Theoretical and experimental basis of biostimulation

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A physical model is described to explain the biostimulating effect of a laser beam. It is proposed that polarization is responsible, with the unidirectional electromagnetic field reorientating the lipid bilayer in the cell membrane. A light source which emits linearly polarized light has been made and used in clinical trials.

KEYWORDS: lasers, polarization, biostimulation, ulcers

Introduction

Biostimulation by laser light

The irradiation of a wound surface with laser light can have, as is widely known, a biostimulating effect Experiments in this field have been conducted since 1967, and the healing effect of the treatment already has a wide range of literature[1].

The healing effect becomes apparent mainly in the healing of refractory wounds and ulcers. Such lingering ulcers develop frequently on elderly people suffering from cardiovascular diseases.

Supposed mechanisms of biostimulation

There are a great number of mutually contradictory theories attempting to explain the biostimulating effect of laser light; however, none of them provide a scientifically acceptable explanation. This is the main reason why laser light has not come into general use to the extent which its efficiency would seem to deserve. Other reasons might be that a continuously operating laser providing the required output and beam diameter is rather complex, and that special skills are required during handling.

The special features of laser light are:

— monochromatism

— coherence

— possibility for producing extremely high output power densities

— polarization.

Immunological tests have shown that where incoherent irradiation by a thermal light source (630 nm) was ineffective, irradiation by linearly polarized, incoherent light proved to be nearly as effective (80%) as that of linearly polarized HeNe laser light at the same wavelength

Clinical results with laser light have shown that the healing effect was independent of wavelength at 694 nm, 628 nm, 514 nm, 488 nm and 458 nm in the visible spectrum, as well as at 1060 nm in the infra-red. Recent measurements on liquid crystals illuminated by laser light have shown that the linearly polarized light can reorder the liquid crystal molecules[3-4].

Knowing that the lipid bilayer of the cell membrane can be considered analogous to a liquid crystal, we assumed that linearly polarized light acts on the lipid bilayer of the cell membrane in the same way as it does on the liquid crystal.

Physical model for biostimulation

Proteins and lipids are the most important units of the cell membrane, with the biologically active proteins incorporated into the lipid bilayer. In living cells the lipid bilayer takes a metastable state similar to that of a nematic phase liquid crystal, which can be reordered by the application of a unidirectional electric field.

In our model linearly polarized light acts on the lipid bilayer of the membrane, whereby structural changes may occur, that is, the random distribution is replaced by a more ordered one. Consequently the surface features (for example, the surface charge distribution) and the lipid protein connections are supposed to be modified. This occurs because the electric field strength of the linearly polarized light changes the conformation of the lipid bilayer as it reorders the polar heads of the lipids.

The reordering of the polar heads is therefore considered to be analogous to the reordering already seen in the case of liquid crystal molecules when being irradiated by polarized light. As in the case of liquid

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crystal molecules, in response to the induced uni-directional field the polar heads tend to rotate in the direction of the electric field[5].

Since at approximately 37°C the state of the lipid bilayer is near to the phase transition point, that is, it is in an unstable state, it is probable that the energy gained during the change of conformation of the lipid bilayer initiates a phase transition of the cell membrane.

As there is very close contact between lipids and proteins making possible energy transfer between them, the conformation change of the lipid bilayer may influence every cellular process connected with the cell membrane, that is, processes related to or taking place through the cell membrane. For example,

— the energy production of the cell,
— the immune processes, and
— enzyme reactions (this may include change of the active transport and activation energy of the enzymes).

The membrane has an important role in a variety of biochemical processes which might be influenced by the above mentioned conformational change of the cell membrane. For example:

— recognition (of antibodies, hormones, etc),
— control,
— active and passive transport (of ions, metabolites),
— transmission and conversion of energy (for example, oxidative phosphorylation),
— information transfer (neurotransmitters),
— membrane fusion (for example, release of materials stored in intracellular vesicles).

Membrane structure can be modified by many agents, for example, plant agglutinins, drugs, hormones, as well as polarized light Very small amounts of some of these chemicals are very effective in causing major changes in cell metabolism. Most probably a very small amount of energy transferred by the polarized light can cause the same effect

In connection with the above agents the membrane acts as a biological amplifier, and can be considered as a dynamic film open to regulatory control.

Materials and methods

Based on the above mentioned ideas a light source has been constructed, called Evolite.

The stimulating effect of treatment with this unit on healing of wounds can be demonstrated by describing the experiences obtained during such treatment applied to chronic wounds lingering for periods of years. Cytological and immunological examinations were made from the wound secretion taken before and after each treatment

Wound healing

To demonstrate the effect of treatment with the Evolite light source 30 patients were treated, to whom most types of traditional therapy had been applied, without even temporary success. The aetiological distribution of their diseases was the following:

— ulcus cruris developed as a consequence of diabetic angiopathy in the case of seven patients, of arteriosclerosis obliterans in six cases, and of varicositas or post-thrombotic syndrome also in six cases,
— there were ten patients suffering from decubitus ulcer,
— and one patient had chronic osteomyelitis.

In the course of traditional therapy, dressings with Mikulitz's ointment, zinc ointment, Peruvian balm, Oxycort, Panthenol spray, Solcoseryl jelly, Debrisan and various other local antibiotic and drying bandages were employed. As a general therapy, the patients took medicaments to improve circulation, vitamins, and they also received corroborant treatment.

Treatment with the aser irradiatioi was started after the failure of the conventional therapy. During the light treatment general basic therapy was continued, but no other kind of local therapy was applied to the wound (except in a few cases where purposive antibiotica in pulver form was given). The general pattern was the application of dry bandages; between two light treatments the patients wore compression bandages. The light treatment was performed once a day, with the area of the light spot being about 3 cm^2, with a power density of 150 mW cm^{-2}.

The wavelength of the light ranged between 400-3000 nm with a homogenous spectral distribution. The emission of the light source is illustrated in Fig. 1, which shows its spectral distribution curve. The emitted light beam was linearly polarized.

For comparison purposes Figs 2a and b illustrate typical absorption distribution spectra of[1] important biological macromolecules of human tissue, that of DNA (chicken erythrocyte DNA) and protein (serum albumin), by which it can be clearly seen that the irradiation produced by the light source is not absorbed by these important elements of human tissue, and consequently cannot have any unexpected side effects.

The whole wound surface was scanned by the light spot for about 30 s on each spot-area, with the front of the lamp 10 cm from the wound surface.

In the course of the examinations the macroscopic parameters of the wounds were measured and the results obtained were evaluated. The changes observed

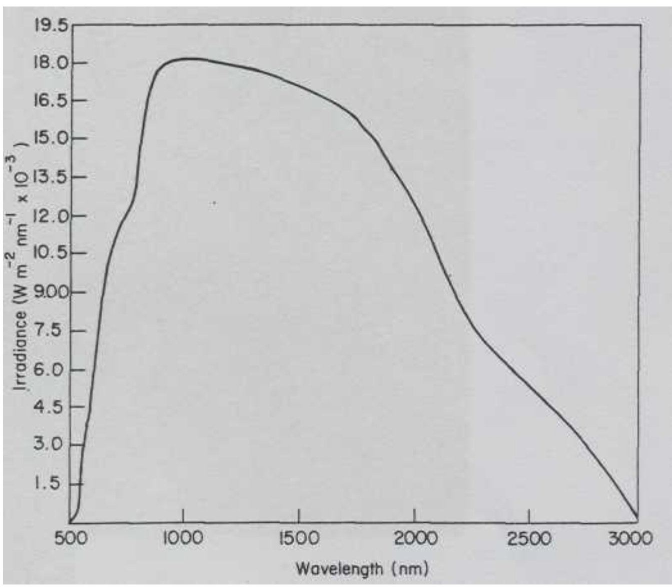

Fig. 1 Emission spectrum of the Evolite light-source

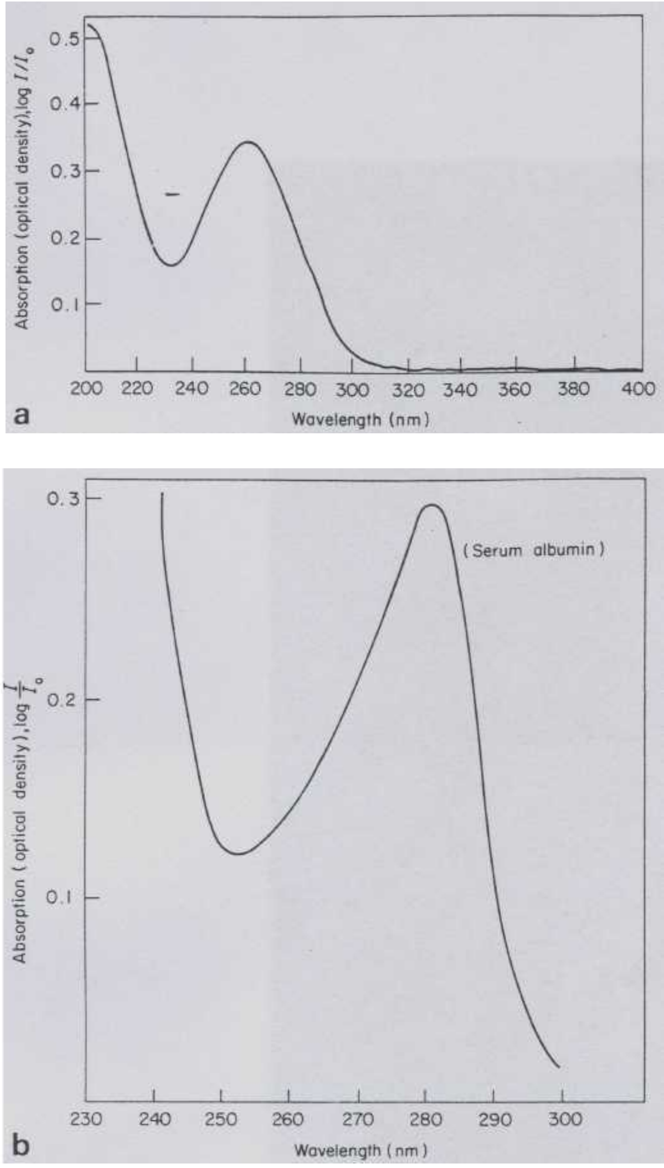

Fig. 2 Absorption spectrum of: a — **DNA; b — protein**

were measured and recorded, including the vertical and horizontal dimensions of the edges of the wounds as well as the depths, and the widths of the newly grown epithelial edges.

Cytology

When the amount of secretion allowed, samples were taken from the wound secretion, before and after treatment, and smears were prepared from the 19 treated ulcera cruris and some of the decubitus ulcera for microscopic evaluation, using the May-Grunwald-Giemsa method. As the treatment progressed the secretion gradually reduced and ceased, and so smears could not be prepared.

The 'pre-treatment' samples were taken immediately after the bandages were removed from the wound, which remained untouched (not cleaned) until the 'post-treatment' sample was taken. The pre- and post-treatment samples originated from the same part of the wound.

Altogether 195 smears were investigated. The results and conclusions are based on the summary of findings gained from these smears.

Immunology

In addition to the samples for cytological investigations (smears), larger samples were taken with a capillary tube, when possible, to determine the composition of proteins, particularly immunoproteins in the serum, by comparative electrophoresis. These investigations were based on 50 immunoelectrophoretic plates prepared from the wound secretion taken from the 19 treated ulcera cruris; 0.4 μl of the secretion was required for each analysis. There were standard samples on each plate, in which the respective areas pertaining to each fraction were known. The measurement could therefore be performed to give not only relative but also absolute values.

During the immunoelectrophoresis the individual protein fractions are separated and the quantitative ratios of these fractions are represented by the ratios of the corresponding areas. By placing the test plate onto an overhead projector and outlining the contours of each fraction projected with the same magnification, the size of the areas obtained can be determined by means of a planimeter. Then the quantities of the fractions are calculated in relation to the standard areas.

Results

Wound healing

During the courses of treatment the wounds began first to purge, and after a few treatments the secretion decreased and became clearer. At the same time the patients reported substantial easing of pain.

Blood vessel endings appeared on the bases of the wounds with white pearly growths around them, and epithelization also started on the edges. This could be observed visually, and was also supported by electron microscopy.

According to our results the beginning and rate of healing largely depend on the age, general condition, and medical and haemodynamic state of the patient. As an example consider the case of a male patient(54). He had had a distinct varicosity for 20 years. His shin split for the first time 15 years ago and since then he had had, almost continuously, ulcers on his leg. The wounds healed spontaneously; he did not want to be operated on.

In over 10 months the current wounds had not healed. He was treated with Venoruton and Padutin, and the local medical attendance involved Oxycort, antibiotics, Neogranormon, Panthenol and Debrisan. When not lying down, he always wore compression bandages. After failure of the above treatment of his ulcus cruris, treatment with the Evolite light source was started.

The treatment began on 1 October 1981. At that time he had a 2 mm deep ulcer of 20 X 24 mm size on the border of the middle and lower third of his lower leg and a 4 mm deep one of 24 X 18 mm in the distal third. After about the fifth treatment, the wounds started to heal rapidly, and became shallower and nervate. Figs 3a, b and c show the process of the wound healing in this case.

The 2 mm deep wound healed completely with crustation and epithelialization after the 40th treatment, the deeper ulcer pullulated by the 57th treatment, and the cure was complete on the 30 November 1981. Since then the patient has had no further problems.

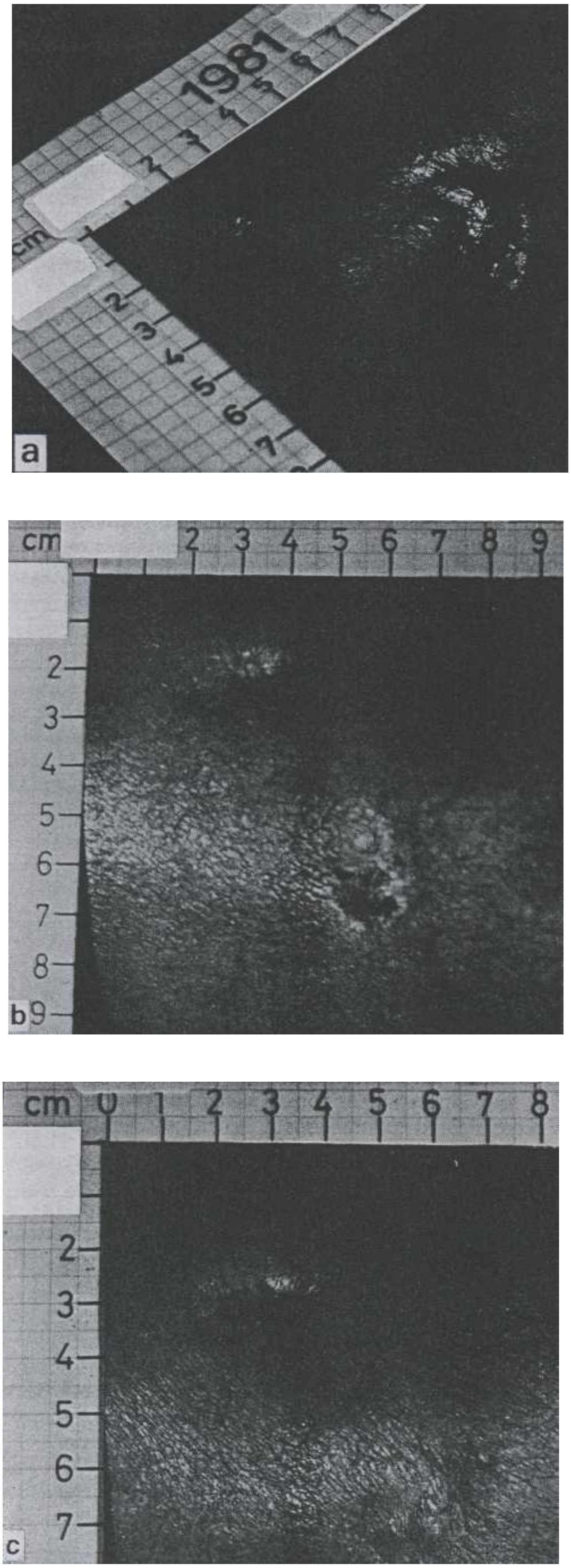

Fig. 3 a — Ulcera cruris of a male patient (54) after a few unsuccessful trials with different traditional treatments, before light treatment (1 October 1981); b — the same ulcera cruris during the treating period (2 Novembers 981); c — state of the ulcera at the end of the light treatment (30 November 1981)

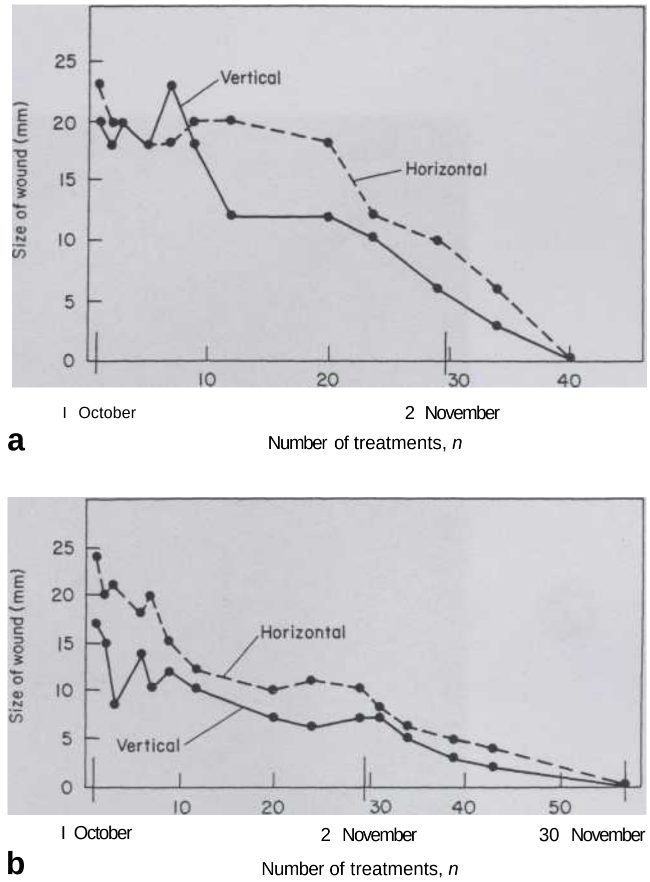

Fig. 4 a — Healing process of the 2 mm deep, 20 x 24 mm size ulcus cruris. It healed with crustation by the 40th treatment; b — healing process of the 4 mm deep, 24 x 18 mm size ulcer. It healed with filling up by the 57th treatment

Figures 4a and b show the healing process of the two ulcera cruris through the changes in the vertical and horizontal sizes of the wounds.

Cytological results

Cellular defence mechanism

i The ratio of the healthy neutrophile granulocytes ready for bacteriophagocytosis to the necrotic ones substantially increased as a consequence of the irradiation. Whilst there were no healthy leukocytes at all in the smear before the treatment, the ratio of healthy neutrophile granulocytes to the necrotic ones increased even up to 50% after treatment. This is illustrated by Figs 5 a and b which show the pre-treatment state, in comparison with the post-treatment state in Fig. 6.

ii Neutrophile granulocytes form the simplest basis of the cellular defence mechanism of the organism. Sacrificing themselves they swallow bacteria in order to protect the organism. This is bacteriophagocytosis.

Before the treatment there was a great number of extracellular bacteria (this is shown in Fig. 5a), and the healing of wounds is impeded mainly by their presence. The intensity of bacteriophagocytosis increased due to the light treatment by different amounts, which were very well demonstrated in the smears. Extracellular bacteria disappeared following the treatment, as is illustrated in Fig. 6.

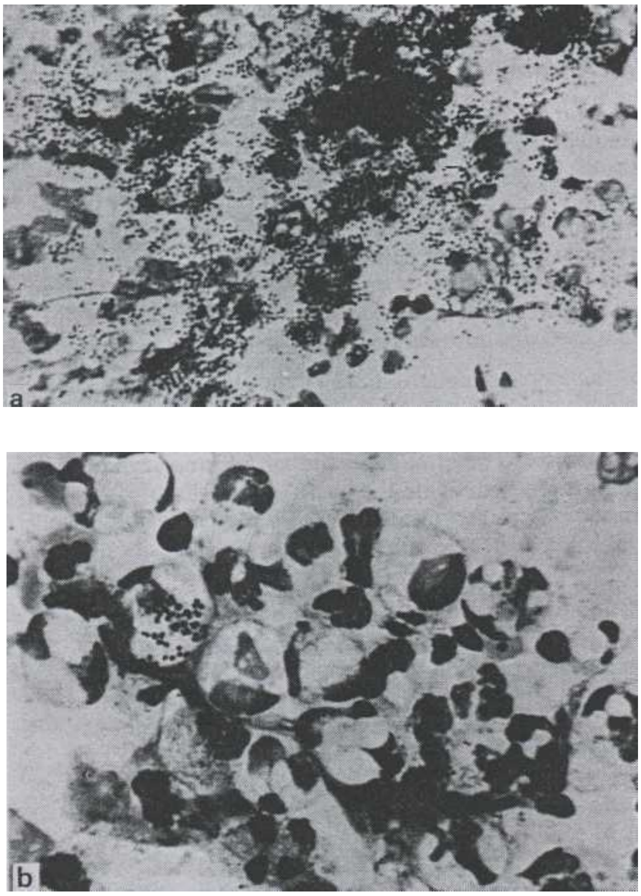

Fig. 5 a — Micrograph of smear prepared from the wound secretion taken before the light treatment. Many extracellular bacteria, with only necrotic neutrophil granulocytes, are to be seen; b — micrograph of smear prepared from the wound secretion also taken before the light treatment, illustrating very sluggish bacteriophagocytosis and the presence of mostly necrotic neutrophile granulocytes

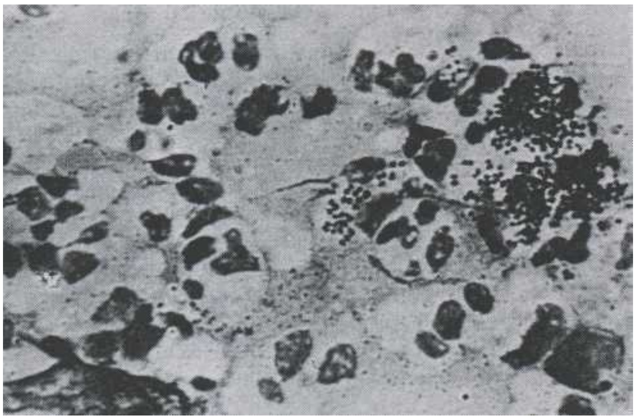

Fig. 6 Micrograph of the smear prepared from the wound secretion taken after the light treatment. Bacteriophagocytosis is very intensive, and a large number of healthy neutrophile granulocytes are apparent

The rather sluggish bacteriophagocytosis before treatment, which meant the swallowing of about 8-10 bacteria by a single cell (see Fig. 5b), became increasingly intensive, the leukocytes phagolysing up to 80-100 bacteria under the effect of the treatment (see Fig. 6, which shows the post-treatment state). Only 5-10% of the healthy neutrophile granulocytes were phagocytic (phagolysed) before treatment; after that this proportion increased to 50-60%.

The appearance of healthy neutrophile granulocytes in the secretion and the types of intensification of bacteriophagocytosis promote the annihilation of extra-cellular bacteria.

iii The other level of cellular defence against bacteria is provided by plasma cells, lymphocytes and monocytes. The appearance of these cells means that the organism mobilizes deeper immunological mechanisms to heal the wound.

While the smear taken before irradiation contained

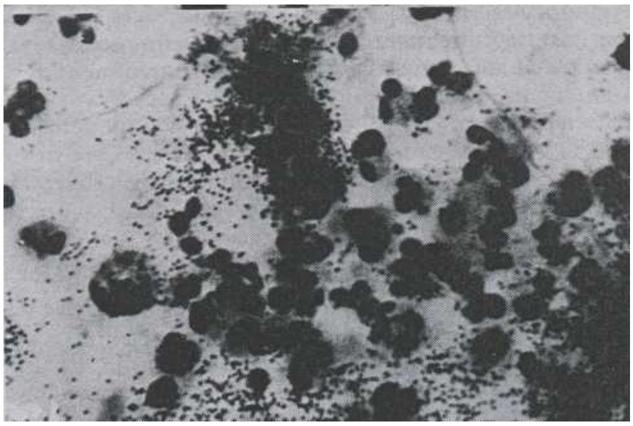

Fig. 7 Before treatment only neutrophile granulocytes are to be seen in the micrograph

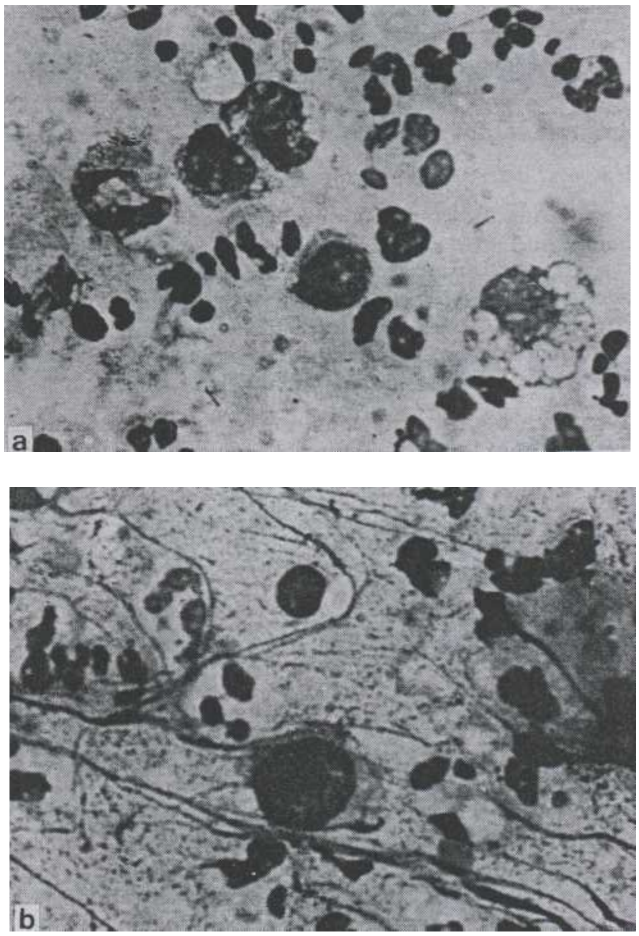

Fig. 8 a — Micrograph of the smear taken after the light treatment showing the appearance of lymphocytes; b — micrograph of the smear taken after light treatment also showing the appearance of monocytes

generally neutrophile granulocytes only, after irradiation other types of leukocytes also appeared. Such cells are the lymphocytes, monocytes and eosinophile cells. Fig. 7 shows the pre-treatment state, where only neutrophile granulocytes are to be seen. According to Figs 8a and b respectively, the lymphocytes and monocytes also appeared in the smear prepared after treatment.

No lymphocytes were found among the leukocytes before treatment, whereas the proportion of lymphocytes increased to 4-10% by the end of the treatment. With increasing numbers of treatments lymphocytes began to be seen in the secretion before the next day's treatment, but the ratio before treatment was 2% which increased to 20% after the treatment A similar increase in proportion of monocytes was detected, from an initial 0% to the post-treatment 5%, and in a later phase from 3% to 6-7%. In the case of the eosinophile granulocytes the ratio changed from an initial 0% to 1-5%, and in a later phase from 1% to 20%.

Granulation

Granulation comprises lysosomatic enzymes; the presence of a definite, large-size granulation in the cytoplasm of the cell is evidence of the appearance and quantitative growth of such enzymes. The appearance of immunochemical protection is verified by the change in the quality and quantity of granules in the cells' cytoplasm due to the treatment This has been demonstrated by the appearance of clearly visible large granules in the smears.

Immunological results

Humoral defence mechanism

Comparing the composition of the wound secretion before and after a single treatment, it was found that the composition of proteins in the secretion had changed, signifying an increase of humoral protection. Irradiation by the light source facilitated the quantitative growth of immunoproteins, to different extents in case of different fractions.

The average growths of the proteins according to the evaluation of the 50 different plates were the following: following:

Ig M	+ 84.83%
Albumin	+ 79.13%
α_1-lipoprotein	+ 73.73%
Ig G	+ 58.53%
α_1-antitrypsin	+ 37.37%
Transferrin	+ 36.29%
α_2-macroglobulin	+ 28.70%
Ig A	+ 20.76%

It was found that, for different patients, the inclination to healing is proportional to the quantitative growth of immunoproteins taking place in response to respective treatments. The more the increase in immunoproteins, that is, the more intensive the response to the treatment, the higher is the rate of healing. Taking into account this relationship, the inclination of the wound to healing and the expected total duration of the cure can already be estimated on the basis of a few treatments.

Discussion

Transition between molecular phenomena and the more complex biological levels

The biological effects described here are presumably related to the effect of light from the Evolite source on the lipid bilayer. They can be explained by the supposition that the antigen structures present in the vicinity of immune cells can provide an immune response under the effect of polarized light They do this by triggering a non-specific (or specific) response in increasing the sensitivity of the immune cells which can contribute to the healing of wounds.

If the electromagnetic field of the light changes the membrane structure of healthy leukocytes, this intensifies the activity of the receptors of the leukocytes. The change in the membrane structure can then directly activate the cyclic adenosin monophosphate[6] (3'5'-cAMP). These two effects can generate a local immune response.

In the course of the triggered immune response there are lymphokins released capable of starting an immunological chain reaction. This chain reaction involves triggering of the factors MCF (monocyte chemotactic factor), NCF (neutrophile chemotactic factor) and ECF (eosinophile chemotactic factor). These factors together attract the neutrophile granulocytes, monocytes, and eosinophile cells to the affected area. This chain reaction involves, furthermore, the generation of MIF (migration inhibiting factor) which inhibits the migration of macrophages. Consequently the aforementioned cells will migrate into and remain in that particular area.

Due to the supposed changes in membrane structure (and the triggered chain reaction) the quantity of SRF (skin reaction factor) can increase. This influences the permeability of blood-vessels, thus helping circulation and in this way the transportation of the protective cells in the blood stream to the wounded area.

The events described facilitate the cellular immune response (with T lymphocytes, killer cells) and the humoral immune response by means of T-helper cells.

Conclusions

The biostimulating effect of the laser light is a well-known phenomenon, for which there was no scientifically acceptable explanation as to which mechanism is responsible. A hypothetical model has been proposed, according to which the polarization is responsible for the phenomenon. The unidirectional electromagnetic field of the linearly polarized light reorientates the polar heads of the lipid bilayer in the cell membrane, and this change may significantly influence the movement and activity of the proteins incorporated into the lipid bilayer, as well as the possibilities of linking the proteins to the cell membrane.

For the purpose of supporting the model a light source was made which emitted linearly polarized light of a definite power — the Evolite, with which different experiments have been carried out

Firstly, using the 'polarized only' light source there were wounds treated which had resisted most types of traditional wound treating methods for months and even years.

The healing process of these wounds was documented.

Cytological and immunological experiments were also carried out, in the course of which the features of the wound secretion (taken before and after treatment) were compared from the cytological and immunological points of view.

The experiments showed that treatment with polarized light stimulates, to a great extent, both the cellular and the humoral defence mechanisms directed to the elimination of the bacteria.

According to our supposition the effect of polarized light on the cell membrane can be considered as an aspecific (or specific) triggering that can induce an immunological chain reaction on some of the few healthy leukocytes on the wound surface. This can lead to the phenomena observed.

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