Regulatory aspects of low intensity photon emission

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Summary. Photon emission from unicellular and multicellular organisms has been a subject of study for many decennia. In contrast to the well-known phenomenon of bioluminescence originating in luciferin-luciferase reactions, low intensity emission in the visible region of the electromagnetic spectrum has been found in almost every species studied so far. At present, the nomenclature of this phenomenon has not crystallized and it is referred to by a variety of names, such as mitogenetic radiation[29], dark luminescence[7], low-level chemiluminescence[20, 36], and biophotons[57]. Particular attention has been focussed on the relationship between photon emission and the regulation of various aspects of cellular metabolism, although in many cases quantitative data are still lacking. Throughout the history of this field of research the question of a functional biological role of the low intensity emission has been repeatedly raised; this is reflected, for instance, in the heterogeneity of the terms used to describe it. The discussion concerns the possible participation of photons of low intensity in intra- and intercellular communication. This paper reviews literature on the metabolic regulation of low intensity emission, as well as the regulation of photon emission initiated by external light. Furthermore, recent data are discussed with respect to a possible biocommunicative function of low intensity photon emission.

Key words. Regulatory aspects; intracellular and intercellular communication; metabolic regulation; biological role; cancer; biophoton review.

Ultraweak photon emission and cellular metabolism

Studies on photon emission have been performed with a large variety of organisms, applying various physical measurements. In 1977, at a symposium on electromagnetic bio-information, Ruth listed data obtained with various cell types and organisms ranging from yeasts to plants and animals[63]. In addition biological indicators have been used to obtain evidence for the existence of ultraweak photon emission of biological origin[29]. Since then there has been a steady increase in cell types from which photon emission has been detected. In the majority of studies only qualitative data have been published, but more recently quantitative data as well as data on the effects of altered environmental conditions have become available. For reasons of clarity the present survey of the literature on ultraweak photon emission from biological sources has been divided in three parts, consisting of observations on tissues/cells of mammalian, yeast and plant origin.

a) Photon emission from mammalian cells

Emission of weak radiation in the visible range of the electromagnetic spectrum by animal tissues and cells has been described for a variety of organs and by many authors. By the use of photomultipliers emission has been readily detected from liver[11, 12, 66], heart[8, 52], lung[14], nerve[5], muscle[9, 68], Ehrlich ascites cells[21, 63], human expired air[79] and blood from healthy persons and patients suffering from various diseases, i.e., cancer, diabetes mellitus and hyperlipidemia[34]. A contracting frog heart in the thorax emits light, and this continues after isolation[52]. Isolated frog muscles obtained from various body locations radiate in low intensity when stimulated[9, 68]. Pulsed electric excitation of frog sciatic nerve caused photon emission[5]. Radiation within the visible range has been reported for mouse liver at physiological temperatures[66]. From in situ and isolated perfused rat liver a spontaneous emission of approximately 7–12 photons/s per cm^2 was registered, depending on the spectral sensitivity of the photomultiplier[11, 12].

Interestingly, the phenomenon of light emission was still present after isolation of individual hepatocytes, amounting to approximately 30 photons/s per 10^6 cells[20]. However, it must be emphasized that the number of photons registered per cell depends on a variety of factors. Among them are instrumental characteristics like the size and shape of the sample cuvette, the use of a mirror, the distance between cuvette and photomultiplier and the spectral sensitivity of the photomultiplier. Other factors relate to the emitting biological material, namely its homogeneity within the cuvette and the number of cells. The latter factors influence the absorption by cells other than the emittor cell. Apparently the actual number of photons registered represents a percentage of photons leaving the cells. Based on quantum efficiency data and light absorption data it can be estimated that mammalian cells emit approximately one photon per cell during a time period of 3–20 min.

Extensive studies consider the underlying mechanism of weak radiation from liver. Emission from intact liver appeared to be oxygen-dependent, increasing by a factor of 30 upon infusion with hydroperoxides[12]. Unfortunately, a quantitative comparison between intact liver and isolated hepatocytes can not be made due to the lack of accurate data, especially on the number of cells contributing to the emission of intact liver. However, also in isolated hepatocytes oxygenation resulted in an increased photon emission, occurring after a lag period of 20–40 min[20]. Several studies were intended to determine

possible intracellular sources of the light emission. In addition to measurements on photon emission by total organ homogenates from heart and liver cells [19], cell fractionation studies showed emission from submitochondrial particles [15, 16] and mitochondria [10]. Furthermore, the microsomal fraction of liver cells was recognized as a major contributor of emission [10, 18, 32, 46, 72, 80]. The light emission of these organelles requires a membrane bound electron transfer system and can be optimized in the presence of oxygen and hydroperoxide.

Spectral analysis has been extensively applied in order to identify more precisely the source of the photon emission in liver, brain, lung and heart cells [14, 16, 19] and hepatic microsomal fractions [18, 46]. In general various emission bands in the range of 400–700 nm were found and the data appeared to suggest singlet molecular oxygen, formed during the free radical process accompanying lipid peroxidation as the major source of light emission (fig. 1).

Thus, free radical decomposition of lipid hydroperoxides leads to the formation of excited chemiluminescent species by the self-reaction of secondary lipids peroxyradicals, producing either singlet molecular oxygen or excited carbonyl groups. Excited carbonyl compounds could in addition be formed by the reaction of the singlet molecular oxygen generated with unsaturated lipids, through a dioxetane intermediate [20]. For a more comprehensive account of lipid peroxidation, including the oxidizing species triggering and participating in this process, the reader is referred to Halliwell and Gutteridge [31] and Boveris et al. [11]. However, it should be emphasized that besides the process of lipid peroxidation additional sources of weak luminescence have been proposed (see later).

Another important target of active research in photon emission concerns the process of phagocytosis by leukocytes. Leukocytes, circulating freely in the blood stream, can move to any site in a tissue which is damaged or invaded by microbes. During acute inflammation circulating leukocytes become attached to the endothelial cells of small blood vessels at the site of the stimulus, move to the endothelium, penetrate through it and pass extravascularly into the adjacent tissue. Many factors have been shown to elicit the chemotactic response, especially interesting are bacterial metabolites and N-formylmethionylpeptides [26],

Photon emission of leukocytes has been measured both by directly applying photomultipliers and by the use of luminol as amplifier of the effect [2, 3, 36, 74]. The intensity and kinetics have been determined. The data suggest that photon emission is related to the process of phagocytosis. Photon emission has been used in order to elucidate the mechanism involved in the elimination of invading microorganisms. Studies revealed that phagocytosis comprises the engulfment of foreign particles, followed by their encapsulation in phagolysosomes [42]. During these processes the particles become exposed to a high flux of oxygen radicals, resulting from an increased non-mitochondrial consumption of oxygen. This respiratory burst appears to be associated with a burst in luminescence. Various oxygen reduction intermediates have been proposed as the species initiating the microbicidal effect, including the superoxide anion, hydrogen peroxide, the hydroxyl radical and singlet oxygen (for review see Badwey and Karnovsky [6] and Halliwell [30]). Furthermore, several mechanisms by which the actual killing of microorganism is effected have been proposed [6, 35]. It is generally believed that the myeloperoxidase–hydrogen peroxide–halide system is the principal oxygen-dependent microbicidal system in neutrophils. However, experiments of Shohet et al. indicate that lipid peroxidation plays a role in the killing process [67].

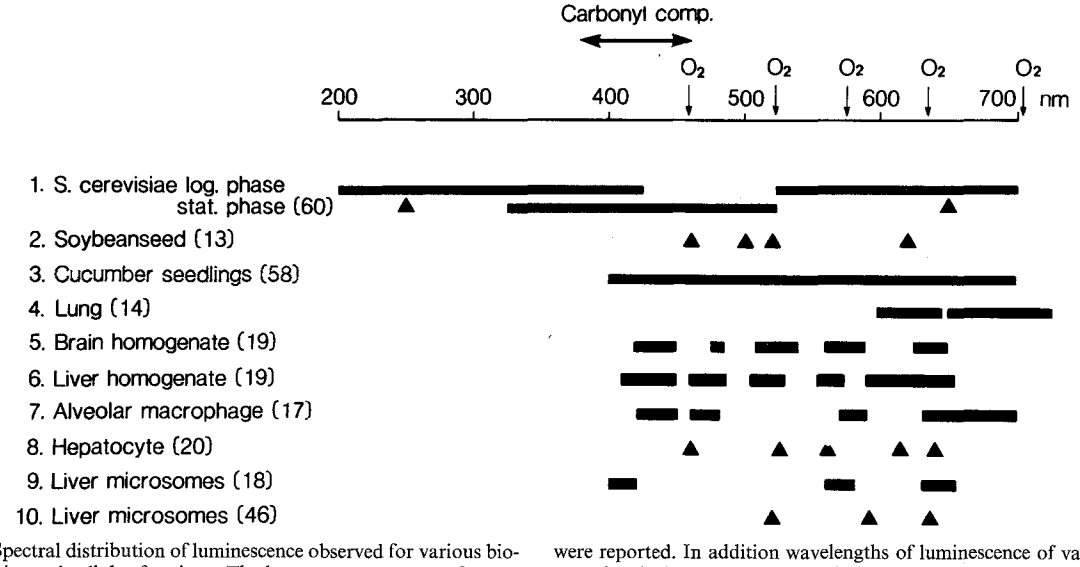

Figure 1. Spectral distribution of luminescence observed for various biological species and cellular fractions. The bars represent range of wavelengths. Optima within these ranges are not shown. The triangles represent specific optima reported in literature. In this later case no ranges were reported. In addition wavelengths of luminescence of various relevant chemical species are given. The numbers in the parentheses represent the reference numbers.

In addition, photon emission of peritoneal and alveolar macrophages has been used to elucidate what is the predominant oxygen reduction intermediate triggering the microbicidal effect[2, 17]. The various results appeared to be conflicting and both singlet oxygen and superoxide anion have been mentioned[3, 17, 35, 36]. However, at present it is not clear whether these differences originate from differences in the cellular source (polymorphonuclear leucocytes or macrophages). Furthermore, the presence of multiple broad bands of emission makes the interpretation of the various results less conclusive.

Despite these difficulties, spontaneous emission in the range of 20–200 photon/s per 10^6 cells, depending on the particular cell type has been reported[17, 36]. The intensity can be modified by immunological factors[64, 70], chemoattractants[26], drugs[51] and the interaction with various solid surfaces[50, 81].

A resemblance between phagocytosis-induced chemiluminescence and luminescence from *Acanthamoeba castellanii* has been found[43]. Studies on the chemiluminescence stimulated by cyanide and dibromothymoquinone suggested that reactions responsible for the production of luminescence are those primarily involved in production of superoxide anions, leading to lipid peroxidation and singlet oxygen formation.

In addition to the studies with isolated hepatocytes and leukocytes several other isolated mammalian cells have been investigated. Thus, Yoshida and Ehrlich ascites tumor cells grown in the abdominal cavity of rats and mice respectively, were found to emit light with a very low intensity[63]. Addition of several cytostatica influenced the emission temporarily. Increasing the concentration of 4-hydroxycyclophosphamide caused an approximately proportional enhancement of the emission of the Yoshida ascites cells. Recently, Cheng et al. also measured chemiluminescence from Ehrlich ascites cells which had an intensity of the same order of magnitude as that of the cells mentioned before[21].

Several attempts have been made by us to measure spontaneous low level chemiluminescence of a liver-derived hepatoma cell line, grown in vitro. In contrast to the reported observations of light emission from hepatocytes[20] these attempts were unsuccessful, although the conditions used for measurement should have permitted the registration of photon emission at a current density of 2 photons/s per cm^2 with a significance level of 99.9% within 6 h[58]. These differences between hepatocytes and hepatoma cells may be of value, because they can present additional circumstantial evidence for differences in radical processes in relation to cellular malignant transformation. In this respect it is interesting that some years ago it has been suggested that the loss of mitochondrial-bound manganese-containing superoxide dismutase (Mn SOD) along with radical production is a cause of cancer[4, 48]. Superoxide dismutase catalyze the conversion of superoxide radicals into hydrogen peroxide and oxygen, neutralizing the potentially damaging effect of the superoxide radical. The observations that tumor cells do produce superoxide in addition to undergoing a loss of Mn SOD, seems to support this hypothesis.

However, recently Galeoti et al. compared the generation of superoxide and lipid peroxidation of the microsomal fraction of various hepatomas with different growth rates and degrees of malignancy[27]. Their results showed that the production of superoxide radicals becomes reduced with increasing growth rate of the tumors. These data seem to be in contrast with those of Oberley and Buettner[48]. However, these results do not exclude the possibility that superoxide or related radicals in combination with a reduced level of Mn SOD play a role in early tumor formation. In this respect analysis of chemiluminescence during malignant transformation could be of great value. In addition, it must be emphasized that decreased photon emission can be due to multiple factors. Besides the possibility of an alteration of intracellular sources of emission, the emission is dependent on intracellular trapping principles (see later).

b) Photon emission from yeast

Photon emission from yeast has been reported in the sixties and seventies[38, 59, 60, 71]. At that time a number of data appeared which are comparable with those described for mammalian cells. Thus, photon emission was increased by blowing oxygen through a suspension of yeast cells. This enhancement was temporary and decreased within 20 min[71]. In addition several other nutritional effects on photon emission were described. Photon emission of *Saccharomyces cerevisiae* during continuous proliferation has been extensively studied by Quickenden et al.[59–61]. After inoculation the emission rises during the logarithmic phase of growth, proportionally to the increase in the number of yeast cells. At the end of the log phase emission decreased. It is interesting, although not yet explainable, that after a short period of time a second, transient increase occurred. Spectral analysis of the luminescence of yeast have shown that in addition to the spectral bands comparable to those observed for mammalian cells, yeast cells emit in the UV part (200–425 nm) of the electromagnetic spectrum (fig. 1). Spectral distribution of luminescence emitted during the logarithmic and stationary phases of growth were shown to be different. Stationary phase cells had a smaller far-UV band, whereas the band in the visible region appeared to be very broad compared to that for logarithmic phase cells. The sources from which the emission from yeast originates are not known. It has been suggested that the far-UV component arises from excited tryptophan[60]. Luminescence in the visible region seems comparable to that observed in mammalian cells.

Yeast metabolism has been studied in great detail, especially the phenomena of glucose repression (catabolite repression) and catabolite inactivation in a large number of yeast physiological processes. Growing yeast metabo-

lizes carbon sources in a sequential way [28]. In the case of glucose (or fructose) metabolism, ethanol is produced while respiration is inhibited, despite the presence of oxygen. Expression of relevant enzymes is either repressed or inactivated. However, enzyme activities readily increase under conditions of glucose limitation and in the presence of oxygen [53, 54, 75–77]. Absence of glucose repression and catabolite inactivation results in a temporary increase in cellular metabolism and ethanol consumption. Furthermore, lipid metabolism and fatty acid composition of yeast varies considerably, according to the growth conditions [49]. Recent experiments with normal and petite (lacking functional mitochondria) yeasts showed clearly the importance of mitochondrial metabolism in photon emission [61].

Taking advantage of the relative ease with which yeast cells can be synchronized by deprivation of an essential nutrient, it was shown that the emission increased during synchronized growth after application of the essential nutrient. Subsequently emission decreased; this was followed by a second increase during budding or cell division [59]. It is essentially unknown whether this fluctuating behavior is the consequence of some oscillatory phenomena initiated by metabolic stimulation or is linked to the process of budding in a causal manner. It might be associated with a unique, underlying metabolic process activated during various phases of the cell cycle, or with two different metabolic processes, each active in a specific phase of the cell cycle. In this respect, spectral analysis of the light emitted during cell cycle progression would be of great value. Furthermore, the alterations in photon emission may be partly due to temporarily active trapping principles. Subsequent analysis of the alteration of gene expression and DNA duplication after growth and synchronization will be of interest in order to prove a coupling between photon emission and DNA structure as suggested by several authors [44, 57, 59].

Although the data from yeast do not permit accurate quantification, photon emission from yeast and from mammalian cells seem to have in general several aspects in common, namely a) the low frequency at which cells emit a photon, and b) the regulation in situations of increased metabolism. Furthermore, as was observed for mammalian cells the existence of fairly broad bands in the emission spectrum may be indicative of the presence of multiple sources of emission, some of them not yet established.

c) Photon emission in plants

In adult plants, seedlings and seeds photon emission of high intensity has been found. For example, cucumber seedlings of about 4 cm emit light at an intensity of at least 6000 photons/s per seedling [63]. Emission of plant material was especially high immediately after placing fresh plants in the dark chamber of the measurement equipment. Subsequently, the emission decreased to a low, but detectable level within a few hours. A great number of experiments have been performed with *Tradescantia* branches with developed roots and seedlings of potato and cucumber [58, 63]. Unfortunately, as in the case of many studies on mammalian and yeast cells, a quantification in terms of photons emitted per cell under conditions of darkness cannot be given. Photon emission from various germinating plants and plant tissue extracts was first reported by Colli and Facchini [25]. Later it was found that similarly to that of mammalian and yeast cells, the emission of seedlings is oxygen dependent [63]. By the use of effective inhibitors and determination of the spectral distribution of intact soybean seeds, homogenates from these seeds and the reaction of pure lipoxygenase, Boveris et al. suggested that photon emission is mainly related to the lipoxygenase reaction [13]. The low photon emission is therefore regarded as mainly originating from excited species such as carbonyl groups, singlet oxygen and/or dioxetane intermediates (fig. 1).

For cucumber seedlings and wheat leaves temperature-dependence of photon emission has been described [37, 58]. Especially interesting is the alteration of emission induced by the transfer from a low to a higher temperature. A transfer from 19 °C to 30, 35 and 40 °C resulted in a transient increase of the emission of cucumber seedlings [58]. Furthermore, the typical temporal course of the response of photon emission to temperature changes appeared to be that after a rise in emission, initiated by the temperature increase, emission subsequently dropped in an oscillatory way. This characteristic response of photon emission has been theoretically described in terms of dissipative structures of thermodynamically open systems, which are far away from thermal equilibrium [55]. These and other results led Popp and co-workers to suggest a major role of DNA in photon emission [57, 62].

In microsporocytes of larch, developmentally linked changes in photon emission, occurring at low frequency, were observed [23]. Large differences were found for the various phases of meiosis, known to cover a process of significant transformation of the nuclear apparatus associated with repeated disappearance and reappearance of nucleoli and rebuilding of cytoplasm [23]. These differences in photon emission have been interpreted in terms of structural changes in chromatin and consequently considered as evidence for DNA as a source of photon emission [22]. Furthermore, additional evidence has been based on the observed correlation between changes in photon emission and DNase activity, measured in vitro, the latter being considered as indicative for DNA conformation. However, this assumption seems at present premature, making this evidence insufficient for definite proof.

Light induced photon emission (IPE)

From the preceding section it was concluded that the photons escape from cells at low frequency. The actual

number of photons generated within the cell may, however, be far higher. In that case a photon has a low probability of escaping from the emitter cell after generation, which means there is an effective intracellular mechanism of photon trapping. One can well imagine that this trapping can influence metabolic and cellular events by triggering amplification mechanisms and promoting photochemical processes, in a way comparable to the mechanisms proposed by Cilento [24].

In this respect it is of interest to consider the events observed after (pre-)illumination of cells. Supposing that cellular photon trapping does occur, it seems likely that as a consequence of illumination the trapping mechanism shifts to a state not capable of photon trapping during some period of time. The duration of the period of latency depends on the characteristics of the trapping mechanism(s). In fact, after termination of illumination, cellular emission may be temporarily at a high level, depending on the recovery time of the trapping process. Furthermore, the maximal amount of photons measured immediately after the start of darkness may be indicative of the actual number of photons that could potentially be released or, in other words, had previously been being trapped.

Experimentally, the plant system has shown the transition between at least two states of photon emission. The first state represents the emission of photons in the presence of external light, experimentally determined immediately after transfer to the dark chamber of the photomultiplier. As mentioned before, during a prolonged stay in the dark photon emission decreased until a second, steady state was reached. The transition between these various states has been studied in detail by way of its decay kinetics, its dependence on the initial light condition and its spectral changes. In essence, the technique of pulse illumination has been used, described as (light)induced photon emission (IPE).

A series of experiments on induced photon emission have been described by Popp and co-workers. The kinetics of the decay curves observed after pre-illumination of cucumber seedlings and leaves of *Bryophyllum* were not found to be easy to interpret. Pre-illumination with quasi-monochromatic light of a wavelength of about 700 nm and higher resulted in decay curves exhibiting a single shoulder shortly after the start of IPE decay [58]. This shoulder was not found after pre-illumination with light of lower wavelength. Furthermore, it was shown that the decay kinetics of IPE were independent of the excitation wavelength and followed a hyperbolic rather than an exponential law. Chwirot et al., using as a model system the microsporogenesis of larch microsporocytes, found that both the intensity of IPE and the characteristics of decay varied considerably during the successive stages of development [23]. Depending on the developmental stage oscillating decay curves were found after excitation with quasimonochromatic light. In contrast, oscillations were not detected after illumination with the full spectrum of a halogen lamp or nonmonochromatic light with a wavelength beyond 350 nm. At 8 out of 13 developmental stages decay of IPE could be described as being essentially hyperbolic. Furthermore, as in other plant systems the process of decay of IPE continues for nearly an hour or even longer. Recently, several mammalian systems have been carefully studied for their decay curves after exposure to light. Up to now only nonmonochromatic light has been used. Contrary to the plant systems, in mammalian systems the time for the decay of IPE is rapid, of the order of a few seconds. The first mammalian system was studied by Schamhart and Van Wijk and consisted of normal, isolated rat hepatocytes and the liver-derived hepatoma cell lines, H 35 and HTC, with different degrees of differentiation [65]. It was found that for any of these cell types the decay curve of IPE could be best described as hyperbolic. In view of the aforementioned hypothesis attention must be paid to:

a) the maximum rate of photon emission immediately after shutting off the external light source (this gives information about the cellular photon emission under conditions of minimal trapping activity), and

b) the rate of decay as indicative for the trapping process, including appropriate excited molecular states.

These aspects were studied in the aforementioned hepatocyte-hepatoma model system in relation to cell density. Comparison of the hepatoma cell lines showed that at high cell densities both the initial rate (measured at about 0.3 s following pre-illumination) and the rate of decay of IPE were more rapid in the poorly differentiated HTC cells compared to the well differentiated H 35 cells (figs 2A and B) [65]. So, in the case of H 35 cells both parameters appeared to be comparable to those of the medium, whereas HTC cells displayed an increasing emission-probability with increasing cell density. Normal hepatocytes exhibited a complex cell density dependency. At low cell density an increase in both parameters with increasing cell density was observed, followed by a decrease of emission-probability at cell densities above 3×10^6 cells/ml. Furthermore, as shown in figure 2C the initial rate and the rate of decay of IPE were found to be correlated in some way. These results were partially confirmed by Popp and co-workers comparing Wish tumor cells and their normal counterpart, human amnion cells (personal communication).

These results may be explained by assuming that under conditions of increasing cell density the proposed photon trapping system becomes inactivated (not susceptible to light) in hepatocytes, whereas on the contrary this system becomes activated in HTC cells. Although there is no doubt that this explanation is at present very incomplete and raises many questions, it could in principle suggest a way of cellular communication.

It is essentially unknown whether this cell density-dependent (rapid) event is regulated by metabolic products, extruded from the cell and active at low concentrations. On the other hand these results may provide evidence for

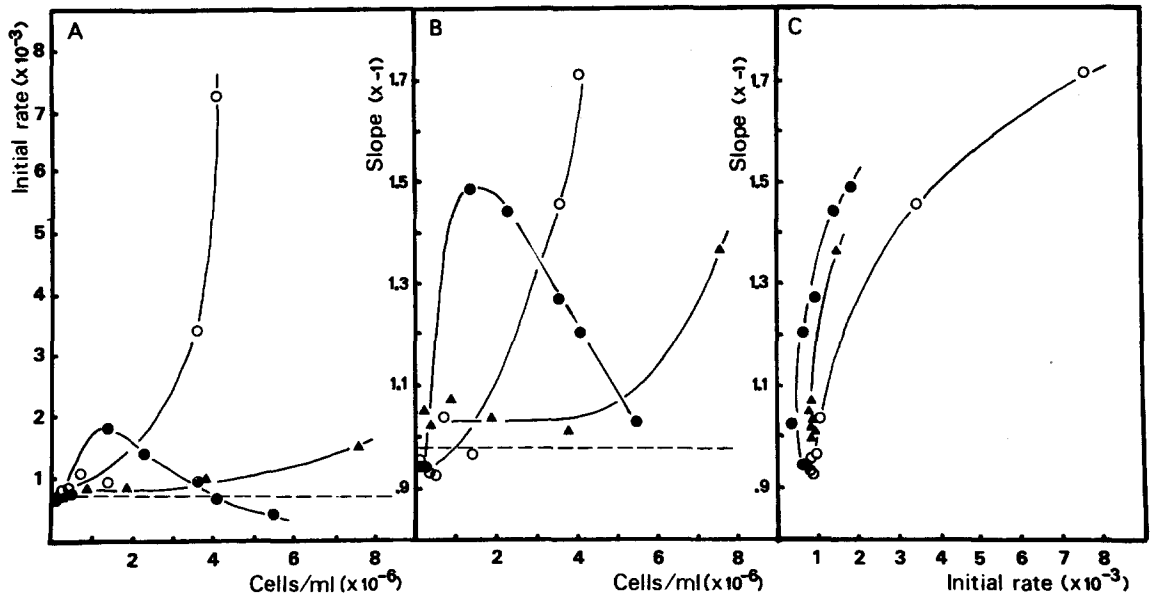

Figure 2. Various characteristics of light-induced photon emission of rat liver hepatocytes and the rat hepatoma cell lines H 35 and HTC in relation to the cell density. Cells in 11 ml suspension were pre-illuminated for 5 min with a halogen lamp prior to the determination of emission. The measurement equipment used has been described before[58]. *A* Initial rate of photon emission as determined 0.3 s after termination of the pre-illumination. *B* Rate of decay of photon emission. *C* Relationship between initial rate and rate of decay of light-induced emission. (●) hepatocytes, (▲) H35, (○) HTC. The dashed lines represent the initial rate (A) and rate of decay (B) observed for the medium.

a recently published model of intra- and intercellular communication[45, 56]. This biophysical model, including the suggestion that the conformational state of DNA acts as a photon storing and emitting resonance device, has a well elaborated theoretical basis[40, 41].

Concluding remarks

In this review a survey is presented of studies concerning the regulation of ultraweak photon emission of biological origin. A variety of organisms has been studied and from the results reported it can be expected that ultraweak photon emission in the visible range of the spectrum originates from various different cellular systems. As clearly pointed out by Slawinska and Slawinski[69] the biochemical interpretation of chemiluminescence is that it originates from the interaction of oxygen with the living cell, resulting in various emissive excited species. There is no doubt that this is one of the major sources. However, in the past few years data have been collected indicating that there are additional mechanisms able to produce photons.

The problem of cellular photon trapping processes other than photosynthesis has until recently received little attention. This trapping process, which is very effective even in etiolated plants, appears also to be present in animal cells. The nature of the trapping is not known, although kinetic data suggest multiple mechanisms. To gain insight into this process, research on IPE seems likely to be worthwhile, since the number of photons emitted by the cell is essentially dependent on the nature and activity of both the photon emitting process(es) and trapping process(es), which may be localized in one system or in two separate systems.

Furthermore, this article stresses the possible consequences of the emission. It may well have a decisive influence on the activity of biological systems, both on the functioning of the emittor cell itself and on its interactions with surrounding cells. Studies in this field of biocommunication are extremely difficult and are at present the topic of vigorous discussion. A recent symposium on photon emission of biological systems was almost completely devoted to this subject (Photon emission from biological systems, IS-86 PEBS, Poland, 1986). In this respect it seems to be of importance to emphasize that evidence has been obtained indicating that the relevant excited species may trigger amplification mechanisms and promote photochemical processes in the dark. This type of intracellular interaction has been named 'photochemistry without light' by Cilento[24]. Sung extended this concept, speculating that this type of interaction may also be relevant at the intercellular level. In this model the 'biophoton' is utilized in promoting some biophotochemical reaction, coupling a bioluminescent reaction in one cell with a biophotochemical reaction in another[73]. Although direct evidence for such a mechanism is still lacking, it should be noted that a number of experiments indicate that cells respond to the presence of weak electromagnetic waves, including light[1, 33, 39, 47].

As mentioned before, in addition to this physicochemical model of cellular communication, a purely physical model for inter- (and intra-)cellular communication has been recently proposed[45,56]. In this model the biophoton is trapped and emitted by a cellular physical resonance

device, presumably DNA; this results in light emission with at least partially a high degree of coherence [40, 41, 57]. Furthermore, the dependence of the emission kinetics on cell density is of special interest. Density-dependent rapid effects on the activity of the trapping mechanism(s) may be present. It seems apparent that the transformed cell is essentially different from its normal counterpart, the parental cell. The altered regulation of the activity of the trapping principle in some cancer cells may be part of the general shift in regulatory systems that characterizes cancer cells [78]. It is essentially unknown whether this density-dependent rapid event is regulated by specific metabolic products, which are secreted and are active at low concentration. On the contrary, it seems possible to consider biophotons at least as a part of the system of cellular communication. Further experiments will have to be designed to gain insight into the use of (coherent) excitations of multicellular systems and their role in cancer.

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