thus require continuous replacement. Whether the rate of histone renewal is related to rapidity or control of RNA synthesis, or strictly coupled with the synthesis of DNA, remains still undetermined.

LUBOMIR S. HNILICA
MING C. LIAU
ROBERT B. HURLBERT
Department of Biochemistry, University of Texas M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Avenue, Houston 77025

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10 February 1966

Bivalve Mollusks: Fluid Dynamics of Burrowing

Abstract. *When bivalves burrow into soft substrates the foot is first extended and then dilated to obtain a firm anchorage before retraction pulls the shell downward. Pedal dilation is principally caused by adduction of the valves. The hinged shell functions as a hydraulic machine in which the strength of the adductor muscles is transferred to the distal part of the foot by means of the body fluids.*

It has long been established that the water contained in the mantle cavity of *Mya arenaria* is used as the fluid of a hydraulic system that enables either contractions of the adductor muscles to extend the siphons, or retraction of the siphons to open the valves (*1*). Past research on the locomotion of Bivalvia (*2*) suggests that burrowing movements of the foot represent the action of the pedal musculature together with the inflow of blood. Recent investigations show that, at adduction of the valves, high pressure occurs in both the hemocoel and the mantle cavity, and that such pressures are utilized in burrowing.

In my investigation, knowledge of the fluid dynamics of bivalves was considerably extended by the use of electronic recording techniques (*3*). Used were a multichannel pen recorder (*4*), isotonic and isometric myographs attached by a thread to a valve to record movement, transducers (*5*) to determine internal pressures, and cine film, sometimes synchronized with the pen recorder, for recording details of digging activity. Film was exposed both at the commencement of burrowing (with the subject on the surface of the sand) and through an aquarium tank filled with sand, when the bivalve burrowed sufficiently closely to the glass side. The pressure transducers were connected to the bivalve by a hypodermic needle (0.9-mm bore) and a short length of pressure tubing; the needle, inserted into the pedal hemocoel or mantle cavity of *Ensis* through the fourth pallial aperture of the mantle, did not obstruct burrowing until the tubing reached the surface of the sand, with at least one-half of the shell buried. In *Mya* and *Margaritifera*, short hypodermic needles were securely fastened with wax in small holes drilled in the valves (Fig. 1, *A* and *B*); specimens kept for several months with the needles attached remained very active. Movement of the valves of clams buried in the sand was recorded by attaching a very light wire electrode to each valve and passing a small a-c signal, from an impedance pneumograph, between them; small variations in current occasioned by movement of the valves were recorded (Fig. 2*c*). The operation of all transducers and the location of the hypodermic needles were checked after each experiment.

Investigation of the fluid dynamics of bivalves was preceded by observations of their burrowing behavior. Genera representative of bivalves living in sand or mud (for example, *Nucula, Glycymeris, Anodonta, Cardium, Tellina, Donax, Mercenaria, Mactra*, and *Ensis*) all show an essentially similar series of steplike digging movements. Each step represents a cycle of activity (Fig. 3) incorporating the following successive actions: extension of the foot (*i*), closure of the siphons (*ii*), adduction of the valve (*iii*), pedal dilation (*iii–iv*), contraction of pedal retractor muscles, causing movement of the shell into the sand (*iv*), followed by a short period of relaxation (*v*) when the valves reopen. Between successive cycles there occurs a static period (*vi*) of variable duration, when the foot is extended, probing more deeply into the substrate, and the shell either remains stationary or is slightly raised (Fig. 3, *L*). Pedal retraction (*iv*) generally occurs in two phases, particularly in bivalves with wide shells: posterior retraction follows anterior, so that a rocking movement is imparted to the shell, making for easier penetration (*6*).

Protraction of and probing by the foot is carried out by the intrinsic pedal

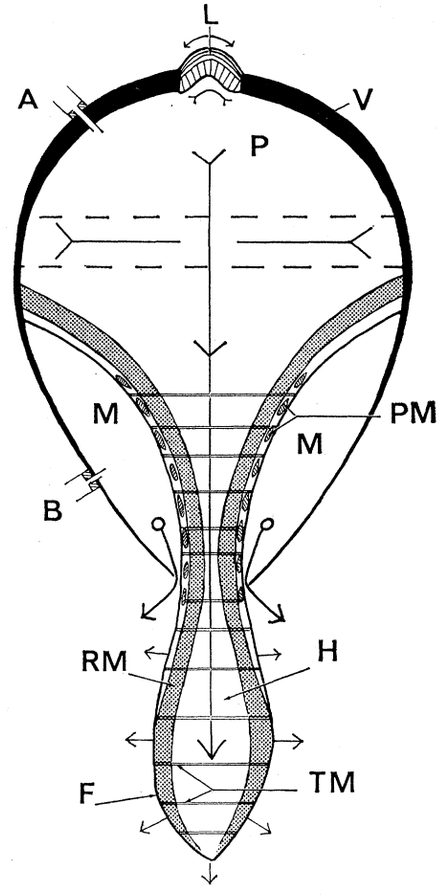

Fig. 1. Diagrammatic transverse section of a bivalve during digging (stage *iii* of Fig. 3), showing: adductor muscle contracted (broken line and >— —<) and tension (<——>) and compression (>——<) of the outer and inner layers of the ligament, respectively; ejection of water from the mantle cavity (o——→); and pressure of blood from the pericardial cavity into the foot (>——→), which causes its extension and outward dilation (——>). *A* and *B*, Location of cannulae; *F*, foot; *H*, pedal hemocoel; *L*, ligament; *M*, mantle cavity; *P*, pericardial cavity; *PM*, protractor muscle; *RM*, retractor muscle (stipple); *TM*, transverse muscle; *V*, valve.

musculature. Contraction of the transverse and protractor muscles (Fig. 1) causes extension of the retractors, generating only low pressures in the pedal hemocoel (Fig. 3). During the digging cycle, adduction of the shell gives rise to a major increase of pressure in the pedal hemocoel; pressure is sustained by retraction of the foot, which occurs immediately after adduction (Fig. 3). Figure 2*a* shows such a pressure peak in the foot of *Ensis arcuatus* and demonstrates that its commencement corresponds to adduction, although the final peak probably represents pedal retraction. When adduction occurs hydrostatic pressure is increased not only in the foot but throughout the whole body enclosed within the shell; pressure recordings obtained from the pericardium and the mantle cavity thus show simultaneous peaks of equal amplitude at adduction (Fig. 2*b*). Blood pressure derived from the heart is low [1.5 cm (water) in the ventricle of *Mya*] compared with that of the body musculature, and greater amplification than that used for Fig. 2 is required to show the ventricular rhythm in recordings of pericardial pressure. In the example shown (Fig. 2*b*), the duration of the pressure peak in the mantle cavity is markedly less than that in the pericardium, for in *Margaritifera*, a freshwater clam, the opposing mantle folds are free and may allow water to escape from the mantle cavity along its entire ventral margin. In such a bivalve, Keber's valve, situated between the pedal hemocoel and the vessel to kidneys and gills, retains the blood in the foot during retraction.

Some bivalves that burrow deeply (for example, *Ensis, Mya*) have the marginal folds of the mantle fused together so as to enclose the mantle cavity apart from the siphonal, pedal, and (in *Ensis*) fourth pallial apertures (*7*). In these bivalves the pressure peaks in pericardial and mantle cavities are of equal duration, and the apertures of the mantle cavity may be used to regulate the pressures generated at adduction and retraction. In *Mya* the blood of the hemocoel and the water of the mantle cavity both act as the fluid of the fluid-muscle system; with the openings of the mantle cavity closed, adduction increases pressure in both pericardial and mantle cavities; this pressure is utilized for siphonal protraction (Fig. 2*c*, *I* and *III*), while, conversely, retraction of the siphon (*II*) produces similar

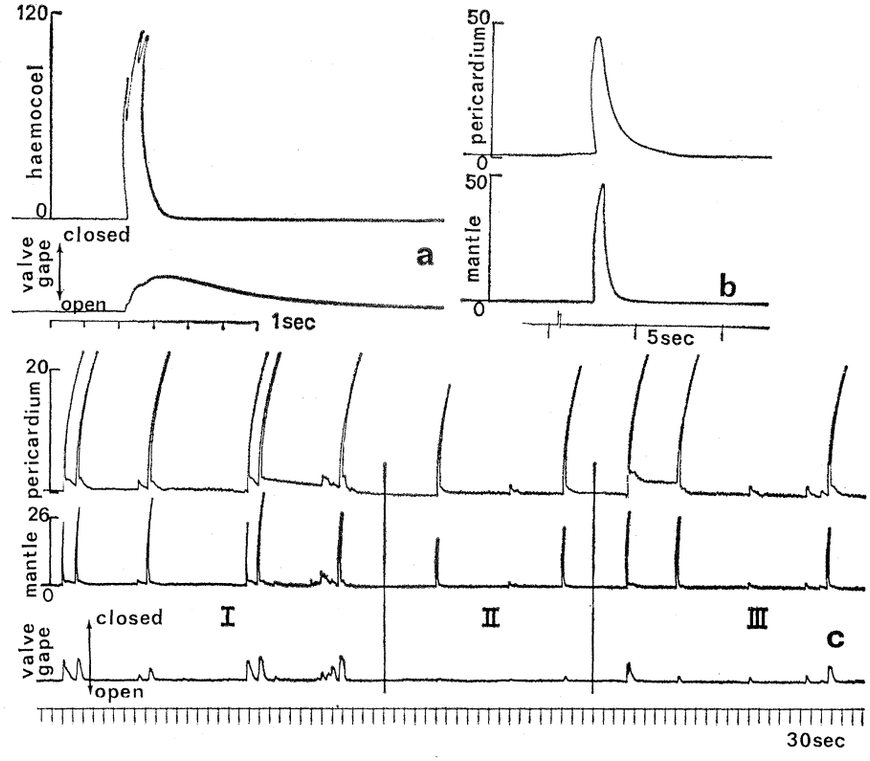

Fig. 2. Recordings of pressure changes in the pedal hemocoel and pericardial and mantle cavities of (*a*) *Ensis arcuatus*, (*b*) *Margaritifera margaritifera*, and (*c*) *Mya arenaria*. *a*, Pressure peak in the pedal hemocoel (top) produced by adduction of the valves (bottom); *b*, pressure peaks of similar amplitude in the pericardium (top) and mantle cavity (bottom) during active digging, recorded simultaneously by cannulation, as shown in Fig. 3; mark above time-trace indicates visual record of siphonal closure; *c*, series of pressure peaks in the pericardial and mantle cavities; adduction of the values corresponds with pressure peaks at the beginning and end of the recording (*I* and *III*, respectively), but in between (*II*) there is little adduction and the peaks are produced by siphonal retraction. Pressure, centimeters of water.

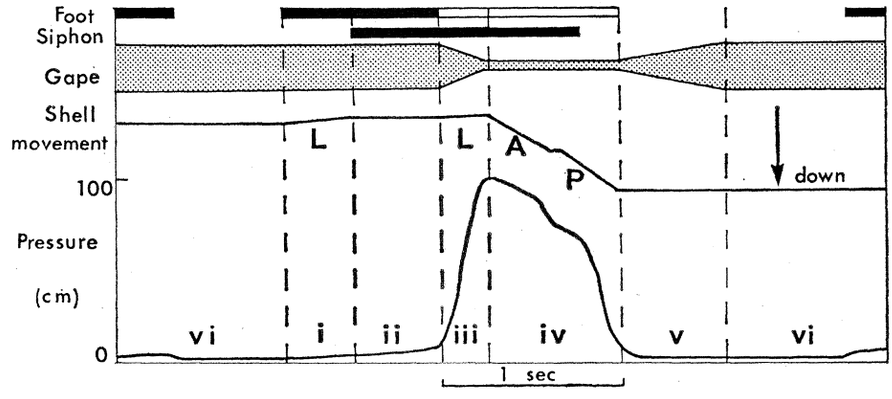

Fig. 3. Diagram showing the principal activities of a bivalve during the digging cycle. Probing of the foot (*Foot*, solid rectangle) and pedal dilation (hollow rectanagle), duration of closure of the siphons (*Siphon*), adduction of the valves (*Gape*, angle of gape being indicated by width of stipple), shell movement, and hydrostatic pressure in the pedal hemocoel are shown in relation to stages *i–vi* of the digging cycle. Protraction of the foot gives a slight lift to the shell (*L*), which is followed by penetration into the sand when the anterior (*A*) and posterior (*P*) retractor muscle contract. Peak pressure in the hemocoel occurs at adduction (*iii*) and is sustained by retraction. Probing by the foot (*i, ii, vi*) corresponds with low-amplitude pressure fluctuations. Pressure, centimeters of water.

pressure changes that tend to increase the gape of the valves (*8*).

The effects of adduction, shown diagrammatically in Fig. 1, are to put the ligament under a strain that subsequently causes the valves to reopen, and to subject the fluids contained within the valves to pressure, ejecting water from the mantle cavity and blood into the pedal hemocoel. Filmed records show that ejection of water liquifies the sand adjacent to the shell immediately prior to retraction, so allowing easier penetration, and that the increased pressure in the foot, together with the relaxation of the transverse muscles distally, causes dilation. Dilation must occur before retraction, in order to give a firm pedal anchorage so that the shell can be pulled down. Thus the bivalve shell effectively operates as a hydraulic machine in which the strength of the adductor muscles is transferred by the body fluids to the distal part of the foot, to be used there during the locomotory cycle.

An important part of the digging cycle is the recovery of the clam during stage *v* (Fig. 3), due to the opening of the valves by the elasticity of the ligament. In the protobranch *Nucula* the ligament is weak and burrowing is both sluggish and superficial, whereas bivalves with more advanced and more powerful ligaments (for example, *Tellina*, *9*) are generally active and deeper burrowers. Some of the hydrodynamic advantages of a hinged exoskeletal structure are thus described; it it hardly surprising that with such a mechanism the Bivalvia have become one of the dominant groups living in soft substrates.

E. R. TRUEMAN

Department of Zoology,
The University, Hull, England

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14 February 1966

Nicotinamide-Adenine Dinucleotide in Tubercle Bacilli Exposed to Isoniazid

Abstract. *There is a decrease in the content of nicotinamide-adenine dinucleotide of tubercle bacilli grown in the presence of isoniazid. In extracts of tubercle bacilli, the activity of nicotinamide-adenine dinucleotidase is nil or very small; after incubation with the drug the enzyme becomes active. Isoniazid also increases the activity of the enzyme after it is partially activated by heating. There may be a correlation between the capacity of isoniazid to activate the enzyme and the decrease in the dinucleotide content of the tubercle bacilli.*

Isonicotinic acid hydrazide (isoniazid, INH) inhibits the activity of mammalian nicotinamide-adenine dinucleotidase. This enzyme is able to bring about an exchange between INH and the nicotinamide moiety of nicotinamide-adenine dinucleotide (NAD) and to form an analog of NAD which has no effect on NAD-dependent dehydrogenase reactions (*1*). Some representatives of the genus *Mycobacterium* produce nicotinamide-adenine dinucleotidase, the activity of which can be detected only after heat inactivation of an inhibitor of the enzyme, present in cell-free extracts of the bacteria (*2, 3*). The bacterial enzyme fails to show analog formation from NAD and INH (*1, 2*), and there is no inhibition by INH of the activity of purified enzyme obtained from tubercle bacilli strain $H_{37}Rv$ (*4*). Isonicotinic acid hydrazide is a potent bactericidal antitubercular drug, and, although the drug induces many biochemical changes in the mycobacterial cells, no correlation between these changes and the bactericidal effect has been shown (*5, 6, 7*).

This report deals with changes induced by INH in the NAD content of *Mycobacterium tuberculosis* $H_{37}Rv$ and with the effect of the drug on the activity of nicotinamide-adenine dinucleotidase of these bacteria.

Tubercle bacilli were inoculated into Dubos-Broth Base Medium (Difco) without added albumin and incubated for 6 to 8 days. After that time, the bacteria were harvested, washed once with saline containing 0.025 percent Tween 80, and inoculated into media containing INH (0.5 μg/ml) and into media without the drug. In some experiments the bacteria were also inoculated into media containing streptomycin sulfate (5 μg/ml). After varying incubation periods the cells were harvested, washed once in saline, and treated with high-frequency sound for 5 minutes in 5 percent trichloroacetic acid. After removal of the protein precipitate, NAD was determined by the fluorescent method with methyl ethyl ketone (*8*), according to a procedure already described (*9*). The activity of nicotinamide-adenine dinucleotidase of the tubercle bacilli was measured by the method based on the cyanide reaction of NAD (*10*). Saline extracts of the bacilli were prepared by treatment in saline with high-frequency sound for about 20 minutes. Activity was assayed in the absence and in the presence of INH (*11*) in both native extracts and extracts heated for 1 minute at 85°C (*4*). In one experiment the effect of INH on the activity of the enzyme was measured in extracts prepared from tubercle bacilli that had been stored for several months at −20°C. The reaction mixture (final volume, 0.9 ml) usually contained 0.8 ml of the extract, having about 9 mg of protein, 0.05 ml of 0.006*M* NAD (*12*), and 0.05 ml of 0.045*M* isoniazid in 0.01*M* phosphate buffer, *p*H 6.5. Before the dinucleotide was added the extracts were incubated with and without INH for 30 minutes, in a water bath at 37°C. After 1 hour incubation with the dinucleotide, 3 ml of cyanide was added. The activity of the enzyme was expressed in millimicromoles of nicotinamide-adenine dinucleotide cleaved in 1 hour by 1 mg of protein.

Assays for the formation of the isoniazid analog of NAD were performed with sound-treated tubercle bacilli, prepared as above for measuring the enzyme activity (*1*). The reaction mixture contained 0.4 ml of extract containing per milliliter: protein, about 8 mg; 0.2 ml of 0.015*M* isoniazid in 0.1*M* phosphate buffer, *p*H 7.2; and 0.2 ml of 0.003*M* NAD. After a 1-hour incubation period in a water bath at 37°C, 2 ml of 0.1*N* sodium hydrox-