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KINGSBOROUGH COMMUNITY COLLEGE TO HOST MACUB CONFERENCE SATURDAY, OCTOBER 24, 2009

CONFERENCE THEME: PANDEMIC INFLUENZA and EMERGING INFECTIOUS DISEASES

Kingsborough Community College will host the 42nd Annual Fall MACUB Conference on Saturday, October 24, 2009 The conference theme will be "Pandemic Influenza and Emerging Infectious Diseases" and will feature a keynote address by a member of the Department of Health and Human Services Center for Disease Control and Prevention.

You are invited to participate in the 42nd Annual Fall MACUB Conference. Abstracts are now being accepted for review for member paper presentations and poster presentations.

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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.

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Out of the Classroom and into the Halls of Science: Breaking New Ground in Students' Acceptance of Evolution?

by

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ABSTRACT

Major and non-majors biology students from our community college visited the new Spitzer Hall of Human Origins at the American Museum of Natural History (AMNH) armed with a series of questions that the authors had prepared for them to use as a guide during their visit. Using the MATE (Measure of Acceptance of the Theory of Evolution) instrument, students were surveyed about their views on evolution prior to their visit to the AMNH and upon their return. The purpose of the study was to determine whether there were any shifts in students' attitude towards evolution as a result of a visit to the museum and classroom discussions on evolution. The results indicate that a visit to the museum, as an addition to regular instruction, produced a positive shift in students' acceptance of evolutionary theory.

Introduction

The purpose of this study was to expose students at the community college level to various methods of teaching evolution so that they could develop a positive view of this all-important biological concept. A trip to a museum of natural history might help change students' preconceived notions or misconceptions about evolution, thereby leading to their acceptance of the evolutionary concept. Similar museum trips and exhibits concerning evolution may be planned and executed by biology faculty in many parts of the country.

One example of the importance of, not only accepting, but understanding the concept of evolution, is the need to explain to students the evolution of human pathogens, such as the HIV or Ebola virus. This generation of biology students, which will give us future scientists, needs acceptance and understanding of evolution in order to be able to fulfill the task of manufacturing drugs that can treat disease caused by human pathogens that can evolve into more dangerous forms[1].

The unity and diversity of life on earth can only be adequately explained by the Theory of Evolution. Biology teachers attempt to teach their students that "…nothing in biology makes sense, except in the light of evolution"[2]. However, biology students come to college with misconceptions that are hard to change. Furthermore, college biology instructors do not always know what students have been taught in high school. For example, a recent article by Randy Moore[3] pointed to the fact that some high school teachers in the state of Minnesota teach creationism (intelligent design) in their classrooms, although the subject is not included in the state's science curriculum. Moreover, he also discovered that only 52% of these high school biology teachers emphasized the theory of evolution. Despite this fact, what is taught in high school and what is incorporated into students' belief systems may not be related to one another.

We believe a more comprehensive way to enhance students' views on evolution is needed for the following reasons: in New York City, community college students come from a diversity of backgrounds, some parochial, some secular. Many community college biology students have alternative high school degrees (GEDs) that do not focus on science concepts. Often, students come to biology classrooms without an open mind towards the theory of evolution. Furthermore, one should not dismiss the reality of teaching evolution within a social, intellectual and pedagogical context. Some students not only enroll in biology courses with misconceptions and skepticism about evolution; they also bring attitudes that are the result of the religious and philosophical environment to which they have been exposed[4].

Often, teachers find themselves in the position of having to address the interface between science and religion, where the two sides may be diametrically opposed. While some instructors advocate the integration of the two positions[5], most scientists utilize the concept of "non overlapping magisteria"[6] to make the persuasive case for teaching science completely

independent of religion and other philosophical positions. Therefore, the task of the biology teacher is to create situations that result in disequilibria, thus causing the students to doubt their initial views and open themselves up to new ideas concerning evolution.

Frequently, one hears students discussing the theory of evolution and saying, "well that's just a theory". It is imperative, therefore, that prior to the teaching of evolution, instructors communicate to students the nature of science[7], where theories are proposed and evaluated as the result of extensive research and accumulation of data. Hopefully, students will comprehend the difference between the meaning of the term theory in every day language and in science. Only then, may students discuss and analyze theories on the basis of scientific evidence.

It is important to point out that in the non-science major college biology courses, faculty are often faced with additional problems such as apathy, lack of interest, a paucity of background knowledge. This puts a burden on teachers to be dynamic and expose students to many types of engaging techniques in order to stimulate their interests. For instance, at Kingsborough Community College, City University of New York, the non-science major biology laboratory curriculum includes a laboratory exercise called Prey and Predators[8]. This exercise gives students first-hand opportunities to understand how populations affect one another, resulting in micro-evolutionary changes over time. During this exercise, which demonstrates the nature of science, students become the "predators" and hunt the "prey" (different color beans spread on a lawn). The red and white "prey" become extinct, while the brown and green "prey" survive. After 3-4 generations, some "predators" with selective adaptations (better capturing implements) survive while the other predators perish. The experience creates a breakthrough situation, leaving students open to discussions concerning the theory of evolution.

In general, acceptance of evolution by the general public in the United States is dismal. In an editorial, Gerald Weissmann[9] pointed out that, while science has undoubtedly increased human longevity, this achievement has been reached in spite of the American public's attitude towards science. The majority of adults in the United States do not believe in Darwinian evolution. In contrast, in the Scandinavian countries and in France, 80% of the populace accepts the theory of evolution. The United States is placed next to the last, followed by Turkey, out of 33 countries surveyed for their acceptance of evolution. In fact, the movement to impose the teaching of creationism has furthered this American decline. Even Iran has pro-science and pro-evolution policies! The Scopes "monkey trial" is still being encountered in certain states in the United States at the present time[9]. Miller believes that creationists want to displace Darwinian evolution from textbooks and curricula, but they also want to undo four centuries of western science[10].

Given these circumstances, we can say that instructors of biology have a difficult task and enrichments, like museum visits should be very helpful in changing students' perception of evolution.

Method

The main question that this study was set up to answer was: Can students' attitudes and acceptance of evolution be changed by utilizing out-of-classroom biology interventions, along with conventional teaching methods? Similar out-of-classroom interventions are not just limited to New York City, and may be found in any town or city across the nation.

In addition to classroom discussions on evolution during the lecture, both science and non-science major biology students were sent to visit the new Spitzer Hall of Human Origins at the American Museum of Natural History (AMNH), in New York City, armed with a detailed guide with questions to answer (Table I), prepared by the authors. This innovative exhibit illustrates the evolutionary links between humans and related organisms, both ancestral and current. Incidentally, another goal of this project was to acquaint the students with the AMNH in its entirety and the variety of other exhibits that it has to offer. Often, following a visit to the AMNH, students exclaimed that "this was my first time going to the Museum" and that they "planned to return again and bring family members with them".

We then used the "MATE" (Measure of Acceptance of the Theory of Evolution) instrument seen in Table 2, (initially developed and used by Rutledge & Warden[11] to assess public high school teachers' acceptance of the evolutionary theory) to assess our community college biology students' acceptance of evolution. The 20-item instrument uses a Likert scale with five points; strongly agree, agree, undecided, disagree and strongly disagree. Of the 20 evolution evaluative items, ten are positively phrased statements and ten are negatively phrased statements. Table 3 includes MATE scoring instructions. Scores may range from 20 to 100 for the total MATE. The higher the score, the more accepting is the attitude towards evolution. This instrument has high validity and reliability. In order to test its reliability, the instrument was administered to university students. The results showed the MATE to be both consistent internally and stable over time[12]. Therefore, we used the MATE to evaluate the effectiveness of our teaching strategy; such as the one used in the present study. Students were given the MATE twice during the semester; first prior to any instruction on evolution and again at the end of the semester after having been exposed to classroom instruction and the museum visit. The subjects were students enrolled in a majors General

Biology course (Bio 13, 4 credits, 6 hours) and in a Biology course for non-science majors (Bio 33, 4 credits, 5 hours).

In addition, the MATE instrument results of a small group of matched students (n = 17), identified by number only, were compared pre-and post-instruction and museum visit. Each section of biology students (n = approximately 24) in both the science and non-science major biology groups was compared pre- and post-museum trip and instruction. Furthermore, the two groups (science and non-science majors) were compared with each other.

Permission from the students was requested in writing. Approval from the Committee for the Protection of Human Subjects was obtained at Kingsborough Community College from the IRB (Institutional Review Board) Committee. In addition, NIH certification for the study of human subjects was obtained by both investigators, using the NIH Office of Human Subject Research computerized certification process.

We also asked an interesting question; Do students view human evolution differently from general evolution? To obtain an answer to that question, we examined students' response to Items # 3 and 15 (a positive and negative matched pair on human evolution) compared to their responses to Items 4 & 16 (a positive and negative matched pair on general evolution). Students' answers to these matched sets of items might better demonstrate whether students accept *general evolution* as compared with *human evolution*. Often students express the misconceptions that evolution occurs for other groups of organisms, but not for humans.

Results and Discussion

A. Major Biology Students

The scores of each section of General Biology (Biology 13) were compared before and after the trip to the AMNH and, as demonstrated in Figure 1, there was an increase in students' acceptance of the theory of evolution as demonstrated by MATE scores, after attending the museum exhibit and answering the questions prepared for them. Subsequently, we compared the total MATE scores of all biology major sections, pre- and post-museum visit. The totals showed that there was an increase in their acceptance of the concept of evolution, as measured by the MATE scores.

As previously discussed, items # 3 and 15 of the MATE dealt with human evolution, while items # 4 and 16 dealt with general evolution. We wanted to determine whether these students viewed human evolution differently from general evolution.

Therefore, we compared the mean scores specifically for these items pre- and post-visit to the museum. Surprisingly, the data showed slightly higher means (7.2) for human evolution as compared to general evolution (7.0), although both aspects increased in value post-museum trip (8.1 and 7.7, respectively).

These findings are confirmed by the scatterplot of Biology major students' scores for these specific items, before (Fig. 2a) and after the museum visit (Fig. 2b). The post-museum scatterplot showed a shift of scores to the right indicating higher scores on both sets of questions. Pearson correlation coefficients were utilized to compare General Biology students' scores on human evolution (Items #3 & 15) versus their scores on general evolution (Items # 4 & 16). The Pearson correlation coefficient (0.270) of the pre-museum trip scores is significant at $p = 0.008$, while the Pearson correlation coefficient (0.436) post-museum trip was even higher at $p = 0.000$. This indicates a closer relationship between acceptance of the concepts of human evolution and general evolution.

B. Non-Science Major Biology Students

For the non-science major biology courses (Biology 33), there was an increase in the majority of the sections' MATE instrument scores post-museum trip, as shown in Figure 3. The total MATE score averages for all Biology 33 sections went from 73, pre-museum trip, to 77 post-museum trip. These results might be explained as possibly due to the attrition of the less motivated, poorer students towards the end of the semester. However, a small group of non-science major students (n=17) who had completed the course, and were individually matched by name (anonymously reported) was also compared in the same manner (Fig. 4). The data for this small group showed the same results, an increase in the MATE scores, as the ones obtained for the entire non-science major group.

Again we posed the same question stated earlier, "Do non-science major students view human evolution differently from general evolution?" The non-science major scores on the MATE instrument for human evolution (Matched Items # 3 & 15) and general evolution (Matched Items # 4 & 16) were different pre- and post-museum trip. There was an increase in the acceptance of both human and general evolution post-museum trip, with a greater increase in general evolution, from 7.1 to 8.1, for general evolution, compared with 7.4 to 7.7 for human evolution. There was also an indication that this non-science group initially scored higher in their understanding of human evolution. This may be due to the fact that in our non-science major course instructors included a complete lecture analysis of human evolution prior to students' visit to the museum. Figs. 5a, b are scatterplots comparing students' score on human evolution (Matched Items # 3 & 15) versus their scores on general evolution (Matched Items # 4 & 16). The Pearson correlation (0.324) of the pre-museum trip scores is significant at $p = 0.005$, while the Pearson correlation (0.455) post-

Table 1

American Museum of Natural History Field Trip Spitzer Hall of Human Origins

1. How far back can we trace the human fossil record?
2. What is name of the human species?
3. What traits do extinct hominids share with modern humans?
4. Look at the three skeletons as you enter the Hall; what are the differences in characteristics between the chimpanzee skeleton, the ancient hominid skeleton and the human skeleton?
5. What is the name of the scientific field of biology that studies fossils?
6. After the extinction of the dinosaurs, mammals evolved and moved into new territories; what are the names of the first primates?
7. Where on earth did the first primates evolve?
8. What are three of the ancient features of primates?
9. What is the name of the last common ancestor of all modern apes and humans?
10. What happened to hominoids about 14 million years ago?
11. Draw a tree (Phylogenetic Tree) of life from the extinct to modern-day primates.
12. Ancient humans have roamed the earth; on what continent did they evolve first?
13. How are fossil bones dated? Explain.
14. Look at the "examining the evidence" exhibit; how can you tell by looking at the teeth what the individuals ate?
15. What features of the modern foot common to Neanderthals are important for standing?
16. What role do "animal remains" play in the life of early humans?
17. What can animal bones tell us about our ancestors?
18. What did our ancestors look like?
19. To what family of primates do humans belong?
20. What is the trait that separates primates from the early hominids?
21. Look at the exhibit "A walk through time"; what findings did scientist use to tell the size, weight and gender of these hominoids?
22. How long ago did Australopithecus afarensis live?
23. How did scientists conclude that the Turkana boy was a growing boy and not a complete adult human?
24. What did hominids eat?
25. Who were the first hominids to leave Africa?
26. What evidence do scientists have to confirm the "Out of Africa" theory?
27. Early hominid tools appeared about 2-5 million years ago; these tools were made of _____.
28. When did the Australopithecus group disappear?
29. Where was the fossil called "Lucy" found? What species was she?
30. How long ago did modern humans evolve?
31. How do scientists retrace the footsteps of humans who died 35,000 years ago?
32. View the movie called "The human bulletin" (you'll find it in a little room on the side) and write your impressions of this movie (just a short paragraph).

museum trip was higher at $p = 0.001$. The comparison of the two scatterplots illustrated a shift to the right, which indicated greater acceptance of both human and general evolution.

C. Comparison of Science and Non-Science Major Biology Students

When comparing the scores of science majors with non-science majors on the paired items (Items # 3 & 15, human evolution vs. Items # 4 & 16, general evolution), the non-science major group exhibited a greater increase in their acceptance of the evolutionary concept. Although we can only speculate as to the reason for this difference, based on the authors' experience of having taught both courses, one could say that non-science majors come to class tabula rasa when it comes to science, therefore they can be more open to new concepts.

This study was presented at the 2007 annual NABT convention in Atlanta, GA. During the Q. & A. that took place after the presentation, someone in the audience, a member of a science museum, made a suggestion for a follow-up study. The authors will therefore, continue to investigate students' acceptance of evolution in subsequent biology courses using a totally matched groups. We plan to use two different groups of students, where one group will visit the AMNH, and the other will

Table 2

The MATE Instrument

For the following items, please indicate your agreement/disagreement with the given statements using the following scale:

A	B	C	D	E
Strongly Agree	**Agree**	**Undecided**	**Disagree**	**Strongly Disagree**

1. Organisms existing today are the result of evolutionary processes that have occurred over millions of years.
2. The theory of evolution is incapable of being scientifically tested.
3. Modern human are the product of evolutionary processes that have occurred over millions of years.
4. The theory of evolution is based on speculation and not valid scientific observations and testing.
5. Most scientists accept evolutionary theory to be a scientific valid theory.
6. The available data are ambiguous (unclear) as to whether evolution actually occurs.
7. The age of the earth is less than 20,000 years.
8. There is a significant body of data that supports evolutionary theory.
9. Organisms exist today in essentially the same form in which they always have.
10. Evolution is not a scientific valid theory.
11. The age of the earth is at least 4 billion years.
12. Current evolutionary theory is the result of sound scientific research and methodology.
13. Evolutionary theory generates testable predictions with respect to the characteristics of life.
14. The theory of evolution cannot be correct since it disagree with the Biblical account of creation.
15. Humans exist today in essentially the same form in which they always have.
16. Evolutionary theory is supported by factual historical and laboratory data.
17. Much of the scientific community doubts if evolution occurs.
18. The theory of evolution brings meaning to the diverse characteristics and behaviors observed in living forms.
19. With few exceptions, organisms on earth came into existence at about the same time.
20. Evolution is scientifically valid theory.

Table 3

MATE Scoring Instructions

Step 1. Scoring of items 1, 3, 5, 8, 11, 12, 13, 16, 18 and 20 is as follows:

Strongly Agree = 5
Agree = 4
Undecided = 3
Disagree = 2
Strongly Disagree = 1

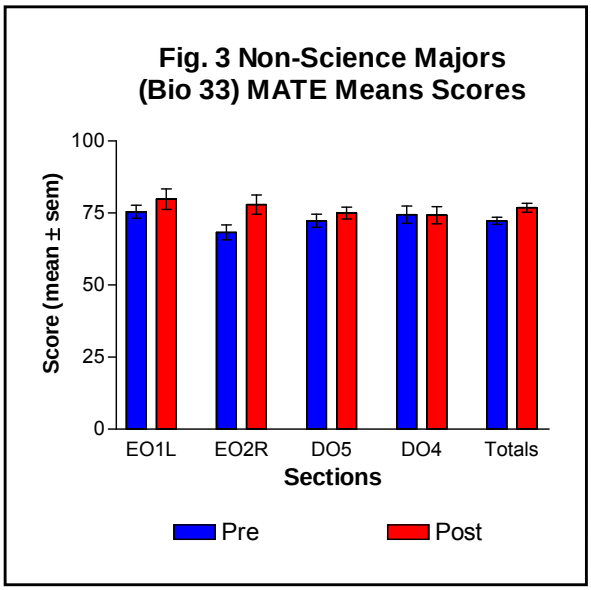
Step 2. Scorings of items 2, 4, 6, 7, 9, 10, 14, 15, 17 and 19 is as follows:

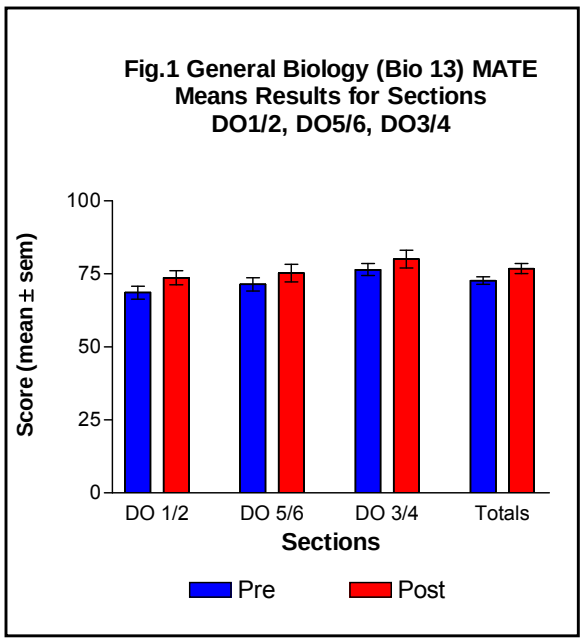
Strongly Agree = 1
Agree = 2
Undecided = 3
Disagree = 4
Strongly Disagree = 5

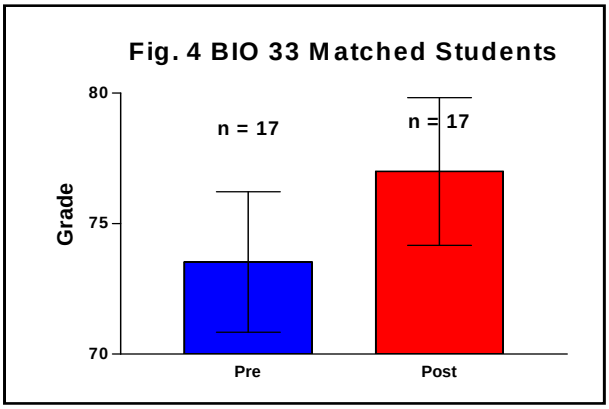
Step 3. An individual's score on the MATE is equal to the sum of the scaled responses to all 20 items.

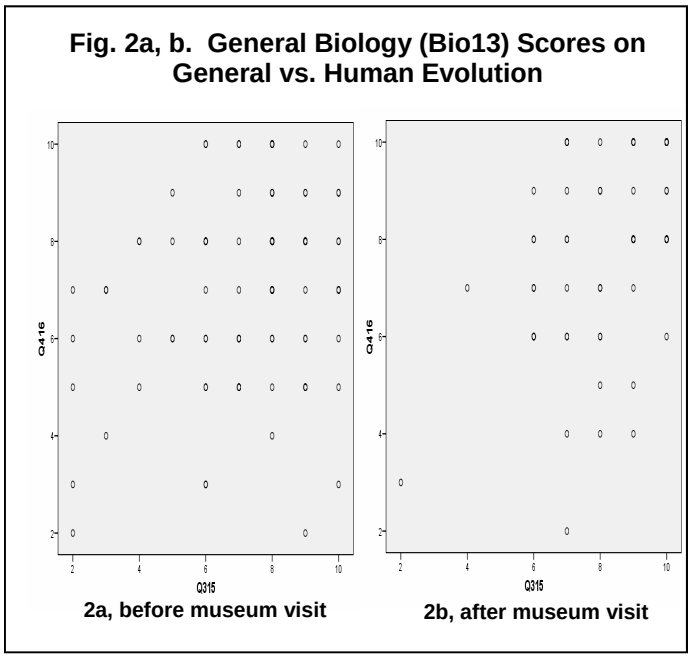
only be exposed to lectures and laboratory experiences on the concept of evolution. Both groups will be administered the MATE instrument at the beginning of the semester, before instruction, and at the end of the semester. Their scores will then be compared to see if the museum visit can be pinpointed as the significant determining factor in the shift produced in students' acceptance of evolution.

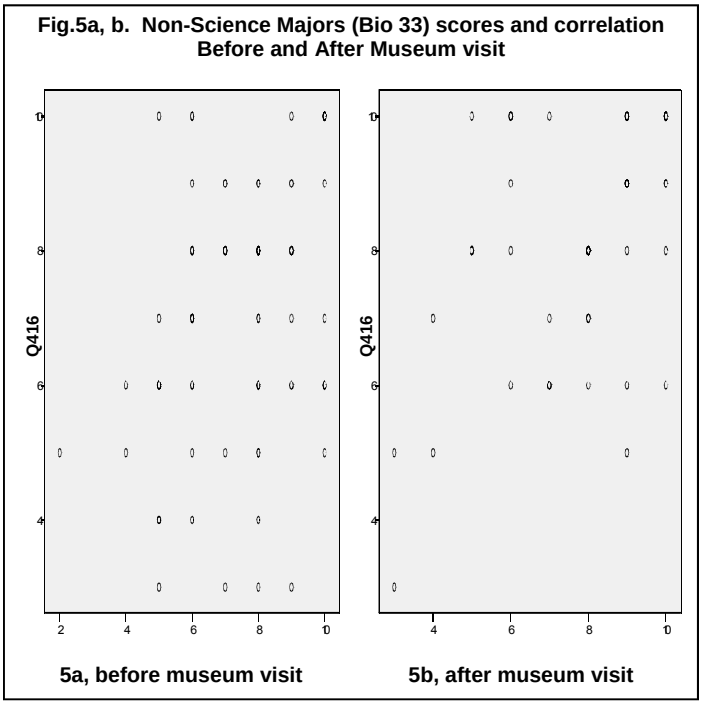
In conclusion, this study has illustrated the impact of enrichments, such as a museum visit, on students' acceptance of complex biological concepts such as evolution. It becomes more and more evident that passive lectures and traditional laboratory exercises alone can no longer be solely responsible for teaching subjects, especially in the sciences, where the fields are constantly expanding and changing.











References

[1]National Academy of Sciences and Institute of Medicine. (2008) *Science, Evolution and Creationism*. Washington, D.C: The National Academies Press.

[2]Dobzhansky, T., 1973. Nothing in Biology Makes Sense Except in the Light of Evolution. The American Biology Teacher **35(3):** 125-129.

[3]Moore, R., 2007. The Differing Perceptions of Teachers and Students Regarding Teachers' Emphasis on Evolution in High School Biology Classrooms. The American Biology Teacher **69(5):** 268-271.

[4]Anderson, R., 2007. Teaching the Theory of Evolution in Social, Intellectual, and Pedagogical Context. Science Education **91(4):** 664-677.

[5]Nord, W.A., 1995. *Educating for Intellectual Belief and Unbelief*. New York: Teachers College Press.

[6]Gould, S.J., 1997. Non Overlapping Magisteria. Natural History **106:** 16-22.

[7]Working Group on Teaching Evolution and National Academy of Science (1998). *Teaching about Evolution and the Nature of Science*. National Academy Press. Washington DC.

[8]Frye, V., 1981. Predator and Prey. *Exploring Biology in the Laboratory*. 2nd Edition. Orlando, FL. Harcourt Brace Jovanovich, Inc. 9-16.

[9]Weissmann, G., 2006. Teaching Evolution, Learn Science: We're Ahead of Turkey, but behind Iran. The FASEB Journal. **20:** 2183-2185.

[10]Miller, K.R., 2008*. Only a Theory: Evolution and the Battle for America's Soul.* Penguin Group (USA).

[11]Rutledge, M.L. and W.A. Melissa, 1999. The Development and Validation of the Measure of Acceptance of the Theory of Evolution Instrument. School Science and Mathematics. **99(1):** 13-18.

[12]Rutledge, M.L. and K.C. Sadler, 2007. Reliability of the Measure of Acceptance of the Theory of Evolution (MATE) Instrument with University Students. American Biology Teacher. **69(6):** 332-335.

Use of Artificial Eelgrass Mats by Saltmarsh-Nesting Common Terns (*Sterna hirundo*)

By

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Abstract

Terns and skimmers nesting on saltmarsh islands often suffer large nest losses due to tidal and storm flooding. Nests located near the center of an island and on wrack (mats of dead vegetation, mostly eelgrass *Zostera*) are less susceptible to flooding than those near the edge of an island and those on bare soil or in saltmarsh cordgrass (*Spartina alterniflora*). In the 1980's Burger and Gochfeld constructed artificial eelgrass mats on saltmarsh islands in Ocean County, New Jersey. These mats were used as nesting substrate by common terns (*Sterna hirundo*) and black skimmers (*Rynchops niger*). Every year since 2002 I have transported eelgrass to one of their original sites to make artificial mats. This site, Pettit Island, typically supports between 125 and 200 pairs of common terns. There has often been very little natural wrack present on the island at the start of the breeding season, and in most years natural wrack has been most common along the edges of the island. The terns readily used the artificial mats for nesting substrate. Because I placed artificial mats in the center of the island, the terns have often avoided the large nest losses incurred by terns nesting in peripheral locations. However, during particularly severe flooding events even centrally located nests on mats are vulnerable. Construction of eelgrass mats represents an easy habitat manipulation that can improve the nesting success of marsh-nesting seabirds.

Introduction

Common terns (*Sterna hirundo*) are colonially-breeding birds that live in a variety of habitats near water[1-3]. Although they are typically thought of as nesting on sandy and rocky beaches, in New Jersey most barrier beaches have been developed and terns nest most frequently on small saltmarsh islands[2,4,5]. Common terns appear to be adapted to nesting in marsh habitat[2,6-8], and saltmarsh islands have important advantages over mainland and barrier island locations, such as a lack of mammalian predation and less frequent human disturbance[7]. However, these islands are vulnerable to tidal flooding and storms, and large numbers of nests are frequently lost[2,7,9-11]. Rising sea levels are expected to exacerbate this problem[10,12,13].

Preferred nesting substrate on saltmarsh islands is wrack, mats comprised mainly of dead eelgrass (*Zostera*) or other dead vegetation deposited by flooding prior to nesting. Nests on wrack are better able to survive flooding than nests on bare soil or in saltmarsh cordgrass (*Spartina alterniflora*), because eelgrass mats provide additional elevation, provide structure, and can float[2,4,5]. The availability of wrack is limited, however, as hundreds of terns may nest on a small island and wrack covers only a small percentage of an island's area. Nests on wrack therefore occur at a higher density than those in other habitat[2,4,9,14]. Nests located in the center of an island are also less likely to experience flooding than those along the edges, but during severe floods an entire island can be under water and complete nesting failure of a colony can occur[2].

Although global populations are large and probably stable, the common tern is listed as "Species of Special Concern" by the New Jersey Division of Fish and Wildlife and is also a focal species for conservation in other states and provinces along the Atlantic coast[15]. In the area where this study took place, the Barnegat Bay ecosystem in New Jersey, the common tern population has decreased since the 1980's in both number of individuals and number of colonies[12,16,17]. This decline may be in part due to increased flooding and lower availability of wrack[16,17]. Other contributing factors include nest site competition with herring and great black-backed gulls (*Larus argentatus* and *L. marinus*)[12,16,17], which can also act as nest predators, and disturbance by personal watercraft[16,18]. These factors do not act in isolation. For example, gulls can increase the effects of flooding by causing terns to nest on lower sites[16,19], and flooding can increase susceptibility to nest predation by gulls[19]. Tern colonies are often actively managed by controlling gulls, limiting human disturbance, and modifying habitat to increase or improve nesting substrate[3,10,15,20,21].

In the 1980's, Burger and Gochfeld[2,22] constructed mats of eelgrass on several saltmarsh islands in Barnegat Bay and documented use of these artificial mats by common terns and black skimmers (*Rynchops niger*), a state endangered species. Because of a lack of large natural mats, the only skimmers to nest successfully in Barnegat Bay in the late 1980's did so on artificial eelgrass mats[12,16,22]. That tern and skimmer nests survive flooding better on wrack than on other substrates[2,4,5,22], also suggests that these mats could be

used as a conservation tool to improve nesting success. Both Safina *et al*.[7] and Rounds *et al*.[10] have proposed placing or manipulating wrack on saltmarsh islands to encourage common terns to nest at higher elevations. The effect of adding artificial mats would be particularly important if suitable wrack becomes less available than in the past, which may have already occurred in Barnegat Bay[17].

I have studied the common terns at one of Burger and Gochfeld's original sites, Pettit Island, in most years since 1996. Skimmers abandoned this site several years before my study began and have not returned, except for one pair that nested unsuccessfully in 2001. In 2002 I began transporting eelgrass annually to this site to create artificial mats. Here I document usage of artificial eelgrass mats by common terns nesting at Pettit Island from 2002 through 2008, document the effects of flooding in this colony from 1996 through 2008, and examine whether the terns benefited from the presence of artificial eelgrass mats.

Methods

This study took place on Pettit Island (39°40'N, 74° 11'W), a 0.3 hectare saltmarsh island in Manahawkin Bay in Ocean County, New Jersey that has been the site of a common tern colony for decades[2]. Manahawkin Bay is part of the larger Barnegat Bay ecosystem, south of Barnegat Bay proper and north of Little Egg Harbor. Excluding permanent and tidal pools, the island is covered almost entirely with *S. alterniflora*.

I collected dead eelgrass in Bayville and Surf City, New Jersey and transported it in large trash bags by boat to Pettit Island annually since 2002. Mats of eelgrass were then constructed, usually near the center of the island. To create a mat I simply dumped eelgrass out of the trash bags and evened out the pile by hand, approximating the height of natural mats. In addition to eelgrass transported to the island, small natural mats located close to the edge of island were occasionally pulled back closer to the center of the island, either alone or to add to an artificial mat. (Pulled-back mats are analyzed together with artificial mats.) The mats were typically placed at a minimal distance of 5 to 20m from the nearest edge of the island, with the majority greater than 12m from the edge. From 2002 through 2007 the artificial mats ranged in size from approximately 2 to 5m^2 and ranged in number from one to four. Total area occupied by artificial mats ranged from approximately 5 to 13m^2 (Table 1). In 2008 I constructed larger mats, measuring 8.4 and 14.4m^2, for a total area of 22.8m^2. Whenever possible, eelgrass mats were constructed in mid-May before terns began nesting (typically late May) and were added to early in the nesting period.

I recorded the number of nests on these mats and estimated the total number of nests in the colony. Flooding events were also documented, as well as loss or survival of nests after flooding, including four years prior to construction of artificial mats in which I also studied the Pettit Island terns (1996, 1997, 1999, 2001). In most years the colony size estimate comes from a nest census before hatching, but in 2003 and 2004 is a minimum estimate based on the number of terns flying overhead during disturbance. In this colony there are often two distinct waves of egg-laying, one in late May and early June and one in late June and early July[23]. Colony size estimates exclude nests appearing in the second wave or later to avoid counting the same breeding pairs twice, because terns losing nests early in the breeding season often re-nest[1-3].

The level of detail recorded varies among years, depending on the focus of my research activity in a given year. The best data is from 1999, 2001, 2002, 2007, and 2008, when nests were individually marked with numbered craft sticks and checked regularly, with information recorded on individual index cards. The cards could include such information as the location of the nest, fate of each egg, date of hatching and fate of each chick (individually marked with metal bird bands), and nesting substrate. Although I constructed mats in 2004, there is little data from this year, because I visited the island only once after mat construction, late in the breeding season.

Results

The total number of nests on the island varied from over 110 nests to approximately 210 nests (Table 1). In 2002 and 2008 I recorded the nesting substrate for a large number of nests (N = 79 and 81, respectively). In 2002 81% of nests were built on wrack (including both natural and artificial eelgrass mats), with 16.5% on *S. alterniflora* and 2.5% on bare soil. In 2008 75.3% of nests were built on wrack, with 14.8% on *Spartina* (including 2 nests on *S. patens*), 4.9% on clumps of root mat and soil, 2.5% on bare soil, and 2.5% on wooden boards. In five of eight years there was little wrack present early in the breeding season, and what was present tended to occur along the edges of the island (Table 2).

Terns used artificial mats in every year in which they were constructed, and the number of nests on artificial mats ranged from 5 in 2002 to 22 in 2003 (Table 1). In five of six years with data, an additional one to four nests were present in *S. alterniflora* immediately adjacent to the artificial mats (within approximately 1m). When constructed prior to the start of nesting, nests on artificial mats were among the first nests on the island (Table 2). With the exception of 2002, when terns began nesting before artificial mats were constructed, all of the nests recorded on artificial mats were present during the first wave of egg laying, with no late nests.

There is a significant correlation between the area of individual artificial mats and the number of nests on the mats (Spearman Rank Correlation, Z = 2.45, P = 0.014, Rho = 0.66). The correlation is not significant if total area

Table 1. Estimated common tern colony size and usage of artificial mats (AM)			
Year	Total Nests	Nests on AM	Total Area of AM (m^2)
1999	160	N/A	N/A
2001	150	N/A	N/A
2002	200	5	9
2003	>110	22	13
2004	>120	No data	No data
2005	200	8	9
2006	200	13	7
2007	210	13	5
2008	125	17	23

Table 2. Status of natural mats and presence of nests on artificial mats early in the breeding season			
Year	Date study began	State of natural mats early in season	Early nests on artificial mats (AM)
2001	23-May	Little wrack, mostly along edges	No AM this year
2002	20-May	Wrack abundant and in from edge	Terns already nesting when AM created
2003	15-May	No wrack present	First 3 and 18 of first 32 nests on AM; 8 of first 9 chicks on AM
2004	4-Jun	Large mats present	Terns already nesting when AM created; 3 nests on remnants of AM from 2003 AM
2005	10-May	Large central mat, little else	1 of first 16 nests on AM
2006	10-May	Little wrack, except narrow mat along edges	2 of first 19 nests on AM
2007	7-May	Little wrack, mostly along edges	2 of first 5 nests and 2 of first 5 chicks on AM
2008	15-May	Little wrack, small clumps along edges	8 of first 23 nests on AM

Table 3. Effects of flooding in the colony and on artificial mats			
Year	Date	Losses to Flooding	Nests on Artificial Mats
1996	2-Aug	Island abandoned after flooding late in season	N/A
1997	5-Jun	Perimeter flooded	N/A
1999	mostly 10-Jun	13% of eggs lost to flooding	N/A
2001	mostly 19-Jul	27% of eggs at peripheral nests lost	N/A
2002	6 and 15-Jun	32% of eggs and 22% of chicks lost, affecting 59 of 111 marked nests	First nest lost; 4 of 4 late nests survive
2003	9-Jun	NE side of island flooded	18 of 19 nests survive
2003	14-Jun	>14% of nests lost to flooding	All 21 nests survive (3 lose an egg)
2004		No data	No data
2005	23 and 30-May	13 of first 16 nests lost	First nest lost
2005	20-Jun	Perimeter flooded	7 of 8 nests survive
2005	7-Jul	Some flooding	All remaining nests survive
2006		No significant flooding	No losses to flooding
2007	8-Jun	All 54 SW peripheral nests lost	All 13 nests survive
2007	12-Jun	Some flooding along NE side of island	All 13 nests survive
2007	14-Jun	Island under water, >75% of nests lost	8 of 13 nests lost
2008	21-May	Nesting delayed ~one week	Nesting delayed ~one week
2008	13-Jun	Approx 15% peripheral nests lost	All 17 nests survive
2008	18 and 25-Jun	Approx 10% peripheral nests lost	All 17 nests survive

Figure 1. A nest on an artificial mat of eelgrass (a) and in nearby *Spartina alterniflora* (b) are shown during a flood.

of artificial mats is used rather than considering each mat separately (Z = 0.99, P = 0.33, Rho = 0.44), but the two years with the largest number of nests on artificial mats were also the two years in which artificial mats occupied the largest total area (Table 1).

Flooding was a major cause of nest loss in most years; flooding events are summarized in Table 3. In most cases flooding affected nests near the edge of the island to a much greater extent than nests near the center of the island (Table 3). Nests on artificial mats tended to survive flooding (Table 3), except in 2007, when flooding was so severe that the entire island was under water. Figure 1 shows a surviving nest on an artificial mat surrounded by water and a nearby flooded nest in *S. alterniflora*.

Discussion

As has been previously reported[2,4-11], common terns nesting on saltmarsh islands clearly prefer wrack, in this location largely comprised of eelgrass mats, as nesting substrate. Approximately 75 to 80% of nests were on wrack, even though *S. alterniflora* occupies a much larger proportion of the island's area[2,4,22]. Terns readily used artificial mats of eelgrass: nests were present on artificial mats in every year of the study and were often among the first nests on the island. That the terns began nesting on the artificial mats so quickly suggests that eelgrass mats are a limiting resource in the colony. Observations across several years (see Table 2) support the suggestion that naturally occurring wrack is less abundant than in the past[17].

Nests on artificial mats typically survived normal flooding, likely due to both their central location and increased elevation. However, few nests survived unusually severe flooding, such as in 2007, regardless of location. In that year the entire island was under water and at least 75% of nests were lost. It is likely that many terns abandoned Pettit Island after this flood, as there was a large decrease in the number of nests on the island between 2007 and 2008 (see Table 1). Effects of flooding were apparent in most years, particularly among nests close to the edge of the island. Losses to flooding were unpredictable, though, as all nests along one side of the island could be washed out while the opposite side of the island was largely unaffected (see Table 3), depending on the direction a storm happened to take.

Burger and Lesser[4] studied habitat selection of common terns nesting on 34 saltmarsh islands in Ocean County, NJ, including Pettit Island. They reported that the terns usually avoided nesting on wrack that was within 5m of the edge of an island, but in my study that is typically where most nests were located. That terns chose nest sites close to the water's edge seems maladaptive, but it is often where eelgrass mats were most abundant. The terns appear to be suffering from an "ecological trap"[24]. They may be trapped by competing characteristics of good nesting sites: a good location may not match with a good substrate because of a recent change in the environment - decreased availability of wrack, particularly in the center of the island. Previous authors have also found that characteristics of good nest sites for marsh-nesting common terns can conflict with one another[10,11].

One of the major benefits of providing artificial eelgrass mats is that preferred nesting substrate becomes more available away from the edges of an island. In addition to transporting additional eelgrass to an island, pulling back eelgrass from the edge, which I did on a small scale, may enhance this benefit. The positive effects of artificial mats may also extend beyond the borders of the mats if they attract terns to form subcolonies closer to the center of the island: some terns built nests in *S. alterniflora* immediately adjacent to the artificial mats. Although these nests were likely to survive most flooding due to their central location, anecdotal evidence suggests that they were less likely to survive major floods than those on the eelgrass mats (Figure 1). For example, in 2007 five of thirteen nests on an artificial mat survived the major flood, while all three neighboring nests were washed out. During the same flood a large natural mat floated nearly 3m inland from its original location largely intact, with several nests surviving.

Artificial mats seemed to be particularly important in years when little wrack was present on the island early in the breeding season. Years with substantial wrack early in the season not only have more substrate available, but what is there is safer from flooding, as it would include material deposited by the highest winter storm tides above the reach of normal tidal flooding[2,4,22]. 2003 is an extreme example, but shows the potential value of artificial mats as a conservation tool. In this year there were no natural mats present in mid-May – it appeared that, rather than creating mats, winter storms were so severe that they washed over the island and removed what was previously present[17]. The first three nests and 18 of the first 32 nests were built on artificial mats, and eight of the first nine chicks hatched on artificial mats. This was also the year with the largest total number of nests on artificial mats, 22. Only one of these nests was lost to flooding, despite substantial losses elsewhere in the colony. The improvement in tern nesting success caused by the presence of artificial mats is probably underestimated by the proportion of nests surviving flooding, because many previous studies have shown that terns that nest early are consistently more successful in raising chicks to fledging[25-27].

The loss of a large number of nests to flooding in a given year does not necessarily mean that the overall population will be affected, because terns are long-lived and have many opportunities to breed[1-3]. Similarly, because flooding does not directly affect adult survival, that fewer terns nested on Pettit Island in 2008 does not mean that the population has decreased, but instead suggests that many adults chose to nest in a different

location within the same metapopulation. On the other hand, if flooding increases in frequency and intensity, which may have already occurred in Barnegat Bay due to the dredging of Barnegat Inlet and reconfiguration of a jetty[12,16] and is predicted to increase further due to rising sea levels[10,12,13], then the Barnegat Bay common tern population will continue the downward trend reported in the late 1990's[12,16,17].

I plan to continue to construct mats in the future, particularly large mats, to increase usage by terns and to possibly attract skimmers, which require larger mats than do terns[2,4,16,22]. Construction of eelgrass mats represents an easy method to modify habitat in a manner that can increase reproductive success of terns and skimmers and may help prevent or slow down further population declines.

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References

[1]Becker, P.H. and J.D. Ludwigs, 2004. *Sterna hirundo* Common Tern. BWP Update **6:** 91-137.

[2]Burger, J. and M. Gochfeld, 1991. *The Common Tern: Its Breeding Biology and Social Behavior*. Columbia University Press, NY.

[3]Nisbet, I.C.T., 2002. Common tern (*Sterna hirundo*). in *The Birds of North America*, no. 618 (A. Poole and F. Gill, Eds.). The Birds of North America, Inc, Philadelphia.

[4]Burger, J. and F. Lesser, 1978. Selection of colony sites and nest sites by common terns *Sterna hirundo* in Ocean County, New Jersey. Ibis **120:** 433-449.

[5]Burger, J. and F. Lesser, 1979. Breeding behavior and success in salt marsh common tern colonies. Bird-Banding **50:** 322-337.

[6]Buckley, F.G. and P.A. Buckley, 1982. Microenvironmental determinants of survival in saltmarsh-nesting common terns. Colonial Waterbirds **5:** 39-48.

[7]Safina, C., D. Witting, and K. Smith, 1989. Viability of salt marshes as nesting habitat for common terns in New York. Condor **91:** 571-584.

[8]Storey, A.E., 1987. Adaptations for marsh nesting in common and Forster's terns. Canadian Journal of Zoology **65:** 1417-1420.

[9]Erwin, R.M. and D.C. Smith, 1985. Habitat comparisons and productivity in nesting common terns on the Mid-Atlantic coast. Colonial Waterbirds **8:** 155-165.

[10]Rounds R.A., R.M. Erwin and J.H. Porter, 2004. Nest-site selection and hatching success of waterbirds in coastal Virginia: some results of habitat manipulation. Journal of Field Ornithology **75:** 317-329.

[11]Storey, A.E.,1987. Characteristics of successful nest sites for marsh-nesting common terns. Canadian Journal of Zoology **65:** 1411-1416.

[12]Burger, J., C.D. Jenkins, Jr., F. Lesser and M. Gochfeld, 2001. Status and trends of colonially-nesting birds in Barnegat Bay. Journal of Coastal Research **SI(32):** 197-211.

[13]Erwin, R.M., G.M. Sanders, D.J. Prosser and D.R. Cahoon, 2006. High tides and rising seas: potential effects on estuarine waterbirds. In *Terrestrial Vertebrates of Tidal Marshes: Evolution, Ecology, and Conservation.* Ed. R. Greenberg, J. Maldonado, S. Droege, and M.V. McDonald. Studies in Avian Biology **32:** 214-228.

[14]Palestis, B.G. and J. Burger, 2001. Development of common tern (*Sterna hirundo*) sibling recognition in the field. Bird Behavior **14:** 75-80.

[15]MANEM Waterbird Working Group, 2006. Waterbird conservation plan for the Mid-Atlantic/New England/ Maritimes region: 2006-2010. Waterbird Conservation for the Americas. http:// www.waterbirdconservation.org/manem.html

[16]Burger, J, 1997. Avian studies on Barnegat Bay. In *Proceedings of the Barnegat Bay Ecosystem Workshop*. Ed. G.E. Flimlin, Jr. and M.J. Kennish. Rutgers Cooperative Extension of Ocean County. Toms River, NJ, pp. 345-350.

[17]Jenkins, C.D., Jr., 1997. Recent trends in colonial waterbird populations of the Barnegat Bay-Little Egg Harbor Estuary. In *Proceedings of the Barnegat Bay Ecosystem Workshop*. Ed. G.E. Flimlin, Jr. and M.J. Kennish. Rutgers Cooperative Extension of Ocean County. Toms River, NJ, pp. 361-377.

[18]Burger, J, 1998. Effects of motorboats and personal watercraft on flight behavior over a colony of common terns. Condor **100:** 528-534.

[19]O'Connell, T.J. and R.A. Beck, 2003. Gull predation limits nesting success of terns and skimmers on the Virginia barrier islands. Journal of Field Ornithology **74:** 66-73.

[20]Kress, S., 2000. Colony site management techniques. Waterbird Conservation for the Americas. http:// www.waterbirdconservation.org/plan/rpt-sitemanagement.pdf

[21]Morris, R.D., H. Blokpoel and G.D. Tessier, 1992. Management efforts for the conservation of common tern *Sterna hirundo* colonies in the Great Lakes: two case histories. Biological Conservation **60:** 7-14.

[22]Burger, J. and M. Gochfeld, 1990. *The Black Skimmer: Social Dynamics of a Colonial Species*. Columbia University Press, NY.

[23]Palestis, B.G., 2005. Nesting stage and nest defense by common terns. Waterbirds **28:** 87-94.
[24]Schlaepfer, M.A., M.C. Runge and P.W. Sherman, 2002. Ecological and evolutionary traps. Trends in Ecology and Evolution **17:** 474-480.
[25]Arnold, J.M., J.J. Hatch and I.C.T. Nisbet, 2004. Seasonal declines in reproductive success of the common tern *Sterna hirundo*: timing or parental quality? Journal of Avian Biology **35:** 33-45.
[26]Arnold, J.M., J.J. Hatch and I.C.T. Nisbet, 2006. Effects of egg size, parental quality and hatch-date on growth and survival of common tern *Sterna hirundo* chicks. Ibis **148:** 98-105.
[27]Burger, J., I.C.T. Nisbet, C. Safina and M. Gochfeld, 1996. Temporal patterns in reproductive success in the endangered roseate tern (*Sterna dougallii*) nesting on Long Island, New York, and Bird Island, Massachusetts. Auk **113:** 131-142.

Age of Red Blood Cells in Three Species of Fowl

by

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Abstract

Electrophoretic patterns were obtained for turkeys, Guinea fowl and pheasants using agarose gel. The three species each have three hemoglobin bands. Turkeys and Guinea fowl have two anodal adult bands and a single cathodal fetal band. Pheasants have three anodal bands, the heaviest band being fetal hemoglobin. The estimated red cell life span for turkeys, Guinea fowl, and pheasants is 13, 120, and 19-20 days respectively.

Key Words: Turkeys, Pheasants, Guinea fowl, red blood cells, hemoglobin, electrophoresis

Introduction

Electrophoretic analysis of fowl hemoglobin (Hb) indicates that several hemoglobins may occur in the same animal. For example, multiple Hb components were found in chickens and ducks[1]. However, the number of hemoglobin components (hereinafter referred to as bands) observed depends upon the age of the animal at blood sampling. Chickens (*Gallus gallus*) have five forms of Hb as determined electrophoretically[1]. Two of the forms are adult hemoglobin, the others are fetal forms (HbFs). One of the fetal forms that occurs in chickens persists for 31 days after hatching[2a,b]. If the chicken's blood is sampled within this time, three bands appear, whereas if their blood is sampled in their fifth week after hatching, or later, only two bands appear. A similar situation occurs in ducks (*Anas platyrhynchos*), but in this species a fetal hemoglobin band persists for almost seven weeks[2a,b]. It appears that since different hemoglobins (fetal and adult) appear in different red blood cells, because their sources differ, the age of red blood cells can be determined in birds if it is assumed that fetal hemoglobin production ceases at hatching, and the die-away (disappearance) of fetal hemoglobin is followed as the bird matures[2a,b].

The purpose of this study was to determine the presence of fetal hemoglobin after hatching, and to follow the die-away of HbF, in three species of fowl, turkeys (*Meleagris gallopavo*), Guinea fowl (*Numida meleagris*), and pheasants (*Phasianus colchicus*).

Materials and Methods

Two turkeys were hatched in a laboratory incubator from eggs obtained from commercial stock. Blood from a single turkey approximately 150 days old was obtained from the State of New Jersey Rockport Pheasant Farm, in Hacketstown. Several guinea fowl were hatched in the laboratory. These eggs were purchased from a commercial guinea fowl breeder. A single adult Guinea fowl (male), more than 150 days old, was purchased from a dealer of live poultry. Three 14 day-old pheasants were obtained from the Rockport Pheasant Farm and a single adult pheasant bird's blood was sampled at the farm. Birds that were returned to the University laboratory were maintained in wire bottom cages and were fed a commercial fowl mash, and had access to water for the duration of the study. Blood from all the birds was collected from the bird's right ulnar artery. Birds were sampled more than once during the duration of the study. At the conclusion of the study the birds were released to local non-commercial farms.

Blood was collected at varying intervals from the three species. Blood sampling for each species was halted when fetal hemoblobin bands (HbF) were no longer detected. Blood samples from adults of the three species were sampled only once to determine if an HbF band was present. Blood samples, obtained in microcapillary tubes, were centrifuged for six minutes (at 5000 rpm). Red blood cells (rbcs) were separated from the plasma, then diluted with deionized water. This mix was mechanically lysed. The protein fractions of the hemolyzed rbcs were resolved using agarose gel electrophoresis (Titan Gel High Resolution Protein System, Ca. No. 3040, Helena Laboratories, Beaumont, Texas). Electrophoresis was conducted at pH 8.7 for 33 minutes at 250 volts. The patterns were

stained with Coomassie Brilliant Blue. Percents of the bands within the patterns were obtained by analysis using an imaging densitometer (Bio-Rad Quantity One Version4.6.3). Regression analysis (Shareware, Curvefit Version 2.10-N) was performed on percents of HbF fractions including all of these observations for each of the three species.

Results

Turkeys had three electrophoretic bands evident until 13 days after hatching (Fig. 1). HbF represented 7.15 percent of the total hemoglobin present on the first day after hatching. The percent HbF declined to 0.4 percent on the 13th day, whereas HbAI increased and HbAII decreased (Fig. 2). HbAI and HbAII, both adult hemoglobins, were anodic. HbF was cathodic.

Guinea fowl had three hemoglobin bands through 120 days (Fig. 3). Neither of two fowl had HbF patterns on the 124th day. The highest percent of HbF, almost 18 percent of the total hemoglobin present, occurred 31 days after hatching. It declined slowly, and unevenly, to the 124th day, when HbF was no longer observed. HbAI and HbAII (adult hemoglobins) were anodic, whereas HbF was cathodic. Both adult hemoglobins increased as HbF declined (Fig. 4). The single adult Guinea fowl (more than 150 days old) that was purchased to obtain a blood sample did not have an HbF band.

Three bands of hemoglobin were present in pheasants up to, and including, their 18th day after hatching (Fig. 5). The percent of HbF on the 18th day was 4.4. HbF was not present in the sample taken on the 21st day. The pheasants were first sampled on their 14th day after hatching (hatching pheasants in this laboratory was not successful). The HbF total on their 14th day was 5.7 percent. All three bands were anodal, with the HbF band closest (heavier than HbAI) to the point of sample application. Both adult hemoglobins increased as HbF declined (Fig. 6).

Discussion

The variation in the age of red blood cells of different species of fowl is larger than that for the age of red blood cells in certain mammal species[1]. The age estimated for guinea fowl red cells in this study is not unusual among vertebrates, and is similar to that reported for human red blood cells of 113 to 118 days[1], and is far less than that reported for box turtles, *Terrapene Carolina Carolina*, with a mean red cell life span between 600 and 800 days, and that of marine toads, *Bufo marinus*, between 700 and 1,400 days[3].

Agarose gel electrophoresis is useful for the determination of the age of red cells in which fetal hemoglobin does not persist, and is based upon the assumption that the red cells that contain HbF have the same life span as the red cells that carry HbA. This appears be the case for the fowl examined in this, and in previous studies[2]. This assumes that the fowl in the studies referred to have red cells that are exclusive to HbF or HbA, but not to both. Thus, as a result of the disappearance of the fetal hemoglobin band using electrophoretic analysis, the age of red cells in these species can be ascertained (Table 1). In certain groups fetal hemoglobin does occur into adult life. For example, in humans HbF occurs in small amounts in adults[4]. In humans, adult and fetal hemoglobin may both be present in some adult red blood cells[5,6,7,8]. Both HbF and HbA were also found in some of the same red blood cells in the African clawed frog, *Xenopus laevis*[9]. However, in other mammals[10], and other amphibians, e.g. the bullfrog, *Rana catesbeiana*[11,12], both hemoglobins do not share the same red blood cell.

Many studies of red cell life span in laboratory animals and man have used radioactive tracers,[3,13-16]. The use of radioactive materials as a method to determine the age of red cells may result in problems associated with the purchase, storage and disposal of these tracer materials. This is not a problem with the agarose gel electrophoretic technique. Other methods to determine red cell life span and their shortcomings are discussed elsewhere[17].

Table 1. A comparison of the age of red blood cells of some fowl (i.e. the life span of the red blood cells when fetal hemoglobin no longer appears in agarose gel electrophoretic patterns).

Species	Age (days)	Source
Turkeys, *Meleagris gallopavo*	13	This study
Pheasants, *Phasianus colchicus*	19-20	This study
Chickens, *Gallus gallus*	31	2a
Japanese quail, *Coturnix coturnix*	36	2b
Ducks, *Anas platyrhynchos*	36	2b

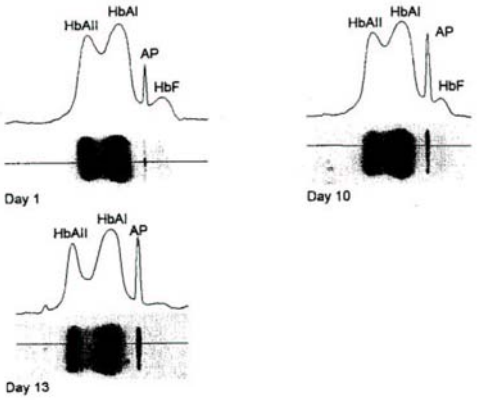

Figure 1. Hemoglobin bands of Turkeys at one, 10, and 13 days after hatching. HBF is cathodic, HbAI and AII are anodic.

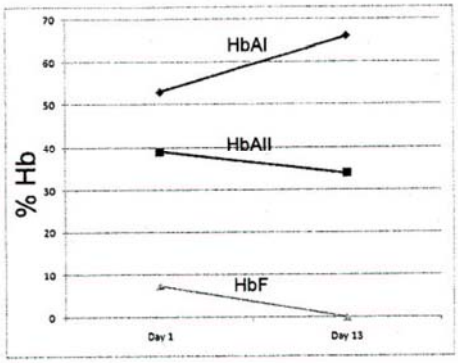

Figure 2. Regression analysis of the three bands of turkey Hb. HbF is not present on the 14th day after hatching.

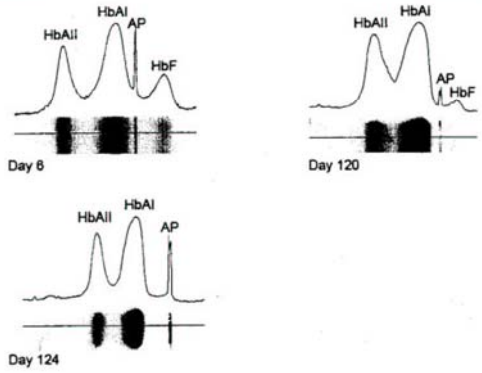

Figure 3. Hemoglobin bands of Guinea fowl at six, 120, and 124 days afer hatching HBF is cathodic, HbAI and AII are anodic.

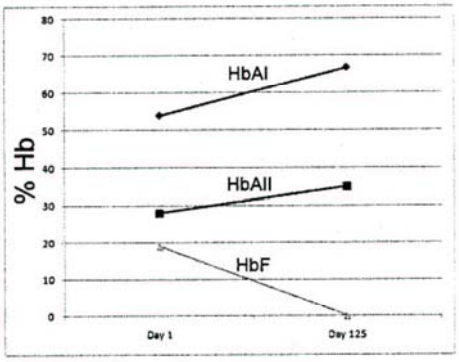

Figure 4. Regression analysis of the three bands of Guinnea fowl Hb. HbF is not present on the 124th day after hatching.

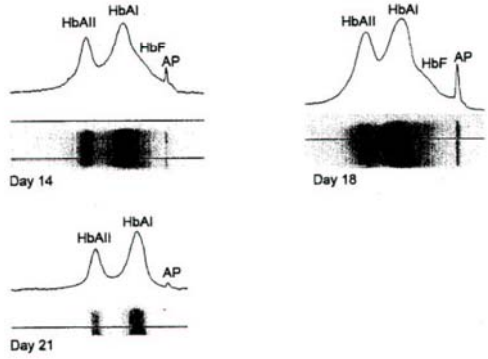

Figure 5. Hemoglobin bands of pheasant at 14, 18, and 21 days afer hatching. All three HBF bands are cathodic. HBF is a heavier protein and trails HBAI.

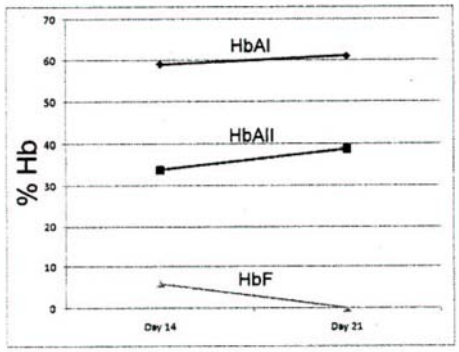

Figure 6. Regression analysis of the three bands of pheasant Hb. HbF is not present on the 21st day after hatching.

Acknowledgments

Thanks are extended to W. Elliott, Instructional Technology Services, and to the Monmouth University Biology Department for their project support.

References

[1]Prosser, C.L., 1973. Comparative Animal Physiology 3rd Edition. W.B.Saunders, Phila. P. 966, plus index.

[2a]Dorfman, D., Fetal hemoglobin and age of red blood cells in fowl. In Vivo In press.

[2b]Dorfman, D., 2008. Die-away in fetal hemoglobin in Japanese Quail, *Coturnix* japonica. In Vivo **29(2):** 26-29.

[3]Altland, P.D. and K.C. Brace, 1962. Red cell life span in the turtle and toad. Am. J. Physiol. **203:** 1188-1190.

[4]Tietz, N.W. editor, 1976. Fundamentals of Clinical Chemistry. W.B. Saunders Co. Phila. 1263 p.

[5]Tomoda, Y., 1964. Demonstration of foetal erythrocytes by immunofluorescent staining. Nature (London). **202:** 910-911.

[6]Hosoi, T., 1965. Studies on hemoglobin F within single erythrocyte fluorescent antibody technique. Exp. Cell Res. 37: 680-683.

[7]Dan, M. and A. Hagiwara, 1967. Detection of two types of hemoglobin (HbA and HbF) in single erythrocytes by fluorescent antibody technique. Exp. Cell Res. **46:** 596-598.

[8]Gitlin, D., T. Sasaki and P. Vuopio, 1968. Immunochemical quantification of proteins in single cells. Blood, **32(5):** 786-810.

[9]Jurd, D.R. and N. Maclean, 1970. An immune-fluorescent study of the hemoglobins in metamorphosing *Xenopus laevis.* J. Embryol. Exp. Morph. **23:** 299-309.

[10]Fantoni, A., A. De La Chapelle, D. Chui, R.A. Rifkind and P.A. Marks,1969. Control mechanisms of the conversion from synthesis of embryonic to adult hemoglobin. Ann. N.Y. Acad. Sci. **165:** 194-204.

[11]Rosenberg, M., 1970. Electrophoretic analysis of hemoglobin and isozymes in individual vertebrate cells. Proc. Nat. Acad. Sci. U.S.A. **67:** 32-36.

[12]Maniatis, G.M. and V.M. Ingram, 1971. Erythropoiesis during amphibian metamorphosis. J. Cell Bio. **49:** 390-404.

[13]Rodman, G.P., F.G. Ebaugh Jr. and M.R.S. Fox, 1957. Life span of red blood cell and blood cell volume in chicken, pigeon and duck as estimated by use of $Na_2Cr^{51}O_4$. Blood **12:** 355-366.

[14]Ebaugh, F.G., Jr., C.P. Emerson and J.A. Ross, 1953. Use of radioactive chromium[51] as erythrocyte tagging agent for determination of red cell survival *in vivo.* J. Cln. Invest. **32(12):** 1260-1276.

[15]Ottesen, J., 1948. Life span of red and white blood corpuscles of hen. Nature **162:** 730-731.

[16]McConnell, K.P., O.W. Portman and R.H. Rigdon, 1953. Intravascular life span of duck red blood cell as determined by radioactive selenium. Proc. Soc. Exper. Bio. And Med. **83:** 140-143.

[17]Berlin, N.I., T.A. Waldmann and S.M. Weissman, 1960. Life span of red blood cell. Physiol. Rev. **39(3):** 577-616.

Pigeon (*Columba livia*) Hemoglobin Patterns and Age of Red Blood Cells

by

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Abstract

Pigeon red blood cell electrophoresis yields a major hemoglobin peak and a minor peak. The minor peak, probably fetal hemoglobin, drops from 5% of the total proteins two days after hatch to less than 3% 24 days after hatch. The minor peak persists in adult pigeons. The estimated age of pigeon red blood cells is 17-23 days.

Introduction

The purpose of this study was to examine hemoglobin (Hb) patterns of pigeons, and to determine the life span of their red blood cells by noting if a fetal hemoglobin is present after hatching, and if one is present, how long it persists in subsequent electrophoretic patterns. Some birds have several hemoglobins, for example three components (hereinafter referred to as bands) in chickens and ducks, two in each of 20 species of wild birds, and one in pigeons and in penguins[1]. The number of bands present depends upon the age of the bird at the time of sampling, since HbF (fetal hemoglobin) does not tend to disappear immediately after the bird hatches, and its persistence apparently depends upon the life span of the red blood cells containing the hemoglobin. The age of red blood cells varies with different bird species[2]. For example, if a hemoglobin pattern is obtained from a 28 day old chicken, three bands appear, after agarose gel electrophoresis, including two HbA (adult hemoglobins), and an HbF (fetal) band. If the chicken is sampled at 35 days of age only the two HbA bands are evident[3]. Guinea fowl bled at 120 days of life have three bands present (two HbA and one HbF) after electrophoresis, but only two HbA bands present when sampled on their 124th day of life[3].

Methods and Materials

Eight pigeons (four males, and four females) were maintained in a cage in the laboratory. Light was provided 24 hours each day. Birds were fed a commercial pigeon feed daily, and they had constant access to water. Several nest boxes were provided for the birds within the cage.

Blood samples were collected from a pigeon more than five years old, and from two pigeons more than two years old. Blood samples were also collected from two successfully hatched pigeons when the birds were two, five, 16, 24, 30, 37, and 50 days old. Blood was collected in heparinized microcapillary tubes from the ulnar artery in the birds' right wing. Blood was centrifuged and the plasma separated from the red blood cells (rbcs).

The red blood cells were lysed mechanically and by freezing, diluted with one to two mls of deionized water, then electrophoresed for 34 minutes using agarose gels (Titan Gel High Resolution Protein System, Ca. # 3040 Helena Laboratories, Beaumont, Texas) at a pH of 8.7. The patterns were stained with Coomassie brilliant blue. Percents of the bands within the patterns were determined using an imaging densitometer (Bio-Rad Quantity Version 4.6.3). The gel obtained from the two day old pigeon was also electrophoresed by a second method. This gel was run for 22 minutes, rotated 90 degrees, and run for 12 additional minutes to provide a two-dimensional pattern.

Results and Discussion

Electrophoresis of lysed red blood cell fluid yielded two distinct bands. Both bands were anodic. The major band, a hemoglobin, constitutes between 94.7 and 97.9 % of the proteins (Table 1). This band migrates a short distance from the point of sample application, in the area of the human plasma gamma region. The second band,

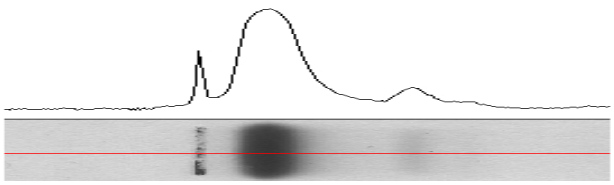
comprising 2.1 to 5.1 % of the red blood cell proteins is a lighter protein and occurs in the approximate equivalent area of human plasma beta proteins. Previous studies indicate that pigeons have only a single hemoglobin[4].

In this study, the periodic examination by electrophorsis indicated the presence of the second band, which persists, and which may be a second pigeon hemoglobin not previously described (Fig. 1). This minor band represents more than 5% of the red blood cell proteins in the two-day old pigeon then declines, between the 17th and 23rd days, to levels observed in the adult electrophoretic patterns (approximately 2 %). Rotation of the gel from the two day old pigeon yielded the major band and the second minor band, with no separation in the minor protein fraction after rotation, so that this band may consist of only a single component. However, the decline of this minor protein, but not its disappearance, is unexplained. This protein may be a fetal hemoglobin produced, for example, by the liver and, after hatching, the production of this protein may be diminished, but does not disappear, similar as In humans, where small amounts of fetal hemoglobin may persist into adult life[5].

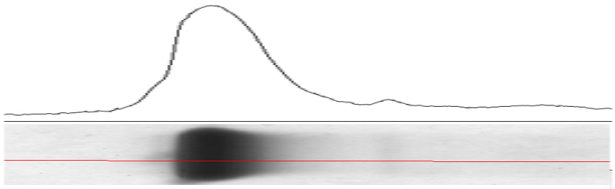
In a study employing radioactivity, an estimate of 35 days was given for the mean life span of red cells of pigeons[2]. In the present study it is estimated that the life span of pigeon red blood cells is 20 days based upon the assumption that the drop in percent in the minor protein fraction, between 17 and 23 days, is due to the disappearance of the red blood cells carrying the minor protein. A comparison of fetal hemoglobins of chickens, ducks, turkeys, guinea hens, pheasants, and pigeons indicates that the minor protein of pigeons observed here is lighter than those of the other fowl, and is anodic. The only other anodic fetal hemoglobin of the aforementioned species is that of the pheasant, and its fetal hemoglobin migrates a shorter distance from the application point than does the pigeon fetal protein. Pigeon chicks are altricial and, unlike the other bird species mentioned, are not

Table 1. Hemoglobin analysis of pigeon red blood cells. Two bands persist after hatching. Both bands, Hbl and HbF (probably a fetal hemoglobin), are anodic.

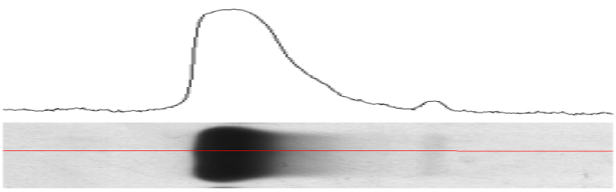
Age	Hbl %	Hbll %
2 days	94.9	5.1
5 days	95.6	4.4
6 days	96.8	3.2
24 days	97.9	2.1
30 days	97.5	2.5
37 days	97.9	2.1
50 days	97.4	2.6
2 years	97.2	2.8
3 years	97.1	2.9
5 years	97.2	2.8



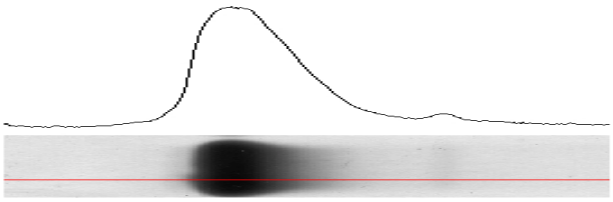
A.) Pigeon 2 Day old chick. Minor band is approximately 5%



B.) Pigeon chick 16 days old. Minor b and is approximately 3.2%



C.) Pigeon chick 24 days old. Minor b and is approximately 2.1%



D.) Pigeon 2 years old. Minor band is approximately 2.8%

Figure 1. Electrophoretic patterns at A) 2, B) 16, and C) 24 days of age and at D) two years. The second lighter band persists, but declines between the 17th and 23rd day after hatching.

readily available. In this laboratory it required eight months before obtaining a successful hatch.

Acknowledgments

Thanks are extended to W. Elliott, Instructional Technology Services, Monmouth University, and to the Monmouth University Biology Department for support of the project.

References

[1]Prosser, C.L., 1973. Comparative Animal Physiology 3rd Edition. W.B. Saunders, Phila. P. 966, plus index.

[2]Rodman, G.P., F.G. Ebaugh and M.R. Spivey Fox, 1957. Life span of red blood cell and blood cell volume in chicken, pigeon and duck as estimated by the use of $Na_2Cr^{51}O_4$. Blood **12:** 355-366.

[3]Dorfman, D., 2009. Age of Red Blood Cells in three Species of Fowl. In Vivo **30(3):** 17-20.

[4]Vanecasserie, C., G. Schnek, and J. Leonis, 1971. Oxygen affinity studies of avian hemoglobins. Chicken and Pigeon European Journal of Biochemistry **24(2):** 284-287.

[5]Tietz, N., 1976. Fundamentals of Clinical Chemistry W.B. Saunders Co. Phila. 1263 p.1

Biochemical and Methodological Consideration in the Study Design: the Selection of a Positive Control for the Hematopoietic Stem Cells Experiments

by

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ABSTRACT

Each experimental protocol requires unique controls. For instance, the controls for prostate cells and cancer experiments are mainly di-hydro testosterone and (R1881). Other positive controls are used in various experiments. Among them are lipopolysaccharide (LPS), 12-O-tetradecanoyl phorbol-13 acetate (TPA). For LPS in a laboratory setting, the concentration is 100 mg/mL LPS for Gram-negative cells. Selecting a control during a specific experiment is important. In our recent study of hematopoietic stem cells, TPA was selected as a positive control[1]. TPA is *a priori* (prototype) tumor promoter. It is used as a biomedical research tool as the *a priori* model of carcinogenesis. This is a significant reason for selecting TPA as a positive control. In clinical and animal experiments when using TPA instead of LPS, there will be no potential risk of fever caused by the positive control, because TPA, unlike LPS, is not pyogenic. Employing TPA as a positive control may prevent the involvement of potentially confounding factors as well.

Introduction

Depending on the nature of an experiment, one may choose different control agent(s) especially as a positive control. For instance, for prostate cells, including their cancer cells, growth is initially induced by androgens (male steroid hormones). In prostate cell growth experiments, controls are mainly di-hydro testosterone and radiolabelled methyltrienolone 17 beta-hydroxy-17 alpha-methyl-estra-4, 9, 11-trien-3-one (R1881). Testosterone is converted into its active form, di-hydro testosterone (DHT) by DHT alpha-reductase. DHT is utilized because it may replicate in *in vivo* conditions. DHT is also metabolized rapidly. Conversely, R1881 is long lasting both *in vitro* and *in vivo*, however, it may over-activate the androgen receptor (AR) downstream signaling. Our recent studies showing that Natural Killer (NK) cells and stem cells/ progenitors in the umbilical cord blood mononuclear cells are affected by chemicals added as treatments[1, 2]. These cells reacted either by proliferation or differentiation. A positive control test was necessary there. There are different positive controls used in various experiments. Among them, there are LPS and TPA. LPS is generally used at a concentration of 100 mg/mL for Gram-negative cells. With LPS, the combination is generally cultured for 7 days before flow cytometry examination, as per our previous experience[1,2].

TPA is an *a priori* (prototype) tumor promoter, in the form of 12-O-tetradecanoyl phorbol-13 acetate. It is used as a biomedical research tool as an *a priori* model of carcinogenesis[1]. It is extremely important that appropriate controls be incorporated during the designing stage of an experiment.

Discussion

The discussions of this manuscript are based upon the methodology and results of our recent articles[1, 2], with which we are using to illustrate the importance of selecting appropriate controls in the design of experiments. In the recent study of hematopoietic stem cells, TPA was used as a positive control. Examples of either LPS or TPA used in studies at our Systemic Biology Laboratory will be presented in this article as a partial list merely for the purpose of illustration in order to help in understanding the potential issues of controls.

Negative and Positive Controls in Our Recent Studies

A brief review of *The effect of grape seed extract on hematopoietic stem cells in the umbilical cord blood* [1] may help understand the issues.

The umbilical cord blood (UCB) is known to possess more progenitor and hematopoietic cells than peripheral blood, and is an excellent candidate for studying the effect of natural health substances (NHSs) on

mononucleocytes (MNC) subsets. Cells capable of initiating human cell engraftment (Severe-Combined-Immunodeficiency-Repopulating cells) are contained in the CD34+ cell fraction, which was the focus of that experiment. The human erythroleukemia cell line K562 (CCL-243, ATCC) was used as a MNCs-sensitive target for cytotoxicity assays. Flow cytometric analysis of UCB was used to trace the phenotypic alterations. In the study, negative controls were treated with an equal volume of phosphate buffered saline (PBS) without natural substances. After 7 days of incubation, the cultures were washed with PBS and then re-suspended in medium containing 20% fetal bovine serum (FBS), while the positive controls were treated with TPA.

Biochemical and Methodological Consideration

A brief review of the current background information of LPS may help to clarify some pitfalls and questions[3].

Why we did not use LPS in this experiment, and why did we use TPA in our experiment of hematopietic stem cells?

It is noted that Porins are proteins that traverse a cellular membrane and behave as a pore through which molecules can disperse. Porins are large enough to permit passive diffusion - i.e. they behave as channels which are particular to dissimilar kinds of molecules. They are common in the outer membrane of the mitochondria and Gram-negative bacteria. Porins of Gram-negative outer membranes and LPS both are the main pathogenic factors implicated in the clinical syndrome of septic shock, especially in disseminated intravascular coagulation. Also, Primary Fibrinolytic Syndrome is associated with subarachnoid hemorrhage. Septic shock has been widely discussed for nearly half a century, while the latter, Primary Fibrinolytic Syndrome associated with subarachnoid hemorrhage, has not been previously reported until 1973[4-6]. The biological activity of Porins and LPS are similar, but have different mechanisms to affect the clinical syndrome of septic shock, as well as to influence subarachnoid hemorrhage. Like LPS, Porin comes from Gram negative bacteria, although Porins acts through different intracellular pathways than LPS (Figs. 1, 2, 3). Gram staining, or also called as Gram's method, is an empirical method of differentiating bacterial species into two large groups, Gram-positive and Gram-negative, based on the chemical and physical properties of their cell walls. The method is named after its inventor, the Danish scientist Hans Christian Gram (1853 – 1938), who developed the technique in 1884 to discriminate between pneumococci and *Klebsiella pneumoniae* bacteria.

Fig. 1. A sucrose specific porin

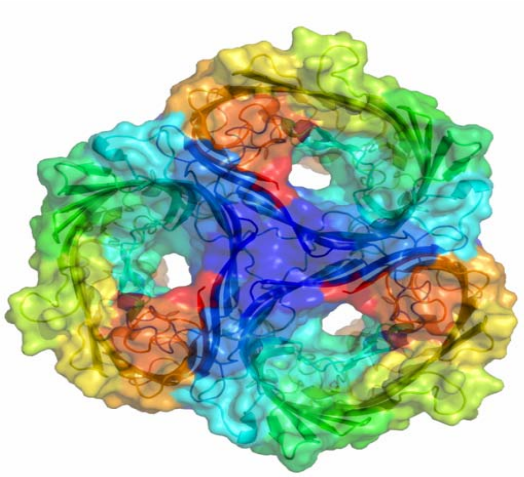

Fig. 1: A sucrose specific porin from *Salmonella typhimurium*, a gram-negative bacterium. Porins are large enough to permit passive diffusion - i.e. they behave as channels which are particular to dissimilar kinds of molecules. They are common in the outer membrane of the mitochondria and Gram-negative bacteria.

Fig. 2 Porins are beta barrel proteins that cross a cellular membrane

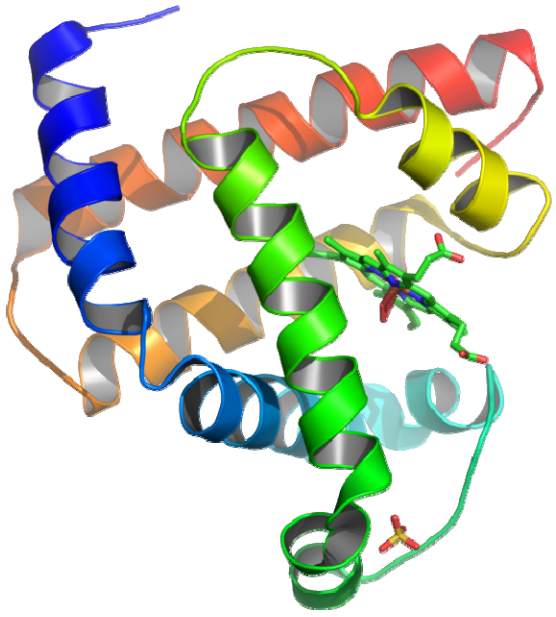

Fig. 2: A single monomer of the same protein in side view, illustrating the antiparallel beta barrel structure. It is noted that Porins are proteins that traverse a cellular membrane and behave as a pore through which molecules can disperse.

Fig. 3 The porin cell wall

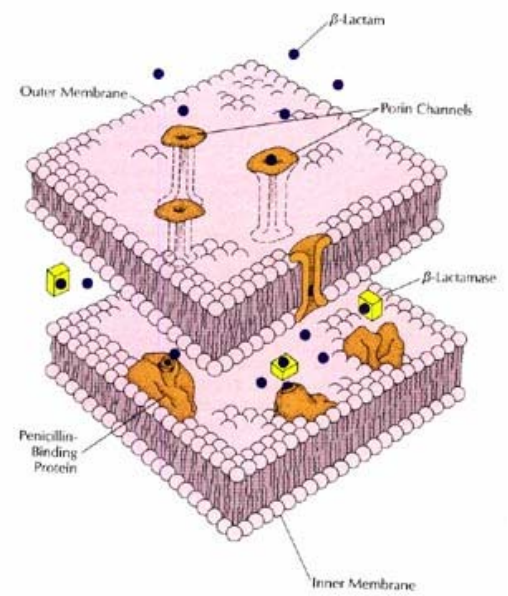

Fig. 3: The poring cell wall in a three dimensional view, with such a perpective, the fine structure of the porin's cell wall can be illustrated.

Table 1. A comparison of positive control in various studies

Articles	Positive Controls
Tang, B., P. McKenna and R.L. Rovit, 1973. Primary Fibrinolytic Syndrome Associated with Subarachnoid Hemorrhage. Angiology 24(10): 627-634.	Gram negative cell outer membrane LSP
Chien, *et al*., 2004. Polysaccharides of *Ganoderma lucidum* alter cell immunophenotypic expression and enhance CD56+ NK-cell cytotoxicity in cord blood. Bioorg Med Chem. 12: 5603-5609.	LSP
Hsieh, J.F and S.T Chen, 2007. Comparative studies on the analysis of glycoproteins and lipopolysaccharides by the gel-based mivrochip and SDS-PAGE. Biomicrofluidics 1 014102: 1-7.	LSP
Tang, B. *et al*, 2007. The effect of grape seed extract on hematopoietic stem cells in the umbilical Cord Blood. In Vivo, 29(1): 10-15.	LSP
Tang, B., J.F Hsieh, C.M. Chien and S.T. Chen, 2008. *In vitro* Maturation of NK Cells in the Human Umbilical Cord Blood Treated with Wheat Extract: A Pilot Study In Vivo 29(3): 9-20.	TPA

LPS is a well-known bacterial pyrogen, which activates several receptors, including but not limited to the Toll-like receptor 4 (TLR4). TLR4 mediates lipopolysaccharide (LSP)-induced signal transduction[7]. In this context, it is important to note that LPS is one of the agonists that can activate TLR4. Activation of such receptors is associated with the occurrence of fever. The mechanisms are still unclear. We do know for certain that fever is an excellent self-defending mechanism of hosts. It is generally composed of multiple phases. Most recently, Steiner *et al* reported that three phases of the classical LPS fever in four mouse chimeras depend on TLR4 signaling[8].

The first phase is activated by TLR4 on the hematopoietic cells, which was the focus of our previous studies[1, 2]. The second and third phases of LPS fever involve TLR4 signaling in both the hematopoietic and non-hematopoietic cells. Notwithstanding the aforementioned, the first phase of LPS fever is definitely triggered by the TLR4 on the hematopoietic stem cells, but not on the non-hematopoietic stem cells. Under such a critical circumstance, it is crucial to foresee any potential bias and confounding factors in choosing the proper agent for the positive controls. The table below is a summary of the use of LSP and TPA respectively as the positive control in studies at our Systemic Biological Laboratory. Among the articles listed below, the first article.

The Summary of Reasons on Using TPA as Positive Control in the Study of Hematopoietic Stem Cells

In our experiment of hematopoietic stem cells, TPA was selected as a positive control because it is a tumor promoter. TPA is often used as biomedical research tool as a model of carcinogenesis. This is a significant reason for decision-making with regard to the selection of TPA as the positive control. In clinical and animal experiments of this nature, there will be no potential risk of fever caused by the positive control. Unlike LPS, TPA is not pyogenic. Conversely, the chance of interaction between two variables, such as LPS and TLR4 on hematopoietic cells can be controlled and eliminated without difficulty. Statistically, whenever and wherever there is an interaction between two or more variables, there is a confounding effect which can be identified with logistic regression[9, 10]. Hence one of the other reasons in employing TPA as positive control is to control potentially confounding factors.

CONCLUSION

As a positive control, if one in the first place, prematurely and erroneously used LPS, which was what we frequently used in the past, we could have faced selection bias, confounding variables, and most of the undesired effects of the interaction between LPS and TLR4 on the hematopoietic stem cells. The use of TPA, but not LPS, in the recent study is an example that illustrates the importance of a sound and mature judgment in contemplating a study design.

Acknowledgments

Thanks are due to Dr. Richard Stalter of St. John's University, Jamaica, NY for his initial reading and to Dr. S. T. Chen, Systemic Biological Laboratory, Institute of Biochemistry, Academia Sinica, Taipei, Taiwan for his encouragement.

References

[1] Tang, B., 2007. The effect of grape seed extract on hematopoietic stem cells in the umbilical cord blood. In Vivo, **29(1):** 10-15.

[2] Tang, B., J.F Hsieh, C.M. Chien and S.T. Chen, 2008. *In vitro* maturation of NK cells in the human umbilical cord blood treated with wheat extract: a pilot Study. In Vivo **29(3):** 9-20.

[3]Chien, C.M., J.J. Cheng, W.T. Chang, M.H. Tien, C.M. Tsao, Y.H Chang, H.Y Chang, J.F. Hsieh, C.H. Wong and S.T. Chen, 2004. Polysaccharides of *Ganoderma lucidum* alter cell immunophenotypic expression and enhance CD56+ NK-cell cytotoxicity in cord blood. Bioorganic and Medicinal Chemistry **12:** 5603-5609.

[4]Tang, B., P. McKenna and R.L. Rovit, 1973. Primary fibrinolytic syndrome associated with subarachnoid hemorrhage. Angiology **24(10):** 627-634.

[5]Tang, B., 1999. Disseminated intravascular coagulation, in Neurotraumatology, Monduzzi, ed. Editore, Italy, pp 137-141.

[6]Tang, B., 2007. Critical analysis of disseminated intravascular coagulation - a scoring system with related literature review. Journal of Ayub Medical College **19(4):** 112-120.

[7]Galdiero, M., A. Tortora, N. Damiano, M. Vitiello, A. Longanella and E. Galdiero, 2005. Induction of cytokine mRNA expression in U937 cells by *Salmonella typhimurium* porins is regulated by different phosphorylation pathways. Medical Microbiology and Immunology **194(1,2):** 13-23.

[8]Steiner, A.A., S. Chakravarty, A.Y. Rudaya, M. Herkenham and A.A. Romanovsky, 2006. Bacterial lipopolysaccharide fever is initiated via T 4 on hematopoietic cells. Blood **107:** 4000-4002.

[9]Tang, B., 2006. Special Communication - Support with clarity: A proper trend in medical statistics. Journal of Ayub Medical College **18 (1):** 81-84.

[10]Tang, B., 2008. Biostatistics with Neurosurgical importance. J Neurosurg. 2008 Jun;108(6):1256-62. PMID: 18518738 [PubMed - indexed for MEDLINE].

The Abstract below was inadvertently omitted from the Member Presentation section of the Winter 2009 issue

A Faculty Survey on the Use of PowerPoint in Biology Courses

Carla Beeber and Carol Biermann from Kingsborough Community College/CUNY (KCC) and Kumkum Prabhakar from Nassau Community College/SUNY (NCC), discussed the results of a biology faculty survey developed by them concerning the use/non-use of PowerPoint (PPT) as a tool to teach biology. The survey had 10-statements for faculty that use PPT. In general, students told instructors that they liked the use of PPT by faculty. Selected survey results indicated that faculty that used PPT made their own PPT slides or modified publisher-prepared slides. In general, faculty was undecided as to whether students obtain better grades in their classes when PPT is used. Survey comments made by instructors were discussed. The authors have developed a student survey on the use of PPT that will be discussed at a future MACUB meeting.

2009 MACUB Conference

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If you have any questions contact Dr. Carla Beeber, at 718 368-5265 or cbeeber@kingsborough.edu. Deadline for submission is October 7, 2009

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If you or any of your students wish to make poster presentations, please register on-line at the MACUB web site www.macub.org.

If you have any questions contact Dr. Mohamed Lakrim, at 718- 368-5107 or mlakrim@kingborough.edu, or Dr. Sarwar Jahangir, 718-368-5743 or sjahangir@kingsborough.edu. Deadline for submission is October 7, 2009

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The Vice President will establish and serve as chairperson of the Advisory Council. In the event the President is no longer able to serve, the Vice President will automatically succeed to the presidency for the remainder of the term.

The Treasurer of the Association is responsible for the preparation of an annual fiscal report, processing of dues, preparing regular financial reports for the Executive Board meetings, income tax reports, and other duties usually pertaining to this office.

The Recording Secretary. shall record Board Members who are present, absent, or excused from Executive Board meetings and shall distribute the minutes of the Executive Board meetings, the annual business meeting, and any other officially sanctioned meetings as advised by the Executive Board. The Recording Secretary is responsible for Election Committee duties as stated in Article VIII - of these Bylaws.

The Members-at Large shall chair committees (Articulation, Exhibition, etc.) and handle other assignments as directed by the Executive Board.

Normally, each candidate for Vice-President, Recording Secretary and Treasurer should have been a Member-at-Large for at least one term and each candidate for Member-at-Large should have attended at least one Annual Conference.

DEADLINE FOR NOMINATIONS is **October, 1 2009**

If you are interested in running for office (or wish to nominate anyone else), please send a letter of nomination to:

Dr. Margaret Carroll
Biology Department
Medgar Evers College
1150 Carroll Street
Brooklyn, NY 11225

Student Membership

We encourage your students to become Associate Members in MACUB. Many of them will go on to graduate and professional schools. Their membership, participation and attendance at conferences such as these can enhance the experiences they include on their applications and discuss during interviews.

2009 MACUB Conference Registration Form
42nd Annual MACUB Conference at Kingsborough Community College
Saturday, October 24, 2009

❑Dr.
❑Prof.

❑Regular Member
❑Full-Time Faculty

❑Student Member[1]
❑Member's Spouse/Guest
❑Adjunct Faculty[1]

❑Mr.
❑Ms.
❑_____

*** Name:** _____ *** School Phone:** _____
*** Department:** _____ *** Fax:** _____
*** School:** _____ *** E-Mail:** _____
*** Address:** _____
_____

***The above information may appear in a Directory of Members unless you indicate otherwise.**

Home Address: _____
_____
_____

I prefer MACUB mailings to be sent to my:

❑School ❑ Home[1]

Home Phone: _____

[1]Student and adjunct mailings will normally be sent to your home address.

	Early Bird by 9/21	In Advance by 10/14	On-Site 10/24	
❑Regular Member	$45	$50	$55	Includes 2010 Membership dues, conference registration, continental breakfast and luncheon.
❑Student Associate Membership	$35	$35	$40	Includes 2010 Associate Membership dues, conference registration, continental breakfast and luncheon.
❑Member's Spouse/Guest	$35	$35	$40	Includes conference registration, continental breakfast and luncheon.

❑I will not be attending the Conference but enclosed is my 2010 membership dues.
❑Regular Member $20 ❑Student Member $10

Return this registration form by October 14, 2009
Please make checks payable to: MACUB
Send registration form and check to:
Dr. Paul Russo
Division of Natural Sciences & Mathematics
Bloomfield College
467 Franklin Street
Bloomfield, NJ. 07003

Registration fees are refundable upon written notification by **October 14, 2009.** The membership fee ($20 for regular members and $10 for student members) will be deducted. *No refunds will be given postmarked after October 14, 2009.*

The Metropolitan Association of College and University Biologists thanks the following Affiliate Members for their support:

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Dr. Edward J. Catapane
Department of Biology
Medgar Evers College
1150 Carroll Street
Brooklyn, New York 11225