Lasers in the Life Sciences 2(1). 1988, pp. 53-74
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Printed in the United States of America

Molecular Mechanism of the Therapeutic Effect of Low-Intensity Laser Radiation

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Quantitative studies have been performed to determine the action of low-intensity visible monochromatic light on various cells *(E. coli,* yeasts, HeLa); also irradiation conditions (wavelength, dose) conducive to vital activity stimulation have been examined. Respiratory chain components are discussed as primary photo-acceptors. The possible ways for photosignal transduction and amplification are discussed. It is proposed that enhanced wound-healing due to irradiation with low-intensity visible laser light (He-Cd, He-Ne and diode lasers) is due to increased proliferation of cells.

KEYWORDS: biostimulation, action spectra, DNA synthesis rates, *E. coli* growth rate

1. "LASER BIOSTIMULATION EFFECT" DOES NOT REQUIRE LASER LIGHT

The low-intensity noncoagulative radiation of He-Ne laser with $\lambda = 632.8$ nm has been used for more than a decade in clinical practice. There are numerous examples where this method has been successful for treating trophic ulcers and indolent wounds of diverse etiology when traditional drug treatment has not been as effective [1, 39, 40].

Sometimes enhanced wound healing has been associated with specific characteristics of laser radiation (coherence, polarization), but there has been no physical basis for such a conclusion [2]. It has been shown that the interaction of laser radiation with molecules in condensed phase at 300°K has coherent character at light intensity $I \geq 2 \times 10^{11}$ W/cm^2 [2]. Usually much

lower light intensities are used in experiments on nonthermal light interaction with a biological system, and in nonthermal (biostimulation) clinical treatment [I], intensities are typically in the range 10^{-2}-10^{-4} W/cm^2. For these intensities, coherence effects associated with molecular excitation is vanishingly small [2]. For low-intensity visible light absorption by biological systems, the interaction is not due to coherence.

The response of mammalian cells to irradiation by relatively broad bands of visible light (mainly blue and red) was known long before the introduction of lasers (see [3-8] among numerous examples and review [42]). Renewed interest in the effects of visible light action on biological objects occurred in the sixties after appearance of the first lasers, particularly the He-Ne laser. The He-Ne laser was the first widely accessible source of coherent light. No wonder that the stimulating effect of light, red in particular, was rediscovered with the use of the coherent light source. Recent literature suggest experiments on the action of incoherent light on biological objects performed in the twenties and the thirties [6-8] were either forgotten or were not known. So, the observed effects were attributed to a unique property of the He-Ne laser light, coherence of its radiation. Actually there were no physical grounds for such a conclusion [2].

Thus, there is reason to believe that the "red light syndrome" has a photobiological nature. Recently we have shown an equal efficiency of noncoherent and laser light in the biostimulation treatment of stomach and duodenal ulcers [9, 33]. Although the therapeutic effect of low-intensity red light is possible with noncoherent light, the lasers are undoubtedly handy tools for laboratory and clinic. The discussion of the importance of specific laser light properties, such as coherence, is essential only as regards the explanation of the action mechanism of low-power laser light in therapy.

2. QUANTITATIVE DATA ABOUT THE ACTION OF VISIBLE LIGHT AT CELLULAR LEVEL

In the last few years, systematic studies have been conducted with objects of various complexity levels—bacteria [10, 11, 17, 51], yeasts [12, 13], and mammalian cells [14-16, 27]—to clarify the molecular mechanisms and quantitative laws of low-intensity visible light action conducive to stimulation of vital activity. The culture growth rate, DNA and RNA synthesis rate, activity of diverse enzymes, cAMP level have been estimated. Some of the results of these studies are summarized in Figures 1 and 2.

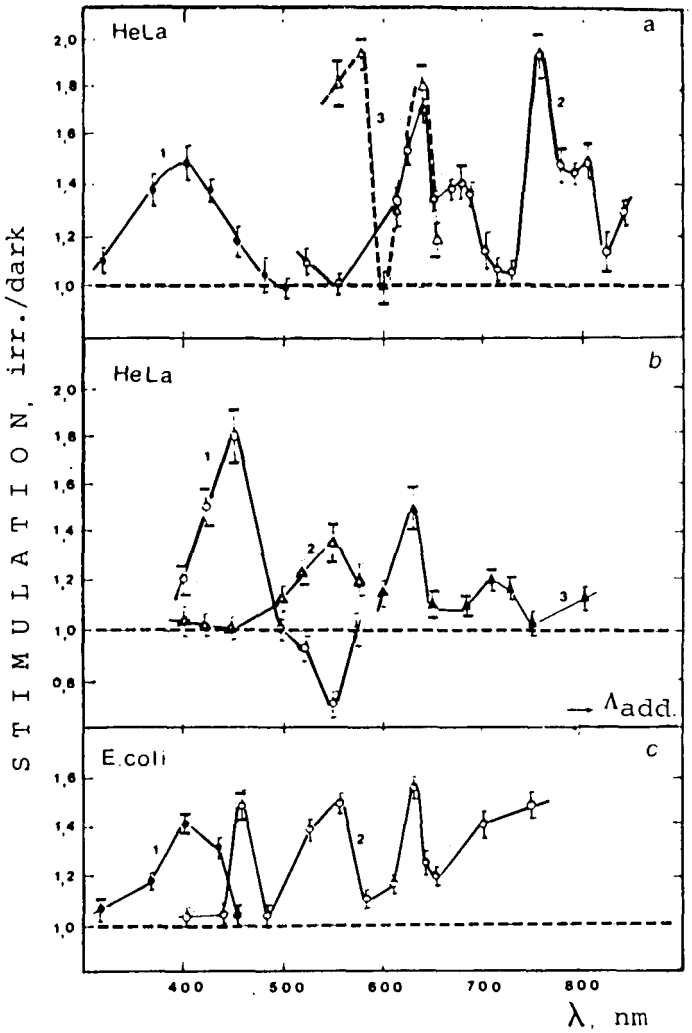

FIGURE 1. Action spectra of monochromatic visible light upon: A, DNA synthesis rate in HeLa cells (1, 0 = 10 J/m^2; 2,D= 100 J/m^2; 3, D = 100 J/m^2 and in conditions of decreased pO_2); B, DNA synthesis rate in HeLa cells at the simultaneous dichromatic irradiation with λ = 632.8nm and λ_{add} (l,D$\lambda_{632.8}$ = 100 J/m^2, Dλ_{add} = 10 J/m^2; 2, Dλ_{add} = l00 J/m^2, Dλ_{add}= 25 J/m^2; 3, D$\lambda_{632.8}$ = Dλ_{add}= 100 J/m^2); C, *E. coli* culture growth rate (1, D=13 J/m^2; 2, D=4 x 10^3 J/m^2. The conditions of irradiation and methods of DNA synthesis rate determination are described in [14]; the *E. coli* growth conditions, irradiation and growth stimulation measure, in [10, 11]; experiment with dichromatic irradiation, in [16]; experiment with decreased pO_2 in [33].

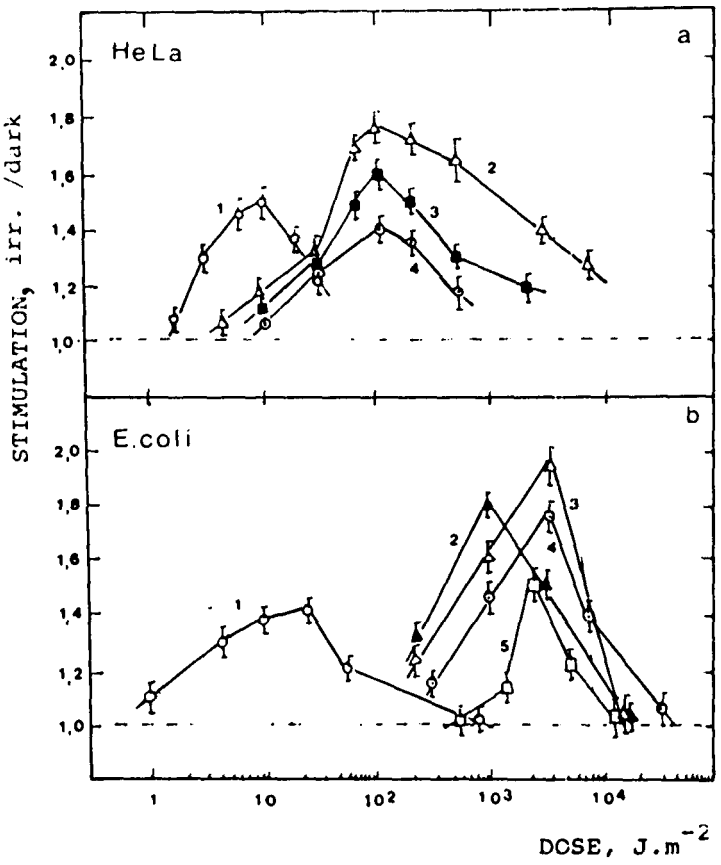

FIGURE 2. The effect of irradiation dose on stimulation of A, DNA synthesis rate in HeLa cells (1,λ =404nm;2, λ = 760nni;3, λ = 620nm;4, λ = 680 nm); B, *E. coli* culture growth rate(l, λ =404nm;2, λ = 560nm;3, λ = 750 nm; 4, 632.8 nm; 5, λ =454nm)[10, 11, 14].

Figure 1 illustrates the action spectra of visible light (A) on the DNA synthesis rate in HeLa cells, and (C) on *E. coli* culture growth rate. The synthesis rate in HeLa cells, and (C) on *E. coli* culture growth rate. The technique and conditions of irradiation are described in [10-14]. One of the maxima of the action spectra occurs about 620 nm; the 632.8 nm wavelength of the He-Ne laser is sufficiently close to the peak to expect significant response. If there exists a correlation between the maxima in action spectra and therapeutic effect of different wavelengths of visible light, the red light near 620 nm must not be the only successful wavelength for "laser bio-stimulation." Indeed, this is supported by the successful clinical use of low-intensity light at λ = 441.6 nm (He-Cd laser) and at λ. = 830 nm semiconductor lasers [39, 40]. The growth stimulating effect of broad band blue light was described in the thirties [6].

All the derived action spectra (Fig. 1, as well as stimulation of protein synthesis in yeast [25], RNA synthesis in plateau-phase HeLa cells [15]) have maxima in several regions of visible light.

Data on the rate DNA synthesis in HeLa cells and culture growth rate off. *coli* as a function of energy density for various wavelengths is shown in Figure 2. From the analysis of these dose curves one can conclude that there are two groups of active spectral regions. The first group—light with wavelengths 365,404 and 434 nm (see Fig. 2 for λ= 404 nm as example)— has stimulative action 10-100 times lower than doses of the second group of wavelengths (454,560,633 and 750 nm). In other words, the same effect can be achieved with light in near UV and blue region using at least Ио the doses required with red or far red light.

In spite of the similarities in different action spectra as well as in dose-response curves, there are great differences concerning the effective dose ranges for every culture. For example, the photosensitivity to red light (λ = 633 nm) is different not only for HeLa cells (D_{max} = 100 J/m^2), *E. coli* (D_{max} = 4 x IO^3 J/m^2), but varies even for different species. This is attested by the relationships between the dose and the biomass accumulation stimulation for different yeast cultures (see Fig. 3). It is seen from Figure 3 that each culture has its own D_{max} the dose for which the biomass accumulation is maximal. The activation percentage at the cells exposure to D_{max} is also dependent on the culture. From these data, the following can be observed from the correlation line shown in the upper section of Figure 3: more photosensitive cultures (lower D_{max}) are activated to a greater degree (higher percentage of the biomass accumulation activation).

A universal photosensitivity nature of various cells—procaryotes, primitive and complex eucaryotes—to low-intensity monochromatic light sug-

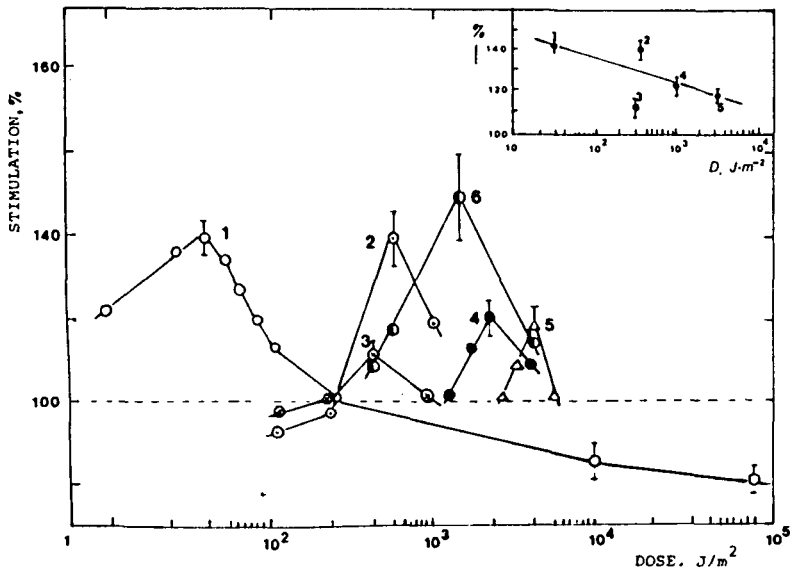

FIGURE 3. The effect of irradiation dose (He-Ne laser, λ = 632.8 nm) on the biomass accumulation stimulation for different yeast cultures (1, *Saccharomycodes ludvigii* (grown with glycose); 2, *Torulopsis sphaerica* (glycose); 3, *Candida boidinii* (glycose); 4, *Candida mallosa* (glycose); 5, *Saccharomyces cerevisiae* 14 (glycose); 6, *Candida boidinii* (grown in methanol). Upper corner: The relationship between the maximal biomass accumulation stimulation and the correspondence D_{max} The conditions of cultivation, irradiation and measurements are described in [12, 25].

gests similar molecular mechanism with the same primary photoacceptors. Complicated action spectra suggest that the photoacceptor might be a set of interrelated molecules. The results of the dichromatic irradiation experiments with simultaneous or short-interval successive exposures to two wavelengths [16, II], support this suggestion (see Fig. 1B). HeLa cells were simultaneously irradiated by light with 632.8 nm and by another wavelength, λ_{add} which is shown on the abscissa of Figure IB. This action spectrum differs significantly from the spectrum for monochromatic irradiation (see Fig. 1A): the blue maximum shifts from 404 nm to 450 nm (at $D\lambda_{max}$ = 10 J/m^2) or disappears (at $D\lambda_{max}$ = 25 J/m^2); a band of inhibition (at $D\lambda_{max}$ = *25* J/m^2) or stimulation (at $D\lambda_{max}$ = 25 J/m^2) appears in the green spectral region. In addition, the maximum at λ = 680 nm vanishes and the maximum at λ = 760 nm becomes unessential.

3. PRIMARY PHOTOACCEPTORS

Modification of the photoresponse by the redox agents suggest that the primary photoacceptor(s) might be component(s) of a redox chain [43]. For example, in the case off. *coli* the reducing agent $Na_2S_2O_4$ prevented the growth stimulating effect of blue, red and far red light [II]. Variations in partial pressure of oxygen (pO_2) of the nutrient medium immediately preceding the irradiation by a short-duration N^ blow (60 sec.) changed the mode of action spectrum in the green region (see Fig. 1A). The growth stimulating effect of red light on *E. coli* was reduced by α-naphtylene-acetic acid (auxin) and KI [17]. These chemicals are believed to be the quenchers of excited flavin molecules, and KI is the uncoupler of oxydative phosphorylation (see [17, 24]). The oxydizing agent methylene blue stimulated DNA synthesis in HeLa cells in the dark, *i.e.,* mimicked the action of red light [24].

It is possible that the respiratory chain could serve as the redox chain. If this is the case, isolated mitochondria must also have the photosensitivity. In fact, red light (600 nm [18], 633 nm [20], and 650 nm [19]) increased the ATP synthesis in mitochondria from rat liver cells [18-20]. Irradiation with blue light (420 nm) increased ATP synthesis while λ = 477,511, and 544 nm were ineffective [18].

For such a mechanism, a correlation has to be found between the bands of action spectra (Fig. 1) and the absorption bands of respiratory chain components. It should be noted, though, despite the universality, general uniformity, and principle of operation of the respiration process, the respiratory chains of procaryotes *(E. coli* included [21]) differ from mitochondrial chains of eucaryotic cells. Respiratory chains of bacteria differ from mitochondrial chain by the set of cytochromes, quinones, and by the chain organization (the presence of alternative pathways). Thus, *E. coli* may have cytochrome o or d as a terminal oxidase, according to the growth conditions [21]. Among the eucaryotes there are some little differences in yeast's respiratory chain compared to the classical mitochondrial chain. In addition to the classical set of cytochromes, the yeast mitochondria have the specific carrier, cytochrome b_2, and a special NADH-dehydrogenase, so the first energy coupling point functions with extreme lability [22].

Absorption bands of most components of the respiratory chain-flavopro-teins and hemoproteins are in the blue (400-450 nm) part of spectrum. Cytochromes absorb in the green region; in the red region (about 630 nm) absorb semiquinone forms of flavo-proteins as well as terminal oxidases-cyt d (it is this branch of the respiratory chain that prevails when bacteria *E. coli*

are grown in the absence of aeration which is a condition in our experiments [11]), and cyt a/a_3 [23] which is the terminal oxidase of mitochondrial chains. The above-mentioned complex absorbs at about 600, 680, 760 and 830 nm [23] and the cyt d absorbs at 630 nm and 683 nm [21]. Thus, a distinct correlation exists between the action spectra (Fig. 1) and the absorption bands of respiratory chain components for the corresponding organisms.

Another argument in favor of the suggestion that the primary photoacceptors can be components of the respiratory chain is an intimate correlation between oxygen consumption activity and culture growth which is especially appreciable for microorganisms. For instance, in our experiments with yeast cultures [13, 25] correlations were found between the respiration intensity of an intact culture and its ability to activate itself after a He-Ne laser irradiation (curve 1 in Fig. 4), and in irradiated with He-Ne laser cultures between the NADH-dehydrogenase activity and the stimulation of biomass accumulation (curve 2 in Fig. 4).

4. ARE THE SAME PRIMARY PHOTOACCEPTORS RESPONSIBLE FOR STIMULATIVE AND INHIBITORY ACTION OF LIGHT?

An analysis of the data "respiration stimulation-culture growth rate stimulation" and "respiration inhibition-growth rate inhibition" makes it possible to contend that in both cases the same molecules can play the role of photoacceptors while the dose and the intensity of the light dictate the sign of the end macroeffect. For example, the action spectra of stimulative (Fig. 1) and lethal action of blue light on *E. coli* [26] both have maxima of 404-410 and 454-460 nm. The stimulating doses of blue light fall within the range between 10 and 10^3 J/m^2 (Fig. 2), while lethal action is caused by doses of 10^6-10^8 J/m^2 [26]. In both cases, the flavin components of the respiratory chain are discussed as photoacceptors [26, 17]. For mammalian cells, data about stimulative and inhibitive action of blue and red light are reviewed in [42]. So, the photocontrol of the cell metabolism from the viewpoint of positive (stimulating) effect takes place only in a narrow dose range of light. As the dose increases, the photoreceptors are damaged and the effect decreases. A further increase of the dose causes destruction of photoreceptors which is accompanied by growth inhibition and cell lethality. The fact that irradiation of cells with visible light of one and the same wavelength and absorption of this light by the same molecules has both a positive effect (acceleration of

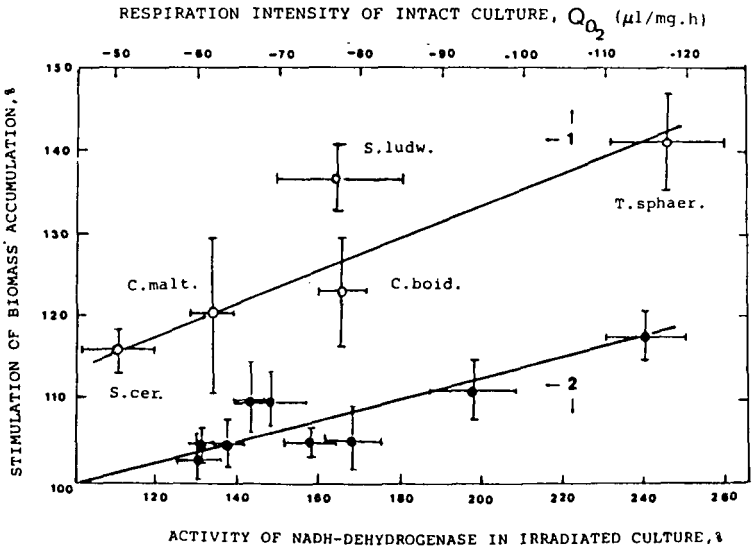

FIGURE 4. Correlations: A, between the respiration intensity of intact yeast cultures and possible maximal biomass accumulation stimulation after irradiation with He-Ne laser in optimal conditions (see D_{max} on Fig. 3); B, between the activity of NADH-dehydrogenases and biomass accumulation stimulation after the irradiation of *Torulopsis spluiericu* yeast with He-Ne laser in different doses (from 3 x 10^2 to 1.,9 x 10^3 J/m^2). Determination of respiration intensity and NADH-dehydrogenase activity are described in [13, 25).

cell division) and a negative effect (damage to intracellular systems and even death of cells) can be explained as follows. There are two processes involving one and the same primary photoprocess-electronic excitation. One of them is the acceleration of the electron transfer in the redox pairs in some sections of the respiratory chain, and the other, the transfer of the excitation energy to oxygen to form $^1O_2$ (as will be recalled, flavins and cytochromes are photosensitizers). At doses causing biostimulation, the former process predominates (the result being the redox control of the cellular metabolism), and at high doses there occurs photodynamic damage.

5. METABOLISM REARRANGEMENT (LONG-TERM EFFECTS OF IRRADIATION)

The experimental data show that comparatively low doses (10^2-10^3 J/m^2) and short periods (10-100 s) of irradiation cause a macroeffect lasting for a long time. Note that in Figures 5 and 6. When the HeLa cell culture in the stationary growth phase was irradiated by the He-Ne laser and the cells were subcultured at various intervals after irradiation (from 5 to 240 minutes), the culture growth stimulation in the exponential phase of growth was observed over 6-7 days (Fig. 5) [27]. Stimulation was noted when the interval between the irradiation and the replantation was 30 min. or more.

The *Torulopsis sphaerica* yeast culture was irradiated by He-Ne laser, incubated during 18 hours (at least 2 divisions), following which the activity of some enzymes was determined (Fig. 6) [13]. The data obtained show that the growth stimulation is accompanied by the respiration activity increase (with no accumulation of toxic intermediates of oxygen metabolism) and by synthetic processes in cell predominance over degenerative ones.

These data indicate that the irradiation causes a cell metabolism rearrangement, the light playing the role of a trigger controller of the cell metabolism.

6. THE POSSIBLE MECHANISM ON CELLULAR LEVEL

It has been known that the proliferation of cells depends on numerous factors of the environment (the nutrient medium ions composition, hormones and growth factors presence, temperature, *etc.).* It is possible that monochromatic light, to which organisms are not evolutionally adapted (as opposed to the white light, to which organisms have been adapted evolutionally), is one of the environment factors (external agents) capable to modify the cells proliferation. In this case the growth stimulation or inhibition by light could be treated as one of the sensory responses for accomodation to the environmental conditions. Accomodation (behavioral) responses of bacteria, including *E.* coli are connected with the phenomenon of proton motive force sensing (pmf-sensing) [28, 29]. The bacteria utilize their respiratory chain as the sensor for a behavioral response *(e.g.,* aerotaxis, chemotaxis, *etc.).* In this case any parameter that alters the pmf can be a stimulus [28, 29]. Thus, the possible role of light as a growth controlling signal for a procaryotic microorganisms can be stated as: the light signal perceived by the cell respiratory chain is a case of the more general class of the sensory reception reactions.

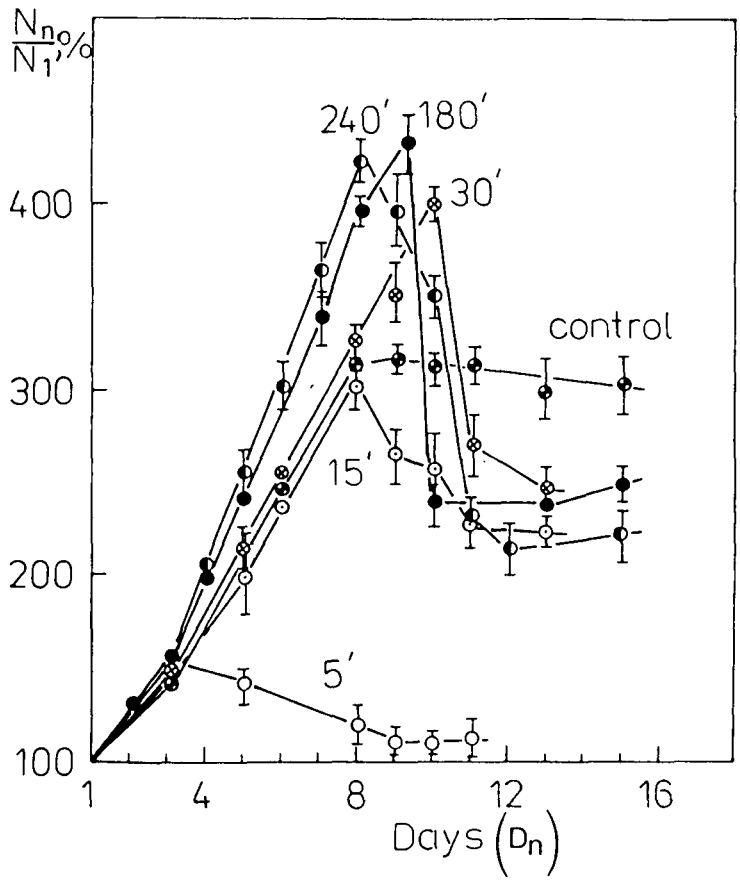

FIGURE 5. The growth curves of HeLa culture after irradiation of plateau-phase cells with He-Ne laser (D = 100 J/m^2) and plating cells into fresh nutrient medium 5, 15, 30, 180 or 240 min. after the irradiation [27]. The abscissa gives the ratio of number of cells in day in (N_n) and at the end of first day (N_1|), the ordinate gives the days (Day_n) after the plating.

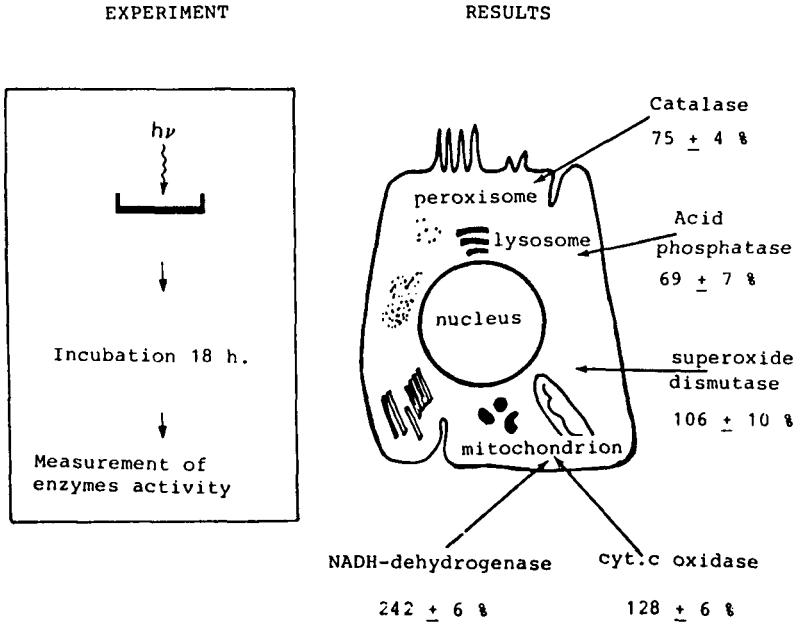

FIGURE 6. Changes in activity of different enzymes 18 hrs. after the irradiation *of Torulopsis sphaerica* yeast with He-Ne laser (D = 1 x 10^3 J/m^2) [13].

These reactions share the same initial stage (a variation in the protonmotive force), while signal realization mechanisms (signal transduction chains) and end results differ. In Figure 7A there is a possible scheme for photosignal transmission in *E. coli* cells.

The sensory transmission chain in eucaryotic cells is a more complicated case. According to the universally adopted standpoint, environmental signals influencing the eucaryotic cell proliferation are perceived and transferred by the cellular membrane and transferred to the nucleus (for instance, action of growth factors, mitogenes of the PHA type, *etc.*). Should our suggestion concerning the respiratory chain as the primary photoacceptor (see part 3 of this paper) be correct, the photosignal transmission must be much more complicated. Figure 7B shows a possible scheme of the photosignal transduction in eucaryotic cells, as it may be thought of based on scanty experimental data available today.

One can imagine the following scheme of interaction between low-inten-

sity monochromatic visible light and a cell. The absorption of light by the respiratory chain components—flavine dehydrogenases, cytochromes, and cytochrome oxidase—causes a short-term activation of the respiratory chain and oxidation of the NAD pool, this in turn leading to changes in the redox status of both mitochondria and cytoplasm [34]. The activation of the electron-transport chain must result in an increase of $Д\mu_H+$ (protonmotive force, $\Delta\psi$ (electrical potential of mitochondrial membrane), and ATP pool and acidulation of cytoplasm. That this inference is true has been confirmed experimentally: changes in $\Delta\mu_H + \Delta\psi$ and ΔpH have been achieved as well as extrasynthesis of ATP [20] by irradiating mitochondria with a He-Ne laser, and by irradiating cells with a wide-band visible light of $\lambda = 400$ nm, they have managed to enhance the activity of ATP synthetase [44].

The rise of the intracellular H^+ concentration controls allosterically the activity of the Na^+/H^+ antiporter situated in the cytoplasmic membrane [45]. This enzyme plays a key part in the alkalization of the intracellular medium. A short-term increase in the intracellular pH is one of the necessary components involved in the transmission of mitogenetic signals in the cell [45]. Earlier we have suggested that low-intensity laser light activates the division off. *coli* by raising the intracellular pH as a result of photoactivation of the respiratory chain in the case of neutral or acid extracellular pH values [17], the necessary intracellular pH level in the case of alkaline extracellular hydrogen ion concentrations being provided by the Na^+/H^+ antiporter [46]. Thus, laser light can, in principle, stimulate division of both procaryotic and eucaryotic cells by controlling the intracellular pH.

The increase of the intracellular hydrogen ion and ATP concentration in eucaryotic cells causes activation of other membrane ion carriers as well, such as Na^+ .Ka^+ -ATPase. The activation of this enzyme leads to an increase in $[K^+]$, and a decrease in $[Na^+$, and E_m. The variation of these parameters is a necessary component in the control of the proliferation activity of the cell [37,38]. It should be borne in mind that changes in the respiratory chain also alter the flows of Ca^+ between mitochondria and cytoplasm, which in turn affects the ratio $[Ca^+]_i/[Ca^{2+}]_o$. Note that alteration of the cellular homeostasis parameters entails a whole cascade of reactions, and the causal relationships are very difficult to establish unambiguously.

The changes in redox level of the cell as well as in concentration of mono-and divalent ions may affect its metabolism by influencing the cyclic nucleotides level [35, 50]. It is well known that cellular metabolism and development are controlled by a system of cyclic nycleotides, particularly cyclic 3',5'-adenosine monophosphate (cAMP), the cAMP production and the

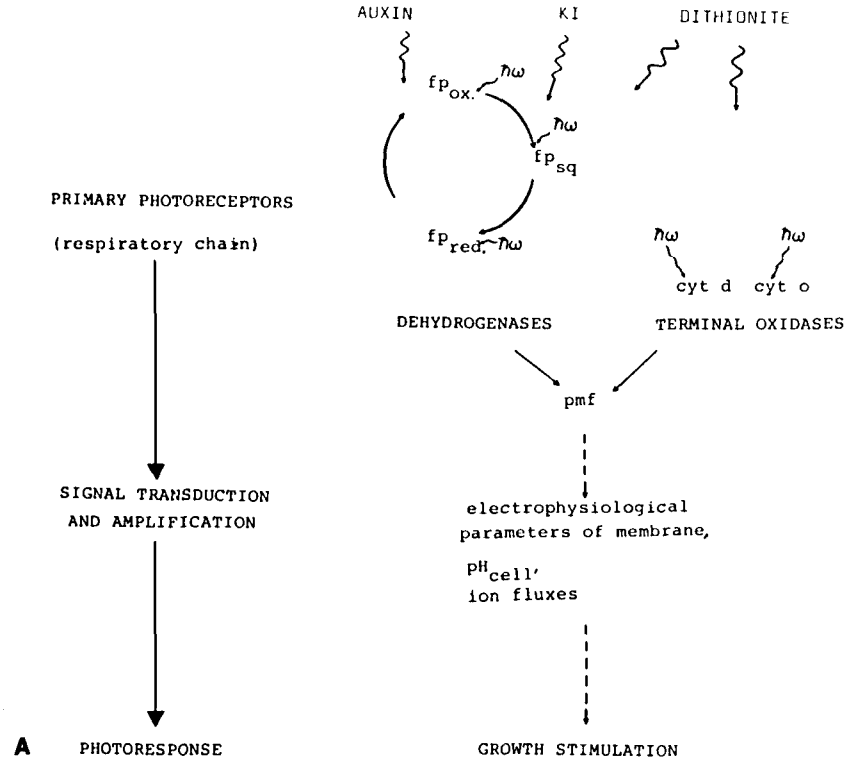

FIGURE 7. Possible pholosignal transduclion chains for growth stimulation by visible light in case of: A, procaryotic (£. *coli};* and B, eucaryotic cells. Possible action of chemicals (auxin, KJ, Na_2,S_2O_4) [11, 17] is shown. $\Delta\mu_H$ + ↑ ΔpH ↑, Δχ ↑ = increase in photonmotive force, proton gradient and electrical potential of mitochondrial membrane; [ATP] ↑ = increase in ATP concentration; E_N↑ = increase in cellular redox potential (change into more oxidized

direction); $\left[\frac{NAD^+}{NADH}\right]_{mit} \uparrow\rightarrow \left[\frac{NAD^+}{NADH}\right]_{cyt} \uparrow$ = oxidation of NADH pool in mitochondrion, causing changes in NADH pool of cytoplasm; Na^+/H^+ antiporter f and $Na^+.K^+$-ATPase = activation of these enzymes; $E_{m}\downarrow$ = decrease of electrical potential of cellular membrane; $pH_I\uparrow$ = decrease of H^+ concentration in cell; $\Delta[Ca^{2+}]_i$, Δ[cAMP] = changes in intracellular concentration of Ca^{2+} and cAMP; DNA, RNA synthesis ↑ = activation of nucleic acids synthesis.

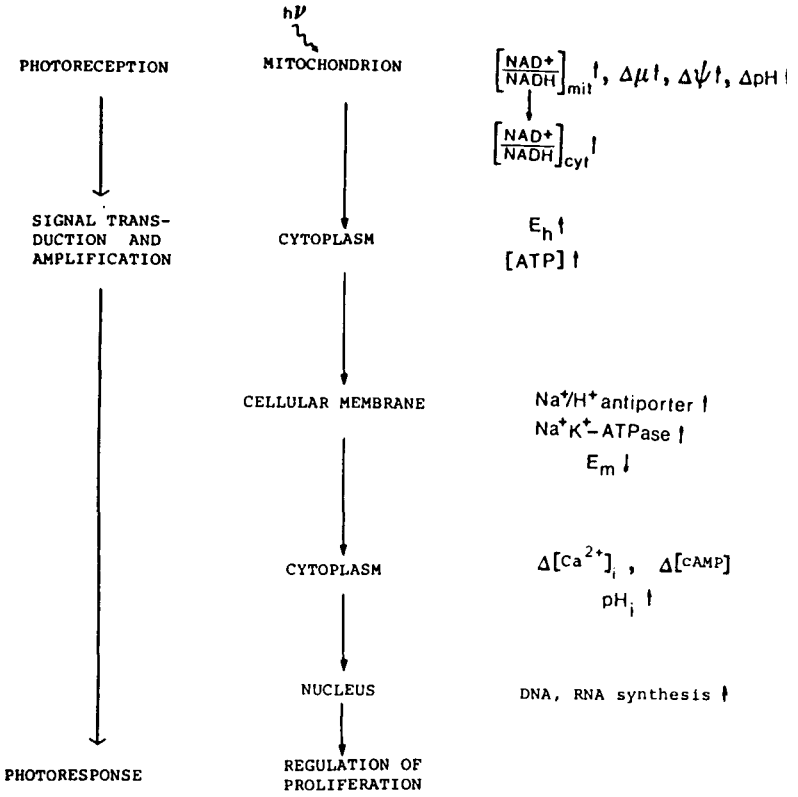

FIGURE 7. *(Continued)*

cAMP-dependent protein kinase activity being involved in the events leading to initiation of DNA synthesis [36]. We have found that the intracellular concentration of cAMP changes in a monolayer culture of Chinese hamster cells following their irradiation with light at 632.8 and 760 nm [30]. These results as well as experimental data about changes in cAMP level in eucaryotic microorganisms after irradiation with blue light [31, 32] provide grounds to believe that the action of visible light upon the cell might be connected with regulation of cellular metabolism via cAMP Variations in

cAMP level, as well as in electrophysiological properties of cellular membranes following blue light irradiation [31], show that the cytoplasmatic membrane participates in the photosignal transfer.

The scheme suggested above is a possible version only. More experimental data are needed to understand how light stimulus is converted into chemical and electrical signs which are understood by the cell, and how these signals can be detected and transmitted by certain cellular components.

7. POSSIBLE THERAPEUTIC MECHANISM

Low intensity visible light may act as a regulating factor of cell proliferation in the local medical effects of low-intensity radiation of He-Ne laser (λ= 632.8 nm), He-Cd laser (λ = 441.6 nm) and far red diode lasers (λ = 830-904 nm) when treating trophic ulcers and indolent wounds. In such injuries, conditions preventing cell reproduction (low values of oxygen concentration and pH, lack of necessary nutrients, *etc.)* develop under these conditions, there is almost no proliferation of cells and they enter Go phase or are found in prologed G|. For such cells, light may serve as a signal to increase the proliferation. Indeed we have noticed the effect in the case of plateau-phase HeLa cells [15, 27], and Go cells like lymphocytes [41]. In case of cells in exponential phase of growth (Figs. 1,2, [14,16, 33]) the stimulation by light may take place only when the culture growth rate is slow. In usual conditions of our experiments the labeling index (percent of cells being in S phase of cellular cycle) of exponential growth phase HeLa culture is 19-21 percent. In this case after the irradiation with maximal stimulative doses *(e.g.* 100 J/m^2 for λ = 632.8 nm) the labeling index increases to 32-34 percent [52]. On this model of HeLa culture with labeling index 19-21 percent legitimacies described above are valid. In the culture, where the labeling index is high, photostimulation appears to be impossible. This problem is discussed in [52].

The same conclusion could be drawn from the growth stimulation experiments with microorganisms. In autumn and winter the culture features relatively slow growth (Fig. 8). In spring and summer, when the culture growth accelerates and the growth rate of the control itself is almost comparable with that of the autumn-winter period after exposure to an optimum dose of red light, the irradiation has little effect (Fig. 8). Thus, there is almost no photostimulation effect in summer, in winter it is maximum, and in spring and autumn the effect is intermediate (Fig. 9). Therefore, the therapeutic

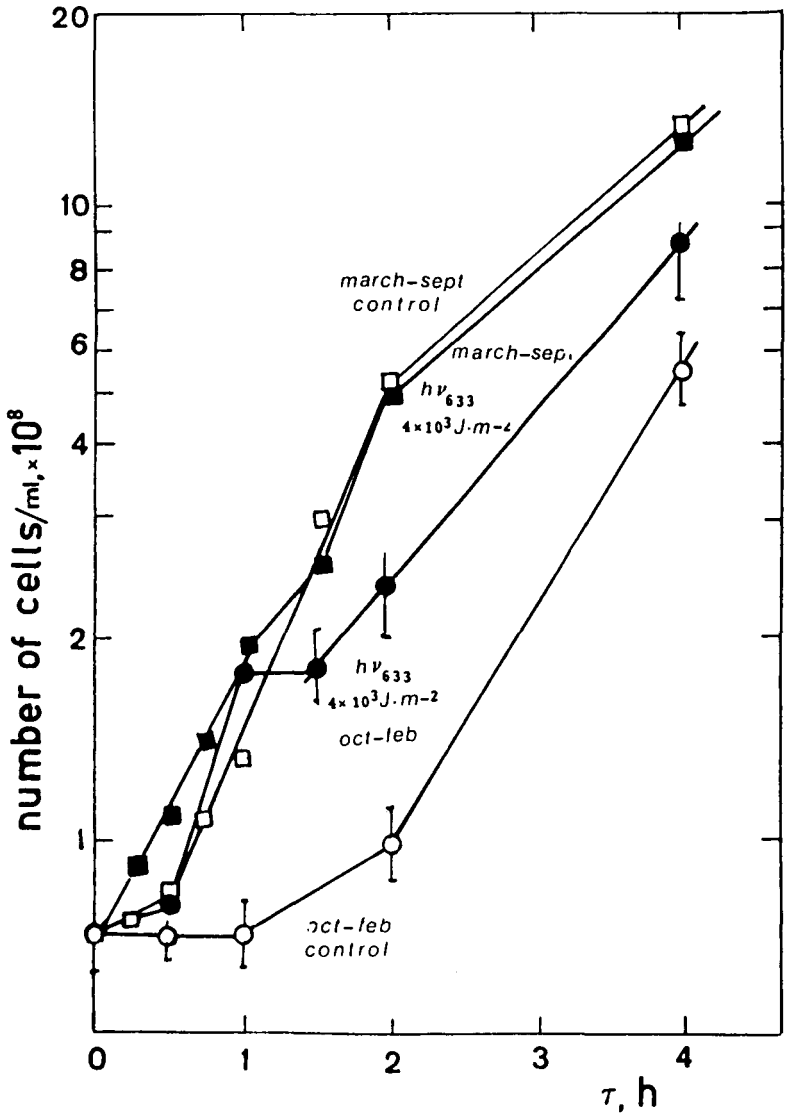

FIGURE 8. Growth curves of *E. coli* culture in summer (March-September) and winter (October-February), and after the irradiation with He-Ne laser (D = 4 x 10^3 J/m^2) in summer and winter. The growth stimulation is denned as difference in the number of cells in irradiated and nonirradiated culture 60 min. after the beginning of incubation.

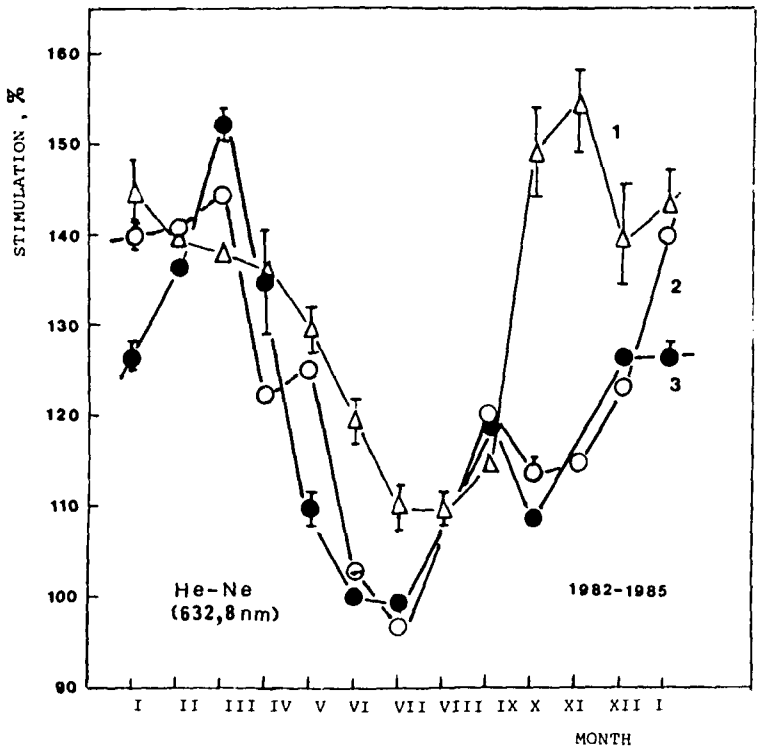

FIGURE 9. The maximal growth stimulation of microorganisms (1, *Escberichia coli; 2, Torulopsis sphaerica,* 3, *Saccharomycodes ludwigii)* after irradiation with He-Ne laser in optimal conditions (D_{max} see *on* Figs. 2, 3). Average date from years 1982-85. The experiments were performed always at the same time (near 11 a.m. with *E. coli* and near 6 p.m. with yeasts).

effect of low-intensity light (blue, red, far red) on irradiation of fresh wounds is minimum, if at all, because in this case the cell proliferation is high and the regeneration of tissue integrity has more or less maximum rate. This may be the reason why the phototherapeutic effect is often not observed by the irradiation of fresh experimental wounds and has been found effective in case of "old and bad" ones. Of course, it should be born in mind that proliferation control in the case of an organism is much more complex process compared to a cell culture. In clinical practice, low-intensity laser radiation is used for treating not

only local lesions (trophic ulcers and wounds, inflammations), but very often also "systemic" effects has been found (the effect is observed at some distance from the irradiation site) [40]. It is exactly this universality of action of low-intensity light (a "cure for all diseases") that gives rise to some distrust, although vast statistical material has been amassed to date proving that such a treatment has a positive effect ([1, 39, 40] and references made therein). The positive effects of phototherapy in the case of "systemic" treatment *(e.g.* pain relief) can be explained by the fact that low-intensity light (blue, red) acts on excitable cells (myocardial cells, neurons) to generate action potentials in them, alter their beating frequency, *etc.* [47-49]. Proof can be found in these works that photoreception occurs on the level of mitochondria and that the intensification of respiratory metabolism of the excitable cell affects the electrophysiological properties of its membrane.

It is quite possible that the universality of low power laser effects both on cellular and organism levels is due to the universal photobiological mechanism-variation of the redox state of the respiratory chain components leading to the changes of physiological state of the cell *(i.e.,* increase of proliferation activity of nonexcitable cells or changes in impulsation frequency of excitable cells).

Acknowledgments

I am greatful to Prof. V. Letokhov for constant support and Drs. G. Fedoseyeva and 0. Tiphlova for helpful discussions.

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