Use of the Vinegar Eel in the Laboratory

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This series of laboratory exercises was developed in the BSCS Research and Development Laboratory at the University of Texas while the author was there. A portion of the work has been published in a modified form in the *BSCS Newsletter*. However, because of the merit of this series, they are reproduced here in their entirety.

Although little use of the vinegar eel has been made in the biology teaching laboratory, the ease of culture and the animal's morphology provide opportunity for a wide range of studies in physiology, embryology, and ecology. The vinegar eel is a free-living, nonparasitic roundworm that is adapted to living in an acidic medium. Because the animal is transparent and viviparous, it is especially useful for demonstration of stages of embryonic development from the fertilized egg to the emergence of the young worm from the body of the female. In fact, within one mature female worm all stages can be observed simultaneously, with the developing embryos arranged in linear order from a single cell to the fully developed young worm. The transparency of the animal allows, as well, studies, *in vivo,* of food digestion and waste excretion. Further, the adaptation of the animals to an acid medium offers possibilities for explorations concerning selection and adaptation for life in media of varying pH values.

The three exercises described here are examples of types of study that can be pursued profitably in either the high school or college biology laboratory.

A starting culture of *Anguillula aceti* (sometimes named *Turbatrix aceti*) can be obtained from a biological supply house. The organism is cultured easily and abundantly in natural bulk cider vinegar that has not been treated chemically to inhibit growth of the bacteria and yeast (mother of vinegar) upon which the worm feeds. The vinegar is obtained at industrial vinegar plants, or it can be purchased in most grocery stores in gallon size containers of a cheap, undistilled grade of vinegar that has a sediment in the bottle. A starting culture designated in a supply catalog as a quantity for a class of 25 students will provide within several weeks a large number of animals. The starting culture is divided between two glass culture bowls, each containing one liter of vinegar. A sheet of plastic film is placed over the bowl to retard evaporation, and ten to fifteen small openings are punched

with a needle in the plastic sheet. Cultures can be maintained indefinitely at temperatures between 20°C and 30°C as long as fresh vinegar is added to replace for evaporation loss. The population of worms may become very dense with a few weeks, at which time new cultures should be started by transferring a portion of the old culture to bowls containing fresh vinegar.

Studies are made with a compound microscope at magnifications of 100X to 400X. At the lower magnifications, it may be necessary to reduce the light intensity. The animals move rapidly with a lateral lashing action produced by the presence of longitudinal muscles and a lack of circular muscles. For observing only the structure of an animal and not its functioning, a glass slide with a drop of culture and a coverslip is held over a flame for a few seconds, or a lighted match can be held under the slide for 3-4 seconds. The heat kills the animals but ordinarily does not distort the structure. Several kinds of stains, such as Delafields hematoxylin, Nile Blue A, crystal violet, or neutral red, make the organs more discernible. Other stains, as well, can be tested for their effectiveness.

I. What is the Nature of Embryo Development in the Vinegar Eel?

Prepare a 0.2% solution of neutral red stain by dissolving 0.2 g of powdered stain in 1 ml of alcohol and adding 99 ml of distilled water. A drop of the medium containing many animals is obtained by placing the end of a capillary tube (inside diameter of bore approximately 1 mm) at the top surface periphery of the vinegar where organisms congregate. It may be necessary to blow gently through the tube to release the drop of animals on to a microscope slide. Add a small drop of the 0.2% neutral red stain to the drop of organisms and cover with a slip. In order to reduce activity of the worms, the 2 drops should be small enough in volume so that the solution does not occupy the complete undersurface of the coverslip. If the animals are still moving too vigorously for observations, it will be necessary to slow them further by withdrawing solution from under the coverslip with a small piece of lens paper or paper towelling placed at the edge of the coverslip. When withdrawing the solution, the animals should be watched at the same time to be certain that only enough moisture is withdrawn to stop the movement of large mature animals. If all solution is removed, the large animals will become distorted in structure.

A mature male is slightly smaller than the mature female. Developing eggs and young worms can be observed readily inside the body of the female. Observe several mature worms and locate all structures that are shown in the diagrams of the male and female, Figs. 1 and 2. Compare the stages of development of a chicken nematode, shown in Fig. 3, with the stages in the vinegar eel. You should be able to find stages in the vinegar eel that are similar to numbers 4-12 of Fig. 3; with diligent searching, the earlier stages can be found occasionally. Complete the diagram of Fig. 1 by adding drawings of the developing worms in the observed positions inside the body of the female. (1) In which position, anterior or posterior, are the most mature embryos located? (2) In which numbered stage is the development of muscles nearing completion? How are you able to determine this? (3) In which stage are the mouth and tail regions first observed?

If you study several slides for a period of 15 minutes or longer, most likely you will observe a young worm in the process of emerging from the body of the adult female. (4) Through what portion of the adult body

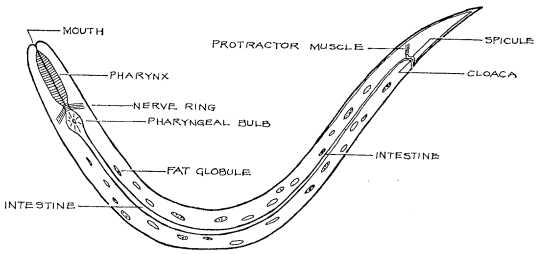

Fig. 1. *Anguillula aceti*, adult male.

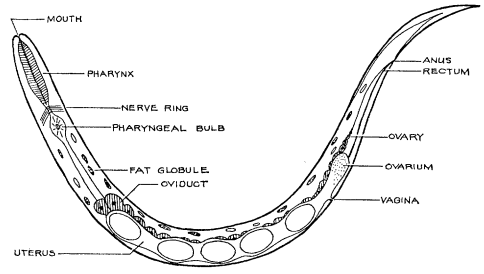

Fig. 2. *Anguillula aceti*, adult female. (Stages of embryonic development are not shown.)

does the young worm emerge? (5) Does it appear that the young worm is expelled by the adult or does the young animal seem to search for a place of escape? Describe your observations. (6) Is the embryo encased in a capsule before birth? If so, is the capsule shed before birth? (7) Does the young animal become uncoiled before or after leaving the body of the adult? (8) What is the length of a newly born worm in comparison to the length of the adult?

To fresh drops of medium containing animals add several dry particles or drops of solution of the following substances: powdered caffeine, powdered adrenalin, chloroform, crushed tranquilizing tablet. Cover each preparation with a coverslip, and after 5 minutes observe the effects of each substance on the worms. (9) Do the worms move about more rapidly or more slowly? Withdraw some of the solution from under the coverslip and observe the developing young worms inside the bodies of females. (10) Does the substance appear to affect the embryos? Describe their reactions. If no reaction is observed to any of the substances, how would you account for this?

References

Barth, L. Embryology, rev. ed. New York: Holt, Rinehart and Winston. 1953.

Buschsbaum, R. Animals without backbones, 2nd ed. Chicago: University of Chicago Press. 1948.

Brown, Frank A. Selected invertebrate types. New York: John Wiley and Sons. 1950.

Hegner, Robert W. Invertebrate zoology. New York: Macmillan Co. 1964.

For the Teacher

In order to obtain abundant mature females, cultures should be maintained for approximately two weeks before the time of the study. Although a class generally needs an outline of procedures, disclosure of results actually denies the student of the personal and often consuming pleasure of making discoveries for himself. Therefore, the suggestion is offered that students be allowed at the beginning of the study to observe the worms with a hand lens held over the culture bowl, and with drops of culture under the compound microscope. After the rapid movement of animals is discovered, the instructions of the first paragraph alone will permit students to discover that because of the animal's transparency the internal organs can be observed. Possibly with only a little questioning, most students will identify males and females. Most likely the first student who discovers the young developing worms inside the body of a mature female will announce his finding—loudly. Only after preliminary observations is it appropriate to distribute the printed exercise with illustrations.

Details of the stages of embryonic development are not included in Fig. 2 of the student exercise in order to allow the student an opportunity to discover that embryos are at various consecutive stages of development with the earliest stage at the anterior end and a fully developed young worm in a posterior location. The details of embryonic stages are provided for the teacher in Fig. 4. Since the embryonic stages of the vinegar eel are very similar to those of the chicken nematode, the teacher may wish to withhold Fig. 3 until near the end of the study period. Note should be made that the chicken nematode deposits eggs outside the body of the female within the chicken intestine where the young hatch from the egg. The chicken nematode, then, is a parasite, and the young are produced in an oviparous manner.

Answers to questions:

(1) The most mature embryos are located in a posterior position in the uterus. The ovary consists of a thread of primary egg cells that arise in a location posterior to the vagina. The egg cells move anteriorally in single-file order through the oviduct to a position where the oviduct joins the uterus. The uterus runs backward to the vagina. Eggs are fertilized near the bend of the junction of the oviduct and uterus, after which the fertilized egg begins to segment as it moves posteriorally through the uterus. Thus, it is possible to observe multiple stages of embryonic development beginning with a fully developed young worm near the vagina to a single fertilized cell at the curvature formed by the junction of the oviduct and uterus.

(2) The development of muscles is nearing completion in Stage 7 of Fig. 3 since movement is first evident in this approximate stage.

(3) A mouth and tail region are first ob-

served in a stage comparable to No. 6 of Fig. 3.

(4) The young worm emerges to the outside through the opening of the vagina.

(5) Quite often a young worm can be observed moving back and forth inside the entire area of the uterus in what appears to be a search for a place of escape. No movements of expulsion on the part of the female have been detected. When observations are extended over a period of 15 to 30 minutes, it is possible to observe the young worm crawling through the opening of the vagina to the outside. Occasionally, a second young worm will begin to move through the uterus before the first worm has emerged to the outside.

(6) Literature references state that the young worm is encased in an embryonic sac. No capsules have been observed by this investigator, nor have any empty capsules been visible after the young worm uncoils.

(7) The young worm becomes uncoiled before leaving the female's body, and at times may become extended full length within the uterus.

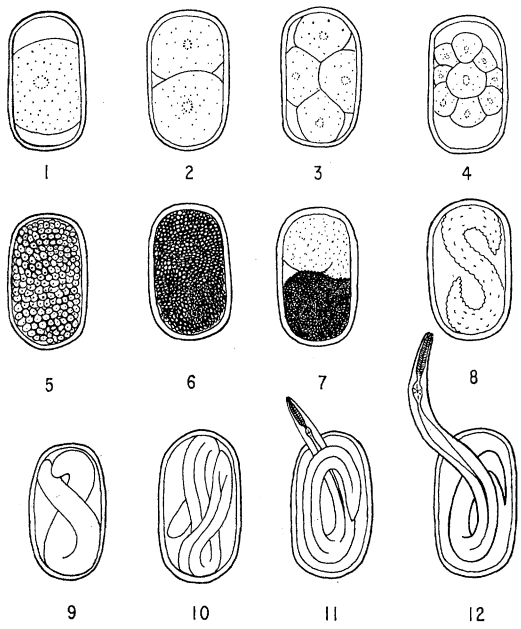

Fig. 3. Development of the egg of the chicken nematode, *Ascaridia lineatus*. 1. Fertilized egg. 2. Two-cell stage. 3. Four-cell stage. 4. Early morula stage. 5 and 6. Late morula stages. 7. "Tadpole" stage. 8 and 9. Vermiform (worm-like) stages. 10. Coiled embryo. 11 and 12. Young worm hatching. (The chicken nematode is oviparous, i.e., the eggs hatch outside the body.)

(8) The young worm is approximately 1/10 the length of a mature female.

(9) In the early experiments performed in this laboratory, no effects from any added substances have been observed. Possibly an external cuticle of the worm results in a slow absorption of solutions, or perhaps stronger concentrations of solutions should have been used.

(10) No affect upon embryos of added substances has been observed, although extensive and prolonged experimentation has not been performed. The subject offers opportunity for further exploration since the addition of foreign substances to an entire culture maintained over several weeks would be a simple operation. A great deviation from normal development or from normal number of offspring possibly could be ascertained quite readily.

II. What is the Nature of Digestion and Excretion in the Vinegar Eel?

Most mature vinegar eels contain many fat globules that cover the digestive tube and thus limit observations. Consequently, animals must be starved for several days before studies of digestion and excretion are performed. To accomplish this, 5 ml of the culture are transferred to a small bottle or beaker containing 10 ml of fresh vinegar. Care must be taken not to remove sediment from the culture bowl when transferring the culture. The bottle or beaker is covered with plastic film that has several openings punched in it. A drop of the culture is examined 24 to 48 hours later at which time the mouth, pharynx and intestine should be visible in most animals.

Part A. What is the pH of the digestive system when the vinegar eel is not digesting food?

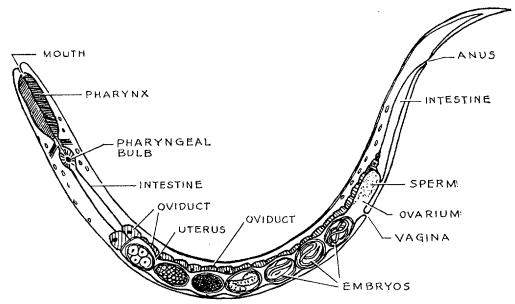

Fig. 4. Female of *Anguillula aceti* with developing embryos.

To a small drop of animals on a slide add one small crystal of Brilliant Green dry stain. Use a toothpick to stir the stain into the drop of vinegar. Allow the crystal to dissolve completely before placing a coverslip over the drop. The solution will be dark green in color. Observe the slide with the low power objective of the microscope. If the animals are moving vigorously, withdraw enough solution from under the coverslip to stop the movement of large animals.

Within several minutes the worms will appear white in color against the dark green background. Use Figs. 1 and 2 to identify the structures of the male and female, if this has not been done previously. Study the animals until one is found that has ingested some of the stain into its mouth region. (1) What is the color of the stain in the mouth and pharynx? Observe this animal or other mature animals until one is found that has a color change throughout the intestine and cloaca regions. (2) What is the color of the stain in these organs?

Brilliant green stain is green at a pH of 4 to 5. Above this pH range the stain becomes blue and then colorless; below pH 4 the stain becomes pink, orange and then yellow. (3) What is the approximate pH of the mouth and pharynx regions of the vinegar eel? (4) Are the intestinal and cloacal regions more or less acid than the mouth? Use a piece of litmus paper to determine the pH of your mouth. This must be done at least 15 minutes after food or drink has been taken into the mouth. (5) How does the pH of your mouth compare with the pH in the mouth of the vinegar eel? From your textbook or from references given at the end of this exercise, learn the normal pH of the stomach and small intestine of the human when no food is present in either organ. (6) How does this compare with the pH that you observed within the intestine of the vinegar eel? (7) Since the vinegar eel lives in vinegar (acetic acid), we might expect the animal to have the same pH inside its body as that of the medium. Is this true? (8) What factors could be responsible for a change in pH inside an animal?

You have observed the pH inside the organs of the animals when no digestion is taking place. In Part B you will supply starved animals with food that has been stained with Brilliant Green to determine if pH changes occur during digestion.

Part B. What is the pH of digestive organs when the vinegar eel is digesting food?

Prepare food for the animals by drawing from the bottom of a jar of fresh vinegar 10 ml of liquid containing sediment. Add 3 small crystals of Brilliant Green dry stain to the 10 ml of sediment and heat gently for 8 to 10 minutes. During the heating, fresh vinegar may need to be added to replace for solution lost in steam. Allow the stained mixture to cool.

Place a small drop of the stained sediment with a small drop of animals that have been starved for several days. Cover with a slip and observe under the microscope. If the animals are moving vigorously withdraw solution from under the coverslip. Study the animals immediately to locate an animal in which yeast cells or other stained particles can be seen moving through the digestive tract. (9) What is the color of the food in the mouth and pharynx regions? (10) What is the color of the food in the intestine? (11) What changes take place in the structure of yeast cells as digestion occurs? (12) In what portion of the intestine does digestion appear to be completed? (13) Do you find any undigested cells at the posterior end of the intestine? (14) Are any cells expelled through the anus to the outside? (15) What is the pH of the mouth region? Of the intestinal region? (16) Does the pH of either the mouth or intestine change when the animal is actually digesting food? (17) If so, how do you account for the change?

Use your text or reference books to learn about pH changes during digestion in the stomach and intestine of the human. (18) How does digestive activity of the vinegar eel compare with that of the human in relation to acidity and alkalinity?

Part C. How Does Excretion of Digestive Products Occur in the Vinegar Eel?

Place a few grains of powdered carmine in a fresh drop of vinegar containing vinegar eels. After approximately 5 minutes you should be able to find animals that have ingested the carmine powder. (19) What is the color of the grains in the mouth and pharynx? In the intestine? Observe one or

more animals during a period of ten minutes or longer. Do you observe the carmine passing through the intestine? (20) Does the animal digest the particles or are the granules expelled through the anus? Describe your observations.

References

Best, C. H. and N. B. Taylor. The living body, 4th ed. New York: Holt, Rinehart and Winston. 1958.

Brown, Frank A. Selected invertebrate types. New York: John Wiley and Sons. 1950.

Carlson, A. J. and H. M. Cavert. The machinery of the body, 5th ed. Chicago: University of Chicago Press. 1961.

Hegner, Robert W. Invertebrate zoology. New York: Macmillan Co. 1964.

For the Teacher, Parts A and B

Occasionally vinegar eels will be filled with oil globules, although generally color changes will be visible in the digestive tube. For best results a good practice is to examine the animals one week before needed and to extend the starvation period if necessary. Cultures of vinegar eels have been maintained in distilled water for a period of two weeks in this laboratory.

If time is available, a much more desirable procedure is to allow students to determine the color changes of Brilliant Green stain by adding dilute sodium hydroxide slowly to a small sample of vinegar containing a crystal of the stain and by adding dilute hydrochloric acid slowly to another sample. Although the pH can be ascertained roughly simply by observation of color changes, a closer pH can be obtained by using pH papers of varying ranges.

Answers to questions:

(1) The stain is a bright green color in the mouth region, through the long, thread-like lumen of the pharynx and inside the circular cavity of the pharyngeal bulb.

(2) The stain changes to a light pink color in the intestine and cloaca region.

(3) The approximate pH of the mouth and pharynx is 4-5.

(4) The intestine and cloaca are more acid, apparently about pH 3.

(5) Generally, the pH of the human mouth is slightly acid, approximately 6.8, although the pH may vary from 6.2 to 7.6. These values are higher than the pH value of the vinegar eel's mouth.

(6) The normal pH of the human stomach is at a range of approximately 5-6 when no digestion is occurring. Normally the small intestine remains approximately neutral except when food first enters from the stomach. The normal pH of the vinegar eel's intestine appears to be approximately 3 to 4.

(7) From early investigations the pH of the mouth and pharynx of the vinegar eel appear to be about the same as that of the vinegar medium in which the worm lives. Apparently the pH value is lower in the intestine.

(8) Conceivably acids may be secreted in the intestine of the vinegar eel which cause a lowering of the pH in that region.

(9) The food is a bright green color in the mouth and pharynx of the vinegar eel.

(10) When the animal is given food, the color of the intestine changes from pink to yellow indicating that the pH is lowered during digestion.

(11) Although spherical or oval yeast cells are observed easily in the pharynx, they have not been observed in the intestine. Apparently the cells are broken down in the pharyngeal bulb, where a grinding action may be applied, although this has not been observed.

(12) Apparently digestion is completed in the pharyngeal bulb and in the anterior portion of the intestine.

(13) No undigested cells have been observed in any portion of the intestine.

(14) No observation has been made of expulsion of undigested yeast cells through the anus.

(15) The pH of the mouth is 4-5; of the intestinal region, approximately 2 or lower during digestion.

(16) A lowering of the pH in the intestine during digestion has been observed regularly.

(17) Possibly an acid or acids are secreted in the intestine during digestion.

(18) In the human, the stomach is acid during digestion, but the food chyme is changed to an alkaline condition upon entrance into the duodenum due to the alkalinity of the digestive juices secreted into the small intestine. According to results of investigations in this laboratory, the entire length of the vinegar eel's intestine remains acid during digestion.

For the Teacher, Part C

If time is available, it is most desirable that students test carmine powder in acid and base solutions to learn if color changes take place. Their tests will show that carmine powder remains a bright red at all pH values. Therefore, carmine will not indicate digestive color changes, as observed with Brilliant Green stain, but students can observe quite easily whether or not the carmine grains are dissolved, thus providing evidence of a possible digestion of the carmine powder.

After 5 to 10 minutes the individual grains will be found in the intestine, where they are washed slowly backwards and forwards. With careful and prolonged observations the red grains can be seen passing through the anal opening to the outside. Because the anus is on the ventral surface of the worm, and because carmine grains will be present in the fluid surrounding the animal, possibly only a few students will be able to observe the actual passage of grains to the outside, although all class members should be able to follow the movement of grains from the anterior region to the posterior region of the intestine.

Answers to questions:

(19) The carmine is a red color in all regions of the digestive tube.

(20) The grains are not digested and are expelled through the anus to the outside of the body.

III. Can the Vinegar Eel Survive at a Higher or Lower pH?

Because the vinegar eel normally lives in acetic acid, a pertinent question can be asked regarding survival of these animals when placed in a medium of a higher or lower pH. A number of procedures can be followed for finding an answer. One possible procedure is described here. Prepare 200 ml each of 0.2N NaOH and 0.2N HCl. Number 8 test tubes and prepare solutions in each tube according to information given in the accompanying table. Measure the pH of the solution in each tube and record in the table.

In order to obtain the survival rate of animals before adaptation, prepare a second set of 8 test tubes in the same manner as before. Place 5 ml of medium containing animals in each of tube 1-a and tube 1-b. After one minute hold each tube before a bright light to determine the quantity of animals that are still moving about. Record the survival rate as *many, few,* or *none* in the table under the column, *Survival Before Adaptation.* Immediately transfer 2 ml of solution containing animals from test tubes 1-a and 1-b to test tubes 2-a and 2-b respectively. After one minute determine the survival rate as before and record. Repeat the transfers and observations through test tubes 3-a, 3-b, and 4-a, 4-b.

Following the determination of survival before adaptation, the animals are adapted over a period of nine days to increasing concentrations of NaOH and HCl. To accomplish this, follow the schedule given below.

Day 1. Place 5 ml of medium containing animals in each of tube 1-a and tube 1-b.

Day 3. Determine the survival of animals in tubes 1-a and 1-b by holding the tube before a bright light. Record the survival as *many, few,* or *none* in the column of *Survival After Adaptation.* Transfer 2 ml of solution with

Survival of Animals Before and After Adaptation Periods

Test tube	pH	Survival Before Adaptation	Survival After Adaptation
1-a 5 ml medium with animals 10 ml vinegar 5 ml 0.2N HCl			
2-a 10 ml vinegar 10 ml 0.2N HCl			
3-a 5 ml vinegar 15 ml 0.2N HCl			
4-a 20 ml 0.2N HCl			
1-b 5 ml medium with animals 10 ml vinegar 5 ml 0.2N NaOH			
2-b 10 ml vinegar 10 ml 0.2N NaOH			
3-b 5 ml vinegar 15 ml 0.2N NaOH			
4-b 20 ml 0.2N NaOH			

animals from tubes 1-a and 1-b to tubes 2-a and 2-b respectively.
Day 5. Observe test tubes 2-a and 2-b and record as before. Transfer 2 ml of organisms from tubes 2-a and 2-b to tubes 3-a and 3-b respectively.
Day 7. Observe and record survival of animals. Transfer 2 ml of organisms from tubes 3-a and 3-b to tubes 4-a and 4-b respectively.
Day 9. Observe tubes 4-a and 4-b and record survival of animals.

Questions

1. What are the highest and lowest pH values at which organisms survive before an adaptation period?
2. Do your results provide evidence that an adaptation to changes in pH may have occurred? If so, at what levels of pH do you find evidence of an adaptation?
3. Considering the fact that about 8 days are required for the development of the embryo from fertilized egg to birth, do you believe that a mutation or recombination of genes were relevant factors in the adaptation of any animals to a higher or lower pH value?
4. Use your text or references given below to learn about physiological adaptations in animals. From your reading present a discussion of homeostasis, or "steady state," as related to a possible explanation of adaptations of the vinegar to various pH ranges.

Further studies

Maintain the tubes with surviving animals for several more weeks and study to determine if a change in pH has modified the growth pattern or reproduction rate. Compare the number of young animals and the number of developing embryos in tubes 1-a and 1-b with those in other tubes.

References

Lerner, I. M. Genetic homeostasis. New York: John Wiley and Sons. 1954.
Overmire, Thomas G. Homeostatic regulation. BSCS Pamphlet 9. Boston: D. C. Heath. 1963.
Selye, H. The stress of life. New York: McGraw-Hill. 1956.
Weisz, P. B. The science of biology, 2nd ed. New York: McGraw-Hill. 1963.

For the Teacher

This study can be varied extensively, particularly with concentrations of acid and base solutions. An extended range of pH and a longer adaptation period may produce a greater adaptation than is obtained with the procedure outlined in this exercise.
Answers to questions:
1. Results of early tests indicate that before the adaptation period many animals will survive for at least 5 minutes at pH 3 and pH 7. After the adaptation period a few animals survived at pH 2.5 and pH 8. More testing is needed. A more gradual change in pH and a longer adaptation period may give more effective results. Students may wish to make their own explorations.
2. Adaptation to increased acid appeared in Tube 2-a with results of early tests. Adaptation to increased alkaline appeared in Tubes 2-b and 3-b.
3. Because the adaptation period extends over only a nine day period, it is unlikely that adaptation will be produced by mutation or recombination of genes with a subsequent selection.
4. Discussion for the subject of homeostasis can be found in the reading references.

Stewardship

A new book called *Stewardship,* beamed directly at owners of open land in the New York metropolitan area, now is available from the Open Space Action Committee, 205 East 42nd Street, New York 10017. The theme of the book is so timely and its message so persuasive and informative, however, that *Stewardship* should be prized by individuals and groups concerned about unregulated urban growth anywhere in the nation. By example and case history it charts what can be done. Illustrated with photographs and designs, it sells for $3 in paper and $6 in hard covers.