LASER APPLICATIONS TO BIOLOGY AND MEDICINE[1]

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Summary The unique physical characteristics of coherence, intensity, and monochromatically offered by laser instrumentation has placed renewed importance on studies in photochemistry and photobiology. Sufficient research experience has now been accumulated to demonstrate the potential usefulness of laser energy to fundamental cell biology, and to the diagnosis and treatment of certain pathological conditions. Current progress in laser applications to ophthalmology, oncology, and dentistry is briefly summarized.

Introduction The laser, as a concept, offers a relatively new experimental approach to biology and medicine, although approximately five years of research has been accomplished, providing certain basic knowledge regarding its action at the cellular and molecular levels. Some practical applications of the laser in medicine have also emerged within this period. It is not within the scope of this report to give a comprehensive review of all of the attempted or anticipated applications of laser energy to biology and medicine, but this brief review will be limited to representative areas in which the laser appears to have achieved some degree of success in its application.

Physical Characteristics of Laser Energy The laser can be considered as a different type of light source. The photons which emanate from this source, as quantum units, are not different from photons from other light sources. However, the emanation of the photons from a laser device collectively show different physical characteristics. Some of these properties include: coherence, monochromaticity, and intensity.

Unlike the photons emerging from non-coherent sources which show different wave forms and different wavelengths (resulting in a scattering of the photons with an associated loss of intensity with the square of the distance), the photons emerging from the laser source are coherent. This permits the maintenance of a beam of relatively

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constant diameter without the benefit of collimating lenses. This has not been conclusively demonstrated to produce a biological effect in itself, but it does permit the ease of alignment and the focusing of the beam to a tiny target diamter. This will be discussed in greater detail in a later section.

The energy from most laser sources is monochromatic. While wavelength-dependent biological activities have been studied with noncoherent light sources used in conjunction with very narrow band filters, this instrumentation cannot compare with the monochromaticity and the intensity provided by laser sources. As a result, much more refined and critical work describing photochemical and photobiological effects can be seen with these newer monochromatic coherent sources.

The biological activity from radiant energy has previously been confined to the X-ray and ultraviolet regions of the electromagnetic spectrum. However, laser sources show such a high degree of intensity that now biological activity can be seen in the visible and near infrared region of the spectrum. The laser can produce a number of physical effects through their interaction with molecules and atoms within a biological specimen. The most common effects which are derived through this intense light source include a marked thermal elevation, the generation of acoustic waves or supersonic shock waves, free radical formation, and the production of high electric field strengths. In addition, the characteristic of intensity can produce a much greater probability of a two-photon interaction occurring within a molecular species than with ordinary light sources.

The duration of the photon train, which is commonly referred to as the pulse-width, varies with the design of the instrument. The first lasers which were made to operate were of a solid state type which contained either chromium atoms within a synthetic ruby rod, or neodymium atoms which were used to dope glass rods. The pulse-width for these lasers were in the 1 to 5 msec range. A number of devices have been utilized to spoil the quality of the laser emission and these “Q-spoiled” or Q-switched lasers can be made to emit their power within a pulse-width of 10 to 50 nsec. A third type of laser, which is, generally associated with gaseous components as a lasing material, emit their power as a continuous wave form so that the pulse-width, under these circumstances, is relatively long.

Specificity of Laser Action The radiant energy from lasers interacting with atoms or molecuTe-s of biological materials could theoretically be expected to show similarities in effect with those produced with noncoherent light sources if one takes into consideration the wavelength, the pulse width, and intensity of the photon flux. Experimentation with relatively low intensity laser sources over the past few years have substantiated some similarities in the photochemical and photobiological responses that have been reported in the pre-laser literature. This includes phototropism of plant seedlings as well as the bleaching of natural chromophores and a variety of dyes. [1]

However, these wavelength-specific effects can be taken to their extreme with the application of laser energy, particularly in the pulsed or Q-switched modes. With the latter system, there appears to be absorption by selective molecular species with the result that the energy is expended on the breaking of molecular bonds rather than the elevation of temperature which can be diffused to adjacent molecules with the resulting thermal coagulation of enzymes and other molecular species. This response was well documented with the application of a wavelength in the green region (5300 A) as applied to rat brain cell suspensions. [2] Under these circumstances, the brain cells were inhibited in their rate of oxygen consumption. Moreover, it was demonstrated spectrophotometrically that the transmission of hydrogen ions along the cytochrome chain was blocked between cytochromes b and c. It was concluded that even though the absorption characteristics of cytochrome b overlapped on those of cytochrome c, the monochromatic intense energy from a Q-switched frequency-doubled neodymium laser was absorbed selectively by cytochrome c without alteration of the function of cytochrome b. This was substantiated with the subsequent application of two wavelengths at 6013 and 6096 A generated by a Raman-shifted ruby laser in the Q-switched mode. Under these circumstances, the orange frequencies were absorbed by cytochromes a and a_3. The irradiated cells showed no morphological change until such time that the inhibited oxidative activity resulted in a loss of energy production and injury and death of the cells.

The coherence characteristic of the laser beam has permitted an ease of beam alignment and focusing, which cannot be achieved with the scattered light from non-coherent sources. A nominal powered ruby laser source, for example , can easily be focused to approximately 1-1/2 to 2μ in target diameter.0 This feature has resulted in the production of the commercial ruby laser microbeam. apparatus for microsurgical manipulation of cells in tissue culture or embryonic preparations. More recently, a pulsed argon ion system has been used in a similar fashion with several advantages. First, the beam can be confined to less than a micron in target diameter (approximately 0.7μ); it is of a wavelength where more chromophores exist within unstained living material, providing a wider application of the beam to cell biology and embryology; the high replication rate of pulses permit the ease of alignment which is provided by continuous wave sources and the opportunity to make linear incisions rather than just perforations; and finally, -a number of common non-toxic vital dyes can be used to photosensitize various organoids within the cell. These include fluorescein and acridine orange.

Applications A number of mysteries still exist with respect to the function of various natural phenomenon in parasitology, embryology, and cell physiology. An effective approach to some ok these questions is through the application of microbeam. irradiation.[4] While many other energy sources have been used for this purpose, laser instrumentation offers particularly effective means of producing microsurgery on parts of single cells.

A particularly satisfactory test object is the specialized mitochondria within beating myocardial cells growing in a monolayer in tissue culture. These structures, called sarcosomes, are hypertrophied and contain an elevated cytochrome content. It is believed that the cytochrome enzymes form the absorbent chromophore within the cell since no other structure shows a specific sensitivity to the focused argon laser power. In contrast, when the beam is focused within the sarcosomes, a small lesion can be seen ranging from a charred spot to an apparent perforation. The response of the injured cell is variable depending upon the relationship of the sarcosome to the nucleus and to the time following laser treatment. If the mitochondrian is adjacent to the nuclear membrane, death often occurs immediately. If the sarcosome is located in the peripheral region of the cell, the beat frequency slows, the cell begins to fibrillate, and death follows after several minutes. Studies of this type can often give much information regarding the importance of mitochondrial metabolism with respect to the beating activity of muscular elements.

Dorsal root ganglion growing in tissue culture demonstrate satellite cells or glial elements which form an enveloping membrane around neuron soma with their cytoplasmic processes. It has been postulated, but not proven, that these satellite cells function as nurse cells by regulating the amount of nutrients or oxygen into the neuron cell body. However, a question of this type might be answered by the selective injury and death of the satellite cell by confining the argon ion laser microbeam to the nucleus of that peripheral element. Such procedures have been demonstrated-to completely denude the neuron of its surrounding cellular envelope.[5] Time-lapse motion pictures following this type of treatment indicated that the neuron remains uninjured for a period of several hours.

The collimated beam of the laser has also been applied in a number of clinical areas. The most successful of these has been in the field of ophthalmology. The Zeiss white light photocoagulator has been demonstrated to be an effective method for treating detached retinas. However, the collimated monochromatic light source in the form of a nominal ruby laser has proven to be more satisfactory in that the lesions can be of a smaller diameter and the pulse-width is normally much shorter. [6,7] As a result, patients treated with laser photocoagulation devices require no anesthetic because they experience no pain during the treatment. Because the laser is monochromatic the energy is absorbed selectively by the pigmented retinal epithelial cells, and where adhesions are formed between the retina and the choroid the nerve fiber layers appear to be uninvolved. In addition to the application of the coagulating of the retinal detachment, the retinoschisis (a longitudinal splitting of the retinal layers) can be walled off before the process also involves the macula. In addition, the microaneurysms of diabetic retinopathy and the neovascular tufts in the retina of diabetics can be destroyed, using a focused laser beam.

This treatment can prevent the subsequent destruction of retinal tissue with glial scar formation, and the production of intractable retinal detachment, thus preserving the patient's vision. A number of diseases of the eye involve edema of the macula; these include central serous retinopathy inflammation of the retina and underlying choroid (chorioretinitis), early senile macular degeneration, and the macular edema which sometimes follows cataract removal. Fluorescein can be applied intravenously to offer excellent retinal angiographic pictures showing leaks of the fluorescein from the capillaries into the edematous areas.[8] While the ruby laser has been demonstrated to be most effective on pigmented epithelium, it is not absorbed efficiently by the blood vessels or nerves. While the sealing of these leaks with the focused ruby laser beam has been effected with dramatic improvement in the visual acuity of such patients, basic knowledge of the wavelength-specific effects are being investigated regarding this application. That is to say, the absorption characteristics of fluorescein within the capillaries absorbs the energy from an argon laser efficiently. As a result, the application of the argon laser for this purpose in preliminary experiments have shown the expected increase in efficiency.

In the area of dermatology, the laser has been applied as an experimental device to decolorize the pigment from tattoos,9 from freckles and epidermal nevi, and from portwine stains.[10] In each of these instances, either the Q-switched or normal modes of the ruby laser have been demonstrated to produce soft white scars rather than the heavily pigmented lesions prior to laser treatment. Extensive studies have been conducted, using transplantable melanomas in mice as well as pigmented cells in tissue culture. The results of these studies have, in some instances, been carried to the human melanomatous patient.[11] While the energy from the ruby or the neodymium lasers is absorbed effectively by the melanin within this pigmented tissue, a number of technical problems have arisen. These include the lack of complete destruction of all of the tumor cells by single impacts of high intensity, and some of the remaining viable cells have been shown to be partially disseminated away from the original locus.[12] These surviving cells appear to grow faster than they were prior to the laser stimulation.[13] However, there remains a promising avenue to utilize laser energy in the treatment of cancer. This involves the combination treatments of ruby laser in conjunction with X-ray[14] or in conjunction with anti-cancer drugs such as cytoxan.[15] While the mechanism of action has not yet been established, it would appear that the ruby laser used in conjunction with ionizing radiation potentiates X-ray treatment of tumors, including non-pigmented tumors, and it also seems to facilitate the treatment of tumors with cytoxan.

An additional potential clinical application of the laser is to the field of dentistry.[16] Current studies are being conducted which suggest that local applications of laser energy can produce a glazing action of the surface of the enamel, which appears to reduce the permeability of the enamel to organic acids. Under these conditions, the irradiation of the tooth can prevent subsurface demineralization which occurs following the incubation of such teeth in a solution of lactic acid in hydroxyethylcellulose at pH 4. 5. Recent experiments, utilizing human dental pulp in tissue culture as well as chimpanzee pulp in vivo, have suggested that superficial treatment of the enamel produce no detectable or permanent damage to the soft pulpal tissue; hence, these studies suggest that the future of the laser in the field of dentistry may be to prevent the carious lesions from forming in those sites where lesions are most frequently found.

Possible Future Applications The laser as a laboratory tool is offering some sophisticated advances in the analysis of various organic and inorganic chemicals. For example, as an extension of standard flame spectrophotometry, the plume coming from the laser-irradiated target can be analyzed to evaluate the kinds of inorganic constituents in the ash.[17] The amount of material being assayed can be extremely small, since the laser can be focused on a very minute target. This permits the evaluation of various regions of a histological specimen, or even the surface of the skin of a patient or an experimental animal without causing undue stress on the test object. In present day tests, this method of evaluation appears to be more sensitive than any existing microchemical analytical system, including the method of neutron activation.[18] The method of getting physical signatures through Raman spectroscopy can also be enhanced with laser irradiation.[19] The stretching of the molecular bonds in a laser-irradiated organic specimen permits a shift to be produced in the monochromatic intense light passed through it. These new signals, which are read from a double spectrometer, produce characteristic signatures representing the sample which was irradiated. While this system is not a new one, the intense monochromatic light produced by laser sources is now making it much more practical, and promises to be a widely accepted method for chemical analysis for laboratories of the future.

The wavelength-specific effect referred to above may permit a more widespread application of laser energy to the area of pharmaceutical preparation as well as the management of parasitic or bacterial infections. For example, as more laser wavelengths become available, it may some day be possible to selectively destroy various species of bacteria in drug preparations, insuring sterilization of such solutions with no risk of molecular breakdown or loss of biochemical activity of the constituents contained therein. Moreover, it may be possible to selectively destroy nests of bacteria which accumulate in the subsurface necrotic tissue resulting from a burn. It may eventually be used advantageously to eliminate contamination from molds or fungi in skin infections. An example of selective destruction of parasitic inclusions in cells has been demonstrated, utilizing malarial parasites contained within erythrocytes. [20] The parasite can be selectively stained with a very low concentration of methylene blue, which results in a photosensitization of the parasite. The host cell of the circulating erythrocyte accumulates a negligible amount of the stain, and the leukocytes accumulate only a nominal amount in relationship to the parasite. Under these conditions, a relatively low dose of ruby laser can selectively hemolyze the erythrocytes which contain the

Plasmodium, but spares the non-infected erythrocytes and leukocytes. When the Parasitic inclusion reaches a maximum size, the metabolic response of that organism produces a breakdown of the host hemoglobin, resulting in the accumulation of small pigment particles which offer a natural photosensitization to the ruby laser beam. As a result, no additional staining is required to produce a selective destruction of the parasitized cells.

The experiences summarized in this report serve merely as examples of the types of applications in biology and medicine which are currently being served by laser energy. The opportunities for future uses of this instrument appear unlimited.

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