IN VIVO

The Publication of the Metropolitan Association of College and University Biologists

Spring 2015 Volume 36, Issue 3

48th Annual MACUB Conference

Hosted by

Montclair State University

Montclair, NJ

Nov. 7, 2015

Instructions for Authors

IN VIVO is published three times yearly during the Fall, Winter, and Spring. Original research articles in the field of biology in addition to original articles of general interest to faculty and students may be submitted to the editor to be considered for publication. Manuscripts can be in the form of a) full length manuscripts, b) mini-reviews or c) short communications of particularly significant and timely information. Manuscripts will be evaluated by two reviewers.

Articles can be submitted electronically to *invivo@mec.cuny.edu* or mailed as a printed copy (preferably with a diskette that contains the file) to the Editorial Board at Medgar Evers College. All submissions should be formatted double spaced with 1 inch margins. The title of the article, the full names of each author, their academic affiliations and addresses, and the name of the person to whom correspondence should be sent must be given. As a rule, full length articles should include a brief abstract and be divided into the following sections: introduction, materials and methods, results, discussion, acknowledgments and references. Reviews and short communications can be arranged differently. References should be identified in the text by using numerical superscripts in consecutive order. In the reference section, references should be arranged in the order that they appeared in the text using the following format: last name, initials., year of publication. title of article, journal volume number: page numbers. (eg. - [1]Hassan, M. and V. Herbert, 2000. Colon Cancer. *In Vivo* **32:** 3 - 8). For books the order should be last name, initial, year of publication, title of book in italics, publisher and city, and page number referred to. (eg. - Prosser, C.L., 1973. *Comparative Animal Physiology*, Saunders Co., Philadelphia, p 59.). Abbreviations and technical jargon should be avoided. Tables and figures should be submitted on separate pages with the desired locations in the text indicated in the margins.

In This Issue:

Save the Date

The 2015 MACUB Conference will be at

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2015 MACUB CONFERENCE

MONTCLAIR STATE UNIVERSITY

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The Department of Biology at Montclair State University has over 1100 undergraduate students taught by 22 full-time faculty members with a broad range of research and teaching interests which are broadly divided into three areas: Molecular Biology, Ecology and Physiology. A number of faculty have research interests that combine two of these areas, including Molecular Ecology, Physiological Ecology and Molecular Physiology. Undergraduates can major in Biology or Molecular Biology. The Department also offers concentrations in Environmental Science and Teacher Education and participates in the interdisciplinary 5-year BS/MS degree in Marine Biology and Coastal Sciences.

Although the Department has a large undergraduate program, it prides itself on providing a small-school experience to the students. Class size is kept small and a strong emphasis is placed on hands-on learning and student-faculty interactions. All undergraduate students, regardless of major or professional interest, are strongly encouraged to fulfill at least one semester of research. To fulfill this requirement, students have the option of working directly with a faculty member or participating in an internship program. Student body diversity is one of the Department's strengths with over 40% of the students being of Hispanic or African-American descent. Another large portion of the students represent a vast array of nationalities ranging from Asia, Africa, South America, Russia and Europe. Students who have graduated from the program have gone on to become physicians, educators, and industry leaders. In 2008, Montclair State University celebrated its 100th anniversary. From its early days as a normal school to its present standing as one of the leading comprehensive universities in the state and region, Montclair State University has seen remarkable change and growth during its first 100 years. During that time, the University has established a solid tradition of educational innovation, inspired teaching and scholarly achievement. Over the next 100 years, about 350,000 students will earn degrees from Montclair State, and the University is committed to providing these bright, ambitious students the facilities, faculty and scholarships they will need to pursue their dreams.

Assessment of Student Learning and Retention of Chemical and Cellular Concepts in Human Anatomy and Physiology

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Abstract

The retention and application of chemical and cellular concepts across the two semester Anatomy and Physiology (A&P) curriculum was assessed using unannounced pre- and post-tests given at the beginning and middle of the semester, respectively. This study revealed that students have limited knowledge in these concepts at the beginning of A&P I and as expected show an increase in their knowledge base gained during the semester, that appears to be retained in A&P II. However, most of their knowledge gain is at the basic (lower) level of learning, where more than 50% of students were able to master 7 of the 10 concepts evaluated. When presented with higher level questions on the same concepts, mastery dropped to a paltry 2 of the 10 concepts. Sadly, students had trouble learning 2 important concepts, namely organic macromolecules and cellular metabolism, where performance on lower level post-test questions hovered around 40-45%. In general, student performance in concepts evaluated from lower level common departmental final questions in A&P I paralleled observations in the pre-post-test study. Students demonstrated a higher level of proficiency in 9 of the 10 concepts tested, presumably attributed to attrition (weaker students dropping course before finals), better preparation and independent-student study, but still failed to master cellular metabolism. This study therefore revealed useful information that may be relevant in redesign of the A&P curriculum, especially on the need to cover certain difficult concepts like metabolism, using more innovative approaches. It also demonstrated an even more urgent need to incorporate more critical thinking skills into the A&P curriculum; not only to help students better comprehend and apply the basic concepts to A&P, but to also develop these essential skills for their future success in allied health careers where these skills are a necessity.

Introduction

Student Background

Gateway A&P courses are one of the major challenges and obstacles for students wanting to pursue allied health careers such as nursing, without the necessary preparation. Degree programs offered at Bronx Community College (BCC) of the City University of New York (CUNY) that require the two semester A&P sequence include nursing, dietetics and nutrition, radiology technology, therapeutic recreation, community health, and nuclear technology, with the majority of students selecting nursing as their declared objective[1]. However, the majority of students entering the A&P sequence have limited biology and science backgrounds[2,3,4]. In addition the A&P curriculum contains so many different concepts that detailed coverage is precluded. As a result, expecting students to learn and comprehend often challenging concepts within a short period may be too demanding.

Many additional factors contribute to community college student success in A&P[5]. For example, the majority of students at BCC are working non-traditional students, who do not have the time[5], or even other resources like textbooks, needed to master the course. Many students also require remediation in English, Reading and Mathematics prior to attempting credit-bearing classes, such as A&P. Their under preparedness quickly affects their opinion of their ability to do well in science courses and their poor study habits combined with their inability to appreciate the relevance of science to their future careers negatively affects them[1]. The result is many students failing to meet the challenging expectations of A&P and ultimately dropping out of their dream allied health careers.

Learning Skills and Bloom's Taxonomy

The most important skills and concepts to reinforce in A&P were recently covered in Miller *et al.* [6], which resulted from joint discussions between, the American Anatomy Association (AAA) and the Human Anatomy and Physiology Society (HAPS). In decreasing order of importance, the four most important skills identified by HAPS were: develop thinking, reasoning, data handling and interpretation skills; apply learning to realistic situations; learn and apply the scientific method; and use homeostasis and control as an integrating theme." [7]

However, higher order skills such as reasoning and analysis cannot occur without first gaining knowledge and comprehension within a discipline. Bloom's taxonomy, which attempts to scaffold cognitive learning on a six point scale from easier to more advanced skills, is commonly applied to most educational settings from kindergarten through college and beyond. In brief the scale[8] (and its revised version[9]) is: 1. Knowledge (Remembering); 2. Comprehension (Understanding); 3. Application (Applying); 4. Analysis (Analyzing); 5. Synthesis (Evaluating) and 6. Evaluation (Creating). Bloom[8,10] and others[9,11] emphasize that remembering knowledge is the foundation to the mastery of all higher levels of learning from comprehension through evaluation that gradually build on each other. The misdirected applications of Bloom's taxonomy, where higher order skills are emphasized and assessed at the expense of basic concepts such as gaining knowledge have been reviewed[11].

Most college level instruction and assessment occurs at the lower levels of Blooms taxonomy. Similar to our A&P common assessment efforts at BCC, about half of the HAPS comprehensive exam is on the first level of Bloom's taxonomy and over three quarters is on the first two levels, with an emphasis on knowledge and comprehension, and only 20% cover other higher order levels[12]. It is important to develop critical thinking skills within the A&P curriculum, to advance reasoning over memorization, especially since most allied health careers require the use of logical reasoning to solve real world problems[6]. However, in agreement with the Greek philosopher Plato, Bloom[10] and most educators would argue that students fail not because they aren't smart, but they need more time to understand conventional wisdom before they can comprehend higher order learning.

Most Important A&P Concepts

Faculty at the college level often wonder about what can be done to help students better learn and retain concepts covered in previous courses, so they can apply these concepts to new scenarios in subsequent courses. Like most other institutions, at BCC we cover most of the basic chemical, biochemical and cellular concepts in the first half of A&P I[13], so as to lay the foundation for application of these concepts to the multiple organ systems covered in the second half of this course and A&P II. Successful students then progress to taking additional science courses such as microbiology before or while taking their chosen allied health courses. Faculty do not have the time to repeat or review, the chemical and cellular concepts covered in A&P I in A&P II or microbiology, and often find themselves doubting if students actually mastered the concepts covered in prior courses.

It's understandable that a two semester A&P sequence cannot fix a decade's long educational gap, but since most students don't seem to have a basic scientific understanding prior to starting the A&P curriculum, every effort should be made to cover basic concepts first. However, while there is agreement on the need to relate structure to function[14], it is not as clear which basic concepts should be stressed. In a 1997 HAPS conference panel discussion with allied health professional school directors, the conclusion was "teach less anatomical detail", but the textbooks and syllabi that most faculty follow still dedicate a significant portion to anatomy[7]. Miller *et al.*[6] identified the three most important systems to cover, in decreasing order of importance, as nervous, cardiovascular, fluid, electrolyte and acid base balance. Similarly, about a quarter of the HAPS comprehensive exam[12] is comprised of questions from nervous and cardiovascular systems in relatively similar proportions, with the next highly represented area being homeostasis and chemical concepts covered in the present study and central to the understanding of all systems. While the concepts to emphasize are subject to debate and may vary from community college setting to medical school, the need to cover basic content in order to build towards higher level understanding is universal.

Study Overview

A pilot study, to determine which cellular and chemical concepts covered in A&P I, if any, were learned and retained in A&P II, was conducted. First, we set out to determine student's prior knowledge of the most important concepts in A&P I in pretests conducted on the first day of the course and followed up with posttests after the midterm. The same pretests and posttests were then given to students in the A&P II course. Questions were designed at both lower and higher levels of Bloom's taxonomy[8, 9] and evaluated by departmental faculty. Results were compared to student performance in departmental common final assessment questions.

Methods

Identification of Common Concepts in A&P I and A&P II

Pre- and post-tests were developed based on chemical and cellular concepts that departmental faculty considered should be mastered in A&P I and retained and applied in A&P II. Mastery of most of these concepts would also be predicted from prior exposure in high school. We had also identified some of these concepts in a previous study involving a pre- A&P workshop[13]. The following ten concepts were considered to be the most important: 1. Homeostasis & Negative Feedback, 2. Ions, Water & Electrolyte Balance, 3. Hydrogen Ions, pH & Buffers, 4. Organic Macromolecules, 5. Enzymes & Chemical Reactions, 6. Organization & Hierarchy, 7. Organelle Function, 8. Protein Synthesis & DNA, 9. Cellular Respiration & Metabolic Pathways and 10. Cell Transport Mechanisms.

Designing a Lower Level and Higher Level Question for Each Concept

For each concept two multiple choice questions were developed in an attempt to address both a lower and a higher level of Bloom's Taxonomy[8,9]. In general, Questions 1-10 were developed at level 1 of Bloom's taxonomy, while questions 11-20 required at least comprehension and/ or application. Faculty were asked to rank the level of difficulty of the questions used on a Likert scale of 1-5, which was broadly adapted from both earlier[8] and revised[9] versions of Bloom's taxonomy, in an attempt to determine how much of an agreement they had with the design of the questions (Figure 1).

Administering the Pre-Post-Tests and Comprehensive Common Finals

The pre- and post-tests were administered at the start of the laboratory period during week 1 and week 8 of A&P I and A&P II. Students were not advised in advance of the tests. As an incentive, students were informed that their scores would count towards their laboratory quiz grades if they improved their overall score. The study was carried out on 8 sections of A&P I and 4 sections of A&P II over several semesters.

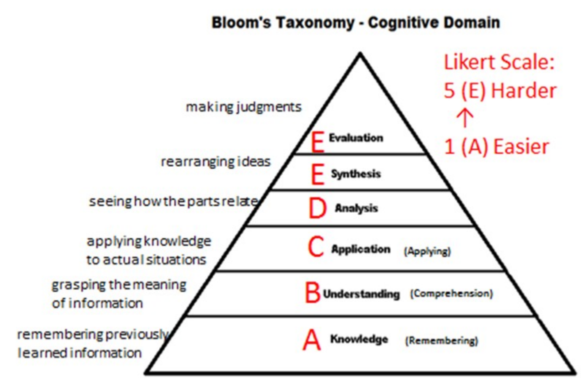

Figure 1: Information and scale used by faculty in ranking of the difficulty of the test questions. *Adapted from references*[8, 9].

The department administers a comprehensive final exam comprised of 25 common multiple choice questions and 50 individual instructor-initiated. We followed the performance of the same cohort of students in the pre- post-test study in the common final questions, so we could compare their performance on the 10 concepts in the two data sets. We also compared the cohort's common finals numbers to the overall departmental performance. Generally, common finals questions were set at a lower level of difficulty and individual faculty included higher level questions at their discretion.

Data Analysis and Statistics

We used student paired t-test to compare the statistical difference between pre- and post-tests as well as lower and higher level questions. Pearson correlation statistics were used to look for any correlation patterns between each of these two data sets. Unpaired t-tests were used to compare performance between independent sections. For the purposes of the present study, the benchmark for student success was set at 50% in pre- and post-tests and 70% in departmental common final questions. All statistical tests were done with SPSS/ PASW version 20 software, where significance was set at 95% confidence level ($p < 0.05$).

Results

There Was About 60% or Better Agreement Between Faculty on the Questions' Level of Difficulty

It was impressive to see departmental faculty consistently rank the questions level of difficulty, in such a way that their average ranking of higher level questions was always at a level that was more difficult than lower level questions (Table 1). Overall, the average faculty ratings of the questions we considered to be lower level was 1.6, which approximates between knowledge (remembering) and comprehension (understanding) on the Likert scale used. Similarly, the average ranking of the higher level questions was 2.5, which falls between understanding and application (Figure 1 and Table 1).

For all ten concepts tested, faculty consistently ranked concept-matched questions such that lower level questions always got a lower ranking (meaning easier on Bloom's taxonomy) than higher level questions (Table 1). However, if we assume an average ranking difference of about 1 indicates a significant difference between lower level and higher level questions, then there was a clear consensus on only 6 of the 10 concepts tested (60%), with one of the concepts (Organelle Function) barely making the cut off by rounding off 0.8 to 1. In other words, faculty rankings of lower level and higher level questions were only different about slightly more than half the time. The average ranking difference of some concepts was as low as 0.3 (Organization and Hierarchy) or 0.4 (Organic Macromolecules, Enzymes & Chemical Reactions, and Protein Synthesis). In general, faculty evaluated most of these latter concept questions at a lower level of difficulty. In addition, there were significant variations between individual faculty evaluations of questions, as indicated by the relatively high standard deviations (Table 1).

Overall, Students Performed Better in Post-tests Relative to Pre-tests in A&P I, But Not in A&P II

Students starting the A&P curriculum performed on average at 40% ± 7 (n=170 students across 8 independent sections) on the twenty questions tested and had higher post-test scores of about 55% ± 8 compared to pretest scores (Figure 2) in A&P I. The overall difference in performance between the pre- and post - tests in A&P I was shown to be statistically significant using paired t-test analysis ($t(7) = 12$; $p = 0.000$). This difference held consistently across 7 of 8 independent sections, with average pre-test scores in these sections ranging from 31-42% and post-test scores ranging between 43-58% (data not shown). The other section also followed the same pattern, but its average pre-test and post-test scores of 56% and 70% respectively were an outlier. As a result of this consistent scores across various sections, there was a significant paired correlation of about 90% between A&P I pre- and post-tests. Such a high positive correlation means that the significant difference observed in pre and posttests paired t-tests wasn't just because of differences in pooled data, but that for a given section, one could predict with 90% probability that students were on average able to learn the concept and thereby improve their post test scores.

The average scores remained about the same in both A&P II pre- and post-tests at about 52% and 53% respectively, which were not significantly different. However, the A&P I pre-test score was so significantly lower than post-test scores that even when combined with pre- A&P II scores, the overall combined data for A&P I & II pre-test score of 44% was still significantly different ($t(11) = 4.6$; $p = 0.001$) than the overall A&P I & II post-test scores of 54% (Figure 2). There was a statistically insignificant Pearson correlation of about 85% between pre and posttest A&P II data, meaning that for a given section, we could predict with about 85% chance that their post scores wouldn't be significantly different. Because the pretest A&P I and II tests had such a different outcome in their correlation, overall combined data had a poor 55% predictive ability that the post test score will be higher.

Students Performed Better in Low Level Questions Relative to High Level Questions

When questions were directed at basic concepts, overall student performance was at about 56% for A&P I and 63% for A&P II (Figure 3) on the

Table 1: Faculty ratings of the questions level of difficulty.

	Faculty evaluation of the level of difficulty of the questions	
Concept	**Lower level questions**	**Higher level questions**
Homeostasis & Negative Feedback	2.1 ± 0.9	3.1 ± 1.0
Ions, Water & Electrolyte Balance	1.5 ± 0.6	3.7 ± 0.7
Hydrogen Ions, pH & Buffers	2.2 ± 1.0	3.3 ± 0.9
Organic Macromolecules	1.2 ± 0.5	1.6 ± 0.7
Enzymes & Chemical Reactions	1.9 ± 0.9	2.3 ± 0.7
Organization & Hierarchy	1.2 ± 0.4	1.5 ± 0.6
Organelle Function	1.1 ± 0.3	1.9 ± 0.9
Protein Synthesis & DNA	1.3 ± 0.6	1.7 ± 0.7
Cellular Respiration	1.4 ± 0.5	2.4 ± 0.9
Cell Transport Mechanisms	2.3 ± 0.7	3.4 ± 0.9
Overall	1.6 ± 0.4	2.5 ± 0.8

Mean ± STD (n = 19). Likert scale used (1 = knowledge, 2 = understanding, 3= application, 4 = analysis, 5 = synthesis/evaluation).

combined pre and posttests. However, students did not perform well on higher level questions, with the performance significantly lower for both A&P I (t (15) = 11; p = 0.000) and A&P II (t (7) = 8; p = 0.000). This trend was consistent across multiple sections, with post-test low level test scores ranging 54-73% and high level scores between 31-47% in 7 of 8 A&P I sections, with the other section having scores of 77% and 64% respectively. There was also a significant paired correlation at about 85% for A&P I, meaning for any given pre and post section, you could predict that their relatively higher scores on low level questions will significantly drop when they attempt the high level questions. However, even though t-tests showed A&P II scores on high level questions also dropped significantly as shown in Figure 3, Pearson correlation was poor and could not give a good predictive ability.

It is worth noting that when pre- and post-test questions were separated by degree of difficulty, the highest improvement in A&P I post-tests was in the low level questions with close to 20% better pre-test performance, compared to only a 13% pre-test improvement in the high level questions (data not shown). This trend was very different in A&P II, where there was an insignificant 4% drop in low level questions in the post test and a 5% increase in the high level post test questions, relative to questions of the same difficulty in pre-tests. Interestingly, there was no statistically significant difference between performance in A&P I and A&P II post-tests, in either low level

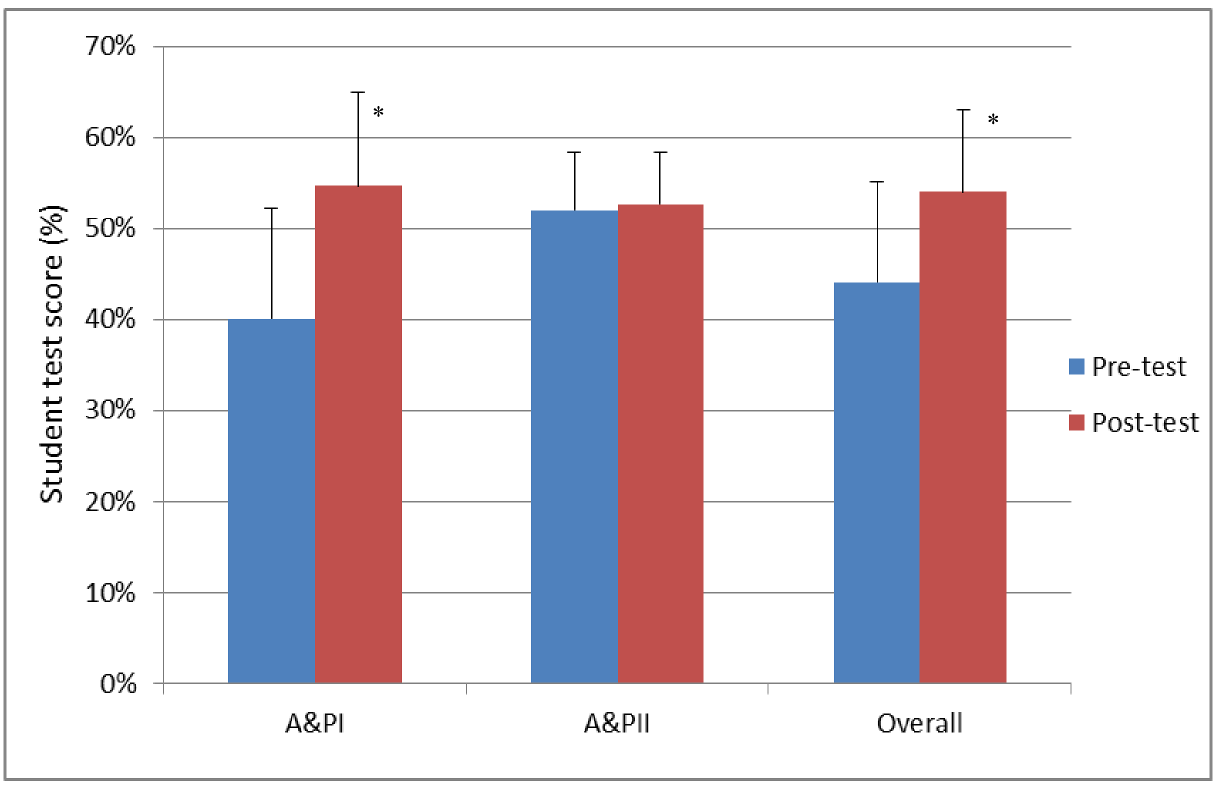

Figure 2: Comparison of overall A&P I and A&P II students' performance on pre- and post-test questions. Mean ± STD. *p<0.05, significantly different from pre-test score.

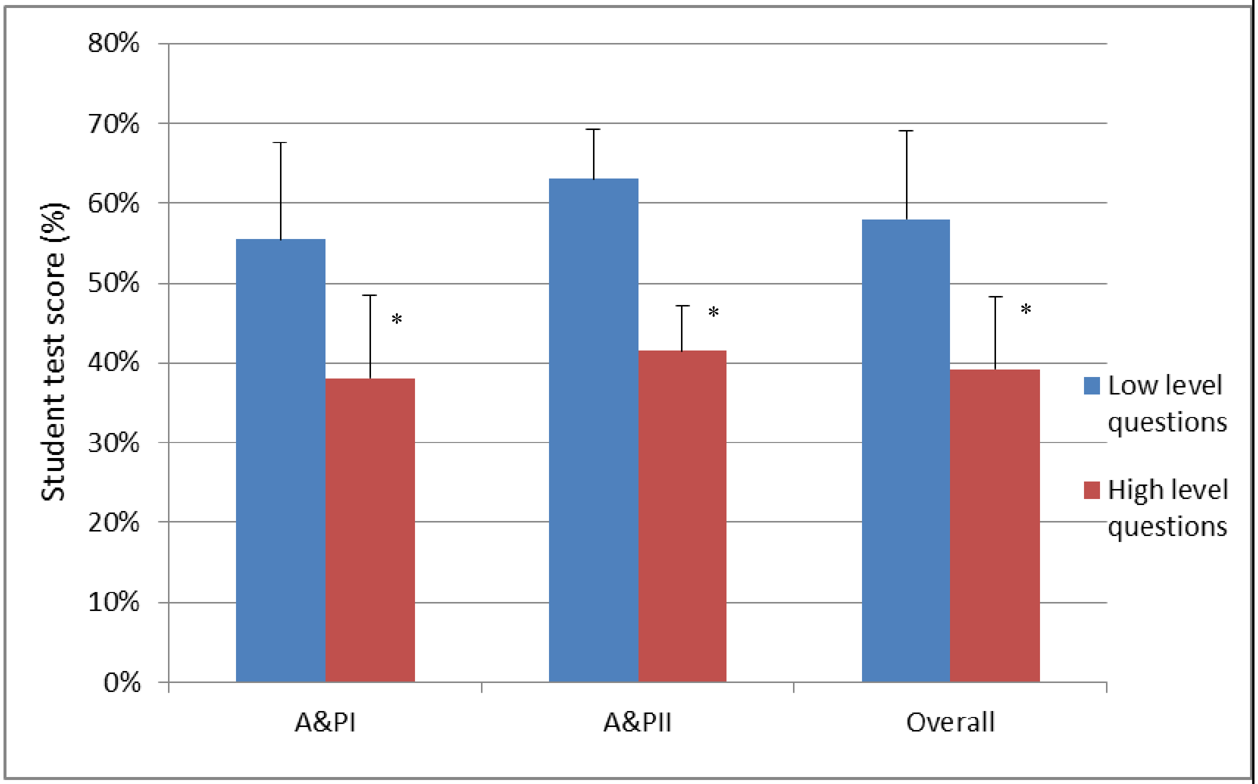

Figure 3: Comparison of A&P I and A&P II students' performance on low and high level post-test questions. Mean ± STD. * $p<0.05$, significant difference between low and high level questions.

or high level questions ($P>0.05$). Given that post-tests scores were similar between A&P I and A&P II, we only further analyzed post-test scores in subsequent sections.

Student Performance Showed Significant Variations Between Concepts

Based on the performance difference between the low and high level questions for each of the 10 concepts tested, we classified the concepts into 3 main categories, using A&P I posttest data (Figure 4 and Table 2). For each concept, if 50% or more students were able to answer a question, we assumed that students were able to learn that concept. In addition, paired t-test analysis of low level and high level questions was done to help with classification into the concept categories (see below). Using this criteria, we concluded that students were able to *learn and apply* 2 of 10 concepts (C2 and C7 in Figure 4; note concept is abbreviated as C henceforth), *learn but not apply* 5 of the 10 concepts (C1, C3, C5, C6 and C10) and *did not learn and therefore were unable to comprehend/ apply* in 2 of 10 concepts (C4 and C9). We were left with 1 concept (C8) that didn't fit into any of these three categories, as students performed better on what we considered to be a higher level question relative to the lower level question. It is worth mentioning that this was one of the concepts that didn't fit with our stringent criteria of about +1 difference between lower and higher level questions in the faculty evaluation.

Paired t-test analysis showed that student performance in low level and high level questions on the 2 concepts classified as *learn and apply* above were barely significant. This shouldn't be surprising because more than 50% of students answered both the low level and high level questions. In fact, in the case of Organelle Function, more than three quarters of students answered both questions correctly (Figure 4 and Table 2). In contrast, there were significant differences between student performance on the relatively easier and harder questions in the next category *learn but not apply.* For example, performance on the lower level sample question for Cell Transport Mechanism (C10; Table 2 and Figure 4) was more than double that of the given higher level question, a result that was very significantly different (t value 9.1, $p<0.05$). In fact, this was the norm in this category, with 4 of the 5 concepts having double scores on the relatively easier questions (Figure 4 and Table 2). The other concept, which is C3 still had a significant difference, with the low level question having about 16% better performance than the high level question. Finally, performance on the two concepts that we categorized as *did not learn, and unable to comprehend* were not only well below 50%, but also had no statistically significant difference between easier and harder questions ($p>0.05$).

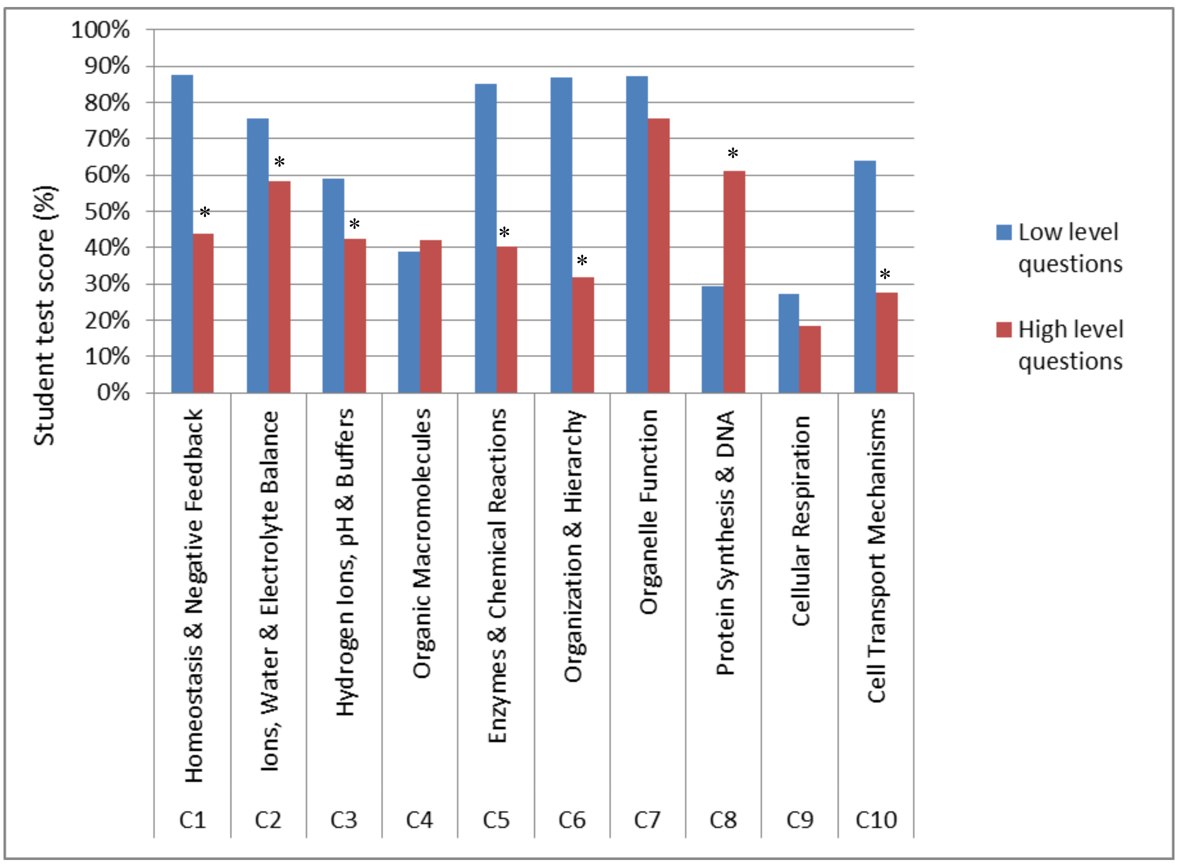

Figure 4: A&P I Post-test **s**tudent performance on low and high level questions in each of the ten concepts. * $p < 0.05$, significant difference between paired concept questions.

Students Performed Better in Comprehensive Common Finals Relative to Pre-Post Test Questions

Finally, we compared A&P I student performance in common A&P I final questions to the pre-post-test study. We followed the same cohorts that undertook the A&P I pre- and post-tests and compared them to department wide performance on relevant finals questions from the 10 concepts. It is worth noting that the departmental final was cumulative over material covered the whole semester and mostly covered lower level questions. Students also knew in advance about this high stakes test and therefore presumably prepared for it. Not surprisingly, students performed much better in the finals than the post-tests (Figure 5 versus Figure 4), exceeding or approaching a 70% benchmark in 9 out of the 10 concepts. Paralleling the post-test study, student mastery of cellular respiration was poor (Figure 5). Our experimental group performed at about the

Table 2. Samples of lower level and higher level questions from each of the three main concept categories.

Concept	Lower level question	Higher level question
Both learn and apply the concept: Example: Ions, Water & Electrolyte Balance	In most organisms, including humans, which of the statements below correctly describes the normal distribution of Na^+ and K^+ ions? a. Na^+ and K^+ levels are both high in ECF (found outside of cells) b. Na^+ and K^+ levels are both low in ICF (found inside of cells) c. Na^+ levels are high in ICF and K^+ levels are high in ECF d. Na^+ levels are high in ECF and K^+ levels are high in ICF	Aldosterone is an endocrine hormone that acts on the kidneys to cause more Na^+ ions to be kept in the blood and more K^+ ions to be excreted in the urine. I.e. it keeps blood Na^+ and K^+ levels within the normal range. In which of the following situations would you expect aldosterone levels in the blood to increase. a. Following a meal, if urine Na^+ levels are low or urine K^+ levels are high b. Following a meal, if blood Na^+ levels are low or blood K^+ levels are high c. Between meals, to decrease blood Na^+ levels or increase blood K^+ levels d. Between meals, to increase urine Na^+ levels or decrease urine K^+ levels
Learn, but not apply the concept: Example: Cell Transport Mechanisms	When a cell is placed in a hypotonic solution a. it will increase in size b. water will leave the cell by osmosis c. it will stay the same size d. none of the above are correct	Antidiuretic hormone increases blood volume by decreasing urine volume. It does so by increasing the permeability of some kidney cell membranes to water. When antidiuretic hormone levels in the kidney increase what will happen? a. water will leave the body, from hypotonic blood to hypertonic urine b. water will leave the body, from hypertonic blood to hypotonic urine c. water will move back into the body from hypertonic urine to hypotonic blood d. water will move back into the body from hypotonic urine to hypertonic blood
Neither learn, nor apply the concept: Example: Cellular Respiration & Metabolic Pathways	Which of the following best describes a metabolic pathway called Glycolysis? a. Glucose → Pyruvic acid b. Glucose → Glycogen c. Glycogen → Pyruvic acid d. Glycogen → Glucose	Red blood cells cannot use oxygen in metabolic pathways making ATP. This means that ATP production in red blood cells occurs by: a. Glycolysis only b. Anaerobic respiration (fermentation) only c. Both glycolysis and anaerobic respiration d. None of the above, red blood cells do not need energy.

same level, or slightly better, than the department and is therefore a good representation of students taking A&P in our department (Figure 5).

Discussion

Overall pre-test scores indicated that students had limited prior knowledge when starting A&P I, which is not unusual[2,4,13]. Student post-test scores indicated an improvement in overall performance, presumably attributed to course instruction and independent student study. However, overall scores were still well below that required to obtain a grade of C+ or better in the course and for progression into an allied health career[13]. On a positive note, student's pre-test scores in A&P II remained about the same as post-test scores in A&P I, especially given that students didn't have prior notice to study for the pre-and post-tests. Overall our results suggest that A&P II students have retained some concepts learned in A&P I.

Based on the current study, what students learned and retained seems to be mostly limited to lower level (basic) concepts, meaning after their initial peak identified in A&P I post-tests, their knowledge seemed to plateau. That their performance was only at 52% during A&P II pre-tests, with not much improvement after going through the A&P II sequence shouldn't therefore be surprising, given that most of these

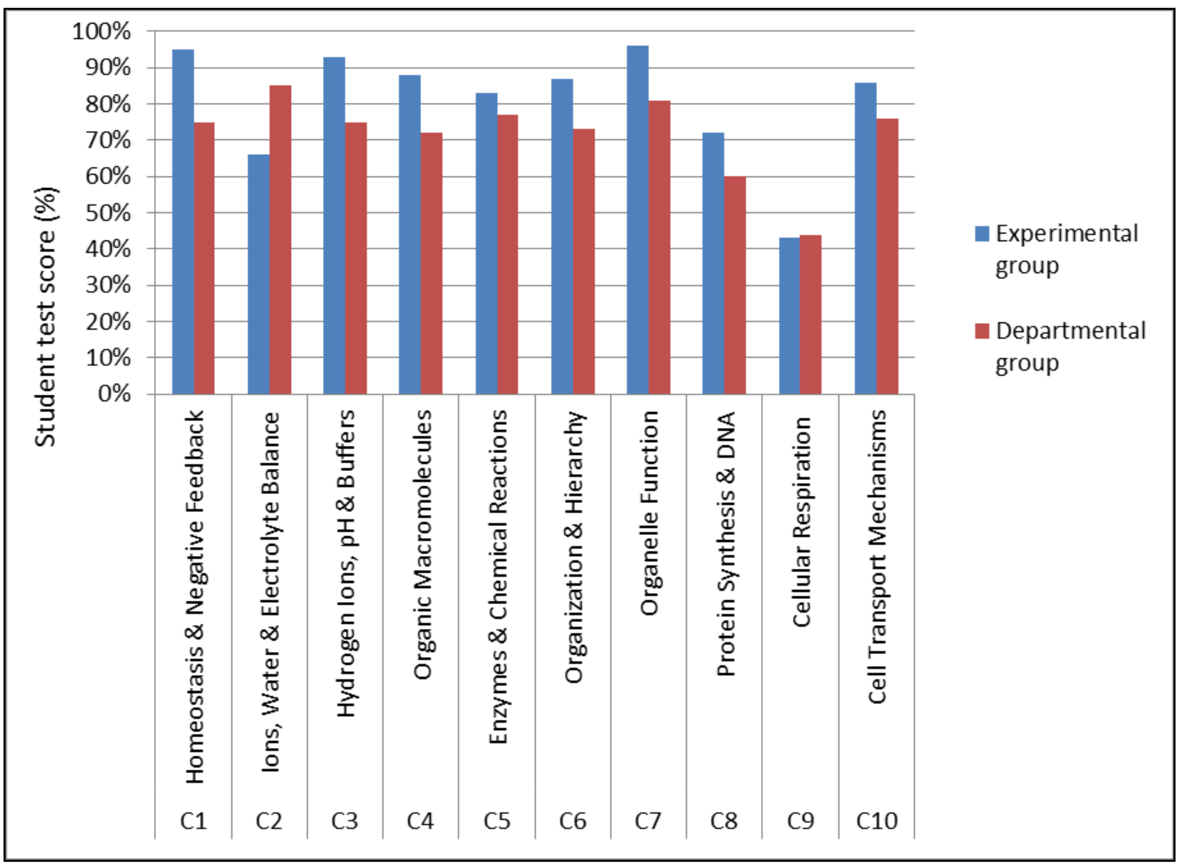

Figure 5: Comparison of A&P I common final pre- and post-test students' performance (experimental group) to department wide group for each of the ten concepts.

concepts were covered in A&P I. It shouldn't also be surprising that they would perform better when given a higher proportion of lower level questions, especially in a high stakes tests that they prepared for, as is evident in both their significantly higher performance on lecture exam 1 (data not shown) and the common part of the comprehensive finals. Questions in the lecture test 1, formally given in week 4 of the course, included a larger range of questions directed at basic concepts in the chemical level of organization, with students scoring about 20% better than they did on post-tests given in week 8 of the course. A similar trend was observed for the cohort's (pre-post-test students) performance in the common finals, which was slightly better than the departmental average. To this end, it is encouraging that students were able to master basic concepts in 7 out of the 10 concepts understudy.

However, in half of these same 10 concepts, they had a tough time applying the basic concept they mastered to a critical thinking scenario. For instance, while students' mastered listing the hierarchy of life they were surprisingly unable to apply this information, when presented with a question asking them to think about the size of cells versus tissues. Overall, the present analysis suggests that students require more training in higher level thinking skills[15]. This goal will prove difficult, given the weak basic concept knowledge of our student population. It is a skill they highly need to perform their everyday duties in most allied health careers and as educators, we should keep incorporating more critical thinking skills into the curriculum[6].

Our data also identified specific concepts in the curriculum that require clarification and/or more in-depth explanation, as students were unable to master 2 out of 10 basic concepts. Students have a limited understanding and/or retention of macromolecules and basic metabolic pathways such as cellular respiration, where there was no significant difference between performances in lower and higher level questions. The latter concept was even more problematic for students that had prepared for a high stakes common final, where performance was expected to be better than a low stakes unannounced post-test. This information is useful in curricular design of A&P, especially when considering how long to cover and what approaches (such as active learning) to take when teaching these difficult concepts[3].

Within our department, a number of faculty utilize active learning strategies, such as flipped classes and concept mapping in our attempts to improve both independent student study skills and higher level thinking. Indeed, we produced an active learning laboratory manual based on our prior experiences in developing a pre-A&P workshop[13]. However, these techniques all rely on the student's ability to independently assimilate and retain basic concepts. Instructors frequently complain that students do not remember concepts supposedly learned in prior classes. The present study shows that students in A&P II retain a few, but not enough, basic concepts covered in A&P I.

The present study also demonstrates that our faculty do not agree on the application of Bloom's taxonomy, or similar models, to their assessment efforts. Earlier more extensive studies, predominantly in K-12 education, also demonstrate that educators trained in the taxonomy do not agree on the classification of questions[16]. While references to Bloom's taxonomy and assessment abound, there is actually very little published in the area of A&P. One study attempted to address classification of science questions by sixth grade educators[17], although there was no reference to individual educator differences. In the present study, although faculty generally agreed in categorizing lower level questions, there was a larger spread in categorizing higher level questions. In informal discussions, faculty did not agree on what a higher level of question means when applied to at risk community college students. For example, the idea that 'easier' questions were actually 'higher' level for second language learners who have to apply new concepts into a weak English background was common. For our student population, mastering A&P I & II may be analogous to asking A&P faculty to master theoretical physics written and taught in Chinese.

Students may also be unable to discriminate between lower and higher level questions. When asked to evaluate lower and higher level questions, A&P II students thought that both questions were of similar difficulty and that they had been taught the necessary information to answer them successfully, but fewer students correctly answered the higher level question (Gannon, unpublished observations). In informal student surveys conducted towards the end of the semester the most frequent complaint is that 'tricky' questions do not test their knowledge of the concepts and are unfair.

It is commonly accepted that higher levels of learning must be preceded by the acquisition of basic concepts. We agree with Booker[11] who states, "Bloom's taxonomy has been used to devalue basic skills education and has promoted "higher order thinking" at its expense." Not surprisingly, the present study identified areas where students were able to master basic concepts, but were unable to apply them. Our observations, while common, do not predict well for student success in allied health careers, or transition to four year programs, where higher levels of comprehension are both expected and assumed.

In conclusion, several studies such as Harris *et al.*[5] have identified lower outside workload and better preparation as key factors in student success in A&P. We have previously shown that a pre-A&P workshop improves student performance in A&P[13], which can presumably be attributed to better preparation for the course. However, many of our A&P students have not planned enough time to study outside of the classroom and expect faculty to extract pertinent information for them. In an attempt to improve independent student study skills, Bronx Community College has developed and piloted a Freshman Year Initiative course. While not specifically directed towards STEM courses such as A&P, early results of this initiative including increases in student retention and credits earned are encouraging[18]. More intervention efforts should be encouraged to try and address the discouraging performance identified in this study.

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The Presence of Histamine and a Histamine Receptor in the Bivalve Mollusc, *Crassostrea virginica*

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Abstract

Histamine, a biogenic amine, is a neurotransmitter in neurons and sensory receptors in invertebrates. Histamine has rarely been reported in bivalves. We used HPLC with pre-column derivatization using 2,3-naphthalenedicarboxaldehyde (NDA) as a fluorescent labeling agent to measure histamine in ganglia, and peripheral tissues of the oyster *Crassostrea virginica.* We also used Western Blot technique to look for the presence of a histamine receptor in the mantle rim. HPLC results found histamine present in ng amounts in both the cerebral and visceral ganglia, as well as the mantle rim and other peripheral tissues of *C. virginica.* The study confirms and quantifies histamine as an endogenous biogenic amine in *C. virginica* in the nervous system and innervated organs. Western Blot technique also identified a histamine H2-like receptor present in sensory tissue of the oyster's mantle rim.

Introduction

Histamine is a biogenic amine found in a wide variety of invertebrates, where it has been found to be involved in local immune responses as well as regulating physiological function in the gut. It also functions as a neurotransmitter, especially for sensory systems[1]. Histamine has been well studied in arthropods and gastropods, but has been rarely reported to be present or have a function in bivalves other than the limited reports identifying it in ganglia and nerve fibers of the Baltic clam, *Macoma balthica*[2,3]. In the gastropod *Aplysia*, histamine neurons are involved in feeding and respiration[4-12]. In insects and other arthropods, histamine neurons have been shown to be involved in photoreception[13-15].

Bivalves, including the oyster, *Crassostrea virginica*, contain dopamine, serotonin and other biogenic amines in their nervous system and peripheral tissues. These biogenic amines serve as neurotransmitters and neurohormones and are important in the physiological functioning of the animals[16-18]. *C. virginica* has a reciprocal dopaminergic and serotonergic innervation of the lateral ciliated cells of the gill, originating in the cerebral and visceral ganglia, which slow down and speed up the beating rates of the cilia, respectively[19]. This neurophsyiological system is a useful model with which to study the actions of these and other biogenic amines. A preliminary physiology study in our lab indicates that histamine may be involved in a sensory-motor integrative response between the animal's sensory system in the mantle rim and beating of the gill lateral cell cilia. The pharmacology suggests the presence of a histamine H2-

like receptor in the mantle rim tissue based on the responses to histamine receptor agonists and antagonists[20].

In the present study we used HPLC to identify and quantify histamine in the nervous system and innervated organs of *C. virginica*, and immunoblotting to determine if histamine receptors are present in the sensory tissue of the mantle rim. Historically, methods to quantify histamine in tissue samples have had low accuracy and specificity. Recently a new HPLC analytical method, using pre-column derivatization with 2,3-naphthalene-dicarboxaldehyde (NDA) followed by fluorescence detection, showed improved accuracy and detection sensitivity for histamine[21,22]. We used this pre-column NDA derivatization technique in the present study.

Materials and Methods

Adult *C. virginica* of approximately 80 mm shell length were obtained from Blue Island Oyster Company, Sayville, NY, and maintained in the lab for up to two weeks in temperature regulated aquaria containing Instant Ocean® artificial sea water (ASW) at 16-18° C, specific gravity of 1.024 ± 0.001, 31.9 ppt salinity and pH of 7.8 ± 0.2. Animals that fully closed in response to tactile stimulation and required at least moderate hand pressure to being opened were used in each experiment.

NDA and histamine were obtained from Sigma-Aldrich (St. Louis, MO). Gemini 5µ C18 reverse phase HPLC columns were obtained from Phenomenex (Torrance, CA). NP-40 lysis buffer, Bradford reagent, Laemmli 2X loading buffer with β-mercaptoethenol (βME), Bio-Rad Mini-Protean TGX gels (10%), Bio-Rad Precision Plus Protein WesternC Standards, Tris/glycine SDS buffer and Bio-Rad Precision Protein StrepTactin-HRP conjugate were obtained from Bio-Rad.

Goat polyclonal anti-histamine H2 receptor 1° antibody (sc19773) and chicken anti-goat IgG-HRP 2° antibody (sc2953) were obtained from Santa Cruz Biotechnology. CN/DAB Substrate, Pierce Western Blot Signal Enhancer and all other reagents were obtained from Fisher Scientific.

Sample Preparation for HPLC Analysis

Right shells of animals were removed and mantle rim, mantle, heart, palps, posterior adductor muscle and gill were dissected, blotted dry, weighed. Approximately 1 g of each tissue was placed in eppendorf tubes containing 2 ml of 0.4M hydrochloric acid on ice. Cerebral ganglia and visceral ganglia were removed and pooled from 6 and 8 animals, respectively, and placed in eppendorf tubes containing 1 ml of hydrochloric acid on ice. Ganglia and tissues samples then were homogenized on ice with a Brinkman Polytron and centrifuged at 2,000 x g for 20 minutes at 3° C. The supernatants were re-centrifuge at 15,000 x g for 20 minutes. The resulting supernatants were vacuum filtered through 0.24 micron Millipore filters and the filtrates kept on ice for the derivatization reaction.

NDA Derivatization Reaction

Histamine standards and tissue fltrates were adjusted to pH 9.5 with NaOH. Aliquots (0.6 ml) of each standard or filtrate were derivatized at room temperature by adding in sequence: 0.2 ml borate buffer (20 mM, 10% v/v acetonitrile, pH 9.5), 0.2 ml potassium cyanide (20 mM) and 0.4 ml NDA (0.3

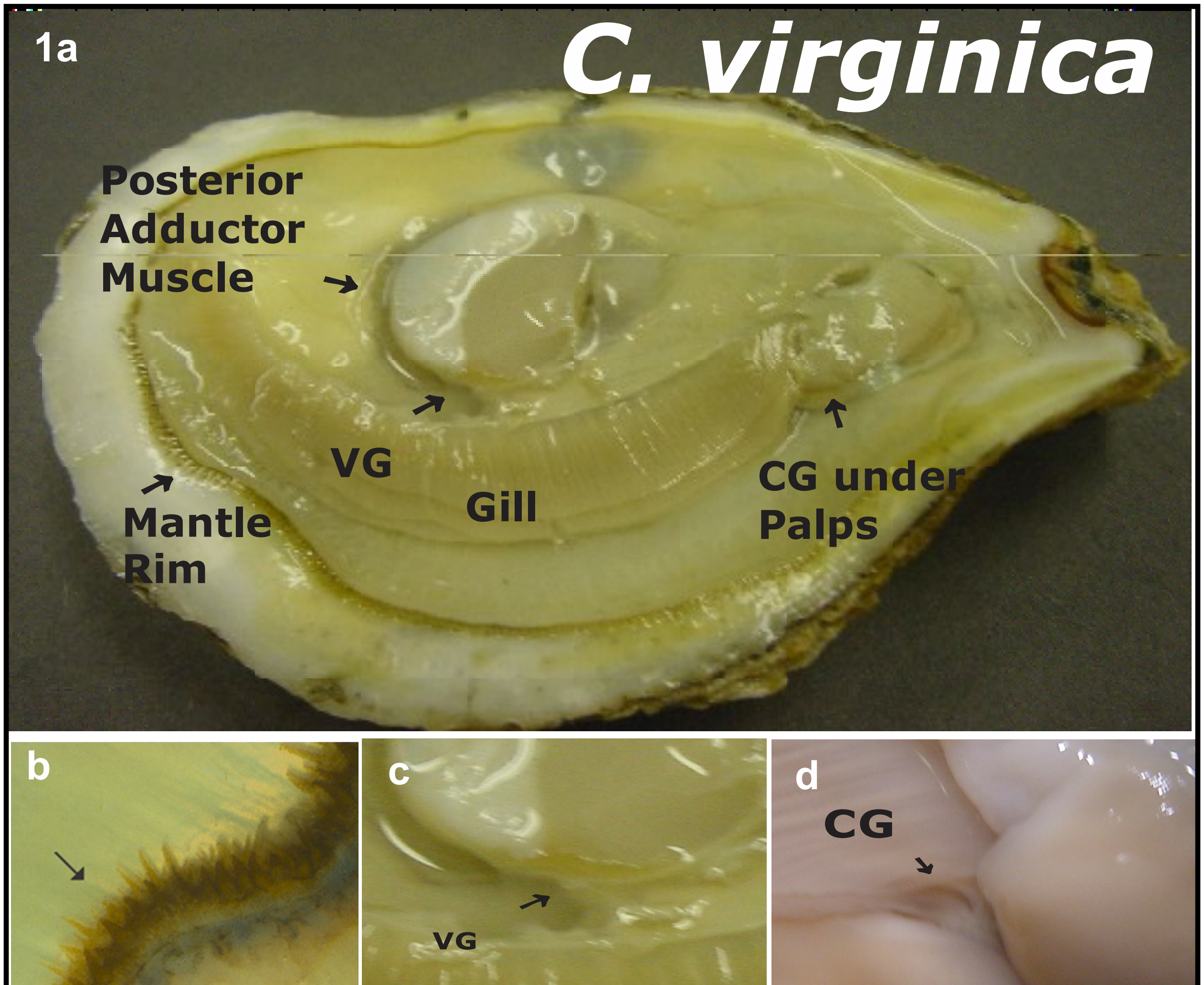

Fig. 1. a - oyster with right shell removed showing tissue; b - enlargement showing sensory tentacles of the mantle rim (arrow), c - enlargement show visceral ganglia (VG), d– enlargement showing cerebral ganglia (CG) after removal of palps .

mM in methanol). After exactly 15 minutes of derivatization an aliquot of each derivitized sample was injected into the HPLC for separation and analysis.

HPLC Analysis and Sample Detection

Aliquots (20 µl) of derivatized samples were injected into a Beckman System Gold HPLC fitted with a Phenomenex-Gemini 5µ C18 reverse phase column and a guard column. The isocratic mobile phase (40/60 v/v acetonitrile /phosphate buffer, 50 mM, pH 6.8) had a flow rate of 2 ml/min. To detect and quantify derivatized histamine, the effluent from the HPLC column flowed through a Jasco FP 2020 Plus Spectrofluorometer fitted with a 16 µl flow cell set for 450 nm excitation and 484 nm emission. A histamine standard curve was generated and used to quantify histamine levels in the samples. Results are reported as ng/g wet weight for peripheral tissues and ng/ganglion for cerebral and visceral ganglia.

Fluorescence intensity of samples produce by NDA derivatization was time dependent. This method was very sensitive at quantifying histamine, but samples had to be derivatized one at a time to ensure that the reaction time was the same for each sample prior to HPLC injection. We found 15 minutes of derivatization was optimal and provided consistent results for both standards and tissue samples. If samples were allowed to derivatize longer than 30 minute before injection there was a marked decrease in fluorescence intensity.

Sample Preparation for Western Blot

Preparation of Tissue Lysate

Mantle rim tissue was dissected, rinsed well in ASW, blotted, cut into ½ inch segments and weighed. Each segment was placed into an Eppendorf tube with 2.5 mL of ice cold NP-40 lysis buffer (containing protease and phosphatase inhibitors), and sonicated on ice for 2-3, 5 sec bursts with a Brinkman Polytron. Sonicated samples were kept for 30 min on ice, then centrifuged at 10,000 x g for 20 min and pellets discarded. The lysate supernatants were pooled and aliquots were analyzed for protein concentration by Bradford assay. Aliquots of the remaining lysate were adjusted to a protein concentration of 4-5 mg/mL and stored at -80° C.

Preparation of Samples for Loading onto SDS-PAGE Gels

Lysate proteins were denatured by mixing aliquots in a 1:1 ratio with Laemmli 2X loading buffer containing βME and allowing the mixture to sit for one hour at room temperature. Laemmli-treated samples (20-40 µg total protein) were wet -loaded into wells of polyacrylamide gels (Bio-Rad Mini-Protean TGX gels, 10%), alongside pre-stained molecular weight markers (Bio-Rad Precision Plus Protein WesternC Standards), then electrophoresed in Tris/glycine SDS buffer (25 mM Tris, 190 mM glycine, 20% methanol, 0.1% SDS, pH 8.3), for approximately 1 hour at 150 v.

Western Blotting

Gels were removed from their plate, and before immunoblotting was started, the pre-stained WesternC standards were visualization to ensure that proteins migrated uniformly and evenly during electrophoresis. Gels were rinsed in transfer buffer (25 mM Tris, 190 mM glycine, 20% methanol, pH 8.3), and sandwiched onto nitrocellulose membranes. Wet-transfer was done in a Mini Trans-BlotR electrophoretic cell (Bio-Rad) under constant current at 20 v for 150 min in the presence of a cooling module to prevent excess heating. After transfer, membranes were rinsed with ddH2O, treated with a Western Blot Signal Enhancer (Pierce), rinsed 5x with ddH_2O, and then blocked with 5% non-fat dry milk in TBS-T (tris buffered saline, 0.1% Tween-20) for 1 hour at room temperature. Membranes were treated with the 1° antibody (goat polyclonal anti-histamine H2 receptor) at 1:300 dilution (TBS-T and 2% blocker) for 24 hours at 4° C, followed by extensive washing with TBS-T. Membranes were then exposed to the 2° antibody (chicken anti-goat IgG-HRP) 1:7000 dilution for 60 min at room temperature. During this time, Precision Protein StrepTactin-HRP conjugate (Bio-Rad) was also present (1:5000 dilution) to resolve protein standards on the nitrocellulose membrane. Membranes were washed extensively with PBS-T (phosphate buffered saline with Tween), followed by chromogenic detection of HRP-conjugated standards and histamine H2 receptor protein using CN/DAB Substrate (Fisher Scientific). After membrane blots were chromogenically developed, images were captured using a Carestream GI212 Pro Molecular Imaging System.

Results

HPLC

The HPLC parameters used were able to resolve NDA derivatized histamine in the standards and tissue samples as a strong peak with a retention time of 6.6 minutes (Fig. 2). The NDA derivatization reaction produced a fairly linear concentration response for histamine standards over the range of 1 femtogram to 1 nanogram (Fig. 3). HPLC analysis revealed histamine in peripheral tissues ranging from about 1.0 - 2.5 ng/g wet weight, with heart having the highest amounts and gill the least (Fig. 4). Histamine also was detectable in both cerebral and visceral ganglia with cerebral ganglia containing about 1.5 ng/ganglion and visceral ganglia about 0.25 ng/ganglion (Fig. 5). Co-injecting histamine standards and tissue samples revealed a single derivatized histamine peak. Other amines tested with this NDA-derivatization procedure, including GABA, glutamine, dopamine, serotonin and histadine, did not have retention times similar to that of histamine.

Western Blot

Western Blot analysis of *C. virginica* mantle rim proteins revealed the presence of histamine H2-like receptors. A protein band of 70 kDa, corresponding to histamine H2 receptors is shown in Fig. 6.

Fig. 2

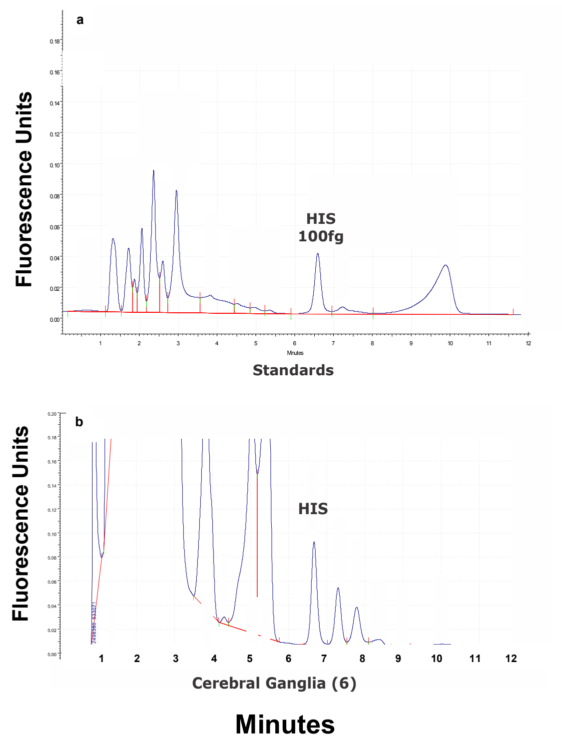

Fig. 2a. HPLC trace of 100 fg of histamine standard; 2b. HPLC trace of 6 (3 pairs) of pooled cerebral ganglia.

Fig. 3

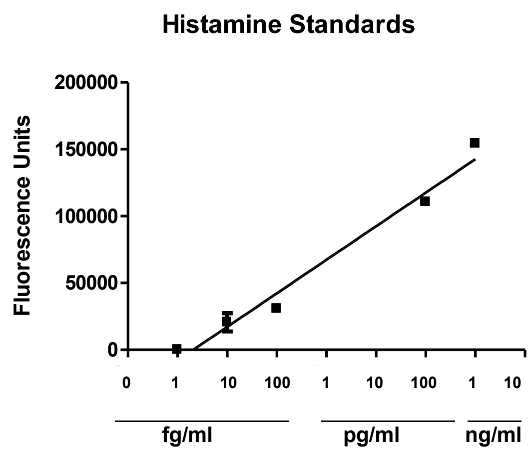

Fig. 3. Standard curve for histamine using HPLC and fluorescence detection with pre-column derivatization with NDA.

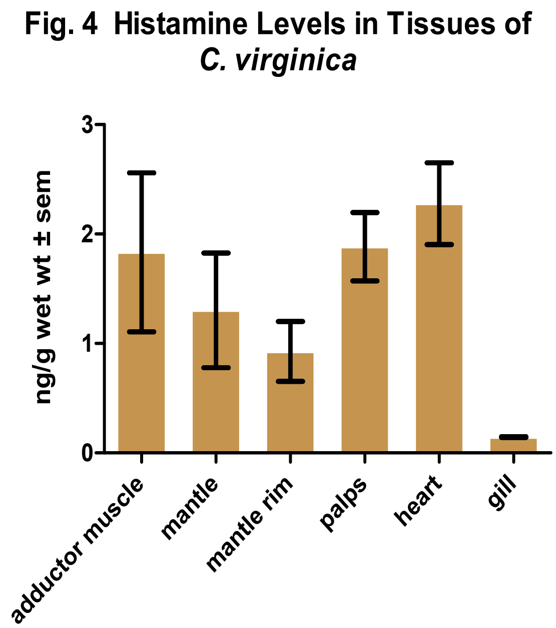

Fig. 4. Histamine levels (ng/g ± sem) in adductor muscle, mantle, mantle rim, palps, heart and gill of the oyster *C. virginica* detected by HPLC with fluorescence detection. N = 2.

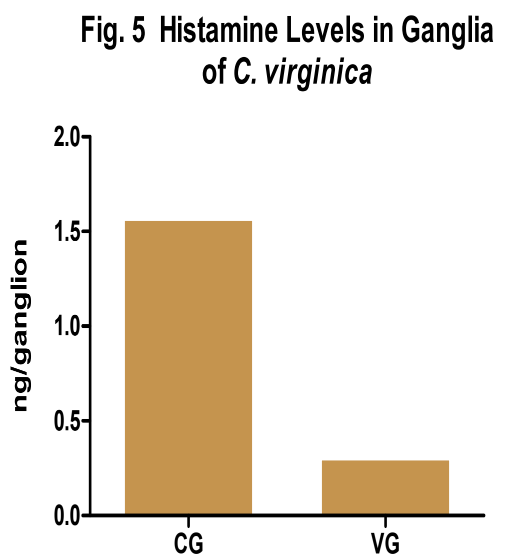

Fig. 5. Histamine levels (ng/ganglion) in the cerebral ganglia (CG) and visceral ganglia (VG) of the oyster *C. virginica* detected by HPLC with fluorescence detection. For the CG, ganglia from 3 animals were pooled for analysis. For the VG, ganglia from 4 animals were pooled.

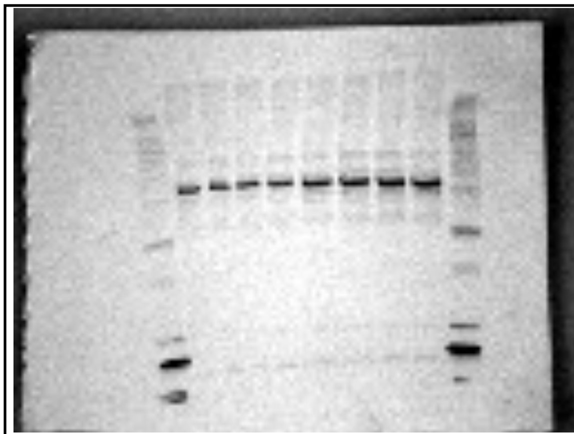

Fig. 6. Western Blot of mantle rim showing a protein band of approximately 70 kDa indicating the presence of an H2 histamine receptor.

Discussion

Histamine is an important bioactive compound, serving as a neurotransmitter and neurohormone in invertebrates, but has rarely been studied in bivalves. Histamine is considered to be a universal neurotransmitter of arthropod photoreceptors[23]. Histamine is a neurotransmitter of statocyst hair cells and involved in graviception In gastropods, and it has been suggested that histaminergic involvement in graviception may be a general feature of many molluscs[24].

Preliminary work with *C. virginica* in our lab showed histamine to be involved in a sensory response of the mantle rim that effects gill lateral cilia beating rates[25]. These findings prompted us to look for histamine in various tissues and ganglia of *C. virginica* using a recently developed HPLC method with NDA pre-column derivatization. In the present study we found histamine in small amounts in oyster peripheral tissues (ng/g amounts) and ganglia (ng/ganglion). For comparison, other biogenic amines present in *C. virginica* such as serotonin and dopamine are about 100 - 200x higher in peripheral tissues and about 10x higher in ganglia[26].

In this study we also were able to use immunoblotting to demonstrate the presence of a strong band at 70 kDa correlating with histamine H2 receptors. Our Western Blot results match well with that of Matsuda *et al.* who found a 69 kDa band identified as histamine H2 receptor proteins in rat and human tissues[27].

The mantle rim of bivalves is a sensory structure containing various sensory receptors. The involvement of histamine in sensory systems of invertebrates, particularly gastropods, coupled with our preliminary physiology research, strongly suggest histamine to be a sensory neurotransmitter in the mantle rim of *C. virginica*. Future work will involve a full pharmacological study of the physiological effects of histamine at the mantle rim, as well as immunohistochemical studies of histamine and histamine receptor types in mantle and other innervated tissues of *C. virginica*.

Acknowledgments

This work was supported in part by grants 2R25GM06003 of the Bridge Program of NIGMS and 0516041071 of NYSDOE.

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