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Water Excretion by Hydra

Abstract. *Hydra were cut so that regenerates consisting only of the central gastric region were formed. This region, which has no natural opening to the environment, is capable of osmoregulation and of removing excess fluid from the gut. The fluid is excreted through a break in the body wall created as a result of a strong contraction when the gut is distended with fluid. A normal hydra, therefore, must remove excess fluid by contracting and expelling it through its mouth.*

Previous experiments suggested that osmotic regulation and volume regulation in hydra involved the active transport of sodium from the external medium into the cells and into the gut in one or more steps (*1*, *2*). It was suggested that water followed passively. However, we were unable to demonstrate in any of these previous experiments how water leaves the animal. It was suggested that water leaves through the mouth, largely because histological studies have demonstrated neither pores nor contractile vacuoles in hydra, and there are no openings to the outside with the exception of the mouth and possibly the aboral pore. However, a regenerating section of an animal lacking a mouth regulates just as well as an intact animal with a mouth (*3*). There is a resting potential across the epithelium of a regenerating animal similar to that of normal animals, indicating that sodium transport is similar in intact and regenerating animals. Thus, hydra regenerates and intact animals are continually transporting sodium from the environment into the gut, and water passively follows.

It was suggested that water enters a hydra by diffusive flow but leaves by bulk flow through the mouth (*1*, *4*). Indeed, it is well known that hydra eliminate waste material after digesting prey by contracting and expelling it out of their mouths (*5*). However, the question remains as to what happens in a regenerate which has no obvious route for bulk flow of water. To answer this question, we began a series of experiments in which various dyes were electrophoretically injected into the enteron of *Hydra pseudoligactus* and *H. oligactus* regenerates, reared as described previously (*1*), 24 hours after cutting.

In virtually every experiment in which dye was injected into the enteron of an animal, the animal was observed to burst. After continual failure, it became apparent that we were observing a normal mechanism.

Twenty-four hours after the animal is cut, the regenerate is turgid and quite transparent due to distention of the body wall. By careful observation of regenerates we noted that, 24 to 28 hours after cutting, the animals would contract repeatedly. Eventually a sufficiently strong contraction caused the body wall to burst, and this was accompanied by an ejection of cellular debris and fluid from the enteron (Fig. 1). Upon relaxation, the turgid appearance was gone and the enteron was reduced in volume. The area where the ejection of solids and liquids occurred as a result of bursting was often at the tip of the animal, but occasionally bursting occurred on the side. In 48 to 72 hours the regenerates had formed normal small hydra, which were able to expel fluid through their mouths. If gastric region regenerates were placed in distilled water 24 hours after cutting similar results were observed (Fig. 2). The contraction and bursting of the animal were very rapid after it was placed in distilled water because of the rapid influx of water with the increased osmotic gradient. In addition, when the animals were placed in distilled water, body cells became notice-

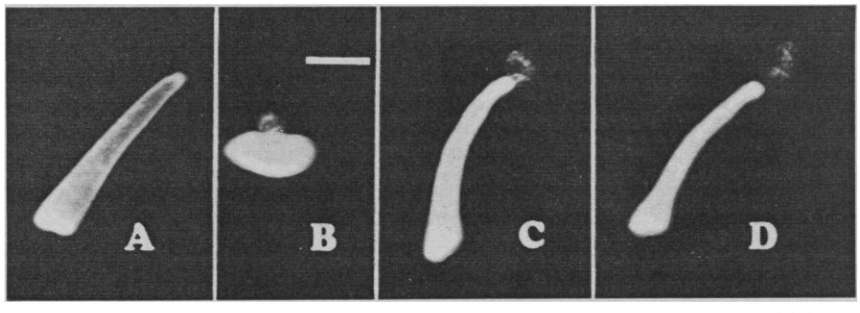

Fig. 1. *Hydra pseudoligactus* regenerate voiding fluid in normal culture water 24 hours after cutting. The turgid, fluid-filled animal (A) contracts until a sufficiently strong contraction (B) causes the body wall to burst. Cellular debris which left the regenerate along with excess fluid can be seen adjacent to the animal. Upon relaxation (C, D) the enteron has returned to a normal volume. The four photographs represent a time span of 15 minutes. Scale line, 1 mm.

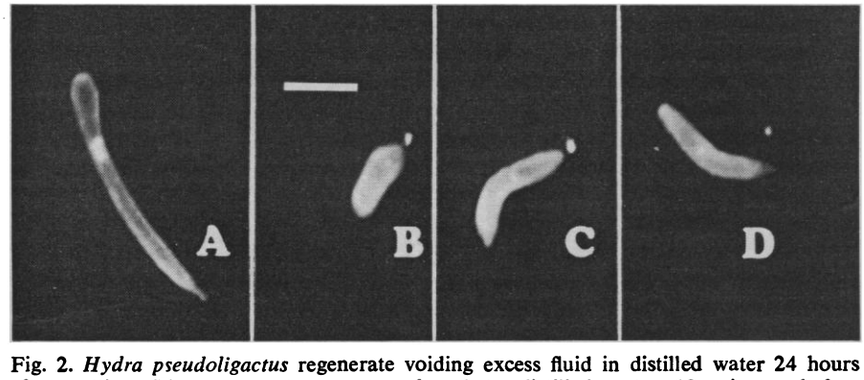

Fig. 2. *Hydra pseudoligactus* regenerate voiding excess fluid in distilled water 24 hours after cutting. The regenerate was transferred to distilled water 10 minutes before it burst (B). The photographs are in temporal order and show the same events as Fig. 1. Scale line, 1 mm.

ably distended. This enlargement of body cells is identical to that reported by Josephson and Macklin (*6*) when the internal gut pressure was increased to 4 to 6 mm of water.

Blanquet and Lenhoff (*5*) observed that tyrosine, when placed in the gut of a hydra, caused a neck to form if glutathione was present in the external environment. Since glutathione specifically elicits the feeding response in hydra and tyrosine is present in the gut because of the breakdown of digested prey, this neck-forming response was suggested to be an adaptive mechanism on the part of the hydra to prevent it from losing its gut contents when ingesting a second prey. We can now suggest why this mechanism is necessary. When a hydra is digesting its prey, its gut becomes turgid because of filling with fluid, and tyrosine in the gut prevents the mouth from opening by increasing the gut pressure required to trigger enteron-emptying contractions. This permits the animal to maintain a higher than normal gut pressure during digestion. After the prey is digested, whatever gut pressure is an adequate stimulus to cause a hydra to contract and expel the gut contents now becomes effective. Thus, tyrosine in the gut is probably not only a stimulus for neck formation but may also modulate the spontaneous contractile pulses (CP's) which could expel the gut contents. The behavior is identical in both cases and, therefore, it might be reasoned that neck formation is related to the mechanism which triggers enteron-expelling contractions.

The turgid condition of the hydra gut has been noted by other workers. Blanquet and Lenhoff (*5*) reported that the animals became turgid just after feeding. Marshall (*4*) reported that tying off an animal just below the tentacles results in the gut cavity becoming turgid. And it is a common observation of workers studying regeneration in hydra that regenerates frequently are noted to be turgid prior to the formation of a new mouth. This is particularly noticeable in regenerates that fail to regenerate a new mouth.

It is now clear how hydra eliminate excess gut fluid. The normal intact animal excretes excess fluid to the environment by opening its mouth and contracting. The presence of tyrosine in the gut is presumably inhibitory to mouth opening, and the stimulus for excreting the fluid is probably enteron pressure. For regenerates that do not have a mouth the response is identical. As the gut becomes distended the internal gut pressure increases and the animal contracts to expel the excess fluid. Since there is no mouth or other natural opening, the contractions may be sufficiently strong to burst the body wall.

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Calcium-Induced Ciliary Reversal in the Extracted Models of "Pawn," a Behavioral Mutant of Paramecium

Abstract. *"Pawn," a genic mutant of* Paramecium aurelia, *cannot swim backward as the wild type can upon proper stimulation. In contrast, after membrane disruption by Triton X-100, the adenosine triphosphate–magnesium reactivated models of Pawns swim backward in the presence of calcium as wild-type models do. Thus, the mutant phenotype is due to an impairment in the membrane and not in the calcium-sensitive motile system.*

The locomotor behavior of ciliate protozoans is largely dependent on a reversal of the direction of the effective power stroke of the cilia (ciliary reversal), which occurs in response to stimuli and causes the cells to swim backward (*1*). Recent evidence (*2*) indicates that ciliary reversal occurs in close association with a membrane depolarization which is mediated by an influx of Ca^{2+} in response to a transient increase in the calcium conductance of the membrane. An increase in cytoplasmic calcium concentration caused by the calcium influx activates a certain calcium-sensitive motile system which governs the orientation of ciliary beating (*3*, *4*).

Mutants of *Paramecium aurelia*, called "Pawn," however, fail to swim backward even in the face of stimuli such as collision with an obstacle and high potassium concentration, to which wild types respond by swimming backward (*5*). This behavioral deficiency in the Pawn can be explained by (i) a defect in the mechanism of calcium conductance increase in the membrane, or (ii) a defect in the calcium-sensitive reversal mechanism in the ciliary motile system, or by both. Recent electrophysiological studies of the Pawn membrane demonstrated a loss of excitability (that is, failure to exhibit an increase in calcium conductance in response to depolarization) (*6*). However, it is still desirable to determine whether any pleiotropic effect in the ciliary mechanism is involved in these mutants. We therefore examined the effect of calcium on the ciliary orientation of adenosine triphosphate (ATP)–Mg^{2+} reactivated,

Table 1. Ciliary reactivation of triton-extracted models of *Paramecium aurelia*. No significant differences in the reactivations were observed between "wild type" (strain 51s) and the mutant "Pawn" (strain d4-95).

Test substances*	Ciliary beat	Ciliary orientation†	Locomotion
EDTA	No	Posterior	No
Mg + EGTA	No	Posterior	No
Ca	No	Posterior	No
ATP + EDTA	No	Posterior	No
ATP + Mg + EGTA	Yes	Posterior	Forward swimming
ATP + Ca	No	Anterior	No
ATP + Mg + Ca	Yes	Anterior	Backward swimming

* Test substances were added into a basic 50 m*M* KCl solution buffered by 10 m*M* tris-maleate (*p*H 7.2 by NaOH). Concentration of each test substance was as follows: ethylenediaminetetraacetic acid (EDTA), 2 m*M*; Mg, 4 m*M*; ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA), 3 m*M*; Ca, 10^{-6} *M*; ATP, 4 m*M*. † Ciliary orientation refers to the direction in which nonbeating cilia point or the direction of effective power stroke in beating cilia.