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PHOTOBIOLOGY OF LOW-POWER LASER EFFECTS

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Abstract—**Quantitative studies have been performed to determine the action of low-intensity visible monochromatic light on various cells *(E. coli,* yeasts, HeLa, Chinese hamster fibroblasts and human lymphocytes); also irradiation conditions (wavelength, dose and intensity) conducive to vital activity stimulation have been examined. Respiratory chain components are discussed as primary photoacceptors. 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 semiconductor lasers) is due to the increasing proliferation of cells.**

1. WHY LOW-POWER LASER EFFECTS SEEM TO BE INCREDIBLE AND MYSTERIOUS?

INTEREST in the "biostimulating effect" of low-intensity red light, particularly He-Ne irradiation, has increased in the last few years, and discussions of this phenomenon have become more urgent.

On the one hand, low-power laser therapy is undoubtedly successful in treating trophic ulcers and indolent wounds of diverse etiology when traditional drug treatments have little effect (Mester 1980; Gamaleya 1977; Kryuk et al. 1986). On the other hand, the method seems to be highly incredible and even mysterious. There is frank skepticism among physicists as to the reliability of the fact that low-intensity visible radiation acts directly on the organism at the molecular level. So a certain disparity has arisen between the active work of doctors and the lack of interest shown by physicists, chemists and biologists.

Indeed, it is incomprehensible how one can explain the special sensitivity of cells to irradiation by red light against a background of systematic irradiation by white light, whose spectrum contains a red component of approximately the same power. A second reason for doubts is that very often the stimulative effects of irradiation are explained by the particular role of coherent laser irradiation acting upon biological objects. This argument is not convincing and makes physicists suspicious when hearing about low-power laser biostimulation. And a third reason for doubts is the universality of clinical use of low-power laser therapy as well as numerous biological and physiological effects. Treatment indications are very broad (see the reviews of Gamaleya 1977; Basford 1986; Kryuk et al. 1986), including even radioprotection (Voskanyan et al. 1985, 1986; Popova et al. 1984; Abvakhitova et al. 1982; Stepanov et al. 1977).

The aim of this paper is to try to explain these uncertainties and to prove the existence of low-intensity visible laser radiation effects at the cellular level.

2. 'LASER BIOSTIMULATION' AS A PHOTOBIOLOGICAL PHENOMENON WITHOUT LASER LIGHT

To support this statement, I will use three arguments. Firstly, curing with red light was used in medicine even in ancient times, and treatment with red light was among the methods used by N. R. Finsen, the father of contemporary phototherapy. Much experimental work was done in the last century and during the first third of this century. One can find these references in the review Karu 1987. It is very likely that by the time the first lasers made their appearance this earlier knowledge was forgotten.

The He-Ne laser was the first commercially available source of coherent light. No wonder that the stimulating effect of light—red light in particular—was rediscovered when use was made of this coherent light source. The observed effects were attributed to the unique quality of the He-Ne laser—namely, the high coherence of this radiation. Actually there are no physical grounds for such a conclusion, as demonstrated in the above-mentioned review (Karu 1987).

Secondly, the coherent effects of light-matter interaction in a condensed phase at 300 K are possible at light intensities $\geq 2 \times 10^{11}$ W/cm^2 (Lobko et al. 1985; Karu 1987). The usual intensity of red light (e.g., those generated by He-Ne lasers or diode lasers operating in the far red region) used in experiments on light interaction with a biological system, clinical practices included, is 10^{-4}-10^{-2} W/cm^2.

And thirdly, both coherent and noncoherent red light were clinically found to be equally effective in treating peptic ulcers (Sazonov et al. 1985; Karu 1986b).

One has every reason to believe that this "laser biostimulation" phenomenon is of a photobiological nature. I would like to emphasize that I do not intend to deny the laser its merit as a handy tool for the laboratory and clinic. A discussion of the importance of specific laser

light properties, such as coherence, is essential only in regard to explaining the action mechanism of low-power laser light.

3. QUANTITATIVE DATA ABOUT PHOTOBIOLOGICAL EFFECTS AT THE CELLULAR LEVEL

During the last six years, systematic studies (Fig. 1) have been conducted with objects of varying complexity levels—bacteria (Karu et al. 1983; Tiphlova and Karu 1986, 1987a, 1987b, 1988), yeasts (Fedoseyeva et al. 1984, 1986, 1987a), and mammalian cells (Karu et al. 1982, 1984a, 1984b, 1984c, 1985a, 1985b; Fedoseyeva et al. 1988) to clarify the molecular mechanism and quantitative laws of low-intensity visible light action at the cellular level.

Some results of these studies are summarized in Figs. 2-4. Figure 2 shows the action spectra (the dependence of the photobiological effect on wavelength) of visible light for stimulation of DNA synthesis in HeLa cells and for growth stimulation of *Torulopsis sphaerica* and *Escherichia coli.* Some other examples of various action spectra are reviewed in Karu 1987. All these action spectra are practically of the same type, having maxima in almost every visible light band (at about 400-500, 570, 620 and 830 nm). If there exists a correlation between the maxima in an action spectra and the therapeutic effect of different wavelengths of visible light, the red light near 620 nm must not be the only successful wavelength for laser biostimulation. Indeed, this is supported by the successful clinical use of low-intensity radiation of He-Cd lasers (λ = 441.6 nm) (Kryuk et al. 1986) and of semiconductor lasers operating at various wavelengths in the far red and near infrared regions (Basford 1986).

Data on the stimulation of metabolic processes in cells as a function of energy doses for various wavelengths are shown in Figs. 3 and 4. From the analysis of these dose-effect curves, one can conclude that there are two groups of active spectral regions. The first group—light with wavelengths of 364, 404, 434 nm (see Fig. 3 for λ = 404 nm as an example) and 890 nm (Fig. 4)—has a stimulative action 10-100 times lower than doses from the second group of wavelengths (454, 560, 633, 680 and 750 nm). In other words, the same effect can be achieved with light in the near UV and blue, as well as the far red region, using one-tenth the doses required, e.g., with red light.

The results of measuring the influence of light intensity (and irradiation time) are presented in Fig. 5. In these experiments, the radiation dose was constant, while the intensity of light and irradiation time were varied.

So, it is possible to conclude that irradiation with monochromatic visible light in the blue, red and far red regions can enhance metabolic processes in the cell. The photobiological effects of stimulation depend on the wavelengths, dose and intensity of the light.

The experimental data show that comparatively low doses ($10\text{-}10^3$ J/m^2) and short periods (10-100 s) of irradiation cause a macroeffect which remains for a long time. When an HeLa cell culture in the stationary growth phase was irradiated as a monolayer with a He-Ne laser and the cells were then plated at various intervals after irradiation (from 5-240 min), the culture growth stimulation in the exponential growth phase was observed to last for 6-7 d (Fig. 6a). Stimulation was noted when the interval between irradiation and plating was 30 min or more (Karu et al. 1984c). Irradiation of cells with stimulative doses of red light can protect them from damaging doses off radiation. In Fig. 6b is shown one result of this

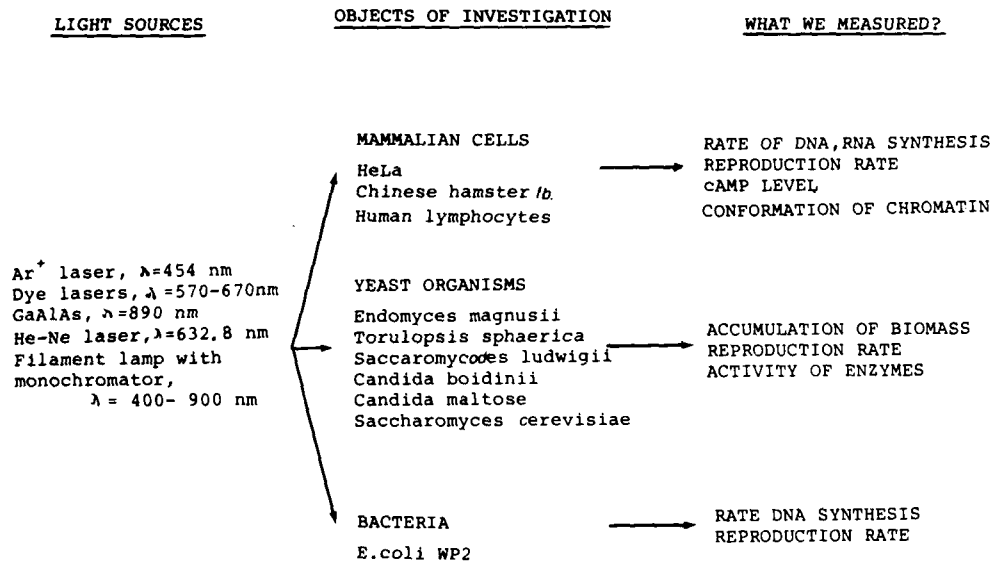


Fig. 1. Experiments performed with various cells for explaining the action mechanism of low-power monochromatic visible light.

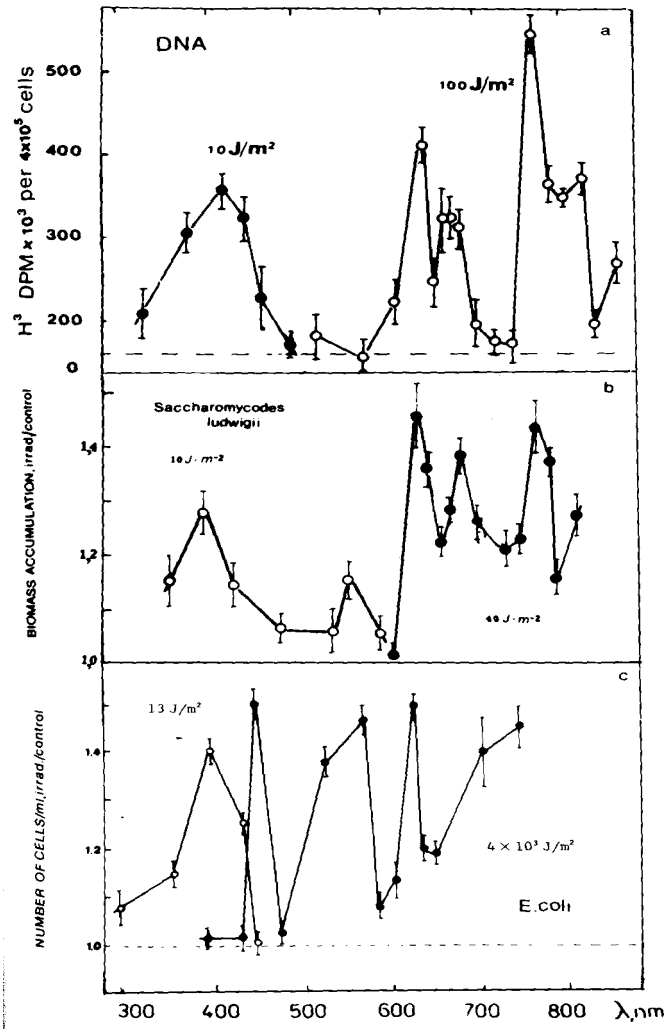


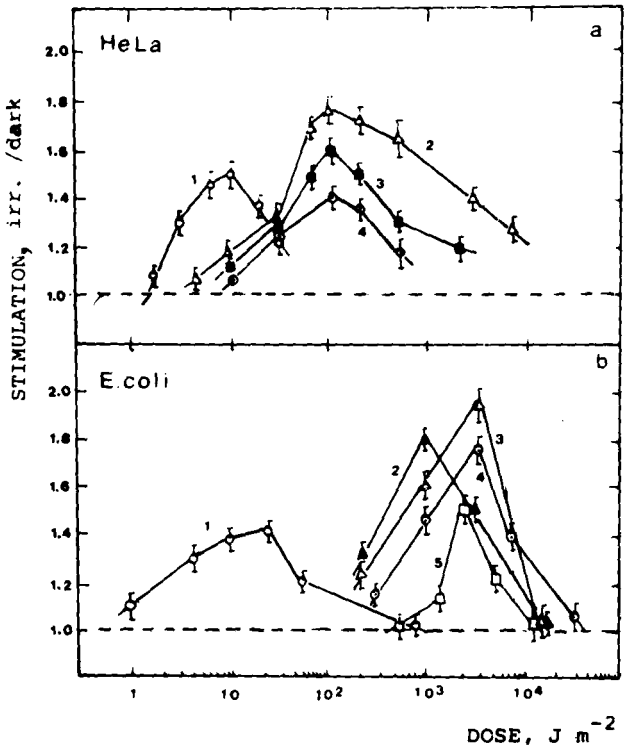
Fig. 2. Action spectra of visible monochromatic light for (a) DNA synthesis stimulation in exponentially growing HeLa cells, (b) protein synthesis stimulation of *Saccharomycodes ludwigii,* and (c) growth stimulation off. *coli.* The experimental conditions are described in papers of Karu et al. 1984a, Fedoseyeva et al. 1987a, and Tiphlova and Karu 1988.

kind of experiment (Karu et al. 1987c). A monolayer of HeLa cells in the stationary phase of growth was irradiated with a He-Ne laser *(D* = 10^2 J/m^2) and after 180 min with γradiation (5.0 Gy). After 15 min, the cells were replated i into a fresh nutrient medium, and the growth curves, clone-forming ability and clone sizes were studied (Karu etal. 1988c). The radioprotective ability of He-Ne laser radiation appeared when the interval between laser and *1* radiation was longer than 30 min.

Autoradiographic studies indicated that irradiation with a He-Ne laser causes an increase in the number of 5-phase cells (Fig. 7), connected with enhanced G'i-5' transition of a part of the population, as well as the increase of grain count on the labeled nuclei, related to enhancement of DNA synthesis in S-phase cells (Karu et al. 1987a).

Irradiation does not influence the proliferation of various subpopulations to an equal degree, as shown by analyzing the clone size distribution after irradiation (Fig. 8). The stimulative effect of irradiation is most noticeable or the proliferation activity of slowly growing subpopulations. Irradiation with a He-Ne laser also caused a decrease in the generation time of yeast cultures in an exponential period of growth (Fedoseyeva et al. 1984; Karu and Letokhov 1985).

In a series of experiments, the activity of some enzymes was measured in a stimulated culture. The culture *Torulopsis sphaerica* was irradiated with a He-Ne laser and incubated for 18 h (for at least three divisions), following which the activity of various enzymes was determined (Fedoseyeva et al. 1986). Irradiation caused considerable



activation of respiratory chain components— NADH dehydrogenases and cytochrome c oxidase. The

Fig. 3. The effect of irradiation dose on stimulation of (a) DNA synthesis rate in exponentially growing HeLa cells (1: λ = 404 nm, 2: λ = 760 nm, 3: λ= 620 nm, 4: λ = 680 nm), and (b) *E. coli* culture growth (1: λ= *404* nm), 2: λ = 560 nm, 3: λ= 750 nm, 4: λ = 632.8 nm, 5: λ = *454* nm). The irradiation conditions are described in papers of Karu et al. 1982, 1984; and Tiphlova and Karu 1987b, 1988.

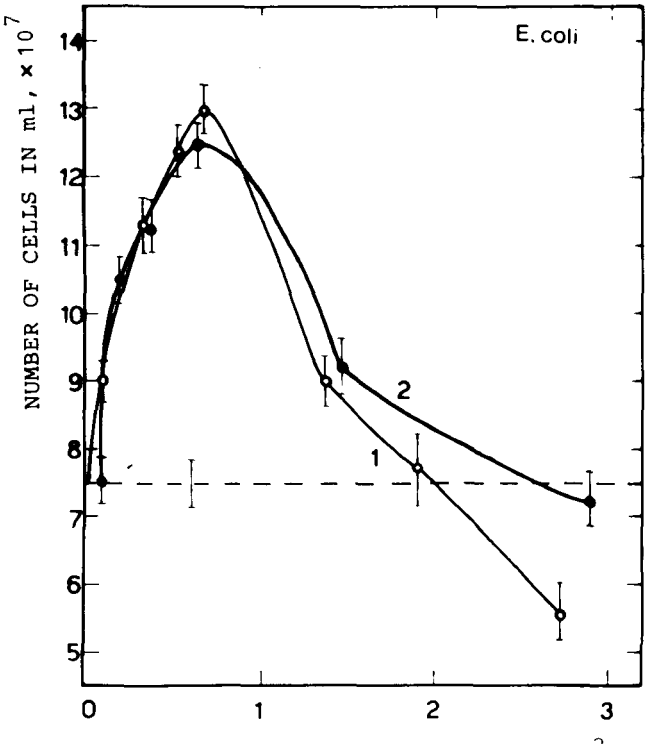


Fig. 4. The effect of irradiation dose (GaAlAs laser, λ = 890 nm, at frequencies of 666 Hz (1) or 3480 Hz (2), J = 1.2 • 10^{-2} W/ m^2) on the stimulation of *E. coli* growth. The irradiation conditions are described in the paper Zharov et al. 1987.

activity of cytosolic dismutase remained practically on the control level, and the activity of acid phosphatase declined (Fig. 9). The data obtained show that the growth stimulation is accomplished by an increase in the respiration activity (with no accumulation of the toxic intermediate of oxygen metabolism) and by synthesis processes in cell-dominating degenerative ones.

To support the suggestion that light can serve as a proliferative stimulus, the resting cells *(Go* cells)—human peripheral lymphocytes—were irradiated with a He-Ne laser and the conformational changes of chromatin (chromatin does not absorb this light) were studied (Fedoseyeva et al. 1988). By measuring the degree of acridine orange binding to DNA, it was found that the changes occurring in the lymphocyte chromatin structure a few hours after irradiation were similar to those caused by mitogen phytohemagglutinin (PHA). The binding of the dye to DNA increased 45-90 min after irradiation, then dropped to the control level, and later increased again (Fig. 10).

Light provides the proliferative stimulus, having some effect on the systems that are known to regulate (or at least take part in) cellular proliferation. The cyclic adenosine monophosphate (cAMP) system has been demonstrated to control both biosynthesis of DNA and RNA and the realization of the biologic activity of these macromolecules (Boynton and Whitfield 1983). The ir

radiation of Chinese hamster fibroblasts with blue (404 nm) or red (632.8 nm) light caused changes in the intracellular cAMP level (Fig. 11). Irradiation with light at 546 or 700 nm had no appreciable effect (Karu et al. 1985b, 1987b). There exists some similarity between the wavelengths effective for DNA and RNA synthesis stimulation (Fig. 2a), which cause the variations in cAMP level.

Our observation of changes in the intracellular cAMP concentration following irradiation may help relate growth stimulation effects to the known regulatory mechanisms of the proliferation activity of cells and may assist in further studies at revealing the mechanism of the biostimulating effect of red light, insofar as it is well known that there exists a causal relationship between variations in the concentrations of cAMP and Ca^{2+} on the one hand and the rate of synthesis of DNA and RNA on the other at early stages of regenerative processes (Martelly and Franquinet 1984).

Summing up, it is possible to conclude that irradiation with monochromatic visible light in the blue, red and far red regions can enhance metabolic processes in the cell and can activate proliferation. The data indicate that irradiation causes a rearrangement of the cell metabolism, light being only in the role of a trigger. This is the reason the doses needed for causing biostimulation effects are comparatively low (10-10^3 J/m^2) and the irradiation times needed are short (10-100 s).

4. PRIMARY PHOTOACCEPTORS AS RESPIRATORY CHAIN COMPONENTS AND REGULATION OF CELLULAR METABOLISM VIA CHANGES IN REDOX POTENTIAL

Complicated action spectra (Fig. 2) 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 (e.g., 404 and 633 nm, 633 and 760 nm) support this suggestion (Karu et al. 1985a; Tiphlova and Karu 1987b). A universal photosensitive nature, similarity of action spectra, and similarity of the shape of dose-effect dependencies of various cells—procaryotes, primitive and complex eucaryotes—to low-intensity monochromatic light suggest a similar molecular mechanism with the same primary photoacceptors. The probability of the respiratory chain components being the primary photoacceptors (i.e., compounds absorbing light at wavelengths effective in bringing about responses for irradiation) has been discussed in Karu 1986a, 1987) using three approaches: (1) Comparison between the action spectra for photo responses and the absorption spectra of respiratory chain components, since an action spectrum should closely parallel the absorption spectrum of the photoreceptor compound. (2) Looking for correlations between the activity of respiratory enzymes and the proliferative activity of cells after irradiation. (3) Use of sub-1 stances which are known to act as quenchers of the excited ' states of the presumed photoacceptor molecules and demonstration that the compounds or procedures known

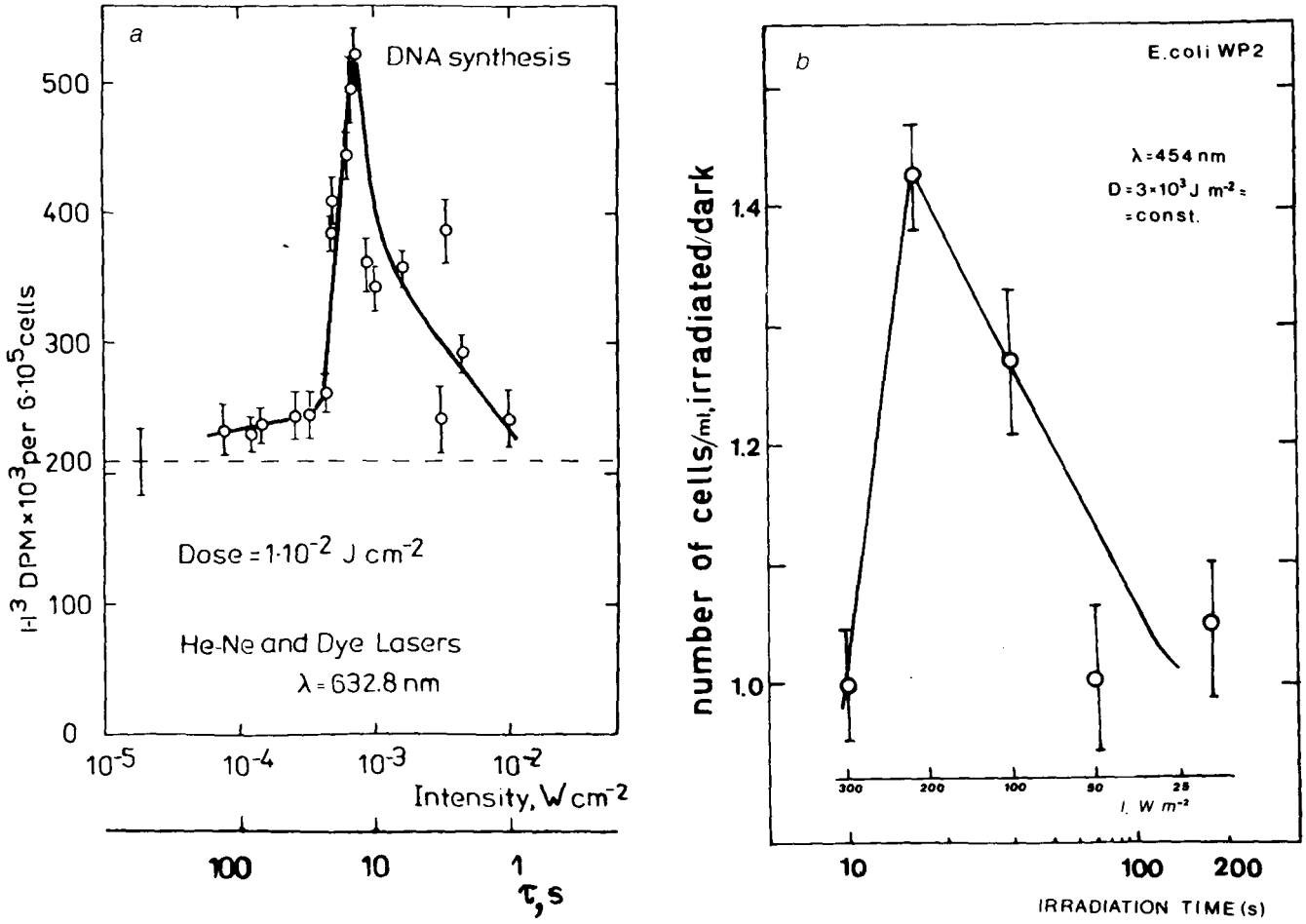


Fig. 5. The influence of light intensity at λ = 633 nm or at 454 nm on the rate of (a) DNA synthesis in exponentially growing HeLa cells, or (b) *E. coli* growth (Karu et al. 1984a; Tiphlova and Karu 1986).

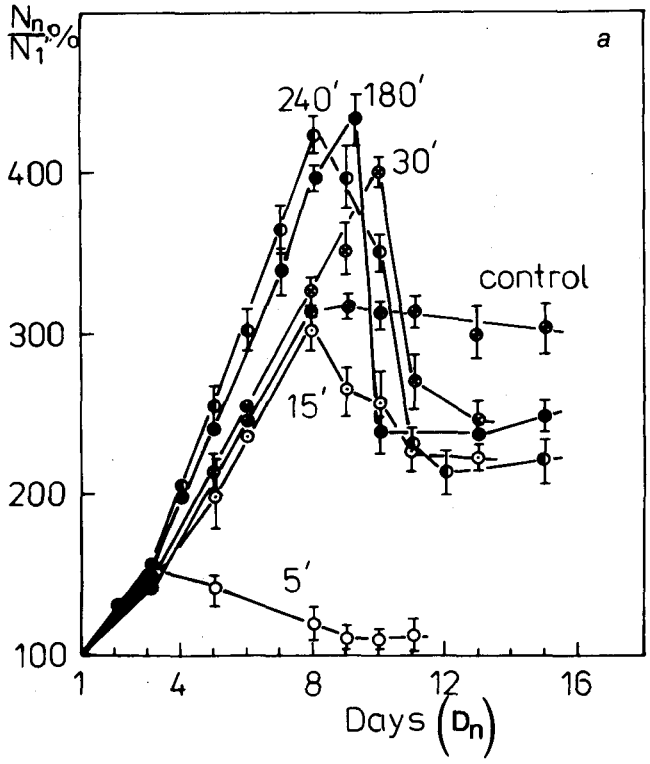
to influence the probable mode of action of potential photoacceptor do, in fact, influence the magnitude of the photoresponse (Karu 1986a, 1987, 1988).

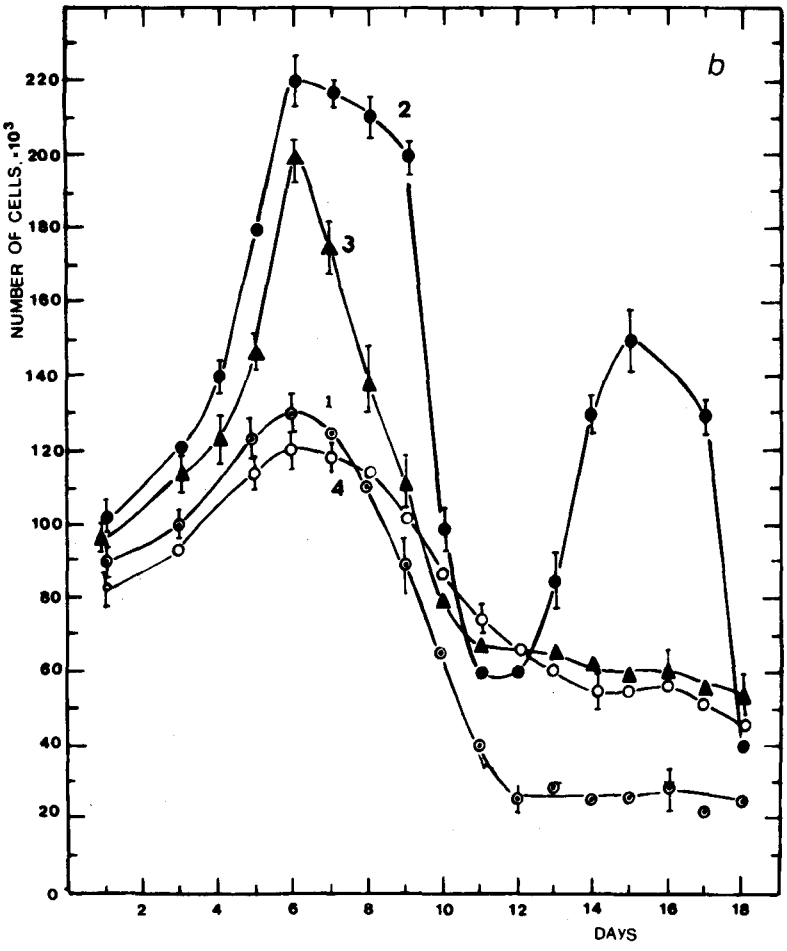
When cells are irradiated with various visible-light bands, light is absorbed by the respiratory chain components, and primary photochemical and photophysical events are believed to occur in mitochondria in the case of eucaryotic cells and in the cell membrane in the case of procaryotic cells. Our present-day knowledge of the subject, moderate as it is, enables us to treat only the, entire respiratory chain and not exactly any of its indi-1 vidual components as a primary photoacceptor. The respiratory chain is a unitary dynamic system which, when I being acted upon at a single point, changes its whole state of response; this in turn determines its respiratory action. The light action is connected with the temporal organization of the respiratory chain whose rearrangement occurs at times commensurate with the irradiation times (Tiphlova and Karu 1987a).

How is it possible to explain the relationship between such distant events as absorption of light by respiratory

chain components and acceleration of growth of the culture?

One can imagine the following scheme of interaction between low-intensity monochromatic visible light and a cell (Fig. 12). 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 NADH pool. This in turn leads to changes in the redox status of both mitochondria and cytoplasm (Krebs and Veech 1970). Activation of the electron-transport chain must result in an increase of promotive force $\Delta\mu_{H^+}$, the electrical potential of mitochondrial membrane $\Delta\chi$ and the ATP pool, and acidification of cytoplasm. That this inference is true has been confirmed experimentally: changes in $\Delta\mu_{H^+}$, $\Delta\chi$ and pH, as well as extrasynthesis of ATP, have been achieved by irradiating cells with a He-Ne laser (Passarella et al. 1984). And by irradiating cells with a wide-band visible light of $\lambda \geq 400$ nm, the activity of ATP synthetase has been enhanced (Nedelina et al. 1985).


a
$\frac{N_n}{N_1}$%
400
300
200
100
240′
180′
30′
control
15′
5′
1
4
8
12
16
Days (D_n)


b
NUMBER OF CELLS. 10^3
220
200
180
160
140
120
100
80
60
40
20
0
2
3
1
4
2
4
6
8
10
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14
16
18
DAYS

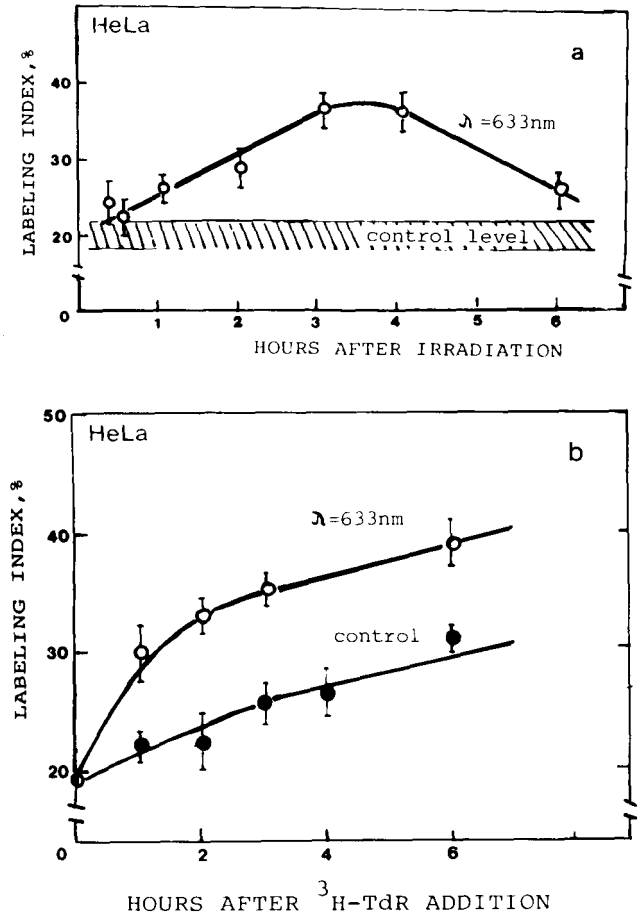


Fig. 7. Changes in (a) labeling index (percent of cells in the S phase of the cellular cycle, measured by pulse labeling with H^3-thymidine at various times after irradiation), and (b) variation of the percent of labeled cells during continuous labeling with H^3-thymidine after irradiation of exponentially growing HeLa cells with a He-Ne laser *(D* = 10^2 J/m^2) (Karu et al. 1987a).

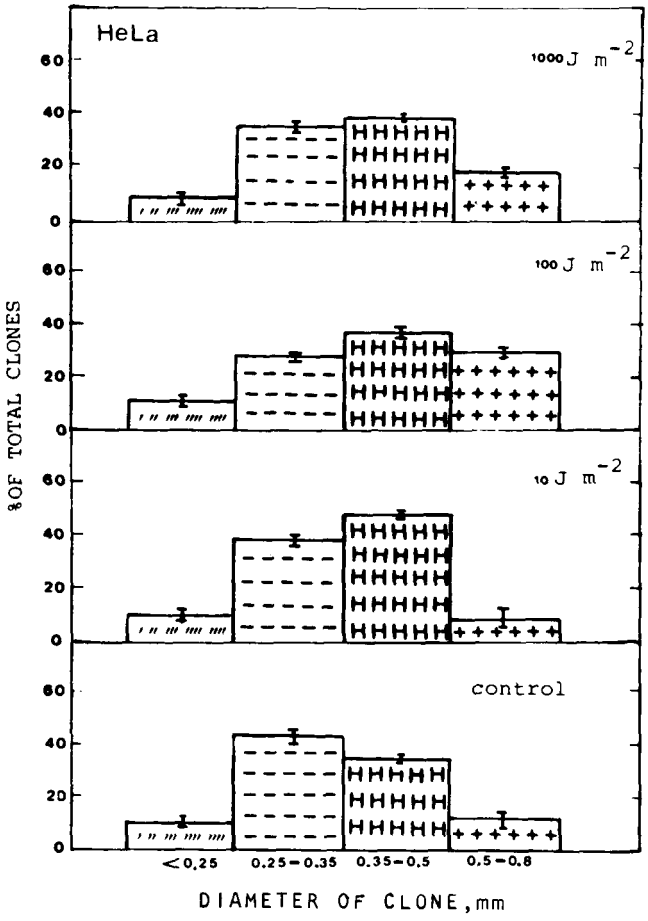


Fig. 8. Histograms of clone size distribution of cell populations at 14 d after irradiation with a He-Ne laser (Karu et al. 1987a).

The rise of the intracellular H^+ concentration controls allosterically the activity of the Na^H^ antiporter atuated in the cellular membrane (Pouyssegur 1985; Zil-berstein et al. 1982). This enzyme plays a key part in the llkalization of the cytoplasm. A short-term increase in the intracellular pH is one of the necessary components involved in the transmission of mitogenic signals in the cell (Poyssegur 1985).

The increase of the intracellular hydrogen ion and ATP concentrations in eucaryotic cells causes activation of other membrane ion carriers as well, such as Na^+, Ka^+ and -ATPase.

Activation if this enzyme leads to an increase in $[K^+]_i$ (intracellular concentration of K^+) and a decrease in $[Na^+]_i$ (intracellular concentration of Na^+) and E_m (membrane potential). The variation of these parameters is a necessary component in the control of the proliferation activity of the cell (Cone 1971; Rozengurt and Mendoza 1980). It should be borne in mind that changes in the respiratory chain also alter the flow of Ca^+ between mitochondria and cytoplasm, which in turn affects the ratio $[Ca^{2+}]_i/ [Ca^{2+}]_0$. Note that alteration of the cellular homeostasis parameters entails a whole cascade of reactions, and the causal relationships are very difficult to establish unam-biguously. The scheme suggested above is a possible version only. For instance, relationships between changes of almost all parameters of cellular homeostasis and activation of proliferation are established.

Fig. 6. The subcultivation growth curves of the HeLa culture after irradiation of the plateau-phase cells (a) with a He-Ne laser *(D* = 10^2 J/m^2) and inoculation into a fresh nutrient medium 5, 15, 30, 180 or 240 min after irradiation or (b) with He-Ne laser *(D* = 10^2 J/m^2) and after 180 (2), 15 (3) or 5 (4) min with 7 radiation (5.0 Gy), and inoculation of the cells into a fresh nutrient medium 15 min after 7 irradiation (one 7 irradiation only) (Karu et al. 1984c, 1987c).

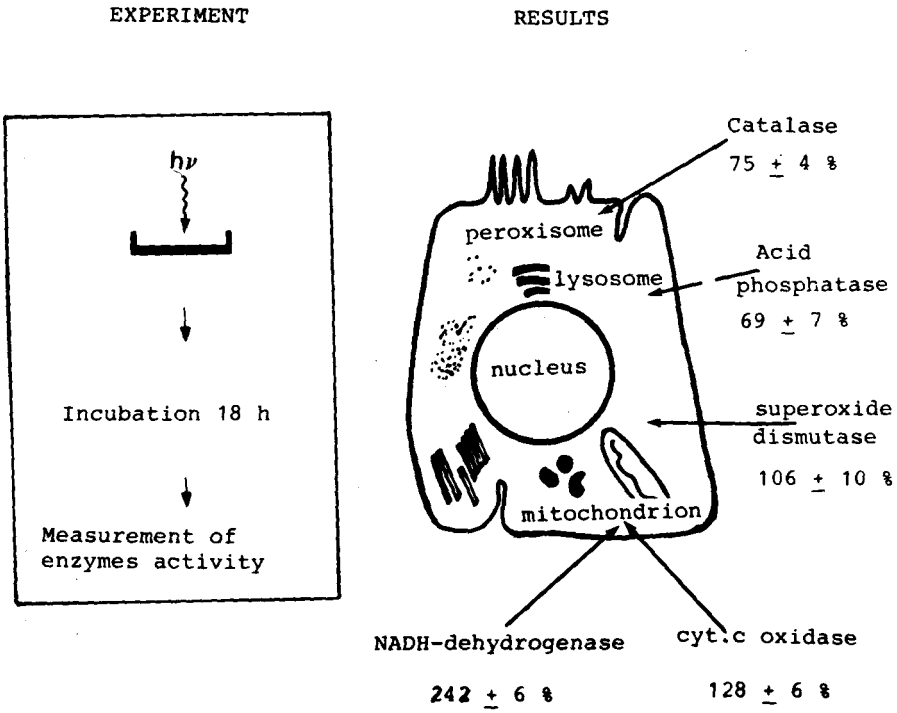


Fig. 9. Changes in the activity of various enzymes 18 h after the irradiation of *Torulopsis sphaerica* with a He-Ne laser ($D = 1 \times 10^3$ J/m^2). Detailed results are given in paper of Fedoseyeva et al. 1986.

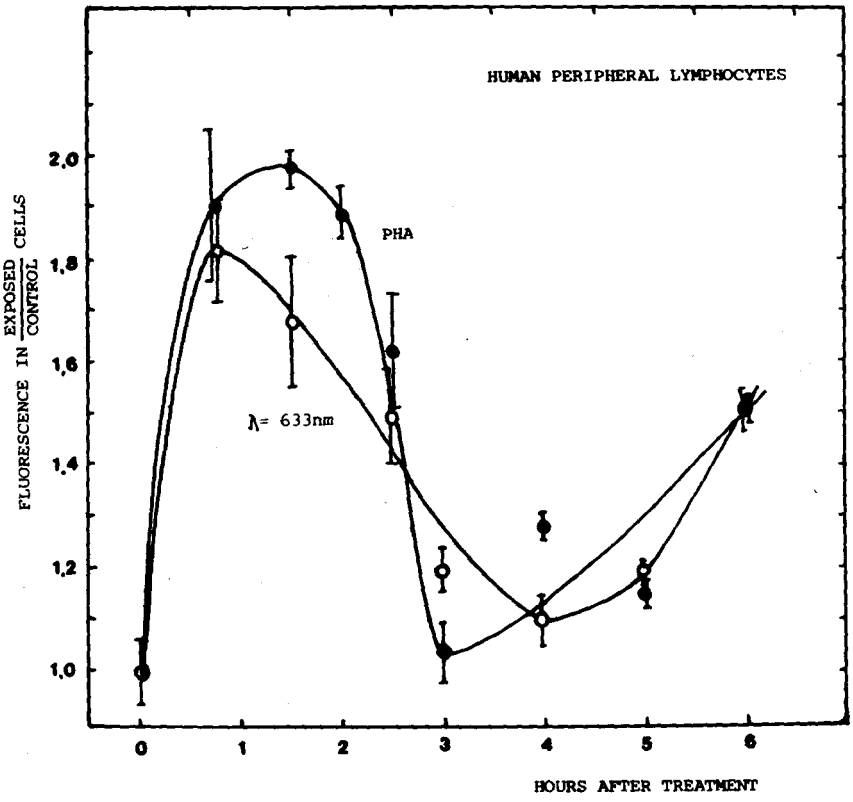


Fig. 10. The binding of acridine orange to the DNA of human peripheral lymphocytes; (1) after irradiation of lymphocytes with a He-Ne laser at a dose of 56 J/m^2, or (2) after adding phytohemagglutinin (4 μg/ml) (Fedoseyeva et al. 1988).

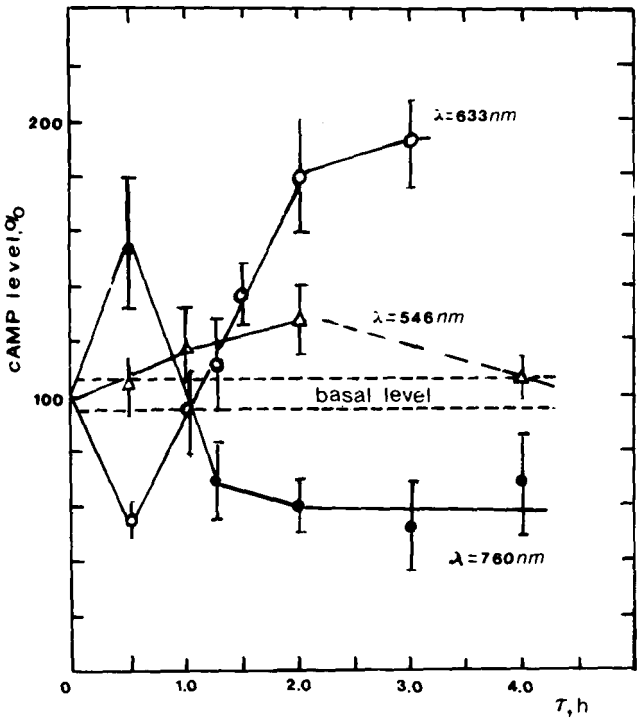


Fig. 11. Changes in intracellular cAMP level after irradiation of Chinese hamster fibroblasts with light at (1) 633 nm, (2) 760 nm, or (3) 546 nm at doses of 1 x 10^3 J/m^2, as described in the papers Karu et al. 1985, 1987b.

Changes in the redox level of the cell, as well as in the concentration of mono- and divalent ions may affect cell metabolism by influencing the cAMP level (Whitfield et al. 1980). Our results (Fig. 11), as well as experimental data about changes in cAMP level in eucaryotic microorganisms after irradiation with blue light (Cohen and Atkinson 1978; Kritsky et al. 1984), 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 the electrophysiological properties of cellular membranes following blue light irradiation (Kritsky et al. 1984) show that I the cellular membrane participates in the photosignal transfer chain. According to the universally adopted standpoint, signals from substances influencing the eucaryotic cell proliferation (growth factors, mitogens of the PHA type) are perceived and transferred by the cellular membrane. Should our suggestion concerning the respiratory chain as a primary photoacceptor be correct, the photosignal transmission chain is longer (Fig. 12) as compared with those for mitogens.

Accommodation (behavioural) responses of bacteria, including *E. coli,* are connected with the phenomenon of proton motive force ($\Delta\mu_H^+$) sensing (Taylor 1983; Glagolev 1984). The bacteria use their respiratory chain as the sensor for a behavioral response (e.g., aerotaxis, chemotaxis, etc.). In this case, any factor (including light quantum) that alters the $\Delta\mu_H^+$ can be a stimulus. This— the possible role of light as a growth-controlling signal for procaryotic microorganisms—can be stated as follows: The light signal perceived by the cell respiratory chain is a case of the more general class of sensory reception reactions. These reactions share the same initial stage (a variation of the $\Delta\mu_H^+$), while signal realization mechanisms (signal transduction chains) and end results differ. The possible scheme of the regulation of the *E. coli growth* rate by visible light is shown in Karu 1988.

The proposal of the respiratory chain as the primary photoacceptor in the cell allows one to explain the problem of positive and negative effects of visible light in the following way. In section 3 of this paper, the positive (stimulating) effects of irradiation were described. But there exists a tremendous amount of data describing an inhibitory or even lethal effect of visible light (especially blue and fluorescent light) on various types of cells. These data are summarized for *E. coli* and mammalian cells in Tables 1 and 2. For these negative (inhibitory) effects, the respiratory chain components as primary photoacceptors are discussed (Epel 1973). An analysis of these data makes it possible to suggest that the dose and intensity of light used determine the sense of the end macroeffect. By way of example, the stimulative (Fig. 2) and lethal (Webb and Brown 1976) action spectra of blue light on *E. coli* both have maxima at 404-460 nm. The stimulating doses of blue light fall within the range between 10 and 10^3 J/m^2 (Fig. 3), while a lethal effect is caused by doses of lO^lO^8 J/m^2 (Webb and Brown 1976). In both cases, the flavin components of the respiratory chain are discussed as photoacceptors (Tiphlova and Karu 1987a, Webb and Brown 1976).

The fact that irradiation of cells with visible light of the same wavelength and absorption of this light by the same molecules have both a positive effect (acceleration of 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 the same primary photoprocess—electronic excitation. One of them is the acceleration of electron transfer in the redox pairs in some sections of the respiratory chain, and the other is the transfer of excitation energy to oxygen to form 102 (as will be recalled, flavins and cytochromes are photosensitizers). At low doses, the former process predominates (the result being the redox control of cellular metabolism), and at high doses photodynamic damage occurs. This problem is discussed in more detail in Karu 1988. The shape of dose-effect dependencies (Figs. 3 and 4) indicates also the possibility of the existence of more than one primary process. It is important to emphasize that the effective dose range for achieving a stimulative effect is rather narrow and is determined for every kind of cell.

5. THE MAGNITUDE OF THE 'BIOSTIMULATION' EFFECT AND ITS DEPENDENCY ON THE PHYSIOLOGICAL STATE OF THE CELL BEFORE IRRADIATION

The experiments with chemicals (auxin, KI, Na dithionite) or procedures (decreasing pO_2 in a nutrient me-

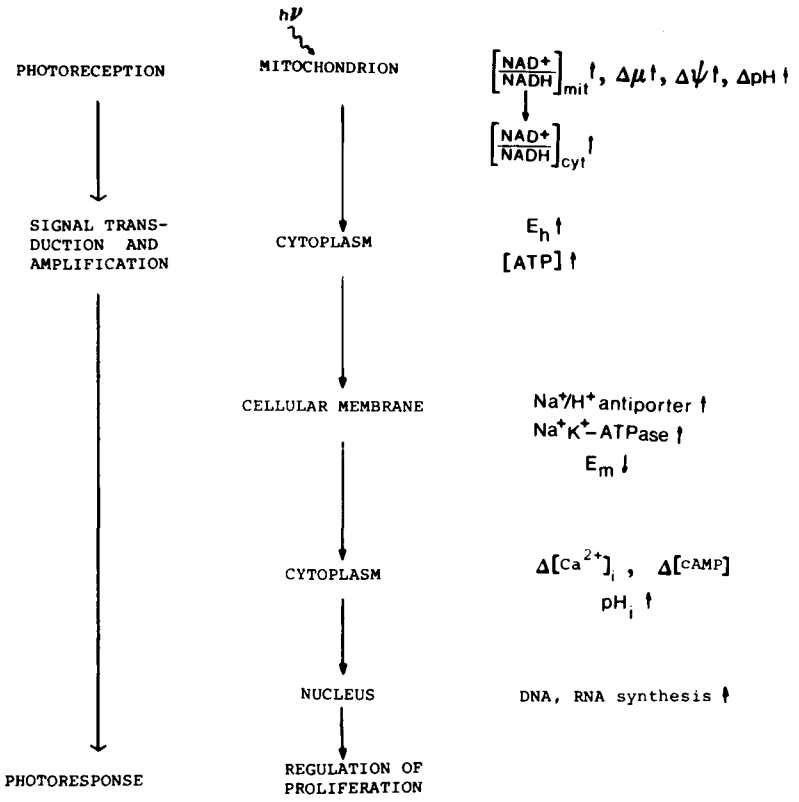


Fig. 12. Possible photosignal transduction chains for proliferation stimulation by monochromatic bands of visible light. $\Delta\mu_H{}^+\uparrow$, $\Delta\mu_H{}^+\uparrow$, $\Delta\chi\uparrow$—increase in photomotive force, proton gradient and electrical potential of mitochondrial membrane; [ATP]↑—increase in ATP concentration; $E_h\uparrow$ increase in cellular redox potential (change into more oxidazed 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^{4}' antiporter ↑, 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; $\Delta[Ca^{2+}]_i$, [cAMP]—changes in intracellular concentration of Ca^{2+} and cAMP; DNA, RNA synthesis ↑—activation of nucleic acids synthesis.

dium, dichromatic irradiation) known to influence the action of potential photo-acceptors indicated that in these cases, when the treatment before irradiation lowers the redox potential in the cell, the magnitude of the irradiation effect is stronger. The opposite is true when the redox potential of the cell is great enough (optimal or near optimal from the functional point of view) before the irradiation and when the effect of light is weak, if even present (Karu 1986b, 1988; Tiphlova and Karu 1987a, 1987b, 1988). It means that the photosensitivity of cells is not an all-or-nothing phenomenon, but depends to various degrees on the physiological state of the cell before irradiation.

In the case of mammalian cellular culture, after irradiation with a He-Ne laser, the growth rate of slow-growing but not fast-growing subpopulations was increased (Fig. 8). In our experiments with microorganisms, we established seasonal variations of possible growth

Table 1. Action of blue and red light on the growth off. *coli* WP 2.

Growth stimulation			Growth inhibition	
Light	Dose (J/m^2)	Reference	Dose (J/m^2)	Reference
Blue				
λ= 400-450 nm	$10\text{-}10^2$	Fig. 2, this paper	$LO^6\text{-}lO^8$	Webb and Brown 1976; D'Aoust et al. 1980; Jagger 1983
Red				
λ = 620-630 nm	$lO^2\text{-}lO^1$	Fig. 3, this paper	$\sim10^8$	Webb and Brown 1976

Table 2. Action of various bands of visible light on proliferation activity of mammalian cellular cultures.

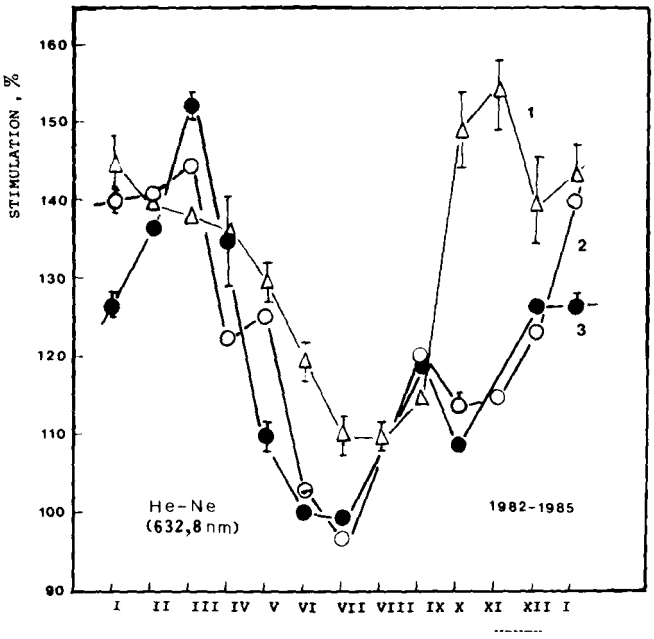
Wavelength (nm)	Culture	Stimulation	Inhibition	Reference
		Dose (J/m^2)	Dose (J/m^2)	
>400	HU-274 WI-38	10^4	lO^5-lO^6	Parshad Sanford 1977a,b
441,6 632,8 694,3 741	Human embryonic skin fibroblasts	LO^3-lO^4	LO^5-lO^6	Stepanov et al. 1977
Cool white fluorescent lamps	Human embryonic diploid lung fibroblasts	Dose not shown. Exposition every day during 150 d for 2 h: cells went through 70 divisions, 4 h: 60 divisions, 6 h: 53 divisions. Dark control group: 53 divisions	Dose not shown. The cells, exposed constantly died within 2-3 days	Litwin 1972
405	Human lymphoblastoid cells	—	10^5	Tyrrell et al. 1984
630-633	Chinese hamster fibroblasts	10^3	10^4	Abvakhitova et al. 1982
632,8	L	7, 5	—	Gamaleya et al. 1983
632,8	Human embryonic foreskin fibroblasts	10^2	—	Boulton Marshall 1986
632,8	HeLa	10^2	—	Fig. 6a, this paper
546-579	HeLa	Dose not shown, dose rate 40-50 W/cm^2	Dose not shown, dose rate 100-300 W/cm^2	Klein and Edsall 1967

stimulation. Illustrated in Fig. 13 are three examples. There is practically no growth photostimulation in summer, and in winter the effect is maximal. It was proposed that the seasonal variation in the magnitude of the effect depends on the growth rate of the culture before irradiation (Karu 1987). This means that in summer when the growth rate of a culture is high (a maximal rate is possible under these fixed experimental conditions), the stimulation of its growth by light appears to be impossible.

Here is an example of how neglect of the physiological state of cells before irradiation can lead to an incorrect conclusion. The main conclusion in the paper of Boulton and Marshall 1986 was that irradiation with a He-Ne laser increased the proliferation rate of a cellular culture, but that with noncoherent light at $\lambda = 633 \pm 9$ nm the proliferation rate did not increase. Comparing the growth rates of the nonirradiated cultures in both experiments, it becomes clear that the culture used for irradiation with noncoherent light divided more rapidly than the one used for irradiation with a He-Ne laser; hence, the light stimulation of the faster growing culture was weaker than that of the slower growing one. This is the real reason for the practically absent effect of noncoherent light, and not the absence of light coherence.

6. THE MECHANISM OF LOW-POWER LASER THERAPY

The "local" effects of phototherapy in treating trophic ulcers and indolent wounds with the He-Ne laser, the He-Cd laser or with diode lasers operating in the far red region may be explained by the action of low-intensity visible light on cell proliferation. In the area of such injuries, conditions are created (low oxygen concentration and pH, lack of necessary nutrients) which prevent proliferation, so that the cells enter the Go phase or remain in the *Gi* phase. For such cells, light may serve as a signal to increase proliferation. When irradiating fresh wounds, the effect of irradiation can be minimal, if even present at all. An effect does happen in cases when the proliferation is active and the regeneration of tissue integrity occurs at a more or less maximal (normal) rate. This may be the reason no phototherapeutic effect is often observed when irradiating fresh experimental wounds and proves effective in the case of "old" and "bad" ones. Of course, it should be borne in mind that proliferation control, in the case of a whole organism, is a much more complex process compared to a cell culture, because in the organism a hierarchy of proliferation control structures exists.



HUNTH

Fig. 13. Variation of maximal growth stimulation of microorganisms during the year (1: *E. coli,* 2: *T. sphaerica,* 3: *S. ludwigii).* Average date from full years 1982-85. The irradiation was always performed at the same time (at approximately 11 a.m. with *E. coli* and at approximately 6 p.m. with yeasts).

In clinical practice, low-intensity laser radiation is used for treating not only local lesions (trophic ulcers and wounds), but very often "systemic" effects have also been found (i.e., the effect is observed at some distance from the irradiation site) (Basford 1986). It is exactly this universality of the action of low-intensity light (a "cure for all diseases") that gives rise to some skepticism, although vast statistical material has been amassed to date proving that such a treatment has a positive effect (Gamaleya 1977; Kryuk et al. 1986; Basford 1986 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, and to alter their beating frequency, etc. (Arvanitaki and Chalazonitis 1947; Salet et al. 1979; Uzdenskii 1982). 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 the cell membrane.

It is quite possible that the universality of low-power laser effects is due to the universal photobiological mechanism—variation of the redox state of the respiratory chain components leading to the changes in the physiological state of the cell (i.e., the increase of the proliferation activity of non-excitable cells or changes in activity potentials of excitable cells).

7. CONCLUSIONS

(1) Laser biostimulation is a photobiological phenomenon. Coherent light is not needed.

(2) Primary photoacceptors are components of the respiratory chain. This explains the universality of low-power laser effects.

(3) The respiratory chain components can be the photoacceptors in the case of cellular metabolism stimulation, as well as inhibition depending on the dose of light. At low doses, irradiation causes redox regulation of cellular metabolism; at high doses photodynamic damage prevails.

(4) Light quantum is only a trigger for cellular metabolism regulation. This explains the low doses and intensities needed.

(5) The magnitude of the biostimulation effect depends on the physiological state of the cell before irradiation. This explains why the biostimulation effect is not always possible.

(6) The therapeutic effects of low-power laser irradiation can be explained by an increase of proliferation of G_o and G_1 cells or by changes in the physiological activity of excitable cells.

REFERENCES

Abvakhitova, A. K.; Grigoryeva, L. M.; Parkhomenko, I. M.Effect of laser radiation on Chinese hamster cells cultured in vitro. Radiobiologiya. 22:40-43; 1982.

Arvanitaki, A.;Chalazonitis, N. Reactions bioelectriques a la photoactivation des cytochrome. Arch. Sci. Physiol. 1:385-405;1947.

Basford, J. R. Low-energy laser treatment of pain and wounds: hype, hope, or hokum? Mayo Clin. Proc. 61:671-675; 1986.

Boulton, J.; Marshall, M. He-Ne laser stimulation of human fibroblast proliferation and attachment in vitro. Lasers in the Life Sciences 1:125-134; 1986.

Boynton, A. L.; Whitfield, J. F. The role of cycle AMP in cell proliferation: a critical assessment of the evidence. In: Greengard, P.; Robinson, G. A., eds. Advances in cyclic nucleotide research. Vol. 15. New York: Raven Press; 1983:193-294.

Cohen, R. J.; Atkinson, A. M. Activation of Phycomyces adenosive 3',5'-monophosphate phophodiesterase by blue light. Biochem. Biophys. Res. Comm. 83:612-621; 1978.

Cone, C. D. Unified theory on the basic mechanism of normal mitotic control and oncogenesis. J. Theor. Biol. 30:151-181; 1971.

D'Aoust, J. Y.; Martin, **W,** G.; Giroux, J.; Schneider, H. Protection from visible light damage to enzymes and transport in *E. coli.* Photochem. Photobiol. 31:471-474; 1980.

Epel, B. L. Inhibition of growth and respiration by visible and near-visible light. In: Giese, A. L,, ed. Photophysiology. New York: Academic Press; 1973:209-229.

Fedoseyeva, G. E.; Karu T. I.; Letokhov, V. S.; Lobko, V. V.; Pomoshnikova, N. A.; Lyapunova, T. S.; Meissel, M. N. Effect of He-Ne laser radiation on the reproduction rate and protein synthesis in the yeast. Laser Chemistry 5:27-33; 1984.

Fedoseyeva, G. E.; Karu, T, I.; Lyapunova, T. S.; Pomoshnikova, N. A.; Meissel, M. N.; Peskin, A. V. The effect of low-intensity red light on the activity of yeast enzymes. Microbiologiya 55:944-948; 1986.

Fedoseyeva, G. E.; Karu, T. I.; Lyapunova, T. S.; Pomoshnikova, N. A.; Meissel, M. N. Sensitivity of yeast culture to low-intensity red light. Microbiologiya 56(5):792-796; 1987a.

Fedoseyeva, G. E.; Smolyaninova, N. K.; Karu, T. I.; Zeienin, A. D. Changes in chromatin structure after irradiation of human lymphocytes with He-Ne laser. Lasers in Life Sciences. 2:197-205; 1988.

Gamaleya, N. F. Laser biomedical research in the USSR. In:Wolbarsht, M., ed. Laser application in medicine and biology. Vol. 3. New York and London: Plenum Press; 1977:1-175.

Gamaleya, N. F.; Shishko, E. D.; Janish, Gu. V. New data about mammalian cells photosensitivity and laser biostimulation. Dokl. Acad. Nauk U.S.S.R. (Proc. of U.S.S.R. Acad. Sci.) 273:224-227; 1983.

Glagolev, A. N. Bacterial A^-sensing. Trends Biochem. Sci.9:397-400; 1984.Jagger, J. Physiological effects of near ultraviolet radiation on bacteria. In: Smith, K., ed. Photochem. Photobiol. Rev. Vol. 7. New York, NY: Plenum Press; 1983:1-75.

Karu, T. I. Molecular mechanism of therapeutic action of low-intensity laser light. Proc. USSR Acad. Sci. (Biophysics) 291 : 1245-1249; 1986a.

| Karu, T. I. Biological action of low-intensity visible monochromatic light and some of the medical applications. In: Galletti, G., ed. Laser (Proc. Int. Cong. on Lasers in Medicine and Surgery). Bologna: Monduzzi Editore, 1986b:25-29.

Karu, T. I. Photobiological fundamentals of low-power laser therapy. IEEE J. Quantum Electronics QE-23(10): 1703-1717;1987.

Kara, T. I. Molecular mechanism of the therapeutical effect of low-intensity laser radiation. Lasers in the Life Sciences. 2:53-74; 1988.

Karu, T. I.; Letokhov, V. S. Biological action of low-intensity monochromatic light in the visible range. In: Martellucci, S.; Chester, A. N., eds. Laser photobiology and hotomedicine.New York, NY: Plenum Press; 1985:57-66.

Karu, T. I.; Kalendo, G. S.; Letokhov, V. S.; Lobko, V. V. Biostimulation of HeLa cells by low intensity visible light. II Nuovo Cimento D 1:828-840; 1982.

Karu, T. I.; Tiphlova 0. A.; Letokhov, V. S.; Lobko, V. V. Stimulation ofE. coli growth by laser and incoherent red light. II Nuovo Cimento D 2:1138-1144; 1983.

Karu, T. I.; Kalendo, G. S.; Letokhov, V. S.; Lobko, V. V. Biostimulation of HeLa cells by low intensity visible light. II. Stimulation of DNA and RNA synthesis in a wide spectral range. II Nuovo Cimento D 3:309-318; 1984a.

Karu, T. I.; Kalendo, G. S.; Letokhov, V. S.; Lobko, V. V. Biostimulation of HeLa cells by low intensity visible light. III. *ft* Stimulation of nucleic acid synthesis in plateau phase cells. g_ D Nuovo Cimento D 3:319-325; 1984b.

Karu, T. I.; Kalendo, G. S.; Lobko, V. V.; Pyatibrat, L. V. Kinetics of tumour HeLa cells growth under subcultivation after irradiation by low intensity red light at the stationary growth phase. Eksperimentalnaya Onkologiya 6(4):60-63; 1984c.

Kapu, T. I.; Letokhov, V. S.; Lobko, V. V. Biostimulation of HeLa cells by low-intensity visible light. IV. Dichromatic irradiation. II Nuovo Cimento D 5:483-496; 1985a.

Karu, T. I.; Lukpanova, G. G.; Parkhomenko, I. M.; Chirkov, Yu. Yu. Changes in cAMP level in mammalian cells after J irradiation with monochromatic visible light. Proc. USSR Acad. Sci. (Biophysics) 281:1242-1244; 1985b.

Karu, T. I.; Pyatibrat, L. V.; Kalendo, G. S. Biostimulation of HeLa cells by low-intensity visible light. V. Stimulation of cell proliferation in vitro by He-Ne laser irradiation. II Nuovo Cimento D. 9:1485-1494; 1987a.

Karu, T. I.; Lukpanova, G. G.; Parkhomenko, I. M.; Tiphlova, 0. A. Effect of irradiation with monochromatic visible light on cAMP content in Chinese-hamster fibroblasts. II Nuovo Cimento D. 9:1245-1251; 1987b.

Karu, T. I.; Pyatibrat, L. V.; Kalendo, G. S. Radiomodification by UV and visible laser light. Radiobiologiya 27:804-809; 1987c.

Klein, R. H.; Edsall, P. G. Interference by near ultraviolet and green light with growth of animal and plant cell cultures. Photochem. Photobiol. 6:841-850; 1967.

Krebs, M. A.; Veech, R. L. Regulation of the redox state of the pyridine nucleotids in rat liver. In: Sund, H., ed. Pyridine nucleotide-dependent dehydrogenases. Berlin, Heidelberg, New York: Springer Verlag; 1970:413-434.

Kryuk, A. S.; Mostovnikov, B. A.; Khokhlov, I. V.; Serdyuchenko, N. S. The therapeutic efficiency of low-intensity laser light. Minsk: Science and Techn. Publ.; 1986 (in Russian).

Kritsky, M. S.; Afanasieva, T. P.; Belozerskaya, L. M.; Chainlakhian, L. M.; Chemysheva, E. K.; Fillippovich, S. Yu.;Levina, N. N.; Potapova, T. V.; Sokolovsky, V. Yu. Photo-receptor mechanism of Neurospora crassa: control over the electrophysiological properties of cell membrane and over level of nucleotide regulators. In: Senger, H., ed. Blue light effects in biological systems. Berlin, Heidelberg, New York:Springer Verlag; 1984:207-210.

Litwin, J. The effect of light on the aging of human diploid fibroblasts. Exp. Geront. 7:381-386; 1972.

Lobko, V. V.; Karu, T. I.; Letokhov, V. S. Is low-intensity laser light coherence essential when biological objects are effected? Biophizika 30:366-391; 1985.

Martelly, J.; Franquinet, R. Planarian regeneration as a model for cellular activation studies. Trends Biochem. Sci. 9:468-471; 1984.

Mester, E. Laser application in promoting of wound-healing. In: Koebner, N. K., ed. Laser in medicine. Chichester, New York, Brisbane, Toronto: John Wiley; 1980:83-85.

Nedelina, 0. S.; Brzhevskaya, 0. N.; Kayushin, L. P. Redox regulation in ATP synthesis. Biophizika 30:179-191; 1985.

Parshad, R.; Sandford, K. K. Proliferative response of human diploid fibroblasts to intermittent light exposure. J. Cell Physiol. 92:481-486; 1977a.

Parshad, R.; Sandford, K. K. Intermittent exposure to fluorescent light extends lifespan of human diploid fibroblasts in culture. Nature 268:736-737; 1977b.

Passarella, S.; Casamassima, E.; Molinari, S.; Pastore, D.; Quagliariello, E.; Catalane, J. M.; Cingolani, A. Increase of proton electro-chemical potential and ATP synthesis in rat liver mitochondria irradiated in vitro by helium-neon laser. FEBS Lett. 175:95-99; 1984.

Popova, M. F.; Zubkova, S. M.; Laprun, I. B.; Bulyakova, N. B.; Domareva, 0. P.; Samokhvalova, N. S.; Azarova V. S. Action of laser radiation on regenerative processes in the conditions of action of ionizing radiation. Proc. USSR Acad. Sci. (Radiobiologiya) 279:1504-1507; 1984.

Pouyssegur, J. The growth factor-activatable Na^+H^+ exchange system: a genetic approach. Trends Biochem. Sci. 10:453-455;1985.

Rosengurt, E.; Mendoza, S. Monovalent ion fluxes and the control of cell proliferation in cultured fibroblasts. Ann. N.Y. Acad. Sci. 339:175-190; 1980.

Salet, C.; Moreno, G.; Vinzens, P. A study of beating frequency

of a single myocardial cell. III. Laser micro-irradiation of mitochondria in the presence ofKCN or ATP. Exp. Cell Res. 120:25-29; 1979.

Sazonov, A. M.; Romanov, G. A.; Portnoy, L. M.; Odinikova, V. A.; Karu, T. I.; Lobko, V. V.; Letokhov, V. S. Low-intensity non-coherent red light in the comprehensive treatment of gastroduodenal ulcers. Soviet Medicine 12:42-46; 1985.

Stepanov, B. I.; Mostovnikov, V. A.; Rubinov, A. I.; Khokhlov, I. V. The regulation of functional activity of human cells by laser irradiation. Proc. USSR Acad. Sci. (Biophysics) 236: 1007-1010;1977.

Taylor, B. How do bacteria find the optimal concentration of oxygen? Trends Biochem. Sci. 8:438-441; 1983.

Tiphlova, 0. A.; Karu, T. I. Action of argon laser irradiation and incoherent blue light on growth off. *coli.* Radiobiologiya 26:829-832; 1986.

Tiphlova, 0. A.; Karu, T. I. Action of low-intensity laser light on transient metabolic processes in cell. Proc. USSR Acad. Sci. (Biophysics) 2955:1002-1005; 1987a.

Tiphlova, 0. A.; Karu, T. I. Action of red and far red low intensity light on the growth ofE. coli. Microbiologiya 56:393-396; l987b.

Tiphlova, 0. A.; Karu, T. I. Action of monochromatic low-intensity visible light on growth of E. coli. Photochem. Photobiol. 48:467-471; 1988.

Tyrrell, R. M.; Werfelli, P.; Moraes, E. C. Lethal action of ultraviolet and visible (blue-violet) radiations at defined wavelengths on human lymphoblastoid cells: action spectra and interaction sites. Photochem. Photobiol. 39:183-189; 1984.

Uzdenskii, A. B. On selectivity and locality of the effects upon the laser microirradiation of cells. Cytologiya 24:1119-11132; 1982.

Voskanyan, K. Sh.; Simonyan, N. V.; Avakyan, Ts. M.; Arutynyan, A. G. Effect of He-Ne laser radiation on x-radiation sensitivity of Escherichia coli K-12 cells. Radiobiologiya 25: 557-559;1985.

Voskanyan, K. Sh.; Simonyan, N. V.; Avakyan, Ts. M.: Arutyunyan, A. G. Modification of the damaging effect of a-particles on E. coli K-12 by low-intensity laser radiation. Radiobiologiya 26:375-377; 1986.

Webb, R. B.; Brown, M. S. Sensitivity of strains ofE. coli differing in repair capability to far UV, near UV and visible radiations. Photochem. Photobiol. 36:425-492; 1976.

Whitfield, J. F.; Boynton, A. L.; MacManus, J. P.; Nixon, R. H.; Sikorska, M.; Tsang, B.; Walker, P. R.; Swierenga, G. H. H. The roles of calcium and cyclic AMP in cell proliferation. Ann. N.Y. Acad. Sci. 339:216-240; 1980.

Zharov, V. P.; Karu, T. I.; Litvinov, Yu. 0.; Tiphlova, 0. A. Biological effect of radiation of a semiconductor laser in near infrared region. Sov. J. Quantum Electronics. 17(11):1361-1362; 1987.

Zilberstein, D.; Agmon, V.; Schuldiner, S.; Padan, E. The sodium/proton antiporter is part of the pH homeostasis mechanism in E. coli. J. Biol. Chem. 257:3667-3691; 1982.