How-To-Do-It

Quantifying Intracellular Water Regulation in a Single-celled Organism

Barton L. Bergquist

Homeostatic regulation of biological fluids, one of the most important concepts in understanding living system functions, has been demonstrated and taught using varied biological models, e.g. dandelions (Bergquist 1981), erythrocytes (Parsons and Schapiro 1975), oyster-plant and potato tubers (Machlis and Torrey 1956). Yet, it is sometimes difficult to teach this idea as a dynamic concept, rather than as factual statements to be memorized. This article describes a relatively simple laboratory experiment that demonstrates the influx and subsequent removal of cellular water by the common ciliate *Tetrahymena*. Students will gain experience in (1) quantitative biological experimentation and (2) observational and descriptive skills.

The techniques described are designed to allow a class of students to collect data, then analyze data by graphical and statistical descriptions regarding the relations within the experiment. The example depicted includes several descriptive-analytical techniques for use as desired. This exercise is directed toward college level undergraduates.

Tetrahymena, a single-celled protozoan, is a relative of the slightly larger *Paramecium*. While widely found in nature (Corliss 1973), it is also commonly used in biological experimentation (Hill 1972; Elliott 1973; Bovee 1979). The general structure of *Tetrahymena* is illustrated in Figure 1. The spherical water expulsion vesicle (WEV) is located near the cell membrane in the posterior portion of the organism. This structure is also known as the contractile vacuole, but WEV more adequately defines its function. The WEV slowly expands as it accumulates water from the cytoplasm of the cell and then suddenly contracts to expel its contents outside the cell. The process is regulated precisely, giving good data for study and analysis.

The water regulating function of the contractile vacuole in protozoans has been reviewed (Conner 1967; Kitching 1967) and its role in ionic and osmotic regulation in *Tetrahymena* is well documented (Dunham & Kropp 1973).

Tetrahymena is hyperosmotic to fresh water, so that continual entry of water occurs in response to the external solute concentration. In fact, water entry may not be based only upon the classical idea of osmosis, since experimental evidence has suggested that both the "Gibbs-Donnan ratio" and sponge-like imbibition of water by cytoplasmic proteins are involved (Organ, Bovee & Jahn 1978). However, in this study, *Tetrahymena* will be placed in phosphate buffer solutions of differing molarities and, as the inward water flux varies, so too will the need to control the intracellular fluid volume. *Tetrahymena* expels excess water by contraction of its WEV (Dunham & Kropp 1973). Functionally, we can liken the WEV to the multicellular organism's urinary bladder because it stores liquid waste products until it contracts and discharges its liquid contents.

Using one of the methods described here, one can retard the motility of the cells and then count the rate of WEV contractions. The buffer concentration or osmolarity can then be compared with the rate of contraction to illustrate the relationship between the two.

Material and Methods

Supplies for each student or student group

1. Five buffer solutions (15 ml/each). Solutions of 0.066 M KH_2PO_4 and 0.066 M K_2HPO_4 are added together with stirring until pH 6.8 is reached (monitor with a pH meter). The stock (pH 6.8) solution is diluted with distilled water to the desired concentrations over the range 0.002 M to 0.0166 M.
2. Vaseline (petroleum jelly).
3. 2-3 glass slides and cover slips per buffer solution tested.
4. Compound microscope with 200X–400X magnification.
5. Conical centrifuge tubes (12–15 ml), one for each buffer solution plus a balancing tube.
6. Five pipettes (or one which is washed between buffers).

Table 1. Raw Data-illustration from 0.0166 M phosphate solution

Cell #	Contractions per 5 min.	Rate per min.	Cell #	Contractions per 5 min.	Rate per min.	Cell #	Contractions per 5 min.	Rate per min.
1	13	2.6	11	10	2.0	21	14	2.8
2	11	2.2	12	14	2.8	22	11	2.2
3	10	2.0	13	13	2.6	23	11	2.2
4	11	2.2	14	15	3.0	24	10	2.0
5	14	2.8	15	10	2.0	25	15	3.0
6	12	2.4	16	11	2.2	26	15	3.0
7	15	3.0	17	14	2.8	27	10	2.0
8	13	2.6	18	12	2.4	28	11	2.2
9	11	2.2	19	13	2.6	29	12	2.4
10	12	2.4	20	11	2.2	30	15	3.0
	30 cell Composite Average				2.46			
	30 cell Composite Std. Dev.				±0.353			

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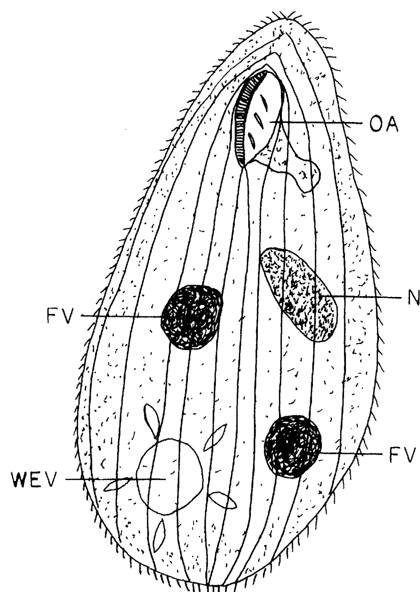

Figure 1. Structure of *Tetrahymena*. FV-food vacuole, N-nucleus (Macronucleus), OA-oral apparatus, WEV water-explusion vesicle.

Supplies for Common Usage

1. *Tetrahymena* in pure culture (may be obtained through biological supply houses). Note: *Tetrahymena pigmentosa* from the author's own laboratory stock was used in the illustrated experiments, other species of *Tetrahymena* are also acceptable.
2. Clinical centrifuge.
3. Measuring pipette (5–10 ml).

Procedure

1. Place a small glob of vaseline (petroleum jelly) in the center of a clean slide. The key here is to get sufficient vaseline in which to entrap organisms, but not so much as to create a mess too thick to be usable. Similar methods of entrapment have been described by Organ et al., 1968 and Organ et al., 1978.
2. Spread the vaseline to form a small raised spot, about 1–2 cm in diameter, on the slide. Make the surface texture of the vaseline spot uneven by alternately touching and withdrawing your finger upward away from the slide.
3. Pipette a small volume of cells (1–5 ml, depending on cell density) from the cell culture into a conical centrifuge tube.
4. Gently centrifuge the cells into a pellet. (Approximately 1.5 min at intermediate speed).
5. Carefully decant the supernatant to leave the cells relatively "dry".
6. Resuspend the cells in 5 ml or more of the buffer to be tested by vortexing or dislodging with squirts from a pipette equipped with a rubber bulb. If the cells have been removed adequately from their culture medium and resuspended in sufficient testing medium, one centrifugation and resuspension is adequate. For more carefully controlled experiments a second centrifugation and resuspension is recommended.
7. Pipette a drop of the cell culture from the bottom of the centrifuge tube into the middle of the vaseline. Be careful not to get too much fluid.
8. Place a cover glass over the spot and gently press it down to squash the vaseline and trap the cells in pockets within the vaseline. Be careful not to overdo this process; this step may require practice.
   Note: An alternate trapping technique can be accomplished by using a small piece of cotton. A dense matrix of the fibers will create enough small cavities in which *Tetrahymena* can be isolated. Care must be taken to use a relatively small amount of cotton which has been flattened on the slide. Chemicals which slow the cells must be avoided to prevent artifacts.
9. Place the slide on the microscope stage and search for cells that are entrapped in a small reservoir of buffer, but are still intact and alive.
10. Find a typical cell and observe the behavior of the WEV (Figure 1).
11. Count the number of contractions of the WEV over a period of five minutes and record your results in contractions/minute.
12. Determine the rate for several cells in each buffer solution. A single slide preparation can be used for several replicates with each experimental solution. Avoid heating by the microscope's light; use minimal light intensity.
13. Repeat using other experimental solutions (0.002 to 0.0166 Molar or other similar concentrations) and record your data. The class may be subdivided into groups each using different molar solutions.
14. Use the t test to identify those rates which are significantly different from each other. Other statistical tests, as illustrated in

Table 2. Composite Data

*Experimental Solution**	*Sample Size (N)*	*Total Counts*	*Rate/Min.*	*Std Dev.*	*t Test Values for pairs*	*Probability*
1. 0.00208 M	30	882	5.88	0.402		
					−1.95	0.056
2. 0.04166 M	30	851	5.67	0.418		
					−8.95	0.0001
3. 0.0833 M	30	678	4.52	0.593		
					−7.15	0.0001
4. 0.0125 M	30	517	3.44	0.532		
					−8.46	0.0001
5. 0.0166 M	30	369	2.46	0.353		

Table 3. Analysis of Variance Table

Source	*Sum Sqs.*	*DF*	*Mean Sqs.*	*F*	*P*
Between	254.735	4	63.68	290.31	0.00001
Within	31.808	145	0.22		

Linear Regression Equation
Form of the Equation
$Y = A + (B \times X)$
Contraction Rate = Intercept + (Slope × Molarity)
Contraction Rate = 6.5247 + (−244.45 × Molarity)

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Tables 2 and 3, may also be used in describing the data.

Treatment of the Results and Discussion

The illustrated experiment may be analyzed using one or more of the following techniques: graphics (Figure 2); paired comparison of groups using the t test (Table 2); analysis of variance and regression (Table 3). A detailed description of the statistics may be found in standard references on the subject (Snedecor & Cochran 1980; Sokal & Rohlf 1981; Zar 1984). Use of these analysis techniques will vary according to the level of the student investigators and purpose involved.

Since the WEV contraction rate depends on buffer concentration, it is appropriate to describe this relationship using a regression; the correlation coefficient (r) would not be suitable (Sokal & Rohlf 1981).

The data show a linear relationship between the concentration of the phosphate buffer solution and the WEV contraction rate (Figure 2). However, nonlinearity occurs at lower molarity. Other experimental data have also shown that *Tetrahymena* changes WEV output in a nonlinear manner at more extreme molarities (Stoner & Dunham 1970). Therefore, use of extreme concentrations in this experimental protocol may also lead to a nonlinear relationship. Other solutes (e.g., sucrose, calcium, potassium, sodium, heavy metals, or combinations of these) might also be usable in alternate types of investigations.

These data were obtained at a temperature of approximately 22°C. Temperature might be a variable to consider for other experiments, but must be held constant in the experiment as defined above.

A class of 20–30 students may be subdivided assigning different ionic concentrations to different groups of students. Like data are then pooled into common groups for analysis.

Variations of this experiment may also be developed as individual student science fair or class projects.

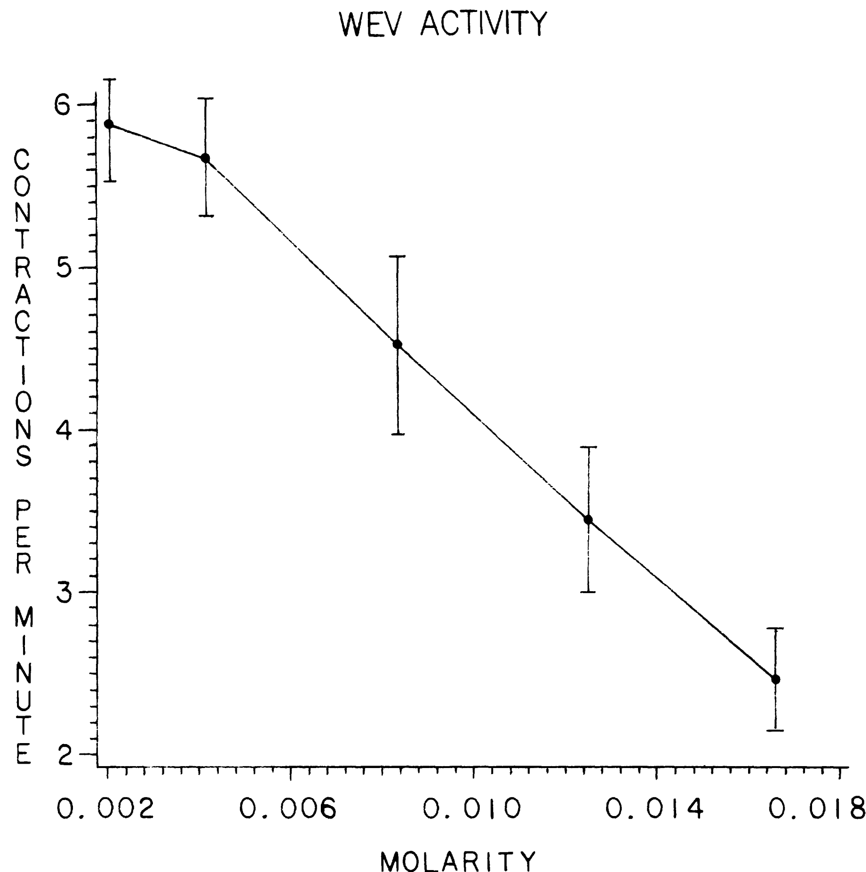

Figure 2. WEV contractile activity in relationship to the molarity of the buffer solutions. Standard deviations for the mean at each tested molarity are indicated by vertical bars.

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