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THE UNIVERSITY OF ARKANSAS
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UNDERGRADUATE RESEARCH JOURNAL

INQUIRY



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Undergraduate Research Journal Fall, 2000 Message from the Provost

The research process involves keen observation, hypothesis development, measurements, analysis of data, and the determination of conclusions. This process will be increasingly needed by future professionals from business managers who apply statistical and other econometric tools to marketing plans, to teachers who tackle the demands for outcome-based education, to computer and software engineers who must assess the potential value of new materials and algorithms on the rates of digital processing.

At the University of Arkansas (UofA), we believe that the process and results of research (the discovery of facts and concepts) and scholarship (the creative organization, criticism, interpretation, and reinterpretation of facts and concepts) are critical to modern undergraduate education and the talents our graduates will bring to the workplace. The results of research and scholarly efforts—represented in this journal—vividly demonstrate how the UofA is contributing to undergraduate education at its best.



**Bob Smith
Provost and
Vice Chancellor for Academic Affairs**

INQUIRY

Undergraduate Research Journal of the
UNIVERSITY OF ARKANSAS, FAYETTEVILLE

Volume 1 - 2000

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Foreword

As the president of the University of Arkansas Teaching Academy, it gives me great pleasure to introduce you to the Academy's new project, *Inquiry: the University of Arkansas Undergraduate Research Journal*.

The Teaching Academy consists of faculty who have been recognized by their peers, colleges, and the larger university for their excellence in teaching. Teaching excellence is generally thought of as excellence in classroom teaching, and certainly classroom excellence is an important component of excellent teaching. But there are several other considerations that are equally important; to be an excellent lecturer is simply not enough. Good teachers must be able to establish a special rapport with their students in order to encourage them to work up to their maximum potential, to instill in them a love for learning, and to encourage them to go beyond the expectations of the classroom and to explore their disciplines for themselves.

This issue of *Inquiry* records the individual research exploration of twelve U of A student/faculty mentor pairs during the 1999/2000 academic year. The projects included here are drawn from disciplines across campus and are representative of the quality of research done by the myriad honor students on campus. These twelve were chosen by *Inquiry's* publication board from nearly sixty abstracts received as a result of a call for papers. They vary in subject, in writing style, and in the manner in which they reference their research sources; but they are uniformly excellent in content. In each case, the paper published herein is a précis of the student's larger research product.

The intent of the journal is to record the depth and breadth of the scholarly activities of the university's best undergraduate students. I believe that it does this. We hope that readers will see that there is no limit to what can be accomplished by the University's best students and teachers working together. I hope you will be as excited as I am with the quality of the work presented here.

Murray Smart, Jr., Editor

University Professor of Architecture, Emeritus

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AN ANALYSIS OF THE THEORY OF FUNCTIONS OF ONE REAL VARIABLE

by Robert Jason Reed
Department of Mathematics
Fulbright College of Arts and Sciences

Faculty Mentor: Dmitry Khavinson
Department of Mathematics

Abstract:

Few undergraduates are aware that the Riemann integral taught in introductory calculus courses has only limited application—essentially this integral can be used only to integrate continuous functions over intervals. The necessity to integrate a broader class of functions over a wider range of sets that arises in many applications motivates the theory of abstract integration and functional analysis. The founder of this theory was the French mathematician Henri Lebesgue, who in 1902 defined the “Lebesgue measure” of subsets of the real line. The purpose of this project is to elucidate the theory of abstract measure spaces and of important spaces of functions (a critical example of which are Banach spaces), and extend the application of this theory. Developing the tools for doing so has been the focus of my advisor Professor Dmitry Khavinson and me over the past three years.

The primary goal of the thesis is to make this highly formal and abstract material accessible to an undergraduate having only a year of coursework in advanced calculus. These concepts are typically introduced at the graduate level, but the ideas require only a familiarity with the analytic style of proof learned as an undergraduate. It would be advantageous to expose advanced undergraduates to this material since these ideas form the foundation for how mathematical research is done at the professional level. The addition of interesting and practical examples (which are scarce in the standard graduate texts) will help to make the concepts more familiar and down-to-earth.

The motivation for a new theory of integration came from the Riemann integral’s apparent inability to operate on functions that fail to be continuous. For example, the Riemann integral of the function that assigns the value 1 to rational numbers and 0 to irrational numbers can be evaluated over the interval $[0, 1]$ with equally valid justification to be 0 or 1. This is because the definition of the Riemann integral depends on partitioning the domain of the function to be integrated, and finding the maximum and minimum values of the function over each partition. The Lebesgue integral, on the other hand, partitions the range of the function to be integrated and then considers the length of the

Jason Reed and Dmitry Khavinson

pre-image of each partition as well as the maximum and minimum values of the function of the partition. The utility of this change of perspective arises when we refine what is meant by “length” in the aforementioned pre-image. The Riemann integral requires that the domain consist of intervals of real numbers (where length makes sense), while the Lebesgue integral can be used with a much broader class of sets. Lebesgue modified the notion of length by defining the measure of a set E to be the smallest possible total length of all collections of intervals that cover E . Using this ingenious method, Lebesgue constructed a theory of integration which forms the most useful example of all general integration theories. The theory has important applications in many areas of science and engineering as well as probability and statistics.

Our approach to the subject has emphasized theory developed in H.L. Royden's classic text, Real Analysis. My project has included analysis of each concept in the text, and I have developed for each major subject a collection of problems solved and applications of major theorems that were explored. The result has been comprehension of many of the foundational ideas in the field. We have used a number of supplemental texts to gain depth of understanding where Royden's text provides only a survey, such as the Riesz Representation theorem, and to extend important ideas, such as the consideration of complex-valued (in addition to real-valued) measures.

The synthesis has been a comprehensive paper which describes the theoretical directions the research has taken, the major results and theorems with proof, and applications and examples which are worked out in detail. The final record of my research will be divided into the following six sections: Lebesgue measure, Lebesgue integral, relationship between differentiation and Lebesgue integration, Banach space theory, abstract measure theory, and general integration theory. The analysis encompasses discussion of the main ideas (what it means for a set function to be a measure, how an integral can be defined in a coherent way with respect to a measure, when the derivative of an integral of a function is the function itself, different ideas about what it means for a sequence of functions to converge to a function, what are the properties of Banach spaces and why they are useful, etc.), as well as important ideas and theorems that interrelate these concepts (i.e., when we can interchange the limit of a sequence of functions and the integral, how we can represent a bounded linear functional, the structure of certain spaces of integrable functions).

The Paper:

The Riemann integral taught in introductory calculus courses has limited application—essentially this integral can be used only to integrate continuous functions over intervals.¹ Even bounded functions such as the characteristic function of the rational numbers on the interval $[0, 1]$ have a Riemann integral whose value can be equally well justified to be 0 or 1. A new theory of integration was called for, and the French mathematician Henri Lebesgue invented it in the early twentieth century. Instead of partitioning the *domain* of the function to be integrated into a number of intervals of fixed length and then sampling it on each partition, Lebesgue's idea was to partition the *range* of the function and consider the length of the corresponding preimages. These preimages, however, were no longer necessarily as simple as intervals (sets whose length is easily defined), and so a new notion of the length of a set was needed. Lebesgue thus defined the "measure" of a set E to be the infimum of the total lengths of all open covers of E . From this springboard he defined a class of "measurable sets" of the real line, over which measurable functions could be integrated. Lebesgue's generalization

permitted vastly more functions to be integrated; and, as E. B. Van Vleck wrote shortly after its conception in 1916, "This new integral of Lebesgue is proving itself a wonderful tool. I might compare it with a modern Krupp gun, so easily does it penetrate barriers which before were impregnable." The purpose of the present project is to explicate the theory of Lebesgue measure and the Lebesgue integral as well as abstract measure theory.

The approach to the subject has emphasized the development of the theory of functions of one real variable in H.L. Royden's classic text, *Real Analysis*. The project has included analysis of each chapter in the text, and for each subject a collection of solved problems and applications of major theorems which were explored. The project has covered many of the major ideas and theorems that form the foundation of the field of what is presently known as "real analysis," a subject typically learned in a one-year graduate course in mathematics. Supplemental texts were used to gain understanding of major parts of the theory that are only surveyed in Royden's text (such as the Riesz Representation theorem) and to extend various important ideas (such as the consideration of complex-valued in addition to real-valued measures).

The synthesis of the project has been a comprehensive paper which describes the theoretical concepts, the major theorems with proof, and applications and examples which are worked out in detail. The final record of my research will be divided into the following five sections: Lebesgue measure, the Lebesgue integral, the relationship between differentiation and Lebesgue integration, Banach space theory, and abstract measure and integration theory. The development of Lebesgue measure includes an axiomatic introduction and analysis of its salient properties (analyzing its vital axioms: countable additivity, translation invariance, and determination that the calculated "measure" of an interval agrees with the established notion of length), the more general notion of outer measure, measurable sets and the construction of a nonmeasurable set of the real line,² measurable functions, and J. E. Littlewood's famous "three principles." Littlewood's principles are the most instrumental notions that form the foundation of functional analysis. Various incarnations of his principles are realized in Egoroff's theorem, Lusin's theorem, the possibility of approximating measurable sets with open sets, and the action of finding a sequence of continuous functions which can uniformly approximate any measurable function. Finally, we demonstrate what it means for a sequence of measurable functions to converge in measure to a function f . This is a notion that is weaker than uniform and even pointwise convergence but has important applications in functional analysis.

Considering Lebesgue's theory of integration, we first review the formal definition of the Riemann integral, and illustrate its limitations. The presentation of the Lebesgue integral then proceeds by construction in successively more complicated settings. At the beginning of this process, we base the definition of the integral on the integral of a *simple function*, which is a

function that assumes only finitely many values on some finite sequence of measurable sets. Its integral is simply the sum of the measures of the sets on which the function is defined times the value of the function on these sets. We proceed to define the integral of a bounded function f on a set of finite measure to be the supremum of the integrals of all simple functions which do not exceed f . We then naturally extend the definition to the integral of any non-negative function g , defining the integral of g to be the supremum of the integrals of f for all bounded measurable functions which do not exceed g . This definition enables us to establish the essential convergence theorems: Fatou's lemma, the monotone convergence theorem, and Lebesgue's dominated convergence theorem. These convergence theorems express the integral of a function f in terms of the limit of a sequence of integrals of functions f_n , when the functions f_n converge to f for each point in their domain (except perhaps for a set of measure zero). Fatou's lemma requires only pointwise convergence but in return concludes only that the limit of the integrals of f_n is less than or equal to the integral of f .

The hypothesis of the monotone convergence theorem is that the functions f_n be increasing to f , then asserts that the limit of the sequence of integrals of f_n is equal to the integral of f . Lebesgue's dominated convergence theorem requires that each f_n be bounded above and below by a fixed integrable function h and concludes equality of the limit of integrals of f_n and the integral of f . These theorems provide powerful tools with which we can determine the integrability of a function and also open the door on the subject of functional analysis. There, we explore the absolute continuity of the integral and approximation of integrable functions by sequences of simple functions, step functions, or continuous functions.

Our consideration of differentiation and integration explores in which settings differentiation and integration are indeed "inverse" operations. The conclusion of this consideration is encapsulated in a result known as the *Fundamental Theorem of Calculus*. We first analyze the application of the derivative to measurable functions and Vitali's covering theorem. This requires introducing the concept of the four derivatives of a measurable function, which must be equal for the derivative of the function to exist. Then we define what it means for a function to be of bounded variation and the relationship of bounded variation to absolute continuity. Absolute continuity, which is stronger even than uniform continuity, is the key with which we establish the Fundamental Theorem of Calculus. It so happens that we can relax the restrictions placed on f in order to be able to recover f by differentiating its integral; but in order to have the converse—to have the integral of the derivative of f be equal to f itself— f must be absolutely continuous. We finally consider the properties enjoyed by convex functions (a special class of measurable functions) and prove Jensen's inequality.

The theory of Banach spaces, of which the p -integrable measurable functions provide the most salient example, is of

vital importance in order to develop a coherent theory of abstract measure spaces. We will cover in detail these so-called L^p spaces, prove the Holder and Minkowski inequalities, and explore the role played by completeness in analyzing the convergence of functions in Banach spaces. In addition, we introduce bounded linear functionals. The Riesz representation theorem is the most important result in this area, allowing us in L^p to find a representation via integration of each bounded linear functional against a function in its dual space, L^q .

Abstract measure and integration theory and functional analysis are the culmination of this project. We distill the most vital properties of Lebesgue measure, and with these define what it means for an arbitrary set function to be a measure. We thus concentrate on "measurable spaces" (an abstract space along with a measure and a collection of measurable sets) and consider which properties of Lebesgue measure remain valid. We then look at a number of general measures that arise in this setting: signed measures (which can take both positive and negative values), complex measures, and product measures. We further consider the decomposition of a measure into disjoint parts (one absolutely continuous and the other singular) and the extension of a measure from an algebra of sets to a σ -algebra. Analyzing the corresponding extension of integration, we prove the general convergence theorems, which allow not only a sequence of functions to vary but also a sequence of measures. We conclude with the Radon-Nikodym theorem, which reveals that each measure can be represented as an integral of a given function against a measure with respect to which the original measure is absolutely continuous. The result gives rise to the Radon-Nikodym derivative, an important function representing a ratio of sorts between the two measures considered.

¹ More precisely, a bounded function f is Riemann integrable on $[a, b]$ if and only if the set of points at which f is discontinuous has measure zero.

² This is a surprisingly difficult task! The fact that this is so seems to justify the usefulness of Lebesgue's definition of what constitutes a "measurable set."

Faculty Comments:

Dr. Dmitry Khavinson, Mr. Reed's mentor, made these comments about the work:

I have known Jason for five years, since he came to the University of Arkansas as a freshman, holding a prestigious Sturgis Fellowship. He has been my student ever since, first in the three-semester Honors Calculus course and, during his third year, in a directed reading course (studying the Principles of Mathematical Analysis by Walter Rudin). He has continued his reading course with me through his fourth year, studying measure and integration theory in Royden's fundamental text, *Real Analysis*. This year he is reading Kai Lai Chung's book, *A Course in Probability Theory*, which forms the basis for his senior thesis.

The thesis consists of exposition some of the most "treacherous" places in the Lebesgue theory of integration—some difficult problems used in graduate courses in Real Analysis and Probability. This work may not be highly original in regard to its scientific merit but is highly so in relation to pedagogy and exposition, and I strongly support this project.

In all my calculus classes he was without a doubt the best student; and, over all, I would not hesitate to place him among the three best undergraduate students I have ever encountered during my twenty years of teaching, both here at the University of Arkansas, and at the University of Alabama, the University of Michigan, and Brown University. Jason is a solid person, a hard worker, and a stable learner. What I find to be his most important quality is that he has a tremendous amount of curiosity for mathematics and science, in general.

Jason has received a number of impressive awards during his undergraduate career. The most prestigious and competitive award that I want to mention here is the Barry Goldwater Scholarship for 1998-99 that allowed him to participate in the Penn State Mathematics Advanced Study Semester with 25 other students selected from throughout the United States. Lately Reed has been actively working on his senior project and has given a number of excellent presentations at our seminars and also at various gatherings of undergraduate students around the country on a rich variety of topics. Also, in 1999 he was awarded an undergraduate fellowship at the Department of Energy at Argonne National Laboratory. In short, Jason Reed is as good a student and a beginning scholar as one can possibly hope for.

Professor **John Akeroyd**, also of the Mathematics Department, echoed Professor Khavinson's comments:

Jason was a student of mine in a course in discrete mathematics and in a two-semester course in mathematical analysis. In all of these courses, Jason defined the top of the class. Though I had a number of bright students in these classes (in fact, more so than ever before), no one was quite able to match Jason's combination of enthusiasm and ability. During my lectures, I was ever conscious that Jason was with or ahead of me in the presentation. His questions and comments in class were insightful and helped other students learn the subject. His test and homework scores were always excellent. I further got to know Jason in his capacity as Pi Mu Epsilon president; I am one of two faculty advisors for this math club at the University of Arkansas. Jason's enthusiasm for mathematics has made my job easy. He organizes talks each semester, asking both students and faculty to participate. He also represents this math club at national meetings and carefully prepares an interesting talk for each of these occasions.

In recent years he has been studying graduate-level mathematics under the guidance of various mathematicians in our department. One of the subjects to which he has been effectively devoting time is Analysis; Professor D. Khavinson has been his supervising instructor in this area. Having a firm grasp of Analysis is an essential first step in understanding many branches of mathematics. Frequently I have had the opportunity to discuss this subject with Jason and witness his development. He is well on his way to gaining fluency in this subject and shows the ability and enthusiasm not only to master it but to make contributions.

Jason has definite goals, and he pursues them with great determination and paradoxically with a joyful and outgoing manner. Among his goals for the 1999-2000 academic year is to continue his studies in Analysis; Dr. Khavinson is on leave, serving with the NSF for a year or two, and I have agreed to fill in. Our goal is to finish readings in a *Real Analysis* text written by H. L. Royden (a very standard text in the field), gain a firm grasp of the fundamentals of Functional Analysis (primarily using well-known texts of W. Rudin) and then proceed into the subject of Mathematical Probability, which is a very natural path to follow after an exposure to Real Analysis. We will budget our time carefully and maintain a rigorous pace. I am confident that Jason is up to the task and indeed will surpass my expectations as he always does; we have already made great strides this year and (if anything) are ahead of schedule. He pushes himself and the instructor with much fervor — such is his enthusiasm for learning. Jason is very easy to communicate with and indeed is an excellent public speaker. The discipline and enthusiasm that he brings to each of his efforts has been an inspiration to other students. I can think of no student in my years here whom I can recommend as highly as Jason. His innate ability and the maturity and determination with which he pursues his goals are quite remarkable.

Serge Tabachnikov, another of Mr. Reed's professors, had this to say:

I know Jason very well: four times he pursued independent studies with me (each project was a semester long; we spent about two hours weekly discussing the subject). The topics included differential geometry and topology of plane curves, "quantum" knot invariants, and differential geometry of smooth and polyhedral surfaces. All these projects went far beyond the standard curriculum and were quite challenging for an undergraduate student. Each of the studies resulted in an essay, written by Jason, and talks, given by him at regional and national conferences. Jason also has the distinctive qualities of a leader. He is the President of the local Pi Mu Epsilon Mathematical Society. It is due to his enthusiasm and organization skills that the Society remains the center of attraction to all undergraduates at UARK who are interested in mathematics.

CULTURAL ATROCITY EXPRESSED IN CONTEMPORARY ART

by Marlie McGovern
Department of Anthropology

Faculty Mentor: Mark Cory
Professor of German and Director of European Studies

Abstract:

Some of the most horrific chapters in human history have involved an ethnic dimension, notably the centuries-long obliteration of traditional Nigerian cultures by European colonizers, the attempted destruction of European Jews in the Holocaust, and the World War II decision to assault the Japanese with atomic bombs. The consequences of the above atrocities are not contained within temporal or cultural barriers, but hold profound and pervasive ramifications within contemporary society in its entirety. More recent conflicts in Africa, Southeast Asia and the Balkans reemphasize the horror and suffering brought about by cultural collisions. One of the most potent reactions to ethnic exploitation, persecution and brutality is the aesthetic response, art as the product of atrocity.

*To demonstrate the powerful implications entailed within the realm of aesthetics, this study explores three contemporary artistic responses to cultural atrocity (Art Spiegelman's *Maus*, Chinua Achebe's *Things Fall Apart*, and Hijikata Tatsumi's *Buto*), and focuses on the concept of art as a refutation of (or resistance to) the aesthetic of the oppressor. Each response probes the tenability and function of art as a means of cultural, social, and political resistance in three ethnically and geographically disparate settings, and attempts to illuminate the potent ramifications entailed within today's society.*

This paper serves as an overview and summation of my Honors senior thesis, which considerably extends and expands the ideas presented here. The larger study examines the history of the cultural atrocity in question and the biography of each artist and offers an analysis of the respective artistic pieces. The greater effect of each work is also assessed--for example, whether the art is viewed by elite or popular audiences, serves to facilitate individual understanding, vindicates an entire ethnic group, or pursues a global objective.

The Paper:

“How should art — how *can* art — represent the inexpressibly inhuman suffering of the victims [of cultural

Marlie McGovern and Mark Cory

atrocity] without doing injustice to that suffering?” asks literary critic Lawrence Langer.¹ In evaluating any artistic medium in reference to cultural atrocity, one must first acknowledge the inherent contradiction between the experience to be expressed and the vehicle for its expression. If art can be described as the ultimate articulation of what it means to be human, acts of cultural atrocity, as the antithesis of humanity, would seem to defeat any attempt to elucidate history through art. Yet, paradoxically, art recurrently emerges from atrocity, often offering deep, multi-layered insight into intense, seemingly impenetrable realms of horror. “To create beauty out of nothingness - this is the dark challenge facing human spirits who sought expression if not renewal, by translating the agony of annihilation [be it human, cultural, or aesthetic annihilation] into the painful harmonies - and discords - of an art of atrocity.”²

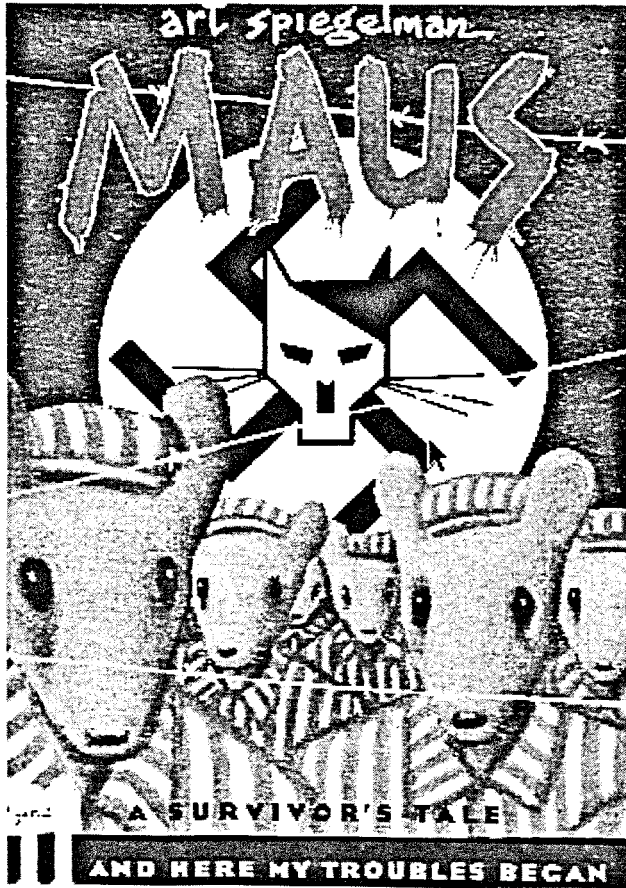


Figure 1. Front cover of Art Spiegelman's 1991 *Maus II: And Here My Troubles Began* (New York: Pantheon)

Aesthetics, specifically in regard to the art of atrocity, entail potent social, cultural, political, and ethical implications which extend far beyond the limits of any singular artistic creation. Interestingly, art may serve as both a forceful means for cultural repression as well as a successful vehicle for defiance and rebellion against oppressive ideology; this is the dual potential of art explored in this study. Art Spiegelman's *Maus*, Chinua Achebe's *Things Fall Apart*, and Hijikata Tatsumi's *Buto*, three contemporary artistic responses to cultural atrocity, utilize art to refute and resist the aesthetics of cultural repression.

In each instance, an overpowering, oppressive force (Nazi politics in Germany, Western colonizers in Nigeria, and U. S. military forces in Japan) asserts ethnocentric dominance over the subjugated cultures in question. The political and social ideologies of the dominant cultures are manifested through their particular aesthetic values. The Nazi aesthetic demands perfection in artistic form, just as the Nazi political agenda requires perfection of the Aryan race through annihilation of the Jews. Western aesthetics celebrate high culture (such as written histories, stories, and poetry), and the Western political mind-set entails bringing "civilization" to (or may justify the exploitation of) the "savages" in Nigeria who possess no written words. U. S.

aesthetics center on modernity and progress and reflect the political desire to promote technology and democracy within Japan, despite the sacred Japanese bond between humans and nature. Through their respective artistic statements, Spiegelman, Achebe, and Tatsumi confront and defy the oppressive ideology of cultural atrocity, offering uniquely valuable insight to modern society.

Art Spiegelman's *Maus* is initially deceptive. What appears at first glance to be an entertaining comic strip is in reality a poignant, multi-layered address to the horrors of the Holocaust. Serving as historical memoir, explanatory biography, and pointed social and political commentary, *Maus* is Spiegelman's challenge to and inversion of Nazi aesthetic and political ideology through comic art. Hitler's 1932 rise to power in Germany brought about long years of unspeakable cultural and political oppression for European Jews. The tenets of National Socialism, aimed directly at reestablishing a sense of German pride and nationalism after the economic and psychological devastation of World War I,



Figure 2. Front cover of Chinua Achebe's 1959 *Things Fall Apart* (New York: Doubleday Dell Publishing Group, Inc.)

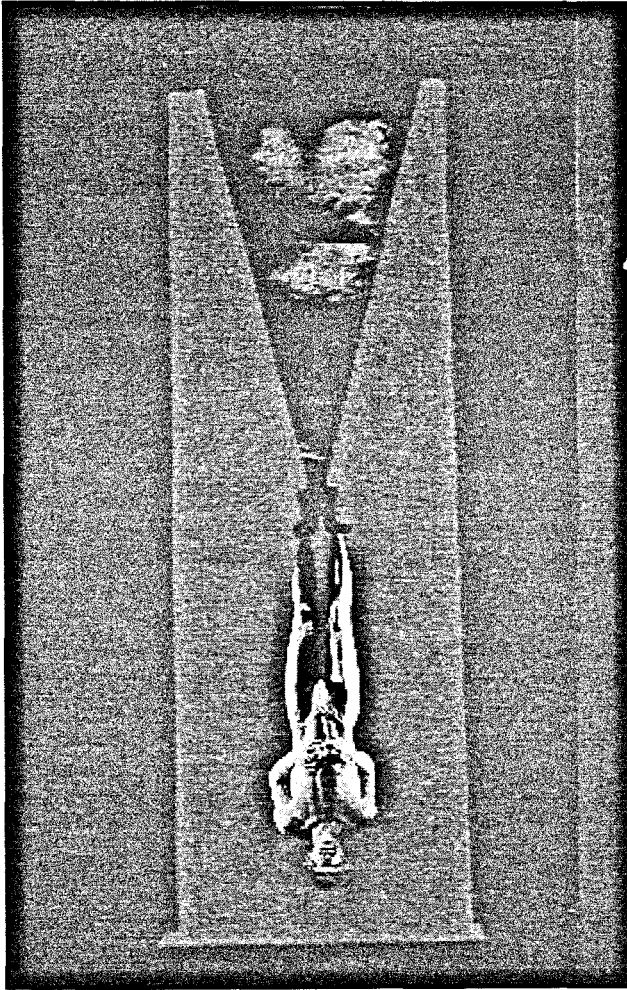


Figure 3. Ushio Amagatso performing Buto. Illustration from J. M. Adolphe 1994 Ballett International, 8:60.

identified the Jewish race as the essential enemy of an ideal Germany. Anti-Semitism pervaded every aspect of life in Germany; art and popular culture were specific vehicles for, and targets of, racial antagonism. In art, as in life's practice, Nazis condemned and exiled the imperfect, the misshapen, and the irregular; aesthetics were state-regulated by the Reichsministry for Popular Enlightenment and Propaganda under Joseph Goebbels.³ Progressing through three stages (1933-1939 defamation and legal discrimination, 1939-1941 restriction and internment, 1941-1945 final solution), German persecution of its Jewish citizens escalated into a state-mandated genocide of unprecedented scale and horror.

The Nazi regime served to divide humanity into various species; Jews were relegated to the status of vermin, unnecessary "pests" to be exterminated. The Nazi representation of the Jewish population was largely confined to editorial cartoons. The anti-Semitic broadsheets and editorial cartoons of the Third Reich depicted Jews as "human vermin," with hideous,

exaggerated rodent characteristics. In reaction against this Nazi symbolism, Spiegelman decided to depict his comic strip characters in animal form (the Jews are mice, the Nazis are cats, the Poles are pigs, the Americans are mongrels, and the French are frogs). Spiegelman states, "*Maus* was made in collaboration with Hitler . . . My anthropomorphosized mice carry trace elements of editorial cartoonist Fip's Jews-as-rats cartoons for *Der Sturmer*, but by being particularized, they are invested with personhood; they stand upright and affirm their humanity."⁴ An encounter with *Maus* leaves one with a deeper comprehension of the Holocaust, and the potent ideology entailed therein, in both historical and contemporary settings.

Chinua Achebe's novel *Things Fall Apart* utilizes aesthetic synthesis to refute inaccurate Western portrayals of Nigerian society through the incorporation of an African cultural practice (elements of oral tradition) within Western literature. The instigation of imperialistic occupation by the capitalist powers in Third World countries, such as the late nineteenth century British colonization of Nigeria, initiated profound and lasting influences on the subjugated cultures. Great Britain imposed foreign systems of government and religion upon the formerly autochthonous regions; consequently, the indigenous aesthetic definition was distorted by altering (and in some instances forbidding) traditional artistic and cultural practices. Among the most prominent changes lies the transition from oral tradition to written language. Audible narratives not only provided entertainment for their respective societies but also served as educational tools in regard to history, genealogy, and religion.⁵ The nineteenth century introduced written language to Africa, forcing previously oral tribes to assimilate alphabetic symbols as an alternate method of communication. Interestingly, the rich, deeply-rooted tradition of orality did not disappear completely; it simply merged with the newly acquired literacy. The modern-day author Chinua Achebe illustrates the partnership between written and oral aesthetics in his fiction, essays, and poetry, and specifically through his novel *Things Fall Apart*.

In *Things Fall Apart*, detailing the story of Nigerian tribesman Okonkwo during the onset of British occupation, Achebe provides an honest, uniquely individual insight into the agricultural, legal, matrimonial, social, and ritualistic aspects of native culture. Achebe's inspiration for the novel came from his desire to dispel the myths and stereotypes about Nigeria constructed by European social scientists. Yet Achebe's fiction is relevant on far more than an anthropological level; his writing transcends the limited boundaries of fiction (largely because of the incorporation of narrative proverbs and other elements of oral tradition) and encompasses the universal themes of ethnographic, social, and political commentary.

Hijikata Tatsumi developed *Buto* dance as a response to the U.S. 1945 atomic bombings and subsequent occupation in Japan. Invoking traditional Japanese spiritual and ritualistic aesthetics in his choreography, Tatsumi's *Buto* serves as a direct refutation

of the prominent and unwanted Western influence pervading Japan from 1945 through 1952. Western occupation in Japan entailed significantly detrimental social, political, and economic ramifications for the Japanese; they were overwhelmed with an inescapable and unwelcome Western presence and ideology within their own country. The pain, loss, and destruction caused by the bombings and occupation were physically and emotionally devastating for at least a generation of Japanese; their lives were forever darkened and fundamentally altered.

Strongly opposed to Western hegemony in Japan, Tatsumi created *Ankoku buto* (the "Dance of Darkness") in 1959, imposing a "particularly Japanese quality"⁶ on physical movement and spirituality. Drawing on the "centuries-old gestures of farmers in their paddy fields, old peasant women, or grotesque prostitutes, and images of his own disabled sister,"⁷ Tatsumi's style was raw, violent, and improvisational.⁸ Through an embrace of the depth and complexity of Japanese cultural history (specifically the elements of theater and religious ritual), Tatsumi's dance served simultaneously to convey the utter grief and suffering of the Japanese people and to contradict the pervasive Western aesthetic influence. Tatsumi's art attested to devastating desolation entailed within cultural atrocity and, in an age of increasing internationalization, serves as a necessary reminder of the horror and suffering which stem from disregard and cultural biases.

The art of Spiegelman, Achebe, and Tatsumi offers testimony to the serious and powerful role of aesthetics within social, cultural, and political arenas. Aesthetics have the potential to delude and confuse (as demonstrated by the Nazis, British colonialists, and the U.S. military) and to bring about destruction; but art also has the potential to elucidate and illuminate history (as seen through the featured artists' works). It is for this reason that art as a reflection of cultural history should be viewed as a viable medium for personal expression, intercultural communication, and global enlightenment.

Endnotes:

¹ Lawrence Langer, *The Holocaust and the Literary Imagination* (New Haven and London: Yale University Press, 1975) 1.

² Langer 1.

³ Thomas Doherty, "Art Spiegelman's *Maus*: Graphic Art and the Holocaust," *American Literature* (1996): 72.

⁴ Doherty 75.

⁵ Isidore Okpewho, *African Oral Literature* (Indianapolis: Indiana University Press, 1992) 110.

⁶ S.J. Cohen, ed., *International Encyclopedia of Dance* (Oxford: Oxford University Press) 18.

⁷ J.M. Adolphe, "Pastime of the Gods," *Ballett International* (1994): 61.

⁸ Cohen 17.

Faculty Comments:

Ms. McGovern's mentor, Mark Cory, had this to say about her project:

When I read Marlie's seminar paper for the Honors section of my course last spring on Literary Reflections of the Holocaust, I knew she had both the basis of an

excellent research proposal and the skills necessary to see a project through to its completion. I was not surprised to learn that she was a candidate for Four Year Honors in Cultural Anthropology, and I was delighted when she conceived of a way to tailor a greatly expanded version of her original study as her Honors thesis. I encouraged her to submit a proposal to the SILO/SURF competition in the fall. We remained in contact over the summer, and by August I had confirmation of Marlie's seriousness about the project. She had secured the support of her advisor in Anthropology, had prepared a substantial bibliography, and had come during the first week of class with her draft proposal! The proposal was funded and she has submitted a significant portion of the SILO/SURF project to the undergraduate research conference in Conway this spring. She will continue to work on the elaboration of the SILO study next year as she completes her program, which she is extending to add European Studies as a co-major. Her present abstract summarizes this larger study.

In terms of method, her thesis examines aesthetic responses to atrocity in a variety of cultures: European, Japanese, African. It involves a variety of art forms: literature, graphic arts, dance. Because of this interdisciplinary approach, it was not an easy combination to sell as an honors thesis, even though our ambitious H2P program prepares students to expect exactly this kind of thinking outside normal departmental boxes. Thankfully, her curiosity and determination have been met by generous support in her major department, where she received excellent methodological training in cultural anthropology. For her insights into Japanese dance, she has been prepared both by her own personal training as a dancer and by the formal guidance of our colleague Terry Brustar in the Drama department. My contribution has been to help apply the lessons of Holocaust literature to the very different kinds of cultural atrocity she explores in the case of colonial Africa and occupied Japan.

In terms of significance, Marlie's study is intellectually courageous and bound to be controversial. The validity of an aesthetics of atrocity is not yet universally accepted even in Holocaust studies (although I believe the best scholars embrace the concept). Where it is accepted, the concept tends to be closely guarded, as if any attempt at wider application could somehow undermine the uniqueness of the Holocaust or lessen the suffering of those victims. In the meantime, the kind of cultural exploitation Achebe addresses has received but a fraction of the attention regularly lavished on the much more prominent European catastrophe. And the attempt by a young American to look with fresh eyes at the aftermath of Allied victory over Japan in these terms is both daring and, in my judgment, long overdue. Finally, I would conclude by pointing out how marvelously well Marlie's entire

program, culminating in this research project, responds to the legacy of Senator Fulbright. I take that legacy to be a commitment to the humanities, an awareness of the dangers inherent in an arrogance of power, an appreciation of the benefits of international study, high intellectual standards, and a resolve to pay back in some future service the privileged opportunities conferred by American citizenship. This, I think, accurately describes Marlie, whose modesty would never permit her to recognize herself in this characterization. Nevertheless, her academic record, her intellectual quest to understand how different peoples have responded aesthetically to the Holocaust, to Hiroshima, to Colonialism, her own study abroad on the short term trips her limited financial resources have allowed (London last summer, Germany, Austria, Holland and the Czech Republic this summer), and her increasing interest in some form of public service after graduate study all mark her in my judgment as a student in whom this institution will take great pride.

Mary Jo Schneider, Professor of Anthropology, made the following remarks about Ms. McGovern's work:

Marlie McGovern has undertaken one of the most ambitious and most important honors projects that I have seen from an undergraduate. Marlie is working under Dr. Mark Cory to explore an important anthropological question: how does art serve as a force of resistance for ethnically oppressed groups. To explore this question, Marlie is comparing three examples of contemporary artistic responses in three settings: Nigeria, Germany, and Japan. Western colonizers, Nazi politics, and U.S. military forces dominated these societies during the middle part of this century. Artistic expressions can give us some insight into how those without power deal with their subjugation.

Marlie is a superb student. She is conscientious, dedicated, and extremely hard working and thorough. She has thought and rethought theoretical and substantive issues as she has tried to come to grips with the complexities of a difficult subject. I have seen a number of drafts and abstracts and I am quite impressed with her sophistication in this final version of her work. Marlie's is an exceptional piece of work that reflects credit both on Marlie and her mentor, Mark Cory. I give Marlie and her work my unqualified endorsement.

I have had Marlie in my History of Anthropological Thought class. She wrote reflective, thoughtful essays that were among the very best in the class. Marlie made connections that others overlooked, and her graceful writing style reflected her training and interest in the humanities and literature.

Professor of Dance, **L. Terry Brusstar**, made the following comments:

My interaction with Ms. McGovern has led me to believe that she is one of the brightest and most capable undergraduate Honors Students the program has had to date. Over the past three years I have had Marlie in two honors dance history courses, and I am presently supporting her research endeavors for her Honors Thesis. During this same period I have taught almost two hundred honors students.

During our teacher-student relationship, I have found Marlie to be both creative and scholarly in the work she has produced. She is consistently motivated, disciplined, and well read, and she produces papers, projects and the like which are well beyond my expectations and at a level far beyond that of her peers.

This project is original in concept and affords Marlie a wonderful opportunity to develop an area of expertise to pursue later in graduate study in Anthropology and/or European Studies. Marlie's research probes the function of contemporary art used as a means of cultural, social, and political resistance. Her study is both timely and significant to the "Popular Culture" discipline under the area of research called "Memory and Representation."

**OLD MYTHS AND NEW REALITIES:
UNCOVERING THE IMPLICATIONS OF SENATOR J. WILLIAM FULBRIGHT'S MIDDLE
EAST PEACE PLAN**

by Angie Maxwell

Faculty Mentor: Sidney Burris

Director, Fulbright College Honors Program

1969 and 1970, and specifically to the increasing alliance of the Soviet Union and Egypt, the Fulbright Peace plan offered a solution not only to the Arab-Israeli conflict, but also to the Cold War rivalry in the Middle East and to the perceived ineffectiveness of the United Nations.

The crucial timing of the release of "Old Myths and New Realities II: The Middle East" reflected Fulbright's awareness that continued attacks between Israel and the fedayeen (Egyptian-trained Arab fighters) would inevitably draw the United States into war with the Soviet Union. Angered by a series of inadequately implemented peace initiatives resulting from the unstable relationship between the Administration and the State Department, Fulbright could remain silent no longer. Moreover, the method of release (the speech was published widely both domestically and abroad before it was delivered on the Senate floor) reflected Fulbright's growing frustrations with the dwindling influence of the United States Congress over foreign policy decisions. Unfortunately, the proposal was rejected immediately by Israel and ignored by the administration. However, it received significant attention from the media.

Angie Maxwell and Sidney Burris

Abstract:

On August 24, 1970, Senator J. William Fulbright presented the speech "Old Myths and New Realities II: The Middle East" to the United States Senate. The intent of this paper is to uncover the significant implications of Senator Fulbright's delivery of this particular speech at this particular moment in American History. In brief, Fulbright proposed a bilateral agreement between the United States and Israel, whereby Israel would return the conquered Arab lands of the 1967 War in exchange for military protection from the United States. The speech, when taken out of context, provides a fairly simple plan to initiate peace in the Middle East. However, in relation to the events of

Old Myths for New Realities

On August 24, 1970, Senator J. William Fulbright presented the speech "Old Myths and New Realities II: The Middle East" to the United States Senate. A lengthy thirty-seven pages, the speech offered a solution to the Arab-Israeli conflict and specifically to the increasing alliance of the Soviet Union and Egypt, an alliance that had peaked in the late summer months of 1970. In brief, Fulbright proposed a bilateral treaty between the United States and Israel, whereby Israel would return the conquered Arab lands of the Six-Day War (1967) in exchange for military protection from the United States. Moreover, the Fulbright Peace Plan, as it was called, would have to be accepted and guaranteed by the United Nations Security Council. The speech, when taken out of context, provides a fairly simple plan to initiate peace in the Middle East. However, when examined

in relation to the long series of American foreign policy blunders in the Middle East, "Old Myths and New Realities II" reflects the evolution of the Senator's thought on the conflict. The manner and timing of the speech's release are significant factors in understanding the Senator's aims and intentions, which included an explication of his philosophy regarding the role of Congress in foreign policy decision-making and an avowal of his dissatisfaction with the secrecy of the Nixon Administration.

Fulbright's involvement in the Middle East stemmed primarily from his reaction to a succession of foreign-policy blunders that angered and frustrated him, the result of each blunder being an increased and alarming Soviet presence in the region. In 1950, the United States, taking a naïve Atlanticist perspective, signed the Tripartite Declaration with Britain and France, in which each agreed to "prevent an arms race among the major local powers in the Middle East, in particular Egypt, Iraq, and Israel."¹ The declaration ultimately backfired because the new, powerful Prime Minister of Egypt 'Abd al-Nassir (Nasser), having knowledge of the agreement, turned to the Soviet Union for military aid. The military aid would prove necessary in the 1956 Suez Crisis. In December of 1955, under the leadership of President Dwight D. Eisenhower, Secretary of State John Foster Dulles offered \$56 million to Egypt to fund the Aswan Dam project, part of Nasser's campaign to modernize the state. Nasser, hoping for a better offer from the Soviets, did not respond to Dulles. Aggravated, Dulles hastily withdrew the offer. In order to fund the Dam on his own, Nasser nationalized the Universal Suez Canal Company, startling the Western allies. Desperate, the British and French joined the Israeli attack on the fedayeen (Egyptian-trained Arab fighters) that was already in progress. The Eisenhower Administration called for an immediate cease-fire; European influence in the region completely disintegrated. The Soviets, sensing an opportunity to extend their influence, offered to participate in the Aswan Dam project which "alarmed Washington officials."² Fulbright blamed Dulles for causing the conflict and called for an immediate Senate investigation.

In 1970, the possibility of a superpower conflict in the Middle East reached a volatile level. The Soviet Union, by this time, had deployed 15,000 military personnel to Egypt. Nasser, now the recognized leader of the Arab Nationalist movement and founder of the Palestinian Liberation Organization, had begun a war of attrition along the Israeli-Egyptian border. In July, Israeli Prime Minister Golda Meir told President Nixon that Israel had installed SA-2 and SA-3 batteries along the border. The Administration panicked, realizing that the slightest sign of aggression by the Arabs could result in a direct confrontation between Israel and the Soviet forces that were now dominant in the Sinai. Within days of Meir's warning, Israeli Phantom jets came under fire in the Canal Zone. Secretary of State William Rogers negotiated a cease-fire that began on August 7, 1970. However, on August 13, 1970, Israeli intelligence revealed that

Nasser and the Soviets had moved additional weapons into the standstill zone. The United States had no evidence to prove that these movements had occurred because the State Department failed to order U-2 reconnaissance planes to photograph the area on the day that the cease-fire was implemented. Israel was outraged by the poor planning and grew increasingly desperate due to the lack of American action against these violations. The mistake increased the urgency of the Arab-Israeli conflict—one factor that prompted the Senator to make his most extensive commentary on the Middle East that August.

Furthermore, Israel and Egypt, as part of the cease-fire compromise, agreed to enter into negotiations under the auspices of the United States Ambassador to the United Nations, Gunnar Jarring. Fulbright did not want to see the Administration force a quick solution to the conflict, as had occurred in the aftermath of the Suez Crisis. Rather, the Senator believed in public discussion of major foreign policy decisions, and he believed that Congress provided the appropriate arena for this type of discussion. Thus, an elaborate effort to release the speech both in abstract form and in its entirety was undertaken by the Senate Foreign Relations Committee staff (Fulbright served as chairman of the SFRC from 1959 until 1974). An advance copy was forwarded to Secretary of State Rogers with a personal note from the Senator that stated flatly, "I have just finished this. I hope it may be helpful to you. If not denounce it."³ A briefing was held for all major media, and excerpts of the speech ran in the *Washington Post* and the *New York Times*. The excerpts ran one day prior to the delivery of the speech on the Senate floor and two days prior to the start of the Jarring negotiations. Indeed, Fulbright intended to exert his influence and encourage a debate on all levels from the mass public, to the media, to the Administration. Fulbright's insistence that any further peace initiatives for the Middle East be discussed in Congress reflected his growing frustration with the lack of communication between Congress and the Administration.

In truth, Fulbright's frustration began with the Eisenhower Administration during the 1950's. On January 5, 1957, the Administration introduced Joint Resolution 19 which authorized the President to employ the Armed Forces at any time to protect the nations of the Middle East from international communism. Fulbright vehemently opposed this resolution because it granted unprecedented freedom to the Executive Branch with regards to foreign policy. As debate continued, Fulbright delivered an impassioned speech against the resolution, calling it a "blank check."⁴ The speech marked the beginning of Fulbright's public campaign against the American policy on the Middle East. However, the Administration implored Congress not to deny the President the ability to protect American national security. On March 5, 1957, Joint Resolution 19—the Eisenhower Doctrine—passed. In the next two years the Doctrine was applied to three separate crises in Jordan, Syria, and Lebanon. The quick succession of these events over a two-year period, without the

consent of Congress, fostered an unprecedented sense of instability and marked a turning point in the history of the Senator's involvement in the conflict. Each of the crises backfired against the United States, increasing Soviet influence in the region, and accordingly, Fulbright marked each event with continued criticism on the Senate floor. As the Arab-Israeli conflict erupted again in 1970, Fulbright resumed his condemnation of unchecked Executive power. The Senate Foreign Relations Committee moved to revoke the Eisenhower Doctrine and the Gulf of Tonkin Resolution—which allowed Johnson to intervene in Vietnam—as a symbolic statement that Congress should not be by-passed in foreign policy making. The revelation of the secret attack on Cambodia in May of 1970 only antagonized the feeling of distrust that Fulbright had for the Nixon administration, in particular. This distrust, in addition to the foreign-policy blunders aforementioned, motivated the Senator to offer the Fulbright Peace Plan, though he knew it would prove controversial.

The plan, as previously stated, consisted of both a bilateral agreement between the United States and Israel and a multilateral agreement through the United Nations. According to the Senator's reasoning, each piece was necessary to a peaceful solution in the Middle East. The bilateral agreement addressed what Fulbright considered to be the root of the conflict—Israeli insecurity. He believed that if Israel's statehood were protected, it would relinquish the conquered territories of the 1967 War. The multilateral agreement would ensure Soviet support of the proposal, since it would have to be passed by the Security Council. Fulbright reasoned that all the Soviets really desired was a role in the decision-making process. Furthermore, if the United Nations was entrusted to secure peace in the region, its reputation could be revived—a reputation that had suffered from several unenforceable resolutions such as Resolution 242 which ended the 1967 War and called for Israeli withdrawal from the occupied Arab territories. Thus, the proposal serves as a clear example of Fulbright's characteristic methodology—his unique way of proceeding—whereby the microscopic focus on a specific problem (the crises in the Middle East) is seen within the realm of a macroscopic goal (the renovation of the United Nations and the sustained balance of power of the Cold War); such a process of thought belongs recognizably to Fulbright.

The bevy of articles that appeared after the release of "Old Myths and New Realities II" ranged from vehement criticism to unprecedented applause. The media focused on two primary points of contention—the proposal's feasibility and its inconsistency with regard to the Senator's position on Vietnam. Critics were correct in their analysis that Israel would not accept the proposal. Fulbright ignored the violation of the cease-fire in his speech, and in an attempt to offer an even-handed policy, Fulbright alienated Israel with his considerate treatment of the Soviets. Other journalists called the Senator a hypocrite for denouncing American involvement in Vietnam, yet offering American troops to protect Israel. However, Fulbright's

supporters in the media proclaimed him to be "The Signalman Senator" who examined each foreign policy situation in its own right, without the influence of the Jewish lobby.⁵ Syndicated columnist Walter Lippmann noted Fulbright's prophetic status, stating that "it has been said of him [Fulbright] that all too often he was right too soon."⁶ Indeed, as the Arab-Israeli conflict continues to claim lives today, Fulbright's proposal was reiterated in his own speeches, as well as in the Brookings Report on the Middle East and the American-Soviet Joint Resolution on Peace in the Middle East.

Endnotes:

- 1 Geoffrey Kemp, "Strategy and Arms Levels, 1945-1967," in *Soviet-American Rivalry in the Middle East* edited by J.C. Hurewitz (New York: Frederick A. Praeger, 1969) 31.
- 2 Robert W. Stookey, *American and the Arab States: An Uneasy Encounter* (New York: John Wiley & Sons, Inc., 1975) 139.
- 3 J. W. Fulbright to William Rogers, August 21, 1970, Senatorial Papers of J. William Fulbright, Mullins Library, University of Arkansas, Fayetteville, Arkansas. Box 40: File 3.
- 4 *Congressional Record*, Senate, February 11, 1957, 1857.
- 5 Louis Heren, "Signalman Senator." *The Times* (London), 25 August 1970, p.8.
- 6 Walter Lippman, preface to *Fulbright of Arkansas*, edited by Karl E. Meyer (Washington, D.C.: Robert B. Luce, Inc., 1963) ix.

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Faculty Comments:

Faculty Mentor Sidney Burris comments:

First of all, Ms. Maxwell has identified an aspect of the Senator's career that still generates much discussion—his opinion on the Arab-Israeli conflict. And she has chosen to analyze one of the central documents that delves into this opinion—his speech, entitled "Old Myths and New Realities II: The Middle East." There is focus to her project; and, yet as she situates the speech within the long history of American relations with the Middle East, there is ample scope as well. The thesis itself, for which the current manuscript is essentially a precis, runs over fifty pages. In reconstructing the background of the speech, Ms. Maxwell has educated herself in the making of a historical narrative, and she has adroitly handled the chronological intricacies, the give-and-take of diplomatic negotiations, that characterized this particular segment of American history. Her work is important because it brings the Byzantine complexity of our Middle East negotiations to bear on the equally

complex structure of thought that lies behind the Senator's speech and so provides a helpful bridge between the informing history and the resulting speech—and this had not previously been done with the same detailed focus that characterizes her approach.

Second, her work has admirably accomplished one of the primary goals that the Honors Program originally envisioned with the required Honors thesis—Ms. Maxwell has plunged herself into the rigors of scholarly research; and, as a glance through her bibliography will show, she has consulted an array of primary sources, including the Fulbright manuscripts in our collection, the Congressional Record in Washington, D.C., interviews with three of the Senator's aides, writers, and press secretaries (Tillman, Williams, and Purvis), as well as the standard secondary sources. And she has carefully collated the material, sifting through the contradictory accounts, and gradually built the coherent picture that characterizes both this project and her thesis from which this project is drawn. She has quite quickly learned the necessity of making accurate historical judgments based on the available evidence, and her approach in each instance has been conservative—no conclusions are drawn where they are not clearly warranted by the material at hand. My training, of course, is in English; and, to direct this thesis and to keep pace with Ms. Maxwell, I have had to do much of the reading that she did, and I can attest to the mountain of information that she has read and digested. It is not that the analysis of a single speech shows extraordinary ambition; it is that the large volume of information that she consulted in reference to the single speech reveals her characteristic ambition to leave no stone unturned. As a researcher, she has been absolutely thorough and uncompromising in her use of sources.

Finally, I would point to the quality of her prose. As the project has progressed through successive drafts, she has moved closer and closer to achieving the kind of limpid style that it seems to me most befits historical narrative of this sort. Never self-conscious, her prose has become more and more adept at deftly handling the date-driven narrative that often characterizes diplomatic history. She has worked hard to effect a balance between chronological accuracy and narrative liveliness. And in my opinion she has largely succeeded—the achievement of a prose style, which is already well within her grasp, is another of this project's accomplishments.

Hoyt Purvis, Director of the Fulbright Institute of International Relations says of the work:

By any measure Ms. Maxwell's study is exceptionally

fine and accomplished work, and for an undergraduate student it represents an extraordinary contribution.

Ms. Maxwell has studied and analyzed an important but relatively unexamined chapter in Senator Fulbright's history as a leading figure in American foreign policy and international relations. In the process she offers some valuable insight into Fulbright's views, his mode of operation, his strategy for attempting to influence the debate on U.S. policy in the Middle East, his efforts to use the media, and his relations with the Nixon Administration.

The historical context, background, and overall perspective of American foreign policy of the era is an especially strong feature of her work. She displays a clear understanding of what was important in the development of U.S. policy in the region and of Fulbright's role. Her analysis and her interpretation are solid, well-founded, and persuasive.

Altogether she has done a highly impressive job of research and writing. Her mastery of the background and context is clear. Her research in the Fulbright Papers, as well as the interviews she conducted with Seth Tillman and Lee Williams, brings an especially valuable dimension to the work.

As a professor of international relations and as one who was involved in working with Senator Fulbright at the time of his 1970 speech, I find this to be a sophisticated, well-written, and insightful work of scholarship. It is clearly worthy of honor.

Fulbright scholar, Distinguished Professor of Diplomacy, and Dean of Fulbright College, Randall B. Woods, remarks:

I am writing to endorse, with great enthusiasm, Ms. Maxwell's project, "Old Myths and New Realities: Uncovering the Implications of Senator J. William Fulbright's 1970 Peace Plan for the Middle East." I have spoken with her at some length about it, and her conception of the senator's fundamental philosophy regarding the United States' relation with Israel and the Middle East is both sound and penetrating. By examining the senator's proposal to send American troops into Israel in exchange for the resumption of their original pre-1967 borders—the senator proposed this while he advocated withdrawing troops from Vietnam—Ms. Maxwell has isolated a sterling example of Fulbright's special brand of pragmatism. Conformity to historical precedent and consistency with an intellectual tradition, while they are worthy concerns for a history professor, can yield disastrous results in foreign policy. But the fact stubbornly remains that many of Fulbright's critics have heretofore missed this fundamental point. Senator Fulbright, however, did not, and Ms. Maxwell's project, while surgically directed toward a specific phase in the senator's career, will shed light on his

entire political philosophy. And it will correct what has become over the years a substantial misconception concerning a vital area of his thinking on international relations. In my judgment, it is a project of real importance that deserves publication.

The research that she has already completed reveals the kind of maturity seldom seen in an undergraduate. Last spring, before she left campus for the summer, she introduced herself to the Fulbright archivist in Mullins Library, obtained the credentials necessary to work with the manuscript collection, and became acquainted with the collection's basic layout—and she did this several months in anticipation of the project's beginning stages. She has now mastered the navigation of the collection. And, while she was in Washington this past summer, she began reading the relevant speeches in the Library of Congress. She returned to Washington during spring break to interview both Dr. Seth Tillman, Fulbright's ghostwriter, and Lee Williams, Fulbright's former Chief-of-Staff. I cannot honestly say that I know of a more disciplined and motivated scholar at the undergraduate level than Ms. Maxwell, particularly in a field where the sources for her work are so voluminous and unwieldy. Many older, more seasoned scholars would be overwhelmed by the sheer amount of material that is available, but Ms. Maxwell has gone through the relevant material with purpose and dedication. Perhaps even more impressive, however, than this substantial preparation is Ms. Maxwell's knowledge of Arabic. She will be able to consult a range of primary materials that previous Fulbright scholars, because of their ignorance of the language, have been unable to examine. This knowledge of Arabic alone gives Ms. Maxwell a clear advantage over many of the reputable scholars who are currently working on Fulbright, and I eagerly anticipate the results of her research into this fertile area.

Since her junior year, Ms. Maxwell has won a Truman Fellowship, the Fulbright College Prize for Distinction in the Liberal Arts, and The Johns Hopkins Essay Contest. Most recently, she was appointed to the USA-Today Academic All-American Second Team, and in December of 1999 she was awarded a SILO-SURF grant to complete her work on Fulbright, an indication of the overall merit of her project.

**JOURNEYS:
THE INTERPRETATION OF MODERN MYTH
THROUGH ART**

by Karen V. Dick
Department of Art
Fulbright College of Arts and Sciences

Faculty Mentor: Marilyn Nelson
Department of Art

Abstract:

"Journeys" primarily investigates the interpretation of one form of expression to another; i.e., the visual interpretation of myth through art in terms of the contemporary individual's psychological voyage. The basis for this study comes from the writings of noted scholar Joseph Campbell and others who place myth in the position of the inward, not the outward journey in the quest for human discovery. As Bill Moyers so eloquently states in The Power of Myth, "myths are the stories of our search through the ages for truth, for meaning, for significance. We all need to tell our story and to understand our story..." This research project is realized in its final form as a body of creative work for exhibition purposes. It is of significance to note that for artists, the exhibition of creative research parallels the publication of written research in other fields. The project is an outgrowth of my past work and as a logical extension of that work, this investigation demonstrates a relevance to art in a historical context, as well as an exploration of contemporary artistic and intellectual activity.

Karen Dick and Marilyn Nelson

Historical Overview

Myth has a long history of interpretation in art, serving as a vehicle for the conveyance of information. According to Gardner's *Art Through the Ages*, one of humankind's greatest feats, the ability to abstract, to make images and symbols, finds its origins in prehistoric cave dwellings, where Paleolithic man was able to fix in place the world of his experience. It is widely speculated that the cave paintings could have functioned in a mythical manner possessing magical meanings for their creators. Since that time, myth has appeared in many forms of art throughout history, yet it is usually seen in the context of the literal; i.e., the ancient adventures of mythological creatures and personae with allegorical references to mankind's journey in the external sense.

As man and technology have evolved, so has myth. Science has disproved many of the literal places and figures of the ancient stories, but the function of myth remains no less important. As written in *The Intellectual Adventures of Ancient Man*, H. and H.A. Frankfort note that the imagery of myth "is nothing less than a carefully chosen cloak for abstract thought. The imagery is inseparable from the thought. It represents the form in which the experience has become conscious."

The impact of myth on man has probably been most fully explored by Joseph Campbell who spent a lifetime investigating the commonality of theme in world myths and their resonance in the search for human consciousness. In fact, the

journey of the hero in myth reflects the journey of every man. As Campbell states in *The Hero with a Thousand Faces*,

"not the animal world, not the plant world, not the miracle of the spheres, but man himself is now the crucial mystery."

The inner world of the psyche and the unconscious, especially the nature of dreams, was explored in the early 20th century by the Surrealist art movement. Surrealism, however, was founded in the basis of composing without any preconceived subject or structure. The element of chance, randomness, and coincidence became the basis for intensive study by the Surrealists. Although myth as a theme was more widely investigated by artists from the early ages through the 19th century, the subject is not relegated to works of the past. Contemporary artists of significance, notably Anselm Kiefer, are intermingling the mythological with the real to explain or interpret the nature of the modern world. Kiefer, who is described by art critic Robert Hughes as "the best painter of his generation on either side of the Atlantic," often uses mythic events as signposts to present his views of physical and human events. My research seeks to creatively explore my concept of modern myth—the adventure of the individual into the unconscious and how that journey parallels that of the ancient tales. For proponents of myth like Campbell, myth serves in the role to reveal a deeper reality and allows man to experience that reality.

Background

My research into the journey of the individual began in 1998 with a multi-part series, *Passage:Reflection*. This project explored the issues of the transformation of the written word to a visual statement. Text, in the form of poetry, functioned as the basis for the series. The poem was written during a transitional passage of my life, and while self-realization was the motivation for writing, the search for self became the catalyst for artistic expression. The primary question investigated was how could a written work be visually stated without losing the identity or the meaning of the words? The objective of the artistic work was not to illustrate the text but to restate the written word in the form of a visual vocabulary, retaining the emotional and psychological sense associated with the poetry.

The project resulted in a 10 print exhibition series. Each piece of artwork dealt with a particular passage of the poem. (See Addendum 1). The prints were digitally printed and related formally and conceptually through development and technical production. Traditional photography and digital processes were employed to integrate image and text into a "visual language," designed to reflect the complex layering of the psychological beings that we all remain.

The Concept and Form

As a student of art history, I have been interested for some time in the study of myth in art. My exploration into the inner self in my previous project in conjunction with a historical concern led me to the current investigation. The concept of interpretation plays a defining role in several aspects of this project. For Joseph Campbell, myth can function as a type of guidepost for the individual in the interpretation of his own life experience. "Anybody going on a journey, inward or outward, to find values, will be on a journey that has been described many times in the myths of mankind..." My objective is not to illustrate stories, but to translate the psychological sense of the inward journey. A point of investigation is how to visually interpret ancient mythology in terms of my own emotional response and my concept of the modern individual's experience. The journey is not the ancient's trek to an unknown land but the individual's quest into the unknown part of his mind.

An approach utilizing painting, photography, and digital imaging is employed. Conceptually, all three mediums have significance to this project. Painting is the oldest of the three—present since the cave dwellers' time. It might be considered the "original" form of abstraction since it was the method in which Paleolithic man represented his place in the world. Artistically, it is also the most "direct from the hand" of the three working methods used. The historical context of painting is relevant to the exploration of myth of all ages, and the directness of its application is important to my interpretation.

Photography provides a working visual vocabulary. The photograph has the unique ability to reference our experience of the world in a direct manner. In the Museum of Modern Art's exhibition catalogue, *Thinking Print*, it is noted that photography possesses that special ability to create narrative subject matter, suiting the desire to reconnect art and life. Photography is an especially powerful medium for communicating meaning or emotion, and on a conceptual level, photography is the referencing medium for modern man.

The use of digital imaging in concert with painting and photography is especially important. The digital process allows for the creation of imagery often not possible in other mediums. The computer is rapidly changing the face of art, and it has certainly become a standard working tool for artists. My work often involves the transformation of the recognizable into the abstract. For this project, such transformation is analogous to the journey of the individual from the external to the internal world. The computer also allows for the integration of language into art. The language selected has relevance to ancient myths whose themes relate to the contemporary individual, thus reinforcing the parallels of the past to the present.

Technical Development

My approach to the technical development of "*Journeys*" remains multi-disciplinary as with my previous work; however the conceptualization of my ideas dictates that I expand my thinking into new areas within the digital imaging field. As a digital artist I am rather non-traditional because I use the computer in an interpretive manner. The majority of artists working in this format are interested in creating special effects, thus allowing the computer and its unique capabilities to become less of a means and more of an end. I view the computer simply as another available tool, much like a pencil, pen, or paintbrush.

In my earlier series, the final production pieces are photographically based, and although highly transformed, the work retains a strong sense of the photographic image. (See Addendum 1). Because of my objective to integrate painting into the digital process, I have challenged myself to develop the images in "*Journeys*" in a painterly manner. By expanding my earlier techniques to include direct photographic scans of such subject matter as my hands, I can translate the work to a new form. An unusual juxtaposition of imagery occurs through this method. The "real" three-dimensional object, a hand, loses its identity as an actual entity to become a symbolic image through electronic means.

The image is actually "built" in the computer rather than transforming an existing one, which is very similar to constructing a painting on a canvas. The colors are all mixed in the computer instead of selecting standardized hues from a software program, and each piece has been digitally painted and drawn upon to create the final image. When text is used, it is layered into the piece as an element of composition while retaining its identity for meaning. Once the image is constructed, traditional painting or drawing techniques may be added as the final application, resulting in a mixed-media piece of art.

The final works measuring 25" x 25" are printed on fine art canvas specific to this process. One of the most significant developments in digital art is the relatively recent introduction of archival inks which are employed throughout this work. When digital imaging processes are presented for exhibition, it is essential to maintain image color and stability through the use of fine art archival products developed for long-life application. Long-life products, although expensive, have rapidly expanded the exhibition potential for digitally produced work.

Project Significance

"*Journeys*" has many areas of significance for me personally and professionally. The project is the culmination of a SILO Undergraduate Research Fellowship, which was initiated outside of course work. The research is fully realized in a body of creative work for exhibition, which is one of the principal avenues available to the professional development of an artist, as

the public display of visual work is equivalent to the publication of an author's written work in other fields.

This research incorporates the various areas of my study as an artist with a multiple media approach. As a Bachelor of Fine Arts major with a concentration in graphic design and painting, I have challenged myself to use the tools normally associated with these mediums in very non-traditional manners. My objective in doing so is to create work that is unique unto itself. In other words, the works do not have an obvious digitally imaged appearance. In fact, a measure of success for me can be measured in the form of a question. If the viewer questions the medium as to print or painting, then I have achieved my goal of establishing the process of digital imaging as a means and not an end.

My choice of subject matter for this research offers contemporary significance. We now live in an artistic environment where pure visual content is integrated with personal, emotional, intellectual, and social content. We are in the Postmodern era where the impulse has shifted from strictly style toward the direction of meaning. As curator Deborah Wye observes in *Thinking Print*, the new art world generation will be "using the word 'strategy' as opposed to 'style' to describe an artist's approach. Strategy implies a complex fabric of thinking and planning." New questions in art include not only "what does the work look like," but "what does it mean?"

Our exploding world of technology provides an appropriate backdrop to the individual's search for internal meaning, and this adventure is and always has been worthy of artistic investigation. To create a body of work through the most technologically advanced methods, while attempting to connect us to the most basic mysteries of human experience, provides a rich field for exploration. This research is designed to challenge my thinking in all of these areas; to create art with visual and emotive content; and to develop work that reflects a personal and universal appeal. Hopefully, this work serves as a testament to my personal as well as creative journey.

It seems to me that the only way in which an artist can make his work durable and great is by seeking to arrive at the most direct expression of something actually felt by himself as a part of his own, and so a part of all human experience.

Edward Carpenter
Angel's Wings

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Examples from the series: "Journeys: The Interpretation of Modern Myth Through Art"

25" x 25" Digital Images on Canvas



Subtle Reality

The place or, medium of realization is neither mind nor matter, but that intermediate realm of subtle reality which can only be adequately expressed by the symbol. The symbol is neither abstract nor concrete, neither rational nor irrational, neither real nor unreal.

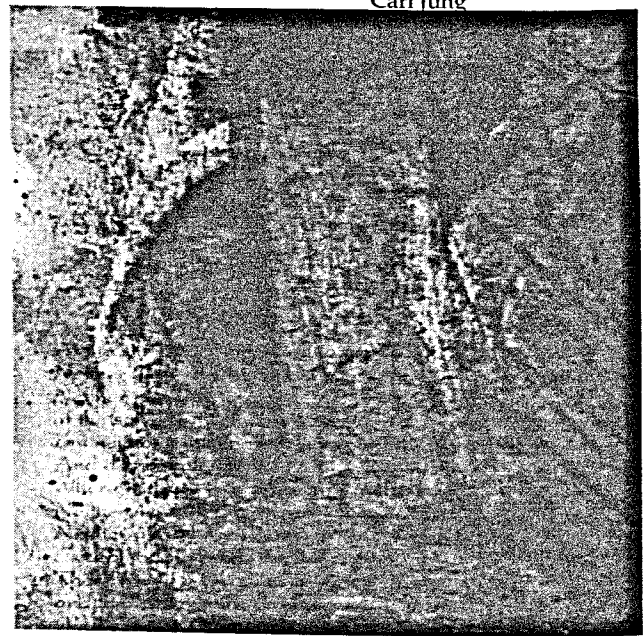
Carl Jung



The Labyrinth

...we have not even to risk the adventure alone, for the heroes of all time have gone before us. The labyrinth is thoroughly known. We have only to follow the thread of the hero path, and where we had thought to find an abomination, we shall find a god. And where we had thought to slay another, we shall slay ourselves. Where we had thought to travel outward, we will come to the center of our own existence. And where we had thought to be alone, we shall be with all the world.

Joseph Campbell

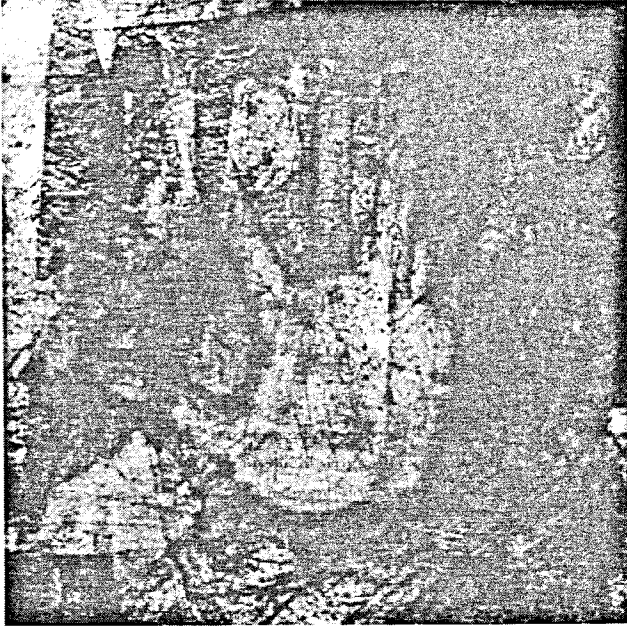


The Still Point

At the still point of the turning world. Neither flesh nor fleshless; Neither from nor towards; at the still point, the dance is, But neither arrest nor movement.

And do not call it fixity, Where the past and future are gathered. Neither movement from nor towards, Neither ascent nor decline. Except for the point, the still point, There would be no dance, and there is only the dance.

T.S. Eliot



The Story-Teller

The depths of the human soul are also "Primordial Times"...For Myth is the foundation of life; it is the timeless pattern, the religious formula to which life shapes itself, inasmuch as its characteristics are a reproduction of the Unconscious. There is no doubt about it, the moment when the story-teller acquires the mythical way of looking at things...that moment marks a beginning in his life.

Thomas Mann

Faculty Comments:

Ms. Dick's faculty mentor, Associate Professor Marilyn Nelson, made these comments about her project:

The Interpretation of Modern Myth Through Art, Karen's newest creative, a series of large scale digitally printed images, extends her work into the intellectual conceptualism of the modern myth, a subject of universal significance for the modern artist. Contemporary artists often strive to integrate pure visual information with emotional, intellectual and social content. Having researched ideas presented by Joseph Campbell and others relative to myths, and their parallels to the journey of modern man, she visually translates the psychological and emotional

sense of the inward journey. This creative quest for self-discovery, Karen believes, functions in both the individual and universal sense.

This research is significant for several reasons. 1) Its multi-disciplinary realization of concepts—juxtaposing and layering her photographs, typography, symbols and color selections and depicting personal as well as universal characteristics and concerns—is timely and important within the realm of contemporary art. 2) This research assumes a multi-disciplinary, non-traditional approach by incorporating photography, painting, and digital imaging. All three media have conceptual significance to the project. 3) The utilization of the computer as a working tool is significant in today's art world as digital images have become accepted as a valid form of art. Karen demonstrates an unusual use of this tool. Where most artists are interested in the effects produced by the computer, Karen is interested in discovering creative uses of software to realize her concepts, to juxtapose her photographs, typography, and color selections to depict expressive characteristics. 4) This sustained and concentrated effort producing high quality work is ambitious and unusual for an undergraduate art student.

In her paper, Karen's understanding of her creative concepts and the media employed for visualization of such concepts is defined in a clear, concise, and organized format. To translate the creative process into writing is a struggle for many artists, but Karen has a strong conceptual and visual reason for each symbol, image, texture, shape, and technique incorporated within her digital images. This understanding of the work does not inhibit the possibilities of spontaneous discoveries during the working process, but provides a knowledgeable background contributing to the ability to discuss the work. The mythological and historical basis, along with the contemporary applications of her work is also well defended.

Publication of Karen's paper in *Inquiry: The University of Arkansas Journal of Undergraduate Research* is a logical extension of the recognition she has recently received. Karen's ability to articulate and defend her creative research won her two prestigious undergraduate awards during the last year. She received one of six university wide Undergraduate Excellence in Research Awards at the University of Arkansas, Fayetteville, and a statewide SILO Advisory Council's Undergraduate Research Fellowship (SURF). These two research papers resulted in two bodies of digital prints reflecting concepts unique for each series of images. Finally, Karen has already demonstrated her ability to successfully formally propose and complete an extensive research paper and project.

Donald Harrington, Professor of Art History, had this to say about Ms. Dick's work:

Karen was ideally suited to undertake this work, not only because of her superior talents as painter, photographer and art historian, but also because of her deep interest in modern interpretations of myth. Mythology, which preoccupied artists from ancient times through the 19th Century, has been largely neglected by modern artists, although the great myths, particularly those having to do with the idea of a journey, have so much to say to any contemporary artist in search of a significant theme.

Karen Dick is not an art history major, but she has taken every possible course that I have offered in the subject over the past five years, and she has far surpassed her peers in each of those classes. Her grades in my classes have always been A-plus. Indeed, I would go so far as to say that in my forty years as an art history teacher, I have never known her equal, let alone anyone more devoted to the subject. Her independent research paper, dealing with pop artist Jasper Johns' treatment of the American flag, remains the single best paper ever submitted in art history at the University of Arkansas.

Kristin Musgnug, Associate Professor of Art, wrote about Ms. Dick's work as follows:

Karen is one of the most outstanding students in the Art Department, and her project is distinguished for its intellectual depth and artistic mastery. Not only is she gifted artistically, but the seriousness of her inquiry makes her a role model for other students. This is a project suitable for exhibition, which in the field of art, is the equivalent of publication.

The project is a thoughtful and sophisticated one which connects new technologies in art making with one of the oldest and most enduring expressions of human culture. Like many art media, computer graphics programs are relatively easy to learn but difficult to master. Karen draws on extensive experience with these media and her work is of professional quality.

Karen's investigations take her to the forefront of what is happening in art today, utilizing some of the many possibilities offered by electronic media. Use of the computer is transforming the art world, and I am excited and pleased to support the work of a student in this area. The field of contemporary art is increasingly dominated by artists who move freely among various media, not confining themselves to just one or two. Combining the media of painting and photography with digital imaging testifies to Karen's abilities to think creatively across the range of media.

I also strongly support Karen's ambitious choice of subject matter. The mythic journey of self-discovery

is a large subject to tackle, but it is also a richly resonant one, allowing for work which is both visually exciting and intellectually demanding. Karen is that rare art student with both artistic and intellectual

Departmental Chairman, **Michael Peven**, also praises Ms. Dick's efforts:

I've known Karen as a student in our Art Department for the last 3 years. While here, Karen has shown exceptional promise as an independently-minded artist with a strong commitment to the creation of meaningful works. Karen has also exhibited remarkable energy and enthusiasm in her pursuit of the B.F.A. degree, taking on numerous extramural projects for the department and others as adjunct to her regular course of studies. These are invariably rewarding and mutually benefit Karen with further professional types of experiences and the clients who receive superior quality artistic designs, posters, announcements and other works. Karen is exceptionally creative and intellectually robust, as manifested in the works that she produces.

Karen's proposal, articulating an project dealing with a body of creative works for exhibition purposes, describes her interest in the interpretation of myth through art and the relationships she hopes to draw between modern and ancient myths, which she defines as the adventure of the individual into the unconscious. Karen's work deals with both written text and visual language in order to translate the psychological and emotional sense of the inward journey she intends to illustrate. This project is tremendously interesting and challenging. Karen's desire and need to realize it through the combination of photography, painting and digital imaging technologies makes it not only a suitable and worthy venture into artistic expression, but one that simultaneously explores the myths of art making itself, thereby adding another intellectual dimension to her pursuit. Karen is an exceptional artist and student of art. This project is an exciting and valuable addition to the contemporary dialog in the field and one that is fully deserving of support

Addendum 1



Fog Has Cut the Passage
From the series, *Passage:Reflection*
Four Color Digital Image
19" x 13"



Deeper Into Nothingness
From the series, *Passage:Reflection*
Four Color Digital Image
19" x 13"

DOUBLE CYSTEINE MUTATIONS IN STAPHYLOCOCCAL NUCLEASE: THE EFFECTS OF ARTIFICIALLY INTRODUCED DISULFIDE BONDS ON PROTEIN STRUCTURE AND STABILITY

by Anna Terry

Department of Chemistry and Biochemistry

Faculty Mentor: Wesley E. Stites

Department of Chemistry and Biochemistry

Abstract:

*Since a protein's function depends on its structure, basic research in protein structure facilitates the solution of many practical problems, such as the synthesis of more effective medicines. With this larger goal in sight, the purpose of this research project is to understand better the chemical principles that underlie protein structure and stability. Disulfide bonds are a potentially stabilizing feature of many proteins. They may form between cysteine residues in close proximity to one another if the orientation is favorable. Often found in proteins produced by organisms that grow at high temperatures, disulfide bonds may anchor side chains together, making a protein resistant to thermal or chemical denaturation. In order to provide a better understanding of the stabilizing effects of disulfide bonds, disulfides are artificially introduced into the protein staphylococcal nuclease to create mutant versions of the protein. Wild-type *S. nuclease* has no cysteine residues, so disulfide bonds must be engineered by substituting cysteines for pairs of amino acid residues in the wild-type protein. To synthesize these double mutants, successive rounds of site-directed mutagenesis are performed on bacteriophage DNA using the Kunkel method. After transformation with the modified DNA, *E. coli* bacteria are used to synthesize the mutant proteins for analysis. Biophysical techniques such as solvent and thermal denaturation provide essential thermodynamic data for characterizing the stabilities of the mutants. On the basis of the data obtained from the *S. nuclease* mutants, generalized predictions about protein structure and stability can be established.*

Anna Terry

Essay

The primary goal of this project is to understand better the chemical principles that underlie protein structure and stability. Since a protein's structure determines its specific function, knowledge of protein structure facilitates the solution of numerous practical problems. For example, the design and synthesis of new medicines, for example, depends in part on basic research into the mysteries of the protein folding process.

The 20 different amino acids are the basic components of proteins. A protein consists of a chain of amino acids in a unique sequence. Depending in part on this sequence, the chain folds into a unique functional form. Between different sections of the amino acid chain, widely separated in the linear sequence but close together in three-dimensional space, forces such as van der Waals interactions, hydrogen bonds, hydrophobic effects, and disulfide bonds help stabilize the three-dimensional structure of the protein.

Disulfide bonds, the primary focus of this research, are a potentially stabilizing feature of many proteins. They form between the sulfur atoms on two cysteine residues that are close to one another in three-dimensional space. Since it is an oxidation, the formation of a disulfide bond often takes place spontaneously in air if the orientation of the cysteines relative to one another is favorable. Disulfide bonds are relatively strong, and the crosslink they make in the chain creates a closed loop that cannot unfold. Thus they may stabilize the protein by anchoring side chains together, making it resistant to thermal or chemical denaturation. In terms of entropy, the presence of disulfide-bond forming cysteines lowers the number of possible final configurations into which a protein may fold during synthesis. Thus they greatly reduce the entropy of the protein in its denatured (unfolded) state. This causes the entropy change for the protein folding process to become more positive, which usually means that the folding reaction is favorable and that the functional (folded) state of the protein is more stable. Of course, this increased stability has an important biological purpose. Disulfide bonds are a common feature of proteins found in thermophilic organisms, such as the unique bacterial species that inhabit the superheated water near thermal vents in the ocean floor. To survive in hot conditions, these organisms need stable proteins resistant to thermal denaturation.

Previous attempts to introduce disulfide bonds into proteins have met with mixed success. It is not clear if the results are unsatisfactory because of poor site choice or because of stability factors. Because they induce permanent "kinks" in the protein chain, disulfide bonds may create an awkward functional form in the mutant protein. Thus they may actually disrupt the overall structure instead of stabilizing it as expected. The basic goal of this research is to determine whether disulfide bonds found at specific sites in proteins from thermophilic organisms can be transferred along with their stabilizing effect to a related protein that is less stable. If so, this would be encouraging information for those who wish to alter proteins to attain higher stabilities. If not, more detailed investigations will be needed to determine what other changes in nearby amino acids are required to reap the full benefit of the stabilizing effect of the disulfide bond.

The protein used for this project is staphylococcal nuclease, which occurs naturally in *Staphylococcus aureus* bacteria and has been cloned and expressed in *Escherichia coli*. *S. nuclease* is a good model protein for studies on protein stability for a number of reasons. It is smaller (149 amino acids long) and easier to purify than most other proteins. Its relatively simple structure allows the generalization of many experimental results to more complex proteins. It folds and unfolds reversibly, a property that is highly conducive to stability studies that utilize denaturation. The gene for *S. nuclease* is in an overexpressing system that makes it possible to produce mutant proteins in large yields. Finally, wild-type *S. nuclease* has no cysteine residues and thus no pre-existing disulfide bonds.

This project involves the mutation of the *S. nuclease* gene to produce proteins with two cysteine residues, which will be studied to provide a better understanding of how disulfides can be engineered into proteins to stabilize them. Disulfide bonds are artificially introduced into *S. nuclease* through the substitution of cysteines for two of the amino acid residues in the wild-type form. By introducing a disulfide bond at a specific site, the effect of the bond at that particular site on the protein's overall structure and stability can be estimated.

Possible mutants were chosen by comparing proteins whose genetic material is homologous to that of *S. nuclease*. Most of these proteins come from thermophilic organisms and have been sequenced as part of various genome projects. They are known to be particularly stable and to have pairs of cysteines, which means that they have the potential to form disulfide bonds, although it is not certain whether their structures actually include disulfide bonds. Since similar genetic sequences imply a similar structure, *S. nuclease* with cysteine mutations at the sites corresponding to those on the homologous proteins should also have this potential to form disulfide bonds, although this is not guaranteed. To ensure that the mutant pairs were favorably oriented for disulfide bond formation, the possible sites were also examined on a computer-generated model of *S. nuclease*.

Eventually, at least six double cysteine mutants will be made: A12C/L25C, L14C/V66C, V66C/V99C, A69C/A94C, I72C/V94C, and V74C/I92C. The letters to the left of the number in each abbreviation stand for the wild-type amino acid, and the numbers stand for the position in the amino acid sequence. More mutants will be added as information is obtained about each possible site of mutation. In order to make these double mutants, ten different single substitutions are needed at the following positions on the chain: 12, 14, 25, 66, 69, 72, 74, 92, 94, and 99. At least one mutant, I72C/V94C, will function as a control, since the mutated sites are too far apart in three-dimensional space for a disulfide bond to form.

In order to change the wild-type codon to the mutant codon, site-directed mutagenesis is performed on bacteriophage DNA using the Kunkel method.² This method makes use of Kunkel DNA, which contains uracil instead of thymine. Modified oligonucleotides (relatively short chains of nucleotides) that contain the desired mutation of a specific site are enzymatically spliced into Kunkel DNA with ligase and polymerase. The resulting mutant DNA templates are transformed into a strain of *E. coli* known as DH5aF'. As the bacteria reproduce, the phage propagates itself by means of the bacterial genetic mechanism, and the desired mutant DNA is copied along with that of the phage. The reason for the presence of uracil, normally found only in RNA, is to ensure a more efficient phenotypic selection of the mutant. In other words, the genetic repair mechanism of the bacteria will not recognize the genetic material as its own DNA, so the mutations will be retained at a relatively high rate rather than reverting to the wild-type form.

After transformation, the mutant phage DNA is extracted, purified, and sequenced to determine whether it has the desired mutation. Preliminary results show that the Kunkel mutagenesis is working well for single mutants, with an efficiency rate of approximately 30% (the expected rate of mutation is between a quarter and a third of samples). DNA sequencing has also shown that the procedure yields samples that contain mixtures of mutant and wild-type DNA. Mutant DNA can be isolated from these mixtures by re-transforming and re-sequencing the phage DNA.

To make the double mutants, successive rounds of Kunkel mutagenesis and sequencing are performed until the desired mutant DNA is isolated. For double mutants, a more sophisticated technique is needed to retain the mutations during transformation. A different strain of *E. coli* called Cj236 is used, along with uridine and the antibiotic chloramphenicol to ensure a more efficient selection of cells that contain mutant DNA.

Finally, the entire *S. nuclease* gene is sequenced to confirm all the mutations and to make sure there are no unwanted abnormalities in the sequence. This is necessary because random mutations such as substitutions and deletions are quite common when the bacterial genetic repair mechanism is suppressed. After the correct sequence is determined, the mutant gene is incorporated into an *E. coli* plasmid, which is used to transform another strain of *E. coli* known as Ar120. The plasmid, along with the gene of interest, is copied with the bacterial DNA as the cells reproduce. At the same time, the genetic mechanism of the bacteria is induced to direct the synthesis of large amounts of mutant proteins for analysis.

After synthesis and purification of the mutant proteins, the effects of the mutations on stability and structure are characterized by various biophysical techniques. Ellman's assay is used to check for disulfide bond formation.³ Ellman's reagent, a spectroscopic indicator, will react only with free thiols (unbonded sulfurs). Since cysteine is the only amino acid that contains sulfur, and since there are only two cysteines in each mutant, the assay will give a negative result if disulfide bonds have already formed between the cysteines.

The stabilities of each of the mutants are characterized by solvent denaturation (with guanidine hydrochloride) and fluorescence-monitored thermal denaturation.⁴ The more stable the mutant protein, the higher the concentration of guanidine hydrochloride needed to disrupt its structure, and the more negative the free energy change for the denaturation will be. Both the oxidized form (the form containing the disulfide bond) and the reduced form (the form containing unbonded cysteines) will be analyzed using these procedures. As mentioned above, exposure to air should naturally produce the oxidized form. To prevent spontaneous oxidation during the analysis of the reduced form, reducing agents such as DTT (dithiothreitol) and TCEP (tricarboxy-ethylphosphine) are used. To provide further

thermodynamic information about the mutant proteins, the solvent denaturation will also be carried out at varying temperatures.

If disulfide bonds fail to form in some mutants, it may be because the insertion of the bond at a specific site is too disruptive to the overall structure of the protein. Thus the single mutants that are required for the synthesis of doubles will also be analyzed as controls, in order to separate the effects of each single-site cysteine mutation from the effects of the disulfide bond formation. After statistical analysis of the free energy data, further predictions about protein structure will be generalized on the basis of the data obtained from the *S. nuclease* mutants. Eventually, the project may incorporate X-ray crystallography as a structural imaging tool.

Endnotes:

- 1 A = alanine, C = cysteine, I = isoleucine, L = leucine, V = valine
- 2 T. A. Kunkel, J. D. Roberts, R. A. Zakour, *Methods in Enzymology*. 1987, 154, 367.
- 3 P. W. Riddles, R. L. Blakely, B. Zerner, *Methods in Enzymology*. 1983, 91, 49.
- 4 Fluoroscopy is possible because *S. nuclease* has a single tryptophan residue, which responds to fluorescence. The method is taken from M. P. Byrne, R. L. Manuel, L. G. Lowe, W. E. Stites, *Biochemistry* 1995, 34, 13949.

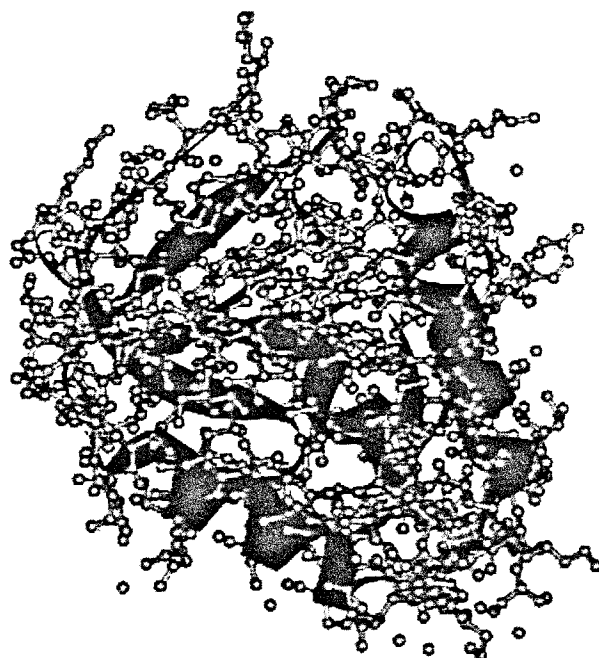


Figure 1. Wild-type staphylococcal nuclease

Faculty Comments:

Wesley E. Stites, Associate Professor of Chemistry and Ms. Terry's mentor, writes:

Anna has been working in my lab since 1997 and I have had an excellent opportunity to evaluate her in that time. Anna is double majoring in two unrelated fields, Chemistry and German, and is doing superbly in her courses. She is also a serious student of music and considered for a while majoring in that area. She has taken an impressively heavy course load during her time here and maintained a 4.00 GPA. Simply put she is an exceptionally bright and multi-talented individual who will succeed in whatever career path she follows.

In her work in my lab Anna is exploring the role of disulfide bonds in stabilizing protein structures. This project is not only intriguing intellectually, but is also deals with a question of considerable practical importance as methods to improve protein stability are of great interest to industry. Anna has made a series of disulfides through site directed mutagenesis and characterized their effects on the stability of our model protein system. This will be of great utility in future efforts to develop models of disulfide effects.

Collis R. Geren, Dean of the Graduate School and Associate Vice Chancellor for Research, has this to say about Ms. Terry:

What should I say about a student who has two perfect scores on the SAT and a perfect score on the ACT, who has maintained a flawless record in her four years at the University of Arkansas majoring in chemistry, who is an impressive concert violinist, who speaks German fluently, and who reads Melville aloud with her friends. To say that Anna Terry is an academic jewel is an understatement.

Ms. Terry combines intelligence with curiosity and industry, a combination that is extremely appealing. She is a Sturgis Fellow, and a good choice it was. Anna Terry excels at everything she does. This sounds like a cliché, but it fits her perfectly. Her reputation as a violinist is such that our chancellor went to a concert of hers during his first year on our campus and was amazed by the quality of the performance. What is more amazing is that Ms. Terry is not pursuing music as vocation. It is simply one of the many things she loves to do and does extremely well.

Faculty members look forward to working with Ms. Terry. John Hehr, Associate Dean of the Fulbright College and Professor of Geosciences, considers himself her mentor, a position he requested, and he is just one of many. She is also very accomplished in her field of chemistry. She has received every campus award we have to offer. She was also recently awarded a Science Information Liaison Office Undergraduate Research Fellowship for her proposed project on

double cysteine mutation in staphylococcal nuclease. As a sophomore she received an honorable mention from USA Today. She has just learned that she has also been selected as a Barry Goldwater Scholar. This is the premier undergraduate award for students in mathematics and science and is given specifically on the basis of the student's research record. Ms. Terry embodies everything such honors stand for: intelligence, integrity, wit, creativity, determination, and industry.

I have known Anna Terry for four years, and I have come to admire her very much. She is meticulous in her preparation. She is celebrated by Professors Sakan and Stites for the work she has done in their labs because she does exactly what she promises to do. She is ambitious in her work and more, and she will persevere until she has successfully and intelligently completed short-term projects or long-term goals. In short, she is terrific.

Donald R. Bobbitt, Professor and Chair of the Department of Chemistry and Biochemistry comments:

I am writing to offer my highest recommendation for Ms. Anna Terry. I have known Ms. Terry for approximately four years, having served as her mentor and advisor. I first met Ms. Terry while serving as a judge at a regional High School Science Fair. It was clear to me then, as it is now, that Ms. Terry stands out in a crowd, even when the cohort is a group of gifted and talented individuals. From my observations of her at the University, it is evident that my initial expectations for her were correct.

Ms. Terry is one of the most intellectually gifted students I have met in over fifteen years at the University of Arkansas. The list I am comparing her to includes former scholarship recipients. In addition to her innate ability, she also has an unusual degree of persistence and competitiveness. She simply will not leave a problem without mastering it. It is obvious from her vita that her abilities are not one-dimensional and limited only to course work; rather, she performs at an exceptional level in a number of different fields including music.

Although I do not have direct knowledge of Ms. Terry's ability in the research laboratory, I am confident she has the tools to be extremely successful. Ms. Terry has the intellectual resources and confidence to attack difficult and involved problems, and the perseverance to carry through the problem to completion. In summary, Ms. Anna Terry is a student of immense promise and ability.

THE LOOP: A PLAN FOR THE FUTURE OF ROGERS

by Rebecca L. Turner
Department of Landscape Architecture
School of Architecture

Faculty Mentor: Judy Brittenum
Department of Landscape Architecture

environment have received the worst of this imbalance as flooding, severe stream-channel erosion, loss of wetland habitat, and riparian ecosystems have become dominating characteristics. Differing principles and goals of Rogers' major municipalities and special interest groups are the catalysts for the overall degradation of the natural systems. As the stream corridors continue to decline, the actual tools needed to stop this decline remain in great debate, varying from engineering tactics to a closed-door, political approach. The greatest challenge to the stream corridors continue to decline, the actual tools needed to stop this decline remain in great debate, varying from engineering tactics to a closed-door, political approach. The greatest challenge to the city of Rogers and to the overall health of the environment, lies in synthesizing the needs, principles, and practices of each group into a harmonious whole. The object of this amalgamation is not only to solve stream quality issues, but also to allow the city to capitalize on the potential benefits of its stream corridors.

The Loop is a multi-objective recreation and urban design project that illustrates the potential of using stream corridor restoration as a springboard into several other realms of city planning and urban design. However, in order to achieve a successful project, extensive studies in civil engineering, local historic and cultural patterns, and land management theories were necessary. A tri-fold, tri-part harmony, The Loop executes an integration of these design principles in the fields of ecology, recreation, and urban design. Using the stream corridors, city easements, cultural and social connectors, and the apparent new development, The Loop serves as medium by which to solve ecological problems, to provide extensive recreation opportunities, and to create a cohesive urban fabric.

The Loop also serves to provide alternatives to typical development practices. By incorporating a task force composed of local elected officials, local land planners, engineers, special-interest groups, and community members, all provisions work together to create positive multiple, mutually reinforcing, long-term benefits, while satisfying the needs of each group.

Rebecca Turner and Judy Brittenum

Abstract:

Storm water management in urban areas is not only a necessary infrastructure, it is also a valuable resource to provide an aesthetic experience for the public while furthering ecological awareness, recreation, and the cultural fabric. Through the restructuring of current development ordinances and through greater attention to the true needs of the natural and built communities, storm water management becomes the tool to breach the gap between man and nature.

Rogers, Arkansas, a city of exciting and alarming growth, maintains traditional planning and environmental practices that have created a center of disequilibrium among the natural and built systems. Local streams and their surrounding

Future development will be governed by these principles and will benefit and strengthen the ecological and social framework of the city. The Loop, through these provisions, strives to establish a balanced system that improves the surrounding environment and provides an ecologically sound, esthetically pleasing experience.

The Loop, a development plan for the future of Rogers, Arkansas, is the catalyst to form a model city in the heart of Northwest Arkansas. By brandishing new techniques that not only improve the quality of life but also guarantee the continuum and health of the natural systems, Rogers establishes itself as a city and a community with a common vision and a brighter future.

The Loop:

The Loop was originally intended to address the growing concerns of ecological and flood water problems caused by rapid development in the city of Rogers, Arkansas. A community growing at the rate of 64% per decade, Rogers' need for expansion and new development has exceeded many of the natural limits and carrying capacities of the surrounding environment. The Loop, using technologically innovative methods, was to provide means of absorbing nutrient loads in water, solve flood control issues, and create guidelines for future development regarding urban watershed infrastructure. However, what was born as a very scientific and technical project, evolved into a social, cultural, and physical study of the city of Rogers, Arkansas.

The waterways of Rogers—eroded, hidden underground, and contaminated with oils, sediments, and hard metals from urban storm water runoff—were the creators of possibility. Three streams and their tributaries create a loop around the City of Rogers, acting very much as a part of the man-made infrastructure that collects, deposits, and disposes of city water. A vital component of this system are the sewer lines that follow along all three of the streams. To protect these sewer lines, easements of fifty feet width on alternating sides of the stream corridors are provided as “no-build” sites, thus are left as open, unused space. These easements provide the city with a man-made green belt. In combination with the natural stream corridors, this green belt—seen as wasted space to the developer and passerby—provided the framework for the evolution of The Loop. The Loop had become the medium in which to address ecological problems, as well as to create a broad-sweeping cultural, social, and recreational connector for the community of Rogers.

A task force composed of city planners, ecological and hydrological engineers, citizens of Rogers, and special interest groups created the third and final component in the evolution of The Loop. Principles and goals established by each of these groups were studied and combined in order to evaluate the needs of the city. Rogers' location within the growing metroplex of

Northwest Arkansas embellished the need to create an identity and to address development. Rather than allowing the city to continue its untended growth, the city needed a strategy to maintain its urban integrity as well as its natural beauty. The Loop, as a connector, established the means by which these problems could be solved. A synthesis of these individual goals became the platform for how The Loop should be approached.

Goal

The evolution of The Loop, as well as an agglomeration of needs, expanded the goals of The Loop from an ecological study to an overall master plan for the city of Rogers. This master plan will result in a realistic strategy for the city to capitalize on its problems and to transform them into potentials. The master plan will act as a seed project that will emphasize the potential of Rogers. The goal became a product that complements the ecology, provides additional recreation for a growing community, connects cultural and social areas of significance, and establishes design principles and guidelines for future development. Through the application of these attributes, The Loop will produce a discrete, personalized identity for Rogers while providing example developments at a variety of levels.

Concept

The concept of The Loop is influenced by a study of the built and natural environments of Rogers. These two intrinsic traits, the former a regimented grid, the latter a free-flowing vernacular, intermingle to form the fabric of the city. Unfortunately, the regimented grid has dominated the landscape, rather than meshing equally with the vernacular. The concept of The Loop focuses on allowing the natural vernacular to supersede the imposing built grid, emphasizing the natural elements as placemakers, place holders, and as directional tools. The physical aspect of The Loop is the ultimate framework and infrastructure by which this reversal would occur. Pulling The Loop and the natural vernacular to the forefront in design decisions re-establishes an equilibrium in the interaction between man and nature.

Program Development

Upon beginning the design process, a thorough inventory and analysis was conducted of the city of Rogers. Just as the concept dealt with natural and built elements, the inventory and analysis also focused on the natural and cultural elements of the city. The knowledge gained from day to day site visits, extensive studies in civil engineering, land management theories, and local historic cultural patterns, was vital to a true understanding of the complex systems of Rogers. These results, teamed with

information gathered from the task force, worked to create a list of long-term goals for The Loop. These program goals use storm water management as the tool to bridge the gap between the natural and cultural elements of Rogers. The Loop also employs the tri-fold roles of ecology, recreation, and urban design to meet these goals. Through this phase, the concept was applied to smaller, site specific areas, where development to achieve the goals would eventually occur.

Identity nodes became the focus of this phase. As a connector, The Loop established major points of intersection and activity along its path. Contextual studies of these nodes, and their functional and physical relationships within the framework, provided applicable and realistic responses to the program goals. It was determined that in order to be successful, The Loop, as a framework must:

- Integrate the surrounding neighborhoods and business districts.
- Provide alternatives to typical development practices.
- Create alternate routes of transportation in the form of biking and hiking paths as well as pedestrian access.
- Unify the city with common visual and physical elements.
- Solve water quality and control issues in aesthetically pleasing and exciting ways.
- Create a common vision for the city of Rogers.

The contextual sections were applied to the individual nodes and developed into several master plans. Later, the alternate designs were rated in their ability to answer the program goals. Using techniques and ideas from each of the design alternatives, the master plan was composed as a synthesis of earlier phases

Master Plan

The master plan achieves a synthesis of design elements that were discovered through the design process. Using The Loop as a framework for a multi-objective recreation and urban design project, the master plan combines the needed design elements and natural and social conditions to reestablish an equilibrium between man and nature. This equilibrium was achieved at many, varied levels to ensure that, like nature, The Loop is a system that relies on its most intricate relationships.

The master plan resulted in the creation of a twenty-three mile recreation trail along the stream corridors in Rogers. Using the trail as the primary foundation, the master plan is able to address ecological solutions and examples of future development in exciting and aesthetically pleasing ways. All of these elements

were intertwined into a single rhythmic system, supporting each other and evolving together. As the needs of one element changed, the entire system changed to accommodate that change.

Ecologically, the master plan provided an additional 8 million cubic feet for water storage during flood periods. These water storage mechanisms also incorporated plantings that absorbed excess nutrients, hard metals, and sediments, while aiding in the slowing of the current within the stream. However, these technical attributes were achieved in a manner that allowed dual uses for the structures—as interactive areas for learning, informal recreation and wetland habitat.

In addition to the twenty-three miles of multi-purpose trails, The Loop provided expanded and more numerous parks and open spaces, and connected the city's schools, neighborhoods, and shopping centers. The program goals were met as Rogers' community and urban fabric were unified through the addition of bike trails, pedestrian walkways along busy streets, and street trees and street furnishings.

Perhaps most beneficial for the future of Rogers was the development of identity nodes. Addressing the growth of Rogers, and the need to continue development for the economy, these nodes provide alternative development opportunities that place the environment at the forefront. Design guidelines, such as building outside the flood way and floodplains—mechanisms that allow water to be captured and used on site—and permeable building materials, were combined with the basics of urban design to create more environmentally friendly, cost-efficient, and attractive new developments. These nodes also encouraged new development to occur in already developed areas, re-focusing growth inward, rather than allowing continued sprawl. By using land more densely, the needed infrastructure can be created within the built environment. This type of land management allows for the maintenance of undeveloped green-strips between cities, allowing separate identities to be maintained. Identity is strengthened as identity nodes are created in a manner that capitalizes on the area's existing characteristics, such as ethnic neighborhoods, or proximity to schools.

The success of The Loop is based not only on its ability to rectify water quality problems and ecological issues, but also by its ability to expand the thoughts of the citizens about their community. More than anything, The Loop, is a "seed project," that enables citizens to see the potential of their growing community. At a time when development and future growth are the focus, The Loop provides a look back at the most intrinsic and important aspects of community: the environment it which it is placed and the wants and desires of the people that compose it. The Loop is the framework to unite these two elements in an harmonious balance. The Loop is the catalyst to form Rogers, Arkansas into a model city—a model community with a common vision—in the heart of Northwest Arkansas.

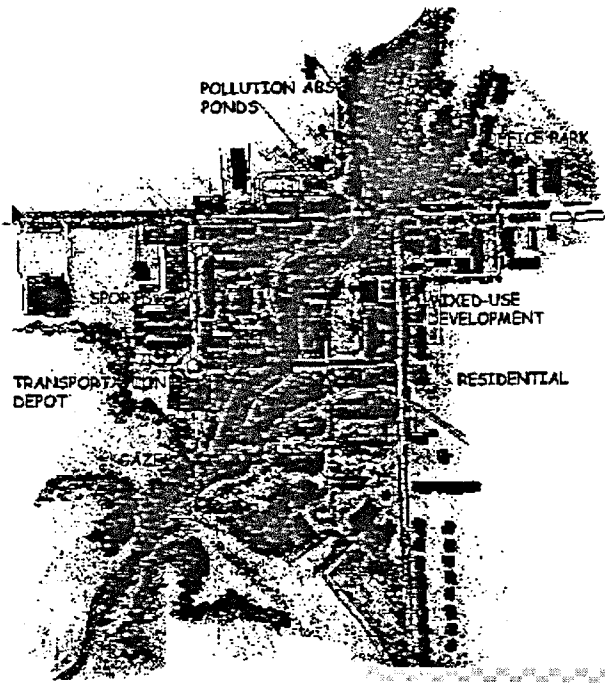


Figure 1. Walnut Intersection

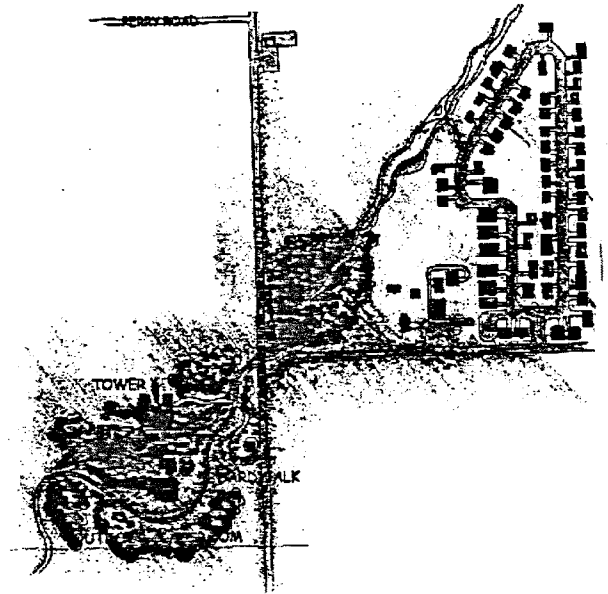


Figure 2. Rogers High School Wetland Education Center

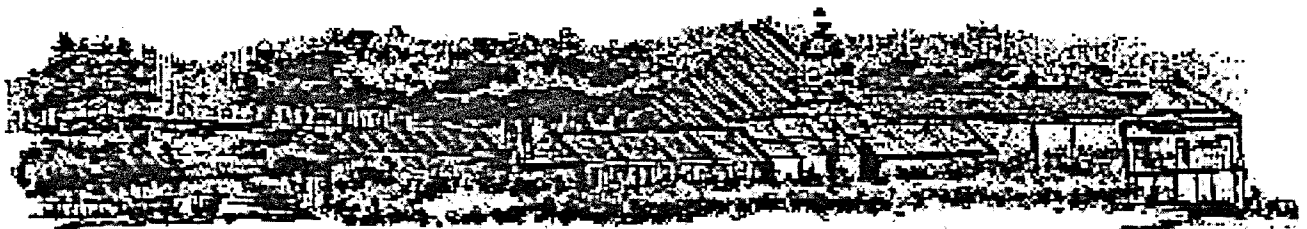


Figure 3. Wetland research Center

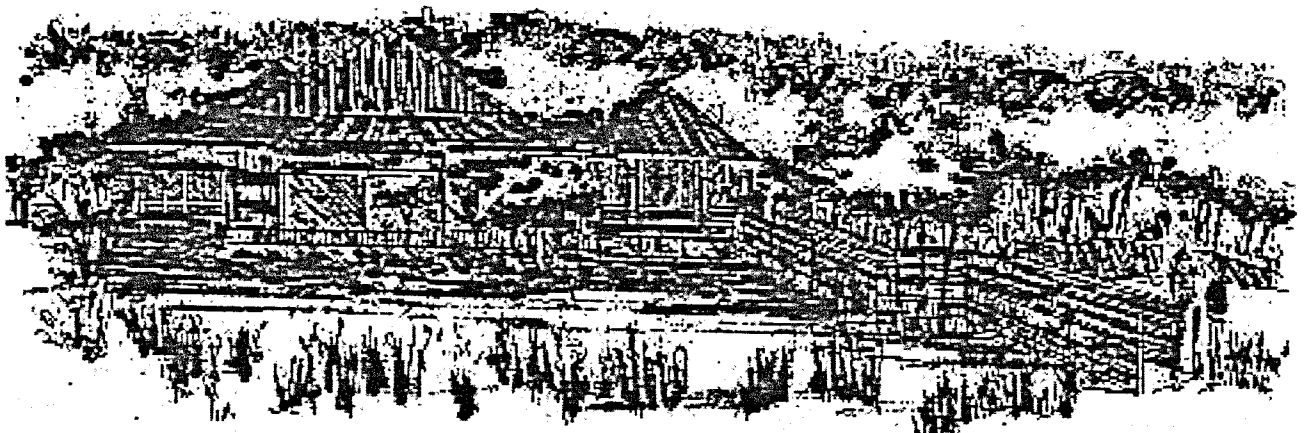
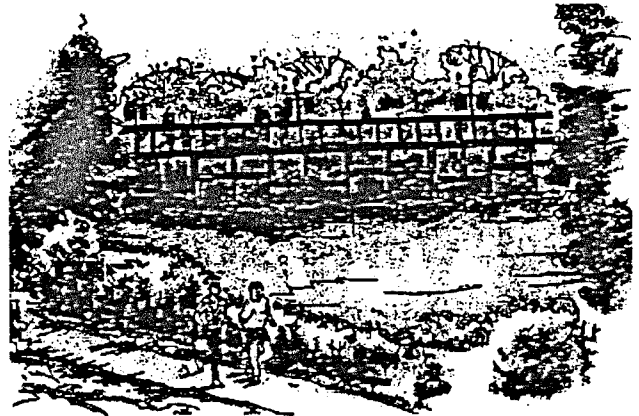
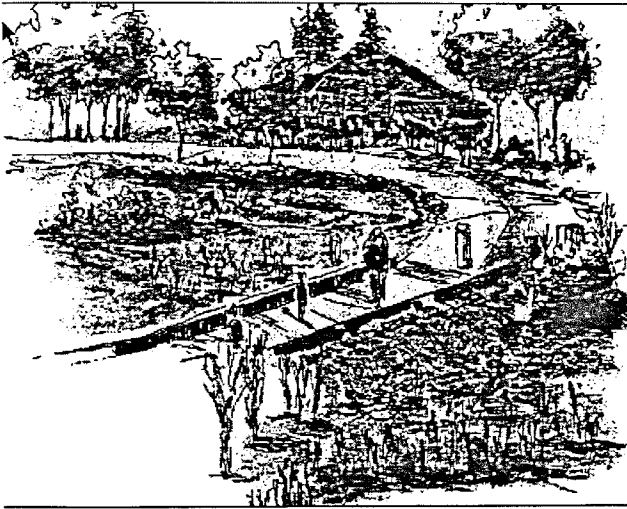


Figure 4. Wetland Lab



Figures 5 and 6. Boardwalk Views

Faculty Comments:

Mark Boyer, one of Ms. Turner's Studio Professors, commented as follows:

As one of Rebecca's advisors on this project I am completely familiar with the breadth and depth to which she worked. As a result of her desire to do more than required, the citizens of Rogers will benefit, even if only a small portion of her design ideas come to fruition. Not only will there be a nice greenway for pedestrian use (typical of community quality-of-life work being done across the country by professional landscape architects) but some of the existing hazardous flooding conditions could be rectified in the process. Rebecca chose to look at those problem flooding areas and try to ascertain some of the causal mechanisms. She went on to incorporate into the design of the public greenway features which would help mitigate those elements. This type of work is no easy task given the political and social framework within which she was working. However, as a true scholar and collaborator, Rebecca contacted experts in varying fields which could provide key information to a successful solution and surveyed residents of the area for their input and ideas. The ready acceptance by the City of Rogers council members and department heads of Rebecca's plan is a testimony to the soundness of her approach and potential success of her design when it is implemented. Rebecca's work is truly high quality to which all students could aspire

Karen Rollet-Crocker, Landscape Architecture Associate Professor and teacher of the first year studio made these remarks about her teaching assistant:

Rebecca Turner's project was very comprehensive and well researched. We ask our students to develop their project independently, and she is exceptionally



Figure 7. Pollution Absorption Pond



Figure 8. Water Access

capable in this respect. She has just competed for a national American Society of Landscape Architecture Honor award by presenting this project to a professional jury. Although the formal decision has not been made public, the jury was particularly impressed with the quality of her work.

Becca is one of the few students I have known who asks for opportunities, rather than waiting until opportunities come along. One example is her current teaching position in our first year landscape architecture studio. Her positive attitude, comments on student work, and time spent helping the students create designs was outstanding. One of her best traits as a teacher is the ability to encourage students to do good work. I could not have asked for a better example to put in front of new students. She has considered getting an advanced degree in landscape architecture in order to teach; I hope she will do so.

Becca has had outstanding references from her summer internships at one of the top firms in the country, HOK of St. Louis. I expect that her career in landscape architecture will be a stellar one, not only because of her skills as proved by her academic work, but because of her concern for people, her leadership qualities, and her drive.

Professional Landscape Architect **Travis Brooks**, who served on Ms. Turner's final project jury said:

The Loop - A Plan for the Future of Rogers Arkansas, prepared by Rebecca Turner, represents a thorough analysis of Rogers Arkansas, its infrastructure, and its potential. I have never seen a more complete project produced by an undergraduate student in the landscape architecture department.

As Rebecca began to research the water patterns in Rogers, she discovered other land use possibilities that could be addressed in her design. After extensive research she realized that her goal of developing an efficient water shed for the city could also provide for the development of needed recreation areas. Instead of ignoring these additional design problems, she welcomed them. This is an excellent example of the importance of landscape architecture in our society. The Loop represents a scientific approach to water sheds and an artistic approach to the development of recreation areas.

Rebecca welcomes all her work with a similar ambivalence to potential enormity. It is always a joy to see her finished projects and realize that she has addressed all of the design issues regardless of their significance or size. Rebecca's fearlessness and ability to tackle abnormally complex ideas are her greatest assets as a future landscape architect.

MELANOCORTIN-1-RECEPTOR (MCR-1) GENE POLYMORPHISMS ASSOCIATED WITH THE CHICKEN E LOCUS ALLELES

by Andrew Ellett
Department of Biological Sciences
Fulbright College of Arts and Sciences

Faculty Mentor: Ronald Okimoto
Department of Poultry Science

Abstract:

The melanocortin 1-receptor (MC1-R) gene has been associated with E locus phenotypes in chickens. Variant alleles of the E locus are important for accurate down color sexing and also for the inhibition of unwanted tissue pigmentation in broilers. PCR (polymerase chain reaction) based tests for various replacement substitutions found in the published E allele sequences gave unexpected results when tested against known alleles of the E locus. To resolve these issues and gain a better understanding of how replacement substitutions in the MC1-R gene are affecting E locus phenotypes, a number of accessions of the e^r, e^b, wheaten (e^{wh} and e^l), and the birchin (E^B) alleles were PCR cloned and sequenced. The published e^r allele sequence is likely to be a wheaten sequence. All three new wheaten sequences produced an inferred amino acid sequence that was identical to the published e^r sequence, but all three of our new e^r sequences had a Thr143Ala polymorphism. All E alleles sequenced except for the new wheaten alleles have Thr143, indicating that the Ala143 mutation may be associated with restriction of black pigment in the feathers. The two recessive e^b sequences had the Lys92 mutation associated with extended black in both chickens and mice, but they also had a His215Pro substitution. The Pro215 mutation is a likely candidate to attenuate the Lys92 mutation producing a brown instead of a black female. The Leghorn birchin allele had the Lys92 mutation, but the Fayoumi birchin allele did not. Instead, the Fayoumi sequence had a Leu133Gln substitution.

Andrew Ellett and Ronald Okimoto

Introduction.

Feather pigmentation studies on the molecular level have shown that melanocortin 1-receptor (MC1-R) gene polymorphisms are responsible for various E locus alleles of the chicken (1,2). The chicken E locus had been tentatively mapped to chromosome 1 (3,4,5), but PAMSA (PCR amplification of multiple specific alleles) tests for specific single nucleotide polymorphisms within the MC1-R gene indicate that the MC1-R gene is on one end of the E30 linkage group of the East Lansing reference population (6). The E30 linkage group is thought to be one of the microchromosomes and is not associated with genetic markers found on chromosome 1. Functional MC1-R is a G-

protein coupled receptor and, when activated by α -melanocyte-stimulating hormone, controls the synthesis and distribution of the two major melanin pigment types (7). An abundance of eumelanin causes brown to black pigment while more pheomelanin results in yellow to red coloration. The alleles associated with the eumelanin abundant phenotypes are usually dominant to those displaying more pheomelanin (8). In chickens, a number of E locus alleles have been identified and described (9). Phenotypic descriptions of some of the E alleles are given in Figure 1 and 2.

Eumelanin is not only deposited in feathers but also in the eye, skin and connective tissue of poultry (9). Dark tissue

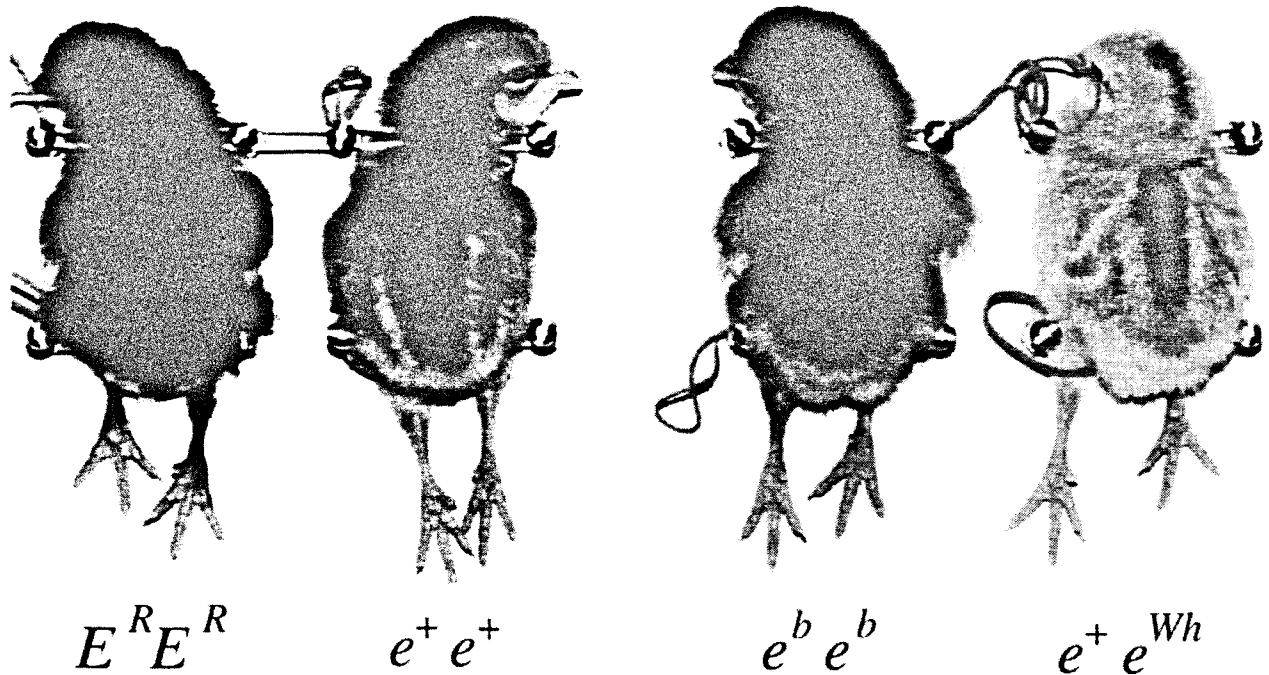


Figure 1. Representatives of various E allele down types. E^R is usually dominant to the other alleles and produces a black down chick. Wild-type (e^+) is dominant to e^b and produces a chipmunk striped down with alternating tan, black and dark brown stripes. The brown or partridge (e^b) allele produces a dark brown down with some light striping often seen in some chicks. The dominant wheaten e^{Wh} allele usually produces a clean yellow down with no striping when homozygous, but is incompletely dominant to some alleles such as the $e^+ e^{Wh}$ chick down depicted in the figure.

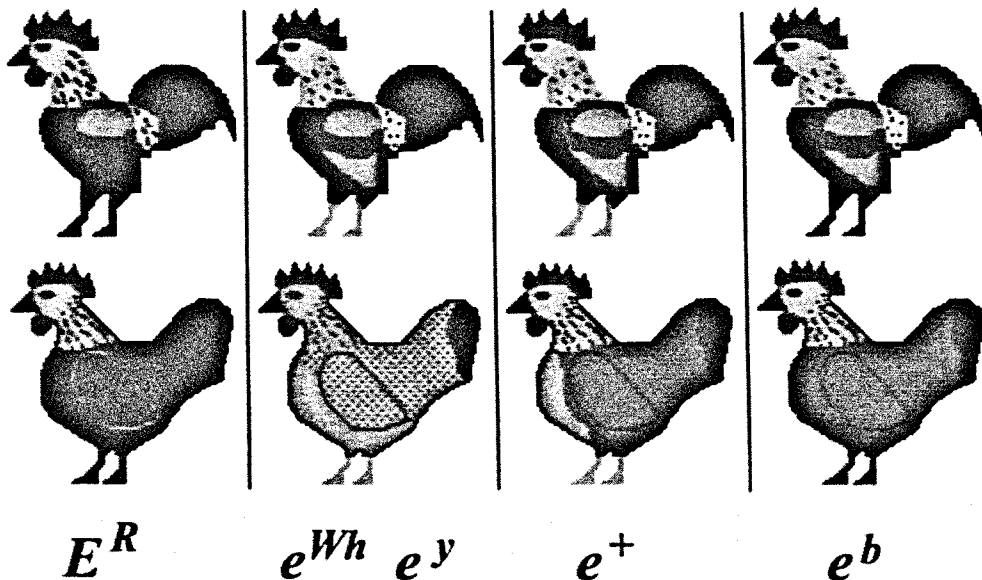


Figure 2. Adult phenotypes of the various E alleles. Females display the most differences in adult feather color caused by the various E alleles. The most dominant extended black (E) allele is not shown, but produces males and females that are all black or nearly all black. Female birchin (E^B) birds are black except in the head and hackle, while males are nearly wild-type, but lack the red in the primary flight feathers of the wing. Wheaten females lack almost all black pigment and show mostly browns and salmon pigmentation. Males are wild-type in coloration, but unlike all other E alleles the wheatens produce a cream not gray feather undercolor (the fluff at the base of the feather next to the body is a light cream color instead of gray). Wild-type (e^+) females have a salmon colored breast with a brown stippled back. Brown (e^b) females are brown stippled over their entire bodies. Males are not distinguishable from wild-type.

pigmentation of broilers is a problem for the poultry industry because consumers mistakenly associate the dark coloration with contamination or poor health of the bird. The *E* and *E^s* alleles are most responsible for the epidermal coloration while *e^a* and *e^b* result in more pigmented abdominal skin fascia (9). Most broiler chickens have white plumage that masks the *E* allele phenotypes affecting feather and tissue coloration which are segregating in a population. In addition, many poultry markets around the world differ in their preference for various skin and plumage color (9), making *E* locus phenotype selection important for an international breeding company. Another important application for the accurate identification of the MCR-1 alleles involves the correct sex determination of day-old chicks. Down color sexing is used extensively in industry for sex determination and certain alleles of the *E* locus are used for the most accurate sexing (9).

The MCR-1 gene of the chicken has been cloned, sequenced and the amino acid sequence of some alleles (*E*, *e^a*, and *e^s*) has been published (1,2). Using the DNA sequence of the published alleles, PAMSA primers were developed and tested on the DNA isolated from birds with known *E* alleles. We obtained unpublished results showing incongruities in the amino acid sequence at position 92. Several polymorphisms in the published sequences could not be verified. In order to address this, several accessions of the various alleles were PCR cloned, sequenced, and compared to the previously published sequences. The ambiguities were resolved and new mutations were identified that could explain the phenotypes of the various alleles.

Materials and Methods.

Chicken breeds and lines and their associated *E* locus alleles are given in Figure 3. DNA was isolated from whole blood using Qiagen, blood kits or IsoQuick, DNA isolation kits.

Polymerase Chain Reaction was carried out using Promega Taq polymerase that leaves A overhangs facilitating the ligation into pCR 2.1 plasmid vector (Invitrogen,). The 5' and 3' primer sequences were -MC1132 (5' AGCCTTTAT TTGGGAG CGCGA) and +MC-117 (5' TGCTGCGGGAGCACTGGT), respectfully, that amplified the entire coding region of the MCR-1 gene. PCR product was then purified by gel electrophoresis and extracted using the dialysis-filter paper method of Girvitz *et al* (10). The purified products were ligated into pCR 2.1 plasmid vectors using the Invitrogen, TA cloning kit and transformed

Line/ Allele	Amino Acid Positions									
	33	37	71	92	133	143	213	215	244	
1 e ⁺	Cys	Asp	Met	Glu	Leu	Thr	Arg	His	Leu	
	TGC	GAC	ATG	GAG	CTG	ACC	CCG	CAC	CTG	
2 e ⁺	Cys	
							TGC			
3 e ⁺	Cys	
							TGC			
4 E?	Thr	Lys	Cys	
			ACG	AAG			TGC			
5 E?	Thr	Lys	Cys	
			ACG	AAG			TGC			
6 E?	Thr	Lys	Cys	
			ACG	AAG			TGC			
7 e ^b	Thr	Lys	Cys	Pro	...	
			ACG	AAG			TGC	CCC		
8 e ^b	Thr	Lys	Cys	Pro	...	
			ACG	AAG			TGC	CCC		
9 E ^R	Lys	
				AAG						
10 E ^R	Gln	
					CAG					
11 e ^{Wh}	Ala	
						GCC				
12 e ^{Wh} / _{e^y}	Ala	
						GCC				
13 e ^{Wh} / _{e^y}	Ala	
						GCC				
14 e ⁺ ?	Ala	
						GCC				
15 e ^y ?	Trp	Gly	Ala	Pro	
	TGG	GGC				GCC			CCG	

Figure 3. Polymorphisms in the chicken MCR-1 gene delineating mutants. Accessions are number coded and specified below. Codons and amino acids are listed for the new *e^e* sequence at the top and under selected amino acid positions. Codons and amino acids are shown for substitutions and dots indicate identical sequences. Sequences 4, 5, 14, and 15 are taken from Takeuchi *et al* (2). 1 - Richardson's Red Jungle Fowl (*e^a*), 2 - San Diego Zoo Red Jungle Fowl (*e^a*), 3 - Welsummer (*e^a*), 4 - Takeuchi Rock Cornish (*E*), 5 - Takeuchi Plymouth Rock (*E*), 6 - Black Australorp (*E*), 7 - Smyth Brown line (*e^b*), 8 - Commercial broiler (*e^b*), 9 - ADOL Line 0 (*E^s*), 10 - Fayoumi (*E^s*), 11 - New Hampshire Red (*e^{Wh}*), 12 - Buff Minorca (*e^{Wh}/_{e^y}*), 13 - Rhode Island Red (*e^{Wh}/_{e^y}*), 14 - Takeuchi Brown Leghorn (*e^a*), 15 - Takeuchi Nagoya Cortin (*e^a*). A ? indicates uncertain allele designation.

into competent *E. coli* cells from Invitrogen,. Four clones from each accession were subjected to plasmid purification using Qiagen, Plasmid Purification Protocol and Kit.

Plasmids were sent to University of Delaware Molecular facilities for DNA sequencing. Sequencing was accomplished using M13 forward and reverse primers on an automated PerkinElmer ABI 377 system. The DNA sequence was assembled and analyzed using the DNASTar, software package.

Results and Discussion.

PAMSA tests were designed using the methodology of Okimoto and Dodgson (11) to differentiate the Cys33Trp, Asp37Gly, and Leu244Pro polymorphisms between the published (2) MC1-R wild-type (e^+) sequence and the recessive wheaten (e^v). We were unable to confirm any of these polymorphism using our three wheaten lines (unpublished results). PAMSA tests designed to differentiate the Glu92Lys polymorphism confirmed that all lines with extended black or birchin black (E or E^R) had the Lys92 substitution. Interestingly, we found two non-black lines that were known to have the recessive e^b allele to also have the Lys92 mutation, and one E^R line (Fayoumi) did not have the Lys92 mutation. To resolve these issues, 12 additional MC1-R genes were PCR cloned and sequenced. The replacement substitutions found in these sequences are listed in Figure 3.

We were unable to confirm the e^v Cys33Trp, Asp37Gly, and Leu244Pro polymorphisms with three new wheaten sequences. In the literature, two of these lines (Rhode Island Red and Buff Minorca) have been found to segregate recessive wheaten (e^v) (9). All three new wheaten sequences had the same inferred amino acid sequences, and one new line (New Hampshire Red) has been confirmed to have dominant wheaten (e^{wh}) by test mating (Figure 1 and unpublished data). It is possible that all three new wheaten sequences may represent dominant wheaten. The published e^+ sequence appears to be a misidentification (sample 14 Figure 3). The amino acid sequence of the published e^+ allele is identical to the three new wheaten sequences. Eggs were obtained from breeders and DNA was isolated from embryos with no down type confirmation of allele identification. None of the three new e^+ sequences have the Ala143 polymorphisms. Instead, they have Thr143 that is found in all sequences except wheaten sequences. Ala143 appears to be characteristic of wheaten and it appears that the published e^+ sequence may be a wheaten sequence. We were unable to confirm the existence of a recessive wheaten (e^v) allele. The Ala143 substitution at a conserved position is a likely candidate to cause the reduction of black pigmentation of the feathers seen in wheaten breeds.

The recessive e^b (brown) allele is likely to have been derived from the dominant E (extended black) allele. The two new e^b sequences have the same amino acid sequence as the published E sequences and the new Black Australorp (E or E^R) sequence with one additional substitution (His215Pro, Figure 3). The Lys92 mutation has been found in mice and causes an extended black phenotype by constitutive activation of the MC1-R (7). The Pro215 mutation occurs at a highly conserved position and may attenuate the constitutive activation of MC1-R producing the brown phenotype instead of black.

Previous test matings indicate that ADOL Line 0 has a birchin like E^R allele, and Malone and Smyth identified the Fayoumi as having the E^R allele. Both E and E^R alleles produce chicks with black down, but the extension of black is less in E^R

birds than in birds with the most dominant extended black (E) allele. The amino acid sequence comparisons indicate that there are two distinct birchin alleles found in chickens. Both ADOL Line 0 and the Fayoumi differed from the published E sequences. Line 0 had the Lys92 mutation of the other E alleles, but lacked the Thr71 mutation. The Fayoumi allele lacked the Lys92 mutation, but had a novel Gln133 mutation. The Gln133 mutation (found in the third transmembrane region) may be another mutation that causes the constitutive activation of the MC1-R. Pig and Fox have been found to have extended black alleles with mutations in the third transmembrane region (8), and various *in vitro* mutations in the third transmembrane region have been found to constitutively activate the MC1-R (7). If sequences 4, 5 and 6 (Figure 3) are representatives of the dominant E allele, the Thr71 mutation that they all share, but that is not found in wild-type, birchin (E^R) and wheaten, may enhance the constitutive activation of the MC1-R.

The new sequences were used to develop PAMSA tests that can be used to genotype animals in commercial populations. With white feathers masking the E locus phenotypes, these molecular tests can be a valuable tool for breeding companies having to use inefficient test crosses to identify unwanted segregating alleles.

Among vertebrates the wheaten (Thr143Ala) mutation, the e^b (combination of Glu92Lys and His215Pro, and the Fayoumi birchin (Leu133Gln) are unique mutations that have not previously been analyzed pharmacologically. The Ala143 mutation of the wheaten allele is particularly interesting because it is a dominant or partially dominant allele. All other MC1-R alleles that decrease the amount of black eumelanin pigmentation are recessive. Dominant restriction of eumelanin does not fit the models of MC1-R function proposed by Lu *et al* (7) and Robbins *et al* (12). It may be that a closely linked modifier gene is affecting the expression of what should be a recessive allele. Carefoot (13) concluded that wheaten could act as a dominant or recessive allele due to modifier genes, but this conclusion was based on chicks produced from a single hen.

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Dr. Carl Schmidt of the University of Delaware for sequencing. We greatly appreciate Dr. Schmidt for his assistance.

Faculty Comments on Mr. Ellett's Research:

Ronald Okimoto, Mr. Ellett's faculty mentor, describes the importance of the research as follows:

My laboratory has been working on the melanocortin 1-receptor (MC1-R) gene for around two years. This is an important gene in the control of melanin biosynthesis. The MC 1 -R gene is important to the broiler industry because it is associated with tissue pigmentation as well as the feather color. Tissue pigmentation is not a desired trait due to consumer preference for processed chicken products. One bird with tissue pigmentation that slips by for further processing can ruin large batches of chicken nuggets and breast patties. The melanin pigment is harmless, but the consumer associates the flecks of pigment with contamination or poor health of the animal.

Andy Ellett's cloning and sequencing work, along with our previous work, has allowed us to create specific molecular tests for the various alleles of the E locus. We can now type a blood sample and tell the genetics of the animal at this locus. This is important because all broilers have white feathers. The epistatic nature of white masks the expression of the E locus alleles. Without these molecular tests the breeders would have to test mate their animals to color testing lines. Test mating is not an economic or efficient means of identifying the animals segregating unwanted alleles.

Andy's paper deals with the basic science of the MCI-R gene. DNA was isolated from individual chickens with the desired E locus alleles. A PCR (polymerase chain reaction) primer set was designed to amplify and clone the entire coding region of the MC 1 -R gene for each allele. These clones were sent to an outside facility for DNA sequencing. The amino acid sequences were inferred from the DNA sequence and compared. This process allows us to clear up some misidentifications in the literature and to correlate the sequences with the phenotypes associated with the various alleles to deduce the effect amino acid substitutions have on the function of this receptor. We can compare our mutations to those described in mammals to get a better understanding of the functional nature of different regions of the protein. MCI-R is a model system for the study of other melanocortin-receptors, and the melanocortin-receptors have been associated with cancer, obesity, and related behavioral traits.

Dr. Douglas Rhoads, Assoc. Professor, Biological Sciences, made the following comments:

I have read the manuscript and am familiar with the subject and the research efforts Dr. Okimoto. This area of research is of prime importance to the poultry industry as down and feather color are associated with consumer estimations of meat quality, and provide a crucial means for sex-sorting chickens at hatch. The impact of feather coloration on the industry is probably measured in the hundreds of millions of dollars annually. Despite this, Dr. Okimoto and his collaborators, including Mr. Ellett, are some of the first to perform truly detailed analyses of the underlying genetics. Previous work by others had been less than complete.

Mr. Ellett's manuscript details the systematic examination of allelic variation in the gene which is believed to be mainly responsible for color determination. Ellett now provides sequences for most of the major alleles which are used in breeding programs. The results are surprising in light of the differences (and lack thereof) between wildtype and the wheaten allele. Therefore, this manuscript provides some key new information and suggests that other genes may be involved. Regardless, the documentation of the allele variations and establishment of easy PCR based screens for the alleles will be of great interest to the industry, to chicken basic research and to research in other animals. Although highly technical in nature the paper is well written and the figures are adequate. This paper easily exemplifies the high quality of research which undergraduates are performing at the University of Arkansas.

PENTOXIFYLLINE MODULATION OF THE CYTOTOXIC T-CELL DIFFERENTIATION PATHWAY TO ENHANCE IMMUNOLOGICAL MEMORY

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Abstract:

Immunological memory, acquired either by previous infection or vaccination, affords vertebrates a large margin of survival. Subsequent exposure to a "remembered" pathogen signals memory cells to undergo a rapid, clonal proliferation into armed effector cells. This rapidly mobilized clone mediates the specific clearance of the trespassing pathogen. Typically, this secondary recall response effectively dispatches the particular pathogen prior to any manifest disease pathology. This is the age-old, enjoyed benefit of immunity. Though this appreciation for immunological memory has been in documentation for nearly 3 millennia, our ability to intentionally increase its normal level of formation is only now beginning. The Durdik lab and collaborators previously demonstrated that the presence of pentoxifylline (PF), a clinically administered phosphodiesterase inhibitor, during priming, produces a decrease in the primary response and a substantially increased memory response within the CD4 helper T-cell contingent. Here it is shown that the presence of PF during priming results in enhanced CD8 cytotoxic secondary responses. CD8 T-cells respond by directly killing cells hosting intracellular infections. A significantly enhanced cytotoxic memory response was demonstrated both 60 and 90 days post-priming to a specific peptide antigen called HY (amino acid sequence: KCSRNRQYL) under a PF-cover during priming, in contrast to groups without PF administration. These findings suggest a substantial potential for the development of enhanced vaccines for intracellular pathogens.

Eric Goodspeed and Jeannine Durdik

Introduction

When the immune system of a vertebrate encounters a new antigenic threat, it responds by entering two mutually exclusive pathways to produce separate populations of dispatching cells, known as *armed effector cells* (Wong, 1996) and *memory cells* (Zinkernagel, 1996). The larger pool of short-lived armed effector cells are responsible for directly clearing the immediate pathogenic threat. The distinct population of long-lived memory cells insure that subsequent threats from the same pathogen will be recognized and cleared with greater expediency (Zinkernagel, 1996). A pharmacological method to intentionally modulate this differentiation pathway to generate more memory cells would be ideal within the scheme of vaccination.

The Durdik lab and collaborators previously demonstrated that the presence of the phosphodiesterase inhibitor, pentoxifylline (PF), during priming modulates the differentiation pathway of CD4 helper T-cells to favor a larger commitment of memory cells at the expense of armed effector cell proliferation (Gupta, 1999). The hypothesis followed that the presence of PF during priming would shift the immune cell differentiation pathway such that the immunological memory CD8 killer T-cell response would be significantly enhanced as compared to normal, untreated levels. The following experimental design provided conclusive data that a CD8 T-cell memory response, both 60 and 90 days

post-priming, can be enhanced with the presence of PF during priming.

Experimental design—Materials and methods

Pentoxifylline

Pentoxifylline (PF), a phosphodiesterase inhibitor, is a clinical agent commonly administered as an anti-inflammatory and blood thinner (McEvoy, 1998). It has been demonstrated that the phosphodiesterase inhibitory function of PF increases intracellular levels of the ubiquitous, secondary messenger, cyclic adenosine monophosphate (cAMP) (McEvoy, 1998).

Antigenic system

Memory CD8 T-cells were generated within an in vivo mouse, HY-antigen system. The genetic variance between genders of the syngeneic inbred C57BL/J6 mouse strain (Jackson Laboratory, 1989), namely, the male possession of the HY-antigen encoding Y-chromosome (Scott, 1995), provided the immunogenic basis for the system. Each experimental time-point, day 60 and day 90, consisted of 2-3 eight-week-old female C57BL/J6 mice that received, on day 0, a priming injection of 10^7 male C57BL/J6 splenocytes. In some experiments, 10^7 B6.THY1.1 male splenocytes were employed as primers. These splenocytes possess two immunogenic differences (HY and THY1.1) relative to the female C57BL/J6 mice. This was performed to increase the overall helper CD4 immune response and subsequent recall cytotoxic CD8 activity. Half of the mice received PF injections (1.5 mg/mouse) once a day from day -1 to day 4. Female C57BL/J6 mice that did not receive PF or priming injections were employed as controls.

In vitro effector:stimulator cultures

At the experimental time-points, day 60 and day 90, splenocytes from each group were harvested and cultured with male C57BL/J6 stimulator-splenocytes in a single cell suspension at a ratio of 5:1 (10^7 effectors: 2×10^6 stimulators). These cultures were incubated at 37°C in 5% CO_2 for 6 days to allow the priming-generated, HY-specific, memory CD8 T-cells to proliferate into armed, HY-specific effector cells. Control effectors, generated from female mice neither receiving priming nor PF, were identically cultured.

Synthesis of HY and LCMV-peptide

With the assistance of Dr. Koeppel's biochemical research lab, the previously identified HY-peptide (amino acid sequence: KCSRNRQYL) (Markiewicz, 1998) and LCMV-peptide (amino acid sequence: KAYVNFATM) (Kyburz, 1993) were synthesized using the solid phase peptide synthesis (SPPS) technique (Merrifield, 1997). These synthetic peptides were designed to fit within the particular sequence motif of the mouse class I major histocompatibility complex (MHC I), D gene locus, b allele (H-2D^b) surface expressed molecule (Markiewicz, 1998). The

LCMV-peptide was employed as an H-2D^b-binding control peptide such that it would bind into the MHC I but not become a recognizable target for the HY-specific effector cells. Each peptide was analyzed for purity by ultraviolet spectroscopy and High Performance Liquid Chromatography.

RMA-S T-cell lymphoma line

The female, immortal T-cell line, known as RMA-S (Ljunggren, 1985), was employed as target cells to test for the cytotoxic killing ability of the previously in vitro generated HY-specific effector cells. The RMA-S cell is incapable of expressing a stable MHC I due to a mutation in the Transporter associated with Antigen Presentation (TAP) gene locus (Kaer, 1992). However, if RMA-S cells are incubated with peptide known to bind within its MHC I (H-2D^b), a stable surfaced expressed MHC I:peptide complex is formed (Townsend, 1996). It is this critical complex that triggers the peptide-specific CD8 T-cell to unleash its arsenal of cytotoxic chemicals upon the target cell. Thus, RMA-S cells were incubated with the previously synthesized HY and LCMV peptides for 16 hours prior to ^{51}Cr labeling (see below) to create target cells. This technique is known as *peptide loading*. RMA-S cells without peptide loading are deficient in MHC class I. These become targets for Natural Killer cells and are therefore not an appropriate target population to serve as a control.

Male Targets

Male C57BL/J6 splenocytes were employed as alternative HY-antigenic target cells.

Chromium-51 cytotoxic release assay

The various RMA-S target cells were incubated with 100 μCi of radiolabeled disodium chromate ($\text{Na}_2^{51}\text{CrO}_4$). The gamma energy emitting chromium-51 (^{51}Cr) radionuclide is readily incorporated into the target cell cytosol via calcium ion channels (Coligan, 1996). Spontaneous release and reuptake is relatively low due to irreversible binding of ^{51}Cr with cytosolic protein (Coligan, 1996). After one hour of incubation (at 37°C and 5% CO_2) the targets were washed three times. Effector cells were collected from their 6 day cultures and cell recoveries determined. Within a 96-well V-bottom microtiter plate effector-to-target ratios of 50:1, 25:1 and 12:1 were created in replicate with target cells constant at a quantity of 10^4 . The assays were designed with nine to twelve effector:target combinations involving an intersecting grid of the three effector treatment groups and the three to four targets types in a total volume of 200 μl . Spontaneous ^{51}Cr release values were established with wells only containing target cells and complete media. The assay plate was centrifuged and incubated for six hours at 37°C and 5% CO_2 . This provided the proper physiological condition for CD8 cell-mediated, cytotoxic lysis of the ^{51}Cr -labeled target cells (Rodrigues, 1992), and the subsequent release of their cytosolic ^{51}Cr into the surrounding media. After incubation the assay plate was

centrifuged and 150 μ l of supernatant of each well was collected for gamma activity detection in a fully automated Packard Cobra® gamma counter. To establish a total, potential gamma count, 75 μ l of each type of ^{51}Cr labeled target cell stock was directly counted. Cytotoxic activity was correlated with gamma counts per minute (CPM) by the following equation:

$$\frac{(\text{Experimental CPM value} - \text{Spontaneous Release CPM value})}{(\text{Total CPM value} - \text{Spontaneous Release CPM value})} \times 100 = \% \text{ specific lysis}$$

Final cytotoxic activity, or percentage specific lysis, is a measure of the *in vivo* generated memory CD8 T-cell contingent.

Results

Figure 1a provides data from the three treatment groups against HY-peptide loaded RMA-S target cells at a 50:1 E:T ratio 60 days post-priming in an HY-antigen system. The control effector cell group generated an expected low baseline specific lysis of 8%. The untreated effector group (hatched bar) did not show any significant increase of specific lysis relative to the control group. The PF-treated effector cell group did show a significant ($p=.042$) increase of specific lysis relative to the untreated group. Figure 1b provides data from the three treatment effector cell groups against male splenocytes at a 50:1 E:T ratio 60 days post-priming in an HY-antigen system. The untreated control effector cell group (checkered bar) elicited an expected low baseline specific lysis of nearly 0%. The untreated effector cell group (hatched bar) did not show any significant increase of specific lysis relative to the control group. The PF-treated effector cell group (solid bar) did show a significant ($p=.037$) increase of specific lysis relative to the untreated group.

Figure 2a provides data from the three treatment groups against the irrelevant control LCMV-peptide loaded RMA-S target cells at a 50:1 E:T ratio 90 days post-priming in an HY-antigen system. All treatment groups elicited specific lysis values consistent with a non-specific target. Figure 2b provides data from the three treatment effector cell groups against HY-peptide loaded RMA-S cells at a 50:1 E:T ratio 90 days post-priming in an HY-antigen system. The untreated control effector cell group (checkered bar) elicited an expected low baseline specific lysis of 8%. The untreated effector cell group (hatched bar) did not show any significant increase of specific lysis relative to the control group. However, the PF-treated effector cell group (solid bar) did show a large significant ($p=.019$) increase of specific lysis relative to the untreated group.

Figure 3a provides data from the three treatment groups against the irrelevant control LCMV-peptide loaded RMA-S target cells at a titrated E:T ratio from 50:1 to 12:1 60 days post-priming in an THY 1.1-antigen system. All treatment groups elicited specific lysis values consistent with a non-specific target. Figure 3b provides data from the three treatment effector cell groups against HY-peptide loaded RMA-S cells at a titrated

E:T ratio from 50:1 to 12:1 60 days post-priming in an HY-antigen system. The untreated control effector cell group (checkered bar) elicited an expected baseline specific lysis of 8% and followed the titration trend. For the first time, the untreated effector cell group elicited a significant specific lysis relative to the control group along the titration curve. This data can be explained by more initial CD4 helper T-cell generated IL-2 during priming due to the additional THY1.1 antigen. The PF-treated effector cell group demonstrated the largest significant increase ($p<.005$) of specific lysis, following the titration curve, relative to the untreated group.

Most recently our lab designed and performed an experiment to determine if the observed enhanced memory cytotoxicity was a direct result of an increased memory CD8 T-cell contingent and not exclusively a matter of help from CD4 memory helper T-cells. Though several crucial steps were altered to bypass any potential CD4 T-cell help at the priming event, the general protocols of the previously described experiments were followed. Mice were primed with syngeneic female C57BL/6J splenocytes loaded with the MHC I-specific LCMV-peptide to insure an exclusive CD8 T-cell recognition of the MHC I:LCMV-peptide complexes. This procedure provided two experimentally relevant functions: LCMV peptide cannot stimulate a CD4 T-cell response and does not bind to the MHC II; and this CD4 bypass insured that any observed recall cytotoxicity was a direct result of a memory CD8 T-cell contingent.

10^7 Female splenocytes were loaded with 200 μ g of LCMV-peptide in a total volume of 1 ml of serum-free media and incubated for one hour at 37°C and 5% CO_2 . The serum-free media allowed the endogenous peptide of the splenocytes to briefly dissociate with their MHC I molecules. The β_2 -microglobulin sub-unit of all MHC I is in a non-covalently bound/unbound state of equilibrium (Rock, 1991). Dissociation of the β_2 -microglobulin sub-unit destabilizes the MHC I which leads to loss of its bound peptide. At this juncture, the thermodynamically advantaged (law of mass action) synthetic peptide usurps the original endogenous peptide position. Fetal calf serum typically employed to make cell culture media "complete" contains large quantities of β_2 -microglobulin sub-units that have been shown to bind to mouse MHC I (Rock, 1991). In terms of attempting to load splenocytes with exogenous peptide, this pushes the bound/unbound equilibrium to the unfavorable bound state. Thus, a serum-free medium is crucial to peptide loading. Each mouse was primed with 10^7 LCMV-peptide loaded cells and treatment-group mice received injections of PF at 1.5 mg per day per mouse on day -1 to day 4, relative to priming. On day 30, post-priming, spleens were harvested and cultured at the 5:1 E:T ratio with LCMV-peptide loaded female splenocytes. *In vitro* generated LCMV-specific effector cells were mixed separately with ^{51}Cr -labeled RMA-S cells loaded with LCMV-peptide or the irrelevant HY-peptide at an E:T ratio of 25:1.

Figure 4 demonstrates that PF-treated effectors exhibited a greater LCMV-specific lysis than the non-treated effectors. This lends support to the claim that the previously observed enhanced CD8 recall cytotoxicity is a direct result of an increased CD8 T-cell contingent. Though this particular experiment requires confirmation by repetition, these data offer a positive trend to the expectation of a PF-mediated memory CD8 T-cell pool.

In toto, these experiments designed with multiple control and experimental conditions provided statistically significant evidence to the proposed hypothesis that the presence of PF during priming enhances CD8 T-cell immunological memory.

Discussion

The potency of the vaccine, as the premiere method of disease prevention and control, is founded on the ability to intentionally induce the generation of long-lived, antigen-specific memory cells (Christensen, 1994). Though the vaccine is a fruitful discovery, it must be understood that it falls short of its potential. Within the vaccine-induced immune response the natural production of antigen-specific effector cells is without justification, for, ultimately, the stimulating agent is non-pathogenic, only immunogenic. To advance the vaccine within the scope of its ideal, we must be able to *shift the fulcrum* of this balanced cell differentiation pathway to favor an exclusive and robust production within the memory cell contingent. PF has previously (Gupta, 1999) demonstrated the ability to *diminish* the CD4 T-cell primary response while enhancing CD4 memory. This work shows that PF enhances CD8 T-cell recall activity. These experiments offer a tantalizing potential to expand the reach of our preventative intentions.

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Faculty Comments:

Mr. Goodspeed's faculty mentor, **Dr. Jeannine M. Durdik**, made the following comments about his project:

This letter is to explain the value and pertinence of the work of Eric Goodspeed, an undergraduate researcher in my lab, who has made a very important and independent contribution.

For successful protection against various infections, the development of an adoptive immune response is necessary. When the same infection is encountered again by the individual, the immune response specifically directed against target molecules of the infectious organism is significantly different from that seen during the first encounter in being much larger. This ability of the immune system to "remember" past history and respond better to reinfection is called immune memory.

However, in response to an infection, cells of the immune system must not only generate this quiescent state called immune memory, but must also mount a response that is immediately effective and useful in combating the ongoing infection. This pathway of immune cell differentiation must thus lead to the development of effector cells. Since effector cells are to be used immediately, a major difference between memory and effector cells is that memory cells must be capable of undergoing proliferation upon antigen re-exposure, while effector cells must be capable of immediate function without proliferating. The immune system, in response to a natural infection, must therefore strike a balance between the generation of memory and effector cells.

However, the requirements are significantly different during vaccination. Vaccination, as commonly understood, is a mock infection, in that it exposes the

body to the molecular targets of a given infectious agent without actually causing disease. Thus, there is no immediate need for effector immune responses to vaccination.

The pathways that strike a balance between effector and memory commitment are mutually exclusive, so that stimulating the immune system too hard may lead to the generation of short-lived effector immune cells to the detriment of the generation of long-lived memory immune cells. A dilemma is thus apparent; overstimulating the immune system by a vaccine may be detrimental to the generation of immune memory against it, but understimulation may also result in poor generation of both effector and memory responses. The solution to this difficulty is the identification and use of immune response modulators that affect the biochemical pathways governing effector versus memory generation differentially. Such modulators should suppress effector generation and skew the balance in favor of memory generation. What Eric has done is describe such an immunomodulatory intervention and how it relates to memory generation for one branch of the immune response, killer T cells. He has discovered that by administering the drug pentoxifylline during immunization that he can demonstrate the persistence of immune memory 100 days later. Without this treatment, there is practically no detectable T cell killing. This finding will have a significant impact on the field of immunology.

Dr. Dwight E. F. Talburt, Professor and Vice-Chair of the Department of Biological Sciences said of Mr. Goodspeed's work:

I feel that Eric is both an outstanding individual as well as a student. I have known him for about 3 years while he has been a student major in the Department of Biological Sciences. Also, he was a student in my senior/graduate level course in Microbial Physiology where he demonstrated clearly his ability to assimilate and gain understanding of a large body of technical information concerning the biochemistry and genetics which underlie microbial growth. In this class he was in the top 5% of about 60 advanced students. Overall, his GPA is about 3.6. In addition to his strong classroom and laboratory abilities, Mr. Goodspeed has been active in various honorary and community service endeavors.

Mr. Goodspeed's work, which he seeks to publish in *Inquiry*, is an outgrowth of work which he began with a "SILO Undergraduate Research Fellowship" under the very able direction of his mentor and research advisor, Dr. Jeannine Durdik. The work is entitled "Pentoxifylline modulation of the cytotoxic CD8 T-Cell differentiation pathway to enhance the memory cell contingent"—which means that he and his advisor have possibly discovered a way to enhance immune responses to disease causing agents. If so, the

application of this agent (pentoxifylline), or some derivative of it, into a vaccine might be an effective approach to eliciting stronger immune responses by the individuals who receive the vaccines.

Dr. Douglas Rhoads, Associate Professor and Vice-chair of the Department of Biological Sciences, had this to say about Mr. Goodspeed:

I have been asked to provide a letter of support regarding the submission by Mr. Eric Goodspeed of an abstract and project for a research award and for publication in the new U of A undergraduate journal, *Inquiry*. I can think of no student that is more appropriate or more deserving of such an award. I had Eric in Cell Biology Lecture and Lab. Eric clearly distinguished himself in this course as one of the brightest and most dedicated and attentive students in the class of 90 students. This is an intensive course in cell metabolism and structure. The exams all require lengthy, hand written answers to questions based on the readings and lectures. Eric sat near the front, attended all but one lecture and asked some of the best questions. The only period he missed he came to me and apologized for missing. He scored extremely well on all the exams and had one of the highest cumulative scores in the class. For that reason I kept in contact with Eric and advised him about getting involved in a research project for his senior thesis requirement. Unfortunately, he selected to work in Dr. Jeannine Durdik's lab. That is unfortunate for me because I was hoping he would choose my lab. It is fortunate for him and for Dr. Durdik that he chose her lab. I am well aware of Dr. Durdik's research program as I have been on several thesis committees for her graduate and undergraduate students. Dr. Durdik molds thinkers and researchers trained in immunology and molecular biology. The students receive a well rounded education and training that is competitive with the best University settings: I think Eric has been very successful in his undergraduate project. I have witnessed him on weekdays, weekends and evenings working in the lab. He enjoys the work and appears quite dedicated to his science. I will add that his personality and attitude make him pleasant to converse with and open to suggestion and discussion. I have read the abstract to his proposal and am excited about the possibility that Dr. Durdik and Eric will be extending the work on pentoxifylline to CD8+ cells. As this effort has the potential to develop new patentable drugs for human health I find it well worth pursuing. I give Eric Goodspeed my highest rating, I think he is in the top 1-5% of all our students in Biological Sciences (class of 600). I highly recommend that you publish his findings and award him for this effort.

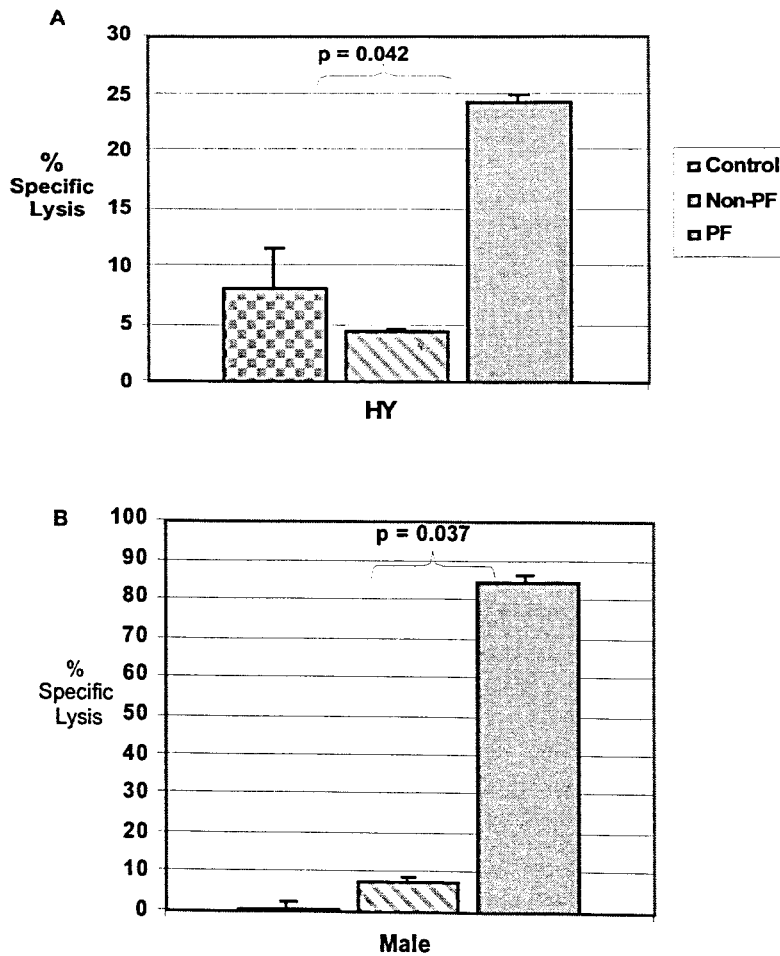


Figure 1. PF-mediated enhancement of CD8 T-cell memory in vivo 60 days post-priming. Female C57BL/6J mice primed with 10^7 C57BL/6J splenocytes were treated either with PBS or 1.5mg PF per mouse per day from day-one to day-four of priming. Splenocytes at day-sixty post-priming from unprimed (checkered bar), primed+PBS-treated (hatched bar) or primed+PF-treated (solid bar) were stimulated in vitro for six days with male splenocytes and used as effector cells at a 50:1 effector:target ratio to lyse (a) ^{51}Cr -labeled RMA-S target cells incubated with HY-peptide or (b) ^{51}Cr -labeled male splenocyte targets in a six-hour cytotoxic assay to determine percentage of cytotoxicity.

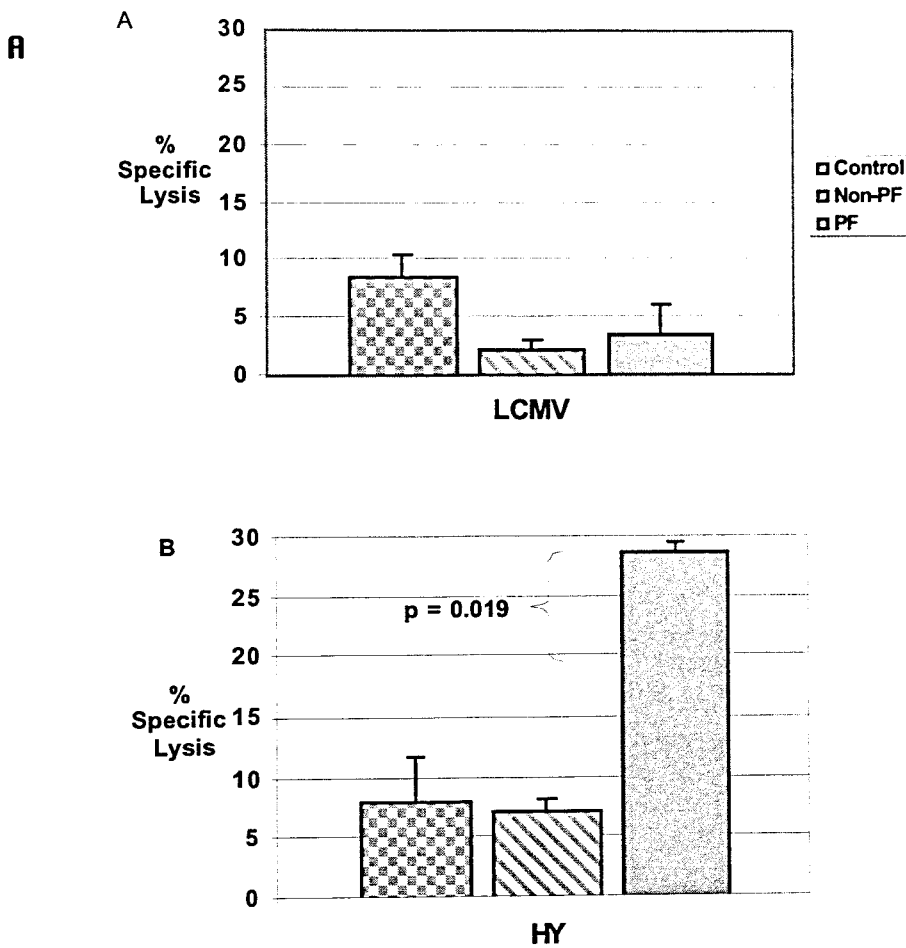


Figure 2. PF-mediated enhancement of CD8 T-cell memory in vivo 90 days post-priming. Female C57BL/6J mice primed with 10^7 male C57BL/6J splenocytes were treated either with PBS or 1.5 mg PF per mouse per day from day -1 to day 4 of priming. Splenocytes at day 60 post-priming from unprimed (checkered bar), primed+PBS-treated (hatched bar) or primed+PF-treated (solid bar) were stimulated *in vitro* for 6 days with male splenocytes and used as effector cells at a 50:1 effector:target ratio to lyse (a) ^{51}Cr -labeled RMA-S target cells loaded with the irrelevant LCMV-peptide or (b) ^{51}Cr -labeled HY-peptide loaded RMA-S targets in a 6-hour cytotoxic assay to determine percentage of cytotoxicity.

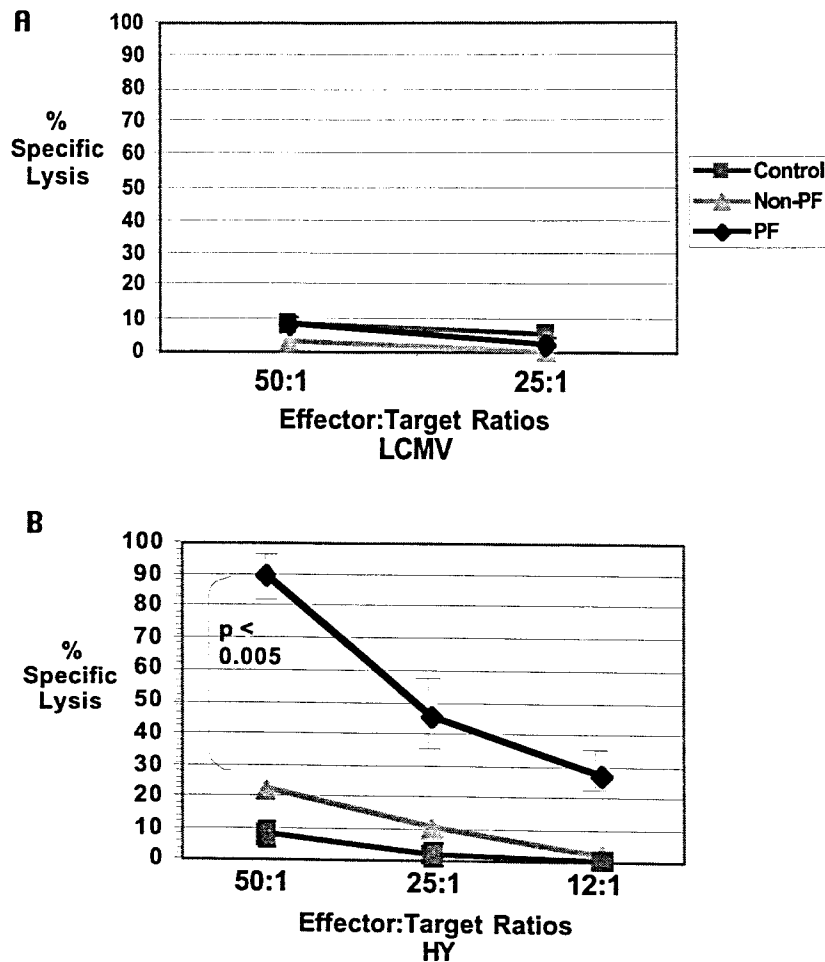


Figure 3. PF-mediated enhancement of CD8 T-cell *in vivo* 60 days post-priming. Female C57BL/6J mice primed with 10^7 male THY1.1 splenocytes were treated either with PBS of 1.5mg PF per mouse per day from day-one to day-four of priming. Splenocytes at day 60 post-priming from unprimed (square), primed+PBS-treated (triangle) of primed+PF-treated (diamond) were *stimulated in vitro* for six days with male splenocytes and used as effector cells at effector:target ratios of 50:1, 25:1 (and 12:1 in B) to lyse (A) ^{51}Cr -labeled RMA-S target cells loaded with the irrelevant LCMV-peptide of (B) ^{51}Cr -labeled RMA-S targets loaded with the HY-peptide in a six-hour cytotoxic assay to determine percentage of cytotoxicity.

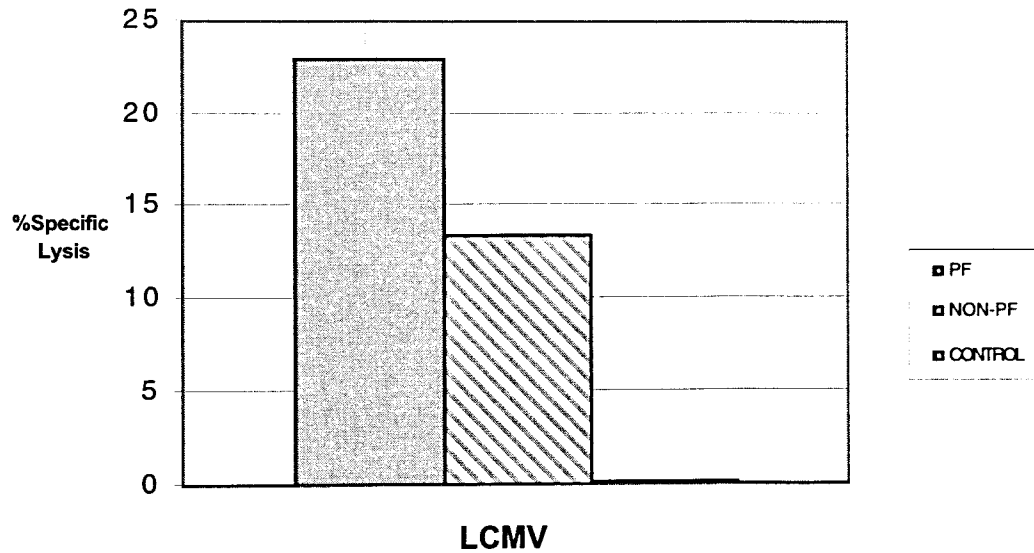


Figure 4. Generation of an MHC class I restricted CD8 T-cell response by priming with LCMV-peptide loaded cells. Female C57BL/6J mice were primed with LCMV-peptide loaded syngeneic female splenocytes. Restimulated LCMV-specific effectors were developed 30 days post-priming and mixed with ^{51}Cr -labeled, LCMV-peptide loaded RMA-S cells at an effector:target ratio of 25:1 ($2.5 \times 10^5:1 \times 10^4$). LCMV-primed, PF-treated mice-generated effectors (solid bar) show a greater cytotoxic activity against the LCMV-peptide loaded RMA-S target cell than the untreated LCMV-specific effectors (hatched bar).

STUDIES OF TRYPTOPHANS IN MEMBRANE- SPANNING "WALP" PEPTIDES BY DEUTERIUM NMR SPECTROSCOPY

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Faculty Mentor: Roger E. Koeppe II
Department of Chemistry and Biochemistry

Abstract.

WALP peptides of sequence acetyl-Gly-Trp-Trp-(Leu-Ala)_n-Trp-Trp-Ala-ethanolamine insert into lipid bilayers as membrane-spanning α -helices and modulate the lipid phase behavior as functions of n and the lipid acyl chain length. A key feature of the WALP peptides is the positioning of tryptophan (Trp) indole rings at the membrane/water interface. For the examples WALP19 with $n = 6.5$ and WALP23 with $n = 8.5$, we have labeled individual indoles with deuterium and incorporated the labeled peptides in oriented, hydrated bilayers of Dimyristoyl-phosphatidylcholine (DMPC). Deuterium NMR spectra from these samples show sharp resonances when the membrane normal is aligned either parallel ($\beta = 0^\circ$) or perpendicular ($\beta = 90^\circ$) to the magnetic field. The factor of two reduction in the deuterium quadrupolar splittings when β is changed from 0° to 90° indicates rapid molecular rotation about an axis parallel to the membrane normal. The magnitudes and ring assignments of the quadrupolar splittings vary with the position of an indole ring in a WALP sequence and indicate different average ring orientations for different locations in the sequence.

Nicole Reed and Roger Koeppe

WALP peptides of sequence acetyl-Gly-Trp-Trp-(Leu-Ala)_n-Leu-Trp-Trp-Ala-ethanolamine insert into lipid bilayers as membrane-spanning α -helices and modulate the lipid phase behavior as functions of n and the lipid acyl chain length. A key feature of the WALP peptides is the positioning of tryptophan (Trp) indole rings at the membrane/water interface. For the examples WALP19 with $n = 6$ and WALP23 with $n = 8$, we have labeled individual indoles with deuterium and incorporated the labeled peptides into oriented, hydrated bilayers of Dimyristoylphosphatidylcholine (DMPC). Deuterium magnetic resonance (MR) spectra from WALP19 samples show sharp resonances when the membrane normal is aligned either parallel ($\beta = 0^\circ$) or perpendicular ($\beta = 90^\circ$) to the magnetic field. The factor of two reduction in the deuterium quadrupolar splittings

when β is changed from 0° to 90° indicates good transmembrane alignment and rapid molecular rotation about an axis parallel to the membrane normal. The magnitudes and ring assignments of the quadrupolar splittings vary with the position of an indole ring in WALP19 and suggest different average ring orientations for different locations in the sequence. With WALP23, a ^{31}P MR spectrum indicates that the DMPC lipids are well aligned (as was also true for WALP19). Nevertheless, the WALP23 deuterium MR spectra are of poorer quality than those observed with WALP19, perhaps because the hydrophobic length of WALP23 is too long for good matching with DMPC. This latter finding only re-emphasizes the high quality of the WALP19/DMPC results.

Introduction

Cell membranes are composed of lipids and numerous proteins that are essential in various biological processes. Membrane proteins catalyze chemical reactions, mediate the flow of nutrients and wastes, and relay information from outside the cell to its interior components (Voet, and Pratt) (Voet, Voet, and Pratt). Interactions between membrane proteins and lipids can affect both the conformation of the protein and the structure of the membrane (Gil, *et al.*; Marsh and Horváth; White and Wimley). Certain amino acids, components of proteins, have particular roles in carrying out protein functions. Interestingly, tryptophans (Figure 1) (and also other aromatic amino acids) have been found to act as 'membrane interface anchors' that hold transmembrane protein segments in a fixed orientation (Chang, and Stevens) (Schiffer, Chang, and Stevens). Although there has been an increasing interest in these anchoring residues, the exact mechanism of anchoring is still unclear. The purpose of this study was to determine the orientation of the tryptophan indole rings at the membrane/water interface in order to gain greater insight into this anchoring mechanism. This information will help to define the boundary between transmembrane segments and exterior domains of membrane proteins, and will contribute to our understanding of membrane protein assembly and topology (Planque, *de Planque, et al.*).

Model systems of synthetic peptides and model membranes are often used to study the interactions between membrane proteins and lipids. The peptides used in this experiment are uncharged and α -helical, designed to mimic the membrane-spanning helices often found in membrane proteins (Figure 2). These "WALP" peptides contain two tryptophan anchors at each end, flanking a central hydrophobic region of alternating alanine and leucine residues, for example acetyl-Gly-Trp-Trp-(Leu-Ala)₅-Trp-Trp-Ala-ethanolamine. WALP peptides have been found to modulate the phase behavior of membrane lipids (Killian, *et al.*). The model membranes are lipid bilayers of dimyristoyl-phosphatidylcholine (DMPC), a lipid with 14-carbon tails without double bonds. Oriented deuterium MR samples of WALP peptides with deuterium-labeled tryptophans in DMPC were measured to determine the orientations of the indole rings.

Experimental Methods

Deuterium labeled L-tryptophan, with five hydrogens on the indole ring replaced by deuterium (Figure 1), was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). The amino group on tryptophan was protected with 9-Fluorenylmethylcarbonyl (Fmoc) from N-(9-fluorenyl-methoxycarbonyloxy)succinimide. The synthesis was tested with an analysis technique called thin layer chromatography. A ninhydrin assay, which tests for free amino groups, confirmed the protection. After recrystallization, product quality was tested by ¹H-MR analysis and UV-visible spectroscopy. The deuterium labeled

Fmoc-tryptophan was used in solid phase peptide synthesis reactions of four different 19-amino-acid WALP19 peptides and two different 23-amino-acid WALP23 peptides, with one deuterium labeled tryptophan in each peptide (Figure 3). Synthesis was performed on an Applied Biosystems 431A Peptide Synthesizer with an extended coupling time for the deuterium labeled tryptophan (Killian, *et al.*; Greathouse, *et al.*). Peptides were then cleaved with ethanolamine in dichloromethane. Peptide purity was confirmed by high-pressure liquid chromatography and mass spectroscopy. To make the oriented deuterium magnetic resonance samples, each peptide was added to DMPC in a 1:20 peptide to lipid ratio. The solution of peptide and lipid was applied to about 40 small glass plates that were dried and stacked in a sample cuvette, then hydrated with deuterium-depleted water. The cuvette was then sealed to keep the lipids from drying out. The sample was then incubated at 40° C for a week, which allows them to form liquid crystalline lipid bilayers on the glass plates (Figure 4). Phosphorus-31 magnetic resonance (³¹P-MR) was used to test for proper orientation of the lipid bilayers. Deuterium magnetic resonance spectra were then recorded for each sample with the normal of the plates at both 08° and 90° with respect to the magnetic field.

Results and Discussion

The ³¹P magnetic resonance spectra indicate the extent of alignment of the lipid head groups. The peak at 30 ppm for WALP23 and 27 ppm for WALP19 (Figure 5) is the signal from the lipid head groups that are properly aligned in the lipid bilayer. Lipids in alternative alignments give the signal for the peaks at -15 and -13.9 ppm. The quality of lipid bilayer alignment is determined by the ratio of the peak sizes. These ³¹P-MR results show that both the WALP19 peptide and WALP23 peptide samples contain well-oriented lipid bilayers.

In deuterium magnetic resonance, characteristics of deuterium nuclei are measured by subjecting the nuclei to a magnetic field and recording the signal at which radio frequency (RF) waves are absorbed. Two peaks are observed separated by a splitting ($\Delta\nu$) that is affected by the orientation and motion of the carbon-deuterium bond. For peptides that rotate rapidly about the helix axis, the deuterium magnetic resonance splittings are related to the orientation of the labeled indole ring by the following equation (Taylor, and Koeppel) (Killian, Taylor, and Koeppel):

$$\Delta\nu_q = \left(\frac{3}{2}\right) \left(\frac{e^2 q Q}{h}\right) \left(\frac{1}{2} [3 \cos^2 \theta - 1]\right) \left(\frac{1}{2} [3 \cos^2 \beta - 1]\right)$$

(Equation 1). Here, θ is the angle between the carbon-deuterium bond and the helix axis, and β is the angle between the magnetic field and the membrane normal, either 0° or 90° (Figure 6). The static coupling constant ($e^2 q Q/h$) is approximately 180 kHz for an aromatic carbon-deuterium bond.

For the WALP19 peptides, reasonably sharp peaks obtained at $\beta = 0^\circ$ and $\beta = 90^\circ$ indicate that the peptides are well oriented in the lipid bilayer (Figure 7). The tilt of the helix axis (which contributes to the angle b) and the structure of the peptide (which could affect the angle θ) must be consistent to give a clear spectrum. Furthermore, the sharp peaks when $\beta = 90^\circ$ indicate rapid rotational motion about the helix axis that allows the signals at various rotational positions to be averaged to a single signal, called motional averaging.

Each WALP19 peptide gives a unique spectrum, indicating that the indole rings of the four tryptophans in the peptide have four different orientations. The distance between symmetrical peaks in these spectra, or splittings ($\Delta\nu$), are compiled in Table 1. Four symmetrical peaks are expected from the five deuterons on each labeled tryptophan, because positions 4 and 7 are geometrically equivalent (Figure 1). The expected result is obtained for WALP19 with tryptophan labeled at position 17. WALP19 with tryptophan labeled at position 2 shows four peaks at $\beta = 90^\circ$ but only three peaks are detected at $\beta = 0^\circ$. For WALP19 peptides labeled at positions 3 or 18, only three peaks can be discerned. The deuterium MR splittings are reduced by a factor of two when the sample is measured at $\beta = 90^\circ$, compared to their values at $\beta = 0^\circ$ (Table 1). This result is predicted by equation 1, and further indicates that the WALP19 peptides rotate rapidly about their helix axis and display motional averaging (Killian, Taylor, and Koeppe).

From the deuterium MR splittings, it may be possible to calculate possible orientations for the indole rings on the labeled tryptophans. This approach has been successfully applied to the membrane channel gramicidin A (Koeppe, Killian, and Greathouse). However, assignment of each signal in these spectra to a particular deuterium label on the ring (Figure 1) must be accomplished first. Future experiments will address this issue by using tryptophan indoles labeled with deuterium at only one position, which requires a more difficult synthesis. Results from those experiments should facilitate the assigning of peaks to the correct positions on the indole ring. The deuterium MR spectra for the WALP23 peptide did not contain sharp peaks (Figure 8). Because the ^{31}P -MR spectrum for this sample indicates that the lipid bilayer is oriented properly, the poor quality of the deuterium MR spectra suggests that the WALP23 peptides may be too long to be stable in a DMPC bilayer. (This behavior of WALP23 contrasts with the very nice deuterium MR spectra obtained using WALP19 (Figure 7) or the shorter WALP16 der Wel, (van der Wel, *et al.*)). The stability of peptide/bilayer systems depends on matching between the hydrophobic acyl chain length of the lipids and the length of the hydrophobic transmembrane domain of the peptide. If the peptide's transmembrane domain is longer than the bilayer thickness, there is an energetically unfavorable exposure of hydrophobic surfaces to the aqueous environment outside of the bilayer. This mismatch can force peptides that are too long to be excluded from the lipid bilayer der

Wel, (van der Wel, *et al.*), which may be the case with the WALP23 sample. Further deuterium MR studies on WALP23 samples may require lipids with longer acyl chains. However, bilayers with longer lipids will not maintain their fluidity unless the lipid acyl chains contain double bonds. A possible model membrane system for WALP23 would be dioleoylphosphatidylcholine (DOPC), a lipid with 18-carbon tails with one double bond.

This project has shown that labeled WALP19 peptides orient well in DMPC bilayers and can be measured by deuterium magnetic resonance. The two-fold reduction in splittings between $\beta = 0^\circ$ and $\beta = 90^\circ$ demonstrates that the peptides are rotating rapidly about the helix axis. Different tryptophans in the peptides yield different spectra, indicating that their indole rings have different orientations. Future experiments using this approach can yield useful information about the particular orientations of the labeled tryptophan indole rings at the membrane/water interface.

Abbreviations

DMPC	dimyristoylphosphatidylcholine
DOPC	dioleoylphosphatidylcholine
Leu (L)	leucine
Ala (A)	alanine
Trp (W)	tryptophan
Gly (G)	glycine
ea	ethanolamine
MR	magnetic resonance
UV	ultraviolet

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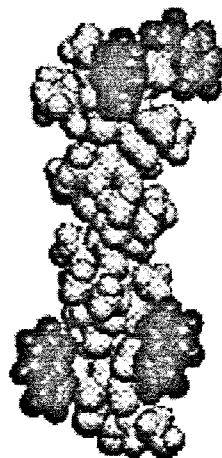


Figure 2. Molecular model of Walp19 peptide obtained using Insight II v. 908.0 (Molecular Simulations Inc., San Diego, CA). Tryptophan indole rings are dark gray and NH groups are black

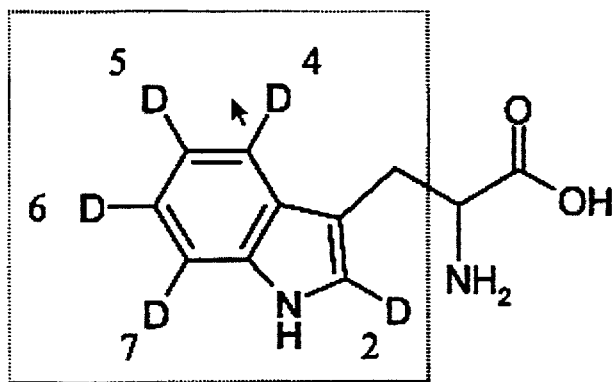


Figure 1. Tryptophan amino acid (the indole ring is boxed). Here deuterium has replaced hydrogen at positions 2, 4, 5, 6, and 7.

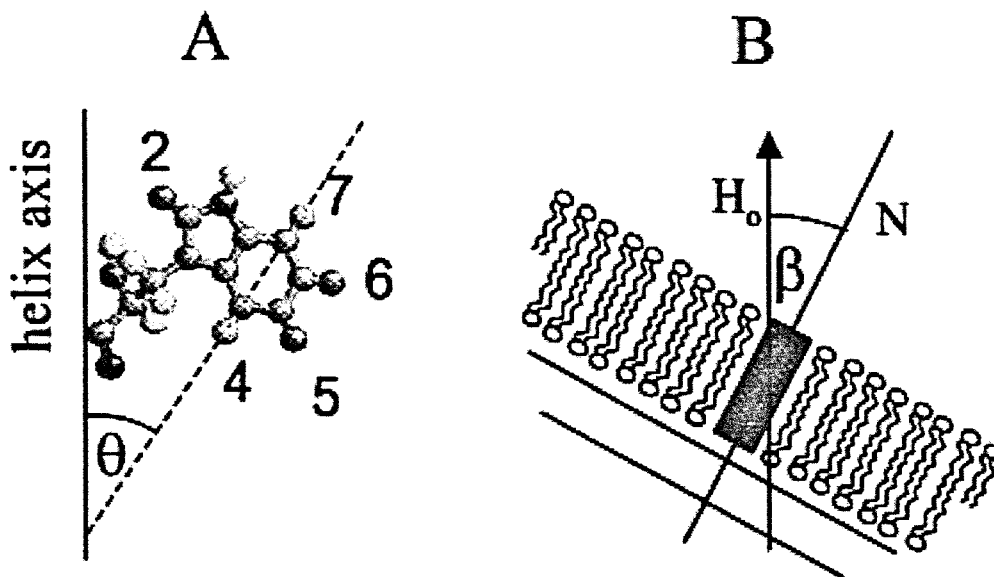


Figure 6. Illustrations of the variables in equation 1. The angle theta is between the axis of the WALP peptide helix and the carbon-deuterium bond (A). The angle β is between the membrane normal N and the magnetic field direction H_0 (B).

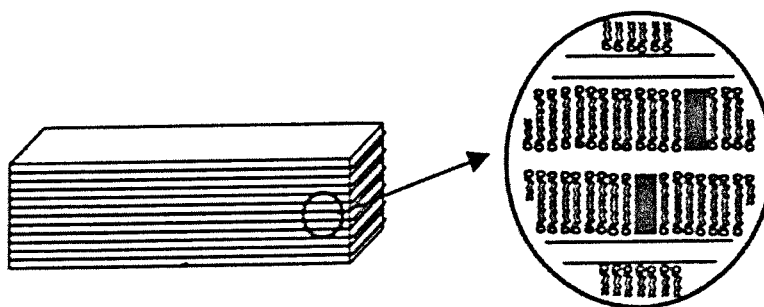
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Ac-G	W*	W	L	A	L	A	L	A	L	A	L	A	L	A	L	W	W	A- <i>ea</i>				
Ac-G	W	W*	L	A	L	A	L	A	L	A	L	A	L	A	L	W	W	A- <i>ea</i>				
Ac-G	W	W	L	A	L	A	L	A	L	A	L	A	L	A	L	W*	W	A- <i>ea</i>				
Ac-G	W	W	L	A	L	A	L	A	L	A	L	A	L	A	L	W	W*	A- <i>ea</i>				
Ac-G	W*	W	L	A	L	A	L	A	L	A	L	A	L	A	L	A	L	A	L	W	W	A- <i>ea</i>
Ac-G	W	W	L	A	L	A	L	A	L	A	L	A	L	A	L	A	L	A	L	W	W*	A- <i>ea</i>

Splittings (Δv) for Deuterium-Labeled WALP19 Peptides		
Labeled Residue #	$\beta = 0^\circ$	$\beta = 90^\circ$
2	81	75
	61	41
	21	10
3	155	78
	85	41
	77	26
17	77	38
	62	28
	44	23
	11	6
18	63	32
	22	10
	11	7

Figure 3 (above). Sequence of WALP peptides. Abbreviations: Ac-Gly = acetyl-glycine, W = tryptophan, W* denotes deuterium-labeled tryptophan, L = leucine, A = alanine, and ea = ethanolamine.

Table 1 (left). Deuterium MR Splittings for WALP19 peptides. Note the factor of two reduction between splitting at $\beta = 0^\circ$ and those at $\beta = 90^\circ$

Figure 4 (below). Liquid crystalline lipid bilayers form on the stacked glass plates with the WALP peptides (represented by shaded rectangles) included.



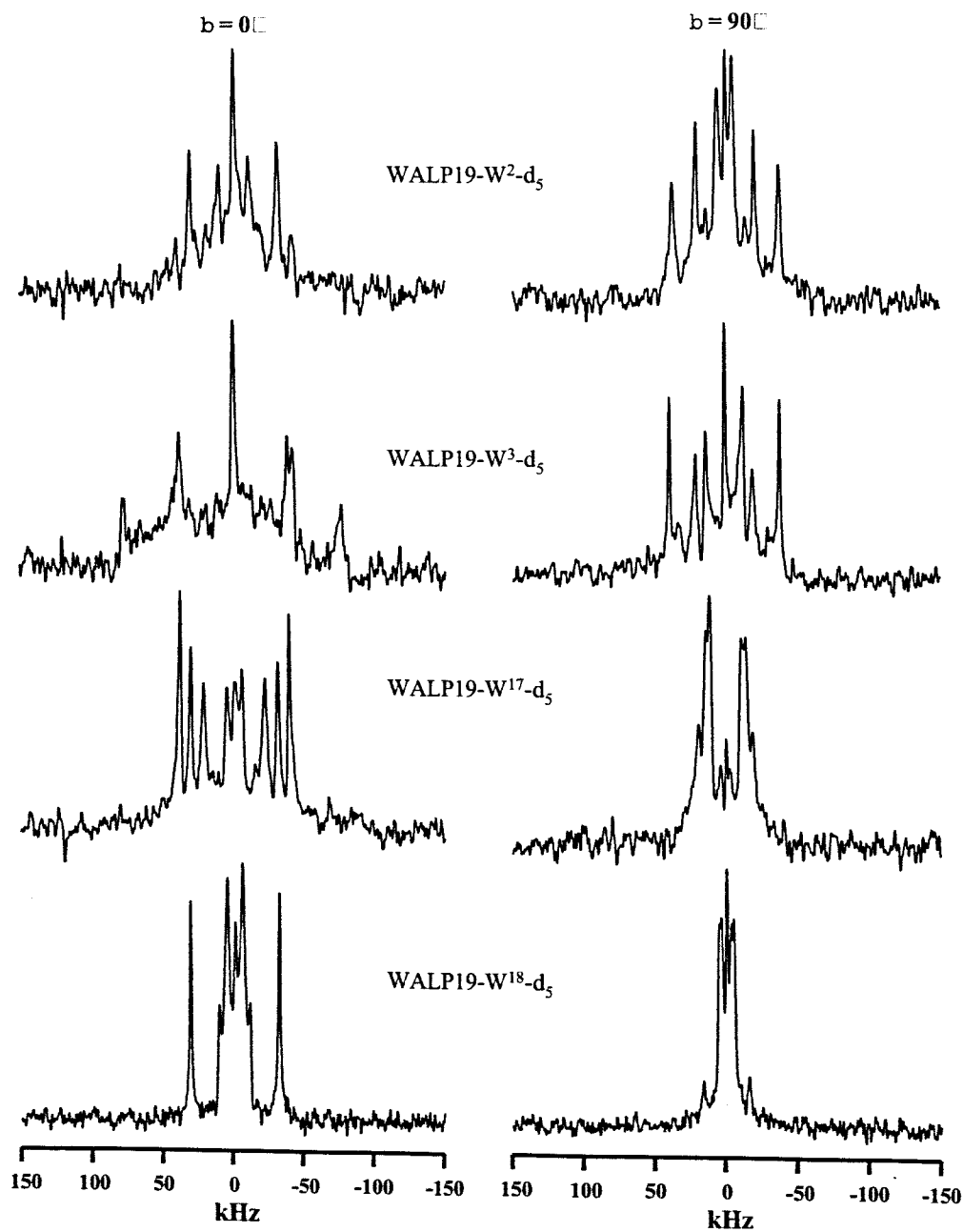


Figure 7. ²H-NMR spectra for tryptophans in WALP23 at $\beta = 0^\circ$ (left) and $\beta = 90^\circ$.

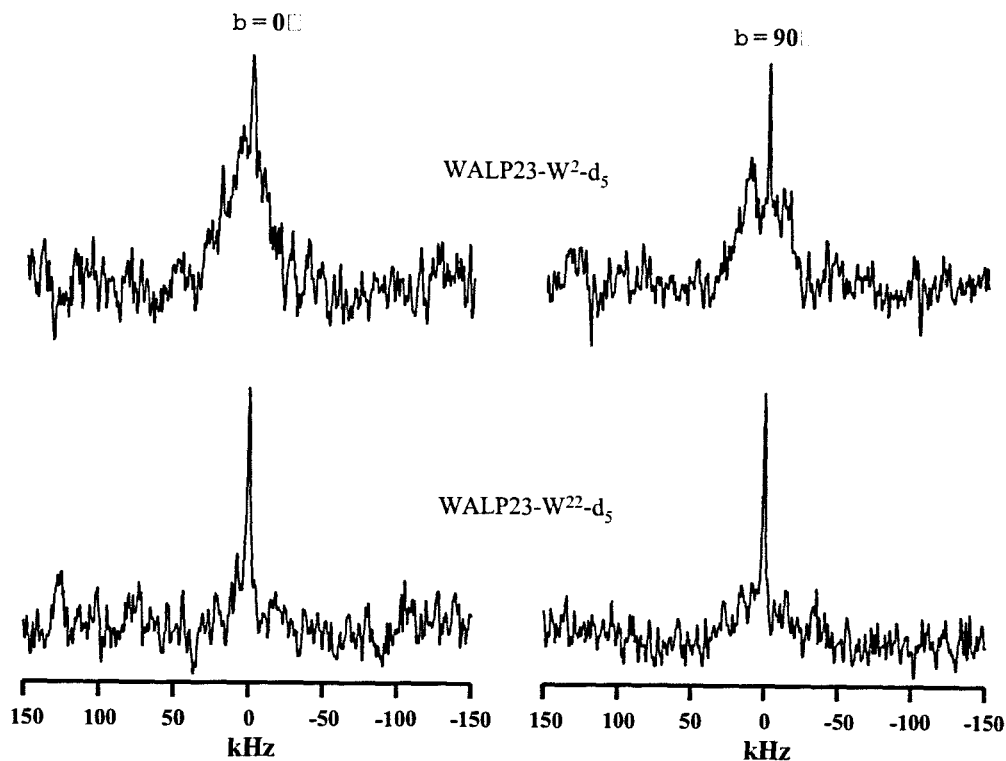


Figure 8. ^2H -NMR spectra for selected tryptophans in WALP23 at $\beta = 0^\circ$ and $\beta = 90^\circ$.

Faculty Comments:

University Professor **Roger E. Koeppel II**, Ms. Reed's mentor, described her work as follows:

Nicole is an outstanding student who has done a superb job on her project. She has used an innovative approach to apply the methods of solid-phase peptide synthesis and solid-state deuterium magnetic resonance spectroscopy to investigate the interactions between proteins and lipids in biological membranes. The approach and Nicole's results have important implications for our understanding of the chemistry of cell membranes and intercellular trafficking.

Nicole is a student leader with a remarkable breadth of interests. She has served for two years as President of the local student affiliate section of the American Chemical Society. Under her leadership, this group has been active in numerous public service projects, including training events for Boy Scouts, and for elementary school students at Springfest each April

and at the Northwest Arkansas Mall during National Chemistry Week each November. Nicole has been awarded and currently holds the prestigious national Morris Udall Fellowship for studies in environmental policy. For her career plans, she will apply her diverse expertise and leadership abilities to combine the fields of chemistry and law into a coherent whole. Approaches to complex problems such as public policy require such interdisciplinary efforts.

The quality of Nicole's research project was evident when she presented her results at the national meeting of the Biophysical Society in New Orleans in February of this year. At this meeting, she defended her research alongside graduate students and postdoctoral fellows who are doing similar or competing experiments to hers in esteemed laboratories around the world. In this setting and as a "mere" undergraduate student, she held her ground well and was a marvelous representative of the University of Arkansas! One version of her abstract has been published in the

Biophysical Journal (volume 78, page 413A), and we will submit a final comprehensive account of her research to a peer-reviewed national journal such as *Biochemistry* (published by the American Chemical Society).

D. Mack Ivey, Associate Professor in the Department of Biological Sciences, commented:

I have had Nicole in class, and I serve on her Honors thesis committee. Her academic skills are top-notch, and her thesis work is excellent. She is investigating the determinants of transmembrane helix formation and stability in membrane proteins. This is a worthy endeavor. Membrane protein structure is a very active field of research, but the traditional methods used to study protein structure are difficult and often unproductive when applied to extremely hydrophobic proteins. Nicole's approach involves the use of deuterium NMR, synthetic peptides, and artificial membrane bilayers in a spectroscopic analysis of structure. This is a highly original and creative approach, which is already producing interesting and readily interpretable results. I predict that this research will have a great impact, and I strongly recommend it for inclusion in the undergraduate research journal.

Suzanne McCray, Director of the Office of Post-Graduate Fellowships and Associate Director of Honors Studies, describes Ms. Reed and her research project in glowing terms:

Recommending Nicole Reed for this recognition is a pleasure. Nicole Reed is a Sturgis Fellow who has greatly exceeded our very high original expectations. She is a very motivated student, who commits completely to each project. She has served as an Honors Council Student Representative and as President of the Association of Honors Students. She is committed to undergraduate research and created a student organization with the goal of interdisciplinary student research. The group has its own web page. They meet monthly with faculty. It has been a wonderful experience for everyone concerned—faculty and students alike. I count Nicole Reed among the top five students I have known in my ten years of working with high ability students at the University of Arkansas

Last year, Ms. Reed received the nationally competitive *Morris Udall Scholarship*, recognizing outstanding achievement in environmental studies. This fall she was awarded a *Science Information Liaison Office Undergraduate Research Fellowship* for the project currently under consideration. When I asked her to explain the project to me in a way that a non-science oriented person could understand, she basically said she wanted to learn more about the interactions of proteins embedded in cell membranes with the lipids that are the main building blocks of those membranes:

"Proteins that are embedded in the cell membrane carry out many functions essential for life," she wrote in an enthusiastic e-mail describing her project. "For example, the reactions that generate the main energy source for all organisms are carried out in membrane proteins. Scientists are trying to learn more about how proteins fold into the membrane, how they interact with the membrane, how they move in the membrane, etc. My projects looks at the edges of transmembrane (membrane-spanning) peptides to find out how they are 'anchored' in the membrane -- what mechanism is responsible for keeping these proteins stretched out across the membrane rather than "'lobbed' up inside the membrane or coming out of the membrane altogether? It has been discovered that tryptophan (amino acid) residues anchor pep/ides/protein segments in the membrane, but the mechanism that allows them to act as membrane anchors is not known. In this project, I am trying to find the orientation of the tryptophan side chains (indole rings), which will help answer the question of how they work as membrane anchors." As I understand it, knowing how the membrane anchors will impact on other studies in the future including those in biomedical research.

Ms. Reed has been the President of the campus American Chemical Society for two years. She was elected in part due to her research interests and because of her ability to make these interests appealing to a wide audience. In celebration of National Chemistry Week last fall, she coordinated a mall event for youngsters, which required several months of planning. The event involved demonstrations and hands-on chemistry reactions geared toward children, but was also popular with their parents. She wanted to attract these children to science and to spark their interest in learning more about their world and its connection to chemistry. The event was an enormous success. Ms. Reed did an excellent job of describing the events and its impact on those involved when she was interviewed at length by KUAF. The national American Chemical Society included a report and a photo of the event in their national publication. She is our undergraduate Stephen J. Gould, bringing science to the masses.

As you can tell from the description of her project, Ms. Reed is majoring in biochemistry, but she will attend *Stanford Law School*. Her focus will be environmental law. Five thousand undergraduates applied to the Stanford's law program; 180 were accepted. Nicole was admitted early, having scored a 171 on the LSAT (a score of 180 is perfect; 163 was the highest mark I had previously seen). Stanford awarded her a full, renewable fellowship to cover her expenses. She will no doubt thrive in law school both academically and personally.

LYMPHOCYTE PROFILES IN BLOOD AND TUMORS OF ARKANSAS ROUS SARCOMA REGRESSOR AND PROGRESSOR CHICKENS

by Brant Ward
Center of Excellence for Poultry Science

Faculty Mentor: Gisela Erf
Associate Professor, Avian Immunology

Abstract.

The Arkansas Rous Sarcoma Regressor (AR) and Progressor (AP) lines of chickens represent an excellent model to study immune responses to tumors. Both lines of chickens initially develop tumors when injected with Rous sarcoma virus (RSV) or with DNA coding for the RSV oncogene v-src (v-src DNA). AR chickens will eventually regress the tumors whereas AP chickens will allow the tumors to progress to a terminal stage. By using both v-src DNA and RSV for tumor induction, we were able to compare the immune response to tumor antigens alone and tumor antigens in combination with viral components, respectively. To study the ability of v-src DNA to induce tumors in AR and AP chickens, 3-day-old AR and AP chicks were injected with v-src DNA, and the incidence and development of tumors was monitored. To gain insight into the role of lymphocytes in tumor regression, 9-week-old AR and AP chickens were injected into the wing web with either PBS (vehicle control), v-src DNA in PBS, or RSV in PBS. Blood was collected prior to injection and during tumor development. Tumors were collected when present. Lymphocyte profiles in blood and tumors were determined by immunofluorescent staining with a panel of antibodies and flow cytometry. Based on tumor induction data, the level of the chicken's immunocompetence is important, not only in the ability to regress tumors, but also in the susceptibility to tumor induction by v-src DNA. Cell population analyses of tumor-infiltrating lymphocytes (TIL) revealed the presence of different types and proportions of immune cells in DNA and RSV induced regressing and progressing tumors. DNA induced tumors were shown to display higher levels of regulatory T cells (CD4+) and a higher ratio of CD4+ to CD8+ TIL than RSV induced tumors, indicating a different response occurs to tumor antigens alone versus tumor plus viral antigens. Additionally, the percentage of CD4+CD8+ TIL, a cell type usually not found in the periphery and whose function is unknown, was higher in AR tumors than AP tumors. Of particular interest was the discovery of CD8a-b+ TIL. This cell type has been recently characterized using transgenic human cells, and appears to be an ineffective cytotoxic T cell subtype. The elevated proportions of this novel cell type in progressing tumors may contribute to the ineffective immune response in AP compared to AR birds.

Brant Ward and Gisela Erf

Introduction

The immune system's response to tumor cells within the body involves a complex series of events that is difficult, at best, to explore and extrapolate. However, a system that allows for precise study of this phenomenon exists in the Arkansas Rous Sarcoma Regressor (AR) and Progressor (AP) lines of chickens. These two lines have been developed based on their characteristic immune responses to virus-induced tumors. When infected with Rous sarcoma virus (RSV), an acutely transforming avian retrovirus, both lines initially develop tumors at the site of infection. Several days later, birds of the AR line begin to show decreases in tumor mass, which eventually leads to the complete regression of the tumor. However, birds of the AP line allow the tumor to increase in size and to progress to a terminal stage.

These traits have been shown in other lines of chickens to be the result of an effective or non-effective immune response, respectively, in which the immune system is either able or unable to recognize and eradicate the tumor cells (Halpern *et al.*, 1993; Plachy *et al.*, 1994; Taylor *et al.*, 1994; Wisner *et al.*, 1991).

The drawback to this system is that when a virus is used to transform healthy cells into cancerous cells, the immune system "sees" two different types of antigens, or foreign proteins: those associated with the tumor itself and those associated with viral infection and replication. In order to overcome this and make sure that the immune response is directed only towards the cancer itself, one must transform cells without using a virus. It has been shown that DNA from the RSV gene *v-src*, which is responsible for causing the transformation of healthy cells into tumor cells, can itself cause tumors to develop in chickens (Halpern *et al.*, 1990; Halpern *et al.*, 1991; Plachy *et al.*, 1994; Taylor *et al.*, 1994). Using *v-src* DNA to induce tumors allows for a direct investigation into the immune response against tumor cells. Therefore, it was the purpose of this project to examine the ability of *v-src* DNA to induce tumors in AR and AP chickens and to compare immune cell profiles in blood and tumors (if present) following *v-src* DNA or RSV injection into (9-week-old) AR and AP chickens.

Methods

The RSV oncogene *v-src* was generously donated by Dr. Michael Halpern of the Medical College of Pennsylvania and Hahnemann University in the form of a bacterial plasmid, which was then cloned into a strain of *E. coli* by Dr. Mark Parcels of the University of Arkansas Department of Poultry Science. These bacteria were then grown in culture to amplify the amount of *v-src*. The plasmid containing the *v-src* gene was purified using a commercially available plasmid purification kit (Promega Corporation, Madison, WI). The purified plasmid DNA was examined by gel electrophoresis and compared to the known molecular weight of the original plasmid containing the *v-src* gene. To ensure proper expression in the chicken cells, the *v-src* gene had to be freed from the bacterial plasmid. This was accomplished by digestion with the restriction endonucleases *EcoR* I and *Hind* III, which cut the plasmid at specific positions flanking the *v-src* gene (Halpern *et al.*, 1991). To prepare the cut DNA for injection into the chickens, it was precipitated in ethanol and then redissolved in phosphate buffered saline (PBS) to a final concentration of 1 mg/mL.

A preliminary study was conducted to test the ability of *v-src* DNA to induce tumors in AR and AP chickens. For this, 3-day-old chicks from each line were injected into the wing web with *v-src* DNA in phosphate-buffered saline (PBS). Tumor development was monitored as outlined below. To study immune cell profiles in blood and tumors, 9-week-old AR and AP chickens were injected into the wing web with either PBS containing *v-src* DNA or RSV, or PBS alone (control). The birds

were kept at the University of Arkansas Poultry Farm in floor pens and were observed for approximately seven weeks after injection. Every two or three days, the birds were examined and the sizes (0.1 mm) of tumors were recorded. For consistency, the largest axis of the tumor was always used for measuring purposes. All studies involving birds followed protocols in accordance with the Institutional Animal Care and Use.

Blood samples were collected from the brachial vein of the 9-week-old chickens before injection with experimental treatments and at ten days post-injection (tumor development phase). Once tumors were observed to be in a state of regression (AR, decreasing in size) or progression (AP, continually increasing in size), a blood sample was collected, the birds were euthanized and the tumors excised. On each tumor collection day, blood samples were also collected from chickens in the control group. Mononuclear blood cell suspensions were prepared by Ficoll density gradient centrifugation. Single cell suspensions from tumor tissues were prepared by mashing minced tumor tissue through a Nylon mesh (60 μ m) into PBS. All cell suspensions were then stained with a panel of fluorescently-labeled antibodies specific to particular proteins expressed on the surface of different immune cells as described by Erf *et al.* (1998). Cell-population analyses were then carried out by 1-, 2-, and 3-color flow cytometry. Statistical analyses (ANOVA and multiple mean comparisons) were performed on the collected data.

Results and Discussion

Due to space limitations, only information from the tumors has been included in this section. Tumor incidence following injection with *v-src* or RSV differed between lines and between age groups. In the 3-day-old chicks, only 30% of the DNA-injected AR chicks developed tumors, while 80% of the DNA-injected AP chicks developed tumors. In the 9-week-old chickens, tumors developed in 11% and 22% of *v-src* DNA-injected AR and AP chickens, respectively, whereas RSV injection caused tumors in all AR chickens and in 90% of AP chickens (Table 1). These data suggest that the level of immunocompetence of the chicken, which is positively related to age, is important in determining the sensitivity of AR, and especially, AP chickens to develop tumors in response to *v-src* DNA. Also, while the DNA induced tumors were visually and tactilely determined to consist of one large mass, the RSV induced tumors appeared to contain multiple small masses that eventually fused together, which may be indicative of the ways that the tumors develop (i.e., infection of new cells with virus, versus division of individual cells transformed with DNA) (Halpern *et al.*, 1993).

Analysis of immune cell populations in tumors from 9-week-old birds by flow cytometry and subsequent statistical analysis led to several unique findings. All tumors contained considerable amounts of tumor-infiltrating lymphocytes (TIL). DNA induced tumors contained a higher percentage of regulatory

T helper cells (CD4+CD8-) than RSV induced tumors in both lines (Figure 1A). Surprisingly, the percentages of CD4-CD8+ TIL (generally considered cytotoxic T cells, which kill tumor and virus-infected cells) were not different in regressing and progressing tumors (Figure 1B). Regressing tumors contained a higher percentage of CD4+CD8+ TIL than progressing tumors (Figure 1C). CD4+CD8+ cells are a unique cell type not usually found in the periphery and whose function is not clearly understood. Our data suggest that these cells may serve an important role in the successful regression of a tumor. The proportions of CD4+ to CD8+ cells were higher in tumors from DNA injected birds than in RSV injected birds, suggesting an immunoregulatory difference in response to tumor antigens versus tumor plus viral antigens. Within the DNA treated birds, this ratio was also significantly different between AR and AP lines (Figure 2). In all tumors, there were more T cells expressing TCR2 ($\alpha\beta 1$ T cell receptor) than TCR1 ($\gamma\delta$ T cell receptor) and TCR3 ($\alpha\beta 2$ T cell receptor). Within AP chickens, there were greater proportions of TCR1+ T cells in RSV induced tumors and a higher percentage of TCR2+ T cells in DNA induced tumors. Within the RSV induced tumors, the percentage of TCR1+ T cells was higher in AP chickens, while AR birds had higher proportions of TCR2+ cells (Figure 3). These observed differences in the proportions of TCR-defined tumor infiltrating cells further suggest a difference in the types of cells responding to tumors in AR and AP chickens. Also of interest were the proportions of cells expressing the molecules CD8 α and CD8 β . AR birds injected with RSV displayed higher percentages of CD8 α + β - TIL, a subset of functioning cytotoxic T cells, than AP birds (Figure 4A). DNA injected chickens displayed higher percentages of CD8 α + β + cells, another subset of functioning cytotoxic T cells, than did RSV injected birds (Figure 4B). Of special interest was the appearance of CD8 α - β + TIL within all types of tumor tissue (Figure 4C). While this cell type has not been defined in chickens, transgenic human cells expressing CD8 $\beta\beta$ homodimers have recently been shown to display ineffective cytotoxicity (Devine *et al.*, 2000), which suggests

that their heightened presence in AP chickens may contribute to the ineffective immune response to tumors in these birds. The proportions of CD8 α - β + TIL were higher in RSV induced tumors than in DNA induced tumors, and were higher in progressing tumors than in regressing tumors.

Through this study, differences in the proportions among TIL have been identified in tumors of AR and AP chickens. These observations suggest that different immune mechanisms were initiated in both lines, further emphasizing the usefulness of this animal model for defining components of an effective anti-tumor/anti-virus immune response. The identification of unique cell-types, namely CD4+CD8+ and CD8 α - β + TIL, in tumors is a novel observation. Although these cells have not been fully characterized, their preferential association with regressing and progressing tumors, respectively, suggests an important role of these cells in the successful or unsuccessful regression of tumors. Future studies will attempt to ascertain the functions of these cell types.

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Table 1: Dependence of Tumor Growth on Age and Chicken Line

Frequency of Chickens Developing Tumors

Inoculum	Total	Regressor	Progressor
v-src at 3 days ^a	11/20 (55%)	3/10 (30%)	8/10 (80%)
v-src at 9 weeks ^a	6/36 (17%)	2/13 (11%)	4/13 (22%)
RSV at 9 weeks ^b	20/22 (91%)	11/11 (100%)	9/10 (90%)

^a Eco R I- and Hind III-restricted plasmid DNA in PBS injected into the right wing web

^b 250 plaque-forming units RSV in PBS injected into the right wing web

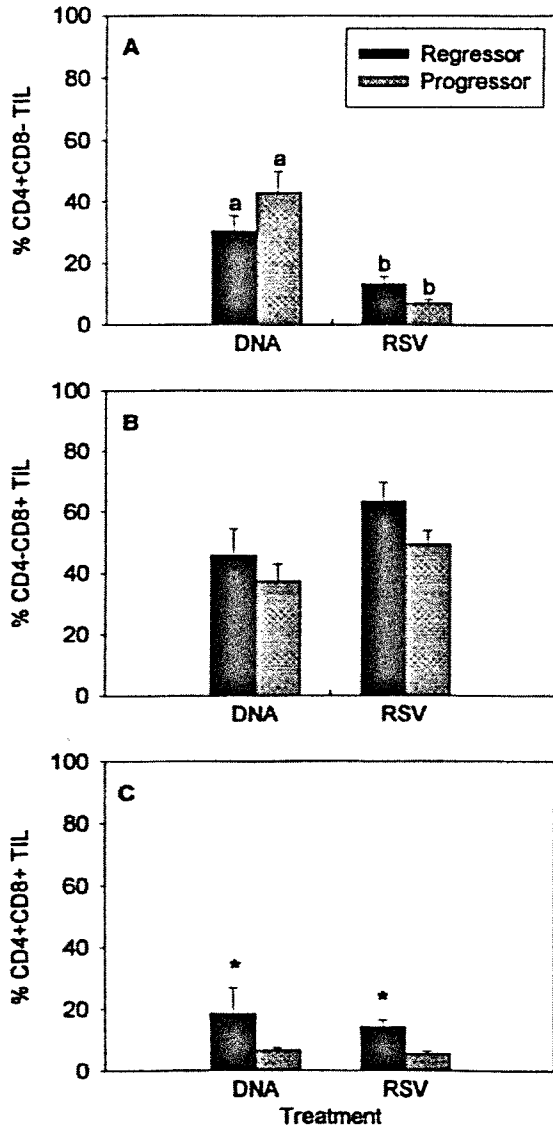


Figure 1. Mean percentages \pm SEM of tumor-infiltrating lymphocytes (TIL) expressing CD4 (A), CD8 (B), or both CD4 and CD8 (C) in DNA and RSV induced tumors in AR and AP chickens. Two-color flow cytometric analyses of tumor cells were carried out using FITC-labeled mouse anti-chicken CD4 monoclonal antibodies (mAb) and PE-labeled mouse anti-chicken CD8 mAb. Numbers shown are percentages of total TIL, which were determined by flow cytometric analysis using mouse anti-chicken K55 mAb (specific to lymphocytes). Within a line, means with different letters (a, b) are significantly different ($P < 0.05$). Differences ($P < 0.05$) between lines but within treatments are indicated with an asterisk.

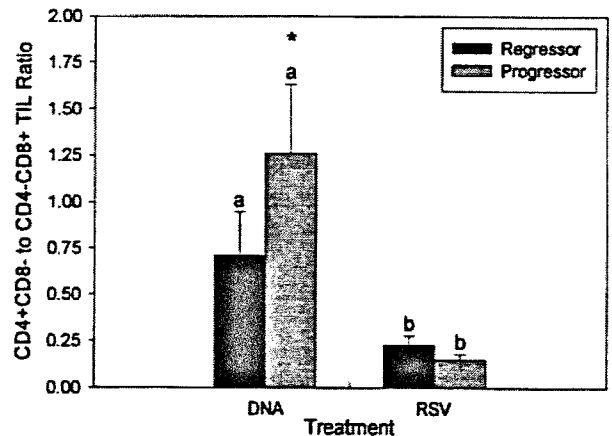


Figure 2. Ratios (mean \pm SEM) of tumor-infiltrating lymphocytes (TIL) expressing only CD4 to those expressing only CD8 in DNA and RSV induced tumors in AR and AP chickens. Ratios were calculated from two-color flow cytometric analyses of tumor cells using FITC-labeled mouse anti-chicken CD4 monoclonal antibodies (mAb) and PE-labeled mouse anti-chicken CD8 mAb. Within a line, means with different letters (a, b) are significantly different ($P < 0.05$). Differences ($P < 0.05$) between lines but within treatments are indicated with an asterisk.

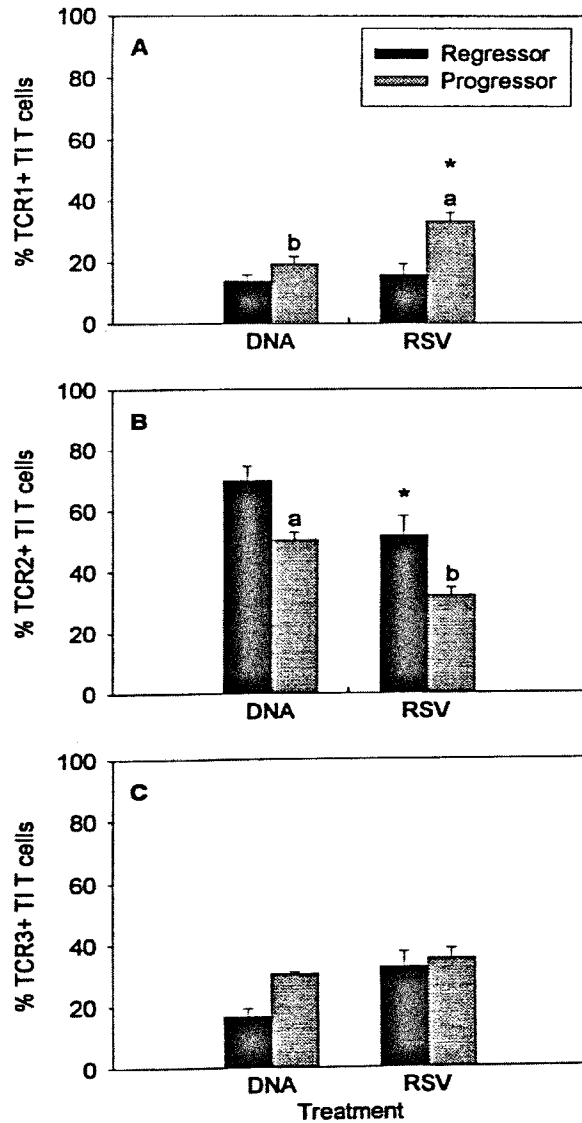


Figure 3. Mean percentages \pm SEM of tumor-infiltrating (TI) T cells expressing TCR1 (A), TCR2 (B), or TCR3 (C) in DNA and RSV induced tumors in AR and AP chickens. Single-color flow cytometric analyses of tumor cells were carried out using biotin-labeled mouse anti-chicken TCR1, TCR2, or TCR3 monoclonal antibodies (mAb). Binding of the biotinylated mAb was detected by quantum-red-labeled streptavidin. Numbers shown are percentages of total TI cells expressing a T cell receptor (TCR1+TCR2+TCR3). Within a line, means with different letters (a, b) are significantly different ($P < 0.05$). Differences ($P < 0.05$) between lines but within treatments are indicated with an asterisk.

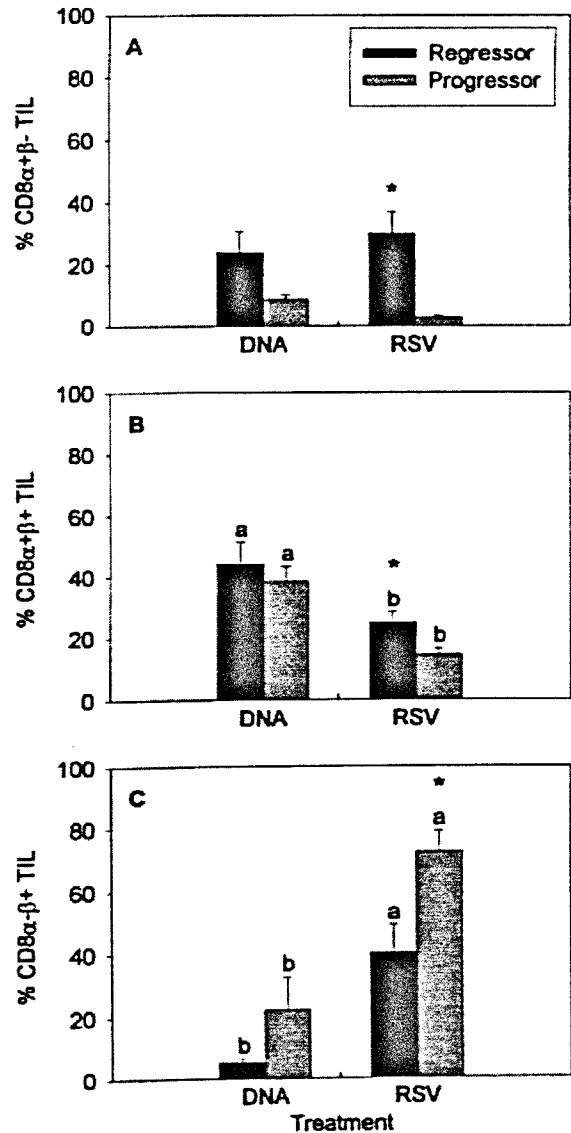


Figure 4. Mean percentages \pm SEM of tumor-infiltrating lymphocytes (TIL) expressing CD8a (A), both CD8a and CD8 β (B), or only CD8 β (C) in DNA and RSV induced tumors in AR and AP chickens. Two-color flow cytometric analyses of tumor cells were carried out using FITC-labeled mouse anti-chicken CD8 β monoclonal antibodies (mAb) and PE-labeled mouse anti-chicken CD8a mAb. Numbers shown are percentages of total TIL, which were determined by flow cytometric analysis using mouse anti-chicken K55 mAb (specific to lymphocytes). Within a line, means with different letters (a, b) are significantly different ($P < 0.05$). Differences ($P < 0.05$) between lines but within treatments are indicated with an asterisk.

Faculty Comments:

Mr. Ward's mentor, **Gisela Erf**, Associate Professor of Avian Immunology, writes about his research as follows:

I am writing this letter in support of the research paper submitted by Brant Ward for publication in the University of Arkansas Journal of Undergraduate Research. Brant has worked diligently on every aspect of this project from the design of the experiment, preparation of V-src DNA, sample processing and data collection, data analysis, literature research, to writing of the paper. Not only has Brant demonstrated excellent technical skills and comprehension of the subject matter, he also demonstrated an excellent ability to condense one aspect of his research into this five page manuscript. This is particularly noteworthy, as Brant collected a tremendous amount of data that were difficult to interpret due to the novel approach used (no comparable published data were available) and to the unique discoveries, such as previously undefined avian cell types (e.g., CD8 α - β + cells), present in the tumors. His findings will form the foundation for continued research in this area.

The immune system is a tightly regulated system orchestrating the function of many types of immune cells in defending the body from disease. The immune system has developed defined approaches to deal with a variety of challenges, such as infection by bacteria, viruses, or parasites, and the development of tumors. In chickens we are in the process of trying to understand the immune mechanisms required to effectively respond to viral infection and tumor cells. With the development and availability of cell-specific markers (e.g., the monoclonal antibodies used here) and state-of-the-art technology (e.g., flow cytometry), we have been able to examine and identify cells involved in various types of immune responses in chickens. The animal model used here (AR and AP lines of chickens) provides an excellent opportunity to study responses to viruses or tumor antigens that are predictably successful or unsuccessful. Knowledge of the mechanisms initiated in a successful response to virus and/or tumor antigens will find direct application in vaccine development, and hence improved health of avian species. Moreover, the ability to examine developing tumors in AR and AP chickens provides insight into events that lead to the natural regression and elimination of the cancer tissue. Although there are lines of chicken similar to AR and AP lines, the type of studies described in Brant's paper has only been conducted in our laboratory (manuscripts in preparation). Brant's project has added a new dimension to our research efforts in that v-src DNA was used as a tumor inducing agent, resulting in tumors in the absence of a virus challenge. Although, the incidence of tumors in the AR line of chickens,

known to mount an effective immune response against tumors, was low when v-src was used, this finding in itself is novel and will open a new venue of studying aspects of tumor prevention and elimination. The discovery of previously undefined cell-types in the avian system will lead to further studies on the mechanisms leading their development, their localization in tumor tissues, and their role in tumor regression. Brant's contributions to our research efforts and those within the field of avian immunology are highly significant.

Nick B. Anthony, Professor of Poultry Genetics, writes about Mr. Ward's research:

I consider Brant Ward's work to be an outstanding contribution to our understanding of poultry immunology and tumor immunology.

Brant is conducting his Honors research project under the guidance of Dr. Gisela Erf who is my colleague and collaborator in the Department of Poultry Science. I am a poultry geneticist and have been maintaining the Arkansas Rous Sarcoma Regressor (AR) and Progressor (AP) lines of chickens since the retirement of Dr. Roy Gyles who originally developed these lines. When injected with Rous sarcoma virus (RSV) into the wing web, these lines of chickens develop tumors at the site of infection. The unique distinguishing feature between chickens from the AR and AP lines is, however, the ability of AR chickens to eliminate the tumor whereas AP chickens allow the tumor to grow. The importance and acceptance of these lines of chickens as animal models for the study of cell-mediated immunity and tumor immunology is demonstrated by the award of a grant from the USDA (Erf, PI) in support of this research area.

The studies conducted by Brant confirm the hypothesis that different immune mechanisms are called into action in animals able to regress tumors compared to those allowing the tumor to progress. Additionally, the fact that Brant was able to induce tumors with viral DNA (v-src) rather than the whole virus (RSV), revealed that different types and amounts of immune cells respond to tumor antigens (v-src-induced tumors) compared to tumor and viral antigens (RSV-induced tumors). Lastly, the observation of a new cell-type present in tumors (especially progressing tumors) of these chickens is a unique and important discovery.

ANALYSIS OF WIRELESS NETWORKING ON THE UNIVERSITY OF ARKANSAS CAMPUS

by Julia Lincoln
Department of Computer Sciences
Fulbright College of Arts and Sciences

Faculty Mentor: Amy W. Apon
Department of Computer Science & Computer Engineering

Abstract.

Wireless communication has already begun to change the way business and research is done today. Development of the wireless network for digital cellular phone has already improved the area of voice communications. The area of portable and mobile connectivity for computers and other devices is beginning to emerge. The research analyzes and compares a few of these wireless networks. Physical aspects such as range, interference factors, and frequency capabilities and restrictions are assessed. Capacity analysis including round trip times, latency, and throughput are done as well. Issues of authentication and addressing protocols are researched to determine optimal performance and convenience depending on the desired functions of a given wireless network. The research is done on three existing wireless networks on campus, each having unique configurations and physical attributes.

Julia Lincoln and Amy Apon

Introduction.

Wireless communication has already begun to change the way business and research is done today. Development of the wireless network for digital cellular phone has already improved the area of voice communications. The area of portable and mobile connectivity for computers and other devices is beginning to emerge. As the popularity of this technology increases, institutions are going to have to make decisions on many factors when determining if and which of these technologies are right for them.

The research analyzes and compares a few of these wireless networks. Physical aspects such as range and interference factors are assessed. Capacity analysis including round trip times and throughput are done as well. Issues of physical implementations are researched to determine optimal performance and convenience depending on the desired functions of a given wireless network. The research will be done on three existing wireless networks on the University of Arkansas campus, each having unique configurations and physical attributes.

Wireless technology works much like it sounds. Instead of physically connecting machines by various combinations of cables, connectors, and bridges, the machines contact each other or the bridges through the air. Much like radio transmission, wireless networks operate using some form of an antenna and receivers. The data is sent via electromagnetic waves to a receiver that then translates the waves back into data.

In the current standard, there are three physical characteristics that categorize wireless networks: diffused infrared, direct sequence spread spectrum (DSSS), and frequency hopping spread spectrum (FHSS). The infrared operates at different bands and has its own set of limitations that are much different from the spread spectrum types. Spread spectrum is used to avoid noise interference that can occur if data was only sent on one frequency. It utilizes different frequencies within the band to avoid the interference. Direct sequence spread spectrum (DSSS) takes the base signal and replaces it with calculated blocks of fixed length codes. DSSS uses multiple frequencies in the band to transmit data, but it only uses one pre-selected frequency for each transfer. Frequency hopping spread spectrum (FHSS) uses up to 80 frequencies to transmit. The signal will start on one channel and then after a designated amount of time, it will "hop" to another channel. The spread spectrum types of wireless implementation are the focus in this paper.

There are two main terms that describe how much and how fast data can be transferred on a network, bandwidth and latency. Bandwidth is the capacity or volume of data that can be sent. Throughput is measured bandwidth and is often referred to in Kbps (kilobits per second), Mbps (megabits per second), or Gbps (gigabits per second). The frequencies used for wireless networks at this time support 1 Mbps and 2 Mbps capacities. Latency can be thought of as the speed of a network. Latency describes how fast the data can be transferred over the network. Latency is referred to in sec (seconds), msec (millisecond; 10^3 sec), usec (microsecond; 10^6 sec), or nsec (nanosecond; 10^9 sec).

The first network analyzed is on Ozark Hall. This configuration consists of a bridge that is physically plugged into the Ethernet in Ozark Hall. On top of the roof there is an 8dBi omni-directional antenna that is wired to the bridge. The receivers are wireless modems that fit into a laptop computer. The laptop can connect through the antenna and bridge to the campus network when it is within a 700' radius of the antenna. This radius is affected by physical interference such as trees, buildings, etc. The connection is limited by line-of-sight. If the receiver is not able to "see" the antenna, then it does not transmit. It uses direct sequence spread spectrum. This wireless network has a 2Mbps capacity and operates in the 2.4GHz frequency range.

The second network is a point-to-point network based from the Graduate Education building to the Speech and Hearing Clinic across Arkansas Ave. There is one parabolic, 23dBi antenna on each building that is connected to a bridge inside, much like the first network. The machines in the Clinic are physically wired to the bridge. The bridge in the Graduate Education building is physically connected to the campus ethernet. This network operates only on a point-to-point basis. It sends from one antenna to the other, using direct-sequence spread spectrum. The network has a 2Mbps capacity and operates in the 2.4GHz frequency range.

The third network is a lab in the first floor of the Science and Engineering building. The network consists of two antennas and three stations. The stations in this case are three AI (artificial intelligence) robots. The antennas are connected to the campus ethernet. A server machine is used to guide the robots at this time, transmitting the data via the wireless connections. This network uses frequency hopping spread spectrum and operates in the 2.4GHz range.

The tool used to test each network's latency was ping. This program is part of the Windows 98 and Linux operating systems, which were the operating systems on the machines tested. The ping program sends a message of specified size from one host to another host machine and times how long it takes from the time it leaves the first host to when the message returns to the first host. This time is called the round trip time (RTT).

For all of the networks, the ping is repeated ten times at each message size interval and the average of those ten pings is taken as the RTT for that message size. As the message size is increased, the RTTs are expected to increase as well. In an isolated environment, the RTTs can be expected to form a relatively smooth curve when graphed. Outside factors such as other network traffic and interference can have an effect on the RTT, however, causing inconsistencies in the RTTs.

The antenna on Ozark Hall was tested first. Pings were conducted between the wireless laptop and a machine, Comet, on the campus network. The only traffic that would alter the RTTs during the primary testing was from any regular traffic on the campus network that might slow the switches' response times. The results were very close to the numbers expected. (Figure 1). The testing was repeated on the same configuration but with traffic purposely introduced between the laptop and comet. Music files were played on the laptop that were physically located on comet. This ensured a continuous stream of data being transferred between the two hosts during the duration of the test. The inconsistency of the numbers reflects the interference caused by the streaming traffic. The longer RTTs as compared to the first test also reflect the added traffic. (Figure 2). The next test was performed on the network between the Graduate Education Building and the Speech and Hearing Clinic. The RTTs were expected to be higher because the messages would have to travel a farther distance to reach their destination with more traffic using the same wire. (Figure 3). The relative closeness of the results between the first two networks is expected. They both use DSSS and have 2Mbps capacities. The point-to-point antennas must transmit over a farther distance. The final test was done on two of the AI robots. When compared to the first two networks, this network had higher RTTs on the smaller messages, but the RTTs did not increase as much as the message size increased. (Figure 4). This can be accounted for due to the difference in spread spectrum methods of the wireless networks. The last network uses FHSS as opposed to the DSSS used on the other two networks.

In conclusion, the wireless networks on the University of Arkansas campus are similar in their speeds and capacities. The physical configuration of each network provides advantages for differing situations. The range of needs met by the networks provides good groundwork for determination of the expansion of use of wireless networking on campus. As the technology advances with wireless networking, I feel that the advantages of wireless networks will very soon outweigh the disadvantages.

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Ping times for the Point to Point Antennas
Figure 2

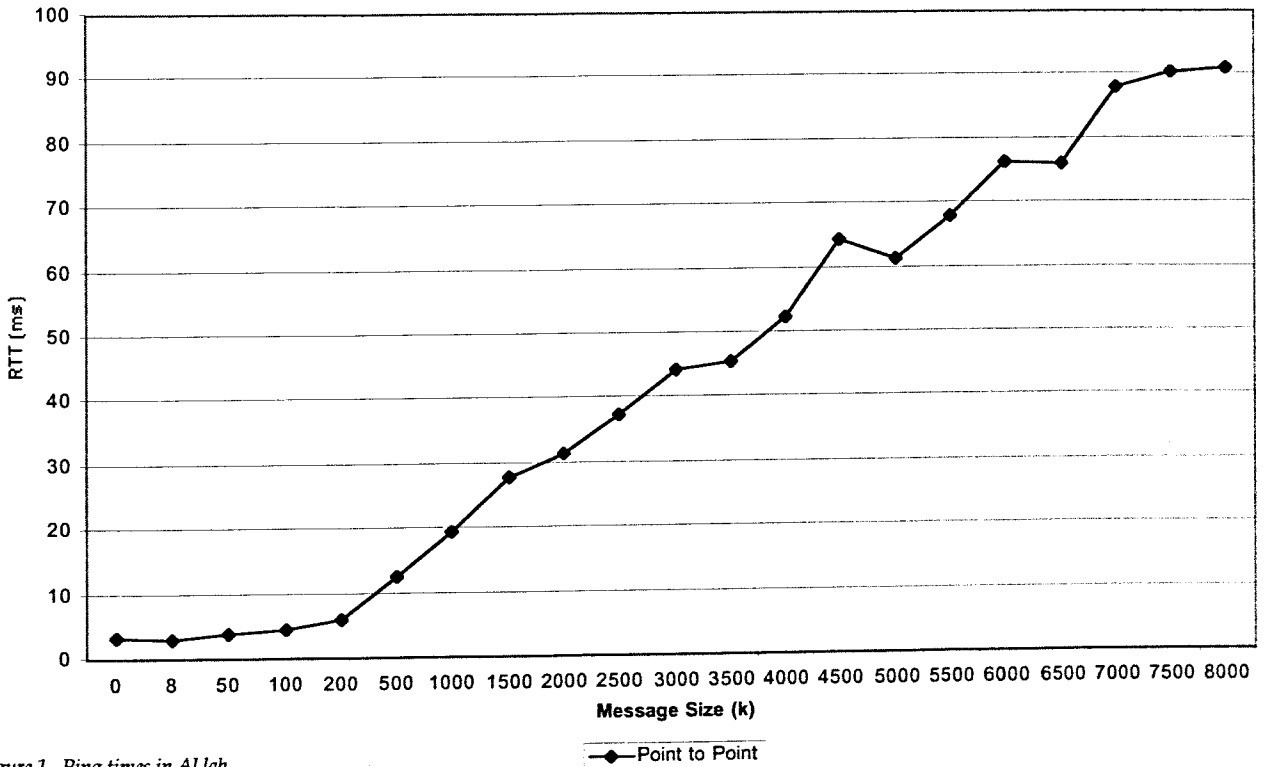


Figure 1. Ping times in Al lab

Faculty Comment:

Dr. Amy W. Apon, Assistant Professor of Computer Science & Computer Engineering, and Ms. Lincoln's mentor remarks:

Julia's project has been to systematically evaluate and compare three different wireless local area networks that are currently installed at the University of Arkansas. Julia has used standard tools to measure the latency and bandwidth of the networks. Latency is the time that it takes for a message to be sent from end to end, and bandwidth is a measure of the amount of data that can be sent at one time. Network engineers at the University of Arkansas can use this information in planning the use of future wireless networks on campus.

The perceived performance of any network can vary tremendously depending on the type of applications and number of computers that use the network. With a wireless shared Ethernet network, as the number of computers attached to the network increases, the contention on the network increases. In this environment many messages must be sent repeatedly so that the performance of the network decreases. Even if only two computers send, but each sends data frequently, the performance of the network can decrease. Julia has experimentally investigated the performance of the three wireless networks under various operating conditions that are expected to affect the network performance.

Wireless networking technology is moving rapidly into network installations, but wireless technology presents new challenges for network engineers and administrators. Many performance and architecture issues of wireless networks in a real operating environment are incompletely understood. The availability of hardware to test and Julia's energy in approaching this project have provided a nice opportunity for an undergraduate to contribute to the understanding of wireless technology for local area networks.

DESIGN, IMPLEMENTATION, AND EVALUATION OF VIRTUAL INTERFACE ARCHITECTURE FOR POWER PC MACHINES

by Ben McKenzie

Department of Computer Science and Computer Engineering

Faculty Mentor: Amy W. Apon

Department of Computer Science and Computer Engineering

Abstract.

The Virtual Interface Architecture (VIA) standard is a low-latency protocol that was designed for use in high-performance networks. VIA improves performance by reducing overhead in messaging. This research has two components. The first part of this research project is the development of a new tool for measuring the performance of a VIA implementation and comparing it to the more traditional high-overhead protocols used on the Internet. The development of the tool represents a significant contribution in and of itself, since the tool has been put into the public domain and will likely become useful by Linux users, both for measuring VIA networks, and as one of the first example codes available for learning how to write programs that use VIA. The new tool has exposed some interesting performance issues of VIA as the number of messages increases that are currently being examined. The second component of this research is the definition and development of appropriate interfaces from the network to the lowest level services in Linux on the Power PC platform, and the testing and evaluation of these functions. The research approach was to port a freely available implementation of VIA that runs on a Pentium platform to the Power PC platform. The architectural differences of the two platforms have raised a number of design and configuration issues that have been investigated and solved.

Ben McKenzie and Amy Apon

Introduction

The Virtual Interface Architecture (VIA) standard is a low-latency protocol designed for use in high-performance networks. VIA improves performance by reducing overhead in messaging. [1] Several tools exist for measuring performance but are unreliable and do not provide accurate evaluation of communication times. [3]

This research investigates and improves the performance of cluster computing. There are two major goals to this project. The first goal is to define, develop, test, and evaluate appropriate VIA interfaces from the network to the lowest level services in

Linux on the PowerPC platform. The second goal of this research project is the development, testing, and evaluation of a new tool for measuring the performance of a VIA implementation, and comparison to more traditional high-overhead protocols used on the Internet. Finally, the research presents ideas of future work which will come from this project.

Background

The background of this project deals with two areas of research. These areas are the history and origins of VIA and techniques of network benchmarking.

VIA is a protocol for system area networks designed by a consortium of industry and researchers including Intel, Compaq, and Microsoft. VIA specifies a low latency zero-copy messaging system designed for high speed, reliable network architectures on system area networks. System area networks, or SAN's are networks in which the nodes, or individual computers, are physically close to each other and are physically and virtually isolated. Typical SAN's are devoted to a single application and have little to no interfering traffic. SAN's tend to be much more reliable and faster than a local area network or LAN. LAN's are commonly used for larger networks such as a campus or building networks. A SAN is the most common type of network for cluster computing. Cluster computing is using a group of computers collectively to work together and solve a problem.

VIA is designed to take advantage of sophisticated hardware platforms that run on a SAN. The native implementation of VIA supports Direct Memory Access (DMA) engines that exist on network cards. A DMA engine allows data to be transferred from the memory of the computer to the network card and then to the network. A DMA engine also allows incoming data to be transferred directly to the computer, or host's memory.

With the network card doing the memory transfers, the host computer is not required to perform costly memory to memory copies.

Locking memory is an operation that interfaces to hardware at a very low level and is not usually performed by user applications. For DMA operations in VIA to work correctly, memory must be "locked". [5] This means that the data will not move to a different physical location during the life of the program. This results in more memory being used during the execution of a program than would otherwise be necessary. Optimally, some of the extra memory could be used for computation. The reason that the memory must be locked is because a physical address of memory is passed to the DMA engine. The DMA engine looks at that physical memory and retrieves the correct data from that physical location.

VIA is designed as a connection-oriented point to point protocol. In this protocol, each Virtual Interface (VI) must connect to another VI and be completely connected before sending any messages. To send to more than one VI from a particular process, multiple connections must be set up within that process.

The industry support of VIA is helpful in assuring that the protocol won't fade into obscurity. Many researchers and industry representatives believe that VIA represents the future of cluster computing. Oracle and Informix both have versions of their database products that rely heavily on VIA. Many high end networking hardware vendors are starting to incorporate support for VIA in their products. There are also several research institutes working on VIA, creating products such as M-VIA and

Berkeley VIA. There are also versions of the Message Passing Interface that utilize VIA for their messaging, such as MPI-PRO, [4] MVICH, [1] and MARK. [2] Message Passing Interface is one of the most commonly used clustering packages.

Existing tools for measuring VIA and TCP are TTCP and vpingpong. TTCP is a benchmarking tool for UDP and TCP performance between two computers [3]. VPingPong is a tool included with M-VIA that is also used to measure the round trip time between two VIA enabled computers [1].

Network Benchmarking

Both VPingPong and TTCP use system calls to access the current time. These calls result in context switches which affect the measurements. They are also two separate programs compiled with different switches. Further accuracy is affected by these programs having different program sizes, different memory allocation approaches, and having different numbers of instructions.

TTCP and VPingPong were used as a baseline for a new testing tool created for this project known as TTCP-VIA. This tool allows "ping pong" testing between two different machines to get a round trip time for a single message. A ping pong test is a good measure of real network speed as it uses a send and receive on both sides. In a ping pong test, host 1 sends to host 2, host 2 receives, host 2 sends to host 1, and finally host 1 receives. The TTCP-VIA tool uses the processor's cycle counter to obtain measurements with microsecond accuracy, a minimum of overhead, and no context switch.

Porting VIA to Power PC

The second goal of the project was to port a version of VIA originally developed for the Intel architecture, to the PowerPC architecture. The baseline chosen for our version of VIA is M-VIA, a modular implementation of VIA available for Intel Linux machines made by the National Energy Research Scientific Computing Center. [1] This implementation was chosen as it allowed for ordinary Fast Ethernet cards to transmit and receive information over VIA.

The problem of not running successfully is greatly heightened by the fact that this code is running in kernel space with direct access to memory and has a very high priority. So, great care must be taken to write the code correctly, or it can and will crash the operating system. The code must also be tested extensively.

There are many issues with porting the M-VIA software to PowerPC machines. One of the critical issues faced was none of the Fast Ethernet drivers included with M-VIA work with PowerPC machines. Second, there is assembly code included in the M-VIA code included to optimize performance. Assembly

code is inherently not portable since all assembly code is machine specific. The final issue confronted is that the Pentium is a little endian machine and the PowerPC is a big endian machine. In a big endian machine the leftmost bytes are the most significant. In a little endian architecture, the rightmost bytes are the most significant. [6]

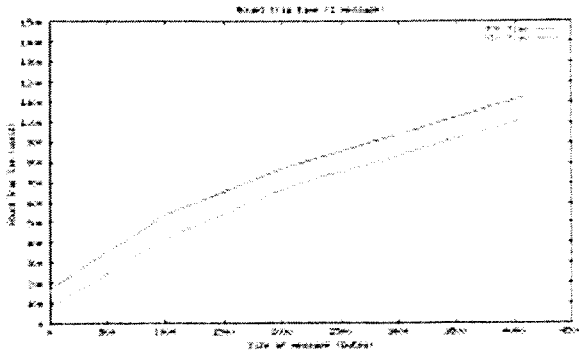
The assembler code contained in the M-VIA code was not necessary for our implementation on the PowerPC. This code is used for what are known to the M-VIA authors as fast operations. Basically, this just allows optimization of the various VIA operations. This code can be bypassed to allow a working but not necessarily entirely optimal version of M-VIA for PowerPC's.

The way that the endian problem was fixed was to typecast the constant values. At compile time, the strings are converted from constant strings to real values stored in memory. Real data values automatically change to the correct architecture form.

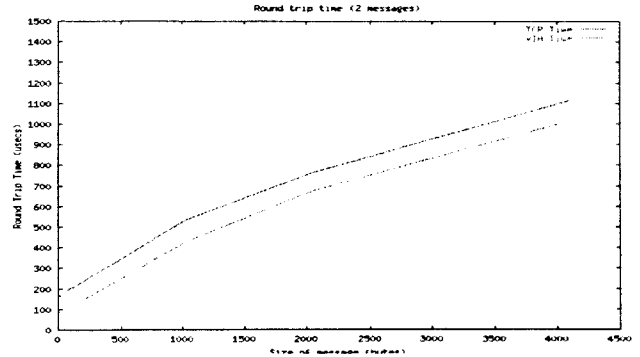
The problem of not having an Ethernet driver is made more simple given the nature of the Linux operating system and the fact that the M-VIA project is an open source project. There are several examples of similar network cards available and these were used to create a new driver. In addition, an existing open source Ethernet driver for this particular card was used as a starting point.

Measurement Results

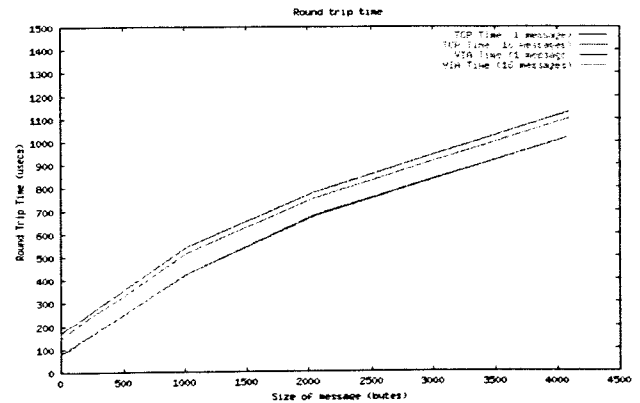
Using the TTCP-VIA tool, we have been able to obtain preliminary performance results on our existing cluster with Intel Linux based machines and have compared the performance of emulated M-VIA to TCP.



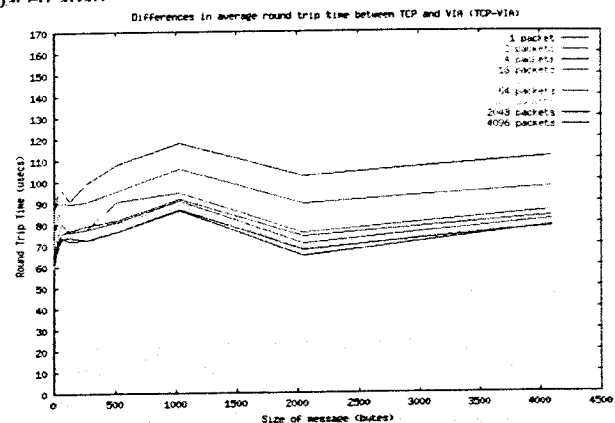
When looking at the data from graph one, the round trip time for a 1 byte message for VIA is 87 microseconds, and for TCP it is 178 microseconds. So, the time to send a 1 byte message is a little less than half the time to send a TCP message for very small messages. This remains true until about 512 byte messages, where the VIA time is 253 microseconds and the TCP time is 263 microseconds. At the largest measurement of 8192 bytes, the VIA time is 1707 microseconds and the TCP time is 2117 microseconds.



Graph two shows very similar results to graph one. All graphs represent average times obtained by dividing the results by the number of messages, so the numbers are more similar to each other. The difference in times for a 4 byte message is 87.25 microseconds/byte for VIA and 156 microseconds / byte for TCP. The difference between the two is a little bit lower than the times for a 1 byte messages, but not much. As you can see the VIA messages are still about twice as fast as TCP messages for very small sizes and for large sizes are still better.



In graph three, TCP's performance inconsistency is shown. The graph shows that the VIA times are very close together, appearing to be one line. The TCP lines, on the other hand, are spaced apart.



Graph four shows the differences in average round trip times between TCP and VIA times. This graph subtracts the TCP time from the VIA time. Notice that the performance difference is greater when sending one message than when sending 4096 messages. This once again shows that TCP performance is inconsistent, and actually changes when sending large numbers. The maximum in the graph at about 100 bytes is due to the change in TCP from using one internal message buffer to using two. The change at around 1200 is due to the Ethernet frame size (1500 bytes).

Conclusion and Future Work

As is shown in the experimental results, there is a lot to learn about network performance measurements. The technique of repeatedly sending messages and dividing by the number of messages sent can result in biased results. Techniques this thesis presented can help to fully evaluate network performance.

With a version of M-VIA created for PowerPC, many new avenues are open for using PowerPC's for clustering. There are some types of hardware which will only work on PowerPC machines. Plus, this will broaden the influence of VIA, and expand its usefulness.

The results of this thesis show many areas for future work. First, a version of VIA will be developed to run on AmpNet, a gigabit speed Fibre Channel based network created by Belobox Networks. [7] Also, areas of VIA have been identified which could be extended to achieve further functionality such as multicasting and broadcasting of messages. Another future goal is to implement a version of MPI that runs over VIA [2].

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Faculty Comments:

Mr. McKenzie's faculty mentor, **Dr. Amy Apon**, says about his project:

Ben's research examines how to increase the performance of applications in a cluster by optimizing the messaging software. A cluster is a computing system that consists of a collection of interconnected

whole computers. The messaging software that Ben is working with conforms to a new standard, the Virtual Interface Architecture (VIA). The VIA standard was published in December, 1997. VIA reduces overhead by interfacing with the operating system at a very low level.

Ben's research project is divided into two components. For the first part of his research project, Ben has developed a new tool for measuring the performance of a VIA implementation and comparing it to the more traditional TCP/IP protocol. Prior to Ben's work, a tool did not exist that fairly compared VIA and TCP/IP. The development of the tool represents a significant contribution in and of itself, since the tool has been put into the public domain and will likely become useful by Linux users, both for measuring VIA networks, and as one of the first example codes available for learning how to write programs that use VIA. Ben's tool, *ttcp-via*, has exposed some interesting performance issues of VIA as the number of messages increases that we are now currently examining as an extension of his thesis. The second component of Ben's work is to define and develop appropriate interfaces from the network to the lowest level services in Linux, and to test and evaluate these functions. Specifically, Ben is taking a freely available implementation of VIA that runs on a Pentium platform, M-VIA and porting it to a PowerPC platform. The architectural differences of the two platforms have raised a number of design and configuration issues that Ben is having to investigate and solve.

Dr. David Andrews, Head of the Computer Science and Computer engineering Department, comments:

Mr. McKenzie is one of the Computer Science and Computer Engineering departments brightest students who is at the beginning of a very promising professional career as a computer scientist. This effort is ideal in fostering Mr. McKenzie's growth by providing him the opportunity to enhance his traditional academic classroom requirements with in depth self directed research in support of communications protocols and implementation for cluster computing. Over the last decade, clusters, or networked collections of computers, have come to be used for many high-performance parallel applications, and have helped to redefine the concept of supercomputing. Cluster computing is particularly relevant for high performance applications used by industries in Arkansas. For example, Axiom Corporation is investigating the use of clusters for their high performance database queries. Wal-Mart Corporation is one of the largest users of data mining in the world, and cluster computing is an ideal approach for speeding up the processing of the enormous amount of data that is collected by Wal-Mart each day.

In addition to allowing applications to execute faster, cluster computing is also being used to build highly available systems. In a highly available system, when a component fails, the workload is redistributed to other components allowing all applications to run without interruption. In the particular case of running a database on a cluster, the system will not lose or damage data when a device fails. For these reasons, virtually all database vendors now target a clusterbased product for systems that require high availability.

Mr. McKenzie has established an impressive academic record within the department, achieving a 3.8 GPA. Mr. McKenzie has the necessary background for this work having enrolled in Dr. Apon's Concurrent Computing class and has traveled to California to learn and work with the company providing the experimental hardware and software that is used in this project. Mr. McKenzie's capabilities can also be seen by the company's' current interest in recruiting him to work after he has completed his education here at the University of Arkansas. capabilities.

