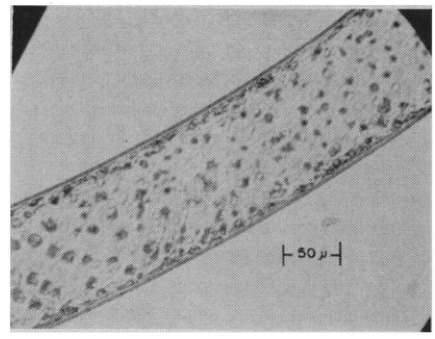
of the electromagnetic spectrum (λ = 694.3 mμ). The dye permitted a large reduction in beam energy and confined burn effects to the localized area (*5*). This permitted puncturing of the cell wall.

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.



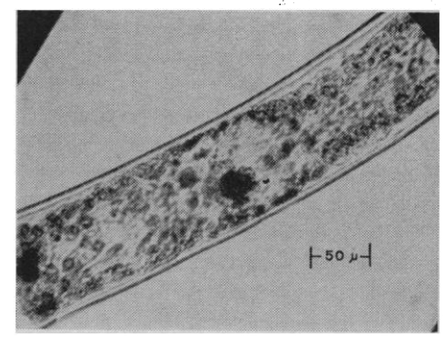


Fig. 1 (left). Filamentous alga, *Spirogyra*. Field of view showing slightly less than one cell. The nucleus is in the center, ovoid shaped, before laser irradiation (× 140). Fig. 2 (right). The same cell as Fig. 1, after laser irradiation (× 140).

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*).

NORMAN M. SAKS
Biology Department,
New York University,
Washington Square, New York
CHARLES A. ROTH
TRG, Inc., Syosset, New York

References and Notes

1. G. C. Dacey, *Science* **135**, 71 (1962).
2. P. O'B. Montgomery, *Ann. N.Y. Acad. Sci.* **97**, 491 (1962).
3. Supplied by TRG, Inc., Syosset, N.Y.
4. C. H. Townes, *Biophys. J.* **2**(2), 325 (1962).
5. S. Fine, E. Klein, R. Scott, R. Sued, A. Roy, *Life Sciences* **1**, 30 (Jan. 1963).
6. Consists of $NaNO_3$, $CaCl_2$, $MgSO_4 \cdot 7H_2O$, KH_2PO_4, NaCl, minor elements, vitamin B_{12}, and distilled H_2O.
7. M. M. Zaret, G. M. Breinin, H. Schmidt, H. Ripps, I. M. Siegel, L. R. Solon, *Science* **134**, 1525 (1961); M. M. Zaret, H. Ripps, I. M. Siegel, G. M. Breinin, *Arch. Ophthalmol. Chicago*, **69**, 97 (1963).

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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

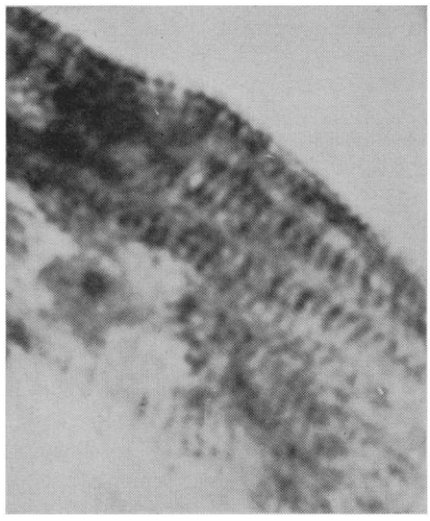

Fig. 1. Bright field photomicrograph of an extracted beating heart cell stained for calcium-activated adenosine triphosphatase activity (× 4000).

rats was cultured in plastic culture dishes as single cells which continue to beat (*5*). After extraction with a glycerol-water solution (*6*), the cells no longer beat spontaneously, but the contractile apparatus is still functional. This preparation is similar to the extracted fibers described by Szent-Györgyi (*7*). Extracted cells were fixed at 0°C for 30 sec with 3 percent formol (*8*), and incubated for 15 min at 37°C in a mixture containing $10^{-3}M$ ATP, $6.7 \times 10^{-3}M$ $CaCl_2$, and $2 \times 10^{-2}M$ sodium barbital at *p*H 9.4. The inorganic phosphate formed at the site of enzyme action was precipitated *in situ* as calcium phosphate. After treatment successively with a soluble cobalt salt and ammonium sulfide the precipitate was converted to a microscopically visible black deposit, CoS. As a control, inorganic phosphate was substituted for ATP in the reaction medium. The result was an almost imperceptible overall graying of the cells with no localization. The cells were mounted wet with polyvinyl pyrrolidine (*9*). This eliminates the need for clearing and dehydration and permits the use of plastic culture dishes which would dissolve in clearing solutions.

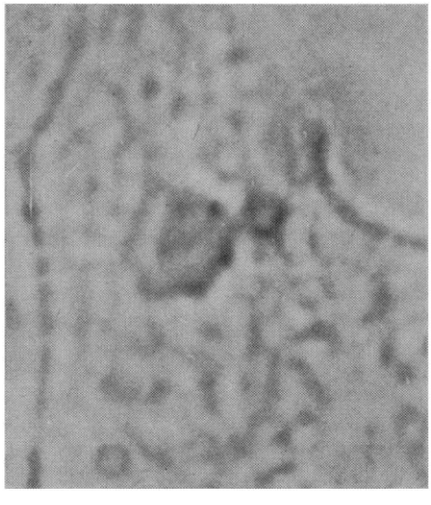

Fig. 2. Bright field photomicrograph of a control cell, which was incubated with inorganic phosphate in place of ATP. Unstained cells are barely visible under bright field conditions (× 4000).

Figure 1 is a photomicrograph of a preparation of a heart cell preparation grown in culture for 2 days. This cell has been stained for the calcium-activated enzyme and the blackened areas indicate the site of action. Of particular interest are the striations which indicate that this activity is discontinuously distributed along the myocardial fibril. Figure 2 is a photomicrograph of a control cell which was incubated with inorganic phosphate instead of ATP. The cell is barely visible and no localized staining occurs. The calcium-activated adenosine triphosphatase activity of isolated myosin suggests that the activity in the myofibril may be associated with myosin, and that the heavily stained band corresponds to the A band while the unstained area corresponds to the I band (*10*). It is not clear whether the alternating, more lightly stained bands are indicative of myosin at a region corresponding to the Z line or of some other calcium-activated adenosine triphosphatase in the sarcomere. During the course of our work a similar observation has been reported (*11*). The excellent electronmicrographs demonstrate that the enzyme is localized only in the A band. This may be further studied with the use of fluorescein myosin antibodies (*12*) in conjunction with enzymatic localization (*13*).

HAZEL LEWIS
ISAAC HARARY
Departments of Biophysics and Nuclear Medicine and *Biological Chemistry, University of California, Los Angeles*

References and Notes

1. H. Kuramitsu and I. Harary, unpublished data.
2. F. Guba and F. B. Straub, *Studies Inst. Med. Chem. Univ. Szeged* **3**, 46 (1943).
3. S. V. Perry, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1955), vol. 2, p. 582.
4. R. D. Lillie, in *Histopathelogic Techniques and Practical Histochemistry* (McGraw-Hill, New York, 1954), p. 202.
5. I. Harary and B. Farley, *Exptl. Cell Res.* **29**, 451 (1963).
6. H. Hoffman-Berling, *Biophys. Biochim. Acta* **14**, 182 (1954).
7. A. Szent-Györgyi, in *Chemistry of Muscular Contraction* (Academic Press, New York, 1947).
8. S. S. Lazarus and H. Barden, *J. Histochem. Cytochem.* **10**, 285 (1962).
9. D. B. MacCallum, *Stain Technol.* **37**, 129 (1962).
10. A. Szent-Györgyi, in *Chemical Physiology of Contraction in Body and Heart Muscle* (Academic Press, New York, 1963).
11. L. W. Tice and R. J. Barnett, *J. Cell Biol.* **15**, 401 (1962).
12. H. Holtzer, J. Marshall, H. Finck, *J. Biophys. Biochem. Cytol.* **3**, 705 (1957).
13. Supported in part by grant No. AM-02135-04 from the National Institutes of Health, and under contract No. AT-04-1-GEN-12 between the U.S. Atomic Energy Commission and UCLA.

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Language and Thinking: Positive and Negative Effects of Naming

Abstract. *Subjects instructed to think of novel shapes in terms of relevant names made fewer errors in recalling a serial ordering of the shapes, but more errors in solving a mental jigsaw puzzle and in drawing the shapes from memory, than subjects instructed to visualize the shapes without using words.*

Is it easier to think about objects, and to manipulate them "in one's head," if one has names for them than if one does not? Spiker (*1*) found that performance in the delayed-reaction experiment, often considered to depend on representational processes, was facilitated when subjects had names for the stimuli. He suggests that during the delay period the subject repeats the name of the baited stimulus, and that the name then serves to guide his choice. This hypothesis assumes that names do in fact perform this representational function more effectively than nonverbal representations, such as images. On the other hand, Saltz and Newman (*2*) found that while learning the names of the parts to a low criterion before doing a mechanical assembly problem led to fewer errors, learning to a high criterion resulted in more errors than no name learning at all. Thus names yielded no consistent advantage in the kind of mental manipulation required in the assembly problem. These findings suggest that the effect of prior name learning on thinking depends on the nature of the problem. Pretraining which leads a subject to think of objects in terms of names, rather than, say, in terms of images, may facilitate performance in one problem but interfere with performance in another.