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Photobiological Fundamentals of Low-Power Laser Therapy

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(Invited Paper)

Abstract—**Quantitative studies of the action of low-power visible monochromatic light on various cells *(E. coli,* yeasts, HeLa) were performed to find irradiation conditions (wavelength, dose, intensity) conducive to vital activity stimulation. The action spectra of visible light on DNA and RNA synthesis in HeLa cells have maxima near 404, 620, 680, 760, and 830 nm. Growth simulation of *E. coli* is at a maximum when irradiated at 404, 454, 570, 620, and 750 nm, and biomass ac cumulation stimulation in yeasts has a maxima at 404, 570, 620, 680, and 760 nm. Absorption of quanta is only a trigger for the rearrange ment of cellular metabolism, with photosignal transduction being effected by standard cellular means such as changes in the cAMP level. Respiratory chain components are discussed as primary photoacceptors. It is concluded that "laser biostimulation" is of a photobiological nature, and low-power laser effects can be related to well-known pho tobiological phenomena.**

I. BACKGROUND

TNTEREST in the "biostimulating effect" of low-intensity red light, particularly He-Ne laser irradiation, has increased in the last few years. Discussions about the problem have become ardent. On one hand low-power laser therapy is undoubtedly successful in treating trophic ulcers and indolent wounds of diverse etiology, when traditional drug treatments are of low efficiency [1]-[3]. On the other hand, the method seems to be highly incredible and even mysterious. There is frank skepticism among physicists as to the believability of the reports 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 seems almost impossible to explain the special sensitivity of cells to irradiation by red light against a background systematic irradiation by white light whose spectrum contains a red component of approximately the same power. Another reason for doubt is that, very often, the stimulative effects of irradiation are explained by the role of the coherence of laser irradiation as it acts upon biological objects. This argument is not convincing and makes physicists suspicious when hearing about low-power laser biostimulation.

There was little quantitative information (dependence of the effect on the irradiation dose, wavelength, regime, and intensity) available in 1981 when we started systematic experiments with different cells in an attempt to demonstrate or disprove the existence of photostimulation by low-intensity visible laser radiation at the molecular and cellular levels. This review explains how we understand the problem at the end of 1986, after six years of experimentation.

The "red light syndrome" was well known in the last century, and much experimental work was done in the first third of this century. These data are briefly reviewed in the next part of this paper. Red light was used in medicine even in ancient times, and curing with red light was among the methods used by N. R. Finsen, the father of contemporary phototherapy. It is very likely that, by the time the first lasers made their appearance, this old knowledge was forgotten. The He-Ne was the first commercially available source of coherent light. It is no wonder that the stimulating effect of light, and red light in particular, was rediscovered when coherent light sources were used. The observed effects were attributed to the unique, high coherence of He-Ne laser radiation. Actually, there are no physical grounds for such a conclusion, as we briefly demonstrate in the next part of this paper. I would like to emphasize that I do not intend to deny that the laser is a handy tool for the laboratory and clinic. On the contrary, I do believe that the laser as a light source offers many benefits for medical applications, as the laser beam is easy to transport by means of fibers. The discussion of the importance of specific laser light properties, such as coherence, is essential only with regard to the explanation of the mechanism of low-power laser light in therapy.

II. PHOTOBIOLOGICAL NATURE OF LASER BIOSTIMULATION EFFECTS

For coherent radiation-matter interaction, the high coherence (both spatial and temporal) of light is a necessary, but not sufficient condition. The coherent properties of matter itself should also be taken into account. A coherent light field can disturb the random distribution of the wave function phases of particles (atoms, molecules); where the radiation-stimulated transition rate W (quantum system phasing rate) exceeds the phase relaxation rate $1/T_2$, the light-matter interaction will be coherent [4]:

$$W = \mu E/h >> 1/T_2 \qquad (1)$$

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where μ. is the matrix element of the transition dipole moment, E is the electrical field strength of the light wave, h is Planck's constant, and T_2 is the phenomenological phase relaxation time for a given quantum transition (in condensed media at room temperature, T_2 = 10^{-11} — 10^{-13} s).

The rate at which coherence is produced in matter under the action of a light field must exceed the rate of coherence lost by the light wave itself:

$$\mu \bullet E/h >> \tau_{coh} \approx \Delta \nu_{rad} \quad (2)$$

where τ_{coh} is the time of coherence and $\Delta\nu_{rad}$ is [tne] spectral width of radiation.

Using relations (1) and (2), the condition for coherent light-matter interaction may be expressed as

$$\mu \bullet E/h >> T_2,\ 1/\tau_{coh} \quad (3)$$

the threshold for interaction being

$$\mu \bullet E_{coh}/h = 1/T_2 \quad (4)$$

The light intensity at which the relation (3) is fulfilled in condensed matter at 300 K for compounds absorbing the He-Ne laser radiation (e.g., metalloporphyrins [7]) was calculated to be $I \geq 2 \cdot 10^{11}$ W/cm^2 [5].

The typical laser light intensities used in clinical practice range from 10^{-4} to 10^{-2} W/cm^2. This means that under normal physiological conditions, the absorption of low-intensity light by biological systems is of a purely noncoherent nature.

Indeed, the stimulative action of various bands of visible light at the level of whole organisms and cells was known long before the advent of the laser.

In a large group of studies [8]-[10], [13], [19], [22], [23], the respiratory intensity in frogs, dogs, birds, and rodents was studied by monitoring the production of CO_2 and the consumption of O_2. The respiration intensity was found to increase under conditions of illumination as compared to in the dark, and to depend on the color of light. An important inference drawn from these studies is that, at the level of a whole organism, the skin possesses light sensitivity. The presence of the eyes only modifies the skin photosensitivity effect [8], [22]. Another interesting conclusion was that the respiration-exciting action of light also occurs at the cellular level as observed *in vitro* [8], [13], [22].

A second group of studies [II], [13]-[16] describes the effect of multicolored beams on the growth and development of various organisms. The data of the efficiency of various regions of the visible-light spectrum are contradictory in some respects (there are differences between the objects of study and experimental techniques), but they agree that various colored beams differ in their biological activity which is also different from that of white light. It should also be taken into consideration that the methods used to isolate the beams were not the same; this means that the spectral widths of individual beams might differ appreciably. Some authors [9], [10] found yellow light to be the most activating, with stimulating activity of the wavelengths decreasing in the following order: green, blue, red, and violet. Others [II], [12] said the stimulating activity decreased in the following order: red, orange, green, white, blue, and violet. Other authors stressed the importance of the light intensity [17], [19], [22].

In the 1920's-1930's, the effect of blue and red light on various biochemical processes was also revealed [17], [18], [25], [74].

Recall briefly the medical application of blue and red light. Ponza [20] observed a rapid and sharp improvement in the mood of melancholies after a few hour's stay in rooms with red windows, and in that of maniacs after a few hours in rooms with blue or violet windows. Nowadays, bright light is used in the treatment of patients with seasonal affective disorders [21]. In this case, it seems the effect of phototherapy takes place mainly through the eyes, but on the other hand, contemporary experimental data indicate that this phototherapy may act via a nonphotoperiodic mechanism, possibly by affecting biological systems other than the retino-hypothalamus-pineal axis [21].

Another example is N. Finsen's method of treating patients suffering from acute eruptive diseases (e.g., smallpox, measles). The suppuration, vesiculation, and fever in patients put in rooms with red windows were negligible [22]. In this case, it seems more reasonable to consider that the photosensitivity of the skin plays a major role in the therapeutic benefits compared to the possible action through the eye.

Summing this up, one can say the following.

1) Photosensitivity to various visible-light bands is documented to occur at the level of both the whole organism and the cell.

2) To achieve the desired effect, it does not matter whether the light used is coherent or not. Both coherent and incoherent red light were found to be equally effective clinically in treating peptic ulcers [26].

3) There is every reason to believe that the "laser bio-stimulation" phenomena is of a photobiological nature.

This historical review does not include the literature about laser biostimulation. To get these data, the review articles [2], [88], and books [3], [46] (and the references therein) are recommended.

III. Long-Term Responses to Irradiation

Photobiological effects can conventionally be divided into short-term (or direct) and long-term (or indirect responses to irradiation. Short-term responses are those in which an effect can be observed a few seconds or minutes after irradiation. The long-term effects are observed hours and even days after the end of the irradiation and usually involve novel biosynthesis.

Some long-term responses in *E. coli,* yeasts, and HeLa cells are illustrated in Figs. 1-3. Fig. 1 shows the action spectra of visible light on the synthesis of (a) DNA and (b) RNA in exponentially growing HeLa cells; Fig. 2 shows the same for stationary-phase HeLa cells, and Fig. 3, the action of visible light on the growth of (a) *E. coli*

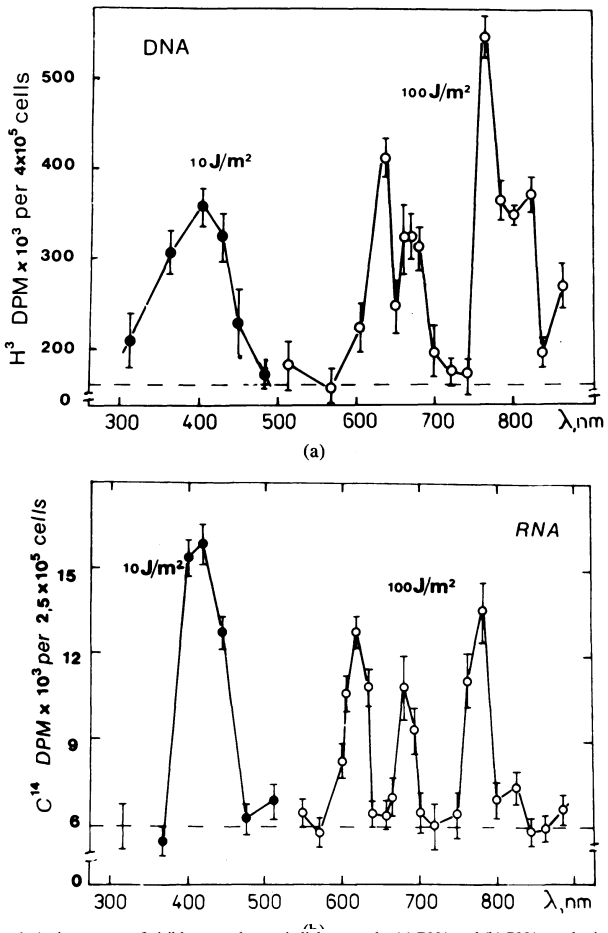


Fig. 1. Action spectra of visible monochromatic light upon the (a) DNA and (b) RNA synthesis rate in exponentially growing HeLa cells for the irradiation doses of 10J/m^2 (-•-•-•-) and 100 J/m^2 (-0-0-0-) measured by 20 min pulse labeling with H^thymidine (DNA) or C^{14} uridine (RNA) 1.5 h after the irradiation, as described in [16], [28], [29].

and (b) *Saccharomy codes ludwigii.* All the action spectra are practically of the same type, having maxima in almost every visible-light band. There is a maximum at about 620 nm, and the He-Ne laser radiation wavelength (632.8 nm) falls within this region.

Fig. 4 presents data on the stimulation of: (a) DNA synthesis in HeLa cells, and (b) the growth of an f. *coli* culture for various irradiation doses and wavelengths. It can be seen that there are two groups of active spectral regions. The first covers wavelengths of 365, 404, and 434 nm (see Fig. 4 for λ = 404 nm) and has a stimulating effect at doses lower by a factor of 10-100 than those of the second group of wavelengths (454, 560, 633, and 750 nm). In other words, achieving a maximum effect with light in the near UV and blue regions requires doses at least an order of magnitude lower than those required to achieve the same effect with red or far red light.

In spite of the similarities between the action spectra, as well as between the dose-response curves, there are rather large differences in the dose ranges effective for

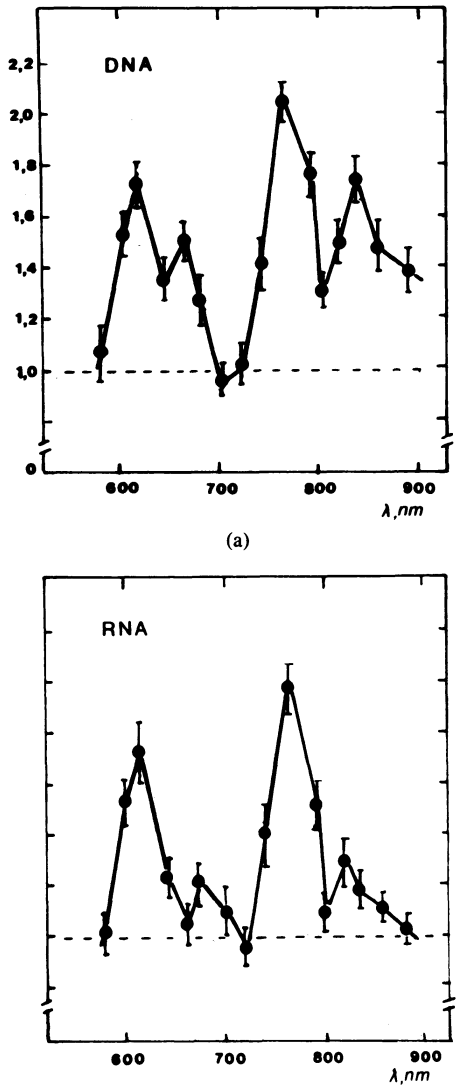


Fig. 2. Action spectra of visible monochromatic light upon the (a) DNA and (b) RNA synthesis rate in stationary phase HeLa cells for the irradiation dose of 100 J/m^2, measured by 20 min pulse labeling with H^3-thymidine (DNA) or C^{14}-uridine (RNA) 4.0 h after the irradiation, as described in [30], and expressed as ratio of radiactivity values of irradiated and nonirradiated cells.

each culture. For example, the photosensitivity to red light (λ = 633 nm) differs not only between HeLa cells (D_{max} = 100 J/m^2) and *E. coli* (D_{max} = 4 x 10^3 J/m^2), but varies even with each culture of the same class. This is attested to by the relationships between the dose and biomass accumulation stimulation for different yeast cultures (see Fig. 5). Fig. 5 shows that each culture has its own D_{max}, the dose for which the accumulation of biomass is a maximum. The activation percentage with the cells exposed to D_{max} is also culture-dependent. From these data, the following correlation can be inferred (the upper section of Fig. 5): the more photosensitive cultures (lower D_{max}) are activated to a greater degree (higher percentage of biomass accumulation activity).

In all dose-response curves (Figs. 4, 5), there exists a threshold, a maximum, and a phase decline.

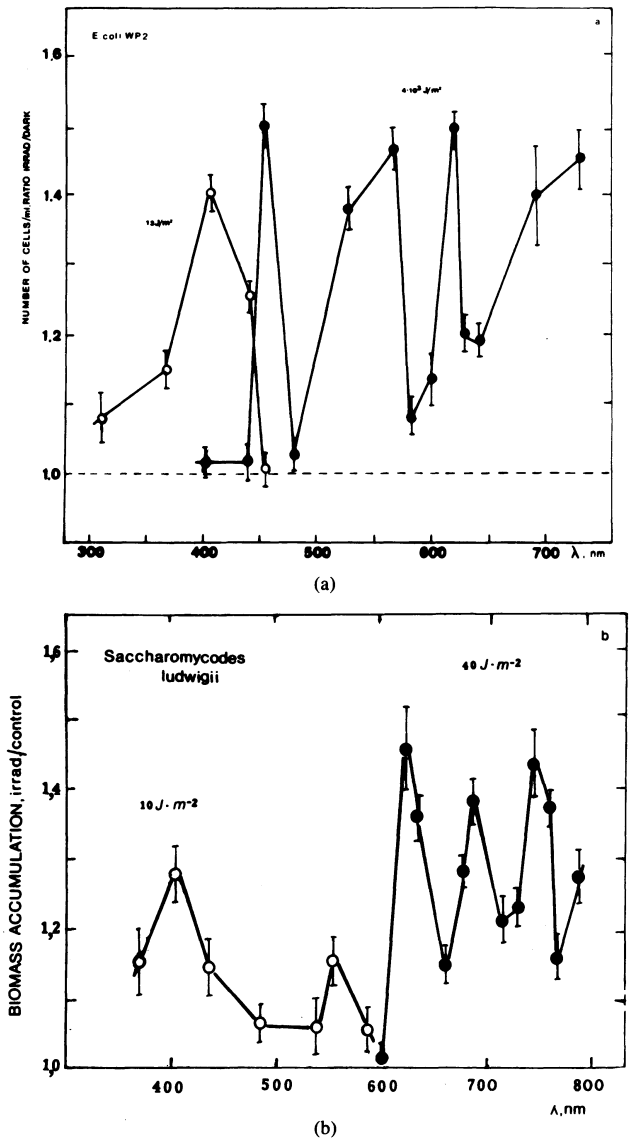


Fig. 3. Action spectra of visible monochromatic light upon (a) *E. coli* and (b) *Saccharomy codes ludwigii* growth expressed as the ratio of the number of cells 60 min after irradiation (a), or amount of synthesized biomass 18 h after irradiation (b), in irradiated and nonirradiated cultures, as described in [31]-[33].

The results of measurements of the influence of light intensity on the stimulation of DNA synthesis in HeLa cells and on the growth of *E. coli* are presented in Fig. 6. In these experiments, the radiation dose was constant, while the intensity of light and irradiation time were varied.

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 macroeflect that remains for a long time. When a HeLa cell culture in the stationary growth phase was irradiated with a He-Ne laser and the cells then subcultured at various intervals after irradiation (from 5 to 240 min), the growth stimulation in the exponential growth phase was observed to last for 6-7 days (Fig. 7)

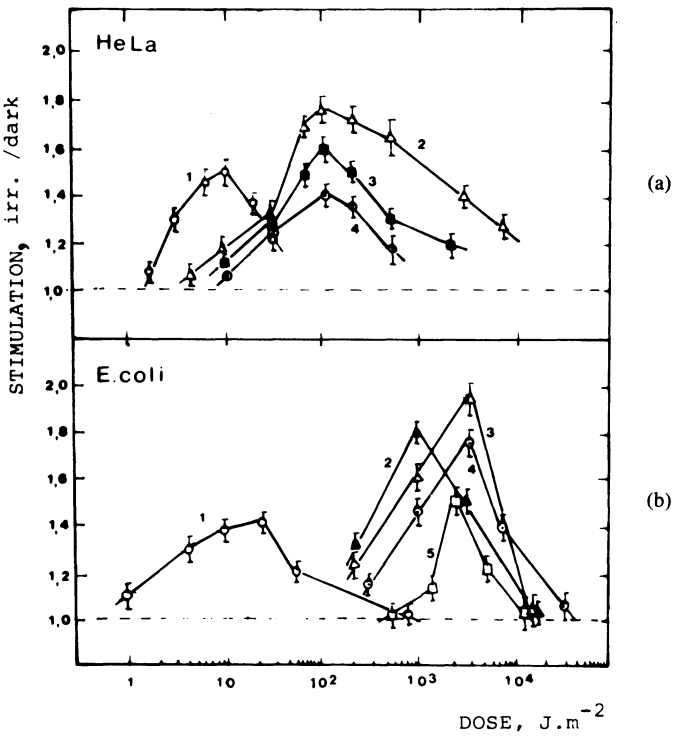
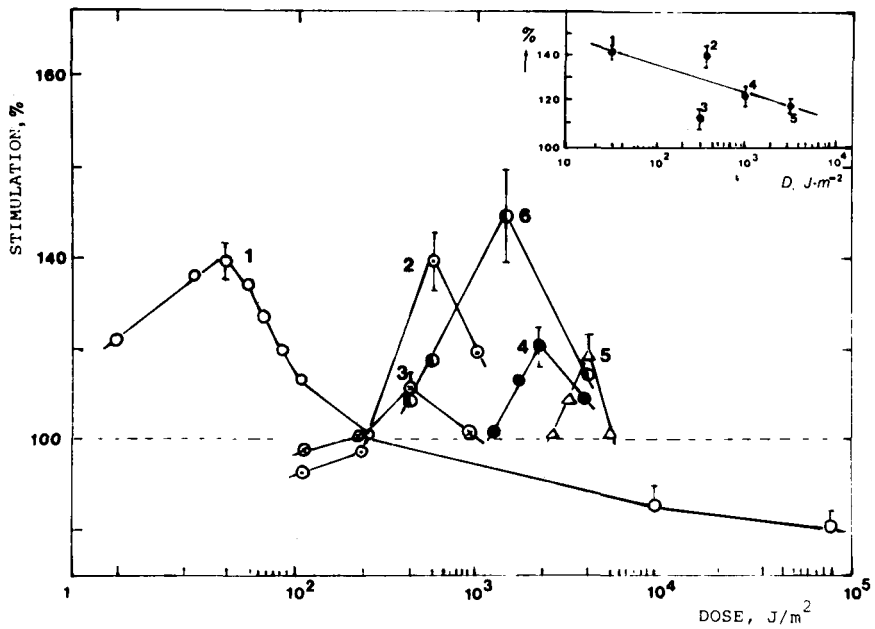


Fig. 4. The effect of irradiation dose on stimulation of (a) DNA synthesis rate in exponentially growing HeLa cells (1—λ = 404 nm, 2—λ= 760 nm, 3—λ = 620 nm, 4—λ= 680 nm, and (b) *E. coli* culture growth (l—λ = 404 nm, 2—λ= 560 nm, 3—λ= 750 nm, 4—λ=632-8 nm, 5—λ = 454 nm) [28], [29], [34]. The conditions of irradiation and measurement are the same as denoted on Figs. 1 and 3.

[37]. Stimulation was noted when the interval between irradiation and subculturing was 30 min or more. Irradiation of human fibroblasts with a He-Ne laser significantly increased the number of cells in comparison with their respective nonirradiated controls [41]. Single or multiple short-time exposures of a human diploid fibroblast culture with fluorescent light reproducibly enhanced the proliferation rate, whereas longer exposures appeared to be cytotoxic [42]. The irradiation of cells with fluorescent light for 2 h daily over a period of 150 days increased their proliferation (irradiated cells went through 70 divisions, while nonirradiated cells went through only 53 divisions [43]). Irradiation of *Pseudomonas fluorescens* with red light (8 W/m^2) or blue light (3 W/m^2) enhanced the division rate by 2-40 percent [44].

In a series of experiments, the activity of some enzymes was measured in a stimulated culture. The culture, *T-rulopsis sphaerica,* was irradiated with a He-Ne laser and incubated for 18 h (at least 3 divisions), after which the enzyme activity was determined (Fig. 8) [38].

Irradiation caused a considerable activation of the respiratory chain components NADH-dehydrogenase and cytochrome c oxidase. The activity of cytosolic superoxide dismutase remained practically at the control level, and the activity of acid phosphatase declined. The data obtained show that growth stimulation is accompanied by an increase in respiratory activity (with no accumulation of toxic intermediates of oxygen metabolism) and by synthetic processes in cells dominating catabolic ones. The


STIMULATION, %
160
140
100
1
10^2
10^3
10^4
10^5
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1
2
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6
%
140
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Fig. 5. The effect of irradiation dose (He-Ne laser, λ = 632.8) on the biomass accumulation for different yeast cultures: (1—*Saccharomycodes ludwigii,* 2—*Torulopsis sphaerica,* 3—*Candida biodinii,* 4—*Candida mallosa,* 5—*Saccharomyces cerevisiae* 14 (all grown in wort), 6—*Candida boidinii* (grown in methanol), measured 18 h after the irradiation and expressed as the ratio of the amount of synthesized protein in irradiated and nonirradiated cells [33].

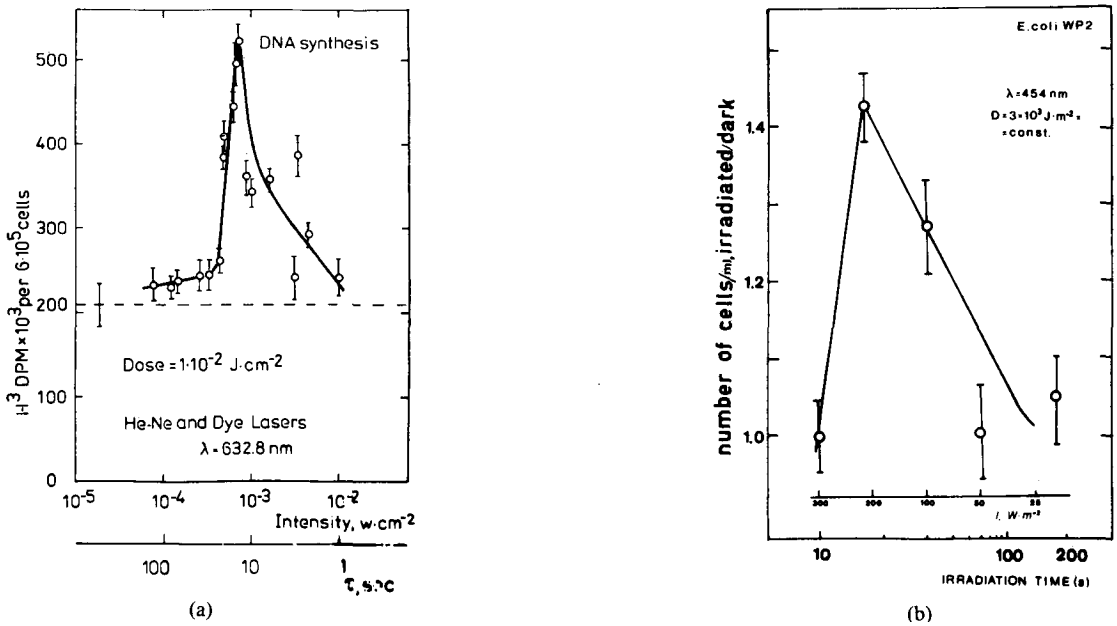

Fig. 6. The influence of the light intensity at λ = 633 or 454 nm (He-Ne and dye or Ar^+ lasers, respectively) on the rate of (a) DNA synthesis in exponentially growing HeLa cells, or (b) the *E. coli* growth [29], [36].

unchanged superoxide dismutase activity after irradiation with the He-Ne laser was also observed in [45].

Irradiation with blue or red light caused an increase in the mitotic index in cellular cultures [27], [46], [47] (Table II). These data indicate that irradiation causes a rearrangement in cell metabolism with light playing the role of a trigger controller [35]. The final response to irradiation is the acceleration of proliferation.

Changes in the cAMP level of Chinese hamster cells after irradiation [48] suggests that light quanta can act as a proliferative stimulus, because cAMP is programmed to stimulate an unkown event or events leading to DNA synthesis in a wide variety of cells and possibly to later event(s) giving rise to mitosis and division [49].

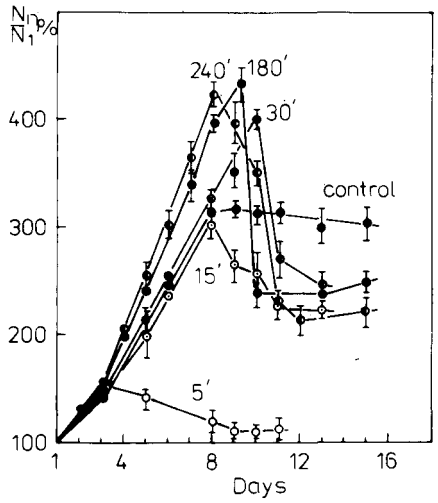

Fig. 7. The subcultivation growth curves of HeLa culture after irradiating plateau-phase cells with He-Ne laser (D = 100 J/m^2) and inoculating of them into fresh nutrient medium 5, 15, 30, 180, or 240 min after irradiation. The *abscissa* gives the ratio of cells in day $n(N_n)$ and at the end of the first day (N_1), the ordinate gives the days (Day_n) after the plating [37].

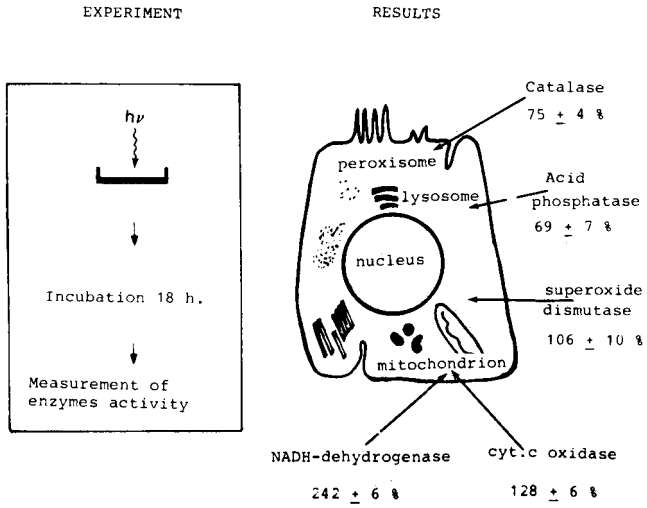

Fig. 8. Changes in activity of various enzymes 18 h after the irradiation of *Torulopsis sphaerica* with He-Ne laser (D = 1 x 10^3 J/m^2). The methods of measurement and detailed results are given in [38].

In all experiments mentioned above, the positive (stimulating) effects of irradiation were described. But there

exists a tremendous amount of data describing inhibitory, and even lethal, effects of light (especially blue and fluorescent light) on various types of cells [50]. Tables I and II summarize the data on light-growth responses of *E. coli* and mammalian cell cultures. The respective data for yeast cultures can be found in [54], [56]. 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. Cells seem to be more sensitive to blue light: positive effects are achieved with rather low doses, negative ones are attained very easily by increasing the fluence. By way of example, the stimulative [Fig. 3(a)] and lethal [51] action spectra of blue light oh *E. coli* both have maxima at 404-410 and 450-460 nm. Stimulating doses of blue

TABLE I
ACTION of BLUE and RED LIGHT on the GROWTH of E. COLI WP2

Light	Growth Stimulation		Growth Inhibition	
	Dose (J/m^2)	Ref.	Dose (J/m^2)	Ref.
Blue λ = 400-450 nm	$10\text{-}10^2$	Fig. 4, this paper, [34]	$10^6\text{-}10^8$	[51] [59] [60]
Red λ = 620-630 nm	$10^2\text{-}10^4$	Fig. 4, this paper, [34]	$\sim 10^8$	[51]

light fall within the range between 10 and 10^3 J/m^2 (Fig. 4), while lethal effects are caused by doses of 10^6-10^8 J/m^2 [51] (Table I). In both cases, the flavin components of the respiratory chain are discussed as photoacceptors [51, part 5]. To get a negative effect with red light is much more difficult. For example, in our experiments with yeast cultures, the growth response was never less than 80 percent of the dark control (Fig. 5), no matter how much the irradiation dose was increased.

Thus, it may be stated that low-intensity visible light causes readily observable changes in cellular biochemical processes involving biomolecules (such as DNA and RNA) which do not directly absorb this radiation.

IV. SHORT-TERM RESPONSES TO IRRADIATION

One well-documented short-term response to irradiation is the photostimulation of the respiration rate and ATP synthesis. The stimulation of ATP synthesis by red light was described for isolated mitochondria [62]-[65] and for *Pieris brassicae* larvae [61]. In the latter case, photostimulation of ATP synthesis was accompanied by acceleration of the larvae development.

Red light at 650 nm [65], 632.8 nm [62], [64], and 600 nm [63] increased ATP synthesis in isolated rat liver mitochondria. An increase in ATP synthesis was also recorded in the case of irradiation with blue light at 420 nm; irradiation at 477, 511, and 544 nm proved to be noneffective [63].

Irradiation with far red light near 760 nm decreased the synthesis of ATP [65].

Photoacoustic spectroscopy measurements revealed that He-Ne laser irradiation causes changes in mitochondrial optical properties and modifies some NADH-mediated reactions of the mitochondria [66].

Irradiation of *Saccharomyces carlsbergiensis* with a diode laser (λ = 904 nm) [67] or of *Torula utilis* with wideband blue light [54] increased the production of ATP and the consumption of glucose in a dose-dependent manner. Increasing the dose caused glucose consumption and ATP production to fall far below the control level. In [67], a sharp change-over of the sense of the photoresponse was demonstrated to occur in a narrow dose range. The dose-response curves in this case resemble in shape the long-term dose-response curves (Figs. 4, 5). In the work of Warnke and Weber [67], complex I (ffavoprotein-FeS system) of the mitochondrial respiratory chain was identified as a primary light absorber.

TABLE II

ACTION OF VARIOUS BANDS OF VISIBLE LIGHT ON PROLIFERATION ACTIVITY OF MAMMALIAN CELLULAR CULTURES

Wavelength (nm)	Culture	Stimulation Dose (J/m^2)	Inhibition Dose (J/m^2)	Ref.
>400	HU-274 WI-38	10^4	LO^5-lO^6	[42]
441.6 632.8 694.3 741	Human embryonic skin fibroblasts	LO^3-lO^4	LO^5-lO^6	[46]
Cool white fluorescent lamps	Human embryonic diploid lung fibroblasts	Dose not shown. Exposure every day during 150 days for 2 h: cells came through 70 divisions. 4h: -"-60div. 6h: -"-53div. dark control 53 div.	Dose not shown. The cells, exposed constantly died within 2-3 days	[43]
405	Human lymphoblastoid cells	—	10^5	[86]
630-633	Chinese hamster fibroblasts	10^3	10^4	[52]
632.8	L	7.5	—	[27]
632.8	Human embryonic foreskin fibroblasts	10^2	—	[41]
632.8	HeLa	10^2	—	Fig. 5, this paper, [37]
546-579	HeLa	Dose not shown. Dose rate 10-50 W /cm^2	Dose not shown. Dose rate 100-300 W/cm^2	[53]

The enhancement of respiration by blue light in both photosynthesizing and nonphotosynthesizing microorganisms is characterized by very low fluence rates, between 0.4 and 1 W/m^2 [69], [70]. All the action spectra reported for this blue-light effect have major peaks between 450-460 nm and a lower peak near 370-390 nm. To enhance respiration, short exposure (even flashes) are sufficient. In the case of photosynthesizing organisms, photosynthetic pigments take no part in the enhancement mechanism [69].

Data on respiratory inhibition by blue light (and the associated growth inhibition) can be found in the extensive review by Epel [50]. It is strongly suggested that in various microorganisms and mammalian cells, the photo-inhibition target site is situated in the respiratory electron transport chain [50], [57]. Inhibitory effects occur at fluences around 10^8 J/m^2. High doses of blue, green, or red light inhibited the respiration of rat cerebral cells [75]. It was suggested that inhibition was the direct result of photodestruction of one or more respiratory cytochromes.

When irradiating isolated rat liver mitochondria with broad-band visible light ($\lambda > 450$ nm) in increasing doses [68], the following temporal sequences of events was observed: stimulation of respiration coupled to ATP synthesis, decline of ATP synthesis, inhibition of respiration, increase in ATPase activity and, finally, loss of membrane potential [68]. Loss of respiration was principally due to the inactivation of dehydrogenases. Among the components of dehydrogenase systems, flavins were most susceptible to illumination, whereas iron-sulphur centers were remarkably resistant. The redox reactions of cytochromes, including the terminal cytochrome oxidase activity, were unaffected.

V. PRIMARY PHOTOACCEPTORS

An analysis of the data on stimulation of respiration and growth rate of cultures on the one hand, and inhibition of these processes on the other, in the preceding parts of this paper makes it possible to contend that, in both cases, the same molecules serve as photoacceptors with the dose and intensity of light used determining the sense of the response.

The photocontrol of cell metabolism from the viewpoint of the positive (stimulating) effect takes place only in a narrow range of light doses. As the light dose is increased, photoacceptors are damaged and the effect decreases as a result. Further increase? in the dose cause the destruction of the photoacceptors, accompanied by the inhibition of metabolism and even the death of cells.

To discuss the probability of some respiratory chain components being the primary photoacceptors (i.e., compounds absorbing light at wavelengths effective in bringing about some or other responses for low-power laser effects), the following three approaches will be used: 1) a comparison between the action spectra for photoresponse and the absorption spectra of respiratory chain components, since the action spectrum should closely parallel the absorption spectrum of the photoacceptor

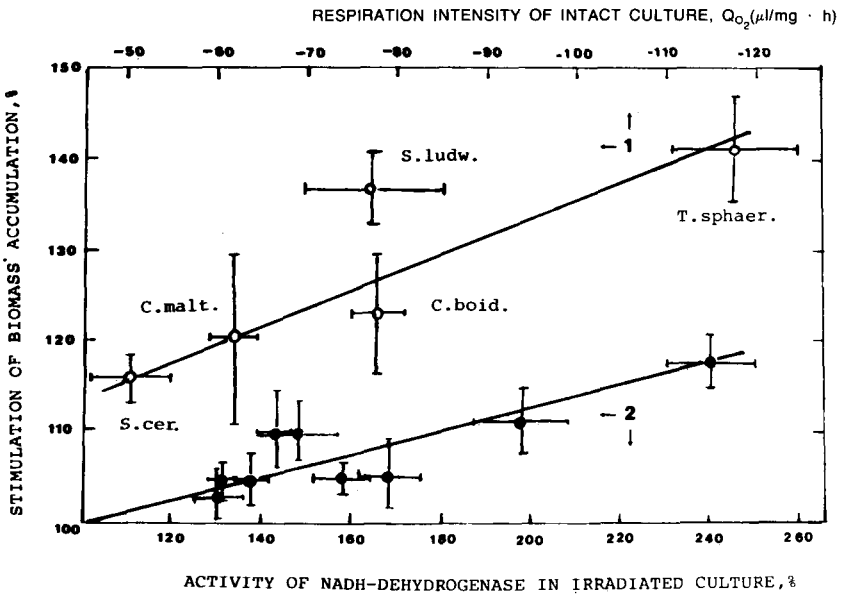

Fig. 9. The correlations: (1) between the respiration intensity of intact yeast cultures and possible maximum biomass accumulation stimulation after irradiation with He-Ne laser in optimal doses (see D_{max} on Fig. 5); (2) between the activity of NADH-dehydrogenase activity and biomass ac cumulation after the irradiation of *Torulopsis sphaerica* 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 biomass accumulation are described in [33], the determination or the NADH-dehydrogenase activity in [38].

compound; 2) the use of substances which are known to act as quenchers of the excited states of the presumed photoacceptor molecules, and 3) a demonstration that the components or procedures known to influence the probable mode of action of a potential photoacceptor do, in fact, influence the magnitude of the photoresponse.

A. Action Spectra of Photoresponse and Absorption Spectra of Respiratory Chains

A great variety of procaryotic and eucaryotic cells have similar long- and short-term photoresponses to irradiation with visible light of the same spectral band (or even wavelength). This suggests that the photoacceptor involved may be common to all the cells. There exists an intimate correlation between the oxygen consumption activity and culture growth, which, as indicated above, is especially manifest in microorganisms. In our experiments with yeast cultures [33], correlations were found to exist between the respiratory activity in a nonirradiated culture and its ability to become activated after irradiation with a He-Ne laser (curve 1 in Fig. 9), and between the NADH-dehydrogenase activity and the accumulation of biomass in irradiated cultures (curve 2 in Fig. 9).

It should be noted that, despite the universality of the respiration process and the principle of operation of respiratory chains, those in procaryotes *(E. coli* included [71]) differ from the mitochondrial chains of eucaryotic cells. Compared with mitochondria, respiratory chains of bacteria present three additional degrees of complexity. First, bacteria possess a variety of electron transfer chains. Second, a given bacterium may change the nature of its respiratory chain in response to changes in growth conditions. Third, many bacterial respiratory chains branch with alternative electron transfer pathways to multiple terminal acceptors. For example, *E. coli* possesses a linear respiratory chain when grown under highly aerobic conditions with cyt. o as the terminal oxidase. Under conditions of oxygen limitation, a second oxidase, cyt.d, is synthesized and the electron flow is divided. The redox carriers of bacterial chains are similar, but not identical, to those used by mitochondria, and include Fe/S proteins, flavoproteins, quinones, and cytochromes.

There are some minor differences in the respiratory chains of yeasts in comparison with the classical mitochondrial chain. In addition to the classical set of cytochromes, yeast mitochondria have a specific carrier, cyt. b_2, and a special NADH-dehydrogenase, so the first energy coupling point functions with extreme lability [72].

Referring to the action spectra of Figs. 1-3, there is hardly a single molecule in the respiratory chain that has a similar absorption spectrum. The complicated shape of the action spectra suggests that here the photoacceptor might be a set of interrelated molecules. The results of the dichromatic irradiation experiments, that is irradiation involving simultaneous or short-interval exposures to two wavelengths (some results of these experiments are discussed elsewhere in the text), speak in favor of this suggestion. In the red region, (about 630 nm), the absorbing compounds are the semiquinone form of flavoproteins, as well as the terminal cytochrome oxidases (cyt.d, which is the respiratory chain branch that prevails when *E. coli* are grown in our experimental conditions in the absence of

aeration [34] and cytochrome-c oxidase complex [73], which is the terminal oxidase of mitochondrial chains). The latter complex absorbs not only at 630 nm, but also at about 600, 680, 760, and 830 nm [73], and cyt.d absorbs at 683 nm [71]. In the blue part of the spectrum (400-450 nm), light is absorbed by flavoproteins which, in the case of the respiratory chain, are represented by dehydrogenases.

B. Modification of Light-Growth Response with Sodium Dithionite and Auxin

Here, we will discuss two series of experiments performed with *E. coli* [39]. In the first series, the influence of a reducing agent, sodium dithionite ($Na_2S_2O_4$), on the light-growth responses of *E. coli* was studied. As can be seen from the data presented in Fig. 10, addition of $Na_2S_2O_4$ to the bacterial suspension just prior to irradiation reduces the stimulation level and suppresses division. The effect depends on the sodium dithionite concentration, no matter which spectral band, red or blue, is used for irradiation. In both cases, 1 mM of sodium dithionite decreases the stimulative effect of light by 45 percent, and 5 mM of $Na_2S_2O_4$ by 65 percent. In our experimental conditions, blue light stimulates growth by 40 percent, and so additions of $Na_2S_2O_4$ in concentrations of 1 and 5 mM cause inhibitions by 5 and 25 percent, respectively. Just as red light under the same conditions stimulates growth by 60 percent, so $Na_2S_2O_4$ decreases the stimulative effect of light by 15 percent (1 mM) or causes inhibition by 5 percent (5 mM).

As the next step, the irradiation dose was varied while the concentration of $Na_2S_2O_4$ was kept invariable at 5 mM. As seen from Fig. 11, the influence of $Na_2S_2O_4$ on the light-growth response features a characteristic dependence upon the irradiation dose. The agent itself has no influence on the division of nonirradiated bacteria or on bacteria irradiated at low doses. If $Na_2S_2O_4$ was added to cells that were subsequently irradiated with doses that caused the maximum effect in the absence of $Na_2S_2O_4$, cell division was observed to be inhibited. As the irradiation dose was further increased, the inhibition of cell division decreased. These results give us reason to believe that blue, red, and far red light cause oxidation of some molecule(s), with the process being the starting mechanism for bacterial cell division. The reduced-minus-oxidized different spectmm of *E. coli* (that is the difference between the absorption spectra of reduced and oxidized bacteria) indicates that one such molecule is cyt.d [34].

In the second series of experiments, the influence of *a*-naphtyleneacetic acid (auxin) on the light-growth response *E. coli* was studied. Auxin is believed to be the quencher of excited flavin molecules [58]. Addition of auxin to the cell suspension prior to irradiation with red light modifies the effect of light in a concentration-dependent manner (Fig. 12). Auxin, in concentrations exceeding 5 mM, decreases the stimulative effect of red light; if its concentration is higher than 10 mM, it even inhibits the growth of the irradiated culture. Auxin was found to

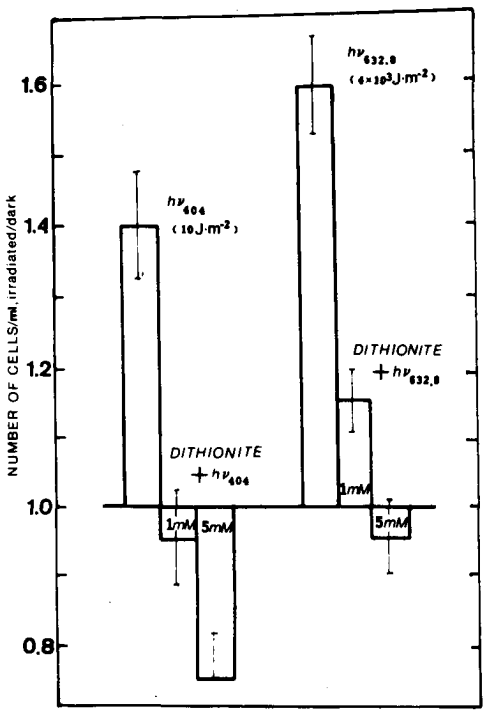

Fig. 10. Changes in *E. coli* growth rate after the irradiation with blue (λ= 404 nm, D = J/m^2) or red (λ= 632.8 nm, D = 4 x 10^3 J/m^2 light in absence or presence or of $Na_2S_2O_4$ (1 x 10^{-3} or 5 x 10^{-3} M) expressed as ratio of values of number of cells in treated and control culture 60 min after irradiation as described in [39].

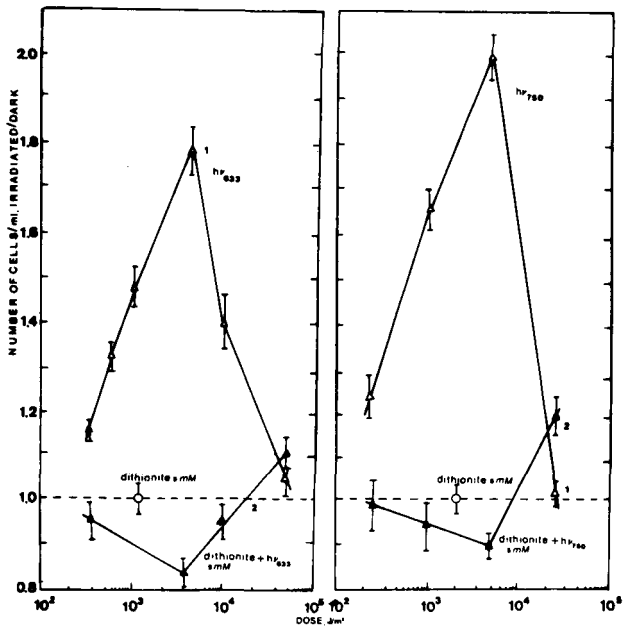

Fig. 11. Changes of *E. coli* growth as a function of irradiation dose by irradiation with (a) red or (b) far red light with or without $Na_2S_2O_4$ (5 x 10^{-3} M), expressed as the ratio of values of the number of cells in treated and control cultures 60 min after irradiation. The sodium dithionite in concentrations of 5 mM did not influence the growth rate of non-irradiated cultures as denoted with a dashed line. $Na_2S_2O_4$ was added to bacterial suspension just before the irradiation as described in [39].

have no effect on the nonirradiated cultures, no matter what its concentration (Fig. 12). On the basis of these results, it many be supposed that flavins either participate

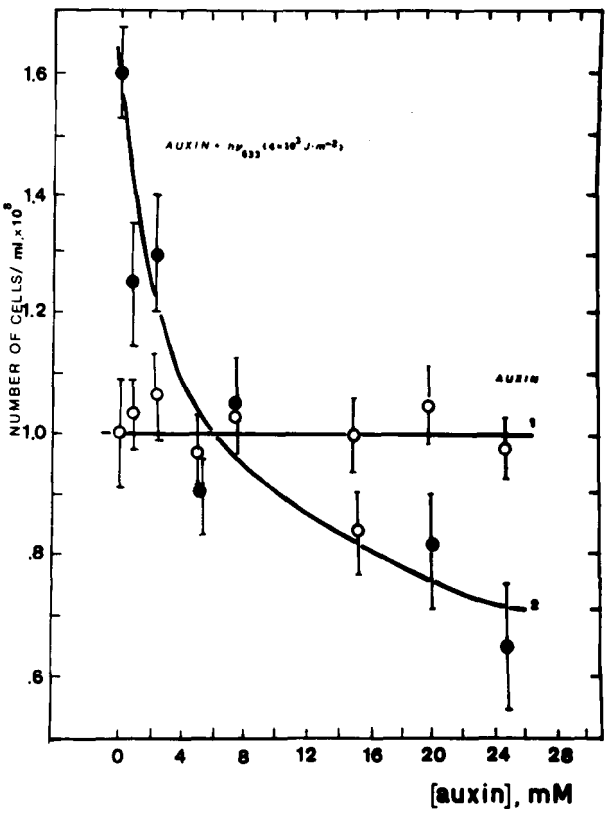

Fig. 12. Changes in *E. coli* growth rate as a function of auxin cocentration when auxin was added (1) to nonirradiated cells or (2) to culture before the irradiation with He-Ne laser *(D* = 4 x 10^3 J/m^2) as described in [39].

directly in the absorption of light (their semiquinone form absorbs red light) or are part of the photosignal transmission chain.

C. Influence ofDicromatic Irradiation on the Final Photoresponse

Referring to Fig. 13, monolayers of HeLa cells were simultaneously irradiated with red light (λ = 632.8 nm) and variable-wavelength light (λ_{add}) [40]. These action spectra of DNA and RNA synthesis stimulation differed significantly from those for monochromatic irradiation (Fig. I): the blue maximum shifts from 404 to 450 nm (at $D_{\lambda add}$, = 10 J/m^2) or disappears altogether at *($D_{\lambda add}$* = 25 J/m^2), a band of inhibition (at $D_{\lambda add}$ = 10 J/m^2) or stimulation (at $D_{\lambda add}$ = 25 J/m^2) appearing in the green spectral region. In addition, the maximum at λ = 680 nm vanishes and that at λ = 760 nm becomes unessential.

In consecutive dichromatic irradiations, the magnitude of the final photoresponse is also influenced by the interval between the two irradiation events [40].

The dichromatic irradiation of *E. coli* was performed in the following way: the bacteria were first irradiated with red, then with far red light or vice versa in one series of experiments, and likewise with blue and red light in another. The results of one of these experiments are shown in Fig. 14. Experiments of triple-exposure irradiation were also performed [39].

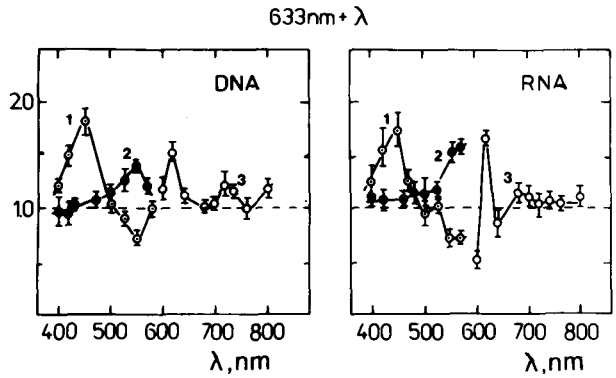

Fig. 13. Action spectra of concurrent dichromatic irradiation on DNA and RNA synthesis rate in exponentially growing HeLa cells, measured by 20 min pulse labeling with H^3-thymidine or C^{14}-uridine, respectively, 1.5 h after the irradiation as described in [40], and expressed as a ratio of radioactivity value of irradiated and nonirradiated culture. The cells were irradiated simultaneously with red light (λ = 632.8 nm, *D* = 100 J/m^2) and with light, the wavelength of which is shown on the abcissa, and doses are (1) 10 J/m^2, (2) 25 J/m^2, and (3) 100 J/m^2.

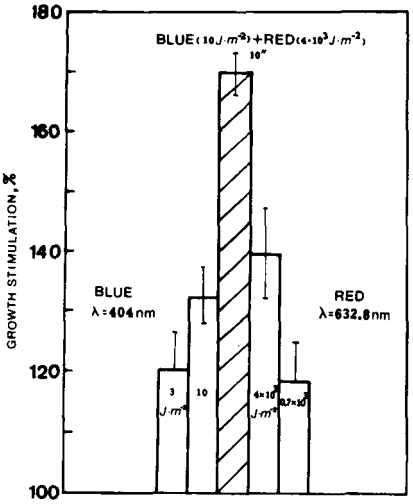

Fig. 14. Changes in *E. coli* growth rate measured 60 min after the beginning of incubation, after concomitant (time between 2 irradiation events 10 s) irradiation with blue (*λ*= 404 nm, *D* = 10 Jm^2) and red light (λ = 632.8 nm, D = 4 x 10^3 J/m^2)—striped column. On the left of the striped column, stimulation was caused only by blue light *(D* = 3 J/m^2 or 10 J/m^2). On the right, stimulation was caused by red light *(D* = 4 x 10^3 J/m^2 or 0.7 x 10^3 J/m^2 [39].

The results of dichromatic irradiation experiments are discussed in [39], [40]. Based on these data, it may be presumed that the photoacceptor is a set of interrelated molecules and that there are some common stages in the realization of the effects caused by the different bands of light.

Irradiation of cells with wide-band light, whether this includes the red interval or not, as well as simultaneous dichromatic irradiation with wide-band visible light and red light, caused practically no stimulative effect (Fig. 15). In these experiments, the red light dose was optimal for the DNA synthesis stimulation (10^2 J/m^2), while that of the wide-band radiation was selected to be about 4 times higher. This dose ratio corresponds approximately to that in natural solar radiation to which biological objects have become adapted through evolution.

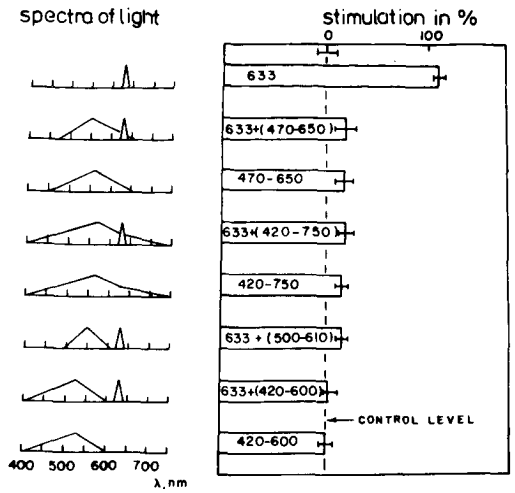

Fig. 15. Effects of widening of the spectrum of the irradiating light on the DNA synthesis rate in exponentially growing HeLa cells when the cells were irradiated simultaneously with wide-band light and He-Ne laser light or only with wide-band light. Simplified spectra of the wide-band visible light and of the narrow-band stimulating red light are shown on the left. The corresponding DNA synthesis rates are shown on the right, measured by 20 min pulse labeling with H^3-thymidine 1.5 h after the irradiation as described in [28], [29], and expressed as a percent of the nonirradiated (taken as 0 percent and denoted with a dashed line) culture.

D. Conclusion

The respiratory chain components are discussed as primary photoacceptors. When cells are irradiated with various visible-light bands, light is absorbed by the respiratory chain. The primary photochemical and photophysical events are believed to occur in the mitochondria in the case of eucaryotic cells and in the cytoplasmic membrane in that of *E. coli.* Incidentally, the possible existence of a transduction chain from photon to the cellular response, the first stage of which is the absorption of light by the mitochondria, is also experimentally documented for some other light effects. The microbeam irradiation of the mitochondrial area of a single myocardial cell [76] or crustacean neuron [77] with green or blue light, respectively, caused changes in the beating frequency of the former and the impulse frequency of the latter.

On the basis of the results of experiments on the modification of photoresponse with $Na_2S_2O_4$ and auxin, as well as with dichromatic irradiation, it is possible to suggest that the absorption of light quanta produces changes in the redox state of the respiratory chain.

Our present day knowledge of the subject, modest as it is, enables us to treat only the entire respiratory chain, and not any of its individual components, as a primary photoacceptor. The respiratory chain is a unitary dynamic system which, when being acted upon at a single point, changes its whole state of response, thus in turn determining its regulatory action.

VI. The Mode of Light Action Upon Photoacceptors

A. Connection with the Temporal Organization of the Respiratory Chain

An ever-increasing number of works have recently been published stressing the nonstationary, periodic character of many metabolic processes and setting up hypotheses that their dynamic organization can probably perform a physiological function [79]. According to these hypotheses, all reactions and processes occurring in a cell or an organism are believed to be periodic and are classified into a series of domains by their rate and period. A periodic process potentially capable of reacting to a low-intensity irradiation (intensity 10-100 W/m^2, irradiation time 10-100 s) must fall within the "metabolic domain." This domain includes processes with a period 1 s to 5 min and covers all intermediate metabolic chains and reactions [79].

The periodic character of intermediate metabolism is confirmed by both mathematical models [80] and some experimental data. For example, such mitochondrial functions as ion transport and respiration, the redox states of NAD, flavoproteins, and cyt.b, the mitochondrial volume and the ATP pool were demonstrated to be periodic and have a period of 0.5-2 min [79]. Under certain conditions, the periodic oscillations of the redox states of flavins and NAD were also observed to occur in *E. coli* [82].

Proceeding from the assumption that the photoacceptors of low-intensity light are the respiratory chain components, and using *E. coli* as an example, we tried to estimate the excitation rate for two monochromatic bands 633 and 454 nm in wavelengths characteristic of absorption of cyt.d, ε_{633nm} = 8.5 x 10^2 1/mole • cm [83] (σ = 1.41 x 10^{-17} cm^2), and flavin enzymes, (ε_{454nm} = 1.1 x 10^4 1/mole • cm [83] (σ = 1.81 x 10^{-17} cm^2), and also the optimal (as used by us) light intensities I_{633nm} = 10^{-2} W/cm^2 (3.15 X 10^{16} photons/cm^2 • s) and *1^^ = 2* x 10~^{2} W/cm^2 (4.54 X 10^{16} photons/cm^2 • s, the excitation rates W_0 can be estimated

$$W_0 = \sigma_{abs} \times I \quad [5].$$

The results obtained (W_{633nm} = 0.43 s^{-1} and W_{454nm} = 0.83 s^{-1}) agree with the periodic energy process parameters and the irradiation times 10-100 s actually required to achieve the stimulative effect.

Thus, the presumption that the respiratory chain is an acceptor of low-intensity monochromatic visible light explains satisfactorily the wavelength and dose dependence of the stimulative effect of such a radiation, as well as the modifying influence of auxin and the reducing agent sodium dithionite on the respiratory process, insofar as the electron transport chain is a dynamic system with an absorption spectrum close to the action spectrum, whose rearrangement occurs in times commensurable with the irradiation times. On the other hand, this presumption makes it possible to explain the relationship between such distant events as absorption of light and acceleration of growth. It is known from the literature that the metabolic activation of both *E. coli* [84] and mammal cell cultures [85] is preceded by the establishment of a certain intracellular pH.

In the case of bacteria, *E. coli* included, with an acid or neutral extracellular pH, the necessary pH gradient is produced as a result of the action of the respiratory chain

situated in the cytoplasmic membrane [84]. So, it can be supposed that the bacterial respiratory chain, once activated by visible light, acquires the ability to produce the necessary pH gradient more rapidly, thus enabling the cell to start dividing earlier.

B. Seasonal Variations of Light-Growth Response

In our experiments with microorganisms, we established seasonal variations of growth stimulation. Illustrated in Fig. 16 are three examples. There is practically no growth photostimulation during the summer. During the winter, the effect is maximum, and in the spring and autumn the effect is intermediate. Seasonal variation in the magnitude of the effect is connected with the growth rate of the culture as follows. In autumn and winter, the culture features a relatively slow growth (Fig. 17). In spring and summer, when the culture growth accelerates and the growth rate of the control is almost comparable to that of the culture exposed to the optimum dose of red light in the autumn-winter period, irradiation has but little effect (Fig. 17). Taking into consideration that the primary photoacceptor is the respiratory chain (see the preceding section), and the fact that fast growing microorganisms respire at their maximum capacity [82], it is clear why it is impossible to stimulate growth in the spring-summer period.

Seasonal variations in the maximum possible photostimulation present evidence that the light-growth responses of microorganisms are connected with their photoperiodical clock, as is suggested, for example, in the case of photoperiodical control of thermogenesis [87].

C. Dependence of the Physiological State of the Cell at the Moment of Irradiation on the Magnitude of Photoresponse

The photosensitivity of cells is not an all-or-nothing phenomenon (as evidenced by the seasonal variations of the light-growth response), and the cell can respond to the light stimulus in various degrees. The magnitude of the photoresponse depends on the physiological state of the cell prior to irradiation which is conditioned by, for example, the amount of nutrients available and the age of the culture. Starving cells are more photosensitive than well-fed ones [56], [59], and the extent of light-inducible respiration varies with the degree of starvation [69]. Usually, cells in the exponential phase of growth are more photosensitive than those in the phase of stationary growth [70].

The proliferative activity of both procaryotic and eucaryotic cells can be altered by a variety of factors; the final effect depends upon how many such factors are at work. Stimulated by some other factor, the cells respond to the next stimulus only a little if at all. For example, phytohemagglutinin, a well-known mitogen, did not increase the division rate of stimulated, fast-growing cells but increased the division of a slow-growing culture tremendously [81].

There could be a number of reasons for changes in the standard proliferation rate of a culture; one of the most important ones in the case of photobiological experiments is light stimulation prior to the experiment during routine laboratory handling. The radiation of fluorescent lamps attached to the ceiling of the laboratory or incubation room may play a role (recall the work [42] where exposures of

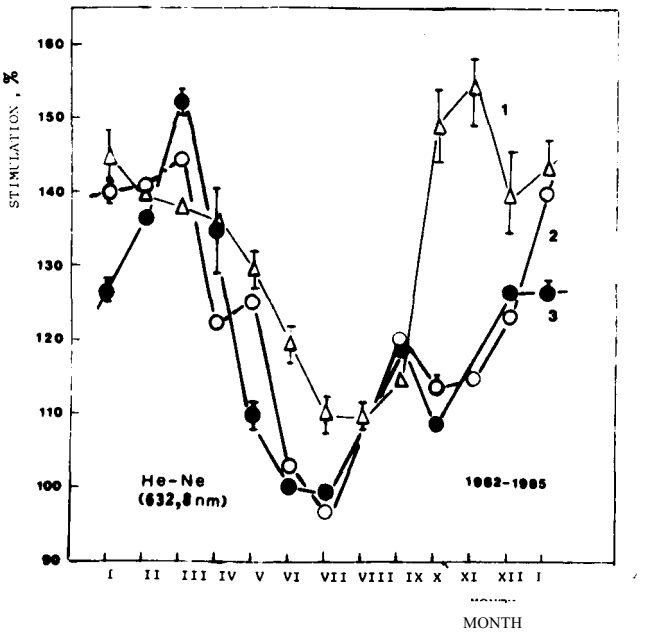

Fig. 16. The average growth stimulation of microorganisms (*1—E. coli, 2—T. sphaerica, 3—S. ludwigii)* after irradiation with He-Ne laser in optimal doses D_{max}, see Figs. 4(b) and 5). Average date from full years 1982-1985. The irradiation was performed always at the same time (near 11 A.M. with *E. coli* and near 6 P.M. with yeasts).

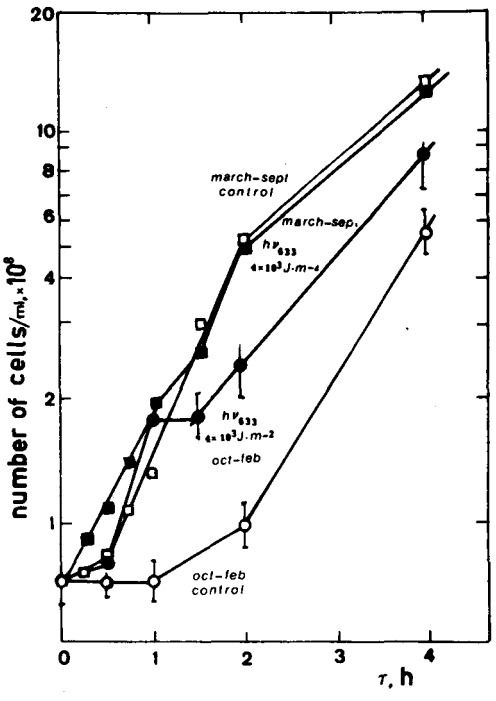

Fig. 17. The growth curves of *E. coli* culture in March-September and October-February without irradiation and after the irradiation with He-Ne laser (D = 4 x 10^3 J/cm^2). The growth stimulation here and in all other figures is defined as the difference in the number of cells in irradiated and nonirradiated cultures, 60 min after the irradiation, and the beginning of incubation when the difference is maximal.

TABLE III

TYPICAL COOL WHITE FLUORESCENT LIGHT DOSE RATES [78]		
Exposure Geometry	Exposure dose rates (J/m^2) (/sec)	(/hr)
Tissue culture hood	3	1.1×10^4
Typical walk-in incubator	1-3	$0.36\text{-}1.1 \times 10^4$
Office fluorescent light	0.7-1	$0.25\text{-}0.36 \times 10^4$

2 h daily caused a significant stimulation of the proliferation rate). Typical dose rates from these lamps (Table III) are sufficient to influence proliferation when the culture is handled in this light even for a very short period of time. Without adequate control of light (and other environmental conditions), serious artifacts can be introduced from low-level fluorescent light exposures.

The following 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 [41] was that irradiation with a He-Ne laser increased the proliferation rate of a cellular culture, but that noncoherent light at $\lambda = 633 \pm 9$ nm did not. Comparing the growth rates of the nonirradiated cultures in both experiments [41, Tables I and II], it becomes clear that the culture used for irradiation with noncoherent light divided more rapidly than the one used for irradiation with the He-Ne laser, hence the light stimulation of the faster-growing culture was weaker than that of the slower growing one. This, not the absence of coherence, is the real reason for the almost negligible effect of noncoherent light.

VII. ON the MECHANISM of LOW-POWER LASER THERAPY

The main aim of this paper is to relate low-power laser effects to well-known photobiological phenomena and, in this way, try to explain the mystery of "laser biostimulation."

The local effects of phototherapy in treating trophic ulcers and indolent wounds with the He-Ne laser [I], with the He-Cd laser [46], 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 the necessary nutrients) which prevent proliferation, so that the cells enter the G_o phase or remain in the G_i phase. For such cells, light may serve as a signal to increase proliferation.

When irradiating fresh wounds, the effect of irradiation can be minimal or nonexistent. This happens in cases where the proliferation is active and the regeneration of the tissue integrity occurs at a more or less maximal (normal) rate, and may be the reason why there is often no phototherapeutic effect observed when irradiating fresh experimental wounds while one is observed in the case of "old" or "bad" wounds. 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 there is a hierarchy of proliferation control structures.

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