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# INVESTIGATIONS ON BIOLOGICAL EFFECT OF POLARIZED LIGHT

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**Abstract**—The biological effects of single and 4-time irradiation of primary human embryo fibroblasts with 4 J/cm$^2$ polarized light emitted by a halogen light source were investigated. The functional state of the plasma membrane was examined by means of lectin-binding and polycationized ferritin-binding techniques. It was established that the Con A binding of the cells did not change, whereas the number of negatively charged binding sites increased to a significant degree in relation to the untreated (control) samples and cell cultures exposed to diffuse (non-polarized) light. The micromorphological examinations showed no ultrastructural deviations. The quantitative increase of negative surface charges may be regarded as an indication of the biological effect of polarized light exerted on the cell membrane. The modifying effect of polarized light on the survival of *E. coli* exposed to the ionizing radiation was manifested in decreased anoxic radiation response.

## INTRODUCTION

The process of wound healing can be influenced favourably by coherent, polarized laser light. In spite of the positive clinical experiences reported in numerous publications, the mechanism of the biological effect of laser is not known completely (Walker, 1985; Fork, 1971). It is supposed that in the cell membranes the polar heads of the lipid bilayer are reordered by polarized light, consequently functional changes take place (Kertesz *et al.,* 1982).

In our laboratory the *in vitro* effect of He-Ne laser on cell membranes has been studied. It was shown that a 1 J/cm$^2$ dose of single laser-irradiation applied on primary human embryo fibroblast culture was not followed either by functional or micromorphological alterations of cell surfaces (Kovacs *et al.,* 1982). On the other hand, repeated laser irradiation induced functional changes in the cell membrane, e.g. the binding of lectin was decreased, though the cells seemed micromorphologically intact (Kubasova *et al.,* 1984a, 1984b).

One of the properties of laser—polarization—is considered to be responsible for biostimulation (Fenyo, 1984). It was suggested that a source of incoherent light emitting polarized light—called EVOLITE—would induce the similar biostimulative effect in living cells as laser. In spite of the many positive clinical experiences with EVOLITE, no references about its biological mechanism are available. The present work deals with the effect of polarized light in a dose of 4 J/cm$^2$ exerted on the plasma membrane of primary embryo fibroblasts after single irradiation as well as after 4-time exposure.

The response of cells towards ionizing radiation can be altered in different ways by physical factors (like the quality of radiation, or temperature), chemical factors (sensitizing and protecting agents) and biological or physiological factors (cell cycle stage, amount of DNA) (Fenyo, 1984). No experimental data are available yet concerning the radiation modifying effect of non-polarized and polarized light. In this work the rate of survival of *E. coli* pretreated with polarized light has also been investigated after the exposure of bacteria to $\gamma$-radiation.

## MATERIALS AND METHODS

*Primary human embryo fibroblast cell culture.* The primary human embryo fibroblasts were obtained from healthy pregnancy interrupted in the second or third month. The cells were cultured in glass Petri dishes of 3.5 cm diameter, in nutrient medium consisting of 2 m Parker 199 solution containing 10% heat inactivated newborn calf serum, at 37°C and 5% CO,. In our investigations 96-h cell monolayers were treated with polarized and diffuse (non-polarized) light.

*Irradiation of cell cultures with polarized and non-polarized light.* The cell cultures were irradiated with polarized light emitted by EVOLITE lamp (West-Germany, Bildsystem AB) from 6.5 cm distance measured between the polaroid filter and the cell layer in Petri dishes. Under such conditions the total surface on the Petri dishes was exposed to the beam of polarized light. The height of the nutrient solution (without calf serum) in the Petri dishes was 0.5 cm. During irradiation the covers were removed. The duration of exposure was 7 min. At the highest intensity and range 5 of the applied source of polarized light, the dose of irradiation of the cell culture surface was equal to 4 J/cm$^2$

The control cells were irradiated with non-polarized, that is, diffuse light of the same light source. To ensure

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†*Abbreviations:* ConA, concanavalin A, a lectin; DRF, dose reducing factor; G, Golgi complex; M, mitochondrium; N, nucleus; SEM, scanning electron microscopy; TEM, transmission electron microscopy; [$^3$H]ConA, [$^3$H]concanavalin A.

equal dose of irradiation, the light source was working without the polaroid filter, at half intensity, range 3 for 7 min. Besides these, experiments were carried out on untreated cell cultures, too.

*Lectin binding by human fibroblasts irradiated with polarized and non-polarized light.* Both irradiated (by polarized and non-polarized light) and the control cell cultures were labelled at room temperature for 10' with a radioactive lectin, [$^3$H]concanavalin A ([$^3$H]ConA)t: Concanavalin A, [$^3$H(g)]-, Net-491, specific radioactivity 50.0 Ci/mmol, New England Nuclear, Boston, MA. Volume of 0.5 m of physiological salt solution containing 0.5 μCi [$^3$H]ConA was added into each Petri dish, then the non-bound radioactive lectin was washed out repeatedly. The lectin binding was performed immediately, 30 and 120 min after the light treatment. The bound radioactivity was measured by liquid scintillation spectrometer, and the results were expressed as percentage of the values for the untreated control cultures. Each experiment was carried out in different layouts, and measurements were performed on 3 parallels each.

*Cationized ferritin binding by polarized and non-polarized light-irradiated human fibroblasts.* The changes in the surface charge of the cell membrane were investigated using polycationized ferritin (Sigma, St. Louis, MO) (Danon *et al.,* 1972) in final concentration of 0.3 mg/m . The cells were treated at room temperature for 1 min. Non-bound ferritin was removed by repeated washing and the cells were prepared for transmission and scanning electron microscopy.

*Electron microscopy techniques.* The methods applied during the micromorphological investigations and performed by means of transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are described in Kubasova *et al.* (1984b).

*Modifying effect of polarized light on radiosensitivity. Escherichia coli* B(r6ATCC No. 23227), further E. *coli* were grown to stationary phase in liquid minimal medium. Cells were washed three times by centrifugation and resuspended at $10^7$/m in buffer (Ewig, 1983). Before exposing to γ-irradiation the suspension was irradiated with polarized light for 10 min (7 cm). The 7-irradiation facility was RH-γ-30 $^{60}$Co apparatus with a dose rate 67.05 Gy/min. Survival curves were constructed from four experimental points. These curves took a linear form and are described by the expression (Powers *et al.,* 1959; Alper, 1960)

$$\ln S/S_0 = \ln n - kD$$

where $S$ is the number of cells surviving a dose $D$, $S_0$ is the original number of cells, $k$ is the slope of the curve and is referred as the inactivation constant, and $n$ is known as the extrapolation number.

Suspensions containing $10^7$ viable cells were equilibrated with nitrogen and air over the bubbled suspension for 15 min before the commencement of irradiation. Gas bubbling continued throughout γ-irradiation.

## RESULTS

### *[$^3$H]ConA binding*

The functional condition of plasma membrane of human fibroblasts upon the effect of polarized and non-polarized light was followed by lectin-binding technique in the course of 2 h after irradiation. It was shown that neither single nor 4-time exposure caused any alterations in the lectin-binding capability of cells (Fig. 1A, B). The binding of [$^3$H]ConA to the mannose-glucose groups on the cell surfaces was not affected neither by polarized nor non-polarized light as compared to the non-treated control fibroblasts.

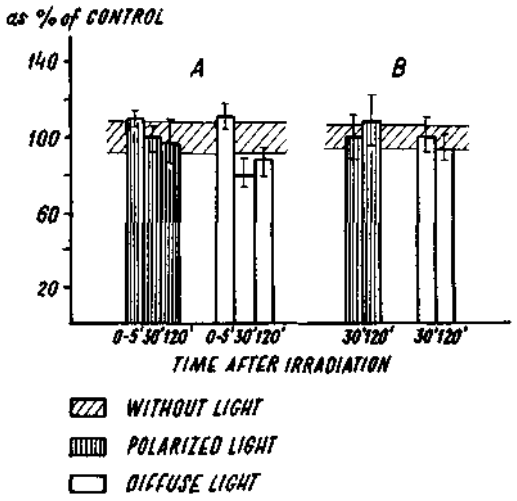


Figure 1. [$^3$H]ConA binding of primary human embryo fibroblasts treated with polarized and diffuse light of 4 J/cm$^2$. (A) single exposure, (B) 4-time exposure. No significant difference in the lectin-binding of the treated and untreated samples was observed.

### *Cationized ferritin binding*

The difference between the exposure to two different kinds of light is unambiguously demonstrated.

The accumulation of negative surface charges 30 min after single irradiation with polarized light was significant (Fig. 2). As opposed to the control sample (Fig. 2A), the polycationized ferritin was evenly bound to the cell membrane in small groups (Fig. 2B), and this phenomenon still persists even after 2 h.

The treatment of cell cultures with diffuse light also increased the binding of polycationized ferritin (Fig. 2C). However, the number of negatively charged binding sites was significantly lower than that of the sample exposed to polarized light, both after 30 and 120 min.

### *Morphological evaluation*

The ultrastructural investigations on fibroblasts did not reveal any significant difference between the untreated control (Fig. 3A) and the cell cultures irradiated with polarized (Fig. 3B) and diffuse (Fig. 3C) light.

Examining the micromorphology of cell surfaces by SEM, it could not be detected any striking alteration in the micromorphological appearance of polarized light-treated fibroblasts (Fig. 4B) as compared with the non-irradiated control (Fig. 4A).

### *Modifying effect of polarized light on survival of* E. coli *γ-irradiated*

When the cells were pretreated with polarized light the anoxic $k$ value was 7.63 $Gy^{-1}$ x $10^{-4}$;

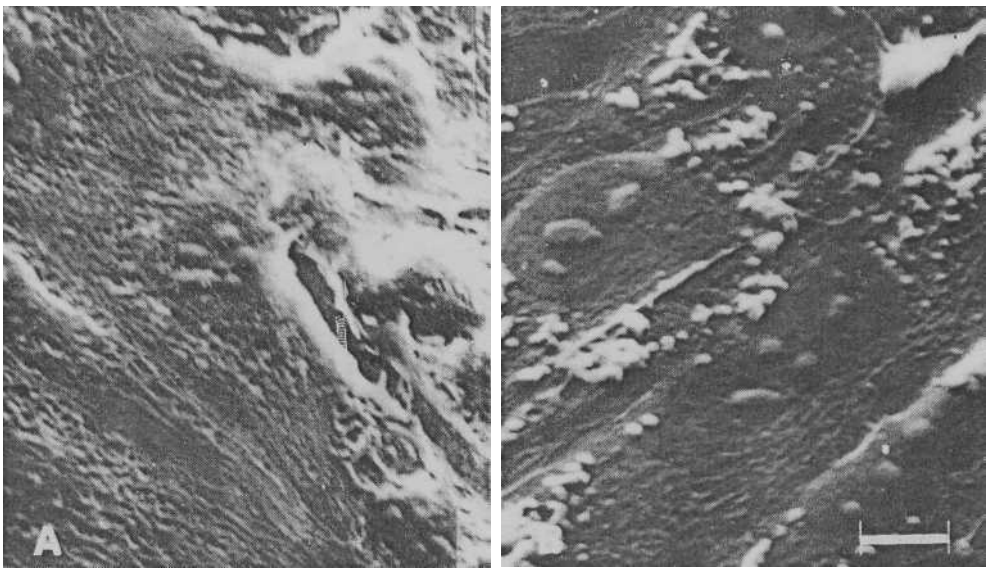


Figure 4. Scanning electron microscopy of primary human embryo fibroblasts. (A) untreated control, (B) 30 min after irradiation with 4 J/cm$^2$ polarized light. Magnification: scale bar = 1 μm.

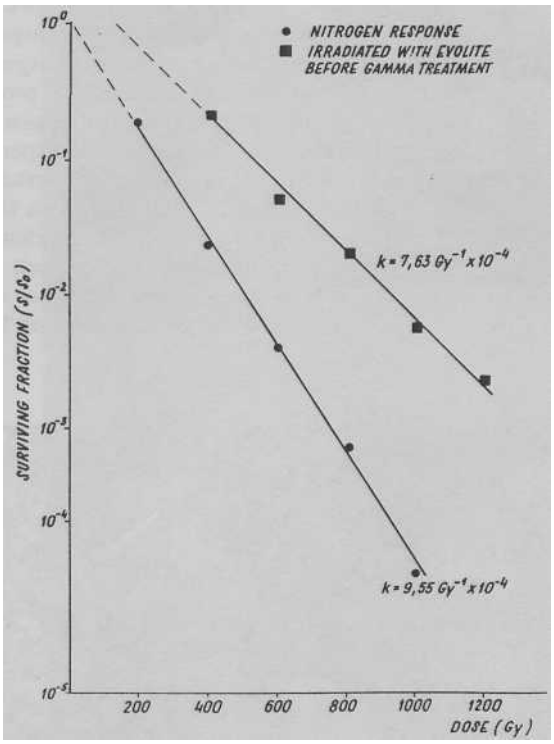


Figure 5. Dose-response curve of *E. coli* irradiated under anoxic condition.

increase in the quantity of negative surface charges on the cell membrane (Kovacs *et al.,* 1982; Kubasova *et al.,* 1984a, 1984b).

Though non-polarized light is made responsible for many biological effects (Boder *et al.,* 1983), the number of negatively charged binding sites of cells exposed to diffuse light was increased only to an insignificant extent in relation to that of untreated fibroblasts. The functional alterations of plasma membrane caused by polarized light occurred in much higher extent than on the effect of diffuse light. Thus, the increase of the quantity of negatively charged sites on the cell surface of human fibroblasts as well as the modifying effect of polarized light exerted on *E. coll* radiation response partly provide the participation of the cell membranes in the biostimulative process already experienced.

## CONCLUSIONS

It was assumed that the favourable healing effects induced by polarized light and experienced in the clinical practice (surgery, rheumatology) were attributable primarily to the changes in the cell membrane.

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Figure 2. Cationized ferritin binding of primary human embryo fibroblasts. (A) untreated control, (B) 30 min after irradiation of the cells with 4 J/cm$^2$ polarized light, (C) 30 min after irradiation of the cells with 4 J/cm$^2$ diffuse light. Magnification: scale bar = 0.1 μm.

without light-irradiation the mean inactivation constant is 9.55 $Gy^{-1}$ x $10^{-4}$ (Fig. 5). It means that a pretreatment with polarized light decreases the anoxic γ-radiation response giving a DRF (dose reducing factor) of 1.25. Similar experiments were carried out using air as the equilibrating gas. In this case no difference was found between the polarized light-pretreated and the control radiation responses.

## DISCUSSION

Biophysical model-experiments have shown the saturation of biostimulative effect induced by polarized light at 4 J/cm$^2$ dose. For this very reason we choose this dose for our experiments in living cells.

The biological effect of polarized light on primary human embryo fibroblasts was investigated by means of techniques sensitively indicating the reordering of plasma membranes, namely the techniques of lectin- and polycationized ferritin binding. The survival rate of polarized light-pretreated *E. coll* was also determined after γ-irradiation of the bacteria. It was shown, that single or 4-time exposure to 4 J/cm$^2$ dose did not influence the lectin-binding capacity of the cells, but the significant increase in the number of negatively charged sites on the cell surfaces was an unambiguous proof of the response of the cell membrane to exposure. Similar results were obtained during our experiments conducted with laser exposure: single irradiation of primary human embryo fibroblasts with He-Ne laser of 1 J/cm$^2$ did not induce significant changes in the amount of [$^3$H]ConA bound to the cell membrane. However, 4-time repeated treatment with 24-h intervals conducted to the decrease of lectin-binding and

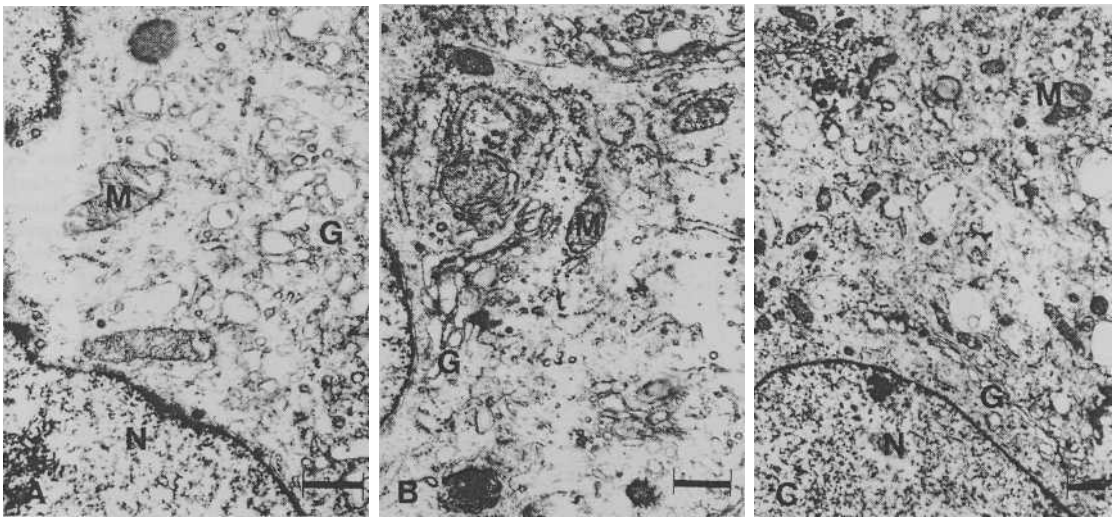


Figure 3. Ultrastructure of primary human embryo fibroblasts. (A) untreated control, (B) 30 min after irradiation of the cells with 4 J/cm$^2$ polarized light, (C) 30 min after irradiation of the cells with 4 J/cm$^2$ diffuse light. Neither polarized light nor diffuse light caused any alterations as compared to the control. G: Golgi complex, N: nucleus, M: Mitochondria. Magnification: scale bar = 0.2 μm.

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