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Luminescence research and its relation to ultraweak cell radiation

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Summary. The fundamental laws of photochemistry and the essential results of experimental research on ultraweak cell radiation are presented. By comparing all the facts it can be concluded that the phenomena discussed may arise from a variety of possible reactions and sources. Recombination reactions of certain radicals actually do release sufficient energy to generate UV-photons of the intensity under consideration. On the other hand, stimulated emission cannot be excluded in view of the distinct deviation of the radiation field from thermal equilibrium. There exist, however, various other candidates, such as direct emitters like flavins, indoles, porphyrins, carbonyl derivatives and aromatic compounds, and molecular oxygen and its various species, as well as collective molecular interactions, e.g. dimole or exciplex transitions, triplet–triplet annihilation, collective hydrolysis, electric field effects in membranes, etc.

Careful biochemical and biophysical experiments are still necessary to find answers to all the questions that remain; not only individual problems have to be solved, but it is important to keep in mind the interrelationships between certain reactions.

Key words. Bioluminescence and weak photon emission from biological systems; excited states; photochemistry; radical reactions; energy transfer; collective interactions; temperature hysteresis.

Introduction

Growing research activity in the field of biological luminescence reflects an increasing recognition of the following: 1) The universality of ultraweak cell radiation (luminescence) which is emitted by all biological systems and is inherently associated with fundamental processes such as cell division and death, photosynthesis, carcinogenesis and possibly growth regulation. 2) The high, yet unexplored diagnostic potential of low level luminescence in medicine, agriculture and other biosciences, and 3) the development of a new biophysical hypothesis assigning particular importance to the informative and organizational role of coherent electromagnetic fields within living organisms.

In this chapter we will consider primarily biochemical mechanisms by which living systems create electronic excited states and photons, and the link between them and physiological processes. An attempt is made here to present the results of the most recent interdisciplinary research in a simple and coherent way. More comprehensive treatments are available elsewhere [5, 7, 13, 19, 30, 35, 37, 51].

Generation of excited states and photons in biological systems

The emission of electromagnetic radiation with the energy

$$E = h\nu \tag{1}$$

and the corresponding wavelength

$$\lambda = c/\nu \tag{2}$$

occurs when an electric charge oscillates at the frequency ν. In the spectral range 180–1000 nm covering the UV, visible and near IR, corresponding oscillation frequencies are 3×10^{14}–1.6×10^{15} Hz. Thus, the generation of photons requires two phases: 1) the energy pumping that promotes an electron to the excited level, and 2) radiative relaxation that creates a photon (fig. 1):

$$E \xrightarrow{1)} P^* \xrightarrow{2)} P + h\nu \tag{3}$$

where P* represents an electronically excited species and ΔE is the difference of the ground and excited states (the pumping energy). Living organisms can utilize a variety of energy forms and transform a fraction of them into an electronic/vibrational excitation. In photosynthetic bacteria and green plants, for example, the photoexcitation of bacteriochlorophyll or chlorophyll takes place and leads to charge separation and storage. A subsequent recombination of charge-separated molecular species results in photon emission in the red part of the spectrum, the so-called photosynthetic or delay luminescence (Strehler's radiation). Heterotrophes employ free energy, G, from the reorganization of chemical bonds of substrates in the consumed food. In this case the change of

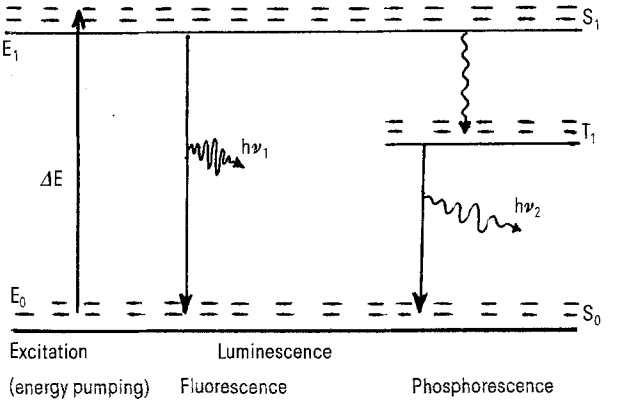


Figure 1. The simplest Jablonski's diagram of energy levels depicting the generation of electronic excited states and photon emission. Electronic levels, ———; vibronic oscillation levels, – – – –; S and T, singlet and triplet states, respectively; radiationless transitions, ~~~~>.

free energy ΔG is a part of the thermochemical effect of the chemical reaction (i.e., a reorganization of bonds):

$$\Delta G = \Delta H - T\Delta S \tag{4}$$

where ΔH is called enthalpy and ΔS is the entropy change. The whole process can be classified as chemiluminescence, since the first phase is a chemical pumping or chemiexcitation. We shall consider this process more exactly, as it is considered to be the main energy-pumping (excitation) source for spontaneous luminescence in biological systems.

Chemiexcitation

In general, three criteria must be satisfied to achieve chemiluminescence:

1) There must be an efficient chemical pathway leading to the formation of product molecules, with an adequate electronic structure facilitating the electronic excitation, high efficiency and ability for luminescence etc. The chemical yield can be represented as a fraction of the total chemical paths:

$$\Phi_c = \frac{\text{the number (or rate) of P molecules formed}}{\text{the number (or rate) of molecules reacted}} \tag{5}$$

2) The sufficient energy amount has to be liberated in one single step:

$$|-\Delta G| \simeq hc/\lambda_{min} \tag{6}$$

where λ_{min} is the short wavelength limit for the excitation of a product molecule P. This process must be faster than the oscillation frequency 10^{12} Hz in order to efficiently produce an electronic excited state of P*:

$$\Phi_{ex} = \frac{\text{the number (or rate) of excited molecules P*}}{\text{the number (or rate) of P molecules formed}} \tag{7}$$

3) Efficient luminescence quantum yield Φ_1

$$\Phi_1 = \frac{\text{the number (or rate) of quanta emitted}}{\text{the number (or rate) of excited molecules P*}} \quad (8)$$

The Φ_1 value and its contribution to the total quantum yield of chemiluminescence are discussed in more detail in the next parts of the chapter.

Values Φ_c and Φ_{ex} are of the order of 10^{-1} and 10^{-5}–10^{-2} for certain model chemiluminescent reactions[51], whereas for low-level biological luminescence they appear to be much lower. In bioluminescence, i.e. the enzymatically controlled chemiluminescence of specific substrates called luciferins, both values approach unity.

Four types of chemiexcitation reactions are presently known which fulfill these requirements, namely: 1) dismutation of peroxyradicals leading to the formation of excited carbonyl compounds or singlet (excited) molecular oxygen, 2) electron transfer reactions, 3) synchronous cleavage of weak C–C and O–O bonds in peroxides, and 4) chemically initiated electron exchange luminescence (CIEEL). The molecular mechanisms of these processes, especially of the enzymatic formation of excited triplet states and electron transfer reactions, are described by Cilento in the next chapter and elsewhere[7, 37, 51]. The question arises, whether these reactions operate in biological systems. To answer this question, a deeper insight into the bioenergetics of living systems seems to be necessary.

Bioenergetics and chemiexcitations

From the most general perspective of bioenergetics, living systems can be considered as chemi-electro-dynamic open systems far from thermal equilibrium. The driving force of life is a reducing potential V_{red} embodied in the reducing equivalent $H^+ + e^-$, i.e., the hydrogen of a substrate. Thus, hydrogen appears to be a common fuel for the whole of the Universe – both for living cells and for stars. The potential realized through transfer of a reducing equivalent reflects the affinity of the reactive component, e.g. the substrate of the redox couple S to donate(S_{red}) or remove (S_{ox}) an electron (e^-):

$$\Delta E = E_0 + \frac{RT}{nF} \ln \frac{[S_{ox}]}{[S_{red}]} [V] \quad (9)$$

where E_0 is the standard potential, R is the gas constant, F is the Faraday constant and n the number of e^- per g-equivalent transferred. The magnitude ΔE is associated with ΔG by the equation (10):

$$\Delta G = -nF\Delta E \quad (10)$$

Biological redox reactions involve transfer of both e^- and H^+ and, as such, V_{red} is influenced by the concentration of protons $[H^+]$, or pH of a solution:

$$\Delta E = \frac{RT}{nF} \ln [H^+] = -0.06\,pH \quad (11)$$

or

$$\Delta E = -0.06 \frac{a}{n} pH \quad (12)$$

at T = 298 K, where a is the number of H^+ transferred. In biochemistry, the pH of the biological environment is assigned an appropriate value of 7.0, denoted as $\Delta E'_0$. Then span of the redox potentials is visualized in figure 2B for the respiratory chain. The stepwise release of energy during oxidoreductions of the substrate and consecutive e^-/H^+-carriers, e.g. NAD/NADH, FAD/FADH etc., is controlled by the spaciotemporal organization of the $H^+ - e^-$ processing within compartmentalized subcellular structures, e.g., the inner mitochondrial membrane, as shown in figure 2. Each step liberates small portions of energy which are adequate for the biosynthetic and homeostatic requirements of the cell, but too small for direct electronic excitation according to the second criterion (equation 6). Thus, the probability of the generation of excited states and photons is very low from the energetic point of view (low Φ_{ex} values). However, experimental evidence leaves no doubt that living systems do create electronic excited states and photons. This fact is interpreted in the framework of contemporary biochemistry as follows; the biochemical pathways of cell metabolism involve some of the four basic types of chemiexcitation reactions. As it is seen from the generalized model (fig. 2), the primary events leading to chemiexcitation are initiated by the electron leakage from the e^--respiratory chain and the consecutive formation of active species such as O_2^-, $OH\cdot$, $^1O_2^*$, H_2O_2, Fe^{2+}/Fe^{3+}. Excited carbonyl compounds $^{1(3)}(>C=O)^*$ and dimoles O_2^* ($^1\Delta_g$) are produced by the dismutation of peroxyradicals and peroxide cleavage (for more information see references 5, 30, 46, 51). This model exemplifies a wide-spread interpretation of low level biological luminescence as an adventitious, nonfunctional event arising in exothermic oxygenation and in radical reactions of a substrate molecule involved in a specific biochemical pathway (the so-called theory of bioenergetic 'imperfections' or 'e^--leakage'[30, 37, 51]). It is worthwhile to emphasize that recombination reactions of certain radicals do release up to 480 kJ/mol energy, and this is sufficient to generate UV-photons of about 230 nm, i.e., close to the mitogenetic radiation range.

Relaxation of excited states-luminescence

An excited atom or molecule P* on the higher energy level E_1 will decay on a lower energy level, e.g. E_0, losing the energy $E_1 - E_0$. When this energy is spontaneously emitted as a quantum of the electromagnetic field, i.e., a photon, we call it spontaneous emission (fig. 1). Its rate is given by (13):

$$\left(\frac{dN_1}{dt}\right)_{sp} = A_{1\to 0} N_1 \quad (13)$$

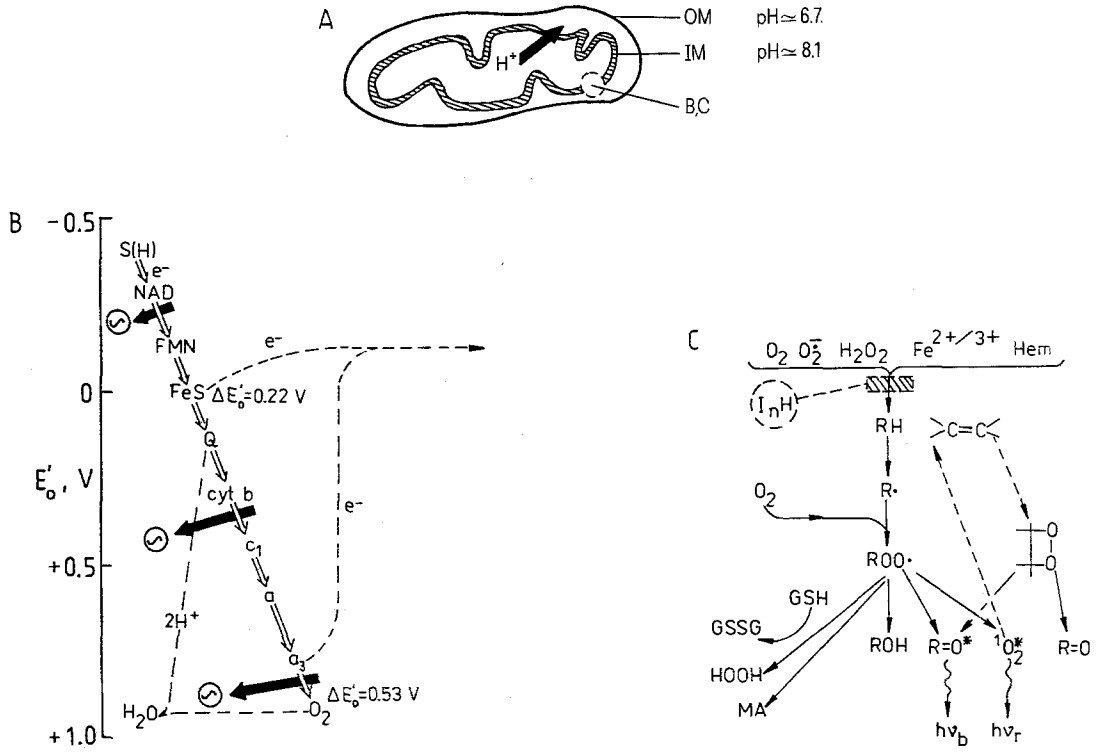


Figure 2. A generalized scheme depicting the link between an electron (e^-) leakage from the respiratory chain and ultraweak luminescence. The e^--transfer system (B) is located within the inner mitochondrial membrane (IM) and coupled to the proton gradient, protomotive force and proton H^+-transport from IM toward outer membrane (OM). Radical reactions (C) involving unsaturated fatty acids and other susceptible compounds (RH) lead to the generation of electronically excited products: carbonyl compounds (>R=O*), singlet molecular oxygen ($^1O_2^*$) and alcohols (ROH, not electronically excited). $^1O_2^*$ can oxidize unsaturated compounds >C=C< such as lipides, phospholipids, ubiquinones, etc., that produce dioxetanes H−C(O)−C(O)− which decompose in chemiluminescent reactions. The excited species decay, with emission in the blue ($h\nu_b$) and red ($h\nu_r$) part of the spectrum. Competitive non-chemiluminescent pathway produces malondialdehyde (MA) and peroxide HOOH via the glutathione redox system (GSH-GSSG). The rate of oxidative chain reactions is controlled in vivo by inhibitors (antioxidants, InH), such as tocopherols, vitamin A, C and K, which scavenge active radicals R'.

where $A_{1\to 0}$ [s^{-1}] is the Einstein coefficient for spontaneous emission, and its reciprocal value $\tau_{sp} = 1/A_{1\to 0}$ is the spontaneous lifetime or radiative lifetime. The decay $E_1 \to E_2$ or $E_1 \to E_0$ may also occur through nonradiative processes which compete with radiative ones decreasing the Φ_1-value. In the biological medium certain substances such as water and molecular oxygen enhance nonradiative processes, diminishing the intrinsic Φ_1-value of an excited state. These quenching processes are partially responsible for very low effective Φ_1-values of biological luminescence.

Stimulated emission can occur when a photon encounters a higher (excited) state. Its ratio is given by the following kinetic equation:

$$\left(\frac{dN_1}{dt}\right)_{st} = B_{1\to 0}\, N_1\, \varrho \qquad (14)$$

where $B_{1\to 0}$ is the Einstein coefficient for stimulated emission, ϱ the radiation density, and N_1 the number of atoms or molecules in the energy level N_1 per unit volume (population density). In thermal equilibrium the higher energy states are less populated than the lower states in accordance with the Boltzmann distribution:

$$\frac{N_1}{N_0} \sim e^{-\frac{E}{kT}} \qquad (15)$$

where k is the Boltzmann constant. Because ϱ is small, events of stimulated emission are far less frequent than those of spontaneous emission. Moreover, the probability for the latter increases with ν^3. This reflects the fact that an excited species can spontaneously decay into many modes ($8\pi\nu^2/c^3$ modes per unit volume, according to the Einstein equation), whereas stimulated emission is prescribed in the single mode of the incident photon. However, in the living cell which is far from thermal equilibrium, the density of excited species and photons is by many orders of magnitude, up to the factor 10^{40}, higher than in thermal equilibrium. Proportionally to this increases the probability of stimulated emission – an

intriguing problem that is discussed later in this and other chapters.

Spontaneous emission from singlet excited states, i.e. fluorescence occurs at a much higher rate than that from triplet excited states, i.e., phosphorescence (fig. 1). This is due to the lifetime of singlet excited states ($\tau^* \simeq 10^{-9}$ s) being shorter than that of triplet ones ($\tau^* \geq 10^{-6}$ s). On the other hand, triplet states are energetically more easily accessible than singlet ones due to the lower-lying energy states, and the ratio of population number N_T/N_S is usually higher than 1 (neglecting quenching processes). Therefore emission spectra of biological luminescence may consist of the superposition of fluorescence (16a) and phosphorescence (16b):

$$^1P^* \rightarrow {}^1P_0 + h\nu_{fl} \quad (a) \qquad {}^3P^* \rightarrow {}^1P_0 + h\nu_{ph} \quad (b) \qquad (16)$$

with some contribution of low-lying vibrational levels $v = 1-2$. Such spectra have been observed in the autooxidation of hydrocarbons[49] which is very similar to the lipid peroxidation in biological membranes[2, 51]. In both cases excited ketones and aldehydes in the lowest excited singlet and triplet states are produced and radiatively decay. The overlapping of fluorescence and phosphorescence spectra of a variety of emitting species in biological samples may account for very broad and diffuse spectra ranging from the near IR to UV[3, 5, 7, 27, 30, 36, 51].

Direct emitters of low level biological luminescence

Efficient emitters should have low-lying excited states and high values of the luminescence quantum yield Φ_1. Therefore most presently known efficient chemiluminescent systems involve large molecules with easily polarizable and thus excitable π-electron systems, such as flavins, indoles, porphyrins, carbonyl derivatives of aromatic compounds, heterocyclic rings like purines and pyrimidines and species-specific compounds evolved in bioluminescent organisms, the so-called luciferins. These compounds have Φ_{fl}-values higher than 10^{-2} due to the short lifetime of the singlet excited state and some of them are shown in figure 3. Good candidates for direct emitters, especially in the near UV range are tryptophan and its oxidative degradation product – N-formylkinurenin[40] as well as nucleic acids[13, 27, 30]. Different ionic and/or radical forms of oxidized/reduced flavins exhibit strong and broad absorption bands as well as emission in a very broad spectral range from near UV to near IR. Flavins are important components of the respiratory chain and their redox reactions produce superoxide ion-radical O_2^- – the species responsible for a number of oxygenation reactions leading to chemiexcitation and luminescence[7, 37, 51].

Experimental evidence accumulated so far indicates that carbonyl compounds $>C=O$ in the excited singlet and triplet states $n-\pi^*$ and excited dimers (dimols) of the singlet molecular oxygen $(^1O_2^*)_2$ are the most common emitters of ultralow luminescence. Carbonyl compounds are a common product of exothermic reactions involved in oxidative metabolism; they have strongly polarizable $>C=O$ groups and relatively low-lying energy levels, especially triplet ones, accessible to chemical pumping. However, in an aqueous solution values of Φ_1 usually do not exceed 10^{-5}. In a hydrophobic and rigid environment, e.g., inside the active center on an enzyme, the rate of radiationless processes decreases. Therefore, in enzymatic reactions involving, e.g., the production of aldehydes or carboxylic acids, the concentration of excited carbonyls and the Φ_1-value drastically increase. Just this situation underlies the so-called 'photobiochemistry without light'[7] and bioluminescence[37].

Molecular oxygen and its various species like O_2^-, OH^-, $OH^\cdot$, H_2O_2 are ubiquitous reactants involved in oxidative metabolism. Figure 4 shows that energy levels of the lowest electronic states of O_2 and its dimole (excimers) require relatively small portions of the excitation energy and reveal many radiative transitions. The emission:

$$(0,0)\ ^1\Delta_g \rightarrow {}^3\Sigma_g^- \quad \lambda = 1.27\ \mu m \qquad (17)$$

can now be recorded with highly sensitive PbS- or Ge-photodiodes. It was found in the enzymatic decomposition of H_2O_2 activated by catalase, lactoperoxidase or peroxidase in vitro systems which model certain biological processes such as, e.g., phagocytosis[7]. This emission is assigned to simultaneous electronic-vibrational transitions involving the electronic $^1\Delta_g$-state and the vibrational transitions $v = 1, 2$ of the solvent molecule[16]. There are also transitions in the UV-region; a very weak emission at 380 and 400 nm, corresponding to the transition:

$$^1\Sigma_g^+ \quad {}^1\Sigma_g^+ \rightarrow {}^3\Sigma_g^- \qquad (18)$$

was observed in a liquid phase[16, 25]. The Schumann continuum

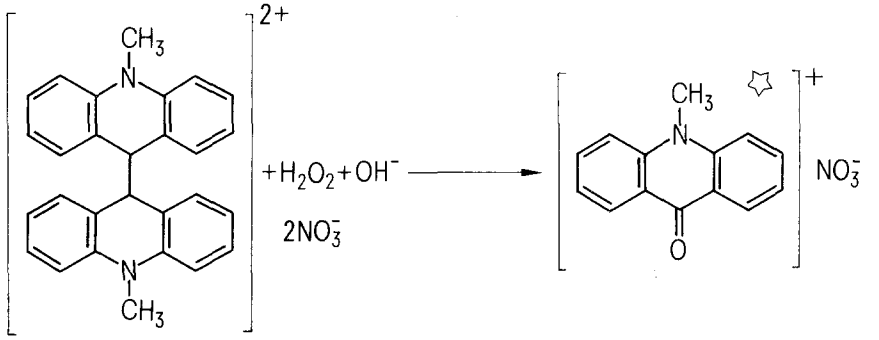
$$^3\Sigma_u^- \rightarrow {}^3\Sigma_g^- \qquad (19)$$

covering the 200–500 nm wavelength range is observed in the gas phase/boundary systems and might be relevant to the spectral distribution of mitogenetic radiation recorded in the spectral range 190–350 nm[11]. Many biological luminescent systems, from expired air of the human breath[50], activated phages[7], and a variety of plant and animal tissues[27, 36, 37, 46] are now being studied from the point of view of possible involvement with $^1O_2^*$.

Energy transfer and indirect (sensitized) luminescence

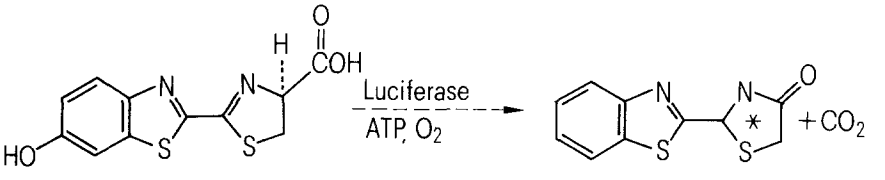
An efficient mechanism of indirect emission may operate when a strongly fluorescent ($\Phi_{fl} \geq 0.1$) molecule A is capable of accepting the excitation energy from the primary excited reaction product P* and to emit its own fluorescence:

$$P^* + A \xrightarrow{k_{ET}} P + A^* \qquad A^* \rightarrow A + h\nu_A \qquad (20)$$



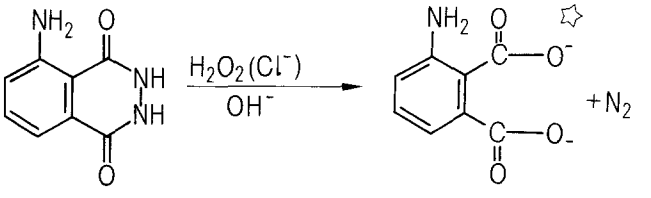
N'N-dimethyl-9,9'-diacridyl nitrate, Lucigenin (**3**)

N-Methylacridone (**3**)
λ_{max} = 470 nm $\Phi_{Cl} \approx 10^{-4}$



Luciferin of firefly *Photinus pyralis* (**1**)

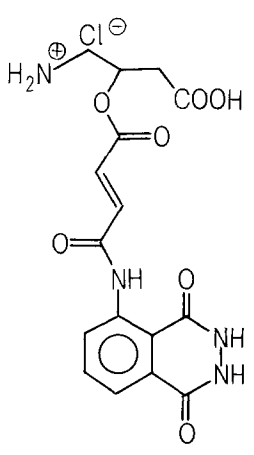
Oxyluciferin (**1**)
λ_{em} = 562 nm $\Phi_{Cl} \approx 1.0$



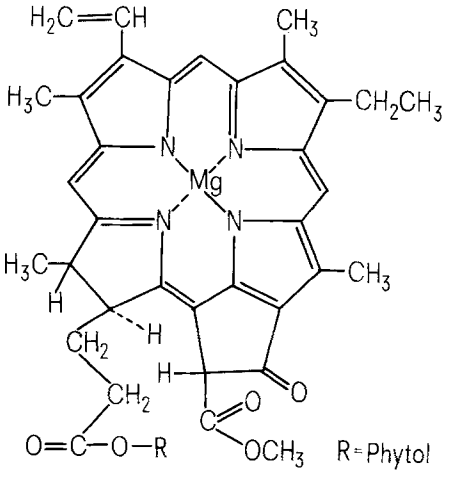
5-Amino-2,3-dihydrophthalazine-1,4-dione, 3-Aminophthalhydrazide, Luminol (**2**)

Aminophthalate dianion (**2**)
λ_{max} = 427 nm Φ_{Cl} = 0,0214

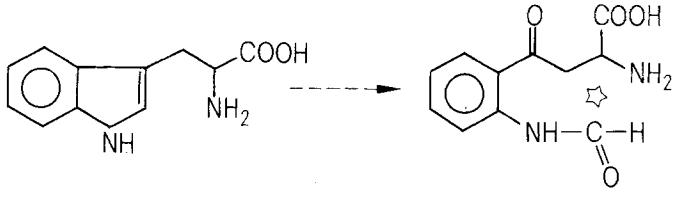
Two modes of this sensitized or activated luminescence may be distinguished: a) if donor P* and acceptor A molecules are not in contact, a long-range Förster-type energy transfer (ET) can take place at the rate constant k_{ET} determined by the distance between P* and A and the spectral overlap between the donor emission and absorption of the acceptor. b) If P* and A come into close contact during the τ^* of P*, then a much more efficient mechanism of ET begins to operate via an electron-exchange. A close packing of molecules in biological structures facilitates this mechanism. Indeed, the highly efficient enzymatic bioluminescence of the firefly, with a total quantum efficiency close to one, involves ET from the excited oxyluciferin to the strongly fluorescent protein of the enzyme luciferase. The observed firefly bioluminescence is a good example of indirect luminescence; the spectrum and Φ_{fl}-value are characteristic of the luciferase (the energy acceptor and emitter). Sensitized luminescence operates also in higher plant tissues. Photon emission from etiolated plants (growing in the dark) reveals maxima at 382, 480, 580 and 640 nm [43]. The spectra are similar to those of model lipid peroxidation [22].



D,L-carnitinyl maleate luminol HCl (**4**)



Chlorophyll a (**5**)
λ_{max} = 682 nm



Tryptophan (**6**)
λ_{max} = 350 nm
Φ_{fl} = 0.55

N-Formylkinurenin (**6**)
λ_{max} = 430-445 nm

Figure 3. Examples of strongly fluorescent compounds involved in bioluminescence (**1**), chemiluminescence (**2**, **3**), and possibly in ultraweak luminescence (**6**) and which can be used as specific chemiluminescent probes; site-specific for the inner mitochondrial membrane (**4**) and for the detection of excited carbonyls (**5**).

and are ascribed to the emission of $^1O_2^*$ and $>C=O^*$. Green plants emit predominantly in the red ($\sim$ 650 nm). The above data and in vitro experiments with chloroplasts [7] provide strong evidence for the in vivo electronic excitation ET from primary P* to functionally important systems, e.g., photosynthetic ones predicted by the theory of 'photobiochemistry without light' (see the next chapter). An important case of the secondary luminescent processes is the so-called triplet–triplet annihilation that leads to the production of higher-energy excited states and photons:

$$^3P^* + {}^3P^* \rightarrow {}^1P^* + {}^1P \rightarrow 2\,{}^1P + h\nu_{fl} \qquad (21)$$

This process, analogous in some respects to the 'higher harmonic modes' acquisition of laser light, is diffusion-controlled and may play an important role in certain cases of biological luminescence which are mentioned in the next section. Here one should emphasize the possible contribution of the triplet–triplet annihilation to the emission in the UV, observed during synchronized cell divisions. Such a possibility has not yet been exploited in the interpretation of mitogenetic radiation.

The overall or total quantum efficiency Φ of a luminescence generated in a chemical reaction is thus given by

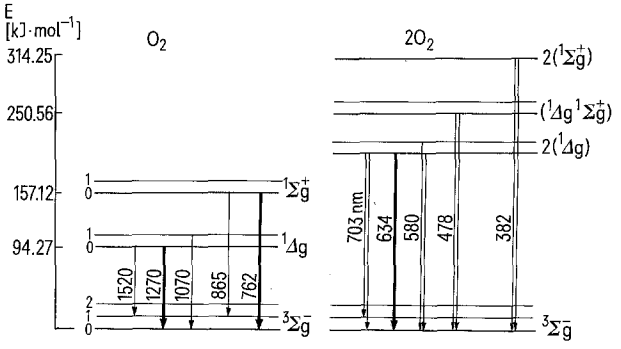


Figure 4. Lowest electronic and vibronic energy levels of the ground-state and excited oxygen molecule and its excimers (excited dimoles). Double and thicker arrows symbolize simultaneous and more probable transitions, respectively.

the equations

$$\Phi = \Phi_c \Phi_{ex} \Phi_1 \text{ (a) and}$$

$$\Phi_{sen} = \Phi_c \Phi_{ex}(P^*) \Phi_{ET}(P^* \rightarrow A) \Phi_1(A^*) \text{ (b)} \quad (22)$$

which are valid for the direct (a) and indirect (sensitized, b) chemiluminescence, respectively. The range of Φ-values is 10^{-15}–10^{-10} for spontaneous ultraweak luminescence, 10^{-12}–10^{-4} for model biochemical reactions, and close to one for bioluminescence. The equation 22 permits the calculation of partial quantum efficiencies, e.g., Φ_{ex} or Φ_1 when values Φ_{ET} and Φ_1 are known. Such calculations are possible only for the simplest model systems and give largely approximated values. The intensity and quantum yield are related by the simple formula:

$$I = \Phi V \left[\frac{\text{Einstein}}{s}\right] \left[\frac{\text{number of } h\nu}{s}\right] \quad (23)$$

where V is the reaction rate of a single energy-pumping step expressed in the number of molecules reacted per s. The formula enables determinations of the concentration of reactants which participate in a light-producing reaction, since V is proportional to the concentration under appropriate conditions. Such determinations are possible for a limited number of well-defined chemiluminescent and bioluminescent systems[9]. In the case of ultraweak spontaneous emission, only very rough estimations of, e.g., the rate of oxidative reactions, or concentrations of peroxy-radicals, are possible[2, 51]. In this respect the use of specific chemiluminescent probes, combining high selectivity with light amplification, seems to be very promising[12].

It is worthwhile to emphasize the uncertainty in determinations of basic parameters of low level luminescence caused by changes of these parameters due to interactions of the electromagnetic field with the extremely complex biological medium. The strong absorption of light in biological tissues prevents the detection of the primary source of the photons. Values of I, I (λ), Φ, etc., measured experimentally, do not necessarily characterize the primary emitter. These considerations are particularly pertinent to the problem of mitogenetic radiation measurements since the biological medium strongly absorbs and scatters light just in the UV-range, and measured values of I and Φ might be underrated by a factor of up to 100. Previous research in this field has not sufficiently analyzed this aspect and the resulting implications.

The possibility of photoemission from collective molecular interactions

The criteria of chemiexcitation described above for the condensed homogeneous phase are not, perhaps, pertinent to a heterogeneous, anisotropic biological medium with high values of gradients. The question arises: is it possible that molecular interactions collectively accumulate small portions of energy until the threshold value $E \simeq hc/\lambda_{min}$ is reached? There are at least two classes of luminescence generated without chemical reactions sensu stricto, i.e., without reorganization of strong chemical bonds, namely: 1) crystallo- and lyo-luminescence, where light emission accompanies growth and solubilization of crystals, and 2) emission associated with water penetration into biopolymers and dry biological objects such as seeds and spores[3, 39, 41]. The existence of photon emission in such processes strongly suggests that small portions of energy can accumulate to the extent necessary for electronic excitation. Hypothetical mechanisms of certain processes involving weak intermolecular couplings probably resulting in photon emission will therefore be briefly discussed.

The relaxation of superhelical DNA

Nearly all naturally occurring circular DNA molecules are negatively supertwisted. Energy is required to convert a relaxed DNA molecule into a supertwisted one (fig. 5). For example, the formation of 15 superhelical turns in SV 40 DNA costs about 420 kJ/mol[47]. Supertwists must be relieved at the replication fork that rotates 100 times per s, and DNA-gyrase-topoisomerase is involved in this process. No ATP is needed to catalyze this thermodynamically favorable relaxation. The relaxation liberates the amount of free energy $\Delta G = -225$ kJ/mol from 9 relieved supertwists. If this process take less than 0.01 s,

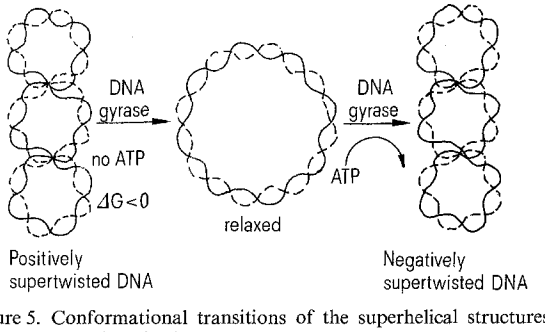


Figure 5. Conformational transitions of the superhelical structures of DNA. Explanations in the text.

the production of the lowest triplet state $\pi - \pi^*$ of thymidylic acid could be possible. There is also the possibility of excimer formation of polynucleotides at room temperature within the lowest long-living triplet states of DNA. The excimer complex is relatively stable and forms a photon trap since its free energy is lower than that of the molecular fragments:

$$M_1 + M_2 \xrightarrow{h\nu} (M_1M_2)^* \quad (24)$$

The natural tendency of exciplexes and excimers to absorb photons and to create excited states associated with ordered and more compact biostructures, e.g., condensed chromatin in the nucleus, fits well to the idea that the relaxation of DNA-superstructures releases photons [23, 27, 30, 33].

Molecular interactions in the electric field of biomembranes

Electric fields in biological microstructures can reach very high values of the order of 10^6-10^8 V/m. The existence of such high field strengths implies that the nonlinear response of molecular interactions should be taken into account. Every electrically charged particle passing the membrane is exposed to this field and its polarization may be dramatically changed. For example, a large change of a dipole moment

$$\mu = \sum_i q_i r_i \quad (25)$$

in a polarizable molecule with q_i charges at r_i distances may be induced and change the probability of the generation of excited states. The energy $E = q\,\Delta U$ which an ion gains in such a field is on average 10 kJ/mol. About 20 accelerated ions have to interact within a limited space and time to accomplish the energy accumulation necessary for the electronic excitation. The probability of such an event would be extremely low but finite, fitting well to the extremely low luminescence yield of the order of $10^{-14}-10^{-10}$ observed, e.g., in the case of crystalloluminescence.

Another source of photon emission might be the inactivation of a neurotransmitter, e.g., acetylcholine (AC) in the synaptic cleft – a gap of about 50 nm separating a presynaptic membrane from the postsynaptic one. The resting potential V of a postsynaptic membrane or a motor plate is about − 75 mV. Other gap-junctions have 15–20 nm diameter holes and $\Delta U \simeq 150$ mV. The full depolarization of the end plate elicited by an action potential results from the synchronous release of about 100 packets (vesicles) of AC from the presynaptic membrane in less than 1 ms. Each packet contains of the order of 10^4 AC molecules with the internal concentration of AC approaching 1 molar. Changes of the μ-value and reorientation of AC dipoles within a vesicle under the influence of the external field can occur only in a collective way. This obviously results from the tight-packing of AC molecules and high gradients of the electric field. The reorientation of dipoles should create electric polarization waves the energy of which might coherently accumulate, leading to electronic excitation. It has been shown in in vitro experiments that the biochemical inactivation of AC after the exocytosis goes along with a photon emission [49]. The relevant process is the hydrolysis of AC at the postsynaptic membrane that restores its polarized initial stage, i.e., the resting potential:

$$AC^+ + H_2O \rightarrow CH_3 - C(=O)(-O^-) + HO-CH_2CH_2-N^+-(CH_3)_3 + H^+ \quad (26)$$

The reaction catalyzed by AC-esterase is very fast (< 400 ns) and releases $30 \leq -\Delta G \leq 130$ kJ/mol as estimated from the energy reorganization of bonds and hydration of ions. The entire depolarization may be considered as 'synchronous' because as many as 10^6 AC molecules undergo hydrolysis during this time. Therefore the probability of an energy accumulation of 3–10 single hydrolysis reactions in close proximity to achieve the threshold of electronic excitation is high [49].

Other weak interactions pertinent to a possible photon emission could be an electrogenic activity of the Na^+-K^+-ATPase pump and a cooperative opening of the cell-to-cell channels associated with the action of Ca^{2+} ions. These events generate an electric current across the plasma membrane with the maximal turnover $\simeq 100\ s^{-1}$ at the time of peak conductance. Indeed, a weak luminescence accompanying nerve stimulation and muscle contraction has been reported [10, 11].

The possibility of population inversion and stimulated emission in electrogenic structures

Endogeneous electrical fields in membranes can create favorable conditions for population inversion and stimulated emission based on metabolically generated electrochemiluminescence. These conditions are the following:

1) Metabolic redox reactions produce ion-radicals $M^{\dot{+}}$ and $M^{\dot{-}}$, donor and acceptor, respectively, the concentration of which can reach the molar range in certain organelles, e.g., in the inner mitochondrial membrane (fig. 2). Recombination of these species generates singlet and triplet excited states.

2) Living systems operate at low redox potential, less than the ΔE of the redox couple H_2/O_2, equal to 1.4 V, but at high-density ionic currents. These conditions are sufficient for both the generation of $M^{\dot{+}}$ and $M^{\dot{-}}$ and their movement across the electric field. Ubiquitous subcellular membraneous structures form slits, hollows, channels etc. with distances 10–100 Å, in which the U-value reaches 10^6-10^8 V/m.

3) The cellular medium contains: a) ionic compounds which may serve as a supporting electrolyte, and b) a variety of dyes with high values of the absorption cross section and Φ_1, which are good candidates for the active material of a laser. Broad emission bands facilitate the

tuning of a laser and ET-coupled amplification of luminescence output.

High density population of excited states arises directly from the recombination of ion-radicals. The reaction goes without activation energy, is fast enough ($k = 10^7$–$10^{10}\,M^{-1}s^{-1}$) and quite efficient ($\Phi_{ex} = 10^{-2}$–10^{0}). The analysis of a four-niveau energy system indicates that triplet–triplet annihilation can effectively contribute to the population inversion[4].

Another favorable possibility in this respect is the formation of heterogeneous excimers (exciplexes):

$$M_1^+ + M_2^- \rightarrow \left(M_1^+ M_2^-\right) \rightarrow {}^1(M_1 \cdots M_2)^* \qquad (27)$$

Free energy $G(M_1^+ \ldots M_2^-)$ stored in the radical ion pair, i.e., in the encounter complex of the solute ion-radicals is adiabatically and isothermally transformed into excitation energy of the heteroexcimer. Calculations and a few pieces of experimental work on electrochemiluminescent lasers indicate that the reversed population can be achieved in the stationary or in the nonstationary (chronoamperometric) approximation. The power of stimulated emission generated by the triplet–triplet annihilation reactions is about 20 kW and the efficiency 20% for the pulse duration 1 µs, the radical-ion concentration $10^{-6}\,M^{-2}$, $\lambda = 500$ nm at minimum cavity loss[4]. A wave-guide laser with the longitudinal propagation of the radiation would meet well the topology of subcellular structures. Analysis of the energy and dynamics of photon field of a four-level excimer open system far from thermal equilibrium shows that a distribution inversion due to a biological pump may bring to the laser-like effects in both bioluminescence and ultraweak photon emission[21].

Collective excitations in perturbed water and cytosol

The analysis of the water-ion macromolecule system in membraneous structures shows that cellular water exists in a physical state sufficiently ordered to exclude solutes, e.g., certain ions, to create an extremely polarizable medium with $H_5O_2^+$ or $H_3O_2^-$ ions in which metastable, dynamic states are possible. The evidence for this comes from a cooperative interaction between the majority of ion-absorbing sites that replace K^+ by Na^+ ions. The sigmoidal nature of the equilibrium distribution isotherm of cellular K^+ and Na^+ is analogous to critical or collective phenomena in general[8, 24]. A strong evidence that collective molecular interactions can produce macroergic effects leading to the electronic excitation comes from experiments with water-induced luminescence of lucigenin in oriented polar solutions (micelles)[26]. It has also been well established that the addition of water to dry seeds[39] or spores of fungi[41] produces instantaneous weak luminescence. Physico-chemical processes of water penetration earlier than the onset of germination, probably involving hydrophilic coherent interactions within a limited space at the water-macromolecule boundary, are considered as a possible source of excitation. Whether a sort of Bose condensation of coherent photons with the same vector of momentum $\vec{p}$ and a constant phase relation ($\Delta\varphi = 0$ or const), or the accumulation of energy in, e.g., 'molecular constellations' might account for these phenomena, remains to be decided. Although the above considerations have mainly heuristic value and require verification, they show that certain conjectural or yet unexplored problems can be solved on realistic bases.

Luminescence response to internal and external factors

Perhaps the most important feature of low-level luminescence is its inherent association with fundamental biological processes such as cell division and death, oxidative metabolism, carcinogenesis, etc., as well as its high sensitivity to environmental stresses. Therefore the intensity, kinetic pattern, photocount distribution and perhaps other parameters of luminescence reflect a subtle balance of the biohomeostasis and the intactness of the organism[45]. The question 'how much information can be retrieved from measurements of various parameters?' is discussed elsewhere[13, 37]. Here we will focus our attention on two effects: 1) luminescence accompanying mitosis and cell death and 2) non-linear response to external temperatures – the two internal and external factors which impose order and disorder upon the biological system.

Mitogenetic and necrobiotic radiation

The emission of the UV component (180–350 nm) of mitogenetic radiation observed during the mitosis of synchronized yeast cell cultures requires a pumping (excitation) process that releases $-\Delta G = 330$–650 kJ/mol. During enzymatic oxidations and other reactions minute amounts of OH·, O·, NH·, NH_2 and HC=O· radicals and atoms may be formed. Their recombination supplies energy enough to excite the UV-levels of the resulting products[34, 42, 46, 48, 51]. One can not exclude the probability that these processes are responsible for luminescence in the UV range of the total spectrum (fig. 6). A maximum in the blue part of the emission continuum of many biological objects indicates that part of the excitation energy is emitted in the UV-range. Perhaps any process enhancing the production of light in the cell also increases the intensity of the UV component. This might occur during mitosis and might fit the recent observation that the intensity of light correlated with the rate of cell division[51]. On the other hand, experiments with cytochrome-deficient yeast mutants suggest the involvement of O_2^-, the amount of which correlates with the total number of viable cells[32].

Thermodynamic analysis of photon emission performed by Popp et al.[27, 28] provides data on the energetic aspects of mitogenetic and necrobiotic radiation. The spectral

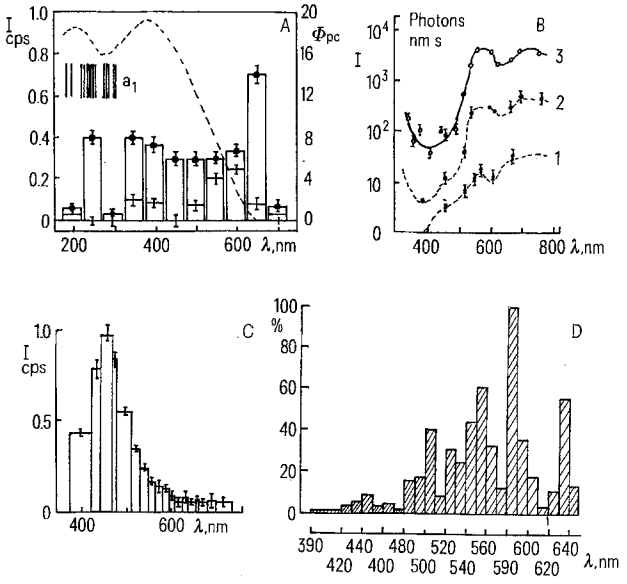


Figure 6. Examples of ultraweak emission spectra from various objects: *A* Synchronized yeast culture (†), medium alone (I), spectral characteristics of the photomultiplier used $\Phi_{pc} = f(\lambda)$ (– – – –)[31]; a_1, emission bands from a rabbit heart muscle under narcosis determined by means of the biological detector – a synchronized yeast culture[11]. *B* Germinating cucumber seeds: native, not perturbed (1), treated with a cytostatic Cialit® (2) and with acetone (3)[30]. *C* Model reaction in vitro between cytochrome c and hydrogen peroxide under physiological conditions[42]. *D* Lipid peroxidation with rat liver microsomes in vitro[22].

occupation number densities $g(\lambda)$ calculated from the emission spectra of several plants[27, 31, 43] obey the condition: $g(\lambda)$ = constant for 10^{14}–10^{15} Hz. This means that the system generating photons is far from thermal equilibrium and implies laser activity in a nonstationary case (see the ratio-value $N_1/N_0 > 0$ in eq. 15). Non-equilibrial constellations postulated by Gurwitsch[11] resemble local metastable excited states of the cytosol mentioned previously, and perhaps the energy-rich superhelical structures of DNA in nuclear chromatin. These states should exhibit absorption and emission spectra in the UV. Thus, the absorption of a UV photon by the excited state results in induced emission (2 hν). Therefore these states or 'nonequilibrial constellations' act like relay stations and may explain the mechanism of the so-called secondary radiation (see the first chapter). Dynamical rearrangements of local excited states to lower energy levels release energy, part of which may be emitted in the UV-range. This mechanism might account for the degradation radiation emitted by cells stressed with cooling, narcotics or mechanical or electrical stimulation, and for the necrotic radiation accompanying the death of cells. The constant $g(\lambda)$ values also mean that biological systems exhibit an optimum response on external influences; for instance 1 or 2 UV photons absorbed by a cell can trigger mitosis or other critical processes. Relevant to this are theoretical considerations[9] showing that the electromagnetic response of the living system depends upon the total load of energy stored in such excited molecular structures. In the extreme case that such a structure was already highly excited, the arrival of just one photon could trigger the discharge of a sizable amount of electromagnetic energy. Thus, the electromagnetic response of a living system depends not only on the incoming signal, but also on the memory of past excitations. As seen from figure 6 A, the degradation spectrum and also the mitogenetic one can be recorded with amazingly high spectral resolution by means of a biological detector; in this case a synchronized yeast culture[11]. Although such sharp resonances cannot be excluded, much more evidence is necessary to prove their existence unambiguously.

The question of whether the mitogenetic effect does exist is still a subject of conjectural opinions in the relevant literature. However, more and more evidence is now available that: 1) natural chronic low-level radiation of cosmic, telluric and technical origin stimulates cell proliferation[20], 2) low-intensity UV-laser radiation stimulates DNA synthesis and increases cell membrane permeability[14], 3) selective killing of malignant cells by postulated UV-radiation from normal yeast cells has been reported[6], and 4) there is an increasing amount of work proving the significant effect of the UV component of weak biological luminescence exerted upon various biological processes[1, 15, 17].

The yield of photon emission appears to be surprisingly high: 0.07–0.7 photons/cell division[31] and about 10 photons per cell during interphase[18]. Similarly, the number of photons emitted by dying single cell cultures is of the same order of magnitude as the number of cells[11, 27, 30]. This is an indication of the association of luminescence with cell division or cell death rather than with accidental chemiluminescence from oxidative radical reactions. However, in multicellular higher organisms, e.g., molluscs, worms and insects, the number of photons emitted in a 'death flash' is lower than the number of cells constituting the whole organism. In this case a strong coupling of molecular oscillatory circuits emitting photons and an internal absorption cause a significant decrease of the number of photons[45].

Temperature hysteresis of low level luminescence

The response of biological systems to alterations of the temperature of their environment has been recently described in terms of the physical model of dissipative structures of thermodynamically open systems which are far from thermal equilibrium[29]. A nonlinear response of radiation to temperature T is manifested by damped oscillations on the $I = f(t_T)$ curves and by non-constant values of activation energy[29, 39]. A significant evidence of nonlinearity and collectivity comes from measurements of the luminescence intensity as a function of cyclically changed T[38, 39]. As can be seen from figure 7, all curves $I = f(T)$ for dT/dt = const exhibit more or less open loops resembling a typical hysteresis phenomenon. The area of the loop calculated as a $\oint I\,dT$ depends on the

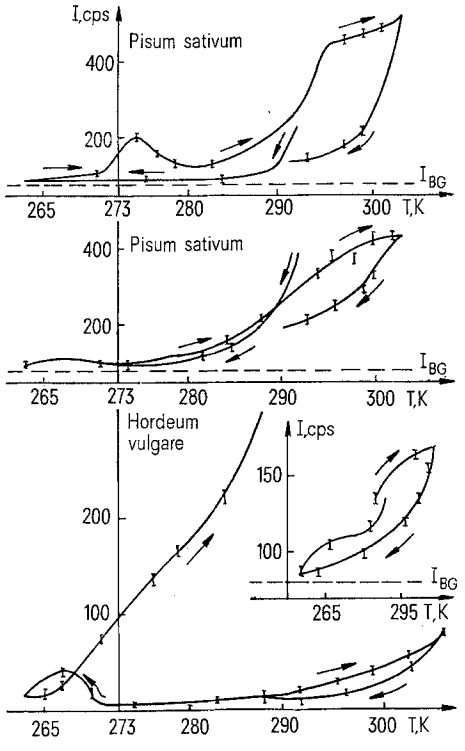


Figure 7. The response of the luminescence intensity to cyclic changes of temperature shows hysteresis-like loops. The rate of temperature (T) changes was 0.5 K · min^{-1} = const. Germinating seeds 4–6 days of green pea (*Pisum sativum*), green and etiolated (the insert) barley (*Hordeum vulgare*) were 30-min dark-adapted in order to eliminate photosynthetic delayed luminescence. Arrows indicate the direction of T-changes. Similar results have been obtained for many other plants[38].

T-range of a cycle. If the range includes the so-called low T-glow limit, i.e., the low-T maximum determining the lower limit of adaptation capacity of a plant (e.g., the maximum at − 8 °C in fig. 7) and/or the upper critical T-point (not shown in fig. 7), the loop is largely open and has a large area. A closer analysis of this phenomenon reveals its analogy to the Curie-Weiss low and the possibility of using certain parameters of the hysteresis loop, e.g., its area and/or the degree of openness, as a relative measure of the deviation from homeostasis. This clearly nonlinear and collective behavior points to the 'memory' of the photon-generating or storing system and cannot be understood in the framework of the classical biochemical model based on compartmentalized reactions[44].

Conclusions

The main purpose of this chapter was to present the biochemical side of processes creating photons in biological systems. As we have seen, the general picture presents a highly fragmented status rerum, largely dominated by the reductionistic paradigm of contemporary biochemistry. Thus, biological luminescence appears to be a heterogeneous phenomenon originating from multiple enzymatic and nonenzymatic reactions occurring in different organelles as a side-product of exothermic processes. Although methodological reductionism may be accepted as leading to a deeper insight into particular structures and reactions, some integrative principle responsible for the coherent and holistic interplay between radiation and matter seems to be necessary for the better understanding of ultraweak cell radiation and its biological role. Therefore, we have tried to emphasize certain aspects of biochemical processes and show examples indicating the collective character of photon generating processes and their possible subordination to more general, unifying factors.

Acknowledgments. This work was supported by the Polish Academy of Sciences, project no. CPBP 01.12.37.1.

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