thus closing the feedback loop. Their hypothesis suggests that biophotons originate from a delocalized coherent field within living matter. When a bioregulatory system is stressed, this is equivalent to a partially disintegrated system with a malfunctioning negative feedback. Gu and Popp review the theoretical research on the non-linear response of photon emission to external perturbation.

The last part of this multi-author review deals with photon emission in relation to disease. Phagocytes (macrophages and polymorphonuclear leucocytes) take an essential part in the defensive attempts of the host. In their article, Lilius and Marnila review the phagocyte activities from individuals under stress or disease. They conclude that photon emission capacity of phagocytes reflects remarkably well the pathophysiological state of the host. But what defence costs has the host to pay? Producing oxygen radicals is dangerous. Cells are unable to completely prevent these compounds from escaping their membranous compartments. Then the question arises as to how, in the diseased state, the chronically adapted cells behave with respect to photon emission. An interesting example is the photon emission of tumors and tumor cells. In the final contribution van Wijk and van Aken review the publications on photon emission in the field of tumor biology.

The assembly of the six contributions in this review offers the reader for the first time a more complete impression of photon emission in the field of stress. In the last decennium the study of stress has moved to center stage in cell and molecular biology. In the past few years HSP's have been the focus of investigations in many areas of cell biology. Several experimental data indicate that the increased production of oxygen-derived free radicals and other active oxygen species are directly or indirectly involved in the regulation of the expression of genes coding for stress proteins [1, 10].

We are beginning to discern the richness of information which can be retrieved from measurements of photon emission. This review on photon emission may offer new insights into the areas of stress and disease.

1 Burdon, R. H., Gill, V., and Evans, C. R., Active oxygen species and heat shock protein induction, in: Stress Proteins, pp. 19–25. Eds M. J. Schlesinger, M. G. Santoro, E. Garaci. Springer Verlag, Berlin/Heidelberg 1990.
2 Cadenas, E., Biological chemiluminescence. Photochem. Photobiol. *40* (1984) 823–830.
3 Jezowska-Trzebiatowska, B., Kochel, B., Slawinski, J., and Strek, W., (Eds), Photon Emission from Biological Systems. World Scientific Publishing, Singapore/New Jersey 1987.
4 Jezowska-Trzebiatowska, B., Kochel, B., Slawinski, J., and Strek, W., (Eds), Biological Luminescence. World Scientific Publishing, Singapore/New Jersey/London/Hong Kong 1990.
5 Nagl, W., and Popp, F. A., A physical (electromagnetic) model of differentiation I. Basic considerations. Cytobios *37* (1983) 45–62.
6 Popp, F. A., and Nagl, W., A physical (electromagnetic) model of differentiation II. Application and examples. Cytobios *37* (1983) 71–83.
7 Popp, F. A., Cilento, G., Chwirot, W. B., Galle, M., Gurwitsch, A. A., Inaba, H., Li, K. H., Mei, P., Nagl, W., Neurohr, R., Schamhart, D. H. J., Slawinski, J., and van Wijk, R., Biophoton emission (Multi-author Review). Experientia *44* (1988) 543–600.
8 Popp, F. A., Li, K. H., and Gu, Q., (Eds), Recent Advances in Biophoton Research and its Applications. World Scientific Publishing, Singapore/New Jersey/London/Hong Kong 1992.
9 Popp, F. A., Warnke, U., Koenig, H. L., and Peschka, W., Electromagnetic Bio-information. Urban & Schwarzenberg, München/Wien/Baltimore 1989.
10 Ropp, M., Courgeon, A. M., Calvayrae, R., and Best-Belpomme, M., The possible role of the superoxide ion in the induction of heat shock and specific proteins in aerobic Drosophila cells after a period of anaerobiosis. Can. Biochem. Cell. Biol. *61* (1983) 456–461.
11 Slawinska, D., and Slawinski, J., Biological chemiluminescence. Photochem. Photobiol. *37* (1983) 709–715.
12 Slawinska, D., and Slawinski, J., Low-level luminescence from biological objects, in: Chemi- and Bioluminescence, pp. 495–531. Ed. J. G. Burr. M. Dekker, New York 1985.

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The effect of stress factors on the spontaneous photon emission from microorganisms

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Abstract. The results of recent work on the photon emission from three yeasts and a bacterium is presented. Both visible region and ultraviolet photon emission is observed; however, no luminescence is observed in the absence of oxygen. The visible region emission is attributed to excited carbonyl groups and excited singlet oxygen dimers formed during the decomposition of lipid hydroperoxides. Possible sources of the ultraviolet photon emission are also examined. The use of microorganisms in the study of ultraweak photon emission and its relation to oxidative, temperature and chemical stress is reviewed and the applications and (or) functions of this photon emission are also discussed.

Key words. Ultraweak luminescence; photon emission; biophoton emission; mitogenetic radiation; lipid peroxidation; yeast; bacteria.

Introduction

Ultraweak luminescences emitted by living organisms at photon fluxes below ca. 10^4 photons cm^{-2} s^{-1} have been detected for a wide variety of organisms which are not normally classified as bioluminescent. Reviews by Slawinska and Slawinski[71,72] and by Cadenas[8] provide useful overviews of this field, while Popp's multi-author review[50] [Experientia 1988] on biophoton emission gives detailed accounts of various aspects of these luminescences.

Two apparently distinct types of ultraweak photon emission have been reported in the literature, one in the visible region and the other in the ultraviolet region of the spectrum. The visible region luminescence is well established and it is generally argued that its source is excited singlet oxygen and excited triplet state carbonyls. These species are formed in vivo by processes such as lipid peroxidation, phagocytosis, enzymatic reactions and interactions of oxygen radicals (especially the superoxide anion) with some metabolites. These processes are often linked with oxidative stress. The ultraviolet (UV) luminescence has still not gained wide acceptance despite its having a longer history than the visible region emissions.

In the 1920s Gurwitsch[28,29] claimed that dividing cells emit a very weak UV luminescence which was termed mitogenetic radiation due to its alleged ability to stimulate cell division when incident on nearby cells. A review of mitogenetic radiation by Quickenden and Que Hee[58] discusses some of the curious features of its history. The controversy surrounding mitogenetic radiation may account for the lack of acceptance of the ultraweak UV luminescence. In addition, many of the modern studies of ultraweak photon emission have not been designed to detect UV light. In some cases[18,22,69] the photomultiplier tubes used were sensitive mainly in the visible region of the spectrum, and in others[33,42,67] the cuvettes used did not transmit UV light. Some recent studies[23,35,73,74], which have employed detectors sensitive in both the UV and visible region of the spectrum, have not carried out spectral determinations or have only monochromated the visible region of the spectrum and hence the extent of any UV component can not be estimated in these cases.

This paper will review the spontaneous ultraweak photon emission from microorganisms with particular reference to the effects of temperature, chemical and oxidative stress.

Early work on photon emission from microorganisms

Very weak ultraviolet photon emission was the subject of frequent reports (over 500 papers) in the 1920s and 1930s under the heading of mitogenetic radiation, which largely originated with the work of Gurwitsch[28,29]. Gurwitsch claimed that dividing cells emit very weak UV light which can itself stimulate division in other cells if incident upon them at an appropriate stage of their life cycle. The early method for measuring mitogenetic radiation from an emitting or 'sender' organism was by its ability to stimulate cell division in a variety of biological detectors such as yeast cultures, bacterial cultures and plant meristems. With biological detectors, the number of cells or cell divisions of the detector organism was compared to that of a control that was not exposed to the sender organism. A statistically significant difference was taken to indicate the mitogenetic effect. Mitotic stimulation was not substantially different when silica was placed between the sender and the detector, but no effect was observed when glass was interposed. It was thus concluded that the radiation consisted of UV light.

Gurwitsch's original mitogenetic studies used onion roots, but yeast and bacterial cultures either on agar plates or in liquid media were found[6] to be the most reliable organisms for studying this effect. Organisms in their exponential phase of growth were relatively insensitive to mitogenetic stimulation, so lag phase or stationary phase organisms were used as detectors. However, exponential phase cultures were usually used as senders since emission was brightest during this period.

Although Gurwitsch is credited with the discovery of mitogenetic radiation, there were several earlier reports of similar phenomena. Thus Scheminzky[70] was the first to report that some high energy radiation was emitted from various biochemical processes. He used cultures of yeast and bacteria to provide the biochemical processes and detected the emanations by means of photographic plates. This work was confirmed in 1918 by Ludwig[45] who also used photographic plates to detect emissions from fermenting yeast. Ludwig found[45], moreover, that these emissions could penetrate optically opaque paper and were absorbed by protein matter, indicating that the emissions could be in the UV region. De Fazi and De Fazi[14–16] studied the effects of UV light on yeast and found that fermentation was greatly stimulated by very weak UV radiation, or by very short exposure to higher levels of UV radiation.

Gurwitsch's work was supported by many Russian workers and several Western workers[6,63,85], but many others[5,32,66] were unable to detect any mitogenetic effect. In view of the contradictory results obtained with biological detectors, some workers[5,65] introduced physical detectors such as the photographic plate and the UV-sensitive Geiger tube in order to detect the UV photon emissions. Unfortunately, the results using physical detectors were as variable as those obtained with biological detectors[5]. In the 1930s, the careful but negative experiments of Hollaender and Claus[31], Gray and Ouellet[25], Lorenz[44] and others challenged the existence of all the mitogenetic phenomena and work subsequently ceased in Western countries. Some early reviews[5,30,63] have described the early mitogenetic work in more detail. Despite this, work[21,26,41] continued in East European countries with surprisingly little acknowledgement of the

negative results of the above Western workers. Biological detectors, mainly yeast cultures, were used through to the 1960s by various East European workers[21,26,41] to support the existence of mitogenetic radiation and there are still occasional reports in both the East European[27,38] and Western[11,24,86] literature describing such experiments.

Detection of ultraweak photon emission with photomultiplier tubes

Very weak visible region (400–700 nm) photon emission was first detected[12,13,76] in the 1950s using the newly developed photomultiplier (PM) tube, which proved to be a very sensitive and reliable method for the detection of very weak light. The first studies[76] involved photon emission from green plants, including three species of algae, following irradiation with visible light. In 1954 Colli and Facchini[12] detected weak visible region luminescence from seeds germinating in the dark. Photon emission from plants has been reviewed elsewhere[1,78]. In the 1960s several Russian groups headed by Tarusov[77], Vladimirov[83] and Zhuravlev[87] studied the visible region luminescence from many plant and animal species.

Konev[39,40] was the first to employ the UV-sensitive PM tube to detect UV photon emission from living organisms. He repeated some of the classical mitogenetic work using synchronized cultures of *Candida utilis* in order to determine if UV photon emission was causally connected with cell division. In a synchronized culture all the cells are in approximately the same metabolic state and hence would be expected to emit a pulse of light correlatable to a given synchronous division step. He detected a UV emission peak which preceded the first wave of cell division by about 1 h and a second weaker peak which corresponded in the same way to the second synchronous division step. Spectral analysis indicated that this emission was in the 250–380 nm wavelength range.

Konev's group studied[46] over 100 different species of organisms covering eight systematic types, including 13 algal, 9 yeast and 8 bacterial species. They detected photon emission from about a third of the algae, bacteria, fungi and insects examined, but in the higher plants and vertebrates all the species investigated displayed luminescence. Only the protozoa gave no detectable photon emission from any of the species studied. However, it is interesting to note that, at least for one of the species of bacteria (*Escherichia coli*) which gave no detectable luminescence, subsequent workers[80,84] have observed significant photon emission. This is possibly due to the greater sensitivity of the more recent PM tubes. No spectral analysis was carried out by Konev's group in these experiments and thus it is not known if the luminescences detected were in the visible or UV region.

Stauff's group[17,75] detected a weak visible (340–600 nm) luminescence from synchronized *Saccharomyces cerevisiae*, but the luminescence did not depend on either the viability or stage of growth of the yeast, but only on the amount of oxygen present. Quickenden and Que Hee[56] reported luminescence from growing cultures of the yeast *Saccharomyces cerevisiae* and found[57] that both ultraviolet and visible components were present in the luminescence emission.

In 1979, Lloyd et al.[42] reported an oxygen dependent luminescence from exponential phase cultures of the amoeba, *Acanthamoeba castellanii*, and suggested that it may be associated with increased intracellular concentrations of superoxide anions, which may in turn lead to the formation of lipid hydroperoxides. The only other reports of photon emission from amoeba have come from the group of Fisher et al.[19,20] who have examined the cellular slime mold, *Dictyostelium discoideum*. They have shown[19] that this amoeba emits very weak light during early differentiation, which is enhanced by heat shock at the beginning of development. They attribute this photon emission to lipid peroxidation reactions arising from reduced oxygen metabolites released by perturbed mitochondrial oxidative metabolism in stressed cells.

Several recent reports of ultraweak photon emission from bacterial cultures have appeared. Roth and Kaeberle[67] have detected luminescence from *Listeria monocytogenes*, but observed none from *Escherichia coli*, *Klebsiella pneumoniae*, or *Salmonella minnesota*. They observed one major peak during the exponential phase of growth of the bacterium, *L. monocytogenes*, and found that this photon emission was inhibited by superoxide dismutase and catalase, but not by hydroxyl radical scavengers. Hunter and Allen[33] observed a similar emission peak from exponential phase cultures of *Streptococcus faecalis*, which was also inhibited by superoxide dismutase and catalase. In addition, they found that oxygen was required for the photon emission. In both of these studies only the visible region of the spectrum was examined and spectral distributions of the luminescences were not obtained. Both reports[33,67] also concluded that hydrogen peroxide and superoxide anion were involved in the production of the light emission.

Although Roth and Kaeberle[67] were unable to detect any appreciable photon emission from *E. coli*, possibly due to the sensitivity of the scintillation apparatus used for the luminescence measurements, the author[80] first observed chemiluminescence from this bacterium using very sensitive single photon counting equipment, the results of which have now been confirmed by Wang et al.[84]. The latter authors detected[84] ultraweak photon emission from three species of bacteria, *Escherichia coli*, *Bacillus subtilis* and *Brevibacterium ammoniagenes*, and have determined their spectral distributions.

Ultraweak photon emission and oxidative stress

It is known[10] that reduced oxygen metabolites participate in reaction cascades leading to photon emission and that these partially-reduced forms of oxygen are cytotox-

ic and may play a significant role in the pathology of age-related and auto-immune diseases and of cellular damage after oxidative stress[53]. Single cellular systems are useful test organisms for chemiluminescence studies of various aspects of oxidative stress, particularly in relation to the major metabolic pathways of living cells.

Work carried out by the author in Quickenden's laboratory[54, 59, 62, 80, 81] has shown that weak luminescence (100–10000 photons $cm^{-2} s^{-1}$) is emitted from oxygenated, liquid cultures of each of the three yeasts (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Candida utilis*) and one bacterium (*Escherichia coli*) studied. In each case two main periods of photon emission were found, one during the exponential phase of growth and one late in the stationary phase of growth. For each species examined the exponential phase emission comprised both an ultraviolet (210–330 nm) band and a visible region (450–620 nm) band, although in differing proportions. The stationary phase emission, however, comprised only a visible region (450–620 nm) band in each case. No luminescence was observed from any of the species when grown anaerobically, despite good growth.

All species were grown as liquid cultures in a sterile, fully defined nutrient medium. However, due to the differing requirements the nutrient medium was different for the yeasts[59] and the bacterium[80]. Luminescence was measured with an E.M.I. 9635 QA photomultiplier tube, sensitive in the wavelength range 200–630 nm, connected to a photon counter. The photon counter comprised a pulse amplifier, a pulse height analyzer and a ratemeter. The output pulses from the pulse height analyzer were also directed to a computer for data storage and analysis. The spectral distributions of the luminescences were determined as previously described[54], by using broad bandpass (50–60 nm) optical filters which covered the wavelength range 210–620 nm and were mounted inside a custom built filter wheel spectrometer.

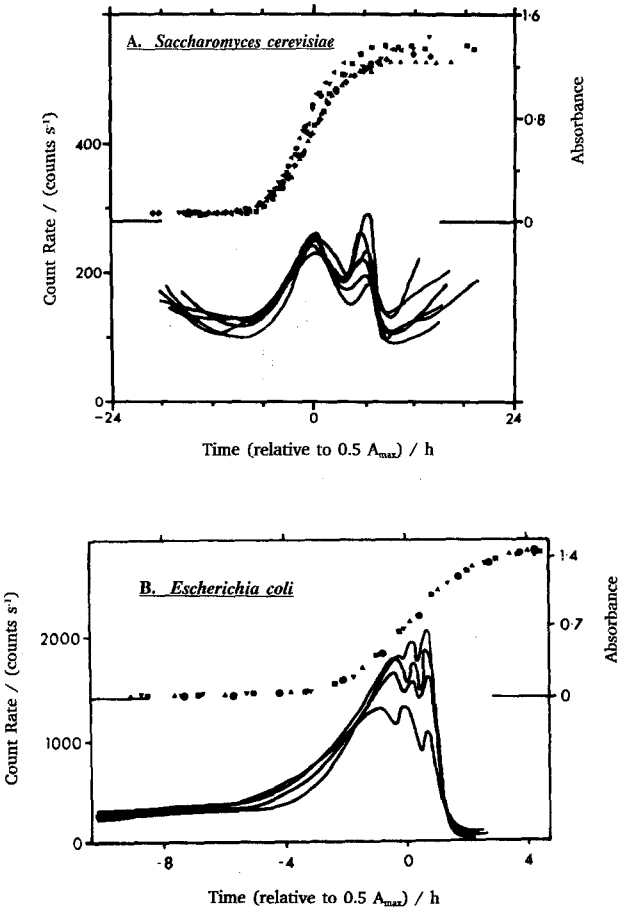


Figure 1. Photon emission intensity (—) and growth (●▼■▲◆◀) curves of oxygenated liquid cultures of yeast and bacteria showing the exponential phase emission. Point sizes and line thicknesses are larger than 50% confidence intervals. Temperature of growth = 306.15 K.
Reprinted with permission from Quickenden, T. I., and Tilbury, R. N., Growth dependent luminescence from cultures of normal and respiratory deficient *Saccharomyces cerevisiae*. Photochem. Photobiol. *37* (1983) 337–344, and Tilbury, R. N., and Quickenden, T. I., Spectral and time dependence studies of the ultraweak bioluminescence emitted by the bacterium *Escherichia coli*. Photochem. Photobiol. *47* (1988) 145–150.

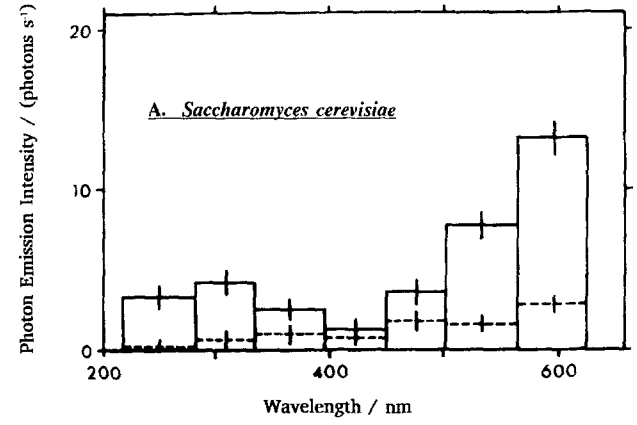
Exponential phase photon emission

Figure 1 shows the exponential phase luminescence from oxygenated cultures of *S. cerevisiae* and *E. coli* plotted together with their growth curves. The luminescence and growth curves of the other two yeasts, *C. utilis* and *S. pombe*, were very similar to that of *S. cerevisiae* (shown in fig. 1 A) and are not shown here. For each of the three species of yeast examined the exponential phase emission consisted of two distinct peaks even when grown under different conditions[81]. All these peaks were very reproducible with respect to the time of growth, when measured relative to the time of half maximum absorbance. This latter measure was necessary to remove the effect of variations in the length of the lag phase from one culture to another.

The peak intensities for each of the species were all reasonably reproducible. The mean peak intensity of both peaks from each of the three yeasts was about 100 counts s^{-1}. The mean intensity (approx. 1650 counts s^{-1}) of each of the three peaks from the bacterium was about 15 times greater than the corresponding peaks from the three yeasts at the same temperature.

Figure 2 shows the corrected luminescence spectra of the exponential phase emissions from *S. cerevisiae* and *E. coli*. Each spectrum was the mean of a total of 50–300 scans from 4–6 different cultures of the organism under study and was corrected for both the filter transmission and the photomultiplier sensitivity. The spectral distribution of the much weaker luminescence from the yeast nutrient medium is also shown in figure 2 A, and that from the bacterial nutrient medium is shown in figure 2 B. Once again the results for the other two yeasts are not shown here, but were very similar in shape to both the *S. cerevisiae* and *E. coli* shown in figure 2.

The spectral distributions of the exponential phase emissions from all the species examined were similar. Each contained both a UV (210–330 nm) and a visible region



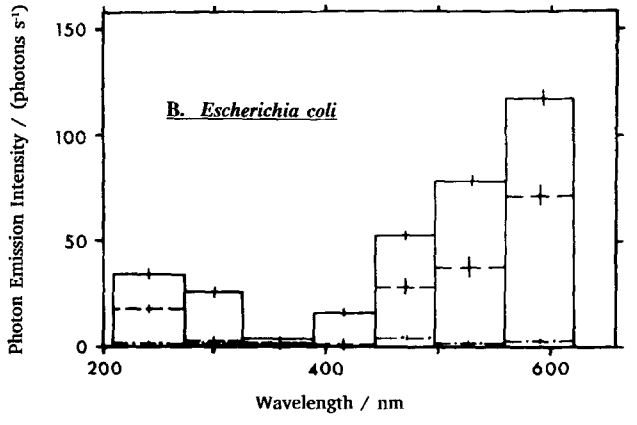
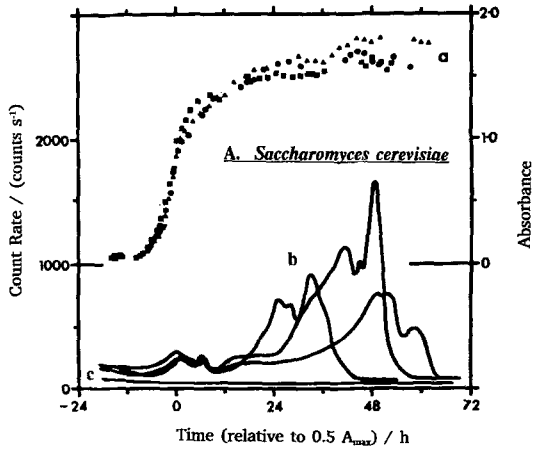


Figure 2. Luminescence spectra of the exponential phase emission from oxygenated liquid cultures of yeast and bacteria.
(——) growth temperature = 306.15 K; (– –) 310.15 K; (- - - -) yeast nutrient spectra; (- · - ·) bacterial nutrient spectra. All spectra have been corrected for the spectral responses of the photomultiplier tube and the various filters used to monochromate the luminescence. Error bars represent 50% confidence intervals.
Reprinted with permission from Quickenden, T. I., Comarmond, M. J., and Tilbury, R. N., Ultraweak bioluminescence spectra of stationary phase *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Photochem. Photobiol. *41* (1985) 611–615, and Tilbury, R. N., and Quickenden, T. I., Spectral and time dependence studies of the ultraweak bioluminescence emitted by the bacterium *Escherichia coli*. Photochem. Photobiol. *47* (1988) 145–150.



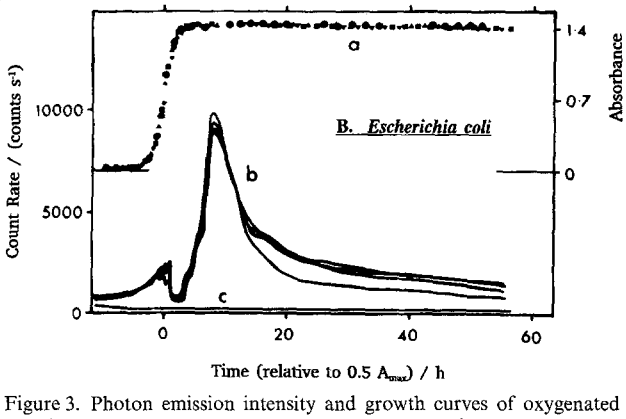


Figure 3. Photon emission intensity and growth curves of oxygenated liquid cultures of yeast and bacteria showing the stationary phase emission.
(a) replicate growth curves; (b) replicate luminescence curves for oxygenated cultures; (c) luminescence curve for a nitrogen saturated culture. Point sizes and line thicknesses are larger than 50% confidence intervals. Temperature of growth = 306.15 K.
Reprinted with permission from Quickenden, T. I., and Tilbury, R. N., Growth dependent luminescence from cultures of normal and respiratory deficient *Saccharomyces cerevisiae*. Photochem. Photobiol. *37* (1983) 337–344, and Tilbury, R. N., and Quickenden, T. I., Spectral and time dependence studies of the ultraweak bioluminescence emitted by the bacterium *Escherichia coli*. Photochem. Photobiol. *47* (1988) 145–150.

(450–620 nm) component, with the latter always being several times more intense than the former.

Stationary phase luminescence

Luminescence emission also occurred late in the stationary phase of growth for each species examined. In all cases, this period of emission was much brighter than the corresponding exponential phase luminescence, as shown in figure 3 for a representative yeast and the bacterium. Figure 3 also shows that no luminescence was detected when these species were grown under anaerobic conditions, which was also the result for those species not shown.

The stationary phase luminescence differed markedly from species to species. The stationary phase emission from *S. cerevisiae*[59] (fig. 3 A) lasted for 20–30 h and was usually composed of two distinguishable emission peaks. However, the corresponding emission from *C. utilis* was comprised simply of a period of continuous emission lasting for more than 170 h, which was also the case for *S. pombe*. The stationary phase emission from *E. coli* (fig. 3 B) comprised only one main peak which decayed fairly steeply at first and then proceeded to decay more slowly over a long period of time (approx. 4–5 days).

In the case of the yeasts, the stationary phase emission was not very reproducible either with respect to the time of growth or with respect to the peak intensities. The emission from the bacterium was, however, quite reproducible (fig. 3 B) both with respect to the time of growth and with respect to its peak intensity. The intensities of the stationary phase emissions from all the yeasts were

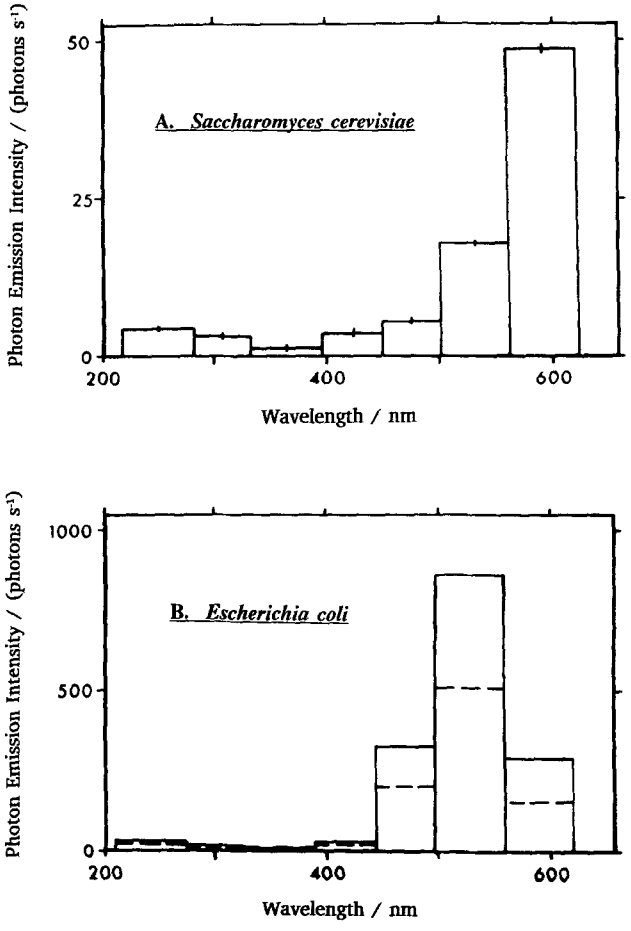


Figure 4. Luminescence spectra of the stationary phase emission from oxygenated liquid cultures of yeast and bacteria.
(—) growth temperature = 306.15 K; (– –) 310.15 K. All spectra have been corrected for the spectral responses of the photomultiplier tube and the various filters used to monochromate the luminescence. Error bars represent 50% confidence intervals.
Reprinted with permission from Quickenden, T. I., Comarmond, M. J., and Tilbury, R. N., Ultraweak bioluminescence spectra of stationary phase *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Photochem. Photobiol. *41* (1985) 611–615, and Tilbury, R. N., and Quickenden, T. I., Spectral and time dependence studies of the ultraweak bioluminescence emitted by the bacterium *Escherichia coli*. Photochem. Photobiol. *47* (1988) 145–150.

similar (ca. 1000 counts s^{-1}), but the *E. coli* emission (ca. 9000 counts s^{-1}) was about ten times greater.
Figure 4 shows the corrected luminescence spectra of the stationary phase luminescence (fig. 3) from *S. cerevisiae* and *E. coli*. The spectral distributions of the much weaker luminescence emissions from the yeast and bacterial nutrient media were too small to be plotted in figure 4.
In contrast to the exponential phase spectra, the spectral distributions of all of the stationary phase emissions contain only visible region luminescence. This visible region band mainly occurred above about 450 nm for all the species examined. In the case of all the yeasts, the visible band was predominantly in the red region, while the visible band from the bacterium was dominated by blue-green emission. The spectra for the three yeasts were very similar to one another, except that the proportion of red : blue emission was less in *S. pombe* than in either of the other two yeasts.
The visible region stationary phase emission from *E. coli* (fig. 4 B) is about 7 times the intensity of the corresponding visible region component of the exponential phase emission (fig. 2 B) but contains a much lower proportion of red and a much higher proportion of blue-green luminescence.

Possible sources of the visible region emissions
Oxygen was necessary for the observed photon emissions from all species examined (fig. 3), indicating an oxidative reaction mechanism. In view of the results of in vitro studies such as those by Vladimirov[83] and Tarusov et al.[77] which show visible region emission during the decomposition of lipid peroxides, it is possible that the visible region emissions from the organisms studied arise from this source. This is consistent with the absence of both lipid peroxide formation and of luminescence under anaerobic conditions. Furthermore, other cellular processes such as respiration and glycolysis are unlikely sources of the visible region luminescences as they are less active during the stationary phase of growth, which is the period of brightest emission.
The lipid peroxide decomposition step[68] is:

$$2 > CHOO\cdot \rightarrow > C = O + > COH + O_2, \qquad (1)$$

in which either the carbonyl group or the oxygen can be formed in electronically excited states. Emission from excited carbonyl groups can then occur at wavelengths which lie between 330 and 550 nm for in vitro model systems[43, 82] and which are in the 400–500 nm range in the case of in vivo sources[83]. In addition, excited singlet oxygen molecules can emit, which in the gas phase[4] is around 780 nm. Other singlet oxygen emissions lie even further into the infrared, but since the photomultiplier sensitivity did not extend into the infrared, these emissions would not have been detected in this study.
The excited singlet oxygen molecules may also excite a secondary emission[9, 10] by reacting with unsaturated lipids:

$$> C = C < + O_2(^1\Sigma_g^+) \rightarrow \overset{+\quad -}{O - O} \rightarrow > C = O^*, \qquad (2)$$

to produce excited carbonyl groups which can then emit as before, where the emission wavelengths would be expected to be in the same vicinity as described above.
Another possible emission is that arising after dimerization of excited singlet oxygen[37], the dimer emitting two broad bands centered on 634 and 703 nm in aqueous systems[3].
The emission from excited oxygen dimers is the process whose wavelength distribution is most compatible with the red emissions observed (figs 2 and 4) from the different organisms. Excited carbonyl groups produced by the reactions in Eqs 1 and 2 may be responsible for the blue-green components of the emissions.
It is noted that, for the stationary phase luminescences, the blue-green emission is a larger fraction (0.70 ± 0.06)

of the red emission from *S. pombe* than from either *S. cerevisiae* (0.37 ± 0.04) or *C. utilis* (0.44 ± 0.07) and that this observation is consistent with the greater proportion of unsaturated lipids in *S. pombe* than in the other two yeasts when they are grown under the same conditions[7,36]. The higher proportion of unsaturation in *S. pombe* would be expected to accentuate the importance of the blue-green carbonyl emission associated with the reaction in Eq. 2, as the emission arises from the scavenging of singlet excited oxygen which would otherwise be available for red (or infrared) emission.

The enzymes involved in the respiratory chain in *E. coli* are located in a region (the lipid membrane) which is richer in lipids than is the case for yeasts, which contain their respiratory processes in mitochondria. If lipids are indeed the source of the visible region emission, there should consequently be a greater amount of lipid peroxidation in bacteria than in yeasts and hence the bacterial luminescence should be more intense than that from yeast. The results in figures 1 and 3 show that this is the case; the *E. coli* luminescences are about 10–14 times brighter than the corresponding luminescences from the yeasts.

Furthermore, the lipid rich environment of the respiratory chain in bacteria also explains the enhanced blue-green emission from *E. coli*, compared with the red dominated emissions from yeasts (fig. 4). This is because in the presence of excess lipids, the excited singlet oxygen molecules formed in the decomposition of lipid peroxides (Eq. 1) tend to be removed by reaction with the unsaturated lipid component forming excited carbonyl groups (Eq. 2), which emit light in the blue-green region. The excited singlet oxygen molecules consequently have less opportunity to form the red emitting dimers. One would thus expect the emission from the organism containing the lipid rich respiratory environment (i.e. the bacterium) to be dominated by blue-green wavelengths and the emission from yeast to be dominated by red wavelengths – as was observed[54,80,81].

Possible sources of the ultraviolet emissions

Significant ultraviolet luminescence was observed (fig. 2) from cultures of yeast and bacteria only during the exponential phase of growth and then only in the presence of oxygen. It is particularly noteworthy that the intensities of the ultraviolet luminescences from all the yeasts and the bacterium studied were about the same. Furthermore, the spectral distributions of these ultraviolet emissions were all similar. Such results tend to indicate that the source of the ultraviolet emissions from the exponential phase of growth might be the same across genera and phyla. This is a most interesting result and suggests that some very fundamental biochemical or biophysical process is responsible for this luminescence.

It should also be noted that these ultraviolet emissions are of similar intensity and wavelength to those designated as mitogenetic radiation by Gurwitsch[29] and the other early workers[63] in this field. Furthermore, like mitogenetic radiation they are also produced at their highest intensity during the exponential phase of growth, when the rate of cell division is at its highest level. The results presented thus provide considerable support for these early mitogenetic observations of ultraviolet cellular luminescence.

Since UV luminescence was detected from each species it was appropriate to test its ability to stimulate cell division when incident on nearby cells, as alleged by early workers[29,63] in the field. However, neither cell division nor growth in *S. cerevisiae* were stimulated or inhibited[60] by the UV emission from optically coupled exponential phase cultures of the yeast. Furthermore, no significant effects on division or growth were observed[55] when cultures of the same yeast were irradiated with continuous or chopped (100 cycles s^{-1}) artificial sources of UV light at 280 nm at various irradiances between 1.2×10^2 and 3.1×10^9 photons $cm^{-2}\,s^{-1}$. These results do not support the claims made by the earlier workers that dividing cells can stimulate mitosis in optically coupled cultures by the emission of UV luminescence, and are also in disagreement with some recent reports[11,24,38,86].

The sources of the UV luminescence from dividing cells are much less certain than are the sources of the visible region luminescences. Several possible causes of the UV emissions will be discussed here.

Early workers[63] in the field of mitogenetic radiation assigned the UV luminescences to various biochemical reactions in the cells, such as glycolysis, proteolysis, and nucleic acid-nuclease reactions. However, all of these biochemical reactions occur in yeast and bacterial cells under both aerobic and anaerobic conditions[47,48], whereas no luminescence was detected under anaerobic conditions (fig. 3). Hence none of the above reactions can be directly responsible for the UV emissions.

Inyushin[34] has attributed the UV luminescences from living organisms to emission from bioplasma. However, there is still controversy[61,88–90] over even the existence of bioplasma in living organisms and hence this proposal is still very much speculation.

Popp et al.[49,51,52] have suggested that DNA is the source of the very weak biological luminescences and that it acts as a 'photon store'. In their hypothesis the existence of a biofield is also invoked, this concept resembling that of Inyushin's bioplasma. The evidence that DNA is the source of this luminescence is only indirect[51] and Popp's research group[64] was itself unable to detect any luminescence emission from pure DNA. At this stage at least, there is still insufficient evidence to support the suggestion that DNA is the source of the UV luminescence.

Konev[39] has attributed UV luminescence from *Torula utilis* (*Candida utilis*) to an excited state of the tryptophan contained in cellular protein. This assignment was based on similarities between Konev's mitogenetic emission spectra and the spectra of the fluorescences produced by

the optical excitation of tryptophan and of cellular protein-containing tryptophan. Proteins are actively synthesized during the exponential phase of growth when the UV emission is observed. However, the luminescence can not arise directly from the major steps involved in protein synthesis, as no UV emission was observed when any of the organisms were grown anaerobically (fig. 3), even though protein synthesis still occurs under such conditions. It is nevertheless still possible that the UV luminescences arise from oxidative side reactions associated with protein synthesis.

It has also been suggested [57, 72] that instead of being the products of luminescent biological reactions, the very weak UV luminescence from living organisms might be cosmic ray excited fluorescence induced in susceptible biological molecules synthesized during the particular stages of growth which produce emission. Thus an investigation of this proposal was carried out [79] in which cultures of *E. coli* were shielded either intermittently or continuously from the soft component of cosmic rays, and examined for changes in growth and in luminescence intensity. No such changes were observed [79] and consequently this explanation is unlikely.

Ultraweak photon emission and temperature stress

The effect of temperature on the photon emission from *E. coli* was examined by the author [80] and it was found that the mean intensities of the exponential phase emissions from *E. coli* decreased by 55(± 9)% when the temperature was raised from 306.15 to 310.15 K. Figure 2B also shows that, although increasing the growth temperature of the bacterium from 306.15 to 310.15 K approximately halves the intensity of the luminescence emission, there is little effect on the structure of its spectral distribution.

When the temperature was raised from 306.15 K to 310.15 K, the stationary phase emission from *E. coli* decreased by 43(± 4)%, which is similar to the decrease observed for the exponential phase emission. As was the case for the exponential phase emission, the spectral structure of the stationary phase emission (fig. 4B) was little changed when the *E. coli* was grown at 310.15 K instead of 306.15 K, despite the reduction by almost a half in the luminescence intensity.

These results support the earlier suggestion that the visible region emissions are attributable to reactions associated with the formation and (or) decomposition of lipid hydroperoxides, since the effect of temperature stress on the intensity of the *E. coli* luminescence (figs 2B and 4B) can be explained in terms of temperature-induced changes in the ratio of unsaturated to saturated lipids. At lower temperatures, mesophilic bacteria such as *E. coli* increase the proportion of unsaturated lipids in the cellular membrane in order to maintain the appropriate membrane permeability [47]. Consequently, the opportunity for lipid peroxide formation and hence for luminescence, should be greater at the lower temperature, which is consistent with the increased emission observed when the temperature is decreased. However, since the source of the luminescence remains unchanged, the spectral distribution of the photon emission should not be altered, which was also the case (figs 2B and 4B).

Fisher et al. [19, 20] have studied the effects of temperature stress on the chemiluminescence of the amoeba, *Dictyostelium discoideum*. They found [20] that starvation of *D. discoideum* induces a chemiluminescence response which is enhanced under heat shock treatment. The chemiluminescence begins to increase dramatically after about 2 h, reaches a peak by about 4 h and declines to base level by 8 h after the onset of starvation. The heat treatment (growing the amoebae at 306 K instead of 294.5 K) produced a 30-fold increase in the photon emission intensity compared to an estimated 3.4-fold increase in the rate of the light yielding reactions. Hence it was concluded that the increase in temperature must enhance the biological process which leads to photon emission by about 10-fold. It is possible that the inducing signal for heat shock gene expression is a product, perhaps an oxygen metabolite, of perturbed mitochondrial metabolism in stressed cells. The authors thus suggest [20] that if an oxygen metabolite, such as superoxide or peroxide, is the inducing signal in stressed cells, then chemiluminescence should occur as a response to stress, which was the case for *D. discoideum*. The authors' observations that chemiluminescence is induced during early development and enhanced by heat shock at the beginning of development indicated a possible relationship between developmental and stress signals, and thus the products of perturbed oxidative metabolism might be inducers not only of heat shock responses, but also of differentiation during early development. Later experiments [19] using many inhibitors, traps or enhancers of chemical species involved in oxygen free radical reactions or oxidative metabolism suggested that the chemiluminescence results from lipid peroxidation reactions following oxygen reduction by leakage of electrons from ubiquinone in mitochondrial electron transport. This is consistent with the view that heat shock causes oxidative stress, which in turn induces both heat shock proteins and production of reduced oxygen metabolites.

The effect of chemical stress on ultraweak photon emission

Yeast

In his early experiments Konev [40] attempted to examine the source of the ultraweak photon emission which he detected from *Candida utilis* by adding propyl gallate, an inhibitor of free radical reactions. The photon emission was completely quenched and Konev suggested [40] that the luminescence may be due to excited species arising from recombination of free radicals. Unfortunately, propyl gallate also completely inhibited cell division in this yeast and thus the results were inconclusive.

Some yeasts, such as *Saccharomyces cerevisiae*, which possess both the respiratory and fermentative pathways of metabolism may lose their respiratory activity completely, even under aerobic conditions, if the glucose concentration is high (> 5%) or if the yeast is grown in the presence of certain dyes or in heavy metal solutions[48]. In the case of high glucose levels, the loss is only temporary and respiration can be easily restored by removing some of the glucose or by transferring the yeast to a lower glucose concentration medium. However, when growth is in the presence of appropriate dyes or metal ions, biochemical mutation takes place leading to a permanent loss of respiratory activity and the formation of respiratory-deficient mutants of the yeast. The author[59,62] has examined photon emission from the yeast *Saccharomyces cerevisiae* when it is grown in the presence of acriflavine. When growth occurs under these conditions, the yeast is almost completely converted to the respiratory-deficient mutant.

The respiratory-deficient mutant of *S. cerevisiae* gave[59] similar emission peaks to that of normal *S. cerevisiae* (figs 1 and 3) except that the exponential phase peak occurred about 3 h later (relative to the time of half-maximum absorbance) and was about ten times as intense. As was the case for the normal yeast, photon emission from the respiratory-deficient mutant occurred only during aerobic growth and varied in intensity with the stage of growth. It was concluded that the photon emissions did not directly arise from either the fermentative or respiratory pathways and that the superoxide anion may be involved in the processes leading to emission. Spectral distributions of these photon emissions were determined[62] and indicated that while the UV component of the luminescence from *S. cerevisiae* is not greatly altered when the respiratory-deficient mutant is used instead of the normal yeast, the visible region component is greatly enhanced in the mutant and its emission tends to be dominated by a band at around 600 nm. These observations are consistent with the suggestion that the visible region component of the luminescence arises from lipid peroxidation reactions.

To confirm that these results were due to the respiratory-deficient mutants and not some specific action of the acriflavine on cell constituents, similar suppression of respiration was obtained by two other methods (Quickenden and Tilbury, unpublished results). Respiratory-deficient mutants obtained by adding ethidium bromide to the medium as well as normal *S. cerevisiae* grown in a 6% glucose medium gave the same results as the respiratory-deficient mutants obtained by the addition of acriflavine. This indicates that the observed phenomena[59,62] are in fact due to the suppression of the respiratory activity of the yeast and not a mere artifact of the method to effect such a change.

Ezzahir et al.[18] have observed a methanal-induced photon emission from lag phase cultures of *S. cerevisiae*. They found that photon emission is increased up to 70-fold and that the initial oxygen consumption of the yeast decreases dramatically when the concentration of methanal is increased. Thus, the authors suggest[18] that the photon emission is not directly connected with the respiration of the cells but with the perturbation of their physiological state. Thermal denaturation of the cells only reduced the photon emission intensity by about 35% indicating that the interaction of methanal with components of the yeast cells is only partially enzymatic. The emission was also enhanced under basic conditions. It was proposed[18] that hydroperoxides could be formed by peroxidation of the methanal and the photon emission assigned to both excited carbonyl groups and excited singlet oxygen formed during the decomposition of these hydroperoxides.

Bacteria

Various workers[2,33,67] have found that superoxide dismutase and catalase inhibit bacterial photon emission indicating that both superoxide anion and hydrogen peroxide are involved in the production of luminescence. In addition, Roth and Kaeberle[67] found that the hydroxyl radical scavengers, mannitol and benzoate ions, did not inhibit the photon emission from *Lysteria monocytogenes* indicating that the hydroxyl radical may not be involved in the reactions leading to light emission. These authors[67] have also stimulated photon emission in *L. monocytogenes* following the cessation of luminescence by the addition of ethanal, similar to that observed above for yeast by methanal. However, unlike the action of methanal on yeast, no significant photon emission was detected[67] by the addition of ethanal to heat-killed cells. Hunter and Allen[33] found further evidence for the participation of hydrogen peroxide in their photon emission from *Streptococcus faecalis*. Addition of myeloperoxidase greatly enhanced the photon emission in this bacterium indicating the generation and accumulation of hydrogen peroxide, a necessary substrate for myeloperoxidase chemiluminescence. Evidence was also obtained for the extracellular production of both the superoxide anion and hydrogen peroxide and therefore that photon emission may arise from reactions on the outer membrane of the microbes or even within the suspending medium.

Amoeba

Lloyd et al.[42] observed photon emission from exponential phase cultures of *Acanthamoeba castellanii* and have shown that cyanide (10^{-3} mol dm^{-3}), which stimulates respiration in the early exponential phase of growth and inhibits respiration in the late exponential phase, enhances the luminescence in both phases of growth. These observations indicate that the photon emission is not directly correlated with the extent of reduction of oxygen at respiratory sites. This was further supported by the failure of two other inhibitors of the main respiratory chain in *Acanthamoeba castellanii*, azide and antimycin A, to affect the photon emission intensity. However, they

did find that both the enhancement of photon emission produced by cyanide and the inhibition of superoxide dismutase produced by cyanide, showed similar dependences on cyanide ion concentration and thus concluded that the luminescence may arise from one of the reactions of the superoxide anion.

Fisher et al.[19,20] observed photon emission from *Dictyostelium discoideum* during early differentiation and have examined[19] the effects of various inhibitors and enhancers of chemical species involved in oxygen free radical reactions and in oxidative metabolism. They have found evidence[19] for the involvement of hydrogen peroxide, superoxide anion and hydroxyl radicals in the processes leading to photon emission. However, unlike *Acanthamoeba castellanii* studied by Lloyd et al.[42], Fisher et al.[19] found that photon emission from *Dictyostelium discoideum* was quenched by some inhibitors of electron transport. They concluded that the photon emission results from lipid peroxidation reactions following oxygen reduction by leakage of electrons from ubiquinone in mitochondrial electron transport. A spectral distribution of the photon emission obtained by Fisher et al.[19] has shown that the emission was mainly in the blue region with a smaller red component, although their photodetector was relatively insensitive in the red region, which was consistent with excited carbonyl groups arising from lipid peroxidation reactions.

Conclusions

This paper has reviewed the ultraweak photon emission from microorganisms and has presented the current evidence for the possible sources and functions of these light emissions. Almost all microorganisms studied have been shown to emit luminescence and some of those species which failed detection of photon emission earlier have since been shown to be chemiluminescent, possibly due to the increased sensitivity of the detection apparatus. Photon emission in both the visible and ultraviolet region has been detected, although very little modern work has been carried out on the ultraviolet emissions and consequently the source(s) of this emission is much less certain than the visible emissions. The visible emissions appear to be due to excited carbonyl groups and/or excited singlet oxygen dimers arising from lipid peroxidation reactions, which in turn are associated with an increase in various reactive oxygen species such as the superoxide anion, hydrogen peroxide, hydroxyl radical and singlet oxygen. There is also indirect evidence for DNA playing a key role in these emissions. The photon emission is affected by all types of stress examined and may be a reliable, non-invasive and extremely sensitive indicator of cellular stress. Furthermore, there are indications that the photon emission may be a product of the stress signals themselves and some biophysical researchers claim that the photon emission may in fact be an integral part of the signalling mechanism itself.

1 Abeles, F. B., Plant chemiluminescence. A. Rev. Pl. Physiol. *37* (1986) 49–72.
2 Allen, R. C., Chemiluminescence from eukaryotic and prokaryotic cells: reducing potential and oxygen requirements. Photochem. Photobiol. *30* (1979) 157–163.
3 Andersen, B. R., Lint, T. F., and Brendzel, A. M., Chemically shifted singlet oxygen spectrum. Biochim. biophys. Acta *542* (1978) 527–536.
4 Arnold, J. S., Browne, R. J., and Ogryzlo, E. A., The red emission bands of molecular oxygen. Photochem. Photobiol. *4* (1965) 963–969.
5 Bateman, J. B., Mitogenetic radiation. Biol. Rev. *10* (1935) 42–71.
6 Borodin, D. N., Energy emanation during cell division processes (M-rays). Plant Physiol. *5* (1930) 119–129.
7 Brown, C. M., and Johnson, B., Influence of the concentration of glucose and galactose on the physiology of *Saccharomyces cerevisiae* in continuous culture. J. gen. Microbiol. *64* (1970) 279–287.
8 Cadenas, E., Biological chemiluminescence. Photochem. Photobiol. *40* (1984) 823–830.
9 Cadenas, E., Arad, I. D., Boveris, A., Fisher, A. B., and Chance, B., Partial spectral analysis of the hydroperoxide-induced chemiluminescence of the perfused lung. FEBS Lett. *111* (1980) 413–418.
10 Cadenas, E., Boveris, A., and Chance, B., Low level chemiluminescence of biological systems, in: Free Radicals in Biology, vol. VI, pp. 211–242. Ed. W. A. Pryor. Academic Press, Orlando 1984.
11 Celan, E., Gradinaru, D., and Celan, B., The evidence of selective radiation emitted by a cell culture which destructively affects some tumoral cell lines, in: Photon Emission from Biological Systems, pp. 219–225. Eds B. Jezowska-Trzebiatowska, B. Kochel, J. Slawinski and W. Strek. World Scientific, New York 1986.
12 Colli, L., and Facchini, U., Light emission by germinating plants. Nuovo Cimento *12* (1954) 150–153.
13 Colli, L., Facchini, N., Guidotti, G., Dugnani Lonati, R., Orsenigo, M., and Sommariva, O., Further measurements on the bioluminescence of the seedlings. Experientia *11* (1955) 479–481.
14 De Fazi, R., and De Fazi, R., Action of ultraviolet rays on alcoholic fermentation. Annali Chim. appl. *4* (1915) 301–329.
15 De Fazi, R., and De Fazi, R., Action of ultraviolet rays upon alcoholic fermentation of the must of India fig. Annali Chim. appl. *8* (1917) 93–101.
16 De Fazi, R., and De Fazi, R., Action of ultra-violet rays on alcoholic fermentation and on yeast. Atti Congr. naz. Chim. Ind. (1924) 449–450.
17 Esser, A., and Stauff, J., Lumineszenz von Hefe. Z. Naturf. *23* (1968) 1554–1555.
18 Ezzahir, A., Godlewski, M., Krol, M., Kwiecinska, T., Rajfur, Z., Sitko, D., and Slawinski, J., The influence of environmental factors on the ultraweak luminescence from yeast *Saccharomyces cerevisiae*. Bioelectrochem. Bioenerg. *27* (1992) 57–61.
19 Fisher, P. R., Karampetsos, P., Wilczynska, Z., and Rosenberg, L. T., Oxidative metabolism and heat shock enhanced chemiluminescence in *Dictyostelium discoideum*. J. Cell Sci. *99* (1991) 741–750.
20 Fisher, P. R., and Rosenberg, L. T., Chemiluminescence in *Dictyostelium discoideum*. FEMS Microbiol. Lett. *50* (1988) 157–161.
21 Gawronski, E., Effeckt mitogenetyczyn drozdzy *Saccharomyces cerevisiae* Hansen rasy piekarnianej AS. Ann. Univ. Mariae Curie-Sklodowska Sect. C *14* (1959) 407–433.
22 Gisler, G. C., Diaz, J., and Duran, N., Observations on the blood plasma chemiluminescence in normal subjects and cancer patients. Arq. Biol. Technol. *26* (1983) 345–352.
23 Grasso, F., Grillo, C., Musumeci, F., Triglia, A., Rodolico, G., Cammisuli, F., Rinzivillo, C., Fragati, G., Santuccio, A., and Rodolico, M., Photon emission from normal and tumor human tissues. Experientia *48* (1992) 10–13.
24 Grasso, F., Musumeci, F., Triglia, A., Yanbastiev, M., and Borisova, S., Self-irradiation effect on yeast cells. Photochem. Photobiol. *54* (1991) 147–149.
25 Gray, J., and Ouellet, C., Apparent mitogenetic inactivity of active cells. Proc. R. Soc. (London) B. *114* (1933) 1–9.
26 Gurwitsch, A. A., Spektry mitogeneticheskoho izlucheniya myshts kak pokazatel' dinamicheskoi molekulyarnoi organizatsii sarkoplazmy. Bull. exp. Biol. Med. (USSR) *62* (1966) 53–56.
27 Gurwitsch, A. A., Livanova, T. N., and Lazurkina, N. N., Effect of glucose administration and increased vagus nerve tone on changes of mitogenetic radiation and structure-energy states of the liver. Bull. exp. Biol. Med. (USSR) *89* (1980) 565–567.
28 Gurwitsch, A. G., The basic laws of mitogenetic excitation. Arch. Sci. biol. St. Pétersb. *31* (1931) 149–159.

29 Gurwitsch, A. G., and Gurwitsch, L. D., Die Mitogenetische Strahlung. Fischer-Verlag, Jena 1959.
30 Hollaender, A., and Claus, W. D., Some phases of the mitogenetic ray phenomenon. J. opt. Soc. Am. *25* (1935) 270–286.
31 Hollaender, A., and Claus, W. D., An experimental study of the problem of mitogenetic radiation. Bull. natl Res. Coun. Washington No. *100* (1937).
32 Hollaender, A., and Schoeffel, E., Mitogenetic rays. Q. Rev. Biol. *6* (1931) 215–222.
33 Hunter, D. J., and Allen, R. C., Oxygen-dependent *Streptococcus faecalis* chemiluminescence: the importance of metabolism and medium composition, in: Bioluminescence and Chemiluminescence, pp. 531–542. Eds M. A. DeLuca and D. W. McElroy. Academic Press, New York 1981.
34 Inyushin, V. M., and Chekerov, P. R., Biostimulation through Laser Radiation and Bioplasma (English transl.). Danish Society for Psychical Research, Copenhagen 1976.
35 Ishida, M. R., Ultraweak photon emission from the root tips of higher plant seedlings. I. The quantitative determination and spectroscopic study. A. Rep. Res. Reactor Inst. Kyoto Univ. *18* (1985) 51–55.
36 Johnson, B., and Brown, C. M., A possible relationship between the fatty acid composition of yeasts and the 'petite' mutation. Antonie van Leeuwenhoek *38* (1972) 137–144.
37 Khan, A. U., and Kasha, M., Chemiluminescence arising from simultaneous transitions in pairs of singlet oxygen molecules. J. Am. chem. Soc. *92* (1970) 3293–3300.
38 Kirkin, A. F., Non-chemical remote interactions between cells in culture. Biophysics (USSR) *26* (1981) 853–858.
39 Konev, S. V., Fluorescence and Phosphorescence of Proteins and Nucleic Acids (English transl.). Plenum Press, New York 1967.
40 Konev, S. V., Lyskova, T. I., and Nisenbaum, G. D., Very weak bioluminescence of cells in the ultraviolet region of the spectrum and its biological role. Biophysics (USSR) *11* (1966) 410–413.
41 Kulin, E. T., Effect of intercellular energy exchange on the metabolism of yeast cultures. Dokl. Akad. Nauk BSSR *4* (1960) 78–81.
42 Lloyd, D., Boveris, A., Reiter, R., Filipkowski, M., and Chance, B., Chemiluminescence of *Acanthamoeba castellanii*. Biochem. J. *184* (1979) 149–156.
43 Lloyd, R. A., Low level chemiluminescence from hydrocarbon autoxidation reactions. II. Thermal decomposition of benzoyl peroxide, cumene hydroperoxide and UV irradiated solvents. Trans. Faraday Soc. *61* (1965) 2182–2193.
44 Lorenz, E., Search for mitogenetic radiation by means of the photoelectric method. J. gen. Physiol. *17* (1934) 843–862.
45 Ludwig, E., Radiation from yeast. Wschr. Brau. *35* (1918) 19–20.
46 Mamedov, T. G., Popov, G. A., and Konev, S. V., Ultraweak luminescence of various organisms. Biophysics (USSR) *14* (1969) 1102–1107.
47 Mandelstam, J., McQuillen, K., and Dawes, I., Biochemistry of Bacterial Growth. Blackwell Scientific, Edinburgh 1982.
48 Moore-Landecker, E., Fundamentals of the Fungi. Prentice Hall, New Jersey 1982.
49 Popp, F. A., Photon storage in biological systems, in: Electromagnetic Bioinformation, pp. 123–141. Eds F. A. Popp, G. Becker, H. L. Koenig and W. Peschka. Urban and Schwarzenberg, Wien 1979.
50 Popp, F. A., Biophoton emission. Experientia *44* (1988) 543–630.
51 Popp, F. A., Nagl, W., Li, K. H., Scholz, W., Weingartner, O., and Wolf, R., Biophoton emission: New evidence for coherence and DNA as source. Cell Biophys. *6* (1984) 33–52.
52 Popp, F. A., Ruth, B., Barr, W., Bohm, J., Grab, P., Grolig, G., Rattemeyer, M., Schmidt, H. G., and Wulle, P., Emission of visible and ultraviolet radiation by active biological systems. Coll. Phenomena *3* (1981) 187–214.
53 Pryor, W. A., Oxy-radicals and related species: their information, lifetimes and reactions. A. Rev. Physiol. *48* (1986) 656–667.
54 Quickenden, T. I., Comarmond, M. J., and Tilbury, R. N., Ultraweak bioluminescence spectra of stationary phase *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Photochem. Photobiol. *41* (1985) 611–615.
55 Quickenden, T. I., Matich, A. J., Pung, S. H., and Tilbury, R. N., An attempt to stimulate cell division in *Saccharomyces cerevisiae* with weak ultraviolet light. Radiat. Res. *117* (1989) 145–157.
56 Quickenden, T. I., and Que Hee, S. S., Weak luminescence from the yeast *Saccharomyces cerevisiae* and the existence of mitogenetic radiation. Biochem. biophys. Res. Commun. *60* (1974) 764–770.
57 Quickenden, T. I., and Que Hee, S. S., The spectral distribution of the luminescence emitted during growth of the yeast *Saccharomyces cerevisiae* and its relationship to mitogenetic radiation. Photochem. Photobiol. *23* (1976) 201–204.
58 Quickenden, T. I., and Que Hee, S. S., On the existence of mitogenetic radiation. Speculations Sci. Technol. *4* (1981) 453–464.
59 Quickenden, T. I., and Tilbury, R. N., Growth dependent luminescence from cultures of normal and respiratory deficient *Saccharomyces cerevisiae*. Photochem. Photobiol. *37* (1983) 337–344.
60 Quickenden, T. I., and Tilbury, R. N., An attempt to stimulate mitosis in *Saccharomyces cerevisiae* with the ultraviolet luminescence from exponential phase cultures of this yeast. Radiat. Res. *102* (1985) 254–263.
61 Quickenden, T. I., and Tilbury, R. N., A critical examination of the bioplasma hypothesis. Physiol. Chem. Phys. med. NMR *18* (1986) 89–101.
62 Quickenden, T. I., and Tilbury, R. N., Luminescence spectra of exponential and stationary phase cultures of respiratory deficient *Saccharomyces cerevisiae*. J. Photochem. Photobiol., B: Biol. *8* (1991) 169–174.
63 Rahn, O., Invisible Radiations of Organisms. Borntraeger, Berlin (1936).
64 Rattemeyer, M., Popp, F. A., and Nagl, W., Evidence of photon emission from DNA in living systems. Naturwissenschaften *68* (1981) 572–573.
65 Reiter, T., and Gabor, D., Zellteilung und Strahlung. Springer, Berlin 1928.
66 Richards, O. W., and Taylor, G. W., Mitogenetic rays – A critique of the yeast detector method. Biol. Bull. *63* (1932) 113–128.
67 Roth, J. A., and Kaeberle, M. L., Chemiluminescence by *Listeria monocytogenes*. J. Bact. *144* (1980) 752–757.
68 Russell, G. A., Deuterium-isotope effects in the autoxidation of aralkyl hydrocarbons. Mechanism of the interaction of peroxy radicals. J. Am. chem. Soc. *79* (1957) 3871–3877.
69 Schauf, B., Repas, L. M., and Kaufmann, R., Localization of ultraweak photon emission in plants. Photochem. Photobiol. *55* (1992) 287–291.
70 Scheminzky, F., Photographic proof of emanations in biochemical processes. Biochem. Z. *77* (1916) 14–16.
71 Slawinska, D., and Slawinski, J., Biological chemiluminescence. Photochem. Photobiol. *37* (1983) 709–715.
72 Slawinska, D., and Slawinski, J., Low-level luminescence from biological objects, in: Chemi- and Bioluminescence, pp. 495–531. Ed. J. G. Burr. M. Dekker, New York 1985.
73 Slawinski, J., Grabikowski, E., and Ciesla, L., Spectral distribution of the ultraweak luminescence from germinating plants. J. Lumines. *24/25* (1981) 791–794.
74 Slawinski, J., Majchrowicz, I., and Grabikowski, E., Ultraweak luminescence from germinating resting spores of *Entomophthora virulenta* Hall et Dunn. Acta mycol. *17* (1981) 131–139.
75 Stauff, J., and Reske, G., Chemilumineszenz der Hefe. Naturwissenschaften *51* (1964) 39–43.
76 Strehler, B. L., and Arnold, W., Light production by green plants. J. gen. Physiol. *34* (1951) 809–820.
77 Tarusov, B. N., Ivanov, I. I., and Petrusevich, Yu. M., Ultraweak Luminescence in Biological Systems. Moscow University, Moscow 1967.
78 Tarusov, B. N., and Veselovksy, V. A., Ultraweak Emissions of Plants and Their Applications. Moscow State University, Moscow 1978.
79 Tilbury, R. N., and Quickenden, T. I., The effect of cosmic-ray shielding on the ultraweak bioluminescence emitted by cultures of *Escherichia coli*. Radiat. Res. *112* (1987) 398–402.
80 Tilbury, R. N., and Quickenden, T. I., Spectral and time dependence studies of the ultraweak bioluminescence emitted by the bacterium *Escherichia coli*. Photochem. Photobiol. *47* (1988) 145–150.
81 Tilbury, R. N., and Quickenden, T. I., Luminescence from the yeast *Candida utilis* and comparisons across three genera. J. Biolumin. Chemilumin. (1992) in press.
82 Vasilev, R. F., Chemiluminescence in solutions. I. Methods of identifying the excited states. Opt. Spectrosc. *18* (1965) 131–135.
83 Vladimirov, Yu. A., Ultraweak Luminescence Accompanying Biochemical Reactions (English transl.). NASA, C.F.S.T.I., Springfield, Vermont 1966.
84 Wang, Y., Zhao, A., Ma, Y., Yu, M., Zhang, Y., Dai, J., and Li, S., Studies on ultraweak luminescence of bacteria. Acta microbiol. sin. *30* (1990) 58–62.
85 Wolff, L. K., and Ras, G., Über Gurwitschstrahlen bei einfachen chemischen Reaktionen. Biochem. Z. *250* (1932) 305–307.

86 Yanbastiev, M. I., Main and additional problems of biophotonics. J. molec. Struct. *115* (1984) 299–302.
87 Zhuravlev, A. I., Aleksandr, I., and Trostkinov, V. N., Luminescence in Living Cells. Nauka, Moscow 1968.
88 Zon, J. R., Physical plasma in biological solids: A possible mechanism for resonant interactions between low intensity microwaves and biological systems. Physiol. Chem. Phys. *11* (1979) 501–506.
89 Zon, J. R., The living cell as a plasma physical system. Physiol. Chem. Phys. *12* (1980) 357–364.
90 Zon, J. R., Physical plasma may exist in biostructures: A reply to the critique by Quickenden and Tilbury. Physiol. Chem. Phys. med. NMR *19* (1987) 295–300.

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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.