Minireview

Spontaneous Ultraweak Bioluminescence in Plants: Origin, Mechanisms and Properties

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Abstract. An analysis of theoretical knowledge and experimental results of ultraweak luminescence (UWL) is provided The role of excited state of molecules and free radicals, formed in various biochemical reactions, in UWL is discussed UWL of model reactions and *in vivo* systems are compared The hypothesis of coherent electromagnetic field as a source of UWL is also discussed. Spectral, kinetic and temporal properties of UWL are summarised, as well as their connection with its origin and role in the organism. Attention is paid to recent progress in experimental methods of low-light detection. The possible use of UWL in environmental studies, selection and other applications is discussed.

Key words: Spontaneous ultraweak bioluminescence — Mechanism of luminescent reactions — Spectral and kinetic analysis — Metabolic implications — Applications

There are several terms, such as ultraweak luminescence (UWL), weak metabolic luminescence (WML), ultraweak photon emission (UWPE), biophoton emission (BPE) and ultraweak bioluminescence (UWBL) to describe emission of electromagnetic radiation in the spectral range from 180 to 1500 nm, from oxidative metabolic reactions of all kinds of organisms and their tissues, cells and sub-cellular components. The wide span of emitting objects makes UWL considerably different from bioluminescence designating emission of light from specialised enzymic reactions or from photoproteins of certain living organisms (Ward 1981). UWL is believed to be a universal natural phenomenon, as a biochemical and biophysical process, involved into the principal biological functions, which are considered as

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its source Lepeschkin (1935) by using photographic plate, detected luminescence while injuring plant tissues in the 1930's It is considered that UWL of living cells, tissues and organisms was discovered during the 50 60's, due to progress in sensitive light detectors Weak light emission from germinating plants was registered for the first time by Colli and Facchini (1954) A few years later, Russian biophysicists discovered UWL in cells of higher animals (Tarusov et al 1962) Subsequent studies showed that UWL was a common feature of living organisms (Tarusov and Veselovsky 1978) and that it was produced during metabolic redox reactions due to which it was named "ultraweak metabolic luminescence" Results obtained in experiments with different systematic groups of organisms showed that UWL intensity increased with the increasing organism complexity (Ruth 1979) The intensity varied within the range of 10 to 10^4 quanta s^{-1} cm^{-2} UWL has extremely low quantum efficiency of 10^{-14} to 10^{-9} photons per activated molecule In comparison with bioluminescence, UWL is weaker by several orders of magnitude

UWL detection

Measurements of UWL intensity and spectral distribution require detectors of high sensitivity The modern devices are primarily based on photomultipliers operating in the single photon counting mode Their sensitivity reaches 2 photons s^{-1} cm^{-2} flow density Due to the inherent weakness of UWL, a filter set is used to study spectral distribution, instead of spectrometers (Inaba et al 1979) Therefore, only UWL spectra of low resolution are known A block diagram showing a single photon counting device used for registration of UWL is presented in Fig 1 Presently, there is no commercially available equipment purposely made for UWL measurement Fig 1 schematically shows common parts of different setups designed in various laboratories Sensitive germanium photodiode with specially designed electronics to eliminate cosmic ray signals, was used to record light emission of singlet oxygen in the near infrared region (Khan 1978)

The newly developed UWL detectors are based on charge coupled devices (CCD) CCD cameras with multi-channels for intensity provide two-dimensional real time imaging of the object (Tsuchiya et al 1985, Ichimura et al 1989)

Experimental objects and UWL localisation

UWL has been studied on different levels of plant organisms Luminescence intensity, kinetics and spectral distribution were monitored in intact roots, shoots, seeds, as well as in single cell fractions isolated from different organs nucleus, mitochondria, cell walls, membranes Experimental objects were subjected to various treatments while UWL kinetics, intensities and spectra were monitored A review of these studies can be found in several papers and books (Tarusov and Veselovsky

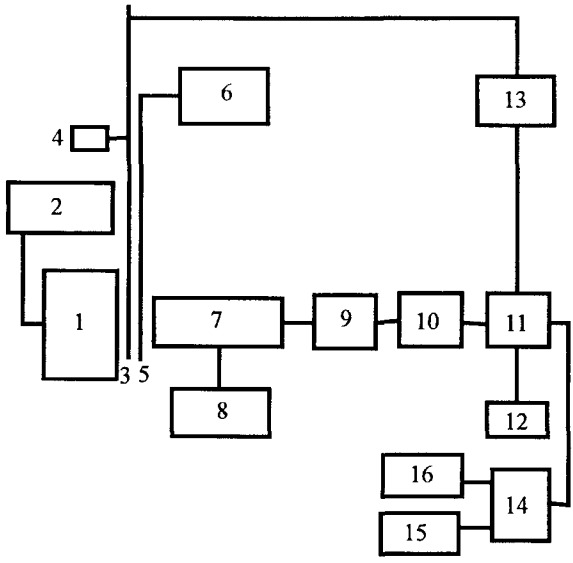


Figure 1 Block diagram of the laboratory-made photon counting system for registration of ultraweak luminescence (for details see Inaba et al 1979 and Popp et al 1981) 1 sample cell 2 temperature controller 3 chopper 4 chopper driving motor 5 filter 6 – filter drive controller 7 photomultiplier 8 – photomultiplier temperature controller 9 pulse amplifier 10 discriminator 11 reversible counter, 12 preset timer 13 phase shifter 14 computer 15 display 16 x-y recorder

1978 Popp et al 1979, Slawinska and Slawinski 1983, Abeles 1986 Radotic et al 1989) In experiments with intact organs, the observed light comes from the organ surface, while luminescence from internal regions is absorbed or scattered in the tissue The strongest UWL intensity is detected in the cell wall compared to other cell parts (Agaverdiyev and Tarusov 1965)

Recently, the relation between morphogenesis and UWL emission has been studied (Kai et al 1994) Seed germination and root growth rates were found to be directly correlated with UWL intensity Highest luminescence intensities were detected in the division zone while intensities recorded in the elongation zone were considerably weaker

The recent application of 2D-photon imaging gives evidence for the localisation of regions of root and shoot luminescence According to some authors, the principal luminescence originates in root apices (Ichimura et al 1989, Scott et al 1989, Kai et al 1994), while others claim that the origin is in the transition zone between the root and the shoot (Schauf et al 1992) According to Schauf et al (1992) luminescence detected in other parts of the root and the shoot is, in fact

transmitted from the transition zone through the tissue like a light guide The phenomenon of light transmission through tissue was used by Schauf et al (1992) to calculate the luminescence intensity of the origin The authors estimated that light losses within the plant were approximately 70 97% and, taking into account total detection efficiency of the equipment, they roughly calculated that the total number of photons emitted from the origin was within the range of 1 3 10^7 1 0 10^8 photons s^{-1} Assuming the volume of the transition zone and the mean cell size they estimated that 0 8 to 8 0 photons s^{-1} per cell were produced

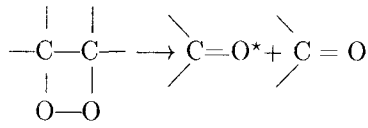
Many experiments were carried out on model-systems in which certain enzymic reactions believed to be involved in UWL emission were investigated Thus, light emission generated in the reactions of peroxidase with different substrates especially with phenolic compounds (Cilento et al 1978 Slawinska 1978, Slawinska et al 1979, Slawinska and Slawinski 1982), alcohols (Haun et al 1980) aldehydes (Soares and Bechara 1982, Dunford et al 1984, Baader et al 1985, Bohne et al 1987) some plant hormones (Augusto and Cilento 1977, Vidigal et al 1979, DeMello et al 1980, Cilento 1982), aromatic pyruvates (Cilento et al 1974, Zinner et al 1980, Dunford et al 1984) was studied Other enzymic, light producing reactions were also investigated, like reactions of oxidases and mono-oxygenase with various substrates, as well as reaction of lipoxygenase with unsaturated fatty acids (Bovens et al 1980 1981, 1983, 1984, Lilius and Laakso 1982) The results obtained in these experiments were used as supporting evidence for the interpretation of *in vivo* UWL measurements However a direct link between any individual *in vitro* reaction and *in vivo* luminescence has not yet been unambiguously established

The mechanism of UWL emission

A great number of molecules in excited state is produced in biochemical reactions They loose energy in different ways Some of them transfer their energy excess to other molecules, initiating subsequent reactions or propagating existing chain reactions Others transfer the energy to their surroundings by collision with neighbouring molecules in so-called radiationless transition A smaller number of molecules release excess of their energy by light emission, and return to the ground state

Experiments with model systems considerably contributed to the understanding of the mechanism of UWL emission in certain metabolic reactions The production of 1,2-dioxethane in enzymic (per)oxidations of aldehydes, fatty acids and some plant hormones (Haun et al 1980, Zinner et al 1980, Abeles 1986) is believed to be one key mechanism for UWL emission The dioxethanes are intermediates giving molecular species containing the C=O group in the excited state When returning to the ground state they produce energy in the form of light (Cilento

1975, Foote 1976, McCapra 1978)



The systems, in which dioxethanes are produced as intermediates, serve as storage of energy that could be used for normal or pathological functions (McCapra 1978)

The second important group of reactions responsible for UWL are reactions of free radical recombinations Radicals may be formed in enzymic and non-enzymic (per)oxidations Some products of radical recombinations are molecules in the excited state Returning to the ground energetic state, they release light energy (Russel 1957, Pryor 1986) e g

$$2ROO^{\cdot} \rightarrow RO^{*} + ROH + O_2$$
$$2ROO^{\cdot} \rightarrow RO + ROH + {}^1O_2$$

Autocatalysed chain reactions, with three stages initiation, propagation and termination (Tappel 1979 Pryor 1986) are prevalent (per)oxidative reactions involved in the formation of free radicals Thus, UWL can be used as an indirect probe of free radical concentrations in the system (Anhstrom and Natarajan 1960)

Singlet oxygen ($^1O_2$) is accepted as one emitter of UWL This form of molecular oxygen is formed in redox reactions Since singlet-triplet (ground-state) transition is spin-forbidden the life time of singlet oxygen is much longer in comparison with free radicals Due to spin-orbit coupling it decays slowly to the ground state by emitting light at 1268 nm Singlet oxygen may also release excess of energy by recombination of molecular pairs, emitting light at 634 and 703 nm (Nakano et al 1975, Khan 1978, 1983, Foote 1979)

Besides the above widely accepted theory, which explains UWL generation in terms of relaxation of excited molecules, there is another hypothesis on the UWL origin, proposed by Popp and co-workers The hypothesis assumes that biophoton emission originates from the de-localised coherent electromagnetic field within living tissue (Popp et al 1981, Popp 1992) This group of authors proposed DNA as the main source of biophoton emission The essential mechanism of the theory of coherence is based on the formation of an array of exciplex (Gu 1992) or exciplex-like coherent excited states in living matter Since the quantum theory shows that fully coherent field in a stationary state is always subject to a Poissonian photocount distribution, the authors also developed a statistical method of UWL analysis, in order to estimate the degree of coherence of emitted photon field (Shen et al 1993) However, experimental evidence in support of the theory is not

convincing Furthermore, the living cell and organism are complex systems and it is hard to believe that only one structure within the cell, such as a DNA molecule, is the only storage of energy for UWL emission This is supported by the data that even cells lacking nuclei (erythrocytes) emit light, as well as by the fact that the highest UWL intensity in plant tissue is detected in cell walls in which DNA is not present Although there is a certain contribution of DNA molecules to UWL emission, which could be even predominant in certain cases due to the specific physical and chemical properties of DNA, its role cannot be generalised Yet, it could help in the exploration of some phenomena which cannot be explained by the theory of excited molecules Moreover, the hypothesis of coherent field might get support from heated debate about conductivity of DNA (Wilson 1997) If it turns out that DNA is a good conductor, the hypothesis will get ground, and more experimental effort should be exerted in order to confirm it

The role of oxygen in UWL

Numerous *in vitro* and *in vivo* experiments pointed out that no UWL emission was possible in the absence of oxygen (Colli et al 1955, Veselovsky et al 1963, Abeles 1986) The intensity of photon emission depends on oxygen concentration and this relation is linear up to 20%, while it insignificantly increases at oxygen concentrations higher than 20% (Colli et al 1955, Veselovsky et al 1963, Abeles 1986) Such concentration dependency is a characteristics of free-radical chain redox reactions It was shown that UWL was not directly related to the normal process of oxidation during respiration The application of inhibitors of certain respiration stages differently affected changes of UWL (Tarusov and Veselovsky 1978) Accordingly, luminescence of roots cannot be correlated with luminescence of mitochondria (Averiyanov 1974)

Oxygen was shown to have an important role in the process of initiation and propagation of free radical reactions resulting in luminescence (Tarusov et al 1962 Tarusov and Veselovsky 1978) Different forms of activated oxygen produced in metabolic reactions are the source of UWL One of these forms, singlet oxygen, has been already mentioned as a direct emitter, but it initiates a number of chain reactions which finally yield UWL This energy enriched form of molecular oxygen produced in metabolic reactions, is more reactive than oxygen in the ground, triplet state and is relatively stable under cell physiological conditions Therefore, the reactivity of $^1O_2$ is higher in comparison with ground-state oxygen (Pryor 1978, 1986, Rabek and Ranby 1978) It reacts with various cell substrates (olefins, aromatic compounds sulphides) by an addition mechanism (Foote 1971) Due to relative stability and reactivity with different substrates, $^1O_2$ is considered to be one of the most important agents in initiation and propagation of reactions yielding UWL The second important form of activated oxygen is superoxide anion radical $O_2^{\cdot -}$ or $HOO^{\cdot}$ the single-electron reduction product of O_2 It is produced in enzymic

and non-enzymic reactions (Pryor 1978, Elstner 1982 Grisham and McCord 1986) Superoxide radical is the driving force and propagator of chain redox reactions Hydrogen peroxide, formed by two-electron reduction of O_2 in different enzymic and non-enzymic reactions is initiator of chain reactions, which can give UWL It is stable over physiological pH and temperature range Hydrogen peroxide participates as a cosubstrate in substrate peroxidation catalysed by peroxidases (Abeles 1986) It is decomposed in Haber-Weiss reaction, catalysed by iron ions and other metals, forming $O_2^{\cdot-}$ and $^1O_2$ (Koppenol et al 1978, Abeles 1986)

Spectral and kinetic analysis of UWL

Due to its inherent weak intensity, no detailed study of the UWL emission spectra has yet been performed Spectra of low resolution are obtained by a set of filters in visible and near infrared regions (Inaba et al 1979) The spectral distribution was different in various objects For example, most light emitted by plants is in blue-green part of the spectrum (Veselovsky et al 1963)

Light produced by dioxethanes, phenolic compounds and products of lipid oxidation (aldehydes and ketones) is within the wavelength range of 459 and 582 nm (Abeles 1986) 400 and 600 nm (Salin and Bridges 1981) 350 and 480 nm (Boveris et al 1981) respectively The emission spectrum of molecule pairs of singlet oxygen has a peak between 634 and 703 nm (red spectrum), while the single molecules of singlet oxygen emit at 1269 nm However, due to non-homogeneity and complexity of biological systems, and particularly because of the impossibility to obtain spectra of good resolution, it is not possible to assign with certainty a contribution of individual reactions to the total UWL spectrum

The time course of UWL emission depends on the conditions of the pretreatment of the experimental object If the object is kept in the dark for 20 60 min there is no initial luminescence (Tarusov and Veselovsky 1978, Salin and Bridges 1983), but if the intact root or shoots are kept in a small amount of water, the light emission shall last several days with periodic variations of intensity (Ruth 1979) Temporal variation of luminescence of the intact object is much greater than in any individual reaction This fact points to the participation of a greater number of emitters and a greater complexity of the processes in the intact organ, i e tissue The intensity and kinetics of UWL vary when the object is subjected to different physical and chemical treatments The effect of applied stress is an abrupt burst of luminescence followed by slow exponential decay The slow decaying part of the temporal curve can be resolved in the semi-logarithmic co-ordinate system (Štrbac et al 1985, Radotic et al 1992) In such a way, several first order reactions have been found to be involved in luminescence emission The corresponding rate constants can be evaluated from the reaction half-times This procedure was applied to different plant and animal objects, where the initial burst of UWL was induced by

different physical and chemical treatments (stimuli) The obtained results indicate that up to three pseudo-first order reactions are involved in luminescence emission Rate constants corresponding to the same group of reactions from different objects were found to be of the same order of magnitude (Table 1) The application of different inhibitors of both free-radical reactions and luminescence shows that the reactions involved in UWL of a single object can be affected independently This suggests that UWL is based on several parallel reactions Different stimuli alter only the luminescence intensity, while the mechanism and reaction rates remain the same It means that the number of excited states and consequently the number of photons emitted, can be changed by alterating the type and the magnitude of the stimulus The analysis of secondary kinetics of induced luminescence of catalase ascorbate and quinone treated root, as well as the kinetic analysis of the exponential decay of free radicals concentration in a tobacco leaf after inhibition show that forms of activated oxygen and peroxy-radicals play the primary role in UWL (Radotic et al 1989 1990a b,c) Some authors claim that the decay of induced luminescence from living tissues in some cases does not follow exponential

Table 1. Kinetics of luminescence induced by different stimuli in several plant and animal tissues, k_1 k_2 k_3 rate constants of the first order reactions included in the luminescent decay (according to Radotic et al 1992)

Object	Stimulus	$k_1/10^{-2}s^{-1}$	$k_2/10^{-3}s^{-1}$	$k_3/10^{-4}s^{-1}$	Reference
Maize roots	0 5 4 5 mol/l H_2O_2	7 5	8 6	8 6	Štrbac et al (1985)
Maize roots	$H_2O_2+10^{-4}$ mol/l ascorbate		8 6	8 6	Štrbac et al (1985)
Maize roots	$H_2O_2 + D_2O$	4 6	15	6 7	Radotic et al (1990c)
Maize roots	$H_2O_2 + 10^{-5}$ mol/l hydroquinone	–	8 2	2 5	Radotic et al (1990c)
Maize roots	H_2O_2 + SOD + catalase	3 8	9 6	13	Radotic et al (1990c)
Cucumber roots	Dehydration	1 8	8	7 8	Veselova et al (1991)
Eucalyptus lignin	Unirradiated	6 9	15 7		Duran and Mansilla (1984)
Eucalyptus lignin	Irradiated	1 6			Duran and Mansilla (1984)
Microsporocytes from Larix	White light	1 5	3 6	8 3	Chwirot et al (1985)
Microsporocytes-male cones	White light		8 2	11 5	Chwirot et al (1985)
Macrophages	Concavalin A 0 37 mg/ml	1 3	4 2	4 3	Cadenas et al (1981)

but hyperbolic law (Popp et al 1981, Popp 1992) These authors use this fact as a proof for the "theory of coherence" They theorise that hyperbolic relaxation is a characteristic of an excited coherent field which maintains coherency in time (Popp and Li 1993)

Factors affecting UWL in plants

In contrast to analytical methods, UWL could be used as a parameter of a global integral effect of stress on a biological system (Slawinski et al 1992)

Temperature

Temperature has a number of effects on a living system It governs the rate at which O_2 or activated O_2 react with substrates Temperature also controls the rate of enzymic reactions through activation of the enzymes The kinetics of free-radical reactions is particularly affected by temperature Low and high temperature extremes have lethal effects on living tissues

The temperature-dependent variation of UWL shows a smooth rising course with two maxima at both ends of the physiological range of temperature The positions of the peaks are identical for roots and shoots The low and high temperature maxima appear between 0 °C and −5 °C, and 45 °C and 50 °C, respectively Thermal death of objects occurs within these regions of luminescence maxima According to some authors, the temperature-dependent variation of UWL between temperature peaks agrees with Arrhenius plot (Veselovsky et al 1963, Agaverdivev et al 1965, Perelygin et al 1966) Other authors report non-linear, hysteretic temperature dependence of the photon emission which can be interpreted by Curie-Weiss rather than Arrhenius law (Slawinski and Popp 1987) Experiments with roots and shoots of different plant species show that the positions of low and high temperature peaks may be used for screening of species on their resistance against chilling and hot weather This is used for selection of resistant genotypes (Tarusov and Veselovsky 1978)

Wounding

Wounding of roots and shoots mechanically results in increased luminescence (Salin and Bridges 1981, 1983) On the basis of the rate of induced luminescence increase it can be concluded that *de novo* synthesis of enzymes does not occur Increased luminescence caused by wounding is interpreted in terms of increased peroxidase activity due to cell damage Phenoloxidases released from cells by the wounding process could be an additional source of increased luminescence Recent studies of UWL in shoots and roots by 2D-photon imaging have shown that wounding does not cause any significant luminescence increase (Schauf et al 1992) However, this conclusion might be incorrect since the applied method is slow

Radiation

Irradiation of objects by UV light in some cases results in UWL intensity decrease (Veselova and Veselovsky 1971), and eliminates certain reactions involved in lumi nescence In some cases, UV light increases UWL, while X-rays radiation inhibits it (Radotic et al 1992)

Osmotic conditions and salts

Dehydration of roots and shoots by dextran results in increased luminescence (Tarusov and Veselovsky 1978), but does not affect the number of reactions involved in UWL emission (Veselova et al 1991, Radotic et al 1992) The rate of UWL increase depends on the degree of water deficit, as well as on plant resistance to desiccation The effect of dehydration on UWL of roots caused by gradual drying in air current is less pronounced than the effects of dehydration caused by sucrose which induces "osmotic stress" (Tarusov and Veselovsky 1978)

Some studies show that after imbibition there is a manifold increase of UWL of both seeds (Boveris et al 1983) and embryonic axes (Boveris et al 1984) The spectral distribution indicates that most of the light is produced by forms of activated oxygen, especially $^1O_2$

Increase of salt concentration in the root environment causes a burst of UWL, whose magnitude depends on the salt concentration Iso-osmotic concentrations of salts are more stimulating than sugars due to greater tissue damage Salt-induced luminescence increase in tissues most probably occurs due to membrane damage, disturbances of the electrochemical gradient, oxidation of cytoplasmatic components and an increase in the amounts of activated oxygen forms The effect of salts on UWL emission is found to be dependent on plant tolerance to salts (Tarusov and Veselovsky 1978)

Toxic compounds

Chemicals causing death of biological tissue induce initial burst of UWL The initial burst of light occurs in all cases Repeated addition of the compounds does not cause a second UWL increase, indicating the death of the tissue Non-toxic concentrations induce a lower UWL increase with a different kinetics than the toxic ones This was used as a test whether certain toxic probes induce damage to or death of plants (Ruth 1979)

Respiration inhibitors sodium-cyanide, sodium-azide, sodium-amytal, as well as heavy metal ions Fe^{3+}, Fe^{2+}, Cu^{2+}, Hg^{2+} (Tarusov and Veselovsky 1978), cause an initial luminescence increase, and then gradually quench it It is assumed that the increase of UWL by respiration inhibitors reflects the increase of O_2 partial pressure in cells, which is caused by reduced O_2 consumption accompanying the UWL increase Heavy metals inhibit enzymic activities, respiration, and cause an increase of free-radical chain reactions (Tarusov and Veselovsky 1978, Abeles 1986)

Alcohols (methanol, ethanol, butanol, propanol), acetone, ether and chloroform induce an initial light burst which increases with the increasing concentration of the agents The effects of methanol and butanol on UWL emission are stronger than the effects of ethanol (Tarusov and Veselovsky 1978) Toxic compounds can simultaneously or independently affect the time course and the UWL spectrum (Ruth 1979) These results are in accordance with results obtained from kinetic analysis i e they provide evidence for the existence of several pseudo-first orders reactions which could be affected independently

Recently the effect of ozone on UWL of biological systems has been studied UWL increased in ozone treated plants (Katziger et al 1994) This increase was considered to be related to the reaction between ozone and lipids, membranes and small molecules, such as cysteine, histidine, tryptophane and methionine

Biological factors

Pathogens cause UWL increase in living tissues due to the activation of protective mechanisms by rising the amounts of H_2O_2 and $^1O_2$ 'pathogen killers' in tissues (Lehrer 1969 Uri and Dunleavy 1974a b Averyanov et al 1978, Uri and Hill 1978 Averyanov and Panayotov 1979 Lehninger 1981) A considerable part of the plant response originates from the cell wall that is from the activities of peroxidase and peroxidase related free-radical chain reactions (Tarusov and Veselovsky 1978)

Conjugation of biochemiluminescence and biophotochemical reactions

Cilento and others have studied a series of reactions including biologically active compounds and enzymes plant and animal hormones, lipids, amino acids t-RNA peroxidase and oxidase Production of compounds in electronic excited state in these reactions was shown through

equivalence of chemiluminescent and phosphorescent spectra of excited products
energy transfer to sensitisers,
detection of photoproducts

A survey of these investigations has been presented in review articles (Popp et al 1979 Cilento 1982) On the basis of theoretical considerations, as well as of some experimental results, Popp and Ruth pointed to the possible regulatory role of photonic emission from DNA (Ruth 1979) On the other hand, Cilento (1982) postulated a concept of 'photobiochemistry without light", not only because of the existence and chemical analogy of many physiological processes being able to produce electronically excited molecular species, but also because of the following facts

the presence of bioluminescent emitters in nonbioluminescent organisms,
the correlation between some biological activities and parameters of the excited state For example, experimental results (Popp et al 1979) confirmed the inter-

pretation that metabolic hydroxylation could not be explained on the basis of electronic characteristics of substrate in its ground state, but substrate has to be in the first electronic excited state to participate in the reaction,

capacity of photon absorption to induce conformational change of proteins (Pieroni et al 1986) and to affect enzymic activity (Comorosan 1974)

Although it is rather difficult to obtain direct experimental evidence it has been proposed on the basis of the above considerations that coupling of chemiluminescent and biophotochemical reactions could be a control mechanism of certain metabolic processes in the cell, as well as a way of intercellular communication

As mentioned previously some experiments provide evidence that plant tissue is able to conduct light practically without losses (Mandoli and Briggs 1982) This fact supports the hypothesis that light could be one of the means of information transfer within the plant An additional evidence of the assumed function of light is the fact that rate constants of UWL relaxation induced by different treatments (stimuli) are of the same order of magnitude in different objects (Radotic et al 1992)

Possible relation between photon emission and control processes

The relation between total number Z of photons emitted in a chemiluminescent reaction and the number of reacting molecules N is

$$Z = q \quad N \tag{1}$$

where q is luminescence quantum yield Strong light absorption in the tissue prevents detection of the primary radiation sources According to theoretical considerations the number of photons $Z(\lambda)$ emitted at wavelength λ is determined by the relative number of excited molecules in the tissue by their optical properties (Ruth 1979) In such a way biochemical and biophysical processes within the cells can be monitored by means of UWL study without cell damage The relation between photon emission and control processes in the cell could be studied Besides photon emission excited molecules can also react with other molecules According to the Arrhenius theory, the reaction rate is proportional to the relative number of excited molecules whose energies surpass reaction activation energy Photon emission is a direct indication of the reaction rates responsible for the formation and relaxation of excited states Therefore, light emission could be used for studies of processes controlling photon emission in the cell These quantitative considerations by Ruth (1979) showed that UWL might be of great importance for biochemical processes within the cells, as well as for their monitoring

Since photonic signals can be transformed into acoustic, chemical and electric processes in the cell, some authors emphasize the possibility that photons emitted within the tissue could be involved in intra- and intercellular information transfer

(Fisher 1979) This hypothesis was stated for the first time by Gurwitsch (1932) whose experiments showed that the low-energy UV light emission might play a role in intercellular communication Although his results were criticised by many authors, introduction of sensitive photomultipliers and photon counting devices revealed the existence of biogenic radiation from processes involved in the mitosis and cell growth, i e processes where intra- and intercellular information transfer occurs (Quickenden and Que Hee 1974 Fisher 1979, Quickenden and Tilbury 1983) The initial results were mostly obtained in experiments on cell cultures (Fisher 1979, Slawinska and Slawinski 1983) Based on these experiments it was postulated that light radiation did not originate from enzymic reactions and respiratory pathway, and that oxygen radicals may have been involved in its emission (Quickenden and Tilbury 1983) It was also shown that some cell organelles such as microtubules could be photon carriers Special local differentiations of the cell membranes involved in the intercellular contact are being considered as the possible two-way transducers of photonic signals (Fisher 1979) Generally it seems that the reactions of the living cell to external light stimulation are based on complex mechanisms For example, it was shown that light stimulation of bacterial membranes induced within milliseconds, changes of membrane potential with a simultaneous change of ion transport (Fisher 1979) However, detailed experimental studies are to be carried out in order to confirm the optochemical mechanism of the cell communication and regulation A bulk of experimental evidence and theoretical considerations are necessary to investigate the information-related significance of biophotonic emission

Application of UWL

Progress in photon counting devices offers an opportunity to use the informative potential of photonic emission for studies of biological systems This potential is contained in the time-space and energetic parameters of the *in vivo* emitted UWL

Correlation between UWL and physiological parameters is a starting point for the diagnostic and analytical applications of UWL Diagnostic applications include the use of UWL in the laboratory investigations of the effects of environmental factors (temperature, osmotic pressure, drought, pathogens) on the plant metabolism UWL parameters (such as intensity kinetics, spectral distribution, temperature dependence, statistical photonic distribution) are used as an integral indicator of the intactness of biological systems as well as a way of rapid, non-invasive study of oxidative metabolism in the organs and tissues In this way it is possible to perform comparative studies on different hybrids and inbreds of the same crop Such non-invasive investigations contribute to a better crop breeding under the given conditions Different cereal and maize varieties have been investigated using this approach (Tarusov 1968, Tarusov and Veselovsky 1978)

The analytical use of UWL is related to basic redox, free-radical processes in the cell It has been used to monitor particular metabolic disorders and pathological conditions of tissues and organs in cases when it was not possible to use other methods, or as a complementary method UWL has also been used as an additional method in the studies of biochemical and biophysical processes in the cell, not yet elucidated but involved in light emission (Abeles 1986)

In order to amplify the light emission, certain coloured compounds, natural constituents of the plant tissue, were used as the acceptors in some studies Chlorophyll was used as the acceptor in studies of light energy transfer through micellar membranes from different outside donor molecules to micellar interior (Brunetti et al 1983) Moreover, chlorophyll was used as an acceptor in studies of UWL of different cell organelles isolated from plant tissues (Hideg 1993)

The use of chemiluminescent probes compounds whose oxidation gives a high percentage of electronically excited products increases the sensitivity of UWL measurements several orders of magnitude The probes most often used for such purposes are luminol (Totter et al 1960, Averyanov 1974 Pogosyan et al 1978 Warm and Laties 1982 Abeles 1986, Bohne et al 1987), lucigenin lophin pyrogallol (Fisher 1979, Quickenden and Tilbury 1983) In systems emitting UWL luminescent probes react with free radicals of oxygen and organic molecules, as well as with singlet oxygen and some metals in certain oxydation state

Directions of the future development

The knowledge gained on bioluminescence points to its role in the ecology of organisms or populations In these terms, bioluminescence seems to have some particular adaptive functions On the contrary, UWL has been much less understood The above discussion shows that neither of the theories about the origin and the role of UWL in the living systems has been able to completely explain this phenomenon The theory of relaxation of excited states is the most suitable to explain the majority of UWL properties, but in some cases, such as hysteretic temperature dependence, the theory of coherent electromagnetic field is more convenient However in order to understand completely the origin and the role of UWL in biological systems a complex consideration of all previous experiments and theories is necessary and then, on these grounds future experiments can be planned which might bring us closer to a complete insight into the UWL phenomenon It shall be necessary to develop devices for the measurement of extremely low light intensities which would enable exact spectral analysis of emitted light Only a precise spectral analysis would offer an explanation of the light emitter in the cell Another important direction in the UWL studies is the investigation of the correlation between luminescence and information transfer within cells and tissues This

kind of investigations is also dependent on the development of the corresponding methodology

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