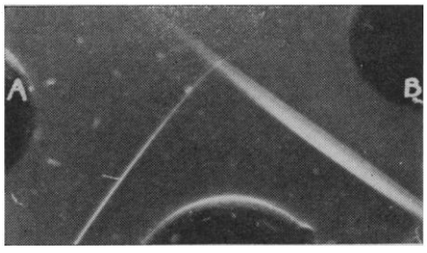

Fig. 2. Double diffusion in agar with anticatalase in center well, "apocatalase" in well *A*, and catalase in well *B*.

diffusion assays with bacterial anticatalase as antibody, the final "apocatalase" preparation was obtained as a colorless precipitate from 38 to 40 percent $(NH_4)_2SO_4$. Redissolved in water and adjusted to *p*H 8.5, the faintly yellow, enzymatically completely inactive solution crystallized as minute plates upon slow evaporation (Fig. 1).

Ultracentrifugation of the "apocatalase" revealed the presence of two components with distinctly different sedimentation constants, a fast moving ($s_{20,b} = 5.3 \times 10^{-13}$ sec) and a very slowly moving one ($s_{20,b} = 0.84 \times 10^{-13}$ sec). In spite of this heterogeneity, single, sharp precipitation bands formed with the homologous antibody and also with anticatalase in double-diffusion plates. Although "antiapocatalase" precipitates the catalase, it does not inhibit its enzymatic activity.

Figures 2 and 3, in which catalase and "apocatalase" diffuse from adjacent wells toward anticatalase and antiapocatalase respectively, show strikingly different patterns of double diffusion. Though puzzling at first glance, these patterns reveal some of the reactant's characteristics and allow certain conclusions. In Fig. 2 the catalase-anticatalase band crosses, apparently undisturbed, the band formed by apocatalase and anticatalase. The latter, however, forms a typical weak "spur" that curves toward the former. Figure 3 on the other hand, shows an unmistakable pattern of fusion or "identity."

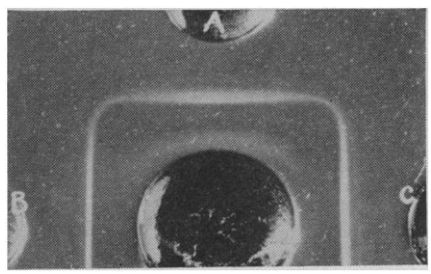

Fig. 3. Double diffusion in agar with "antiapocatalase" in center well, catalase in well *A*, and "apocatalase" in wells *B* and *C*.

In consideration of the notorious heterogeneity of even the most specific antibodies, these immunodiffusion patterns suggest the following interpretation. Figure 2 reflects the presence of antibody molecules (anticatalase) that differ in their specificity for the whole antigen and for smaller and larger parts of it (subunits?). Their different relative concentrations are responsible for the different quality of the "spurs." However, the anti-apocatalase (Fig. 3), produced in response to an antigen that is presumably a mixture of antigenically similar subunits (none larger, but many smaller than half the size of the catalase molecule), cannot contain antibody molecules that are highly specific only for the whole catalase molecule or major parts of it. Thus, a pattern of identity is to be expected.

In conclusion, the presence of at least large parts of the protein moiety of catalase, a classical hematin enzyme, has been demonstrated in catalase negative cells. The apoenzyme is apparently synthesized before the incorporation of the prosthetic group and independently of its presence. Whether our crystalline "apocatalase" represents a mixture of antigenically similar, true precursors of catalase, or whether extraction and purification procedures gave rise to "subunits" originating from a fully synthesized, native apocatalase cannot be decided at present (*8*).

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2 April 1963

Ruby Laser as a Microsurgical Instrument

Abstract. *Morphological changes at the cell level have been produced experimentally by a pulsed ruby laser (optical maser). A pulse duration of approximately 500 microseconds caused discrete damage to structures in the cell without irreversible damage to the surrounding area.*

By the use of a focused ruby laser (*1*), openings up to 25 μ in diameter have been produced in the cell walls of the alga, *Spirogyra*, without causing irreversible damage. The relative transparency of the cell wall to laser beams permitted microsurgery to be performed on the internal structures of the algal cell without damage to the exterior cell wall. It was possible to sever the chloroplast helix, disrupt crystals, and produce localized coagulation of the chloroplasts and cytoplasm. Focusing the laser beam on the cell nucleus caused coagulation and a reduction in nuclear volume. Ultraviolet irradiation of the nucleus of a cell also caused a slight reduction in volume and a condensation of ultraviolet-absorbing material at the nuclear membrane (*2*).

Instrumentation for the experiments consisted of a low-power ruby laser (*3*) incorporated and integrally mounted with a triocular biological microscope. This permitted precise positioning of the laser beam on the specimen. The laser has a maximum output of 20 mj per pulse. The pulse duration is 500 μsec. It is possible to view the specimen immediately after irradiation with the beam. Cross lines in the ocular permit fast and accurate positioning of the target, since the laser beam is coincident with the optical path of the microscope.

Laser spot size incident upon the specimen is varied by changing the power of the microscope objectives. The greater the magnification, the smaller the focused spot becomes. The energy which is incident upon the specimen may be measured with the use of a thermopile (*4*). Irradiating energy is controlled via metallized neutral density filters of known transmittances which are interposed between the laser and the focusing objectives.

The experiments indicated that, unless relatively high beam energies were used, the laser beam passed through the cell wall without causing any visible effect on it. Methylene blue chloride was applied to the cell wall, since the laser emission is in the deep red part

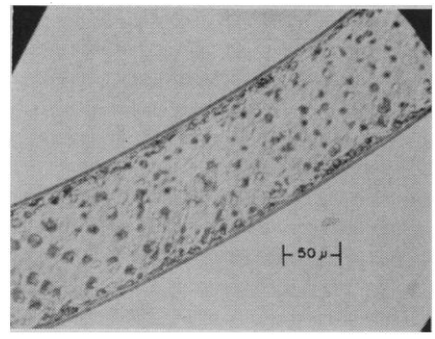
of the electromagnetic spectrum (λ = 694.3 mμ). The dye permitted a large reduction in beam energy and confined burn effects to the localized area (*5*). This permitted puncturing of the cell wall.

Spirogyra filaments containing many cells were placed on slides in drops of Bristol's solution (*6*) and protected with a cover slip. The cells were then subjected to various amounts of laser beam flux in selected areas of the cell.

Initially, the full energy of the laser beam was focused on the cell wall. This caused a disruption of the target cell and affected adjacent cells. Cell walls were split open violently with nuclei and chloroplasts ejected into the surrounding medium. Smaller beam energies ranging from 1.4 to 2.5 mj per pulse produced gradations of effects on the cell. The cell wall showed brown or blackened areas about 25 μ in diameter. Exudation of a plug of cytoplasmic material closed the burned area. The chloroplast and cytoplasm in the burned area became coagulated and globular. The single nucleolus in the nucleus became hyaline at the same time. The end walls of the two adjacent cells bulged into the affected cell, indicating loss of turgor pressure in the target cell. A beam energy of 0.3 mj per pulse produced similar but somewhat lessened effects. However, there was no visible damage to the nucleus at this energy level. Cells were stained with 0.01 percent methylene blue chloride. The cell wall retained the dye, whereas the other structures in the cell retained little, if any, dye. A beam energy of 0.16 mj per pulse appeared to be the most effective level for producing burned areas in the cell wall by using the dye technique for greater absorption. Burned areas ranging from 15 to 25 μ in diameter in the cell wall were produced by this method. The cells appeared to be normal except for the burned areas and openings produced by the laser beam.

The chloroplast helix in the cell was subjected to beam energies ranging from 0.3 to 0.61 mj per pulse. This resulted in coagulation of the chloroplast and cytoplasm along with the loss of turgor pressure in the cell. The nucleus rounded up as a result of irradiation of the chloroplast. With the beam energy reduced to 0.2 mj per pulse, there was no visible effect on the chloroplast or any other structure in the cell.

Normal cells were photographed prior to irradiation (Fig. 1). The nucleus of the cell was also directly subjected to beam energies ranging from 0.16 to 0.61 mj per pulse. This resulted in a blackish colored burn of the nucleus and a decrease in nuclear volume with the nuclear membrane assuming a sinuated appearance (Fig. 2). The filaments which normally anchor the nucleus in the cytoplasm were either damaged or destroyed. Loss of turgor pressure and plasmolysis resulted at the higher energies utilized above. A beam energy of 0.08 mj per pulse had no visible effect on the nucleus of the cell.



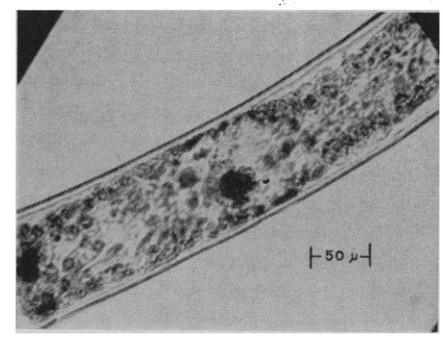


Fig. 1 (left). Filamentous alga, *Spirogyra*. Field of view showing slightly less than one cell. The nucleus is in the center, ovoid shaped, before laser irradiation (× 140). Fig. 2 (right). The same cell as Fig. 1, after laser irradiation (× 140).

The physiological effects of heat and possible mechanical agitation will have to be investigated further to determine the usefulness of the laser in single-cell studies. However, specific portions of the cell can be subjected to low levels of energy without too much damage other than to the immediate area. The laser has a sufficient range of energies to produce various effects on this species of alga, including total destruction.

The data obtained in these initial experiments, although somewhat cursory, indicate that laser microsurgery should have application in biological research, especially in such areas as cytogenetics, as well as the biomedical sciences (*7*).

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Calcium-Activated Adenosine Triphosphatase Localization in Cultured Beating Heart Cells

Abstract. *The calcium-activated adenosine triphosphatase activity of cultured single beating heart cells of the rat was localized and visualized by incubating the cells, after extraction in a mixture of glycerol and water, with adenosine triphosphate and calcium. The calcium phosphate, precipitated at the site of the enzyme reaction, was converted into a visible precipitate. The enzyme activity appeared in bands perpendicular to the myofibrils.*

The amount of myosin in cultures of beating heart cells has been followed (*1*) by extraction of the cells (*2*), and by measuring the calcium-activated adenosine triphosphatase associated with myosin (*3*). The relation of myosin changes to growth and dedifferentiation may further be studied in the single cell. We have devised a histochemical technique which is valuable for visualization of the distribution of this enzyme in the single beating heart cell. The adaptation consists of a selective extraction of the single cell and a substitution of adenosine triphosphate (ATP) for glycerol phosphate in the Gomori technique (*4*).

Heart tissue from 2- to 7-day-old