro Cell. Devl Biol. *25* (1989) 1039–1045.
52 Souren, J. E. M., and van Wijk, R., Fibroblastic cell cycling in collagen gels. Cell Proliferation (1992), in press.
53 Sunta, C. M., and Watanabe, S., Thermoluminescence of LiF LD-100 by phosphotransfer. J. Phys. D appl. Phys. *9* (1976) 1271–1278.
54 van Rijn, H., Bevers, M. M., van Wijk, R., and Wicks, W. D., Regulation of phosphoenol pyruvate carboxykinase and tyrosine aminotransferase in hepatoma cell cultures. J. Cell Biol. *60* (1974) 181–191.
55 van Wijk, R., Souren, J., Schamhart, D. H. J., and van Miltenburg, J. C., Comparative studies of the heat production of different hepatoma cells in culture. Cancer Res. *44* (1984) 671–673.
56 van Wijk, R., and Schamhart, D. H. J., Regulatory aspects of low intensity photon emission. Experientia *44* (1988) 586–593.
57 van Wijk, R., Souren, J., and Zoutewelle, G., Immune-modulation by the extracellular matrix, in: Matrixforschung in der Praeventivmedizin, pp. 41–49. Ed. H. Heine. Gustav Fischer Verlag, Stuttgart/New York 1989.
58 van Wijk, R., van Aken, H., and Schamhart, D. H. J., Delayed luminescence of rat hepatoma cells, in: Biological Luminescence, pp. 460–469. Ed. B. Jezowska-Trzebiatowska. World Scientific Publishing, Singapore/New Jersey/London/Hong Kong 1990.
59 van Wijk, R., and van Aken, H., Light-induced photon emission by rat hepatocytes and hepatoma cells. Cell Biophys. *18* (1991) 15–29.
60 van Wijk, R., and van Aken, H., Spontaneous and light-induced photon emission by rat hepatocytes and by hepatoma cells, in: Recent Advances in Biophoton Research and its Application, pp. 207–229. Eds F. A. Popp, K. H. Li and Q. Gu. World Scientific Publishing, Singapore/New Jersey/London/Hong Kong 1992.
61 van Wijk, R., Souren, J., and Ovelgonne, H., The extracellular matrix, fibroblast activity and effect of *Echinacea purpurea* extract, in: Normal Matrix and Pathological Conditions, pp. 77–93. Eds H. Heine and P. Anastasiadis. Gustav Fischer Verlag, Stuttgart/New York 1992.

62 Volman, H., A morphologic and morphometric study of the mitochondria in several hepatoma cell lines and isolated hepatocytes. Virchows Arch. Abt. B. Zellpath. *26* (1978) 249–259.
63 Volman, H., and van Wijk, R., Protein content of mitochondria from cultured hepatoma cell lines and from isolated rat liver cells, Archs int. Physiol. Biochim. *88* (1980) B215.
64 Voyevodina, T. V., Kovalsvskii, A. N., Kratasyuk, V. A., Schultz, V. R., and Nifantyev, O. E., Bioluminescent technique to analyze degree of endotoxication in peritonitis, in: Biological Luminescence, pp. 573–581. Ed. B. Jezowska-Trzebiatowska. World Scientific Publishing, Singapore/New Jersey/London/Hong Kong 1990.
65 Wicks, W. D., van Wijk, R., Clay K., Bearg, C., Bevers, M. M., and van Rijn, J., Regulation of growth rate, DNA synthesis and specific protein synthesis by derivatives of cyclic AMP in cultured hepatoma cells, in: The Role of Cyclic Nucleotides in Garcinogenesis, pp. 103–124. Eds J. Schultz and H. G. Gratzner. Academic Press, New York 1973.
66 Wicks, W. D., van Wijk, R., and McKibbin, J. B., Stimulation of enzyme synthesis and inhibition of DNA synthesis and growth rate by cyclic AMP derivatives in cultured hepatoma cells. Adv. Enzyme Reg. *11* (1973) 117–135.
67 Zhuravlev, A. I., Spontaneous superlow chemiluminescence and creation of quantum biology, in: Biological Luminescence, pp. 19–48. Ed. B. Jezowska-Trzebiatowska. World Scientific Publishing, Singapore/New Jersey/London/Hong Kong 1990.

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Reviews

Ouabain – a link in the genesis of high blood pressure?

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Abstract. Hypertension or high blood pressure is a risk factor that increases risk of myocardial infarction, renal failure or cerebral stroke. The pathogenesis of hypertension is due to a variety of causes, including inherited predisposition, dietary habits, especially salt intake, smoking, and also 'general lifestyle'. But for the scientist interested in the complex interplay of physiological and molecular factors, the actual causes of high blood pressure remain uninvestigated. The following article is concerned with new reports that ouabain, a plant derivative, occurs in human beings, in whom it appears to have a hormonal function; ouabain may even play a key role in the pathogenesis of hypertension. We are thus brought a step closer to the background of cardiovascular disease; we may also be afforded a lead to a new therapeutic principle.
Key words. Excretion; muscle cell; natriuretic hormone; glomerulus; tubule; Na^+,K^+-ATPase; ouabain; digitalis; blood pressure.

Cardiac glycosides are produced by plants

A large number of rare plants produce cardiac glycosides. The most familiar example is foxglove ('digitalis' in Latin). Preparations of digitalis have been used in medicine for more than two centuries to treat congestive heart failure, for one of the compound's predominant actions is to stimulate the force of myocardial contraction. However, the action of digitalis preparations is not confined to the heart alone but also affects other organs, a point to which we shall revert later.

The action of an extract of the foxglove plant was first described in 1785 by William Withering in his book 'An Account of the Foxglove'. The property he noted first was the ability of digitalis to increase the flow of urine (diuretic action). In the same work he also called attention to the hazards of an overdose of digitalis.

The therapeutically most important compounds contained in the digitalis plant, digoxin in digitoxin, are chemically very similar. They consist of a sterol skeleton attached to three sugar residues. Steroid hormones, which occur in animals and man, also have a sterol skeleton as their basic structure. Ouabain is a vegetable derivative with a marked cardiac action and is obtained from the waba yo tree (*Strophanthus gratus*) which grows exclusively in East Africa. Ouabain contains only one sugar residue, but it is one that isn't commonly found in the animal world, namely rhamnose (see diagram).

The action of ouabain in human beings has been known for very much longer than its chemical structure. Both the structural analysis and chemical synthesis of ouabain were described in the 40s and 50s of this century by the team working with Professor T. Reichstein at the University of Basel.

The present article is concerned with this 'African' ouabain. Ouabain was recently detected in human blood and indeed in concentrations capable of eliciting a reaction. Therefore, it is legitimate to ask whether ouabain is a hormone.