11.2 Possible primary mechanisms

In order to facilitate an understanding of the mechanisms of laser therapy, we have chosen to separate what we call the primary mechanisms and the secondary mechanisms. The primary mechanisms relate to the interactionbetween photons and molecules in tissue, while the secondary mechanismsrelate to the effect of the chemical changes induced by the primary effects.

The fact that the biostimulative effects are dose dependent indicates that there may be thresholds involved in different mechanisms - a certain photon density is needed. There can be many reasons for this, one of which may be multiple photon action. If the effects simply were due to electron excitation and ionisation, there would be no thresholds - single photons would do the job. The table below shows examples of dose levels for different cell types and different wavelengths in vitro.

Wavelength in nm	**Culture type**	**Measured activity**	**Stimulation dose, J/cm²**	**Inhibition dose, J/cm²**	**References**
633	Chinese hamster	cAMP	0.01		Karu [F16]
630-633	Chinese hamster fibroblasts	Proliferation	0.1		Abdvakhitova [1230]
632.8	Human embryonic foreskin fibroblasts	Proliferation	0.01		Boulton [268]
632.8	Red blood cells	Deformability	-	>1	Yova [1229]
632.8	HeLa	Clonogenicity	0.01 ~ 0.1	>1	Karu [1228]
632.8	Mouse mast cells	Cell granule release	2 - 4		Trelles [1227]
660	Hypertrophic scar-derived fibroblasts	Proliferation	2.4 - 4		Webb [615]
660	Human neutrophils	Bacteria killing	2.4 -4.8		Yu [1231]

Table 11.3 Examples of different dose levels in vitro

Wave-length in nm	Culture type	Measured activity	Stimula-tion dose, J/cm²	Inhibition dose, J/cm²	References
694.3	Murine mela-noma cells	Growth rate	< 0.01	> 0.2	Carney [520] Hardy [1226]
812	Human buc-cal fibroblasts	Proliferation	0.45		Loevschall [75]
860	Human fibro-blasts	Succinic dehydroge-nase activity	2	16	Boulton [419]
904	Keratinocytes	Proliferation	0.25 - 4		Steinlechner [4]

Table 11.3 Examples of different dose levels in vitro

11.2.1 Polarisation effects

One important property of light is the polarisation. Any kind of light can be made polarised, simply by letting it pass a polarisation filter. It is easy to show that when non-coherent polarised light is penetrating and being scattered in tissue, the degree of polarisation rapidly decreases as a function of penetration depth.
(In a non scattering medium the polarisation can be kept high, see chapter 11.2.1.3 “Cell cultures and tissue have different optical properties” on page 358) When tissue (or any scattering medium) is illuminated with coherent light, polarised light will occur due to the formation of laser speckles (se below). This is independent of whether the incident light is polarised or not. If the incident coherent light (laser light) is polarised, this polarisation is evenly distributed. This degree of polarisation rapidly decreases as a function of penetration depth, but instead, laser speckles are formed by interference and then an uneven polarisation distribution has occured.

It has been documented that ordinary broadband non-coherent polarised light can give biostimulative effects on superficial problems like wounds and ulcers, but not deeper down in tissue. The Bioptron lamp is such a device. This further supports that some of the mechanisms are laser specific. See chapter 11.2.1.2 “Porphyrins and polarised light” on page 357.

11.2.1.1 What characterises the light in a laser speckle

The phenomenon of speckles is a form of optical noise. It was observed long before the laser arrived. As early as 1877 Exner [741] reported granulations in filtered mercury lamp light. When the first lasers came, the speckles became not only much more noticed but also a problem. Dennis Gabor, the "father of holography", published an article in 1970 with the title: "Laser

Speckle and Its Elimination" in which he described different ways to get rid of speckles.

Speckles can be real or virtual. The three-dimensional structure of real speckles is manifest not only in an apple but also in a patient's tissue during irradiation with laser light. It arises as a result of interference between different beams with a random direction, amplitude and phase. In laser speckles, (See Figure 1.5 “Laser speckles” on page 14.), which have a higher intensity than the surrounding environment, the light is linearly polarised, or partially polarised (a mathematical description of partially polarised light is found in reference [850]), because the higher intensity has come about as a result of constructive interference, which occurs only if the interfering waves have the same polarisation. In this way, islands of polarised light appear in the tissue with an average size of a few tenths of a millimetre, that is, generally larger than the cells they surround. Interestingly enough, these islands occur regardless of whether the irradiating laser emits polarised or unpolarised light.

Literature

Hode [743, 745] investigated 1972-1973 the possibility of using the inherent information in speckle fields to observe surface movements and deformation in real time.

Horvath [223] has actually measured the light distribution in tissue when illuminated with coherent and incoherent light. He used a small detector and could verify that there is a three-dimensional speckle structure of the light in the tissue if illuminated by laser light but not by incoherent light. This proves that the laser light, after penetration of tissue, is spatially coherent.

Literature: [744, 746]

11.2.1.2 Porphyrins and polarised light

If polarisation is important, can we not simply use polarised normal light? The answer is yes and no. If we illuminate cell cultures, the polarisation remains unchanged throughout the thin layer of cells. However, in the case of a highly scattering medium, such as living tissue, the polarisation is lost after a penetration of a millimetre or so. Therefore, if we polarise light from a pocket torch and use it to irradiate skin, the polarisation will disappear before it encounters the deeper-lying tissues. However, we could use polarised normal (broadband incoherent) light to treat open wounds and improve healing [272] (if we filter off all wavelengths shorter than 600 nm, since these have a negative effect on cells already stimulated). The light would directly encounter the cells in the wound, where there is no overlying skin to reduce or eliminate the polarisation. The positive effect of polarisation has been shown by Bolton [1284]. When macrophages were irradiated by visible 95% polarised light the fibroblast proliferation was greater than when irradiated with 14% polarised light.

Now, accepting that laser light gives rise to areas of polarised light in tissue (as earlier described), we might also ask the question: what is there in the body/tissue/cells that reacts to the light's polarisation? Are there polarisationsensitive elements?

Yes, there are. It is known that matrix-fixed chromophore molecules (e.g. the body's porphyrins) possess absorption dipoles and both absorb and emit (e.g. through fluorescence) linearly polarised light [20] of a determined polarity. Porphyrins are just one of the elements in the mitochondria's respiratory chain and are the molecules chiefly responsible for the absorption of blue and red light. The polarisation in the speckles created by laser light is significant here, and this could explain why the studies mentioned above showed different results with lasers and incoherent light sources. Some persons have the opinion that the respiratory chain is at the base of all effects that laser therapy might have. However, there are effects of e.g. HeNe laser irradiation on red blood cells in which there are no mitochondria [987]. As the cytochrome system in the mitochondria is influenced by photons [1312], it is logical to assume that other chromophores in the cell could be influenced by photons as well.

The conditions we have described above are in no way a complete list. We simply want to show, by looking at the physical conditions in more depth, that it is inappropriate (in the way chosen by some authors) to try to prove that laser therapy cannot work. It is reminiscent of the well-known proof, as deduced by mathematicians and physicists, that a bumblebee cannot fly because its wings are too small in relation to its body weight.

11.2.1.3 Cell cultures and tissue have different optical properties

In a number of studies in which the biological effects of monochromatic light from various sources have been compared, the following has been found:

1 In cell cultures (low scattering medium) laser light gives almost the same effect [33] as incoherent light (i.e. colour-filtered light from a light bulb or an LED of the same wavelength).

2 When tissue (highly scattering medium) is irradiated, laser light gives a stronger effect than incoherent light in all the studies so far conducted. In some of the studies, certain effects were also achieved with LEDs, but the studied LED-light-influenced phenomena were not as clear as with laser light of the same wavelength and the same dose. It should be noted that in four of these studies, adverse effects from LED light were seen [332, 493, 659, 825].

3 In a comparison between light of different kinds, cell cultures with T- and B-lymphcytes were irradiated with (a) polarised HeNe-laser light, (b) polarised narrow band incoherent light and (c) non-polarised narrow band incoherent light – all three with the same wavelength and dose. If the effect of the laser light (a) is set to 100%, the polarised narrow band

incoherent light (b) gave 81% and the non-polarised narrow band incoherent light gave less than 1% effect [23].

4 Even polarised broadband light [272, 1284] has a clear effect on cell cultures and also affects open wounds and certain skin problems; however, it is not as effective as polarised laser light.

5 Under certain power density conditions, non-polarised broadband light can also have an influence on cells in cultures [252, 374]. Biostimulative effects have been noticed after treatment with so-called IPL devices (Intensive Pulsed Light, originally used for hair removal) with a spectrum limited to 600-1200 nm, power densities of 20-50 J/cm^2 and peak power density in the order of 1 kW/cm^2. This can indicate that multi photon actions may be of importance. (See chapter 11.2.6 “Multi-photon effects” on page 365.)

Karu [33] has demonstrated stimulating biological effects in cell cultures from monochromatic incoherent light. However, she has also shown that cell cultures which are first irradiated with laser light, and have consequently exhibited biological effects, and which are then irradiated with broadband (that is, non-monochromatic and incoherent) and laser light simultaneously, subsequently have their laser-produced biological effects reduced to almost nothing [22]. This indicates that there are more mechanisms at work here than simply the excitation of polarisation-sensitive chromophores.

It is important to understand the purely optical difference between irradiating tissue, which spreads light very diffusely, and a thin transparent cell layer in culture. If a thin layer of cells in culture is irradiated with polarised light, the polarisation is maintained right through the whole layer. This means that the cells are entirely surrounded by polarised light. Mester has shown that leukocytes in culture are affected by both polarised laser light and polarised incoherent light, but not by unpolarised incoherent light [23]. Our opinion is that many of the phenomena that hundreds of research scientists around the world have been able to establish as a result of laser irradiation of cells and tissue, are laser-specific in vivo, and that this is due to the coherence and/or narrow-band nature of the light, but not always laser specific in vitro.

11.2.2 The effect of heat development in the tissue

It has occasionally been asserted that the "possible" effects of laser therapy are due to the laser heating the tissue, and that one could just as well use a blanket, hot shower or a heat-lamp. Heat can of course be valuable many times, but in this context we have to look a bit closer into the matter.

11.2.2.1 Macroscopic heating

A heat-lamp has an output of 50-100 watts, while a therapeutic laser often has an output of 5-100 milliwatts (one milliwatt is a thousandth of a watt), that is, thousands of times weaker. All light that is absorbed by tissue is converted to heat, but it is not the heat itself that is of importance here. A blanket, hot shower or a heat lamp causes macroscopic heating of the skin and tissue - a rather even and smooth temperature distribution. Therapeutic lasers cause no perceptible heating, which heat-lamps obviously do and still there is a clear biostimulative effect, also on chilled tissue. So if it were just a question of heating the tissue, heat-lamps would give just as good or even better therapeutic results! It is true that a GaAlAs laser in the >100 mW range can cause sensations of heat on sensitive areas such as the lip, and on pigmented areas, but more than 95% of the laser therapy described in the literature is performed with lasers in the <100 mW range.

11.2.2.2 The microscopic heat effect

However, the uneven, speckled light distribution in tissue causes local temperature differences. These have been calculated by Horvath [223]. Such temperature differences lead to local gradients in certain concentrations of substances, which in turn bring about transport of materials in the tissue in the manner described by Fink's equations. In other words, when tissue is irradiated with laser light, a microcirculation will be initiated, which is not the case during irradiation with non-coherent light sources, such as LEDs for example. Spanner [224] has shown that a temperature difference across a cell membrane of 0.01 °C causes a difference in pressure of 1.32 atmospheres, and this can mean that the distribution pattern of Na^+ and K^+ can be considerably influenced [225]. The local transient rise in temperature of absorbing biomolecules may cause structural (e.g., conformational) changes and trigger biochemical activity (cellular signaling or secondary dark reactions). [1373], [1374]

11.2.3 Mechanical forces

A very interesting experiment has been performed by Rubinov [1417]. He brings about a new approach to the understanding of biological activity caused by low-intensity laser radiation, in which coherence is a factor of paramount importance. This is based on the dipole interaction of gradient laser fields with cells, organelles and membranes. The laser intensity gradients in an object arise due to the interference of the light scattered by the tissue with the incident light beam (speckle formation). Apart from speckles, different types of light spatial modulation can be created deliberately, using different schemes for beam interference. It is shown that gradient laser fields may cause spatial modulation of the concentration of particles and increase their “partial temperature”. Rubinov presents the results of experimental observation of trapping of different types of particles, including human lymphocytes,

in the interference fields of the HeNe laser. The sweep-net effect on particles of different sizes when moving the laser field is demonstrated and crystal-like self-organisation of particles in the laser gradient field is observed. The influence of gradient laser fields on erythrocyte rouleaus, on the apoptosis of human lymphocytes as well as on their chromosome aberrations is demonstrated.

It may be concluded from the experimental studies that the influence of an interference laser field with a correctly chosen period can stimulate the repair system of a cell, increasing its viability.

Rubinov concludes further: *"Illumination of biological tissue by coherent laser light unavoidably leads to strong intensity gradients of the radiation in the tissue due to speckle formation. This causes the appearance of inter- and intracellular gradient forces whose action may significantly influence the paths and speeds of biological processes. In contrast to the photochemical action of light, which is accompanied by absorption of quanta and has a specific character (i.e. is characterized by a specific spectrum of action), the action of the gradient field is of non-resonant type. It is not accompanied by photon absorption and has a universal character, i.e. it depends weakly on the radiation wavelength, but requires a high degree of coherence. The use of different schemes of interference allows us to obtain different configurations and periods of spatial modulation of the laser light intensity. The application of such interference fields opens new possibilities for controlling fundamental biological processes and may lead to new technologies in laser therapy."*

No doubt that these effects of gradients are very essential. However, they can not be the only important effects occurring in laser illuminated tissue. Also photon absorption and other photon energy specific effects occur, see e.g. the reference [1279] below, but the effects of field gradients are laser specific and can also be (at least part of) the explanation of why so many different laser types (wavelengths) give similar biologic effects. The more we learn about this, the more we realize that the mechanisms behind laser biostimulation are very complex and the authors of this book doubt that we will ever know all details. Why are for instance about 10% of humans and animals resistant to laser therapy?

11.2.4 Excitation effects

The most obvious photochemical and photo-biological effects are due to excitation of photon absorbing molecules. In this field, a lot of work has been done by professor Tiina Karu. In the following section, we have, with her permission used some of her material and ideas.

Literature

The stimulation of cellular ATP production has been suggested as one of the most important effects of laser therapy. In a study by Mochizuki [1279] the effect of 830 nm laser irradiation on the energy metabolism of the rat brain

was observed. A diode laser was applied for 15 min with an irradiance of 4.8 W/cm^2. Tissue adenosine triphosphate (ATP) content of the irradiated area in the cerebral cortex was 19% higher than that of the non-treated area, whereas the adenosine diphosphate (ADP) content showed no significant difference. Laser irradiation at another wavelength (652 nm) had no effect on either ATP or ADP contents. The temperature of the tissue was increased by 4.4 - 4.7 °C during the irradiation of both wavelengths. These results suggest that the increase in tissue ATP content did not result from the thermal effect, but from a specific effect of the laser operated at the 830 nm wavelength.

Further literature: [398, 460, 497]

11.2.4.1 Primary reactions due to excitation

There are several such possible primary reactions. When a photon is absorbed, it can transfer its energy to an electron. If the photon energy is high enough, it can change the energy state of the electron, e.g. from level S_0 to S_1. Also triplet states can be involved. It has also been shown that excitation can occur by means of multiple photon action.

The mechanisms that have been proposed are:

1 Changes in redox properties and acceleration of electron transfer. ("Redox properties alteration hypothesis" [1349]. Photo-excitation of certain chromophores in the cytochrome-*c*-oxidase molecule (like Cu_A and Cu_B or hemes *a* and a_3 [1351] influences the redox state of these centers and, consequently, the rate of electron flow in the molecule. [1349]

2 NO release from catalytic center of cytochrome *c* oxidase. ("NO hypothesis") [1369]. It is thought that laser irradiation and activation of electron flow in the molecule of cytochrome-*c*-oxidase could reverse the partial inhibition of the catalytic center by NO and in this way increase the O_2-binding and respiration rate.

3 Superoxide generation. ("Superoxide anion hypothesis"). It has been suggested [1411] that activation of the respiratory chain by irradiation would also increase production of superoxide anions and that the production of $^{-}O_2$ primarily depends on the metabolic state of the mitochondria. [1412]

4 Photodynamic action. ("Singlet oxygen hypothesis") [1367]. Certain photo-absorbing molecules like porphyrins and flavoproteins (some respiratory-chain components belong to these classes of compounds) can be reversibly converted to photosensitisers.

5 Changes in biochemical activity induced by local transient heating of chromophores. ("Transient local heating hypothesis") [1374]. When electronic states are excited with light, a noticeable fraction of the

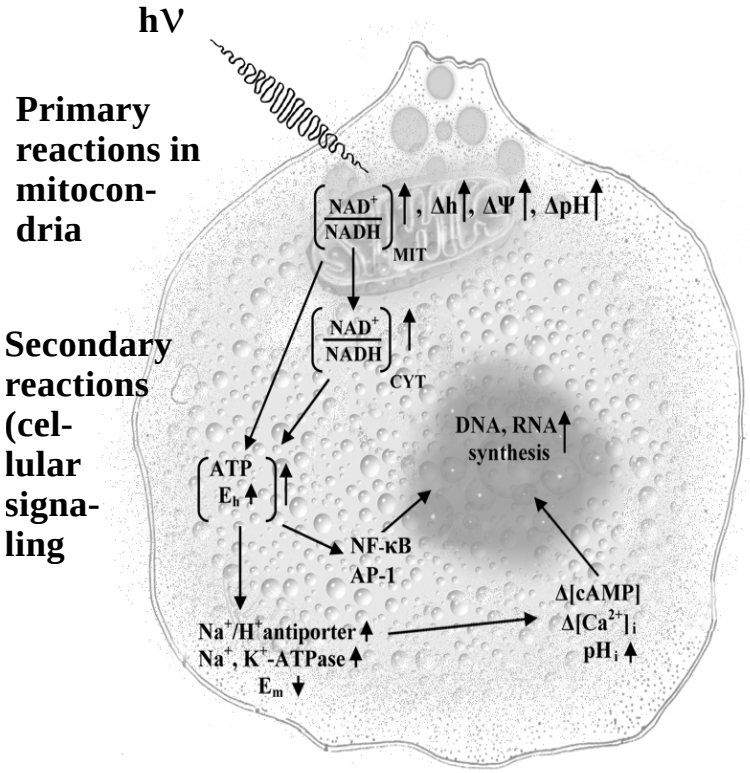
excitation energy is inevitably converted to heat, which causes a local transient increase in the temperature of absorbing chromophores.

The first two processes are of Redox type and the next two give rise to reactive oxygen species (ROS). The belief that only one of these reactions occur when a cell is irradiated and excited electronic states are produced is groundless. Rather, it is likely that more or less all of them take place. The question is, which mechanism is decisive?

Also; it is quite possible that all the mechanisms mentioned above lead to a similar result - a modulation of the redox state of the mitochondria (a shift in the direction of greater oxidation). However, depending on the light dose and intensity used, some of these mechanisms can prevail significantly. Experiments with *E. coli* provided evidence that, at different laser-light doses, different mechanisms were responsible - a photochemical one at low doses and a thermal one at higher doses. [1413]

11.2.4.2 Secondary reactions due to cell signalling

After the primary reactions in the mitochondria, a scheme of cellular signalling cascades (secondary reactions) occur in a mammalian cell. In the figure below, from Tiina Karu, $E_h \uparrow$ means a shift of the cellular redox potential to more oxidized direction. Further, the arrows ↑ and ↓ indicate increase or decrease of the respective values, brackets [ ] indicate the intracellular concentration of the respective chemicals.



Cytochrome-c-oxidase and flavoproteins like NADH in the respiratory chain in the mithocondria can act as photoreceptors. This can cause a short time activation of the respiratory chain and oxidation of NADH pool leading to changes in the redox state of both mitochondria and cytoplasm, changes in pmf (proton motive force), $\Delta\Psi$, Δph and leading to extra synthesis of ATP. Also a rise of Na^+/H^+ antiporter in the cytoplasmic membrane which causes a short term increase in pH_i and causing a change in the redox state of the cytoplasm, i.e. the redox state in the whole cell.

Figure 11.7 This figure is from Karu 1988. The process as described is of course simplified, but all steps are verified through experimental work.

These processes have been further verified. Yaou Zhang et al. [1416] used the cDNA microarray technique to investigate the gene expression profiles of human fibroblasts irradiated by low-intensity red light. Proliferation assays showed that the fibroblast HS27 cells responded differently to different doses of low-intensity red light irradiation at a wavelength of 628 nm. An optimal dose of 0.88 J per cm^2 was chosen for subsequent cDNA microarray experiments. The gene expression profiles revealed that 111 genes were regulated by the red light irradiation and can be grouped into 10 functional categories. Most of these genes directly or indirectly play roles in the enhancement of cell proliferation and the suppression of apoptosis (see also Rubinov, chapter 11.2.3 “Mechanical forces” on page 360). Two signaling pathways, the p38 mitogen-activated protein kinase signaling pathway and the platelet-derived growth factor signaling pathway, were found to be involved in cell growth induced by irradiation of low-intensity red light. Several genes related to antioxidation and mitochondria energy metabolism were also found to express differentially upon irradiation. This study provides insight into the molecular mechanisms associated with the beneficial effects

of red light irradiation in e.g. the acceleration of wound healing by laser therapy.

11.2.5 Fluorescence - luminescence

Most people have at some time seen fluorescence. In discotheques, for instance, a UV-lamp is often set up to illuminate the guests. When the ultra-violet light hits our white shirt or our teeth, the invisible ultra-violet light is converted to visible light by means of fluorescence. Nice, bright porcelain crowns could suddenly "disappear", to the dismay of the owners. Modern ceramics have now adjusted to this embarrassing situation. In the old type of alarm clock, light energy from the bed lamp excited the phosphor molecules and the energy from the light was then stored for minutes or more and slowly emitted in the form of greenish light. This long-term storage is called phosphorescence.

Many animals have the ability to convert chemical energy into light energy - a process called luminescence. From this it is clear that light can change not only chemical processes in our bodies, but also that our cells can create light by a variety of different processes. It is also known that cells can communicate with each other by means of emitted and absorbed photons [753, 852].

Allan [853] discovered in 1972 that polymorphonuclear leukocytes emit photons during phagocytosis with a spectral maximum close to 600 nm. Nelson [854] found in 1976 that macrophages also emit light. Rosen [855] found experimentally that singlet oxygen is involved in the process; and Andersen [856] compared the emission spectrum from singlet oxygen with the spectrum from polymorphonuclear leukocytes in 1977 and found that they were more or less identical.

Part of the mechanisms of biostimulation can include fluorescence, phosphorescence or luminescence as possible means of communication between cells in different places.

Further literature: [448, 1000, 1001]

11.2.6 Multi-photon effects

Multi-photon effects are today more and more used in PDT, see chapter 11.6 “Photodynamic Therapy - PDT” on page 374 and in two-photon laser scanning microscopy by using a Ti:sapphire laser (superpulsed like the GaAs laser).

One possible explanation of the efficiency of the GaAs laser is action through multi-photon effects. The photon energy of the HeNe laser is 50% higher than the photon energy of the GaAs laser. However, in the extreme pulses of the GaAs laser, the photon density is very high, which is necessary for multi-photon action.

Literature

Shear [859] studied non-linear excitation of the neurotransmitter serotonin by means of Ti:sapphire laser light pulses. The results indicate that serotonin is photochemically transformed as a consequence of four-photon absorption to a photoproduct that then emits in the visible region via two-photon excitation. A minimum bound for the two-photon emission action cross-section was observed at 830 nm.

Maiti [860] measured serotonin distribution in live cells with three-photon excitation. Three-dimensionally resolved images were made along with measurements of the serotonin concentration from about 50 mM and up.

These investigations and many others, show that serotonin works as an optical target, especially for high photon density light pulses. With laser light (coherent) in particular, it is possible to achieve extra high intensity peaks due to interference.

11.2.7 Lasing effects in tissue

According to what is known as "Kendrick Smith's First Law of Photo Chemistry", light must be absorbed before photochemistry can occur. This is true if we are talking about energy transfer. But this does not mean that light cannot influence matter/tissue unless it is absorbed, since light can act as a catalyst. The existence of the laser is a proof of that. Lasing occurs when an excited atom is stimulated to emit a photon before emission occurs spontaneously. This takes place when a photon of the right energy (wavelength) enters the electromagnetic field of the excited atom: the incident photon triggers the electron energy shift and the energy difference is converted into electro-magnetic radiation and released by means of an identical second photon.
And **nota bene:** the trigger photon is not absorbed.

For a laser to work - i.e. to emit more photons of a certain energy than comes in, we must have an inverted population. In normal matter this is never the case. Living matter, however, has a very complex structure with a multitude of continuously ongoing chemical reactions where any conceivable level of energy is found. There are constantly excited molecules at every level of energy and it is not too unlikely for them to be triggered by incoming photons to release energy in the form of a photon while the triggering photon continues as it does in a laser. Hence, it seems possible that stimulated emission (laser action) can take place in tissue and that tissue itself can act more or less like a dye-laser.

There are currently thousands of different types of laser, and these produce light, UV or IR radiation of various wavelengths. Though a laser usually has one characteristic wavelength, it is sometimes possible to choose within a range of wavelengths. There are tuneable lasers where the wavelength can be changed, even during operation. Dye-lasers have a lasing medium that can be liquid and has a broadband amplification profile. Rhodamin, for example, has an amplification profile between 560-650 nm

which covers both the wavelength of the HeNe laser and the InGaAlP lasers. Rhodamin is similar to porphyrin in optical property - it can easily cause fluorescence.

So, it is not unlikely that excited molecules in the tissue act like the medium in a dye laser and that this is triggered by the laser therapy. This could perhaps be part of the explanation of the rather deep effects of laser therapy. In particular, the CO_2-laser biostimulatory effect is very difficult to explain as the light is absorbed so superficially and also has such low photon energy. The wide-band action of tissue working as a dye laser can also make it easier to understand why biostimulation occurs for so many different wavelengths. Wide-band radiation, such as that from the sun, exhausts a lot of excited levels, thus inhibiting the effect of the laser therapy. Such sunlight induced extinction will only occur at depths that can be reached by that light. Secondary effects caused by laser treatment cannot be extinguished by sunlight or light from other sources.

11.2.8 Non linear optical effects

Theoretically, it is possible that non-linear optical effects can also occur in tissue. An example of a non-linear optical effect is the KTP (Kalium Titanyl Phosphate) crystal in the KTP laser (frequency-doubled Nd:YAG with 532 nm wavelength) which causes harmonic overtones, of which the first is used (532 nm is half the wavelength of the pumping light, in this case, Nd:YAG laser light - 1064 nm).

In order to achieve non-linear effects, high power density is needed. How high depends on the matter in question. We regard it as theoretically possible, but unlikely, that such phenomenon will take place in tissue. But if so, it is most likely to occur if lasers with high peak power, such as the GaAs laser is used, further intensified in bright speckle points due to interference.

11.2.9 Opto-acoustic waves

If intense light pulses are absorbed, acoustic waves may occur. In chapter 7 “Veterinary use” on page 283, we have noted that horses can "feel" GaAs laser light. It is possible that this is due to opto-acoustic waves.

11.3 Secondary mechanisms

11.3.1 Effects on pain

Pain is very complex in its nature. Since we are not specialists in this field, we have chosen to show that laser therapy influences many of the transmitter signal substances that we know are involved, such as endorphines, nitric oxide, bradykinine, serotonine etc, and also direct effects on nerves, e.g. C-fibres.

Literature

Mizokami [247] has studied the change of serotonin in plasma in 63 patients with chronic pain. He used a GaAlAs laser, 830 nm, 60 mW output power. Patients achieving good pain relief from laser therapy were selected. On the first day of therapy, the change rate of plasma serotonin had a stable tendency to give a positive ratio. The treatment was applied every other day, resulting in a negative ratio from the tenth day of treatment.

Serotonin production was found to be enhanced when rat brains were irradiated with HeNe laser. In this study by Rossetti [502], the rats were exposed to stress. In both irradiated and control animals the enzymes aspartate transferase (AST), both cytosolic and mitochondrial, glutamate dehydrogenase (GIDH), and total superoxide dismutase (SOD) were monitored spectrophotometrically. In the brain of the irradiated rats there was a marked increase of total SOD, together with an appreciable decrease of cytosolic AST, and insignificant variations in mitochondrial AST and GIDH. In rats exposed to stress alone, the SOD decreased and the cystolic AST increased.

Montesinos [40] has shown that laser light affects the production of endorphins.

Honmura [188], by blocking opiates with naloxone, has been able to demonstrate that the pain-relieving effects of laser therapy do not depend solely on endorphins.

In a study on rabbits, Labajos [499] found an increase of ß-endorphine levels when a pain stimulus was given simultaneously with GaAs or HeNe laser.

Wakabayashi [190] has demonstrated that GaAlAs laser has a suppressive effect on injured tissue by blocking the depolarisation of C-fibre afferents.

Vizi [246] has demonstrated that ruby laser can enhance the release of acetylcholine.

A study by Mrowiec [432] indicates that nitric oxide is involved in the mechanism of laser therapy-induced analgesia. The analgesic effect of GaAs laser light in rats was prevented by an injection of I-NAME, an inhibitor of nitric oxide syntheses.

As mentioned earlier, Lubart [29] has demonstrated that singlet oxygen is produced in cells irradiated by HeNe laser. Singlet oxygen, in small amounts, is very important in biochemical processes and may be important in biostimulation. It is proposed that singlet oxygen is photo-produced by the natural porphyrins in the cells.

According to Lubart, only red (632 nm) and green (540 nm) light has an effect on the Compound Action Potential of the nerve. 904 nm laser light failed to produce this effect, supporting the theory that light with a wavelength in this region activates enzymes in the cell membranes while porphyrins act as photoreceptors in the visible range of the spectrum.

Friedman [375] reports that non-linearity in photo-biostimulation is a process where linear optical absorptions produce active chemicals such as cytoplasmatic H^+ and Ca^{2+}. These chemicals participate in chemical reactions, the rates of which depend on non-linearity of the concentration of these photoproduced chemicals, thus allowing very sensitive light control of non-linear biological reactions. Important contributions to neural excitability and growth include photostimulation of ATP production, which fuels the action potential and fills the synaptic ATP vesicles. ATP plays an important role as an extracellular neurotransmitter. With non-linear process, we do not mean non-linear optical effects as described in, (See chapter 11.2.8 "Non linear optical effects" on page 367.)

In another study [377], the same author suggests a mechanism of stimulation of damaged cell cultures: Laser irradiation is assumed to intensify the formation of a trans-membrane electrochemical proton gradient in mitochondria. This enhances ATP production, which activates the Ca^{2+} pumps, depleting the Ca^{2+} concentration in the cytoplasm and increasing the Ca^{2+} concentration gradient of the surrounding medium relative to the cytoplasm. This triggers enhanced Ca^{2+} influx into the cells via the Ca^{2+} ion channels of the cell membrane. In addition, with sufficient irradiation, the proton-motive force (pmf), due to the proton gradient, causes more Ca^{2+} to be released from the mitochondria by an "antiport" process. The additional calcium transported into the cytoplasm, together with other factors controlled by the pmf triggers mitosis and enhances cell proliferation. At higher doses, too much Ca^{2+} is released. This causes hyperactivity of Ca^{2+} ATP-ase and exhausts the ATP reserves in the cell.

Brill [999] suggests the system of guanilate cyclase - cyclic guanosin monophosphate - NO-synthase as a primary photoacceptor.

Further literature: [1014]

11.3.2 Effects on blood circulation

Thermographic studies have shown that laser therapy can indirectly cause a higher temperature in the tissue, which is due primarily to an increased blood flow [170, 189]. In a number of studies [109, 190, 221], this rise in temperature has been measured in tissue irradiated with laser light, with the result that the temperature can rise by an average of 0.9 degrees over the area, and up to four degrees at certain points [14].

Literature

Miro [301] has measured the effect of laser therapy on blood circulation in the nail bed and the mesenteric capillary flow. The increased blood flow continued for 20 minutes after the cessation of the laser treatment, even when the tissue was cooled.

Meada [460] measured the thermic changes after laser therapy using thermographic and thermometric methods. Excised skin in rats showed an increase of 0.4 °C, which remained constant during 30 minutes and was

noted equally on both the irradiated side and on the unirradiated contra lateral dorsum. There was no change in histology.

Haina [497] reports a maximum temperature rise of 1 °C in human skin after HeNe irradiation of a density of 600 mW/cm², spot size 2 mm.

Sato [398] measured an increase in regional body temperature of 0.7 °C in a group of patients receiving active laser treatment for postherpetic neuralgia. Interestingly enough, there was no increase of temperature in the placebo group.

11.3.3 Stimulatory and regulatory mechanisms

In 1981 Mester [34] presented an article in which he summarised the research his group in Budapest had published: "The following model is proposed in order to explain the stimulating effects of polarised light (100% from a laser, 75% from a thermal light source): the electrical field intensity from the linearly polarised light changes the conformity of the double lipid layer in the cell membrane by means of electron polarisation of the lipids' electrical dipoles. One of the consequences of this is a change in the distribution of charge on the surface of the cell membrane, which can lead to changes in the lipid-protein bonds. Because the membrane acts as a biological amplifier, changes in the cell membrane affect every process associated with the cell membrane: the cell's energy production, its immunological processes, enzyme reactions, transport factors, etc."

11.3.4 Effects on the immune system

In the same article by Mester, the effects on the immune system were presented. In a study of the changes of the immune defence components by means of measurements before and after laser treatment, e.g. the alpha-I-lipoprotein content increased by 120%. The effect of laser therapy on macrophages is an indication of this claim [291].

Literature

Dima [372] compared the activation of macrophages using HeNe laser, interferon and corynebacterium parvum. All three methods activated an intense phagocytic activity of the macrophages.

Yamaya [458] found that 904 and 830 nm laser enabled a rapid activation of the superoxide system, NADPH-oxidase.

Stadler [1031] irradiated human whole blood with 660 nm laser by using fluences between 0.1 and 5 J/cm². The lymphocytes were isolated after irradiation of the whole blood. As a control experiment, lymphocytes were first isolated and then irradiated with the same fluences. Lymphocyte proliferation was significantly higher in samples irradiated in the presence of whole blood compared with lymphocytes irradiated after isolation from whole blood. Free radical and lipid peroxide production also increased significantly when samples were irradiated in the presence of red blood cells.

Thus, the reaction of light with haemoglobin seems to be one of the keys in biostimulation.

Further literature: [1, 2, 214, 262, 273, 373]

11.3.5 Other interesting possibilities

An interesting hypothesis about the effect of laser therapy has been put forward by Reznikov and Pavlova, based on three separate studies [728, 729, 730]. Reznikov [857] gives the following description:

"Laser light could be considered as a trigger of an adaptive reaction because during evolution, this kind of "irritator" (stresser) is unusual, uncommon. There is not any physical agent besides laser light which we never experienced evolutionary - radiation, gravity, temperature, light, pressure, CO_2 or O_2 saturation, etc. Because of multiple exposures to changes of those conditions (effectors) during the evolution, listed effectors may induce stress only if they are approaching to damage (hazardous) range. The real therapeutic effect related to stress/adaptation, however, may be expected only if the effector does not add its own hazardous effect. Among all known cases it is possible only if laser light is applied at the low dose. Why?

Unlike other physical factors, the light with narrow spectral band is absolutely unusual for our nature. There is no situation where bio-organisms on Earth could be exposed to this kind of light and develop unresponsiveness to that during evolution. Because of that, we believe that the most important for laser effects is not the wavelength but the monochromatic nature, especially with narrow spectral band.

If we will excuse ourselves from the discussion on differences in light absorption and energy of photons (the important aspect of optimisation in laser therapy) and will focus on the induction of adaptive reaction: the more "odd" light we are exposed to, the more response we can expect. If the light's bandwidth increases, the effector loses power to induce adaptive reaction (even if this light still is considered monochromatic, at some degree, it may be not so unique evolutionary as the light with less band-width).

In other words, extreme monochromatic light (such as laser light) exposure was not experienced evolutionary. As such, it is a stimulus that is unique to the experience of the organism. The more unique the light, the more the organism is required to resort to adaptive mechanisms, to resolve the stimulus. For this reason, laser light is considered a unique adaptogen, generating adaptive reactions. The less band-width of the light, the more unique the light is to the experience of organism resulting in greater efficiency of laser therapy."

In the book "The science of low-power laser", Karu pinpoints some typical features of laser therapy, based on extensive in vitro studies:

* Patient response to laser therapy depends upon their physiological status;
* Laser therapy effects are truly dosage and wavelength dependent;

* Biological responses may be maximised within certain "action spectra". A number of action spectra may apply for a particular biological response, and the response may be maximised at a specific wavelength within each action spectrum.
* The biological responses of cells to pulsed laser therapy can be different (but not necessarily better or worse) from responses to continuous wave, and there is a strong dependence on pulse repetition rate, pulse duration and duty cycle, as well as dosage and wavelength.
* Visible and infrared light affect biomodulation in different ways - visible light elicits photochemical changes, whilst infrared can only produce physical changes. However, the end result may - in some cases - be the same.
* The biological response to laser stimulation can be significantly different according to the sequence in which different wavelengths are applied, and even non-existent if two or more wavelengths are used simultaneously.

Further literature:
The stimulation of ATP synthesis [140, 149, 241, 257, 378, 590, 946, 1258]
DNA replication [21, 22, 33, 75, 121, 181, 220, 249, 251, 510, 587, 675, 754, 947, 948, 950].

11.4 Summary of mechanisms

In an effort to summarise the mechanisms to some degree, in the manner we believe we understand them, and in accordance with other researchers we have contacted, we have created the block diagram shown below. There is, of course, a lot more published about the details in the different stages of the diagram, but including all these beyond the scope of this book.

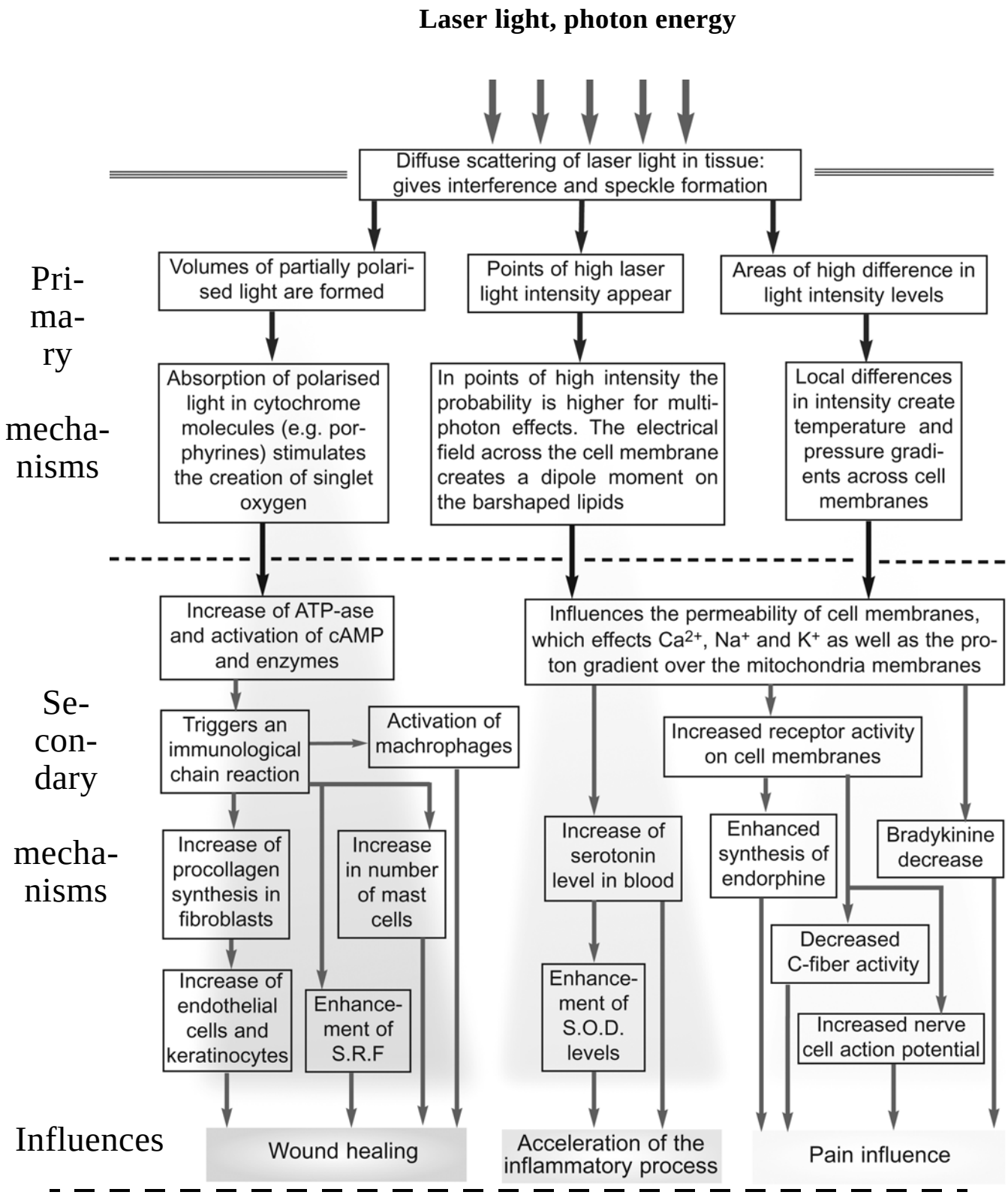


Figure 11.8 The mechanism of laser therapy