with the aid of beads has also been applied successfully to manipulation and cloning of other mammalian cell types, namely HeLa carcinoma cells and Detroit-98 bone-marrow cells. Two methods have been employed: (i) beads carrying single cells were immediately transferred to suitable tissue-culture media, or (ii) beads carrying single cells were permitted to remain in the cups until clones of sufficient size had established themselves on each of these particular beads. The latter procedure made it possible to obtain progeny from known single cells in the presence of other cells, a condition that in exploratory trials has proved favorable for the initiation of clones. For the purpose of recording the location of beads initially carrying only single cells, a photographic recording procedure was employed: first the location of every bead in the cup was mapped by projection onto photosensitive paper; 24 hours after the cups had been inoculated with a trypsinized cell suspension, the positions of all beads carrying single cells were marked on this photographic record. It should be added that provisions for the carbon dioxide tension required during the manipulation and growth of these mammalian cells were made in a simple manner: cups were incubated, unsealed, at 37°C in a CO_2-incubator (or in a closed jar in which CO_2-rich conditions were produced by burning a candle to extinction), and were inspected in a CO_2-rich atmosphere that had been created by CO_2-exhaust from a funnel placed adjacent to the microscope stage.

This procedure should prove useful for the primary isolation, manipulation, and cloning of many different cell types, particularly if initial maintenance of selected cells in the presence of other cells is prerequisite to the successful establishment of a clone (*6*).

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Cytochemical Adenosine Triphosphatase of Vorticellid Myonemes

Abstract. A highly specific adenosine triphosphatase has been found to be localized in the contractile apparatus of vorticellids. It is most prominent in the cilia as well as in the myonemes which course the latitudes and longitudes of the cell and spiral in the flat peristome. The stalk displays more activity in the spasmoneme sheath than in the spasmoneme itself. The spasmoneme canal appears negative. A delicate fiber coils around the outside of the stalk in a helix. All these structures may be seen in the living protozoan by phase-contrast microscopy.

A very rich literature supports the hypothesis, originally presented by A. Szent-Györgyi (*1*), that contractility is dependent upon some interaction of adenosine triphosphate (ATP) and actomyosin. An important aspect of this interaction is the adenosine triphosphatase which contractile protein possesses by virtue of its myosin content (*2*). Extracts made from vertebrate (*2*) and invertebrate muscle (*3*) have this enzymatic property. Of fundamental importance is the fact that similar extracts of cells (*4*) or their isolated motor apparatus, sperm tails, (*5*) also display this enzyme activity.

Recently, direct demonstration of adenosine triphosphatase in intimate association with motor organelle was made for the fibers within sperm flagella by a cytochemical method (*6*). It is the purpose of this report to describe the cytochemical locus of adenosine triphosphatase in myonemes, the motor organelle of vorticellids, and other protozoans.

Vorticella convallaria were used throughout. They were derived from a clone cultured for several years by a previously described method (*7*) with some modifications. A cerophyl extract was substituted for the lettuce in the egg-lettuce medium. It is prepared by boiling 1 mg in 1 ml of distilled water for 5 minutes and filtering while hot into flasks which are then plugged and refrigerated. Equal parts of the egg and cerophyl are mixed to provide the culture medium. Routinely, petri dish bottoms were lined with non-nutrient agar before they were filled with the culture medium. Vorticellids detach from such a surface after 3 to 4 days of incubation at 25°C, thereby making for convenient harvest by light centrifugation after filtering through a No. 25 plankton net. They were then washed three times in sterile culture medium or Chalkey's solution before use in subculturing or in cytochemical study.

For assay, 20-μl aliquots of cell suspensions were placed on lightly albuminized cover slips (No. 0, 22 mm) and frozen directly on a block of Dry Ice. They were then thawed and dried with the aid of a fan. When dry, the cover slips were immediately immersed in substrate medium contained in Columbia staining dishes which had been previously equilibrated at 37 ± 0.2°C for 1 hour.

The substrate medium was essentially that of Padyluka and Herman (*8*). It contained 5 mmole of ATP (*9*), 2.5 mmole of cysteine-HCl, 20 mmole of $CaCl_2$, and 25 mmole of Veronal. The *p*H was adjusted to 9.4 and the solution was filtered before equilibration. In some experiments, 5 mmole of ADP, 12 mmole of muscle adenylic acid, (AMP), beta-glycerophosphoric acid, glucose-1-phosphate (*10*), and fructose-1,6-diphosphate (*11*) replaced ATP as substrate.

All substrates were added as the dry powder, and demineralized water was used throughout. All experiments included parallel preparations incubated in control media which lacked substrate. In addition, vorticellids that had been steamed for 10 minutes before incubation were also tested.

After incubation for 15, 30, 60, 180, or 240 minutes, inorganic phosphorus was visualized as black CoS by 3-minute immersion of cover slips in control medium saturated with $(Ca)_3(PO_4)_2$ by the addition of 0.1*M* Na_2HPO_4. They were then placed in 2-percent $CoCl_2$ for 3 minutes, followed by three washes in distilled water. Both the $CoCl_2$ and distilled water were brought to above *p*H 8 by the addition of 25 mmole of Veronal, as recommended by Danielli (*12*). Following this, cover slips were dipped in 1-percent $(NH_4)_2S$ for 2 minutes, washed

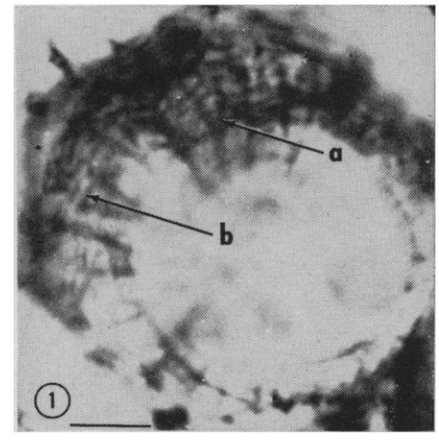

Fig. 1. Sites of adenosine triphosphatase in head. Incubation time, 60 minutes. *a*, Longitudinal myoneme; *b*, latitudinal myoneme. Magnification marker at lower left, 5μ.

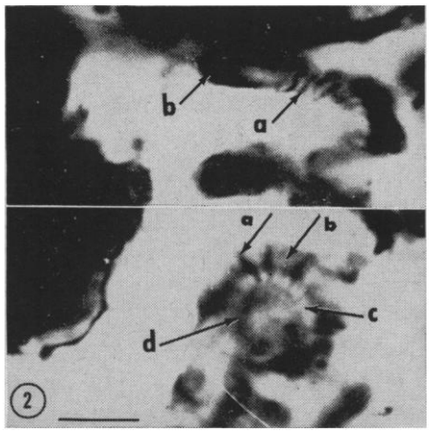

Fig. 2. Loci of adenosine triphosphatase in stalks. Incubation time, 15 minutes. (Top) Lateral view of upper coil of loosely wound stalk. (Bottom) Anterior view of coil of tightly wound stalk. *a*, Cortical helix; *b*, sheath of spasmoneme; *c*, spasmoneme canal; *d*, spasmoneme. Magnification marker at lower left, 5μ.

in three changes of tap water, and dehydrated in 80-percent ethyl alcohol for 1 minute, in 95-percent for 2 minutes, in 100-percent for 3 minutes, and were finally cleared rapidly in xylol and mounted in Clarite.

After 15 minutes' incubation with ATP as substrate, prominent fibers appear along the longitudes of contracted heads (Fig. 1). Some of these course in furrows along their entire length, while others seem to bend to join opposite fibers at the peristome, thereby forming characteristic arcades. The area between these fibers is dotted with numerous granules which presumably are mitochondria. In some cells, other, more delicate, fibers course around the latitudes. These become more prominent after 30 minutes, and together with the longitudinal fibers, give the pellicle an over-all basket-weave appearance. The peristomial shelf contains another fiber which follows a flat spiral beneath the adoral ciliary membranes. The cilia, which form the membranes, are also positive. A similar pellicular net of longitudinal, latitudinal, and spiral fibers were described by Entz (*13*), who used conventionally fixed and stained materials. They are the myonemes of the head and impart its characteristic motility.

Another fiber associated with an adenosine triphosphatase winds around the stalk as a helix in the outer membrane of the spasmoneme sheath (Fig. 2, *a*). It has the same order of activity as the myonemes in the head, as it becomes prominent after 15 minutes of incubation, and presumably is a structural embellishment of these. This cortical helix resembles one figured by Faure-Fremiet (*14*), and may be analagous to the helix in sperm tails (*15*). The sheath which surrounds the spasmoneme (Fig. 2, *b*) appears densely positive in many individuals after 15 minutes. The spasmoneme canal is always negative (Fig. 2, *c*), whereas the spasmoneme (Fig. 2, *d*) displays only moderate activity in the shorter incubation periods.

It should be mentioned that the spasmoneme usually appears darkened in occasional individual stalks of control preparations, therefore making it difficult to evaluate cytochemically. I believe this darkening to be the result of hydrolysis of tightly bound endogenous substrate because the spasmoneme of steamed vorticellids is lightened to its inherent refractility.

The adenosine triphosphatase associated with myonemes is highly specific since the latter appear most intensely when ATP is used as substrate. The enzyme does not attack AMP, beta-glycerophosphoric acid, glucose-1-phosphate, or fructose-1,6-diphosphate. Only slight visualization occurred with adenosine diphosphate.

All myonemes may be seen by phase-contrast microscopy of the living organism. Longitudinal and latitudinal myonemes and the granules between them appear in compressed heads. When the stalk is actively contracting or relaxing, striae appear along its entire length. I believe these to be identical with the cortical helix. Such demonstration confirms the reality of the structures found cytochemically.

The evidence presented indicates that the myoneme is intimately associated with a specific adenosine triphosphatase and thereby is provided with the equipment necessary to make up the free energy deficit incurred in the contractile process. It is suggested that the detailed morphology of myonemal systems as revealed cytochemically may serve to provide further biochemical basis to demonstrate and confirm taxonomic relationships (*16*).

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Penetration of Lead by the Wood Piddock Martesia striata

Abstract. An attack by *Martesia striata* Linnaeus on the lead sheathing of a power cable is described. This is the third such attack recorded and all have occurred in Florida waters. A guide for describing future attacks is recommended with the hope that the specific conditions under which attacks occur can be learned.

On 15 August 1959 an electrical power cable extending 1060 feet across Boca Ciega Bay beside the Treasure Island Causeway, St. Petersburg, Fla., shorted out. The cable had been placed in service during the spring of 1953 and had no previous history of malfunction. After the cable was raised it was found that the protective sheathing of a section about 4 feet long, approximately 200 feet from the west shore of the bay, had been damaged. The sheathing consisted of an outside layer of asphalt-impregnated jute over a closely coiled wrapping of heavy steel armor wire over a second layer of asphalt-impregnated jute which surrounded a tube of pure lead that had an outside diameter of about 55 mm and walls 3 mm thick. A three-conductor, paper-insulated cable lay inside the lead tube.

In the damaged section (at a depth of about 8 feet) the steel wires had rusted, snapped, and exposed the layers beneath. In some areas the jute had been eliminated, and the lead sheath beneath was exposed. There was no evidence of abrasion or oxidation on the lead surface. It was found, however, that the exposed lead was both pocked and riddled with small holes in each of which could be seen a small molluscan shell. It is not known whether the jute layer was penetrated before the lead, or whether any exposed areas of lead were not attacked.

The mollusks proved to be young pholadids, *Martesia striata* Linnaeus (*1*). The specimens appeared to be identical with *Martesia funisicola*