THE LASER AS A POTENTIAL TOOL FOR CELL RESEARCH

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ABSTRACT

Freshly prepared hemoglobin solutions were successively irradiated up to five times with 1 MW (monochromatic wavelength) of green (530 mμ) laser power. Oxygenated hemoglobin showed no detectable change, but the spectral absorption of reduced hemoglobin showed a shift toward the characteristic curve for the oxygenated form. Intact human erythrocytes exposed to a power density of 110 MW/cm² of green laser radiation showed no appreciable change in diameter or mass, but they became transparent to a wavelength range from 400 to 600 mμ. A similar power density from a ruby laser failed to produce this bleaching effect. This response in the erythrocyte demonstrates a principle which suggests the laser as a tool for cell research: specific molecular components within a cell may be selectively altered by laser irradiation when an appropriate wavelength and a suitable power density are applied.

Cytologists have enjoyed the recent development of a variety of technical procedures which have helped to bridge the gap between morphology and the biochemistry of the cell. Preliminary investigations with focused ruby laser beams (3, 18) have suggested that this system has potential for performing microsurgery. The major limitation to this device, however, is that either the target area must contain some component which will absorb a wavelength of 694.3 mμ or extremely high power densities must be used. Few natural cellular substances other than pigment granules show this absorption characteristic.

New developments in laser instrumentation and applications of non-linear optics phenomena are providing a wider range of wavelengths which are of potential value to the experimental cytologist. These new coherent and monochromatic wavelengths (MW) can be obtained by adding to to the basic unit of the Q-switched ruby orneodymium laser appropriate non-linear media (*e.g.*, potassium dihydrogen phosphate) for the purpose of frequency doubling (7) and quadrupling (11) and/or specific liquids for producing new coherent Raman shifted frequencies (5, 14, 8). Moreover, a combination of both techniques will permit a large selection of intense, coherent, narrow band frequencies to be produced at almost any region of the spectrum from the ultraviolet to the infrared, as needed. The biologist can now begin to select wavelengths which are maximally absorbed by such biochemically important entities as coenzymes, vitamins, the cytochromes, or nucleic acids. A preliminary study (12) with the green wavelength (530 mμ) obtained from a frequency-doubled neodymium Q-switched laser demonstrated the feasibility of applying this energy to cells in tissue culture. The study reported here describes the specificity of the effect of a single selected wavelength, within a controlled energy range, on human erythrocytes.

MATERIALS AND METHODS

The unfocused 10 MW output from a neodymium laser was passed through a Fabry Perot mode selector and a potassium dihydrogen phosphate (KDP) crys-

tal which was properly index-matched (9, 13) for maximum energy conversion from 1060 mμ to 530 mμ. (The exact wavelength was measured by a Jarrell Ash spectrometer to be 529.3 mμ). Suitable filters (KG3) were placed in the light path to eliminate the infrared energy. The green radiation was deflected downward 90° from a high quality, front surface mirror. The 12-mm diameter beam passed either through a spectrophotometer cuvette containing 3 ml of a hemoglobin solution or through a biconvex lens to be focused on a 1-mm diameter target area (Fig. 1). The power was monitored by measuring the energy incident on a calibrated thermocouple. A fast diode photodetector was used to measure the pulse width by means of an EG&G traveling wave oscilloscope. The measured pulse width at 530 mμ was 20 nsec., and at 1060 mμ it was 30 (nanoseconds).

RESULTS

Repeated exposure of oxygenated hemoglobin solutions to 1 MW of green laser power from one to five times produced no detectable effect. However, a similar procedure altered the shape of the absorption spectrum of the reduced hemoglobin to one which was intermediate between the reduced and oxygenated curves (Fig. 2). More convincing evidence of oxidative activity was obtained from the monitoring of reduced hemoglobin in both the "reference" and "sample" cuvettes at 570 mμ. The span was adjusted to read 90.5 per cent transmission of light under unirradiated control conditions. Following the irradiation of the "sample" solution with two exposures of 1 MW

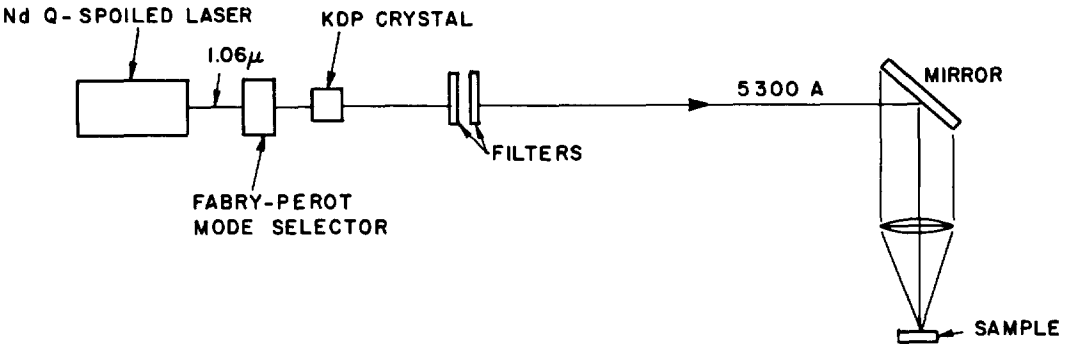

FIGURE 1 Schematic diagram of experimental arrangement of Q-switched laser.

Hemoglobin solutions were prepared by adding a drop of freshly drawn blood to about 3 ml distilled water. The erythrocytes were cytolyzed from the resulting osmotic pressure and the ghosts were allowed to settle to the bottom of the tube. The hemoglobin solutions were bubbled with either 100 per cent oxygen or wall gas (methane) to induce either an oxygenated or a reduced form of the molecule. The optical densities of these solutions were measured with a Beckman Model DB scanning spectrophotometer.

Blood smears were made on microscope slides and irradiated, while still fresh, with either a focused green or Q-switched ruby laser beam. Both laser sources had a pulse width of about 20 nsec, an output of about 1 MW, and were focused to a target diameter of 2 mm. The cells were allowed to air-dry, and then were examined with brightfield and interference microscopy. Individual cells within and outside of the target area were monitored for optical density throughout a 400 to 500 mμ range with the aid of a Leitz cytophotometer and a Model 520M photovolt meter.

green laser power each, the sample showed a transmission of 84 per cent with respect to the "reference" solution. Two additional exposures of green laser light decreased the transmission at 570 mμ to 77 per cent (Table I). Thus, the 570 mμ peak characterizing oxygenated hemoglobin increased with respect to the unirradiated solution.

Unirradiated red blood cells on the unstained, air-dried slide showed the typical biconcave morphology of the human erythrocyte. Within the target area of the focused green laser beam (110 MW/cm^2), the erythrocytes were seen to have intact membranes and appeared somewhat flattened although the concave appearance was retained by many cells (Fig. 3). Several others (Fig. 3, arrows) showed the typical morphology of the target cell found in many hypochromic anemias, but particularly in Cooley's anemia (2). Although the variable surface configuration of the red blood cell led to non-reproducible measurements with

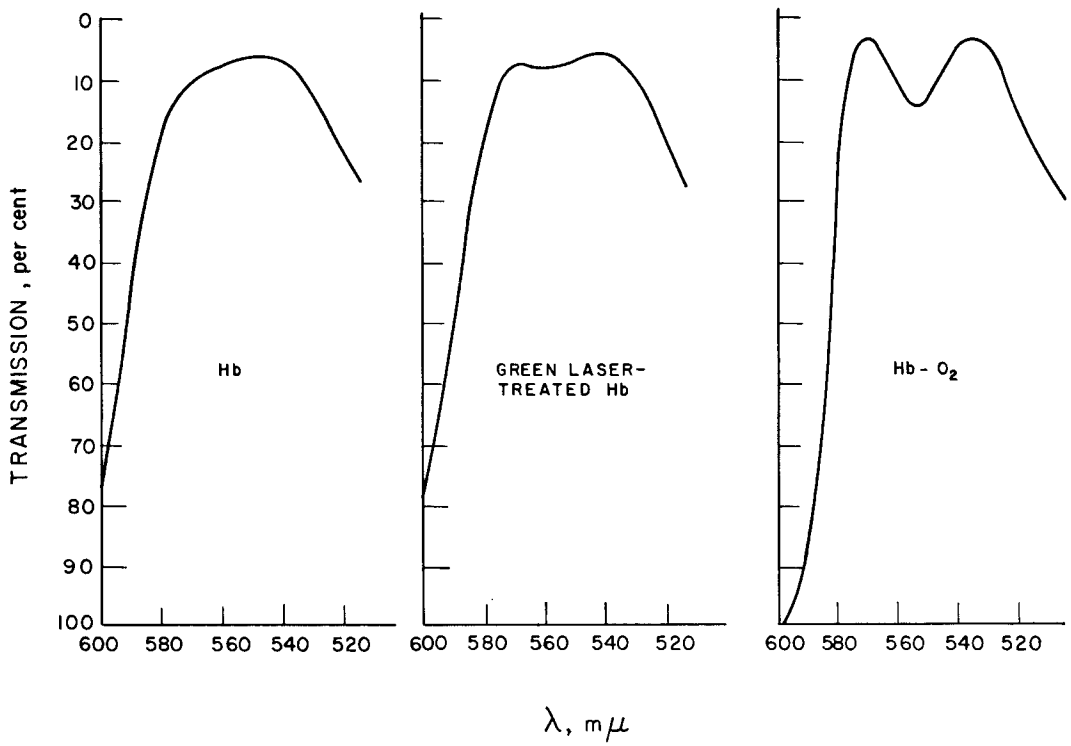

FIGURE 2 Comparative strip chart recordings of the spectral absorption of control, laser-irradiated, and oxygenated hemoglobin solutions.

the interference microscope, no appreciable difference in mass was observed. Because of an apparent optical reinforcement in photons at the margin of the focused laser beam, a ring of crenated and partially cytolyzed cells was formed which outlined the target area. Some of the cells along the margin of this ring showed a crenated edge, illustrating the sharp demarcation between the irradiated and control areas.

Images of 43 cells each were photographically enlarged for measurement of representative control and green laser-irradiated areas. The mean diameters of all cells were virtually identical. The control cells were found to be 7.50 $\mu \pm 0.37$, while the irradiated elements showed a diameter of 7.45 $\mu \pm 0.39$. Exposure to a power density of 110 MW/cm^2 produced no effect on the size of the erythrocytes. Their appearance under the light microscope suggested they were colorless with respect to the unirradiated cells.

The cytophotometric measurement of individual cells in the region of 500 to 600 mμ indicated an insufficient optical density (OD) to describe their state of oxidation or reduction. However, the presence of hemoglobin was demonstrated in isolated control cells by maximum OD readings of 0.3 to 0.4 observed at 415 mμ (Fig. 4). Cells close to the edge of the target area showed lower optical density values, while irradiated cells throughout the bulk of the target showed no capacity to block light in a 400 to 600 mμ range (Fig. 5). The application of light from a Q-switched ruby laser at an equivalent power density showed no such change in light absorption by the cells.

TABLE I

The Effect of the Green Laser on a Hemoglobin (Hb) Solution

Total number of exposures	Light transmission
(1 MW green laser)	*(per cent)*
0	90.5
2	84.0
4	77.0

The per cent transmission of light (570 mμ) was monitored with respect to an unirradiated reference Hb solution.

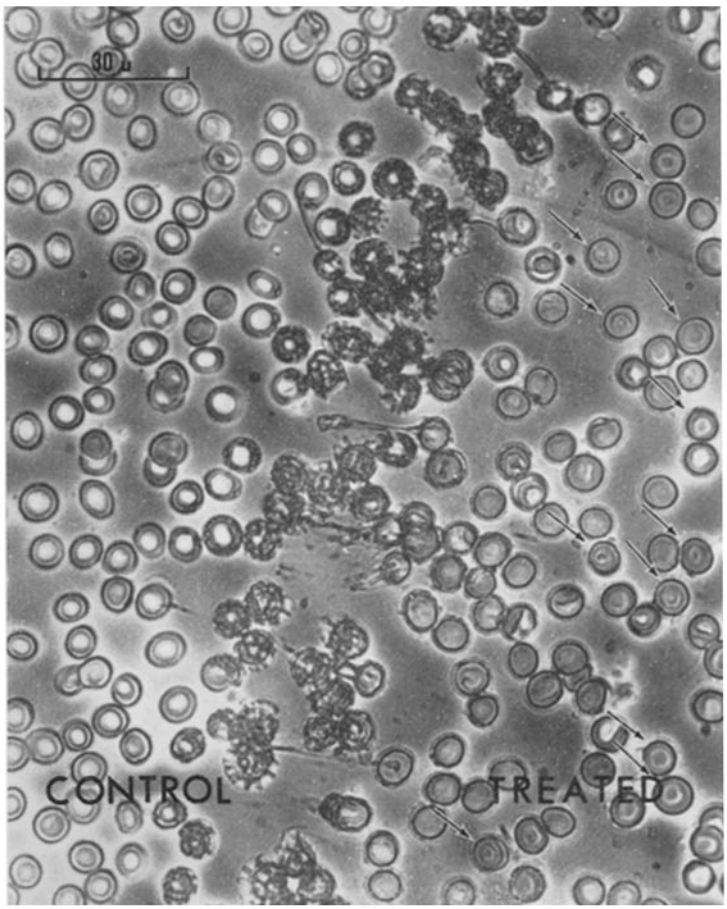

FIGURE 3 Normal and green laser-irradiated human erythrocytes as observed with an interference microscope. The central band of crenated cells marks the edge of the target area. The arrows draw attention to elements showing target cell morphology. × 533.

DISCUSSION

No attempt was made in this study to define the effect of the green laser on the hemoglobin solution, other than its altered spectral absorption. If this could be interpreted as true oxygenation, it might be postulated that a sufficient amount of laser power could facilitate the binding of hemoglobin with oxygen by the displacement of electrons from the heme portion of the molecule either by direct action or indirectly by the formation of oxidizing free radicals. Derr *et al.* (4) have offered support to the latter hypothesis by demonstrating free radical formation with a pulsed ruby laser. However, the demonstration of hemoglobin oxygenation does support the prediction that an erythrocyte would be sensitive to green laser irradiation by virtue of the absorption characteristics of its hemoglobin.

The loss of optical density of green laser-irradiated erythrocytes suggested a marked alteration in the molecular configuration of the hemochromogen portion of the hemoglobin and likely its oxygen binding capacity. This response appeared to be wavelength-dependent since it could not be duplicated with the ruby laser. The fact that the cell membranes remained intact indicated that lipoprotein structures of the cell are not sensitive to 530-mμ laser energy. Johnson *et al.* (12) reported that this wavelength did not alter the lipoprotein structure of mitochondria. The measurement of cell diameters and a qualitative evaluation of mass with an interference microscope suggested that

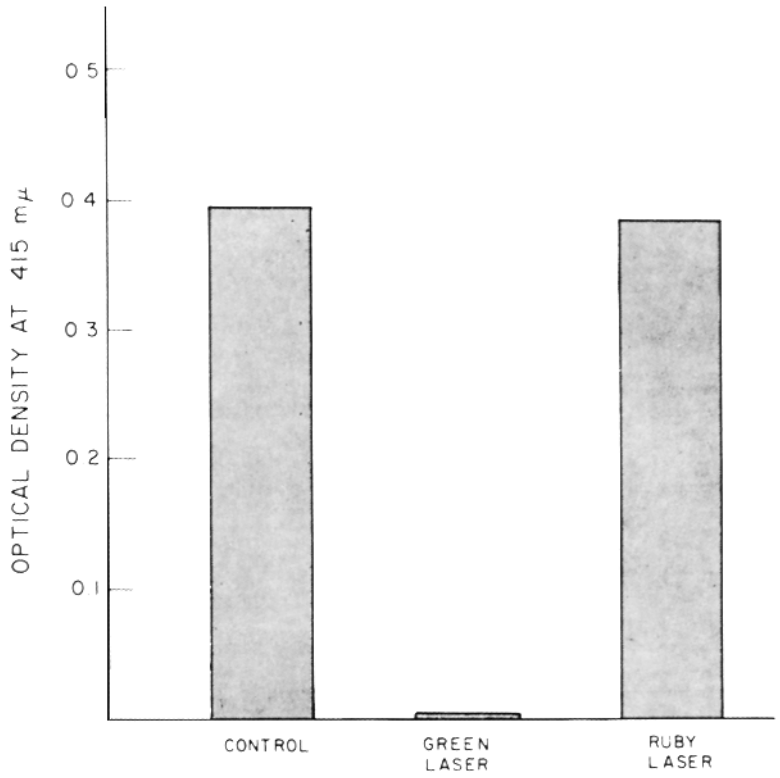

FIGURE 4 The average optical density of control, green laser-irradiated, and ruby laser-irradiated human erythrocytes as measured with a cytophotometer, using a wavelength of 415 mμ.

green laser irradiation produced no appreciable effect on the mass of the erythrocyte. Behrendt (1) stated that 97 per cent of the dry weight of erythrocytes is composed of hemoglobin protein (globin), so the primary damage may have been restricted to the hemochromogen portion of the molecule.

This demonstration of laser sensitivity, as related to the absorption of specific wavelengths of the spectrum, confirms and extends the conclusions of earlier studies using cells in tissue culture (12, 16, 17). However, this relationship is apparently pertinent only within a limited energy range. Low power densities may produce no effect or a reversible change such as the oxygenation of hemoglobin. Extremely high power densities appear to produce damage to structures, such as cell membranes, which normally would not be injured. This was observed not only in the crenation of erythrocytes in this study, but in the report by Helsper *et al.* (10) that retinal epithelial cells are lysed by exposure to the ruby laser only if they contain pigment granules. Bessis *et al.* (3) demonstrated lysis of the erythrocyte using a focused ruby laser with a high power density in spite of the fact that hemoglobin absorbs a negligible amount of the energy at 694.3 mμ.

The selective alteration of a molecular component, with an appropriate wavelength within a suitable energy range, offers great potential to gain new information regarding the metabolism of the cell. Analogous to using chemical blocking agents (15), the use of the green laser might prove helpful in elaborating the role of cytochromes *b* and *c* in oxidative phosphorylation. In addition, the relationship between the cytochromes and the elementary particle, as postulated by Fernández-Morán (6), could be attacked with new vigor. The recent availability of a few kilowatts of laser power (7) at 265 mμ offers additional potential for studying cellular structures containing nucleic acids in a manner analogous to that described by Uretz and Perry (19). Current and future studies are being extended in these directions.

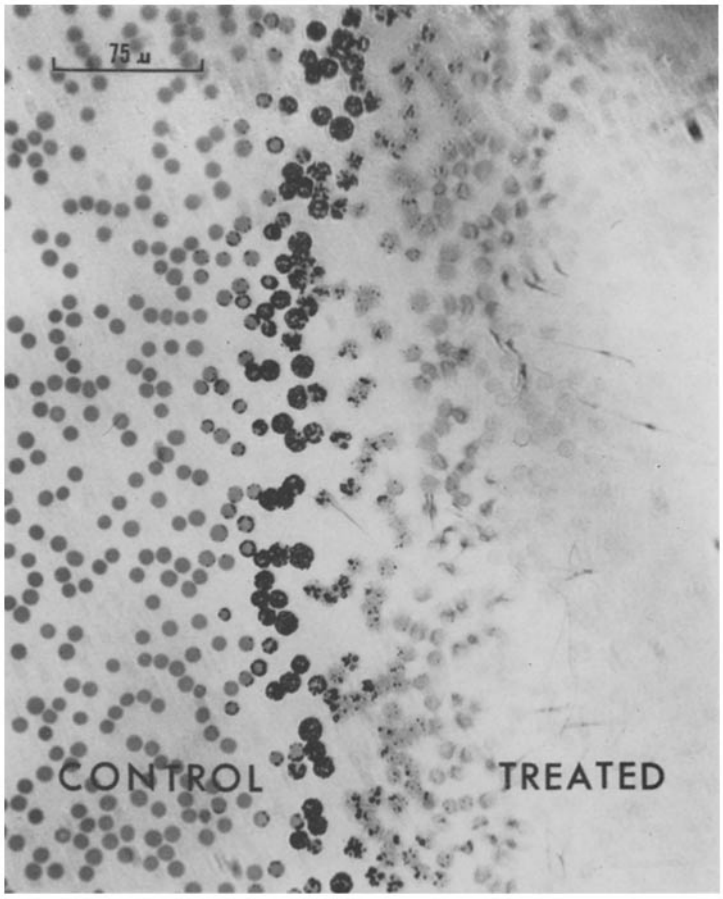

FIGURE 5 Unirradiated (left) and green laser-irradiated (right) human erythrocytes, photographed with a light source adjusted to emit a wavelength of 415 mμ. The row of light-scattering crenated cells mark the edge of the target area. × 267.

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