Use of invertebrate animals to teach physiological principles

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Deyrup-Olsen, Ingrid, and Thomas M. Linder. Use of invertebrate animals to teach physiological principles. Am. J. Physiol. 260 (Adv. Physiol. Educ. 5): S22-S24, 1991.—Experiments with invertebrate animals offer valuable opportunities in the teaching of physiology. In some cases, exercises that use these animals may demonstrate physiological principles more clearly than experiments that use vertebrates. Other experiments are easy to perform because of the latitude of conditions in which the tissues of many invertebrates function. Experiments with invertebrates can also illustrate a far wider range of physiological mechanisms than occurs in vertebrates and are especially suited for opportunities for independent original investigation by the students. Since at this time invertebrates are underutilized in teaching, much can be gained by the design, testing, and dissemination of innovative experimental protocols for student use.

Laboratory studies of living animals have long been recognized as central to meaningful instruction in physiology and are indispensable even in today's environment with its pressures to process students as efficiently and inexpensively as possible by substituting workbooks, videotapes, computer simulations, and other devices for experience with actual living forms. Much that is valuable can be lost in this process, including the essence of the physiology. Students at all levels should do more than learn seeming "facts," no matter how creatively presented. They should also observe nature and practice science. Only through work with real animals can students come to appreciate fully the exquisitely controlled, interactive complex of biological processes that constitute life. Moreover, the results of real experiments are rarely as clear-cut as one might imagine, and animals are more variable than textbooks imply; living forms offer many surprises and unanswered questions.

Regrettably, the study of animals by students is becoming increasingly difficult, not only because of external pressures imposed by the "animal rights" movement against the use of vertebrates but also because of environmental changes. Amphibians and reptiles, including the familiar frogs and turtles of conventional physiology teaching, are dwindling in numbers and even becoming endangered.

The use of invertebrates offers solutions to some of these problems. Invertebrates are readily available, there is an immense range of species, and these animals have physiological processes both shared with and different from those of vertebrate animals. Experiments with invertebrates, long championed by great researchers and teachers, including W. S. Hoar, C. L. Prosser, and J. H. Welsh, contributed to important developments in present day physiology.

We do not wish to imply, however, that vertebrates should not be used in physiological laboratories, nor that invertebrates can replace vertebrates in every particular. In the present paper we would emphasize, rather, the expanded possibilities that invertebrates bring to teaching. We offer examples based on our own teaching experience and cite published protocols where possible. In some cases, however, such materials are not available and we have had to improvise, adapting research data directly for use in our teaching laboratories. This has proved to be a highly rewarding and stimulating experience for both ourselves and our students.

Advantages Offered by Studies with Invertebrates

Laboratory exercises using invertebrates may illustrate a physiological principle with greater clarity than exercises using vertebrates alone. The use of the frog sciatic nerve to demonstrate action potentials is a case in point. The student dissects the nerve from a frog and lays it on wires in a chamber. If the dissection has been done skillfully, an electrical stimulus to one end of the nerve will trigger all-or-nothing action potentials that travel along the axons. However, what does the student observe in the electrical recording? As the student increases the intensity of the stimulus, the active response appears to increase in a stepwise fashion. The single most important characteristic of an action potential, its all-or-nothing character, is masked in this preparation. Indeed, a naive and uncoached observer would deduce that the active response of the nerve is graded. In the laboratory we wish above all to ask a student to observe and draw conclusions. With the frog sciatic nerve preparation, this process leads to false inferences.

In contrast, when nerve activity is recorded from certain nerves in arthropods, the important characteristics of the action potential are obvious. With the cockroach (Periplaneta sp.) leg preparation, for example, the student can observe discrete pulselike potential changes when individual sensory spines are moved and can verify that the action potentials of each neuron are of uniform size (8; T. M. Linder, and J. M. Palka, unpublished observations). Also, increasing the displacement of a spine increases the frequency of action potentials, illustrating the coding of intensity by frequency. Finally, the action potentials are observed in a biological context; they are linked in an obvious fashion to the sensing of...
mechanical movement. In this instance, then, exploring an invertebrate system leads not just to a substitute experiment but to results more informative than those obtained in the simple vertebrate system.

A second and perhaps more familiar example is the study of the effect of changing environmental temperature on metabolic rate, typically assessed by measuring oxygen consumption (rarely, carbon dioxide production) as a function of time (6). In many invertebrates, metabolic rate increases with rising temperature throughout the physiological range. In other cases metabolism is quite stable despite shifts in temperature. Ectothermic vertebrates show similar patterns. These observations naturally open up discussions about the effects of temperature on the rates of biochemical reactions and what compensatory mechanisms could allow animals to stabilize metabolic rates. Even more significantly, the observations provide a background against which to interpret effects of temperature change on the metabolic rates of mammals, including humans. In these animals changes in metabolism seem to run counter to general expectations of the effects of temperature change on the metabolic rates, typically assessed by measuring oxygen consumption (rarely, carbon dioxide production). If one begins at the lowest physiologically tolerable temperature and gradually increases the temperature, the energy expenditure of the mammal declines, rather than increasing, until the "thermal neutral zone" is reached. This result points clearly to the intervention of thermogenesis as a regulatory mechanism and provides an excellent starting point for exploring the importance and consequences of homeostasis. In this way, the observations on ectothermic animals supply a background that moves the study of effects of temperature in the direction of understanding regulation. Without this background, students tend to stay in the "we shiver to stay warm" mode of thinking.

Through further work, students can learn that ectotherms often have at least some capacity for regulating their body temperature. However, the regulation is behavioral, since the animals manipulate their temperature by responding to thermal gradients and selecting relatively favorable environments. Such behavior is readily demonstrated in the student laboratory, for instance, using crayfish in aquaria with thermal gradients established with heating and cooling devices. Thermal preferences of crayfish change with metabolic states, and these animals respond to bacterial infections by raising body temperature through the selection of warmer-than-normal environments, a phenomenon termed behavioral fever (2). Behavioral fever appears to be adaptive in ways similar to physiological fever in endothermic vertebrates suffering from bacterial infections. A controlled rise in temperature, whether behavioral or physiological, is advantageous to the host as it copes with bacterial parasites and their toxins. Observations of this type with invertebrates illustrate vividly that the fever response is active and not merely a passive consequence of infection. The concept can then be generalized to endotherms, where the principle would be difficult for students to uncover by direct observation.

Invertebrates extend the range and variety of physiological processes available for examination by students. The use of invertebrates reinforces themes dear to comparative physiologists. When a mechanism is observed to be present in all animals, one appreciates the fundamental importance of the process and the interconnectedness of life. However, even those mechanisms that vary in different animals stimulate insights, because they show the variety of solutions that have evolved to deal with common physiological requirements.

Invertebrates, for example, solve problems of circulation by using a variety of pulsatile organs, including myogenic and neurogenic hearts. Clams (Mercenaria sp., Saxidomus sp., and other species) and amphibians provide the classic myogenic preparations (3a, 4). The widely available crayfish (Procambarus sp., Pacifastacus sp.) supply an easily obtained, hardy neurogenic preparation (3b). In the crayfish, both inhibitory and excitatory nerves from the central nervous system can be stimulated, and pharmacological experiments with transmitters and blockers can be performed. It is also possible to set up a demonstration recording the electrocardiogram of the intact crayfish as it moves about an aquarium and in this way observe the neural control of the heart as the animal interacts with its environment.

Muscles also provide interesting comparisons because they are so diverse. Near one extreme is the classic frog gastrocnemius muscle, which is highly specialized for fast contractions used in jumping. At the other extreme is the byssus retractor muscle of the mussel (Mytilus) (7). This muscle exerts slow steady tension on the byssus threads attaching the animal to its substrate. Mussels are widely available, and the preparation is comparatively simple and reliable. In between these two extremes are a variety of other useful muscle preparations.

Many experiments with invertebrates are easy to perform. Frequently the tissues of invertebrates, like those of amphibians, function well at room temperature. This allows the use of simple equipment without temperature control or an external oxygen supply. For example, the crayfish hindgut illustrates many of the same principles as the rat intestine (3c). The hindgut shows spontaneous rhythms and offers good opportunities for studying excitatory and inhibitory transmitters and their blockers. The crayfish hindgut has the advantage, however, that it works well at room temperature and does not require special oxygenation.

Cilia are important effectors in most animals. In mammals, for example, they play crucial roles in the respiratory and reproductive systems. However, because cilia are difficult to study in mammals, students have long used the frog palate/pharynx preparation as a model system. Other equally available models are offered by the gills of molluscs, such as mussels (e.g., Mytilus sp.) and clams (6). The preparations are simple to set up. First, one of the halves of the shell is removed, exposing the underlying gill. Then the gill is immersed in sea water, or another appropriate medium, and ciliary activity is observed by following the movement of small particles over the tissue surface. Alternatively, the beating cilia may be viewed with the microscope. The preparation also yields interesting results with changes in variables, such as temperature, ionic composition, and osmotic pressure. Students may also investigate how crude oil and other environmental toxins affect the cilia.

There are unlimited possibilities for student research with invertebrate systems. One of the most valuable ex-
practices for an undergraduate is to work independently on a project. Here invertebrates can be especially useful. In many cases they provide an ample source of specimens to be used for developing techniques and collecting data. Also, invertebrates often are not nearly as widely studied as vertebrates, allowing a student rich opportunities for striking out in an original direction.

For example, students in our physiology laboratories observe active transport of ions in the midgut of the tobacco hornworm (Manduca sexta), an insect that is readily available from major suppliers (5, 11). The preparation is somewhat more delicate than the frog skin but yields results on ion movements that are as clear-cut and informative as those from studies with frogs. There is the advantage, moreover, that a student who wishes to explore the Manduca preparation is confronted by numerous unanswered questions related to the biology and ecology of leaf-eating insects. The work may even take an applied turn, as in examining the practical issue of insect control.

The use of invertebrates in teaching physiological principles also yields a broader perspective on research. As a profession, physiology today tends toward an even narrower focus on a relatively few “model” species. This restriction is unquestionably productive in the short run but leads to neglect of the vast array of animals and the many novel solutions that have evolved to deal with physiological problems. Original experiments with invertebrates in undergraduate laboratories may alert students early in their careers to possibilities for research beyond the standard vertebrate species. Further, many problems of human biology involve invertebrates, ranging from parasitic species such as protozoans and nematodes to members of the great animal phyla, Arthropoda and Mollusca. These animals interact with humans as competitors for food and fiber resources and as vectors of disease. They are also crucial to the survival and reproduction of many plants and participate in various other positive ways in plant-animal relations. We would hope that students working with invertebrates in physiology laboratories may gain some insight into the relationships of other species with our own.

Practical aspects of teaching with invertebrate animals. Useful background information on experimental procedures with invertebrates in the teaching laboratory can be found in a few current laboratory manuals (6, 8) and occasionally in journals (7a).1 Animal supply companies offer many invertebrate species, and animals may also be collected from the environment, as in the case of crayfish, which are commonly found in freshwater lakes and streams, and mussels and clams, which are found at seashores. The latter species also are readily available in fish markets. Generally both procurement and care of invertebrates are relatively simple and inexpensive compared with maintenance of vertebrate animals. Most conventional teaching equipment can be adapted directly for studies with invertebrates.

Nevertheless, there are difficulties in implementing the effort to increase the use of invertebrates in physiology teaching. Perhaps the foremost is the fact that many physiologists, trained somewhat narrowly at today’s cutting edges of research, have had little or no experience in working with invertebrate animals. Additionally, laboratory manuals concentrate primarily on animals of a few familiar vertebrate species, chiefly frogs, turtles, rodents, and humans. Although the research literature abounds with interesting experiments that potentially could be transferred to teaching, many physiology instructors do not have the time to adapt and test these experiments for easy use by students. There is need, therefore, for development and publication of appropriate experiments based on invertebrate species. The present paper is written to call attention to this need, as well as to the richness, variety, and intellectual stimulus of laboratory instruction using invertebrate animals.

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REFERENCES