

Tapping Recent Alumni for the Development of Cutting-Edge, Investigative Teaching Laboratory Experiments

Mark R. Brodl

Department of Biology
Knox College
Galesburg, IL 61401
e-mail: mbrodl@trinity.edu

ABSTRACT: This project presents a model for the development of an innovative, highly-experimental teaching laboratory course that centers upon collaborative efforts between recent alumni currently enrolled in Ph. D. programs (consultants) and current faculty. Because these consultants are involved in cutting-edge research, their combined talents represent a much wider range of expertise than any individual faculty member could bring to teaching laboratory development. Furthermore, the consultants' understanding of the context of the institution and its curriculum uniquely qualifies them to serve in this capacity. In this particular project, this model was applied to laboratory course development for Biology 308, a course in cellular vertebrate physiology. Consultants were selected who were involved in research in the areas of renal cell physiology, cell motility, hormonal signaling, immunology, and neurology. Each consultant's experimental system was used to develop a teaching exercise that investigates topics relevant to the course. These are areas covered in Biology 308 that were either not previously represented in the laboratory course, or the original exercise in these areas was highly routine and unengaging. The project has significantly increased the breadth of expertise at Knox. In addition, course evaluations have indicated that the students find the lab course more interesting and significant. Students are more thoroughly engaged with the exercises because they feel they have a greater stake in their outcome.

KEYWORDS: graduate student-undergraduate student interaction, investigative teaching labs, sodium/potassium ATPase α -subunit, teaching laboratory course development, undergraduate

INTRODUCTION

Given the limited size of small liberal arts colleges, faculties are often asked to teach classes outside of their formal training so that a department can cover its discipline. At Knox we have a biology department of only five faculty members. Though I am a plant cell and molecular biologist, I was asked to teach an upper-level course in vertebrate physiology with a decidedly cellular focus. The course had a legacy of being very popular with our majors, and it was seen as essential for students pursuing graduate or medical school. With prerequisites of three introductory biology courses in ecology and evolution,

organismal form and function, and cell and molecular biology and with introductory chemistry strongly recommended, it was mostly populated by juniors and seniors. The course I initially developed and taught in my first years at Knox took a traditional lecture-and-lab approach. I felt much more comfortable in the classroom, because the lecture portion covered material well within the grasp of any respectable biologist, but the laboratory section was an altogether different challenge. In constructing a lab experience, I dug through countless lab manuals, and after making what I felt were necessary modifications and improvements, I had what I thought was a set of lab experiences that

supported the lecture well. But as I taught the course, I became greatly frustrated by how superficial the experiences seemed to be. For students the next requirement in the biology major after Vertebrate Cellular Physiology is independent research, and the experiences obtained in my teaching lab were not preparing students as well as they might to make the most of an opportunity to work independently. The biggest problem was that the laboratory section asked the students to do exercises rather than experiments. I knew well that the best learning would result from open-ended experimentation (National Science Foundation, 1996; Rothman and Narum, 1999). Furthermore, I did not feel invested in the laboratory course. In my other courses, I had developed the exercises from my own expertise in the field. This could not be easily accomplished in this course; I simply did not have the training.

It was a conversation with a former student of mine, then enrolled in a Ph. D. program in cell biology, that got me thinking about an answer. After hanging up the phone from our conversation, I found myself wishing there was some way to get her to help me develop a lab based on the work she was now doing in hormonal signaling. From here, I made a list of other former students enrolled in Ph. D. programs in fields encompassed by the course. I got in touch with each of them about the work they were doing and asked them if they might be interested in working with me to use their experimental systems to develop cutting-edge investigative teaching experiences for the Vertebrate Cellular Physiology course. Recruiting former Knox students would help insure that the laboratories were appropriately challenging yet “do-able” at Knox. The National Science Foundation (NSF) had just announced a new Leadership Project under their Improvement in Laboratory Instruction Program that was an ideal funding source for the project (this program is currently known as Curriculum, Course and Laboratory Improvement). Eight months later I had NSF funding and the project was under way.

Details of the Approach

Five Knox alumni (graduate student consultants) and their Ph. D. thesis advisors signed onto the project. I visited each of their research laboratories to see first-hand how their experimental system worked. This visit also gave us an opportunity to lay out preliminary ideas for teaching laboratories. For two to three months following each visit, the consultant and I kept in close contact via phone, e-mail and fax. We brainstormed through several possible teaching experiments, with the goal of focusing our efforts on the one with the best potential for success. We also sometimes bounced ideas off of the thesis advisors. The first requirement was that the exercise had to be investigative. It was also important that the experiment was structured so that it made full use of the scheduled laboratory period, which is 150 minutes in duration; though returning to

the laboratory outside of formal meeting times was possible, we tried to keep “off hours” at a minimum. To insure the experiments could be sustained once the grant expired, we also worked to keep costs down as much as possible. Also, we put a premium on experiments that approximated, as much as possible, the real excitement of the research laboratory. We looked for experiments that utilized research instrumentation available in the department and gave the students a sense of investment in their work and challenged them to bring concepts they were learning in class to the research bench.

Once focused on the candidate experiment, we developed a step-by-step protocol from which we made a list of the necessary material and equipment. We also developed a bibliography, including references for background, methods, and general principles. After we had worked through the logistics, and the supplies and equipment were secured, the graduate student consultants visited our campus to run through the experiments on a trial basis. We made notes about any errors or lack of clarity in the protocol, including potential bottlenecks where students may likely make a mistake, and what the “pre-lab” lecture would have to emphasize. Finally, we discussed how student performance in the laboratory would be evaluated.

A total of five exercises were developed, covering hormonal signaling through the G-protein cascade, interferon in the immune response, actin-based cell movement, nerve growth factor (NGF) in neuron-neuron synapse formation, and Na^+/K^+ ATPase expression and nutrient uptake in nephrons. The exercises were two to three laboratory periods in duration (i.e., 2 to 3 weeks). In some cases an exercise was initiated the same day that another exercise ended. In addition to time spent in the scheduled laboratory period, most of the experiments did require some time outside of class. Most of this time was spent on growing, maintaining, and manipulating cell cultures. The NSF grant provided funding to equip a tissue culture facility (The Center for Cell and Tissue Culture) that is located directly adjacent to the teaching laboratory. It also provided funding for site visits and modest stipends for the project participants as well as supplies for the first two years. The total cost for running the labs was estimated at \$165 per student.

An Example Laboratory

The experiments conducted on Na^+/K^+ ATPase expression and nutrient uptake in nephrons are presented as an example of the sort of laboratory experiments we developed and how the students were engaged in the laboratory. This exercise was introduced with assigned readings on the expression of the alpha subunit of the Na^+/K^+ ATPase (α -SU) in Maiden-Darbey Canine Kidney (MDCK) cells and its dependence on Ca^{2+} for the establishment of the cadherens junctions necessary for establishing and maintaining cell polarity in epithelial tissues (Cantley,

1981). The first reading(s) in each unit was discussed as a class, setting an example for how to approach and analyze a paper. It also provided an opportunity to begin building a model of how the system operated, which became a model, in turn, for talking about potential experiments. Following this, the students read two additional papers in the field (Caplan *et al.*, 1986; Hammerton *et al.*, 1991). They were encouraged to discuss these papers among themselves. Our next discussion entailed adding details from their readings to our working model and then identifying reasonable questions that could be asked (Fig. 1). In this particular case, the students had read that the polarized expression of α -SU could be established within 24 h of cell confluence if Ca^{2+} were present; without Ca^{2+} , polarized expression could be established in cultured cells 18 h following its addition. They asked many

good questions with solid rationale for asking them. It was then important to help them realize that there are constraints about what types of experiments can be done in science to answer those questions (and that at Knox there are even more constraints) (Fig. 1). To obtain answers to the questions, the students identified the sort of experiment that would have to be done. Through this process we determined that as a class we would investigate what happens to the expression of α -SU when confluent cells grown in Ca^{2+} -containing medium are shifted to Ca^{2+} -free medium (with EGTA). This process was not left completely to chance. The papers the graduate student consultants and I selected for the students to read were chosen to point to fairly obvious experiments that could easily be done at Knox. In addition, I did play a role in steering the discussion toward the most feasible investigations.

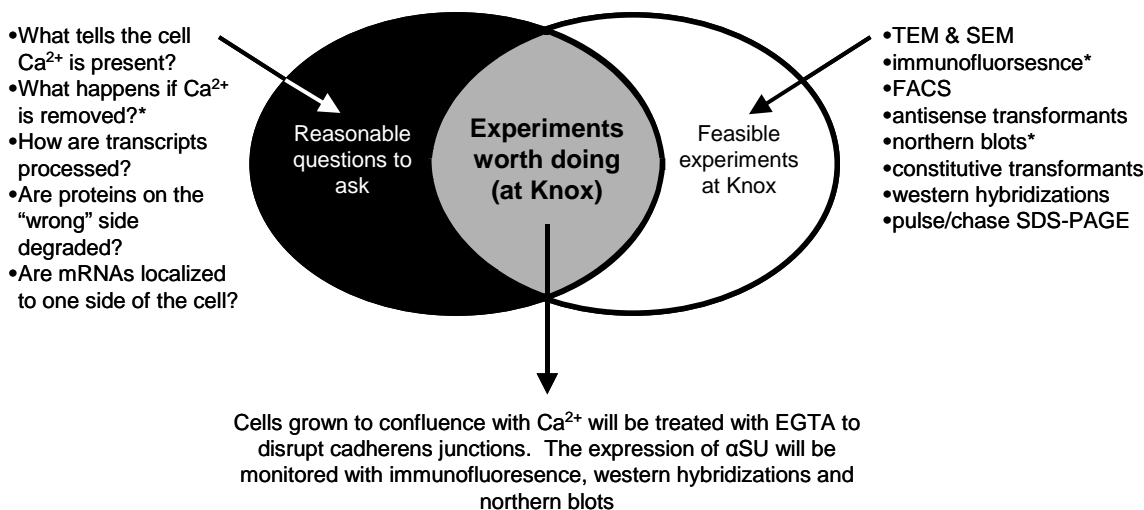


Figure 1. Process for focusing class discussion to a workable experiment. Discussions generated a list of worthwhile experimental questions as well as a list of feasible experimental approaches to answer those questions. Where there was overlap, students chose a specific experiment, then worked out the experimental details. *Indicate experiments easily done at Knox.

Once the students agreed upon the general experiment, class discussion turned to the practical issue of defining experimental and control treatments. The students constructed a timetable for organizing their work. Students worked in pairs, and each pair of students had responsibility for obtaining data for a given sample of the overall experiment. This approach highlighted the collaborative nature of science, and it helped to make each student feel invested in the experiment. Each pair of students ran the sample in duplicate, with each student in the pair responsible for one of the replicates. Running samples in duplicate insured that each student had an opportunity to manipulate a sample, and it also served to provide confirmatory or back-up data. In addition, I prepared cells to have at-the-ready should there be a problem

with a given sample. It was important that in the end we had a complete set of interpretable data.

During the remainder of the laboratory period, the students began growing their cells and preparing the solutions they would need for executing the experiment. Each group was given a flask of MDCK cells, which, over the course of a week, they would on their own time, split into three sub-samples and grow to confluence (T-75 flasks for northern blots, T-25 flasks for western hybridizations, and 6-well plates with coverslips for immunofluorescence). At confluence, the students came in to switch their cells to Ca^{2+} -free medium for the designated time interval of their assigned sample. The timing of the switch was such that all of the samples were ready for harvesting during the laboratory period of the following week.

After this, the students executed their own RNA and protein extractions and prepared their cells for immunofluorescence. The electrophoresis was begun during the laboratory period, but was terminated in the evening. The RNA and protein in the electrophoretic gels were electroblotted to nitrocellulose hybridization membranes overnight, and non-isotopic detection of RNA and protein on the blots was done the following days during the normal lecture period. Students signed up for time slots to use the fluorescence microscopes. Photographs of northern, westerns, and immunofluorescence images were acquired digitally and posted on the class web site so that everyone in the class would have access to all the results.

The final step involved writing a paper describing their findings. The students had five samples in their northern hybridizations (Fig. 2A), western blots (Fig. 2B), and immunofluorescence images (Fig 3). With this rich set of data, we discussed their results as a class before the students began writing their co-authored papers. They could see that the removal of Ca^{2+} affected protein levels (Fig. 2B) and protein

distribution (Fig. 3) but not mRNA levels (Fig. 2A). Furthermore, they could see that after protein distribution was affected (within the first 4 hours), the levels of proteins in the cell decreased. Each student of each pair independently wrote drafts for the introduction, materials and methods, results, and discussion sections. They then were required to critique each other's drafts (within the pair), making detailed and helpful comments on what was done well and what needed improvement. They then collaborated on writing all the sections of the report, building upon what they had originally drafted. The final manuscript was written according to the instructions for authors for the *Journal of Cell Biology*, including the preparation of figures and tables. They handed in their final manuscripts and their drafts so that I could assess the contributions and progress made by each student. The graduate student consultants were in contact with the students via e-mail during the experiments as a resource for problem solving and discussing interpretations, etc. In some cases they also contributed to the evaluation of the final reports.

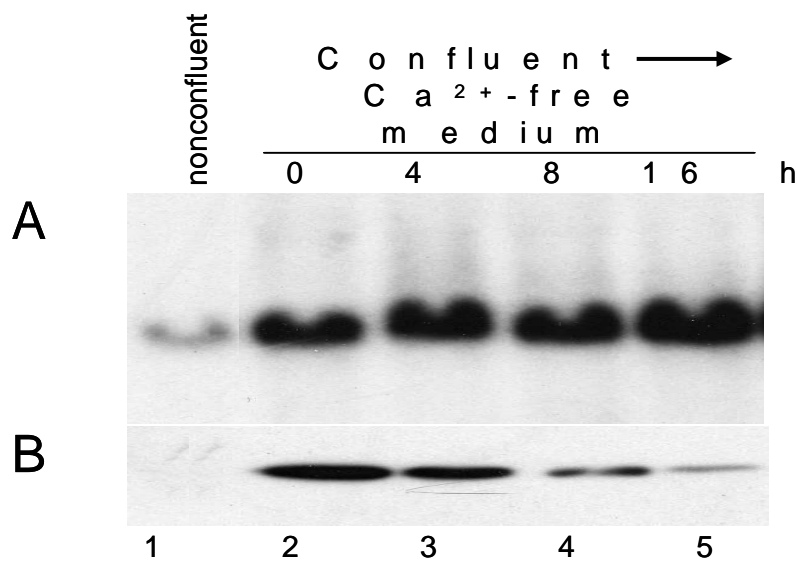


Figure 2. Student northern and western blot data examining the effect of Ca^{2+} removal on the expression of the α -subunit of the Na^+/K^+ ATPase (α -SU) of confluent MDCK cells. A) Transcript levels were analyzed by northern blots. B) Protein levels were analyzed by western blots. Cells were either grown to subconfluence (lane 1) or confluence followed by incubation in Ca^{2+} -free medium for 0 (lane 2), 4 h (lane 3), 8 h (lane 4) or 16 h (lane 5) according to the procedures of Grindstaff et al. (1996). RNA was isolated from cells grown in T-75 flasks using the TRI Reagent method according to manufacturer's instructions (Molecular Research Center, Cincinnati, OH) and quantified using A_{260} . Twenty μg of total RNA per sample were electrophoresed into 1.2% agarose gels (Maniatis et al., 1982) and capillary blotted to Gene Screen Plus membranes (New England Nuclear, Boston, MA). Northern blots were probed according to the procedures of Church and Gilbert (1984) by Stratagene's (La Jolla, CA) Illuminator chemiluminescent method. Proteins were extracted from cells scraped from the bottoms of T-25 flasks and homogenized in 1-mL glass tissue homogenizers. Thirty-five μg of total protein per sample were separated by 12% SDS-PAGE and electroblotted onto nitrocellulose following BioRad (Hercules, CA) Mini Protean instructions. α -SU was detected using Amersham's (Piscataway, NJ) ECL western blotting system with anti- α -SU antibodies (Grindstaff et al., 1996). Confluence increased α -SU mRNA levels, and protein accumulated. Shifting to a Ca^{2+} -free medium progressively diminished α -SU protein levels, though its mRNA levels remained high.

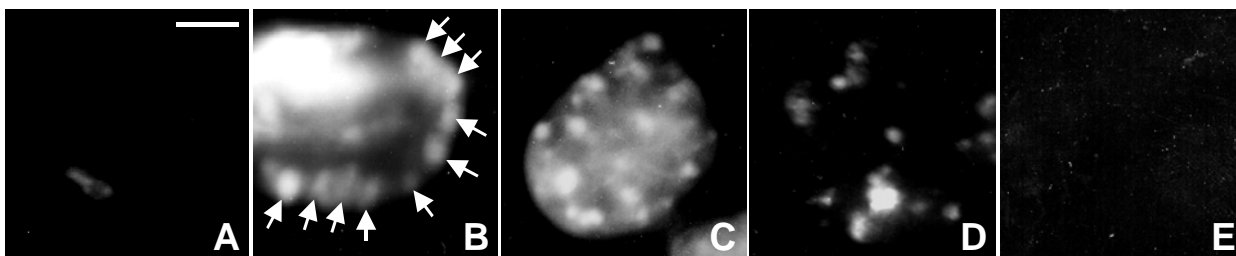


Figure 3. Student immunofluorescence images showing the effect of Ca^{2+} removal on the distribution of Na^+/K^+ ATPase α -subunit in confluent MDCK cells. Cells were grown according to the procedures of Grindstaff et al. (1996) to A) approximately 80% confluence (nonconfluent control sample), B) confluence, C) confluence then shifting to Ca^{2+} -free medium for 4 h, D) 8 h, and E) 16 h. Cells were grown on coverslips in 6-well plates and then fixed and immunofluorescently labeled according to the procedures of Welch and Suhan (1986) using anti- α -SU antibodies (Grindstaff et al., 1996). Confluence resulted in α -SU protein localization to basolateral membranes (arrows) (bright fluorescence in upper left is noise also visible under normal light). Shifting to a Ca^{2+} -free medium rapidly delocalized α -SU from its basolateral membrane localization and overall signal diminished rapidly. Bar = 25 μM . Micrographs are at equivalent magnifications.

Following the discussion of the results, we began the next set of experiments in the second half of the laboratory period with discussions of the next paper and the initiation of cell cultures. In each successive laboratory, the discussions progressed more quickly to the identification of a research question and the initiation of experiments. In some years, the questions evolved from the results of investigations conducted by students in previous years. In some years after learning what the students before them had done, questions evolved from the results of earlier investigations done in the class.

OUTCOMES

At this point, ten years after the start of the project, the exercises I developed with the graduate student consultants have each been used several times. Knox evaluates each lecture and laboratory course with a standard course evaluation form. In addition, I have developed a more open-ended qualitative survey to gain more critical insights. The student responses on the standard Knox evaluation forms showed marked improvement for the laboratory section (Table 1). In general, questions relating to the impact of the course and how challenging or stimulating it was, and the overall quality of the laboratory, showed substantial increases, yet there were not substantial differences in organization, preparation or effectiveness on my part. My original intent had been to have the students do all five exercises in each year. However, it became apparent in the first year that this was not feasible, and student feedback on my own evaluation forms indicated that the laboratory course was requiring too much work and time. This was especially the case when it came to writing up the results of one lab while

starting the next; there simply was not enough time devoted to writing. I modified the course and used three to four exercises each year during the 10-week quarter. Each laboratory experiment is intensive, and students are learning critical thinking skills that require time for development. What is sacrificed with regard to content, I feel is more than compensated for by an in-depth understanding of investigative process and by a more in-depth understanding of the research problem (National Research Council, 1997). Students are better prepared to do well in their independent research projects. The techniques they learned were immediately applicable to the research programs directed by colleagues in Biology and Biochemistry. The Center for Cell and Tissue Culture is available for use by research students and faculty whenever it is not reserved for course use.

Although each pair of students ran duplicate samples and I had prepared back up material in the event of cell culture contamination, there were occasions when a “data hole” resulted because a group failed to process a sample correctly. Fortunately, in most years, enrollments in the course were such that two groups processed at least some of the key experimental or control samples. In spite of this, for most of the experiments done over the years, at least one of the samples had some sort of problem even in the very best of the various replicates (e. g., Fig. 3B). There were relatively few years, however, when all replicates of a given sample were absolutely unusable, and in such events it was often possible to “borrow” data from previous years. A full set of data was important in allowing the students to learn the most from their writing experience.

Table 1. Results from student evaluations using Knox College's standard evaluation forms. Only questions in the laboratory section of the Knox College course evaluation form are shown. For 1987-1991, N = 98; for 1992-1999, N = 142

Statement	% Responding	
	Strongly Agree / Agree / Disagree / Strongly Disagree	
	1987-1991 Average	1992-1999 Average
This laboratory made a significant contribution to my education	13 / 52 / 24 / 11	45 / 42 / 6 / 7
For my preparation and ability this laboratory was appropriately demanding of my intelligence	23 / 57 / 17 / 3	63 / 34 / 0 / 3
This laboratory stimulated my personal initiative to thought and learning	10 / 45 / 31 / 14	51 / 43 / 6 / 0
Generally, the instructor was well prepared for the lab	86 / 11 / 3 / 0	83 / 12 / 5 / 0
This laboratory was well organized	78 / 18 / 2 / 2	73 / 22 / 2 / 3
Criterion	% Responding	
	Excellent / Good / Satisfactory / Fair / Poor	
	1987-1991 Average	1992-1999 Average
The overall quality of the laboratory	29 / 34 / 30 / 7 / 0	73 / 14 / 13 / 0 / 0
The effectiveness of the instructor	78 / 20 / 2 / 0 / 0	82 / 18 / 0 / 0 / 0

In addition to the problem of data holes, other technical difficulties cropped up from year to year. For example, in the case of the sample lab exercise presented in this present paper, Ca^{2+} removal caused the cells to separate and lift off from the coverslips, making it difficult for the students to obtain quality micrographs. This was partially remedied by using poly-lysine-coated slides. Although frustrating, these sorts of unanticipated technical difficulties provided opportunities to build problem-solving skills. They also presented a more realistic view of the obstacles inherent in research.

In addition to the laboratory exercise developed specifically for the upper-level Vertebrate Cellular Physiology course, the brainstorming process we went through in the early stages of the laboratory development process generated several interesting "spin-off" exercises that were appropriate for the introductory Cell Biology course. For example, the MDCK cell lines were grown to confluence on filters to monitor the rate of vectorial transport of candidate solutes (amino acids, proteins, disaccharides, monosaccharides, urea, etc.) across a polarized epithelium. Students could investigate a wide range of substrate-containing solutions and incubation conditions. These experiments helped to enrich the

introductory laboratory sections by introducing new investigative exercises.

As the exercise became dated over time, I obtained internal funds to bring in other alumni to develop teaching exercises using the same approach. These later exercises had some funding constraints that limited what sort of equipment could be used in the project, but some modest equipment purchases were still possible. Instead of bringing in a fleet of alumni, these exercises were developed at the more modest rate of one every year or two. Recently an alumna of the course became a graduate student consultant.

For the Vertebrate Cell Physiology students, the laboratory experience was empowering. The experiments in which they were engaged had clear links to ongoing research. Feedback on my evaluation forms indicated the students greatly appreciated this. These links were underscored by the fact that their lab work was based on work from a research lab in which a Knox College graduate was pursuing cutting-edge research. I know that, for many students, this was an important indicator to them about their own potential, especially for students who were not performing as well in the lecture section. Furthermore, the experimental component in each laboratory exercise helped to diminish the perception that the student must

correctly come up with some answer already known to the teacher. This small but significant shifting of the power base helped the students begin the process of imagining themselves as investigative scientists. The work in the classroom began to feel more like that of a research team. Finally, students felt far more invested in their writing. They had ownership of original data, and student comments indicated that it was, “the first time [they] really cared about writing really well.”

Indeed, the quality of the student writing was significantly improved from what had been before the course had been redesigned. Students commented that they learned from their lab partners as they critiqued one another’s drafts. In addition, students felt that the collaboration required for the final paper was very beneficial to honing writing skills. They also found it useful to follow real-world instructions to authors for manuscript preparation. However, some student pairs did not work well together. In some cases this was due to different writing abilities between the group members. I found this could often be mitigated if I arranged for a stronger writer (not necessarily the partner) to help the weaker writer produce a better draft. In such cases I made certain I could identify a particular strength in the weaker writer that I could call upon later. This was all done discretely, but for the students involved it helped them to see that they each brought their own strengths to the research. In other cases it was a problem of one member of a group being too dominant in the partnership. I allowed students to swap partners from one experiment to the next, and I found the students to be fairly good at self-sorting. However, with some students I needed to intervene more directly and ask them to give their partner a chance to participate more fully in the science.

The graduate student consultants also gained some insights from the project. First of all, because many of the experiments were “what if?” experiments that would have otherwise been too risky for the graduate student to invest much time, there were occasions when the graduate students used the data generated by the class as preliminary data for

subsequent follow up experiments when the class results looked promising. In addition, the graduate students gained unique insights on the connection between teaching and scholarship and why scholarship is valued at primarily teaching institutions, as well as why quality laboratory instruction might be important at research institutions (National Research Council, 1997). Graduate student training typically develops a sense of teaching and research as opposing obligations competing for limited time. This sense is reinforced by what they hear from their research mentors as their mentors complain about teaching “loads.” For graduate students fellowships provide research “opportunities,” while assistantships come with teaching “obligations.” As a result, the impression is set early on in the life of a prospective faculty member that the teaching laboratory is a low priority chore distinct from and at odds with research interests. For the students involved in this project, they developed a better appreciation for the synergy between research and teaching.

Bringing back recent graduates who have gone onto Ph. D. programs in the sciences can serve as a mechanism for invigorating teaching laboratories and keeping the laboratory experience relevant and more experimentally-driven in virtually any science or engineering discipline. Our former students are exposed to up-to-the-minute research, and they represent a broad, excellent and untapped source of expertise for keeping undergraduate laboratories current and exciting.

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In Memoriam -- George O'Connor

A long-time, sporadic, member of ACUBE (going back to AMCBT) died on December 17, 2004. George O'Connor was a Professor of Biology at Rockhurst University for 35 years. He was 64 years old and looking to retire in two years. His on again, off again relationship with ACUBE began in 1969 when he came to Rockhurst, and was interrupted as his family grew in number. He was very devoted to his family and they took precedence over many aspects of his life. He taught Invertebrate Biology, an offshoot of his love of raising tropical fish, as well as a means of working his way through college. He also taught General Biology, Anatomy and Physiology, and in later years added Research Techniques and Evolution to his responsibilities. In the past few years George with John Koelzer of the Mathematics Department at Rockhurst developed a course in Mathematics of Biology. They presented a paper on this course to ACUBE at the last meeting. Since his children moved onto their own careers he was planning to continue and expand his contacts with his ACUBE friends. He was a good friend and colleague, and will be missed by many.

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