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# Stress-induced photon emission from perturbed organisms

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*Abstract.* This paper reviews an ultraweak luminescent response of selected biological systems (lower and higher plants, insects and spermatozoa) to certain kinds of detrimental mechanical, temperature, chemical and photochemical stress and to lethal factors. The enhancing effect of white light and formaldehyde on the ultraweak luminescence of yeast and spermatozoa cells is described for the first time. An increase in the percentage of long wavelengths ($\lambda > 600$ nm) with an increase in reaction time, and a significant influence of the suspending medium on the ultraweak luminescence, were observed. The vitality and motility of bull spermatozoa and the vitality of yeast cells were drastically decreased by treatment with white light, water, formaldehyde and iron-ions. Successive irradiation of intact bull spermatozoa cells with white light caused an increase in the intensity of delayed luminescence. An attempt has been undertaken to find stochastic models of non-stationary photon emission. The quasi-relaxation descending stage of non-stationary processes can be modeled as the Integrated Moving Average process IMA(0, 1, 1), and memory and transfer functions can describe the degree of perturbation in the yeast *Saccharomyces cerevisiae*. The relation of the ultraweak luminescence response to perturbations of homeostasis is discussed in the framework of biochemical and physical models.
*Key words.* Photon emission; perturbation of homeostasis; stress; peroxidative damage.

## *1. Introduction*

Ultraweak photon emission (UPE), also called 'biophoton emission' or ultraweak luminescence, is the emission of electromagnetic radiation in the spectral range 180–1500 nm, from all sorts of organisms and their tissues, cells and subcellular components in the living state. It is known to occur in conjunction with various vital processes, and is now considered to be a universal phenomenon in nature. The flux of UPE is in general less than $10^{-15}$ W/cm$^2$ ($\lambda = 500$ nm), which is much lower than that of enzymatically-controlled bioluminescence. In non-bioluminescent organisms, the sources of luminescence in the visible region are singlet oxygen and excited triplet state carbonyls. Perhaps the most important feature of UPE is its inherent association with fundamental biological processes such as cell division, fertilization of an egg, photosynthesis, stress, and the death of an organism.

Unlike analytical methods UPE gives holistic information about the biological effect of stress factors, providing an indication of the global, integrated effect of a stress on a biological system (see table 1).

The level of perfection reached in time-resolved photocounting, and in spatial and spectral analysis, offers the necessary prerequisite for studying the information-potential of biogenic radiation. This potential is related to the spatio-temporal and energetic parameters of in vivo-generated electromagnetic (EM) fields. One can arbitrarily classify this information potential into the two following categories:

Table 1. Main features of the biophotonic response to detrimental environmental and internal stress factors

| | |
|---|---|
| Holistic response | Combines the effect of stresses on the homeostatic system (a synergetic superposition). |
| Multiparametric information | Contains information about: species-specificity of a stress factor, the rate and energetics of process, its heterogenity and dynamics. |
| Homeostasis-dependent sensitivity | High or low depending on the efficiency of regulation mechanisms, quantum yield and the rate of processes. Can be enhanced by e.g. fluorescent probes or reversible perturbation of homeostasis. |
| Discrete character of the photonic signal | Inherently quantified signal is ideal for statistical (numerical) procedures, e.g. analysis of stochastic processes, correlation analysis etc. |

*I. Macroscopic*
Integral (holistic) macroscopic response of a biosystem to stimuli (stresses). Correlation between the UPE response and physiological indices may serve for analytical/diagnostic purposes. Possibility of evaluation of the capacity of homeostasis and degree of its perturbation by stress.

*II. Microscopic*
Physically regulated quanta (a discrete spatio-temporal structure of the EM field) may act as an information signal in extra/intracellular communication and regulation of cytobiological processes. Photonic signals can be transformed into acoustic, chemical or electric process within the cell. Possibility that stress may modify the signal (introducing error).

The macroscopic information may be extracted from such characteristics of UPE as its intensity I, kinetic pattern $I = f(t)$, spectral (energy) distribution $I = f(\lambda)$ and statistical photocount distribution $P(n, \Delta t)$, which were previously found to be correlated with biological indices. Monitoring UPE in such cases may serve as a fast holistic method for the evaluation of the effect of detrimental environmental factors on individual organisms, cell populations and biocenoses (ecosystems).

## 2. *Some examples of stress-induced ultraweak emissions*

### *Mechanical stress*

A mechanical factor such as pressure or other injury causing damage to plant tissues increases the I of UPE and brings about kinetic changes from a quasi-stationary (intact tissues) to a non-stationary emission[44, 51, 53]. Plant tissues subjected to mechanical stress emit more radiation in the red (660–800 nm) than intact ones (440–650 nm)[52]. It has been shown that mechanical stress activates peroxidative enzymatic systems[43] and increases the rate of oxidation of plant polyphenols[52]. The latter reactions are accompanied by relatively strong chemiluminescence in the red part of the spectrum[50]. Thus, both these processes contribute to the observed enhancement of UPE. Tryka and Koper[69] have shown that cereal grains with microinjuries caused by a mechanical stress exhibit stronger UPE when soaked with water than intact grains. A reciprocal relationship between microinjuries and germination ability expressed in UPE intensity may serve as a method for the determination of injuries to grain[68].
An immobilization stress applied to rats during 2 h first decreased the intensity of UPE of blood serum in vivo and after 1 day increased it[49].

### *Temperature stress*

The dependence of UPE intensity (I) from plants on temperature (T):

$$I = f(T) \quad dT/dt = \text{const} \simeq 1\ \text{K/min} \qquad (1)$$

has two characteristic local maxima. The first one is the so-called 'low T-glow limit', i.e. the low T-maximum determining the lowest limit of the adaptation capacity of a plant. The second 'upper critical T-point' corresponds to the highest thermal adaptation capacity of a plant species[66]. Very interesting is the oscillatory alternation of luminescence induced by the transfer of plant seedlings from a low T (292 K) to a higher T (303, 308 and 313 K)[36]. The so-called 'T-hysteresis luminescence' which relates the I of luminescence to a cyclically varied T:

$$I = f(T_{cyc}) \quad dT/dt = \text{const} \qquad (2)$$

has been analysed[61, 64]. Certain parameters of the hysteresis loop, e.g. its area and 'openness', might be used as a rough evaluation of the deviation of a plant organism from homeostasis. The oscillations and hysteresis are clearly nonlinear and collective behavior of a living plant tissue, pointing to the 'memory' of the photon-generating biosystems.
Temperature stress not only influences the I of UPE, but also changes its spectral distribution[4, 22]. Figure 1 shows emission spectra from wheat leaves at various temperatures. When leaves are heated at 35 °C, the relative luminescence intensity $I(\lambda)$ in the range $\lambda = 650–720$ nm decreases. A distinct increase of the 650–670 nm emission band and the decrease of the 670–800 nm band correspond to the elevation of T from 35 to 40 °C.
A cold stress decreases the intensity of blood serum UPE from rats in vivo[3] by almost 200%. According to these authors, immobilization and cold stress activate the secretion of catecholamine neurotransmitters (adrenaline, noradrenaline, hydrocortisone) in order to mobilize the energy-producing potential of the organism and to restore homeostasis. The authors claim that these effects are associated with a decrease in the lipid peroxidation rate and a concomitant decrease of the generation of electronically excited molecules P* and subsequent luminescence. However, one has to realize that catecholamines quench ultraweak luminescence of peroxidative enzymatic and nonenzymatic reactions in vitro[55] and in vivo[49].
An attempt has been made to use the probability of photocount-time series (PCTS) distribution:

$$P(n, \Delta t), \text{ where } \Delta t = \text{const} = 0.1 - 1\ \text{s} \qquad (3)$$

as a relative measure of a stress-induced perturbation of homeostasis[22, 37]. Calculations show that the PCTS-statistics of wheat leaves obeys Gauss and Pascal distributions, and that variance increases significantly with the increase of T. The recorded stationary UPE from wheat leaves and camera together is multimodal and chaotic (a 'white noise'). In contrast, distributions from germinating cucumber seedlings have a narrower half-width than those from camera plus seedlings, which suggests a significant degree of coherence of luminescence from the seedlings. In other experiments[21, 62] it has been shown

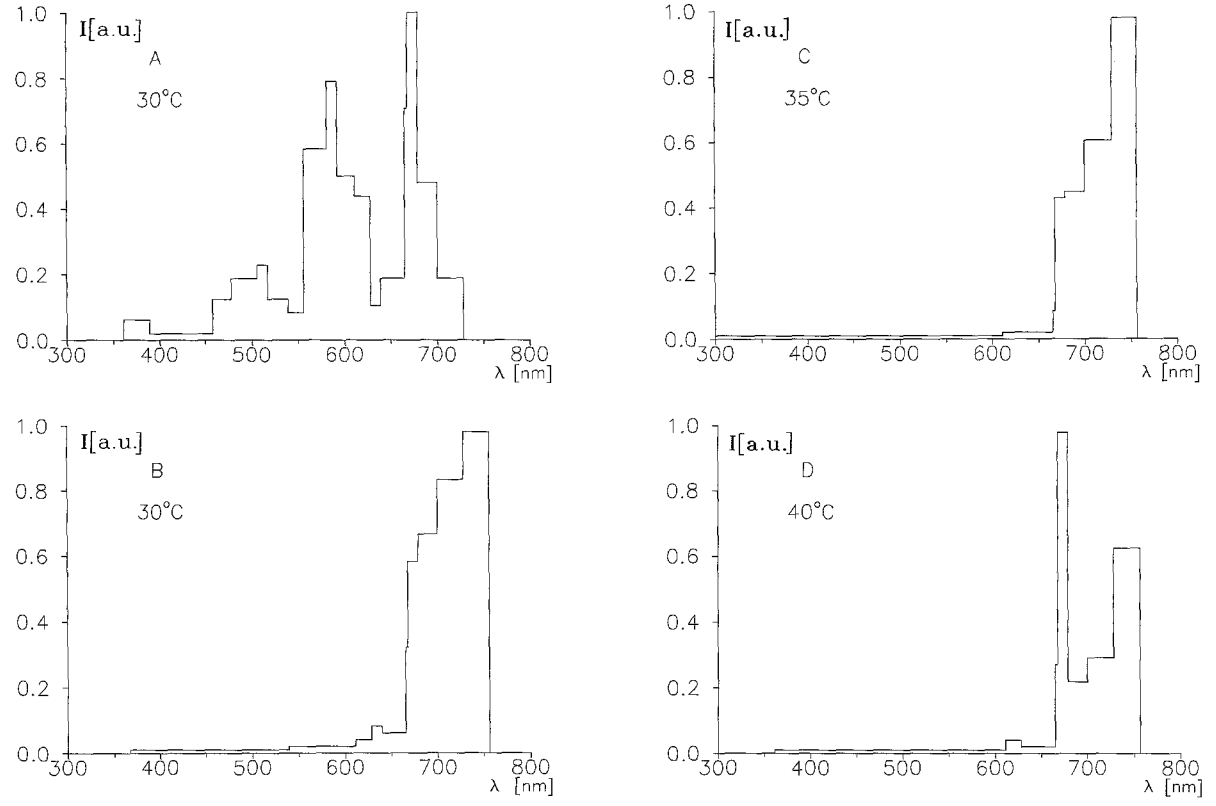



Figure 1. Low level luminescence spectra from etiolated (A) and green (B, C, D) wheat leaves at different temperatures. The maximum error of the relative intensity I($\lambda$) does not exceed 8% and 12% in the range 380–450 nm and over 450 nm, respectively. The width of the rectangles is equal to the difference in wavelength $\lambda$ between two successive cut-off filters. All spectra are corrected for the spectral response of the PM T, transmittance of filters and the background of camera + filters[22].

that above the critical T (e.g. 318 K for wheat leaves), the UPE represents a nonstationary strongly correlated process that does not obey the Gauss distribution[20]. Thus, the results obtained are controversial and this statistical approach does not lead to an unambigous interpretation of PCTS from perturbed and intact plants. Recently it has been shown that nuclei emit extremely weak PE at the phase transition in lipid membranes, of the order of $I = 10^{-5}$ photons/s per nucleus. Measurement of such weak light was only possible with the use of the most advanced SPC image technique. The above process is very sensitive to temperature, so the phase transition point can be determined from the I = f(1/T) dependence with a high accuracy, not achievable with other methods[46].

*Chemical stress*

Various chemical substances cause a considerable change, in the most cases an increase, in the intensity (I) of UPE from plants[42, 51, 66] and animals[33, 57–59]. The change of luminescence pattern may occur immediately after the treatment with the chemical or may be delayed by some minutes or even hours. Until recent Japanese work[45, 72] was carried out, very little was known about concomitant change in the spectral distribution of luminescence; previous published results were controversial. Effects of chemical stress on yeast cells, bull spermatozoa, plants and lower animals are described in the next sections.

*Influence of protein denaturating and redox cycling agents on ultraweak photon emission of yeast*

Our previous research[9] has shown that unperturbed yeast cells produce extremely weak UPE while cells treated with certain chemicals show strong emission. Thus yeast cultures may be used as a simple model organism which give a strong 'contrast' in the UPE-response. We used formaldehyde because it is the simplest carbonyl compound, very reactive and strongly cytotoxic. It occurs in the polluted environment and is formed as a transient unstable metabolite in numerous biochemical transformations, especially of the C1-compound pool. In proteins, the reaction with formaldehyde leads to intra- and intermolecular cross-linking by $-CH_2-$ bridges, and subsequent denaturation[9].

*Media and growth. Saccharomyces cerevisiae* strain SP-4 and lyophilized preparations were used for experiments. Standard liquid YPC medium containing 1% yeast extract, 1% bactopeptone and 2% of glucose was used. Cells were grown at 28 °C to stationary or logarithmic phase.

*Experimental procedure.* The yeast culture was twice centrifuged and resuspended in 0.9% NaCl, pH = 6.5. For

each measurement, suspension containing $10^7$ cells/ml was used. The cells were maintained in complete darkness to avoid the additional photoinduced delayed luminescence. The sample was injected into a scintillation vial placed on the photocathodes of a photomultiplier within a light-tight camera. Oxygenated or anaerobic conditions were maintained during the experiment by passing a stream of argon or air, respectively, through the suspension medium [9]. All measurements of ultraweak luminescence were performed by means of the single-photon counting method. Pulses were counted by means of a 200 MHz PFL-28 type counter. The number of pulses counted in successive time intervals (count rate as a function of time, PCTS) were stored in the memory of an 'on line' connected computer.

UPE emission spectra were determined using a Hamamatsu R1333 PMT and a set of cut-off filters. Fluorescence emission and excitation spectra were measured with a Perkin-Elmer MPF 44a spectrofluorimeter. Oxygen consumption was measured in a thermostated vessel with a Radiometer electrode of the Clark type coupled to an appropriate amplifier and recorder.

*Results and discussion.* Oxygenated yeast cells in normal conditions emit an extremely weak spontaneous UPE. Anaerobic cultures emit no detectable luminescence. Emission intensity from yeast cells treated with formaldehyde depends strongly on the oxygen concentration (fig. 2). The intensity of UPE increases with the increase of the concentration of formaldehyde in the range 0.06–7% by a factor of up to 70, while oxygen consumption by yeast cells decreases [9]. Thus, UPE is not directly connected with the respiration of the cells, but probably with the perturbation of their physiological state. Formaldehyde in concentrations 0.01–7% added to the suspension of yeast cells in PBS or 0.8% NaCl solution strongly enhances the intensity and total output of the photocount (integrated intensity or light sum $\sum n$) independently of the growth phase. When $CH_2O$ is added to living cells, the increase in UPE is slower than in the case of dead cells, but the intensity and $\sum n$ are higher [9]. In the case of dead cells (heated 1 h, 80 °C) the formaldehyde-induced UPE arose mainly from the supernatant [9]. As can be seen from the results presented in figure 3 the spontaneous UPE of native yeast cells in aqueous suspension covers predominantly the red region (approximately 70% of emitted light). In the presence of lethal doses or formaldehyde a blue-green transient emission appears, then it weakens with time and a strong emission in the red region above 600 nm appears.

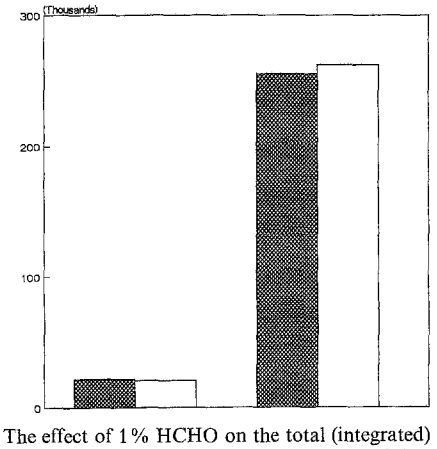



Figure 2. The effect of 1% HCHO on the total (integrated) intensity of photon emission from yeast cells under anaerobic (left) and aerobic (right) condition in the logarithmic ▩ and stationary □ phases.

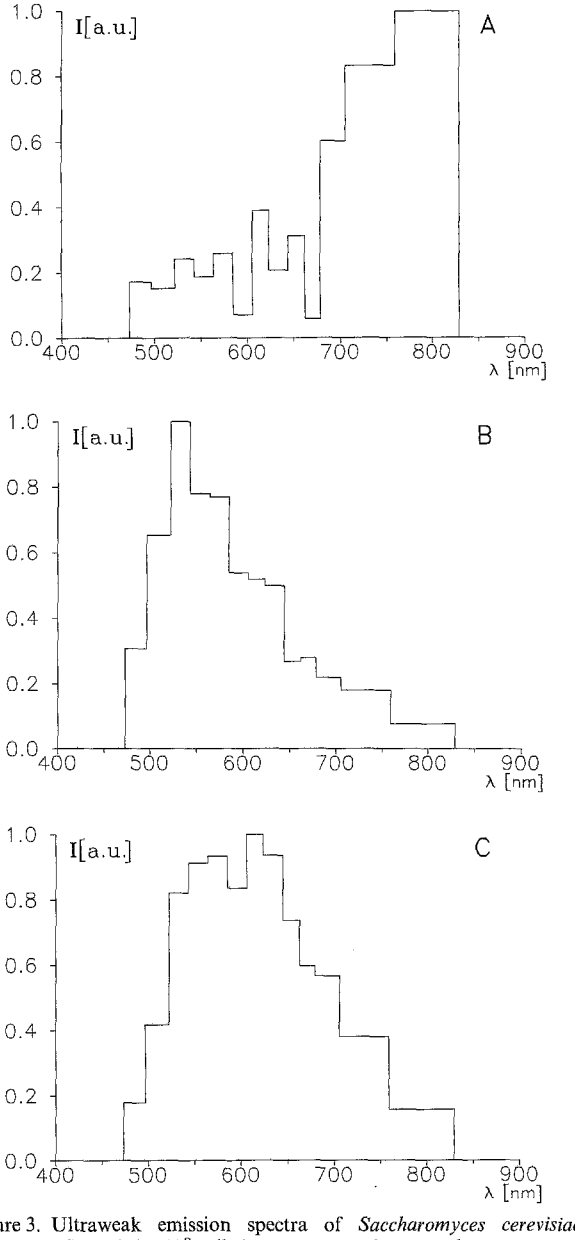



Figure 3. Ultraweak emission spectra of *Saccharomyces cerevisiae* Hansen CBS 5926, $2 \cdot 10^8$ cells in water + saccharose + lactose.
*A* Aerated, 9 min after adding water,
*B* treated with 3.5% (final concentration) $CH_2O$ after 5 min,
*C* treated with 3.5% (final concentration) $CH_2O$ after 472 min.
The spectra were measured using an R1333 Hamamatsu PMT with 13 cut-off filters and counting time 5 s, and corrected for PMT and filter characteristics.

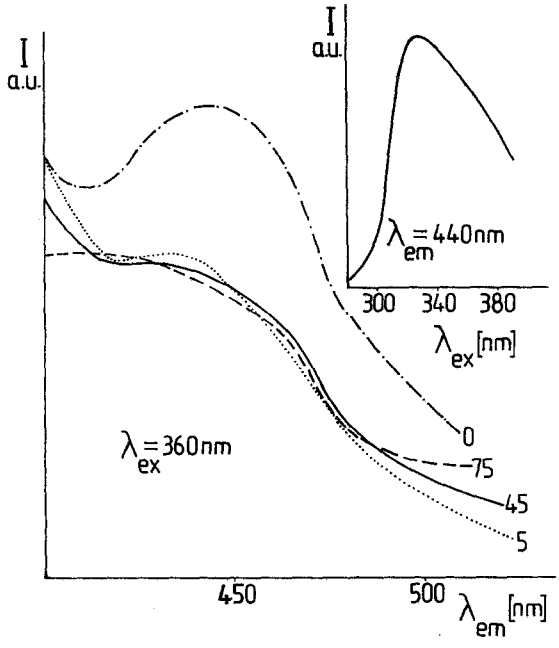



Figure 4. *A* Fluorescence emission spectra ($\lambda_{ex}$ = 360 nm) from a suspension of intact yeast cells (0) and after different times (as indicated by numbers in min) of interaction with 3% HCHO.
Insert: excitation spectrum from the intact cell suspension recorded at $\lambda_{em}$ = 440 nm.

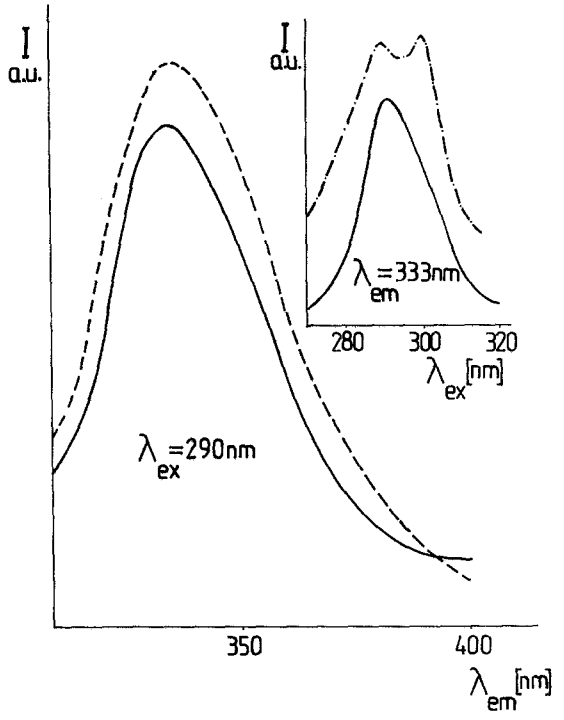



Figure 4. *B* Fluorescence emission spectra of the yeast (*Saccharomyces cerevisiae*) recorded at the $\lambda_{ex}$ = 290 nm and cell density 2 · $10^8$/ml. Intact cell suspension in water ----, in 3% formaldehyde after 1 h of interaction ——.
Insert: excitation spectra ($\lambda_{em}$ = 333 nm) of yeast cells suspension in 3% HCHO after 30 min of interaction -----, and from supernatant ——.

In our experiments a formaldehyde-sensitive weak fluorescence at 435–440 nm ($\lambda_{ex}$ = 360–375 nm) is observed (fig. 4A) in intact cell suspensions and supernatants. It might originate from the fluorescence of thiamine derivatives, e.g. from thiochrome ($\lambda_{em}$ = 425–435 nm, $\lambda_{ex} \cong$ 370 nm) which is the product of thiamine oxidation. N-formylkynurenin derivatives which are products of the metabolic oxidative degradation reactions of tryptophane also emit in this region. Other components of yeast fluorescence are at least 10 times weaker than tryptophane emission/excitation. Among them a clear though low-intensity emission band at 528 nm ($\lambda_{ex}$ = 220, 362 and 440 nm) undoubtedly arises from $^1$S of flavins. No marked changes in intensity are found for intact, formaldehyde or thermally-inactivated yeast cells either in cell suspensions or in supernatants. After a long period of interaction between yeast cells and formaldehyde, only a very weak decrease of the emission band at 333 nm ($\lambda_{ex} \cong$ 290 nm) is observed (fig. 4B), without measurable changes in $\lambda_{max}$, $\Delta\lambda$ or its shape. Therefore one may conclude that the polarity of tryptophane residues is not significantly changed during a relatively short interaction time. This would be in agreement with the formation of hydrophobic $CH_2$-bridges between protein chains in the reaction between the NH- and/or $NH_2$-groups of the proteins and formaldehyde. A strong emission band with the $\lambda_{max}$ = 333 nm, spectral band width $\Delta\lambda$ = 56 ± 2 nm and $\lambda_{max}$ = 285–290 nm, is the predominant component of the fluorescence from suspensions and supernatants (in this case about 40 times weaker) of yeast cells. This band is similar to the spectrum which is considered to arise from the $^1L_a$ state of tryptophane in protein when the tryptophane is buried in the hydrophobic environment. The position of the emission maximum, 333 nm, is at a shorter wavelength than for tryptophane residues which are exposed to water (≅ 350 nm). The excitation spectrum of cell suspensions displays two maxima at 286 and 298 nm rather than at 280 nm, which is the absorption maximum of tryptophane. Its shape can be qualitatively explained in terms of an inner filtering effect. The majority of these fluorescence emissions originate from the surface of the yeast cells (from the cell pellet after centrifugation). Nevertheless, a small fraction of fluorescence can also be detected from the solute (supernatant), which indicates that $CH_2O$-perturbed yeast cells excrete certain fluorescent degradation products to their environment. According to Quickenden et al.[38, 67] the blue-green component of the UPE is to be attributed to excited carbonyl groups, and the more intensive red one to excited singlet $O_2$-$O_2$ dimers, both species arising from the decomposition of lipid peroxides. Because fatty acids with more than one double bond have not been found in yeasts and bacteria, the proposed interpretation[38, 67] may not be valid. In the case of yeast cells, singlet oxygen cannot be formed by lipid peroxide decomposition and cannot excite a sec-

ondary emission by reacting with polyunsaturated fatty acids:

$$2{>}CHOO^{\cdot} \longrightarrow {>}C{-}OH + {>}C{=}O + O_2$$

$$>C=C< + {}^1O_2 \longrightarrow -\underset{\underset{O-O}{|}}{\overset{|}{C}}-\underset{\underset{O-O}{|}}{\overset{|}{C}}- \longrightarrow >C=O^*$$

Thus, the observed photonic response from stressed yeast cells does originate from the increased rate of reactions generating electronically excited, light emitting species. This conclusion is relevant to the forthcoming discussion and the proposed radical-reactions model connecting UPE with perturbations of homeostasis.

We have also studied the effect of another well known protein-denaturating compound, trichloroacetic acid (TCA) and its sodium salt (TCNa) on yeast cells. TCA used in the concentration range 0.1–22% elicits a UPE which is distinct, although much weaker than the $CH_2O$ UPE. The emission spectrum covers the region 460–870 nm with maximum 620–660 nm and is similar to that of yeast + $CH_2O$ shown in figure 3. It can be seen from figure 5 that the UPE signal elicited by TCNa or NaOH (pH = 8.0) is higher than that with TCA (pH ≅ 2) for the same concentration of TCA and TCNa. The degree of ionization and/or $OH^-$ concentration play a role in the photon generating processes. In order to discriminate between these two possibilities, the effect of NaOH alone (pH = 8.0) was measured. As can be seen from figure 5, the increase of $OH^-$ concentration predominates over that of TCNa in the stimulation of UPE. This is valid for both live (intact) and thermally deactivated cells and may be interpreted as a 'trivial' CL accompanying oxidative reactions involving $O_2^{-\cdot}$ at higher pH values.

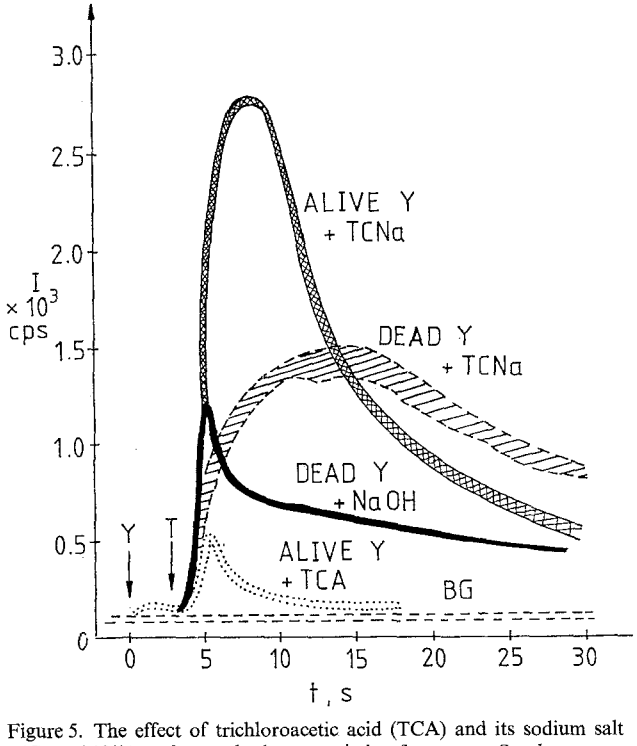



Figure 5. The effect of trichloroacetic acid (TCA) and its sodium salt (TCNa, 16%) on ultraweak photon emission from yeast *Saccharomyces cerevisiae* (Y) (6 · $10^7$ cells/ml) at pH = 1.8 (TCA) and 8.0 (TCNa), NaOH.

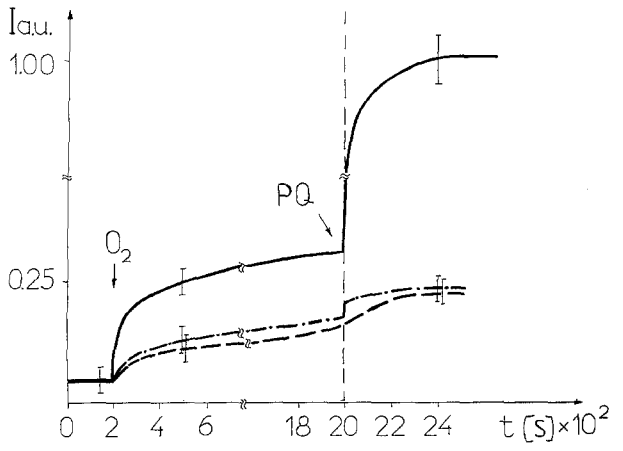



Figure 6. Ultraweak photon emission induced by the addition of 0.0125% paraquat (PQ; 1'1-dimethyl-4,4'-bipyridinum dichloridae) to the suspension of yeast *Saccharomyces cerevisiae*. —— wild strain, .-.-.- catalase-deficient and ----- superoxide dismutase-deficient mutants. A liquid, $O_2$-oxygenated cell culture with the cell density 7 · $10^8$ cells/ml in the logarithmic phase was used.

The effect of the well-known electron acceptor and redox-cycling compound, paraquat (PQ) on the intensity and kinetics of UPE is shown in figure 6. As can be seen, the SOD-lacking mutant, even in the logarithmic phase, gives a distinct enhancement of the I and $\sum n$. In the case of a wild strain when the probability of electron leakage from the respiration chain is much lower, the effect of PQ is also low.

Acetaldehyde $CH_3CHO$ induces instantaneous sharp UPE signals which have a higher amplitude and much faster decay than those induced by formaldehyde. The concentration-dependence I or $\sum n = f([CH_3CHO])$ exhibits a maximum for $[CH_3CHO] \cong 12\%$. It is interesting to note that an analogous dependence for UPE of soybeans studied by Watanabe et al.[75] is reciprocal. The observed effects must depend on the rate of penetration of a toxin into the membranes of cells and organelles (i.e. hydrophobicity and the size of molecule) as well as on the cytotoxicity. A lack of knowledge about detailed mechanisms of the toxin-yeast cell interaction make it difficult to propose any reliable interpretation.

*Effect of other chemical stress factors on UPE in plants and animals*

Our experiments with the unicellular organisms *Chlorella vulgaris*, *Paramecium aurelia*, *P. caudatum* and *P. bursaria* treated with trichloroacetic acid (TCA), $CH_2O$, $H_2O_2$ or UV-light showed that the very low UPE of these organisms is not sensitive to these chemical stressors at low concentrations. *Chlorella vulgaris* and symbiotic *Parame-*

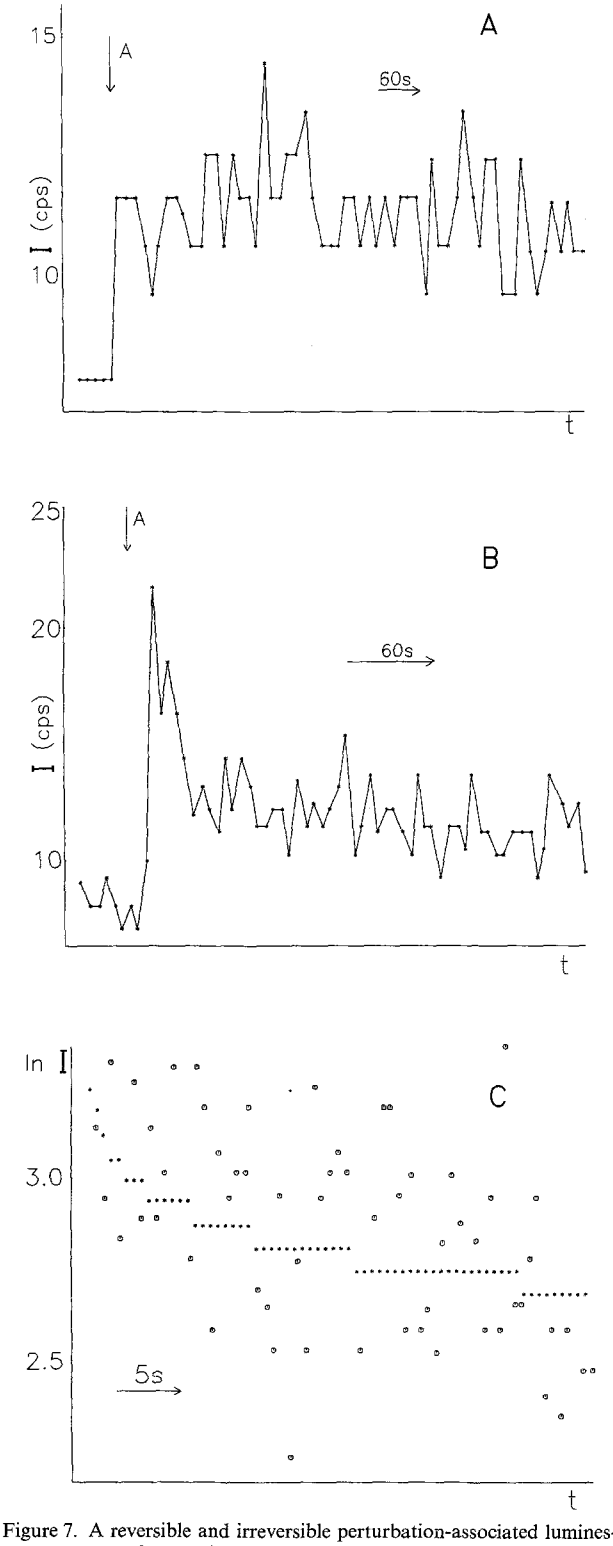



Figure 7. A reversible and irreversible perturbation-associated luminescence response from a single caterpillar (*Vanessa atalanta*) treated with sublethal (0.2 ml) (7A) and lethal (0.2 + 0.3 ml) (7B) doses of acetone (A). A fit of the descending part (quasi-relaxation) of the second curve to the theoretical hyperbolic (+ + + +) decay on a semilogarithmic scale is shown at the bottom[59] (7C).

*cium bursaria* (containing green algae) showed photoinduced delayed luminescence associated with PS II, which appeared to be sensitive to low doses of UV light. Oxygen stress (a pure oxygen atmosphere) decreases UPE of *Hibiscus* leaves; a deficiency of $O_2$ or a nitrogen-atmosphere increase UPE in the spectral region, 660 nm[40]. Experiments with lower animals, mainly insects intoxicated with various chemicals, showed that stressed organisms emit more photons than non-perturbed ones. Comparison of the luminescence response from live and dead organisms shows that the dead organism responds to the second treatment only with spurious luminescence. Figure 7 gives a typical picture of the kinetics of UPE from insects[59, 62]. However, these experiments are very preliminary and their results cannot be generalized.

The effect of catecholamine neurotransmitters on the I of UPE of blood serum of higher animals is of particular interest as the central nervous system and the immune system have special relevance for controlling homeostasis. Catecholamines and glucocorticoids influence UPE accompanying free radical reactions in blood serum. The physiological level of these hormones corresponds to the 'optimum I of luminescence' i.e. to the 'homeostatic luminescence level'.

The relationship between the luminescent response of higher organisms to stress and the level of catecholamines is also relevant to neuroimmunomodulation. Bronchodilators, such as adrenalin, histamine or dl-isoproterenol, decrease luminescence of blood serum of rats in vivo[3], and inhibit zymosan-stimulated chemiluminescence of alveolar macrophages[73]. Such a decrease in UPE response would correlate with a decreased ability of treated macrophages to secrete reactive forms of $O_2$. An increased level of catecholamine and cortisol may also lead to depression and anxiety, which, in turn, weaken immunity. On the other hand, relaxation techniques have been found to enhance immune parameters[19]. For these reasons, one may expect that UPE can be used as an auxiliary tool for studying neuroendocrine and immune processes.

Two simultaneous stress factors may exert a synergetic effect, as demonstrated in the case of electronically-stimulated cooled liver[16] and cucumber seedlings stressed with $CH_2O$ and then treated with drugs[56]. Luminescence from the surface of liver, skin, heart or lungs and plant tissues may serve as a sensitive non-damaging tool to evaluate the oxidative stress and to monitor the rate of metabolic, mainly radical reactions[70, 71]. UPE of brain cells associated with changes in the oxygen supply (ischemia)[18] and the level of ascorbic acid in oligodendrocytes[39] fits well to the biochemical model of a stress-induced UPE response presented below. Taking into account the diversity of living creatures and of detrimental chemical factors, it becomes obvious that research on the influence of chemical stress on UPE is in a very preliminary stage.

*Photochemical stress*

*Influence of light and formaldehyde on ultraweak luminescence of yeast cells*

Our previous research showed that *Saccharomyces cerevisiae* cells illuminated with white light exhibit a short-lived photoinduced luminescence, probably connected with photooxidation/reduction processes of endogenous yeast cell components such as flavins[11]. Moreover, recently an enhancing effect of white light – an important physical and environmental factor – on the $CH_2O$-induced UPE from yeast cells has been found.

*Experimental procedures. Saccharomyces cerevisiae* cells (10 mg dry mass/ml) were incubated in 1 ml of water and/or 0.9% NaCl without and with formaldehyde at room temperature. After 30 min incubation of yeast cells with formaldehyde ($CH_2O + H_2O$ or $CH_2O$ + 0.9% NaCl), the suspension was centrifuged three times and resuspended in water or 0.9% NaCl. The yeast suspension was irradiated with a 150 W halogen lamp equipped with an antisol heat filter cutting off the radiation of 320 nm > $\lambda$ > 800 nm. The illuminated suspension was injected into a scintillation vial or was kept in the dark. After the appropriate time, preilluminated intact cells (not treated with $CH_2O$) which had been kept all the time in darkness were injected into the cuvette and 5.7% of formaldehyde (final concentration) was added. Air or nitrogen was bubbled through the cell suspension. Luminescence was measured with a FEU-38 photomultiplier sensitive in the range 300–800 nm or a Hamamatsu R928, sensitive up to 850 nm.

*Results.* The data in figure 8 show the effect of light on the formaldehyde-induced UPE from yeast cells. Yeast cells which had not been exposed to light showed a faint formaldehyde-induced UPE. Following exposure to light, formaldehyde caused a 14-fold increase in UPE 30 min after illumination compared with non-illuminated yeast cells (fig. 8 B). Our data not only indicate that light and formaldehyde induce UPE of yeast, but also show a significant influence of the environment of the cells, mainly of the atmosphere ($O_2$) and suspending medium. It is worth noticing that buffers are not inert and must be considered as a crucial component of the light-generating system[7,17]. Undoubtedly, in cells incubated in water the normal function of ion gradients across the membrane is modified, and the cells are more easily affected by light irradiation and formaldehyde. It appears reasonable to assume that the combined effect of medium, light and formaldehyde in our experiments alters the permeability of membranes. Such a functional change (for example in an ionic pump) might be necessary for the efficient generation of photo- and formaldehyde-induced UPE.

Formaldehyde treatment has a significant influence on the kinetics of photo-induced delayed luminescence. Successive periods of illumination of yeast cells (incubated in water and treated with formaldehyde) from 1–6 times

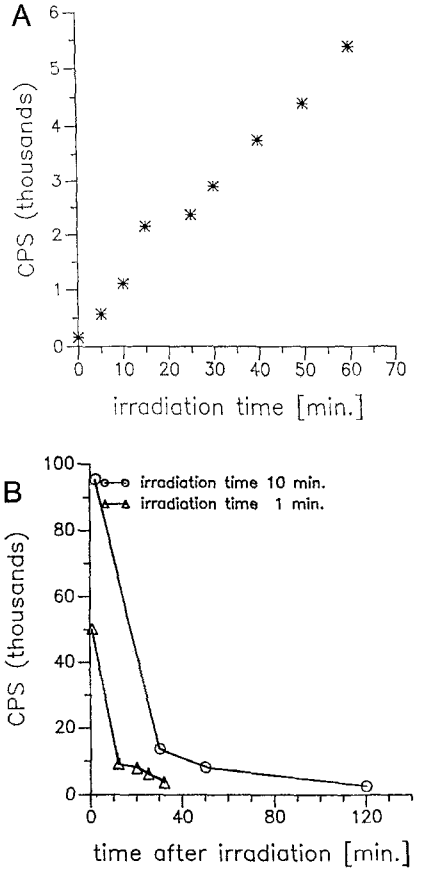



Figure 8. *A* Formaldehyde-induced luminescence of preilluminated yeast cells suspended in water. After illumination for the time specified on the x-axis, formaldehyde was added and the maximum photon emission intensity was measured. *B* Effect of irradiation time on the formaldehyde-induced chemiluminescence. Formaldehyde was added 1 min after the end of irradiation.

resulted in a two-fold increase in UPE of yeast cells. After the first illumination we could observe an additional slower forming luminescent peak. Successive illumination produces a two-peaked response (fig. 9 A). The second peak becomes higher as the number of periods of illumination increases. It is interesting to notice that the second peak response is not observed in the case of yeast cells incubated in physiological solution. In addition to that, there is no change in the kinetics of delayed luminescence (fig. 9 B). A similar induction of UPE was observed in the interaction of formate and/or cyanide with yeast cells (data not shown), but in this case the intensity of photoinduced delayed luminescence is not so significant as it is in the case of formaldehyde. A short heating (5 min, 100 °C) of the cell suspension leads to the complete loss of its photo- and formaldehyde-induced UPE, which proves the enzymatic origin of photo- and formaldehyde-induced luminescence. As is seen from the results presented in figure 10 the spontaneous chemiluminescence of native yeast cells was predominantly in the red region (approximately 70% of emitted light). In the presence of formaldehyde the blue-green emission weakened with time and a strong emission in the red region

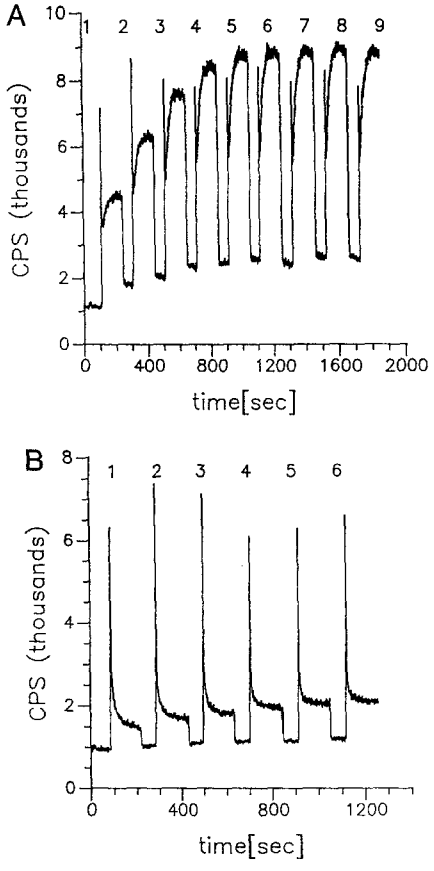



Figure 9. *A* Effect of successive periods of irradiation on the chemiluminescence of yeast cells in water and formaldehyde. Yeast cells were incubated 30 min in formaldehyde and washed three times before irradiation. *B* Effect of successive irradiation on the chemiluminescence of yeast cells in 0.9% NaCl and formaldehyde.

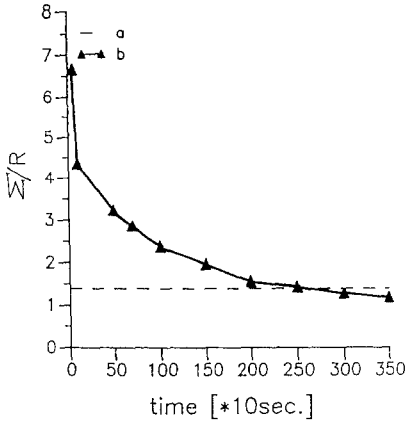



Figure 10. The ratio of total emission intensity (Σ) to the intensity for $\lambda > 620$ nm (R) for:
a, spontaneous emission from native yeast cells;
b, photoinduced emission from cells incubated in formaldehyde.

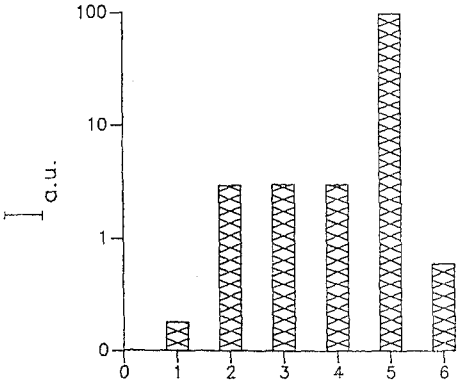



Figure 11. Effect of white light on the formation of the active form of oxygen in the system FMN + NADH. 100 mM KCl, 20 mM $KH_2PO_4$, $1 \cdot 10^{-4}$ M luminol*, 50 μM FMN, 50 μM NADH, 1.5 mM $NaN_3$ (pH = 7.5).
*1* background;
*2* background + luminol + $KH_2PO_4$ + KCl;
*3* background + luminol + $KH_2PO_4$ + KCl + NADH + hν;
*4* background + luminol + $KH_2PO_4$ + KCl + FMN + hν;
*5* background + luminol + $KH_2PO_4$ + KCl + FMN + NADH + hν;
*6* background + luminol + $KH_2PO_4$ + KCl + FMN + NADH + $NaN_3$ + hν.
Maximum error 7%.
$I_{a.u.}$, arbitrary units of chemiluminescence intensity.
* In all experiments luminol was not irradiated.

greater than $\lambda = 600$ nm appears. Since there is an increase in the percentage of long wavelength light, it is suggested that there are many different systems or emitters for ultraweak luminescence, with different emission spectra. Light emission extends a long way into the red and infrared. This fact may be ascribed to the emission of singlet molecular oxygen. A hypothetical mechanism which at least partially explains experimental findings may be proposed, as follows. Under photo-oxygenation conditions, photoreduced flavin undergoes a series of reactions in which oxygen-free radicals such as singlet oxygen, hydroxyl, perhydroxyl and superoxide anions may be formed [14]. Alternatively, singlet oxygen may be formed from the reaction of superoxide anion and hydrogen peroxide [31]:

$$Fl + NADH + H^+ \xrightarrow{h\nu} FlH_2 + NAD^+ \quad I$$

$$FlH_2 + O_2 + \xrightarrow{h\nu} FlH^{\cdot} + O_2^{\cdot -} + H^+ \quad II$$

$$FlH_2 + O_2^{\cdot -} + H^+ \longrightarrow FlH^{\cdot} + H_2O_2 \quad III$$

$$FlH^{\cdot} + O_2 \longrightarrow Fl + O_2^{\cdot -} + H^+ \quad IV$$

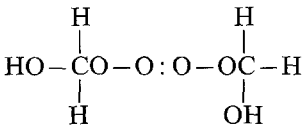
$$O_2^{\cdot -} + H_2O_2 \longrightarrow OH^- + OH^{\cdot} + {}^1O_2 \quad V$$

To check this possibility we have used a sensitive chemiluminogenic probe luminol + $Cu^{2+}$ for the formation of active forms of oxygen [1, 2]. Chemiluminescence measurements amplified by luminol detect the generation of different reactive oxygen species. When a solution of FMN and/or NADH is injected into a buffered solution of luminol no flash of light is produced, while an irradiated solution of FMN + NADH produces a strong luminescence. Sodium azide provokes a strong inhibition of luminescence, so that the increase in luminescence provides an estimate of quantity of the active form of oxygen formed during the irradiation of the system FMN + NADH (fig. 11). Low-molecular-weight alde-

hydes are known to form α-hydroxymethylperoxides with hydrogen peroxide[54] (reaction VI):

$$CH_2O + H_2O_2 \longrightarrow HOCH_2OOH \qquad VI$$

$$HOCH_2OOH + FlH^{\cdot} \rightarrow HOCH_2O^{\cdot} + FlH_2 \qquad VII$$



$$\longrightarrow CH_2O + HCOOH + H_2O + O_2 \qquad VIII$$

According to thermochemical calculations[54] the enthalpy of reaction VIII is $-\Delta H = 545$ KJ/mol. Some fraction of the total amount of oxygen may be formed in the excited singlet states $^1\Delta_g$ and $^1\Sigma_g^+$. In reaction II reduced flavin reacts with oxygen yielding a flavin semiquinone and superoxide anion free radical. The flavin semiquinone reacts with oxygen to give superoxide anion free radical in reaction IV, which forms hydrogen peroxide in reaction III. In reaction VII α-hydroxymethyl peroxide with semiquinone flavin gives reduced flavin. Successive periods of illumination, in the presence of donors and oxygen, increase the amount of hydrogen peroxide available to react with formaldehyde via reaction V. The conversion of the formaldehyde to formate in cells is catalyzed by formaldehyde dehydrogenase and S-formylglutathione. Formate inhibits cytochrome c oxidase, NADH-cytochrome reductase and catalase activities[30]. The inhibition of catalase activity provokes the accumulation of hydrogen peroxide. Cellular iron, chelated as ADP-$Fe^{3+}$, catalyzes a Haber-Weiss reaction as follows:

$$O_2^{\cdot -} + \text{ADP-}Fe^{3+} \longrightarrow O_2 + \text{ADP-}Fe^{2+}$$

$$\text{ADP-}Fe^{2+} + H_2O_2 \longrightarrow \text{ADP-}Fe^{3+} + OH^- + OH^{\cdot}$$

$$H_2O_2 + OH^{\cdot} \longrightarrow H_2O + O_2^{\cdot -} + H^+$$

$$H^+ + O_2^{\cdot -} \longrightarrow HO_2^{\cdot}$$

$$H_2O_2 + OH \longrightarrow H_2O + {}^1O_2$$

$$O_2^{\cdot -} + H_2O_2 \longrightarrow OH^- + OH^{\cdot} + {}^1O_2$$

These various forms of oxygen can indirectly initiate chemiluminescence reactions:

$$2\,O_2(^1\Delta_g)_{v=0} \longrightarrow 2\,O_2(^3\Sigma_g^-)_{v=0} + h\nu \quad \lambda = 634\text{ nm}$$

$$2\,O_2(^1\Delta_g)_{v=0} \longrightarrow 2\,O_2(^3\Sigma_g^-)_{v=1} + h\nu \quad \lambda = 703\text{ nm}$$

Thus, analyzing photo- or chemically-induced luminescence one can come to the conclusion that the luminescence method can serve as an indicator of the influence of such perturbants on biological systems. Of course, the data presented in this review provide no unambiguous answer concerning the mechanisms of light- and formaldehyde-induced UPE. The nature of the light emitters and details of reaction mechanisms remain essentially unknown. Nevertheless, yeast cell cultures appear to be a sensitive and versatile model system for further study of stress-induced UPE.

*Influence of formaldehyde and light on the ultraweak photon emission of bull spermatozoa cells*

It is well known that the duration of daylight influences the fertility level in cattle, altering initial progressive motility of sperm[41], and thyroidal and other metabolic functions. Giese and Wells[15] found that glycine (50 nM) protected the spermatozoa of *Strongylocentrotus purpuratus* from the detrimental effect of light. Exposure of spermatozoa to visible light can result in two diametrically differing effects. 'Photoimmobilization' occurs when bovine spermatozoa are exposed to white light over a period of some hours. An intermediary peroxide is suspected to play a role in this inhibition, and the inactivation effect of light is believed to be the result of a photosensitized oxidation which involves one of the sperm cytochromes as the photosensitizing agent[29]. We have attempted to employ UPE as a possible indicator of photochemical stress on bull spermatozoa[13].

*Experimental procedures.* Fresh bull ejaculates were evaluated for volume, sperm concentration and initial progressive motility. Ejaculates were then stored for 90 min at room temperature and then kept at 4 °C for 60 min. The percentage of live sperm was 70.5%. Spermatozoa were separated from seminal plasma by centrifugation and resuspended in 5 ml fresh trisodium citrate (2.9%). In some samples, the washing and centrifugation was repeated in 0.9% NaCl and/or water. The spermatozoa obtained were suspended in 5 ml of the appropriate medium to obtain approximately $144 \times 10^6$ cells/ml. Motility was expressed as the percentage of motile sperm, moving in any direction and any speed. Vitality was determined by staining spermatozoa with eosine-nigrosine and expressed as the percentage of live sperm. After each experiment, percentage viability and percentage motility were determined. Irradiation of cells and formaldehyde treatment were performed according to Ezzahir et al.[10]. Luminescence changes in bull spermatozoa due to peroxidation of lipids were measured by treating the spermatozoa with ferrous ions. The extent of lipid peroxidation was estimated by the induction period and maximum intensity of the Fe-induced chemiluminescence measured by an FEU-38 photomultiplier.

*Results.* The photoinduced luminescence was dependent on the vitality of bull spermatozoa cells: cells incubated in physiological solutions (0.9% NaCl and/or 2.9% sodium citrate) were sensitive to light, whereas cells incubated in water or treated with formaldehyde were not. Data presented in figure 12 indicate that successive periods of illumination of native bull spermatozoa cells with white light caused an increase in the intensity of delayed luminescence. After the seventh illumination, the enhancement of delayed luminescence was about 400%. The vitality and motility of bull spermatozoa were drastically decreased by white light, water and formaldehyde. The spermatozoa themselves can produce hydrogen peroxide in vitro during the oxidation of three L-amino acids, namely L-tyrosine, L-phenylalanine and L-tryp-

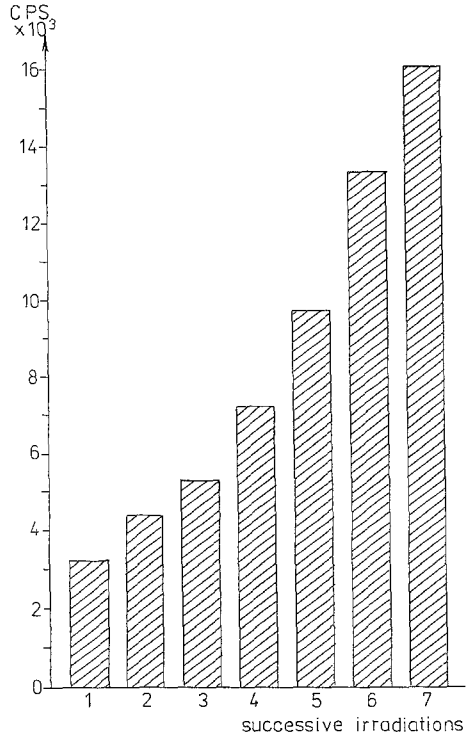



Figure 12. Effect of successive irradiation on the chemiluminescence of spermatozoa incubated in sodium citrate. For each measurement the cell suspension contained $14 \times 10^7$ cells/ml.

tophan[29]. The hydrogen peroxide-forming aerobic process in bull semen is an oxidative deamination catalyzed by the L-amino acid oxidase of spermatozoa. Two types of dehydrogenases, both flavoproteins, can carry out oxidative deamination. One is specific for L-amino acids and is called L-amino acid oxidase. The other flavoprotein is D-amino acid oxidase. They contain FMN and FAD, respectively, as prosthetic group.

$$\mathrm{L{-}R{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}{-}COOH + E{-}FMN \longrightarrow R{-}\underset{\displaystyle O}{\underset{\|}{C}}{-}COOH + NH_3 + E{-}FMNH_2}$$

$$\mathrm{D{-}R{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}{-}COOH + E{-}FAD \longrightarrow R{-}\underset{\displaystyle O}{\underset{\|}{C}}{-}COOH + NH_3 + E{-}FADH_2}$$

The oxidative deamination reaction liberates a relatively large amount of energy $-\Delta H$ that can be stored in triplet carbonyl products.

The reduced flavin nucleotides can react directly with molecular oxygen to form hydrogen peroxide

$$E{-}FMNH_2 + O_2 \longrightarrow E{-}FMN + H_2O_2$$

$$E{-}FADH_2 + O_2 \longrightarrow E{-}FAD + H_2O_2$$

or an energy transfer process is possible to excited oxygen:

$$^3\left[\mathrm{R{-}\underset{\displaystyle O}{\underset{\|}{C}}{-}COOH}\right]^* + {}^3O_2 \longrightarrow {}^1\mathrm{R{-}\underset{\displaystyle O}{\underset{\|}{C}}{-}COOH} + {}^1O_2({}^1\Delta_g)$$

In eucaryotic cells L-amino acid oxidase and D-amino acid oxidase, and also urate oxidase, are localized in peroxisomes, which may be regarded as specialized oxidative vesicular organelles. They also contain catalase, which catalyzes the decomposition of peroxides generated by these oxidases[24]

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 .$$

The lack of catalase in mammalian semen explains the harmfulness of hydrogen peroxide and pure oxygen for spermatozoa. It is interesting to notice that an extract from 200 mg sperm (wet weight) required 20 min at 18 °C to decompose a quantity of hydrogen peroxide which would have been decomposed in 2 min by 1 µl blood[29]. The enhancement of luminescence of illuminated bull spermatozoa cells may be explained by the formation of oxygen free radicals (see reactions I–VII, above) and the lack of catalase.

*Iron-induced chemiluminescence reveals peroxidative damage to spermatozoa cells*

Iron-induced chemiluminescence (CL) gives us direct information about free radical interactions accompanying lipid peroxidation. A high level of CL indicates the formation of lipid free radicals and hydroperoxides[8, 29]. According to our investigations[12], the addition of iron ions to bull spermatozoa incubated in water in contrast to spermatozoa cells incubated in physiological solutions, enhances the CL by almost 300%. The stimulation of CL with the iron ions is dependent on the incubation medium. The data obtained indicate that the perturbation caused by water (osmotic shock) leads to membrane damage. The membranes of mammalian spermatozoa are susceptible to damage by oxygen as a consequence of lipid peroxidation, which could be mediated by free radicals. The level of lipid peroxidation is known to be influenced by antioxidants such as α-tocopherol, ascorbate, and glutathione[44, 74]. The seminal secretion in bull contains 14 mg ascorbic acid/100 ml, and other reducing substances[29]. Probably, antioxidants, amino acids and proteases present in the seminal plasma play a role in the survival of spermatozoa. The beneficial action of the amino acids is believed to depend primarily on their metal-binding capacity[29]. Excessive dilution of semen has a deleterious effect on spermatozoa[29]. The enhancement of iron-induced CL in the case of spermatozoa cells incubated in water may be related to the absence, or too low a concentration, of chelating agent in the incubation medium. Moreover, parameters of the iron-induced CL,

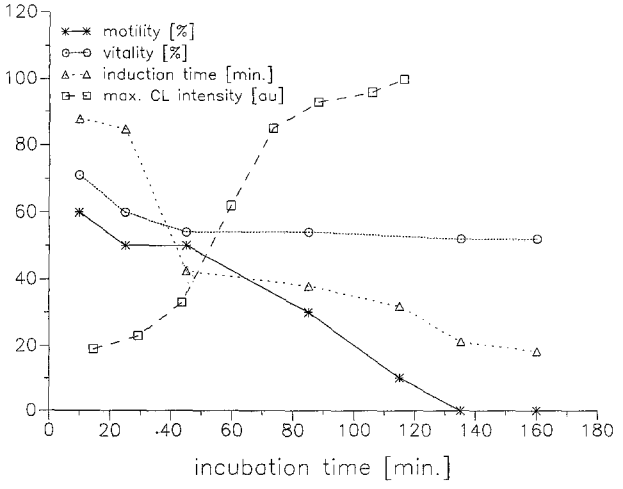



Figure 13. The influence of incubation time at 43 °C on motility, vitality, and iron-induced chemiluminescence parameters: induction time and maximum intensity of chemiluminescence of bull spermatozoa cells.

such as peak amplitude and induction period, were measured and compared for vitality and motility. The increase of incubation time was found (fig. 13) to enhance CL. In contrast to this, the vitality and motility decreased. This result supports the hypothesis that motility and vitality are affected by free radical formation and concomitant lipid peroxidation initiated by environmental osmotic and chemical stress factors.

### *3. Interpretation of the stress-induced ultraweak photon emission*

#### *'Living state' as a photon-generating open system*

Biological organisms are 'open systems' as they exchange energy, information and matter with their environment. Open systems achieve the degree of spatio-temporal order (infrastructure) necessary for survival through the use of a flow of metabolic energy. To maintain this order and a steady state (a dynamic equilibrium) requires that the rate of entropy S production inside the open system $dS_i/dt$ is balanced by the rate of external entropy ($S_e$) production:

$$dS/dt = dS_i/dt + dS_e/dt = 0 \quad (4)$$

where $dS_i > 0$. The most economical steady-state is characterized by the minimum value of the entropy source $\sigma$:

$$\sigma = dS_i/dV\,dt = \text{const} \rightarrow \min \quad (5)$$

for $\sigma > 0$. Here V is the volume of the entropy-producing compartment. After a stimulus exceeding a threshold value, every living system will respond by stress (perturbation of homeostasis), producing a reflex (reaction) with the effect of cancelling any perturbation produced by the stimulus. This process consumes free energy $\Delta G$, the source of which is catabolic reactions which are frequently associated with the generation of photons (UPE). Therefore the rate of these reactions $J = \zeta$ and flux $J_i$ of reactants (i) increases:

$$\sigma = \sum_i J_i X_i > 0 \quad (6)$$

where $X = A/T$ is a thermodynamic force or gradient (chemical affinity $A = \sum \nu_\gamma \mu_\gamma$ and $\mu_\gamma = (\partial G/dn_\gamma)_{n',p,T}$, n being a molecular fraction of a reactant $\gamma$). Any perturbation of homeostasis, in turn, increases energy dissipation $\sigma$. One may expect that in a perturbed state, a biological system may become more sensitive to external and internal stimuli than in the non-perturbed (intact) state, and that this behavior is reflected in parameters of UPE. The above expectation is of the highest significance from the methodological point of view and will be discussed further.

#### *Perturbations of homeostasis and luminescence response*

The question arises how perturbations of the homeostasis are related to parameters of UPE. In other words, how and where the sensed stimulus is processed to bring about changes in a) the energetics, b) the kinetics of reactions generating electronically excited molecules and c) the radiative de-excitation (relaxation) processes? Two alternative models, biochemical and biophysical, may be proposed to account for the stress-induced UPE from organisms.

#### *a) Biochemical model: Oxidative radical reactions in biomembranes*

At present the role of biomembranes in processing the energy/information of a stimulus seems to be well established. The structural and functional stability of membranes has been shown to be a sensitive function of external and internal factors such as temperature T, osmotic pressure, pH, electric charge density, concentration of chemical toxins, irradiation etc. All disturbances affect the rate and energetics of those exergonic reactions which produce excited species P*, owing to the following relationship:

$$I = dn/dt = \dot{n} = [k_f/(k_f + \sum k_d)]\,(k_1\,[A]\,[B]) \quad (7)$$

Here $\dot{n}$ stands for the number of counts per sampling time $\Delta t$ and $k_d$ for the rate constants of quenching processes (non-radiative de-excitation). Kinetic and energetic aspects of P*-generating reactions are well described by the following general equation:

$$-k_B\,T \ln K^{\#} = \partial G^{\#}/\partial\zeta = \partial H^{\#}/\partial\zeta - T\,\partial S^{\#}/\partial\zeta \quad (8)$$

$\zeta$ is the degree of advancement of the chemical reaction. The derivative $(\partial G/\partial\zeta)_{p,T}$ is the difference in free energy $\Delta G$ between product(s) and reactant(s) at a given point during the course of a reaction, and as such is an instantaneous rate of $\Delta G$ with respect to $\zeta$. $K^{\#}$ is the equilibrium constant, associated with the equilibrium between reactants in the transition state (#). $\Delta H^{\#}/\partial\zeta$ and $\Delta S^{\#}/\partial\zeta$

represent enthalpy and entropy changes associated with motion into the transition state. It seems appropriate to use the transition-state (activated complex, #) theory of reaction rates, as the transition state is a molecular aggregate in chemical flux. The notion of fluxes will be used frequently for the description of the association between chemical reactions (molecular fluxes) and photon fluxes (intensity) of UPE. The value of $\Delta G^{\#}$ contributes to the excitation energy (*) and its distribution among the products of an elementary light-producing reaction according to the obvious relationship:

$$/\Delta G/ = hc/\lambda_c \tag{9}$$

where $\lambda_c$ is a short wavelength limit of the emission spectrum of luminescence. Values of the total quantum yield $\Phi$ of a chemiluminescent reaction

$$I = \Phi J \tag{10}$$

and $I(\lambda)$ depend on $\Delta G$ ($\Delta G^{\#}$) and $\Delta H$-distribution among the reaction products as well as on their spectroscopic properties (energy levels configuration, radiative lifetime, rate constants of quenching processes etc.). The least reliable links in the whole bioenergetic system of a cell are lipids and phospholipids in membranes. The electrochemical properties of the lipid bilayer, and the ability to undergo cooperative structural (conformational) phase transition, are essential factors contributing to the weakness of this system. Moreover, the oxidation of lipids proceeds by radical chain reactions with an autoacceleration, and has a high $\Delta H$-value. Among these chain reactions the dismutation of peroxy radicals ROO· liberates enough energy ($\Delta H$, $\Delta G$ or $\Delta H^{\#}\Delta G^{\#}$) to generate electronically excited states of carbonyls $=C=O$ and singlet molecular oxygen:

$$\mathrm{ROO^{\cdot} + ROO^{\cdot} \xrightarrow{k_6} \begin{matrix} RH + {}^{1,3}(=C=O)^* + O_2(^3\Sigma_g^-) \\ RH + {}^1(=C=O) + O_2^*(^1\Delta_g\,{}^1\Sigma_g^+) \end{matrix}}$$

where 1,3 denote spin multiplicity. The reaction rate $J = k_6\,[ROO]^2$ is proportional to the ROO radical flux $J_r$, while the generalized force $X_r$ is a chemical affinity of ROO·. Under homeostatic conditions the flux $J_r$ is minimalized due to the ROO·-scavenging reaction:

$$\mathrm{ROO^{\cdot} + InH \xrightarrow{k_7} ROOH + In^{\cdot}}$$

where InH is a inhibitor or radical scavenger (e.g. an antioxidant such as ascorbic acid, $\alpha$-tocopherol, $\beta$-carotene, glutathione, superoxide dismutase SOD) delivered to the reaction site at the rate $J_i$. The stationarity condition $dS/dt = \text{const} \rightarrow \text{minimum}$ requires that the fluxes $J_r$, $J_i$ and $J_l$ (unsaturated lipids which undergo peroxidation reactions via ROO· accompanied by light emission), are counter-balanced:

$$\begin{aligned} J_r &= L_{rr}X_r + L_{ri}X_i + L_{rl}X_l \\ J_i &= L_{ir}X_r + L_{ii}X_i + L_{il}X_l \\ J_l &= L_{lr}X_r + L_{li}X_i + L_{ll}X_l \end{aligned} \tag{11}$$

The simplest model of the weakest link among the three components ROO·, (r), InH (i) and lipids (l) includes thermodynamic forces X (chemical affinities or gradients of ROO· with InH and lipids, InH with lipids, etc., coefficients $L_{ik}$ characterizing fluxes $L_{jj}$, $L_{kk}$,...) and the interaction between them ($L_{jk}$,...). According to the Onsager principle, under stationary conditions

$$L_{jk} = L_{kj} \text{ etc.} \tag{12}$$

and

$$d[ROO^{\cdot}]/dt = d[InH]/dt = d[L]/dt \longrightarrow \text{minimum} \tag{13}$$

It is important to realize that the luminescence flux $J_l$, i.e. the # of photons per s per $cm^2$ emitted from a living organism is related to fluxes $J_r$, $J_i$ and $J_l$. The rates of ROO· production and consumption of lipids and InH are minimalized owing to protective enzymes such as catalase, superoxide dismutase SOD and antioxidants such as $\alpha$-tocopherol and glutathione. The solution of the simplest case – two $L_{jk}$ and $X_k$ equations (11) gives a quadratic equation. After inserting it into the equation (6) for $\sigma$ one obtains a parabola, and such a geometrical representation seems to be a good model of homeostasis perturbations induced by external stress factors[58, 60, 65] (fig. 14). The most stable system is represented as being at the bottom O of a paraboloid valley or $\sigma_2$ of a cross-section parabola (dashed area). Small perturbations caused by weak stimuli (stresses) cannot raise or lower it much. On the side of a steep hill, a small stress can initiate a large perturbation. When a regulatory system is stressed, it is pushed up the hill-side, and will give larger responses to stimulus (stress).

The stationarity condition for a chemical reaction in the stationary state not very far from thermal equilibrium is[32]:

$$\sum_i \delta J_i \delta A_i \geq 0 \tag{14}$$

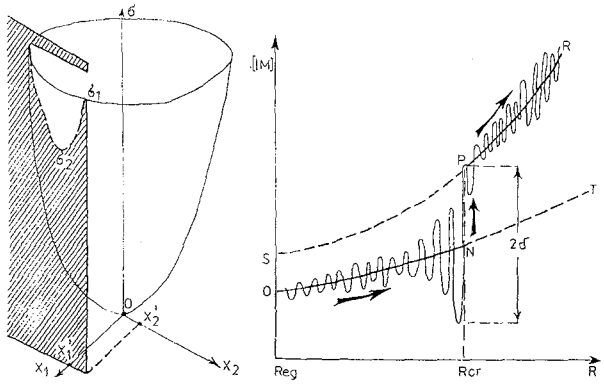



Figure 14. A three-dimensional representation of homeostasis in a regulatory system (left) and a non-linear non-equilibrium dissipative system with fluctuations and extra-entropy production (right). $X_1$, $X_2$, R, space coordinates; O, S, substrates; P, product; [IM], concentration of an intermediate; d, amplitude of fluctuations[60]. Explanations in the text.

$\delta$ is the fluctuation of J, A and reactant, intermediate and product concentration. Far from thermal equilibrium $\delta$ and an extra entropy production, i.e. an energy dissipation $\sigma$ increase:

$$\delta\sigma = 1/T \sum_i \delta J_i \delta A_i . \quad (15)$$

When values of $\delta$ exceed the threshold values and the evolution of a reaction system O ⇌ N approaches the critical value of a generalized reaction coordinate $R_{cr}$, an instability arises (fig. 14, right). The reaction system jumps (N ⟶ P) into a new stationary regime PR. Photon generating reactions are coupled to nonlinear nonequilibrium dissipative structures (systems) because: a) there is a permanent metabolic energy flow (supply), b) there are square terms in equations describing the rate of photon-producing reactions, e.g. $[ROO^{\cdot}]^2$ or a donor-acceptor interaction in certain $e^-$-transfer redox processes and triplet-triplet annihilation or excimer (exciplex) formation, e.g. $(O)_2^*$, c) the excitation temperature $\Theta(\nu)$ corresponding to the photochemical potential within the living cell always exceeds the thermodynamic temperature T:

$$\mu(\nu) = h\nu(1 - T/\Theta(\nu) - k_B T) . \quad (16)$$

This has been inferred from measurements of the absolute spectral intensity $I_a(\nu) = dI/d\nu$ of UPE[37,63]. The above considerations account well for observed intensity fluctuations from organisms subjected to stress factors. Levine and Kidd[25] write of a 'Unified Stress Hypothesis' for disease causation and consider biological stress to include chemical, physical, emotional (psychological) factors and infection. All these factors can cause an increase in endogenous free radical production and possibly overwhelm the body's various antioxidant defences (InH and protective enzymes like SOD, catalase, glutathione oxidoreductase etc.). Figure 15 shows a simplified picture of the chemical stress hypothesis. Free radical reactions are only destructive to the organism when they get out of control. This risk is the price organisms must pay for evolving beyond the anaerobic single cell by using $O_2$ as an acceptor to increase the efficiency of their bioenergetics.

The above model is based on the heterogeneous chemiluminescence associated with compartmentalized peroxidative reactions and cooperative processes in biomembranes. Its origins go back to the 'Imperfection Theory' of Zhuravlev[77,78] and the 'Thermal Transition Theory' of Seliger[47,48] which are both based on spontaneous adventitious chemiluminescence.

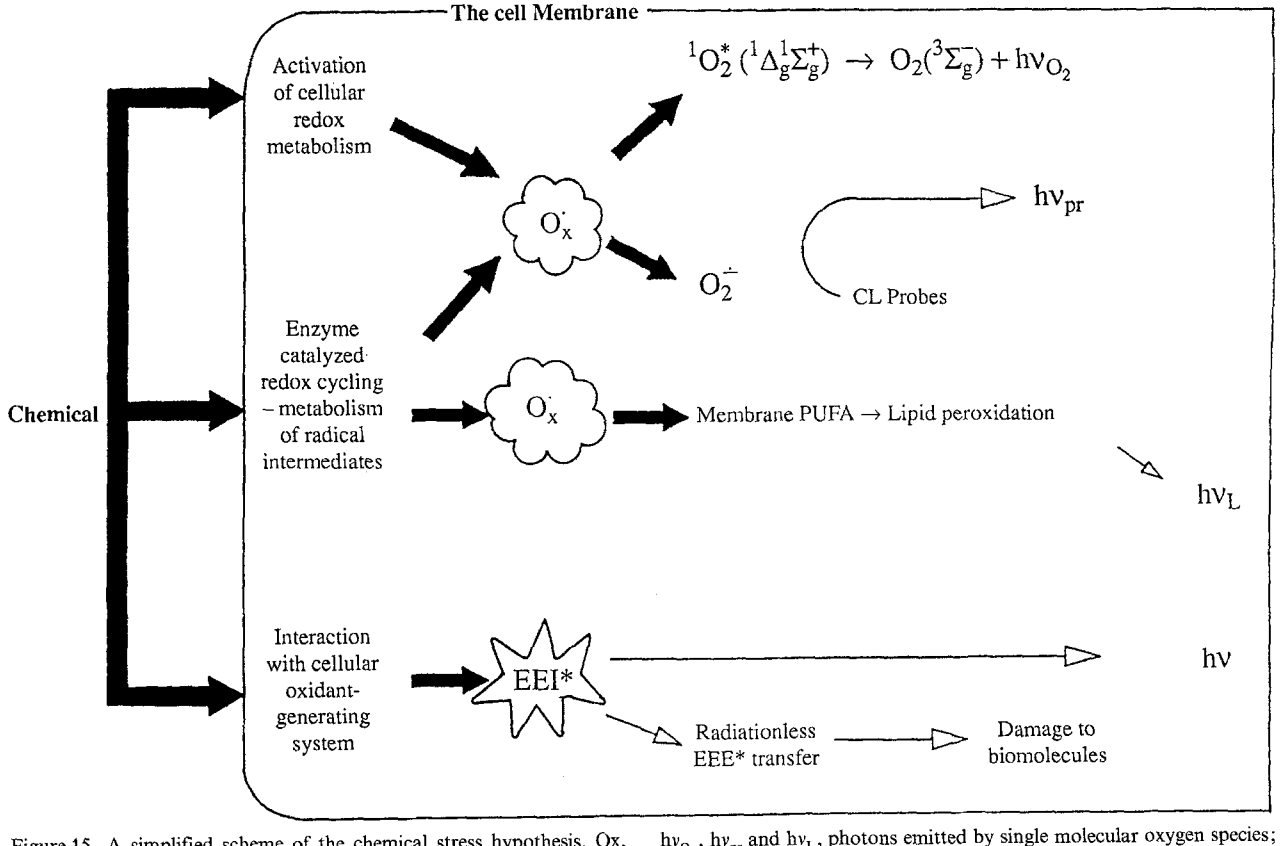



Figure 15. A simplified scheme of the chemical stress hypothesis. Ox, radical oxidation intermediates; PUFA, polyunsaturated fatty acids; EEI*, electrically excited intermediates; EEE*, electric excitation energy; $h\nu_{O_2}$, $h\nu_{pr}$ and $h\nu_L$, photons emitted by single molecular oxygen species; chemiluminescent probes and lipid peroxidation products, respectively[73]. Further explanations in the text.

*b) Biophysical model: Photon storage hypothesis and Q-factor*

The following quantum biophysical model offers an alternative to the biochemical one and provides a more fundamental basis for the understanding of the photonic response from stress-perturbed biosystems. The simplest description of the biophysical 'Photon Storage Hypothesis' is based on the existence of a delocalized coherent electro-magnetic field (EMF) within the tissues[26 - 28, 34, 35]. According to this hypothesis, photons play the role of basic information messengers in intra- and inter-cellular communication. Coherent oscillations of the EMF can act as carriers of information controlling homeostasis in living systems. The situation of a regulatory (homeostatic) system may be illustrated by an electrical oscillator RLC or a resonator cavity. The response of the resonator (oscillator) to a stimulus ('stress') depends on the level of its 'figure of merit' or 'homeostasis' and may be quantified by the value of the 'quality factor':

$$Q(\nu) = \frac{2\pi E_{\text{stored in oscillator}}}{E_{\text{spent per period } \tau_o}} = \tau(\nu)/\tau_o = c\,\tau_o/d \qquad (17)$$

where d is the diameter of a resonator cavity and c is the velocity of light. In other words, Q is equal to $2\pi$* # of periods $\tau_0$ during which the oscillation process lasts before its total energy (E) has dropped to 1/e of its initial value. Thus, it is the oscillation time in radians:

$$Q = 2\Pi * 1/[1 - \exp(-\tau_o/\tau)]. \qquad (18)$$

Moreover, the Q-factor accounts for the sharpness of a resonance, as it is the reciprocal of the fractional bandwidth $\Delta\nu$ or 'selectivity':

$$Q = \omega_o/\Delta\omega = \nu_o/\Delta\nu. \qquad (19)$$

It is also related to photochemical potential $\mu$ (eq. 16) by the relation:

$$Q = \mu/\Delta E \quad E = nh\nu. \qquad (20)$$

This means that the Q-value is the potential of an oscillator normalized with respect to the half-width of the spectral energy distribution. A stable resonant system has a high Q-value. Such a system loses only a very small amount of its energy E. This feature agrees well with a low intensity luminescence from intact (non-perturbed) organisms (the bottom of a valley, fig. 14). When a bioregulatory system is stressed, it is pushed up the hillside, and will give larger responses to a stimulus (amplification of a signal). Such a situation is equivalent to a partially disintegrated system in which the Q-value is diminished due to the malfunction of the negative feedbacks (e.g. an L-C decoupling in a RLC electric oscillation circuit). Then it loses, or radiates out, more E. Indeed, stressed biosystems usually emit a stronger UPE than intact ones. However, the biophysical model does not predict a decrease of UPE intensity as has been observed for certain cases of stress factors (e.g. osmotic stress[66]; or catecholamines[49, 56]). Despite the opposite interpretations, both models lead to similar predictions of the I-change and its statistical fluctuations. Hence, one would expect that increased perturbation (disorder) will enhance UPE owing to both: 1) the increase of 'imperfections', and 2) the decrease in the Q-value. Indeed, in the majority of cases, stress enhances the I of UPE and the amplitude of the fluctuations $\delta I = \delta n$'.

*4. Evaluation of the perturbation of homeostasis from the stress-induced luminescence response*

*The simplest semiquantitative model*

Magnitudes of emission and/or functions derived from the total number of photons emitted under stress conditions have recently been discussed[58 - 60]. Experimentally measured values of UPE intensity $I_i$ correspond to the total photon fluxes ($J_i + J_r + J_l$). In the case of a reversible perturbation of homeostasis by low doses of chemicals, the I slowly increases ($dI/dt = \ddot{n} > 0$) and usually reaches a flat long-lasting plateau ($\ddot{n} \to 0$) (fig. 16). This novel steady-state UPE level is characterized by a large amplitude of biphasic fluctuations and reflects a new, reversibly perturbed state of homeostasis (compare fig. 14). However, when homeostasis is irreversibly perturbed, which leads to the death of the organism studied, a rapid and strong increase of UPE occurs. Its amplitude and duration are related to the rate of dying, depending on the type of stress factor, its strength, and the permeability of a tissue to the factor. From the measurements of luminescence response as depicted in figure 16 one can construct a very simplified luminescence level diagram and calculate the relative effect of a stress-induced perturbation of homeostasis ($_p$) or a synergetic effect of an additional factor ($_d$) imposed on the perturbing one (stress)[56].

$$E_p = (I_p - I_s)/I_s;\ E_{pd} = (I_{pd} - I_p)/I_p$$

$$E_{pd'} = (I_{pd'} - I_p)/I_p.$$

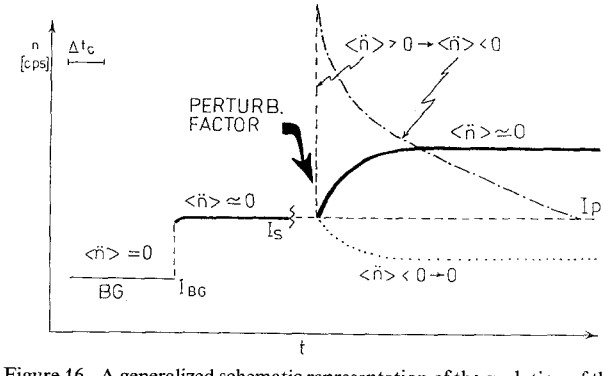



Figure 16. A generalized schematic representation of the evolution of the luminescence response to stress factors. BG, background; IBG, IS, IP, bioluminescence intensity from BG; sample and perturbed (stressed) sample, respectively. $\Delta t_c$, sampling (counting) time[60].

Such an evaluation was used to quantify the effect of homeopathic drugs on formaldehyde-stressed plant seedlings[56].

*Multiparametric measurements*

A more precise quantitative characterization of the UPE-response might be achieved by using 'multiparametric measurements of UPE response' for a single procedure and sample. For example, one can measure simultaneously $I_{max}$ of the T-stress induced increase of photosynthetic delayed luminescence, and the corresponding temperature $T_{max}$ to evaluate the effect of thermal stress and the adaptation capacity of cultivable plants[5,6]. A statistical distribution P(n,Δt) and kinetic parameters have been calculated from a single time-resolved photocount series (PCTS) of human breath[76] and stressed plants and animals[20-23]. A good example is the Fe-induced luminescence from spermatozoa cells where two parameters $I_{max}$ and $\tau_i$ are measured and correlated with physiological activity (see fig. 13). In most studies of stress-induced UPE an uncertainty exists regarding changes of spectral distribution. If a function $I = f(\lambda)$ is different for luminescence of the perturbed ($_p$) and unperturbed (intact, $_i$) sample, then the changes in the measured I = n of UPE partially result from the different spectral responses of the photocathode. Results obtained so far indicate a diversity of 'spectral behavior' of different perturbed biological objects. Distinct spectral changes are observed in certain cases, e.g. $CH_2O$ + plant tissues, mechanical and T-stress[4,51-53,75]. On the other hand, not statistically significant, spectral distributions are reported in other cases (e.g. acetone, cialite, saline + plant tissue[42]).

*Stochastic models of UPE-response to stress*

A new methodological approach employs the correlation analysis of time-resolved photocount series (PCTS) in order to determine the linear discrete stochastic models of the stationary and non-stationary ultraweak luminescence from organisms perturbed by physical and chemical stress[20,21,23]. Analysis of PCTS {n(t), n = 1, 2,..., N = 200–3000; $\Delta t_c$ = 0.1–1 s} has shown[20-22,62] that the spontaneous photon emission from unperturbed living organisms is a stationary uncorrelated process, while the emission from perturbed organisms is a non-stationary correlated process. The photon emissions from the perturbed organisms were recorded from the moment of intoxication (t = O) till the moment when a stationary level of UPE was reached (t = N). The non-stationary emissions being investigated here involved solely a descending stage resembling the relaxation process[62]. The photon emission process occurring after perturbation consists of two stages following in succession, the ascending and descending stages. The ascending stage is described by the Autoregressive Integrated Moving Average (ARIMA (1, 2, 1)) model and the descending stage by the IMA (0, 1, 1) or the ARIMA (1, 2, 1) model. Analysis of the temporal behavior of the perturbed organisms may be performed by means of a memory function approach[20,21,23]. This approach allows the expression of the dependence of any given state of an organism, represented by the number of photoelectron counts n(t), at the moment t, on its previous states represented by the $n(t-\tau)$ values. This dependence takes the form[21]:

$$n(t) = a(t) + \sum \pi_\tau^* n(t-\tau), \quad \tau = 1, 2, \ldots$$

where n(t) and a(t) are values of the photon emission and white noise processes at a time t. The $n(t-\tau)$ value contributes to this linear combination with a weight $\pi_\tau^*$ which determines an influence of $n(t-\tau)$ on n(t). The memory time $T_m = (2\tau^o - 1)$ ($\Delta t_c$, $\Delta t_c$ is the counting time) $\tau^o$ is a time parameter which splits the whole history of a {n(t)} process, $X = \{n(t): \tau = 1, 2, \ldots\}$ into the two subsets $\{n(t-\tau): \tau = 1, 2, \ldots, \tau^o\}$ and $\{n(t-\tau): \tau = \tau^o + 1, \tau^o + 2, \ldots\}$. The linear stochastic models of the non-stationary photon emission processes permit the effect of perturbing factors on biosystems to be determined. For organisms whose photon emission is described by the IMA (0, 1, 1) model $\psi = 1 - \vartheta$, where $\psi$ is the perturbation homeostasis coefficient. For this model the $\vartheta$ parameter belongs to the interval (0, 1) and consequently, $\ln(1-\delta)/\Delta t_c \ln \vartheta$, for $\Delta t_d = 0$ ($\Delta t_d$ denotes the dead time of a recorder):

$$T_m = \{[2\ln(1-\delta)/\ln\vartheta] - 1\}\ \Delta t, \text{ for } \Delta t_d = \Delta t_c$$

At a given $\delta \in (0, 1)$, where $\vartheta \in (0, 1)$,

$$T_m = \ln(1-\delta)/\ln\vartheta \ \{\Delta t_d \cong 0,\ \Delta t_c = 1 \text{ s}\}$$

is a monotonically increasing function of $\vartheta$. Assuming that a magnitude of perturbation P is proportional to the concentration of, e.g., formaldehyde (c), the collation of the memory time $T_m$ with P via c is possible[21,62]. Since $T_m$ as a function of c has the form:

$$T_m(c) = A/c^a$$

where A and a are constants and consequently

$$\theta(c) = \exp\{[c^a \Delta t_c \ln(1-\delta)]/A\}$$

$$T_m \longrightarrow \infty \text{ and } \theta \longrightarrow 1 \text{ for } c \longrightarrow 0,$$

which is consistent with the previous thesis about the behavior of these quantities in the absence of stress (perturbation). In this case the IMA (0, 1, 1) process becomes transformed into a white noise, a(t) (having $T_m \longrightarrow \infty$) corresponding to the photon emission process from unperturbed organisms. Therefore one should expect that the smaller the perturbation, the greater the values of $\theta$ and $T_m$. In this way, the more perturbed a system is, the shorter will be its memory time.

The experimental results with yeast cells treated with formaldehyde show (table 2) that $\theta$ and $T_m$ (at $\delta = 0.99$) decrease in the ranges 0.55–0.02 and 7.7–1.18 s, respec-

Table 2. Memory time ($T_m$) and coefficient of homeostasis perturbation $\psi$ (P) for yeast cells treated with formaldehyde

| Formaldehyde (%) | $\vartheta \mp SD(\vartheta)$ | $T_m \mp SD(T_m)$ | $\psi$ (P) |
|---|---|---|---|
| 0.03 | $0.55 \mp 0.03$ | $7.7 \mp 0.7$ | 0.54 |
| 0.06 | $0.47 \mp 0.03$ | $6.1 \mp 0.52$ | 0.53 |
| 0.25 | $0.33 \mp 0.04$ | $4.14 \mp 0.46$ | 0.67 |
| 0.5 | $0.3 \mp 0.04$ | $3.82 \mp 0.43$ | 0.7 |
| 1 | $0.17 \mp 0.04$ | $2.6 \mp 0.35$ | 0.83 |
| 2 | $0.09 \mp 0.04$ | $1.91 \mp 0.36$ | 0.91 |
| 4 | $0.03 \mp 0.04$ | $1.31 \mp 0.38$ | 0.98 |
| 8 | $0.02 \mp 0.04$ | $1.18 \mp 0.6$ | 0.98 |

tively, when the concentration c of formaldehyde decreases in the range 8–0.03%. Therefore one can admit that $T_m$ is a unique measure of perturbation, i.e. there exists a one-to-one correspondence between the memory time and the magnitude of perturbation; obviously $T_m$ is inversely proportional to perturbation.

Acknowledgments. This work was supported by the Polish Science Research Council (KBN) Grant No. 553369102 and the National Animal Research Institute. We are also indebted to Prof. H. Inaba and Dr M. Usa for their kind support and permission to use the spectral analysis system at the Biophoton Laboratory in Sendai.

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