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# From the Editor

Dear Readers,

As many graduating seniors turn in their last research papers, or finish their last design seminars, we have, over 4 years, acquired a set of tools more powerful than any specific knowledge from their respective classes. The ability to reason, research, and formulate our own questions is invaluable, regardless of what path we take after college. While we continue with our ever-specializing educational training – formally or informally – we must be vigilant that we do not detach ourselves from a larger social context. With the opportunities afforded by this institution, we are obligated to use our skills to affect change from the local to international levels.

This second edition of the *Journal of Undergraduate Research* (*jur*) is grounded in an academic context, but demonstrates some of the relevant methods and tools that will be helpful beyond the college setting. Even though scholarly vernacular certainly has its place in academia, the style presented in this journal has been designed to reach a diverse audience while being mindful of placing it in a larger context. This issue again offers a broad look at undergraduate research interests ranging from Linguistics and Maths to Neuroscience and English.

Encouraged by the reception of our last issue by students and faculty, we hope that this issue will continue to inspire deeper and broader inquiry. An increase in interest has allowed our staff to rapidly expand, as well as encouraged high quality research submissions.

Again, we invite the University of Rochester undergraduate community to contribute their original research, letters, or reviews to the Fall 2003 issue of *jur*. Also, we are actively looking to expand the *jur* staff. Please e-mail *journal@rochester.edu* or visit our website *jur.rochester.edu* for further information.

Sincerely,

Prabhjot "Jot" Singh Dhadialla '03
Editor–in–Chief

# Faculty Interview

## *jur* gets a lesson in medieval history from Dr. Thomas Hahn, English, and Dr. Richard Kaeuper, History

A medieval artist's depiction of siege warefare, common during the Crusades.

**jur:** Why study medieval history?

**Kaeuper:** Well, we study medieval history because it's the origins of our own society, in many ways that are obvious but also some that are not. We have a world of universities, government, law, vernacular languages, etc.it is our parent civilization, and yet it's also a world that's so very different from us. It's a hierarchical world, a world that's religious in ways that the modern world is not – you can see what connects us and what forms us, and yet still step outside when looking at the medieval.

I think that's a very important point – one of the benefits of studying any old period is that it gets us outside of the present. The danger is that we might begin to think that the world has always been the way it is, and that the way we do things is natural and inevitable. I fear that modern students think that democracy is the natural form of government and that if you remove the restraints from people they will come to democracy, naturally. I think that's as far from the truth as anything I can imagine, and I say that not because I'm opposed to democracy but because I value it so highly. It's something that's so carefully crafted, so getting back to a time that people naturally thought not in democratic terms but hierarchic terms I think is tremendously important.

**Hahn:** So at the risk of there seeming to be a party line I have to agree precisely; my answer would have been framed in exactly the same way. It does seem to me that we oscillate betweens these notions of same and other. As we look at the past and we look at its surviving artifacts, one of the things we need to do first is get some sense of how these things made sense to their original audiences and producers on their own terms, but also how we are motivated by our own interests. It is those interests that we can best relate to in the 21st century, and that relation allows us to make sense of that past. It seems to me that we're continually in this dynamic process of both trying to see the past and its artifacts, its productions, and the experience of the people living then in its own terms, but also to see them in terms that serve our interests right now.

I think the one disciplinary difference that I'm struck by is the strong emphasis on institutional and public connections that Dick made. One of the things that separates English from history is that I would probably end up talking about things much more in terms of a focused study on particular artifacts, stories, texts, circulations of manuscripts, whatever. And of course I want to see them in some larger sort of systems, but I think that it's a kind of macro-micro vision here where literary studies is much more focused on particular subjects while historical study on larger patterns and institutions.

**jur:** What are your personal attractions to the material?

**Kaeuper:** I guess I was attracted to the color and romance of the medieval past. It's what attracts so many of us – it is other, and bright, and pre-Raphaelite, and all these other things. But as a historian, to underscore Tom's point, I always think that in studying the Middle Ages, one can see a civilization put together, and it's almost in slow motion. The modern world goes so fast, but time on a medieval scale is more relaxed. Sometimes you come upon something remarkable and think "Ohhhh, look at this," in a positive way, although also sometimes in a negative way. I mentioned the latter because I don't romanticize the Middle Ages at all. I don't wish to. Sometimes students say to me, "You should see this film, it's really dirty." They don't mean scandalous, they mean it's full of dirt that would imply realism, because they know I don't have a romantic view of the Middle Ages anymore.

We can see aspects of the world we live in really taking form. You can watch the constituent elements of the civilization, and then you can watch what kinds of choices and sacrifices are made, what kinds of problems emerge, and what kinds of oppressions take place in order to have the evolution that goes on. Anyone who takes my courses knows that I think we've got our historiography quite wrong, that the really fruitful and creatively building time in European history is just as likely to be found in the 12th century as in any other century with names like Renaissance attached to it.

I like these people, I wish I could get closer to them, and that is one reason that I turn to a lot of literary sources because we lack the memoirs and day-to-day kind of evidences from the Middle Ages that historians of later periods can rely on.

**Hahn:** In some crude paraphrase of Marx, "People choose their careers, but not in circumstances they have any control over." I think I have a sort historical curiosity that stemmed from a lot of language study in Catholic school. I think that there was also a strong emphasis on continuity there, and the more I became interested in the Middle Ages the more I began to think about differences between then and now.

I think the thing that continues to attract me to it most, however, is that Medieval Studies, in a way that stands out from other disciplines, has been interdisciplinary. This has been true for the last 75 years or more since the founding of the Medieval Academy. In this way you can take a look at something that survives from the Middle Ages and approach it through a whole series of interdisciplinary perspectives, looking at not only its connection to religion or art, but its economic situation, what kinds of material conditions motivated its production, and so on.

I think we study these things because it is so difficult to recapture a past that is so removed from us, and particularly in the Middle Ages because there are so relatively few sources – we have to scramble to be able to make sense of the past. It's almost as if people who work in more recent periods are overwhelmed by the amount of data they have to look through in archives or elsewhere. Now, I don't want to say that we are so lucky not to have more information; I would indeed like to have more information, but not having it is actually one of the things that produces a very inventive response to the medieval past. In many ways, I think that as my own history as a teacher and a scholar at Rochester has unfolded, it's been a history of finding more and more ways of how to inventively think about the past.

**jur:** What kind of questions about medieval material do you most frequently deal with?

**Kaeuper:** Well, I'm always trying to understand how this society works, and in that I follow in what Tom just said about the past being forever closed: it's like putting together a puzzle with lots of pieces missing, and there's a fascinating element of detective work in that, sometimes at very precise levels with very particular questions and at sometimes very general levels with very large questions.

I started out asking how medieval British government financed itself and basically went from there. I was surprised in that work to find out how small the escorts were for such large sums of money, which led me to ask, 'What's the status of public order?' My assumption was that it must be pretty good because the king is shipping around king's ransoms with very small forces, but there were a lot of things I didn't take into consideration, and my assumption ended up being wrong. In the search for an understanding of public order, I discovered that public order was in crisis in the later Middle Ages, a subject that Tom and I have taught together in classes that we've done together.

From there I thought, "Well, where's the crisis of public order in France? Is it something structural, is it peculiar to England?" That took me across the channel, and I started asking comparative English-French questions, a thing that's very hard to get medievalists to do, I think. It's unfortunate that there's not been a lot of comparative work because suddenly things snap into focus when you get two societies as parallel as England and France in the later Middle Ages. They shared two high cultural languages, Latin and French, and though the French of England was going off in a different direction, the societies are linked by marriage, trade law, war, etc. Those connections, however, haven't been closely studied, for the most part.

In studying public order I got interested in chivalry, which I believe was a very mixed force in public order, and then looking at chivalry led me to questions of popular religion, because "Can one imagine Western chivalry without the infusion of religious ideas, regardless of what happens to them once they arrive?" is a very interesting question.

So there's been this trajectory of one question leading to another, and I think that's one of the wonderful things about our work - you just keep asking questions. A former neighbor met me in a supermarket a few years ago and said "Hi, I bet you're still doing medieval history," and I said "Yep, I haven't solved it all yet." In one sense, I think the questions are sort of eternal, over and over again, I don't think we always have to ask questions, or formally do, but as Tom said, finding new ways to come after questions. I always tell students "You've found a good topic when you ask 'What's the basic book on this,' and you discover there *isn't* a basic book on it, and it's a question that seems so important that you wonder where it is."

**Hahn:** I'd start with the contention that knowledge is social, that it's something that inhabits living bodies, and from there one of the questions then becomes how to do justice to the lives of the people of the past. But we must recognize that we have to sustain a dialogue with our own students, with our colleagues, and some wider world that we reach through scholarly publication. It seems to me that one of the things that one continuously negotiates, at least in literary studies, is this sense that one wants to do justice to those lives, and to the documents that express the experiences of the people of the past, and yet at the same time recognize that the questions that we ask really arise out of the kinds of dialogues we're having right now, not only with our students but also with our colleagues, both present and in a wider world.

I've just recently finished an essay on Arthurian romance that took me much longer than it ever should have. One of the difficulties with the subject is that it's just so big – Arthurian romance is everywhere. But also, part of the purpose of the essay was to talk about what's "new" in Arthurian romance in the last quarter century, and I wound up talking about this notion of "Arthurity," that is, the way in which earlier scholarship imagines that there's almost a literary DNA, Arthurness, as it were, and that you can find this in some particular

The funeral of Raymond Diocries of Notre-Dame de Paris.

sources. However, it seems to me that this notion has been displaced by some much more historically acute sense that stories of Arthur are produced with these particular interests of constituencies in mind. So, what ends up coming across in Arthurian romance is not a portrayal of a previous mythos of Arthur but rather a reinvention of it; each reader who comes to the text, including readers of the 21st century, are in this process of reinventing. The sense of reading these texts against the experiences of generations of readers right down to our own time seems to be one of the things that I find myself thinking about and wanting to write about pretty continuously.

**jur:** In writing on medieval topics, what sort of sources make up the bulk of your research?

**Kaeuper:** Well, the sources of course depend on the questions asked, and I'm always urging students to remember that questions that aren't asked cannot get answered. I'm overly fond of quoting Frederick William Maitland, the great Victorian legal scholar who said, "If we can only ask the right questions, we shall have done something toward a good end." So, you get your questions lined up, then you go for the sources. Some questions can't be answered, and students find that very discouraging. "But I want to know this," they'll say; well, it's very hard to know that because you probably can't get the evidence for it.

I began by using government records, parchment records from the public records office, but more recently I have turned to medieval literature as a historian, and it's been the link between the Tom's and my work, because as I've said, we lack other kinds of sources. I think that these texts were written by writers for patrons with points of view, and trying to understand what those are is another important thing to do. I think there's spin in this, there's ideology, there's valorization and demonization going on, so I teach courses, as does Tom, in which we try to get back to the issues of real people in the Middle Ages by using these texts. A student said something to me once that I liked very much: "You don't want to look at texts, you want to look through them." Guilty as charged – I always imagine these texts as great sheets of glass, and that I'm looking back through them; there are real people back there, and sometimes they're doing things that we're troubled by. But I want to look through these texts to the world that produced them.

I think one of my beacons as a historian, however, is to hew to evidence in the most precise way possible. That's why I'm less enthused about heavy theorizing because I think there's a danger of it turning out to be painting by numbers – you can decide what you're going to find, and that danger is always present in scholarship. The riposte to that, of course, is that you're fooling yourself if you think you're objective, but I think we can try very hard; I don't want to throw out the baby with the bathwater, because while I think that texts have spin and are representative of points of view, that's really what I'm after in a text. I've started into more than one subject with one point of view and the evidence has turned me to another point of view, and so I don't think that my views are just pure subjectivity, and that's probably true of most views. I think there is an irreducible element of close scrutiny of evidence that may change your mind, and without that, I don't see how we're writing anything other than propaganda, if we're not insofar as we are able as human beings responding to real evidence and looking at it carefully and as objectively as possible. A student said to me once, "Look, I know what's right, I'll just select the evidence to support what I know is right." I said, "What would you say to me if a Nazi propagandist said that to you?" That's crude, but it's clear that the evidence changes with the questions asked, and I hope that we can keep asking fresh questions, or freshly asked questions, and looking at new evidence.

**Hahn:** One of the projects I'm working on right now is the first book in English on the New World. This is a very small book, published in Antwerp by a minor printer, one that no sane literary scholar would consider to be a literary text. It reminds me when I attend to such things as this book that it is quite a scramble for us people who deal with earlier fields to find ways to make these texts interesting; it's not simply to decipher or read through those texts themselves in terms of the interests that motivate them, but also to decide how to try to engage people who are alive right now in my classes and through my writing with these things that might otherwise seem uninteresting.

Part of what's interesting about this is to try to figure the ways in which this text feeds into the kinds of literary and cultural traditions among the things that interest me. One of those ways is in the sense of continuity, that is, we've got here a text that's primarily about novelty - the book's title is "Of the New Lands" - but it also includes several other medieval texts. So, to try to make sense out of what a text published around 1510 is doing in relation to these medieval legendary stories and a mass-produced audience reading in English while having been published in Antwerp, all of these things come into play in trying to offer a historical and cultural explanation of why this book is important. Part of what interests me here is to look through these texts at the motives that drive their publication, to look at their originals, and to look at other languages that this printer was working from.

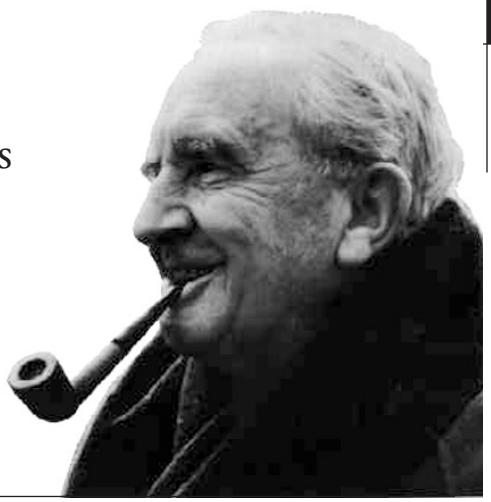
Even in deciding to publish such a book, there's obviously a kind of spin on the printer's side: he's thinking that there's an audience in England, he's going to set this in type (a very labor-intensive thing to do), he's going to produce X number of copies, and then he's going to take them over there and try to sell them. In any case, it seems to me that this business of attempting to both decipher spin but also to put some spin on this in terms of making this an interesting text is also worth talking about. For example, when one embarks on a project like this, to think of this in the crudest possible materialist 21st century terms, you have to convince a publisher that this is worth doing. Just saying, "Hey, I found this old book which nobody seems to be interested in, would you like to publish it?" will usually get an answer of "No." So, we need to find out what kinds of perspectives we can bring to it that actually suggest why it was important in its own time and why it remains important to think about it now; for example, in terms of this book, how does it respond to (and challenge) current global studies arguments? Those are the things that we are continuously thinking about, and thinking about in terms of our daily classes: that is, how do you walk into a classroom and convince the people sitting there that they need to take their precious time and energy and devote it to the things we're obsessed with and care about? □

Freshman Scholarship:

# Fairy Stories

## A psychological examination of the dual audiences for fantasy literature

Laura Cantone, 2006
Advisor: Michael Livingston
Department of English



**A glimpse of how psychology can explain the appeal and continuity of fantasy literature.**

Although fantasy literature as a genre is usually thought of as having children as its core audience, there is a good case to be made to the contrary. The natural source for the opposing argument would be a fantasy writer such as J.R.R. Tolkien, especially given that he has written on the question of child versus adult audiences for fantasy literature as well as having written his own famous fairy story, The Hobbit. However, another source can be found by looking to psychology. Since the time of Tolkien's writing, a number of psychological theories have been conceived that serve to substantiate his hypotheses.

Tolkien's ideas on the topic are put forward in his essay called "On Fairy Stories," in which he examines these proposed dual audiences for fantasy literature. He acknowledges the common association of fantasy literature with children directly by saying that "it is usually assumed that children are the natural or the specially appropriate audience for fairy stories." [1] These associations persist into today, a hardly surprising fact given that most fairy tales are found in the children's section in libraries, and also that such stories are often made into children's animated films. But Tolkien questions whether there is "any essential connection between children and fairy stories," [2] and continues on to theorize that adults, in fact, have just as strong a draw to fairy stories as children do, if not stronger. It is this idea that Tolkien presents that has a number of psychological theories supporting it.

One such psychological theory, according to Saul Kassin's book Psychology, states that cognitive development "is not a process by which children merely 'copy and paste' what they are told."[3] Rather, the creation of intellectual notions occurs "through a complex interplay between preexisting knowledge and new information gathered through interaction with the external world."[4] It seems clear, then, that children can process thought with a degree of complexity, and so the many adults Tolkien alludes to in "On Fairy-Stories" are not completely correct in arguing that "it is easier to work the spell with children" because children immediately accept everything in the tales they are told.[5] The naïveté of children, according to Tolkien, is "an adult illusion produced by children's humility, their lack of critical experience and vocabulary, and their voracity (proper to their rapid growth)."[6] Children are intuitive enough to take the experiences from their real lives and juxtapose them against the make-believe events that occur in fantasy literature; they are similar to adults in their ability to distinguish between the two and accept fairy stories for their entertainment value rather than completely believe in them. As a result, authors can afford to put highly unrealistic events and characters into their stories knowing that both children and adult audiences will be able to accept the fantasy not for its authenticity, but for its enjoyable, outrageous nature.

While children may be capable of separating fact from fiction, that does not necessarily mean that they are capable of adult-level thought processes. Kassin explains that "even though concrete operational children (children between the ages of seven and twelve) appear to reason like adults do in response to specific problems, they do not think on an abstract level."[7] So, while many children will realize that The Hobbit is fictitious and are likely to take away from it the entertaining plot, they probably will not glean any more than that. Adults, on the other hand, may realize that there is much more behind The Hobbit because of their capacity for abstract thought. They may read it as a story stereotyping British aloofness, a tale re-defining the medieval hero, an object lesson about life, or even a unique representation of the Anglo-Saxon poem Beowulf.[8] Tolkien's characters and the situations they encounter are entertaining enough to sate the appetites of children, but his story is also sufficiently deep and creative to capture the interest of adults. Because children are able to reason like adults in their ability to sort fact from fiction yet are unable to think abstractly, Tolkien's tale attracts for both children and adults because it places adult concepts within an entertaining children's plot.

The concept of a dual audience for fantasy literature is further supported by psychological tests that equate fantasy to imagination. One such test, The Thematic Apperception Test, "requires the individual to create imaginative stories in response to a series of pictures; the content of his fantasy is then analyzed to reveal the underlying aims of his behavior."[9] The test operates under the principle that the imagination produces fantasy, so it stands to reason that imagination is the essential quality that readers of fantasy both possess and utilize. In The Hobbit one must imagine the fan-

Just as a fragile object left to a child is likely to be broken, a fairy-tale, likewise, will cease to exist if not fostered by adult guidance.

tastic worlds and characters that Tolkien creates, yet he does not overly develop his characters and settings so that they must be imagined in only one way. Instead, Tolkien allows each individual reader to personalize his or her own picture of Middle-Earth and its inhabitants. For example, the only physical description Tolkien gives of the dwarves is the color of their beards, belts, and hoods: "It was two more dwarves, both with blue hoods, silver belts, and yellow beards; each carried a bag of tools and a spade."[10] The author says nothing about the size of the creatures, who they are, or where they came from; he leaves much of the description of the dwarves and other characters up to the reader's imagination.

Tolkien intentionally omits details to stimulate the imagination but is not specifically targeting children when he does. While it is a common assumption that children are more imaginative than adults, many psychologists think that this is not necessarily the case. In his book The Child's World of Make-Believe, Jerome L. Singer sees "the young child's cognitive structure as limited with a corresponding paucity of fantasy life."[11] Singer says that children are too cognitively undeveloped to have a complex imagination, but he pinpoints a change in adolescents, going on to state that "the richness and elaborateness of the fantasies of adolescents point to the manifestation of their cognitive maturity."[12] In other words, while imagination is an inborn attribute, children do not fully develop it until adolescence or adulthood. It would seem, then, that there is a "cognitive skill of imagination" that becomes more refined with age.[13]

As a result of this development, fantasy appears to take on different meanings as people reach adulthood. The more abstractly they can think, the better they use their imagination and experience fantasy literature. Singer points out that the imagination grows stronger after childhood because the adolescent has had more experiences to draw information from and has more overall knowledge of the world: "The implication here is that imagination requires stimulation from the environment, peer contact, parental approval and contact, and adequate time and space, in order to flourish."[14] This leads Singer to draw the conclusion that "one way children might learn fantasy behavior is through observation of models."[15] Children eventually develop into adults who are able to combine abstract thought with imagination, in part because of adult example and guidance. For this reason, fairy-stories are passed down from generation to generation: the child first observes the adult who encourages a growth of imagination, then the child gains a more advanced "cognitive skill of imagination," enjoys fairy-stories on a deeper level, and becomes the adult model for future children. Because of this dependency on adults for the encouragement of an interest in fantasy literature, both children and adults play vital roles in assuring the future existence of fairy-stories. Fairy-stories must appeal to both audiences or the cycle of imagination will stop and fantasy literature may cease to exist.

Another venue in which psychology corroborates Tolkien's theories lies in the debate on nature vs. nurture. Joel Paris examines the conflict between the biological and environmental perspectives in his book Myths of Childhood, asking questions like, "Why are people so different from each other?. . .Does the explanation lie in nature or in nurture?"[16]In other words, are people biologically predisposed to act and think a certain way, or do life events and the role of the environment shape a person?He provides one explanation in saying that "the commonsensical answer to this question is, of course, both. . .Although each child begins life with unique and heritable temperamental characteristics, complex personality traits do not emerge de novo, but are shaped by a multitude of life experiences."[17] If Paris' ideas are compared to Tolkien's theorizing, the two are actually revealed to be quite similar:

> *In fact only some children, and some adults, have any special taste for [fairy stories]; and when they have it, it is not exclusive, nor even necessarily dominant. It is a taste, too, that would appear, I think, very early in childhood without artificial stimulus; it is certainly one that does not decrease but increases with age, if it is innate.*[18]

Should Tolkien have been familiar with the debate over nature vs. nurture in cognitive and emotional development, it seems that he would probably fall on the nature side, given his statement about a basic predisposition to fairy stories. However, Tolkien also points out that this inclination tends to increase with age, denoting a more nurture-based perspective. It follows from Tolkien's statement (and Singer's earlier one as well) that adults must have learned through environmental influences to cherish fantasy literature even more so than they did as children. That it is adults who insist on reading and adapting the stories for their children just as it is adults who foster the development of a child's imagination is cause to assume that the "taste" for fantasy literature increases with age as a result of encouragement by adults. While some parents might read the stories to their children with only the intent to entertain, personal adult interest is another reason for the survival of fairy-stories. Tolkien states: "Fairy-stories…cut off from a full adult art, would in the end be ruined."[19] Just as a fragile object left to a child is likely to be broken, a fairy-tale, likewise, will cease to exist if not fostered by adult guidance; what begins as a natural disposition develops through external encouragement.

Psychological theories can also provide some explanation for how unrealistic characters and actions in The Hobbit may be so easily accepted by both children and adults;

the theory of animism is one in particular that provides such an explanation. Animism is defined as a tendency to endow non-living (or made-up) physical objects and events with biopsychological attributes, such as life, consciousness, and will.[20] From the age of two to twelve, concepts having to do primarily with the external world – physical objects, space, time, physical causality – are less important to children than those having to do with humans.[21]As a result, children at this age tend to imagine that all things, especially those that are inanimate and unrealistic, have living human characteristics. In The Hobbit, it is not hard for children to accept talking birds and other anthropomorphized creatures, exemplified by the characterization of the Eagles:

> *And though the Lord of the Eagles became in after days the King of All Birds and wore a golden crown, and his fifteen chieftains golden collars (made of the gold that the dwarves gave them), Bilbo never saw them again—except high and far off in the Battle of Five Armies.*[22]

Tolkien gives birds the human ability to speak and reason in English, even imparting the Eagles with a social class, a respected royal leader, and a system of government. This extreme anthropomorphism is very attractive to children, as is the way Gollum treats his ring as though it were a person. When he realizes he has misplaced it, he hisses: "Where is it?Where iss it?. . .Losst it is, my precious, lost, lost!Curse us and crush us, my precious is lost!"[23]The manner in which Gollum names his inanimate Birthday present "my precious" has psychological appeal to children as they often name toys or presents as well. Since two to twelve-year-olds are at the prime of both animistic thought processing and fairy-story familiarity, fantasy literature like The Hobbit keenly attracts the child. But animism does not lose its appeal after age twelve by any means; in fact, Miller argues that:

> *Animism persists in an attenuated form into adulthood. Thus we refer to boats and cars as "she" and describe them as having human qualities. The golfer talks to his putter as though it has a will of its own. Many adult religions are animistic, most obviously pantheism, which believes that God is everywhere and in everything, including inanimate objects.*[24]

**Anthropomorphism at its best:** The raven is supposed to be old and wise, so the illustrator makes him bald. Furthermore, he is pictured in mid-sentence, indexing his capability for speech.

While adults do not actually believe that their boats are beautiful women, or that their putters will obey their commands, they continue to treat lifeless objects as though they possess certain human qualities. In this respect, they can relate to the humanized characters and objects in fairy-stories, as well. It is not a matter of believing the out-of-reach to be real; children, after all, do understand that birds cannot truly speak. Rather, it is the enjoyment and imagination of animism and fairy-stories that continues to attract both adults and children.

Tolkien believed that the value of the fairy-story is not for children alone, but for adults, as well.[25] As his own popular fairy-story, The Hobbit, shows, both children and adults can be entertained by fairy-stories. In the intervening 65 years, modern theories of psychology have tended to support Tolkien and give a scientific explanation for the purpose of his work. Even though most writers, Tolkien included, are probably not aware of psychological ideas such as the debate between nature and nurture, the mind's ability to process thought abstractly and concretely, and the tendency to endow non-living objects with human attributes, these elements are nevertheless present in their stories, supporting their validity as works for both children and adults. Psychology truly is a useful science that can be applied to even the most remote, seemingly unrelated issues in other areas of study. In this case, psychological ideas applied to literature confirm a possible purpose for Tolkien's creation of The Hobbit. □

*Laura Cantone is a sophomore pursuing her degree in English at the University of Rochester. Her interests in fairy-tales and child-psychology stem from her aspiration of becoming an elementary school teacher.*

# Cell Adhesion Molecules in Asthma

## VLA-1 and VLA-2 Expression on CD4+ T cells in OVA Induced Asthma

David Messenheimer, 2003
Advisor: David J. Topham, PhD.
Department of Microbiology and Immunology

**A study concerning potential roles of key adhesion molecules in asthmatic immunity.**

Asthma remains one of the major respiratory diseases in the world, affecting millions of humans each year. With its prevalence in Westernized, developed countries steadily increasing over the last 25 years, asthma will continue to be a major focus of respiratory research in the future.[1] The causes of this increase remain unknown, but both genetic and environmental sources have been blamed. It has also been noted that children who contract respiratory infections at a young age have a much lower prevalence of asthma later in life.[2] But oddly, similar children who are vaccinated against influenza do not retain this same immunity. Early infection, and the consequent altering of the immune system, could be the key to asthmatic immunity. This alteration occurs at the cellular level, meaning that the molecules that signal and bind immune system cells to the lung during asthma are the crucial determining factors in the mounting of an immune response. Other studies have shown that children in daycare and children in large families have a lower incidence of asthma, possibility due to greater exposure to respiratory viruses that would modify their immune systems.[3] A vaccine to help prevent asthma attacks would be very useful and may now be possible, as studies of these fundamental adhesion molecules, which change the immune system's response to the disease, are highly promising.

Asthmatic symptoms, such as restricted breathing and airway inflammation, can be linked to two specific cytokines (chemical signals), Interleukin-4 (IL-4) and IL-5. IL-4 induces Immunoglobulin E (IgE) synthesis, mast cell activation, and eosinophil recruitment. Immunoglobulins, commonly called antibodies, are the proteins involved with antigen recognition and cell-cell interaction. IgE levels correlate directly to asthma, and it has been shown that IgE induces the acute phase of asthma. The acute phase occurs 2-5 minutes after exposure to an allergen and involves a non-specific process, such as histamine, to induce a very broad, general response. The chronic phase occurs later and involves more specific cells, such as CD4+ T cells and B cells, which are sometimes recruited as memory cells if the allergen has previously been detected in the body. The findings described in this article pertain to the chronic phase for asthma.

Mast cells activated by IL-4 produce histamine (an inflammatory molecule) and IL-5. Eosinophils respond to an allergic asthma reaction, and are thought to be important chiefly in the defense against parasitic infections. The airway inflammation caused by IL-5 and histamine molecules leads to hyperreactive airways and bronchoconstriction. This reduction in airway diameter causes the common symptoms of coughing and wheezing, and also means the airway is much more sensitive to small amounts of the specific allergen. An increase in the secretion of mucus makes breathing difficult by trapping inhaled air in the lung.[4] Mice that lack the gene for IL-4 production (called IL-4 knockout mice) have no inflammation in the lungs and little airway hyperreactivity, proving the cytokine's important role in the asthmatic response.[5] IL-5 has a different, but nonetheless important, role in producing the symptoms of asthma. IL-5 induces eosinophil differentiation and activation. Studies done with IL-5 knockout mice, lacking IL-5, had similar results to the IL-4 knockout experiments. The mice were shown to have reduced airway hyperreactivity, lung damage, and eosinophil levels.[6] However, there was no decrease in IgE production, which suggests that IL-5 has no role in the activation of IgE.

Allergic asthma is caused by the inhalation of a simple allergen, usually a common, non-toxic protein found in nature. In mice, chicken Ovalbumin protein (OVA) has been used successfully as an allergen to model allergic asthma. Mice were first immunized with two very dilute OVA injections in the abdominal cavity 7 days apart. After 7 more days, they were challenged with a much stronger dose of OVA (40mg/ml of saline) inhaled through the nose. The immune response was then measured on day 5 after the challenge, which has been proven to be the peak time point of the CD4+ T cell allergic response.

CD4+ T helper cells are the major lymphocyte responders in an allergic response and are the main cells responsible for the memory of an allergen. CD4+ T cells are activated by short segments of the Ovalbumin protein, called antigens, and migrate to the lung in an attempt to destroy the allergen by activating macrophages and B cells. These allergen-specific CD4+ T helper cells produce IL-4 and IL-5, and are thus very important in the study of allergic asthma.[7]

The adhesion molecules responsible for CD4+ T cells' attachment to the extracellular matrix of the lung during allergic

asthma are still relatively unknown. However, recent studies point to Very Late Antigen 1 (VLA-1) and VLA-2 as likely candidates. These two heterodimeric integrin adhesion proteins both appear very late after T cell activation, with VLA-1 specifically binding to Type IV collagen on the extracellular matrix and VLA-2 binding to Type I collagen. Both of these types of collagen are found in the lung. Recent studies show that VLA-1 is the pivotal adhesion molecule in the retention and survival of $CD8^+$ T cells recruited to flu infections in non-lymphoid organs, such as the lung. But VLA-1 and VLA-2's role in the retention of $CD4^+$ T cells during allergic asthma has yet to be established. The expression of these adhesion molecules could be the vital factor in the ability of the immune system to mount a successful memory response against asthma-inducing allergens. With this knowledge, these T cells' adhesion molecules could be altered, leaving them unable to bind to the lung, thus causing little damage during an allergic response. This development would significantly reduce the effects of asthma, and in some cases even eliminate the disease.

In a typical immune response, lymphocytes are initially present in the lymph nodes and spleen and not the periphery tissues, such as the lung. Within the first 2 days after the challenge, however, the number of cells in the lymph nodes peak, as Antigen Presenting Cells present the OVA antigens to naïve T cells. These T cells (mostly $CD4^+$) then travel to the site of infiltration, in this case the lung, peaking at day 5 of the response. Presumably, some cells begin to slowly die, apoptosing (actively committing suicide) as the OVA proteins become scarce. Some cells will also leave the site of inflammation and travel to other organs of the body, although the destinations, such as the liver and other periphery organs, and the recruitment of these cells are still being researched.

T cell populations in the lung and the BAL (a wash of the lungs) were predominantly $CD4^+$ on day 5 after the initial OVA challenge (Figure 1). T cells in the lymph nodes (MLN) and spleen were unaffected by the influx in $CD4^+$ cells in the lung (Figure 1). The higher than normal percentages of $CD8^+$ cells observed outside of the lung (specifically in the MLN) can be attributed to the increase in growth factors such as IL-2 and IL-4, which stimulate both $CD8^+$ cells and non-specific $CD4^+$ cells. Thus, all of the lymph nodes will not have elevated $CD4^+$ populations, while the $CD8^+$ cells will be slightly increased. Due to its large size, the spleen sees little change in either cells' population.

EliSpot assays were performed in order to check the specificity of these cells, and to ensure that they were truly specific for and induced by the OVA allergen. This assay attaches antibodies specific for the cytokine IL-5 to a filter. Live cells from the animal are then stimulated with these antibodies, and the allergen (OVA) is added to the experimental group, while the control group receives no allergen. Cells that are activated and specific for OVA will produce IL-5 in the presence of OVA. The IL-5 binds to the attached antibody. Another type of antibody is then added to this complex which binds to the top of the IL-5, creating a sandwich-like structure around the cytokine. This top antibody contains a specific marker, which creates a clear blue spot on the filter after further development.

The obtained results show that the cells stimulated with OVA produced much more IL-5 than the control group without the allergen. Therefore these cells recognize OVA and are specific for it. Although the presence of OVA specific cells in the lung appears small (Table 1), a fraction of one percent of T cells in an organ is, in fact, a significant population. If we consider that there are approximately $10^{18}$ different T cell receptors present in the human body, populations 0.11% and 0.23% of all $CD4^+$ cells are not insignificant.[8] On average a given specificity of T cells in a mouse only constitutes 0.001% of the total population.

Having answered the question of specificity, we now move ahead to the expression of the adhesion molecules VLA-1 and VLA-2 on these OVA specific $CD4^+$ T cells. BAL and lung $CD4^+$ T cells from day 5 mice were stained to show the expression of VLA-1 and VLA-2 adhesion molecules. The $CD4^+$ cells in the BAL expressed more surface VLA-2 (62.89%) than VLA-1 (19.26%) (Table 1). A significant population (15.70%) of these $CD4^+$ cells also expressed both integrins. $CD4^+$ T cells in the lung showed a similar pattern of expression, although the $CD4^+$ cells were present at a lower percentage (Figure 2). However, this does not completely rule out VLA-1's importance in the retention of $CD4^+$ cells; although, due to its low

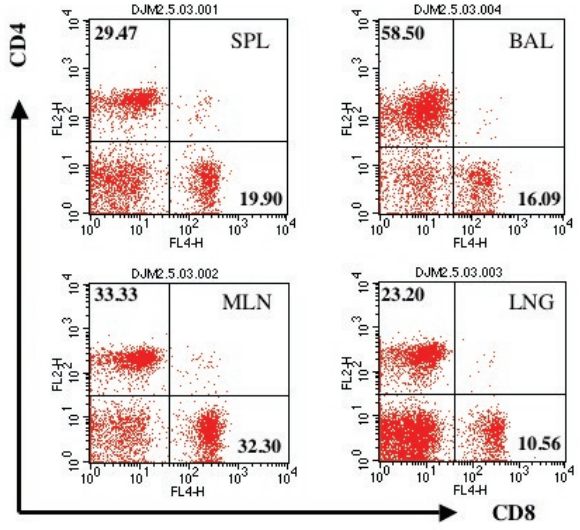
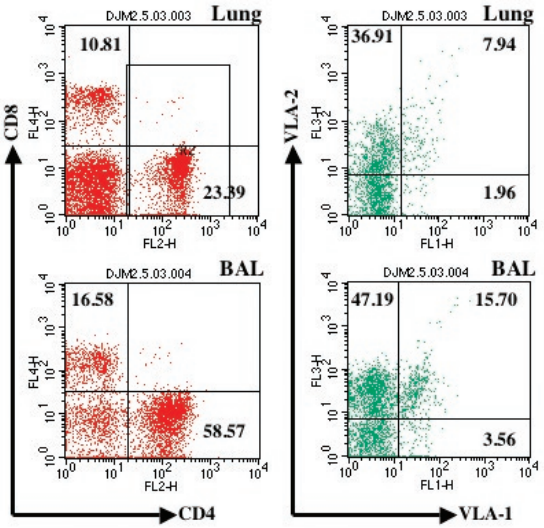



**Figure 1.** Populations of $CD4^+$ and $CD8^+$ T cells in four organs of day 5 OVA induced asthmatic mice. $CD4^+$ cells can be located in the upper left quadrant, while $CD8^+$ cells can be found in the lower left. Numbers indicate percentages of the cells. The larger difference between $CD8^+$ and $CD4^+$ T cell populations in the BAL was expected as $CD4^+$ T cells are heavily recruited to the site of inflammation, and are the main responders in an asthmatic attack. CD8+ T cells remain inactivated in the lymph nodes, but are increased due to the presence of the cytokine IL-2, which is produced by the proliferating $CD4^+$ cells. Interestingly in the lung, the $CD4^+$ population is not, as great, but is still much greater than the $CD8^+$ population.

| | Organ Cell Counts per Mouse | IL-5 Producing OVA Specific Cells per Organ |
|---|---|---|
| BAL | 3.53 x $10^5$ (58.57 % of cells are $CD4^+$) | 237 (.011 %) of $CD4^+$ T cells are OVA specific) |
| MLN | 3.33 x $10^6$ (33.73 %) | 320 (0.03 %) |
| Spleen | 7.5 x $10^7$ (29.99 %) | 3.9 x $10^3$ (0.02 %) |
| Lung | 1.38 x $10^6$ (23.93 %) | 745 (0.23 %) |

**Table 1.** The expression of VLA-1 and VLA-2 by $CD4^+$ T cells in the BAL and lung of day 5 Asthmatic mice. **Figure 2.** The plots to the left (CD4 versus CD8) show the dominance of $CD4^+$ T cells during an allergic asthma response, especially in the BAL. The plots to the right (VLA-1 versus VLA-2) have been gated on $CD4^+$ T cells. VLA-1 can be seen in both right quadrants, while VLA-2 can be seen in both top quadrants. Both organs show high expression of VLA-2, with a significant population expressing both integrins. This high expression of VLA-2, with less expression of VLA-1, is similar to the expression of $CD4^+$ T cells in the lung during an influenza infection.




expression, it is doubtful that VLA-1 plays a major role in $CD4^+$ retention. Retention of the T cells in the lung cannot be made by simply comparing the percentages of the loose cells of the BAL to those "stuck" in the lung, as one might guess. Presently we do not know the exact strength with which the BAL tears adhered cells away from the extracellular matrix, so we assume that the BAL cells were previously attached in some way to the lung.

Initial experiments, where influenza (strain A/HK/x31) was used to induce a $CD4^+$ T cell response concur with these findings on VLA-1 and VLA-2 expression. At the peak of an influenza response (day 8), the BAL $CD4^+$ T cells showed significant expression of VLA-2 (62%), while a less substantial population expressed VLA-1 (41%), and 30% of the population expressed both integrins.[9] This suggests VLA-1 may play a more important role in activating $CD4^+$ T cells during viral infections than it does in allergic asthma (due to the lower expression [41% compared to 19%] in the asthma model). In contrast, VLA-1 is the major integrin (66%) on CD8+ T cells in the influenza model.[10] The differences between a viral infection (T helper type 1 reaction) and an allergic reaction (T helper type 2 reaction) likely explain the differences in the expression of what can be assumed to be the "minor" integrin, VLA-1. Alternatively, this may also reflect the difference between $CD4^+$ and $CD8^+$ T cells.

Thus, we have demonstrated the OVA specificity of significant populations of $CD4^+$ T cells. We have also shown that $CD4^+$ T cells express predominantly VLA-2 and, less significantly, VLA-1. However, the link between the two findings, that the OVA specific $CD4^+$ T cells are the same cells that are expressing mainly VLA-2, still needs to be proven. One way to test this hypothesis is to transfer cells that are transgenic for the OVA antigen receptor into naïve mice where their expression of VLA-1 and VLA-2 can be tracked. This process can be used to determine if the OVA specific cells are in fact the same cells expressing these adhesion molecules. Additional experiments with VLA-1 and VLA-2 blocking antibodies will be performed to see whether airway hyperresponsiveness or allergic asthma is reduced with hindered expression of VLA-1, VLA-2, or both integrins.

Our research suggests that $CD4^+$ T cells express predominantly VLA-2 during allergic asthma adhesion. We have also shown that very significant populations of OVA specific $CD4^+$ cells can be found in the lung after an asthma response to OVA. $CD4^+$ T cells are the most important and prominent cells found during and after an asthmatic attack. By understanding what holds a T cell in the lung (VLA-1 and VLA-2), scientists can find a way to alter this adhesion and to prevent T cells from causing damage due to inhalation of a harmless allergen. Certainly this adhesion pattern holds true for other types of infections whose main responders are $CD4^+$ T cells, as suggested by similar data derived from a similar influenza model. Thus, increasing adhesion of these same cells could lead to stronger and quicker immune responses to more dangerous infections. Regardless of whether cell adhesion should be increased or decreased, this further understanding of cell surface molecules may prove invaluable in the reduction of the prevalence of respiratory illnesses worldwide. □

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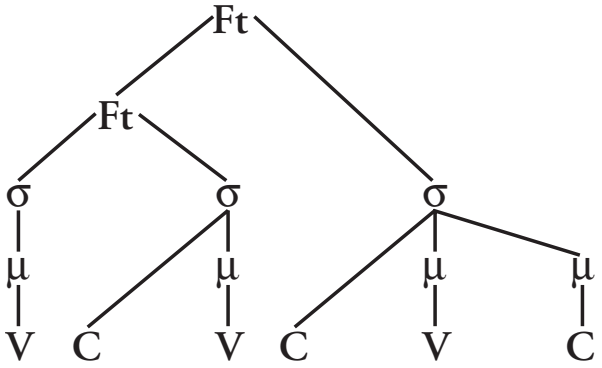
# Insights into the Patterns of Sounds

## Stress in Tondano




Douglas Ball, 2003
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**An analysis of the placement of stress in the Indonesian language Tondano employing a constraint-based theory of sound patterning.**

In recent work into the nature of natural language sound systems, much research has been in the area of stress, a sound system phenomenon that occurs above the "letter" level.[1] This paper will examine the distribution patterns of stress in a hitherto unanalyzed language with a rather complicated stress pattern—Tondano— and will offer an explanation of these patterns. This analysis will be within the framework of a newer phonological (sound system) theory called Optimality Theory. Despite Optimality Theory's initially formidable-looking formal apparatus, it offers a very insightful view of the stress patterns of Tondano, and this paper explains the concepts of the Optimality Theory analysis, yet without much of the mathematical formalisms. However, before charging into the analysis, I first want to give some background on the phenomenon of stress itself, the Tondano language, and Optimality Theory.

### Stress

The study of stress, as alluded to above, falls in the subfield of linguistics known as phonology, the study of sound systems. In particular, it falls into the study of phenomena above the segment (above the "letter" level) known as prosody. People familiar with the study of meter in poetry will find many similar terms in the study of prosody (including the term prosody itself). This similarity is not accidental; indeed, it stems directly from the insight that poetry involves manipulation of the prosodic qualities of language.

The definition that I will adopt here for stress (modified from Crystal 1997) is that it is the realization of prominence given to particular syllables. It is different from tone in that tone involves solely the manipulation of pitch, whereas stress has tied to some notion of strength or emphasis (which can include pitch or not, see below). This notion of emphasis has traditionally lent itself to the thought that stress involves one or more of the following: increased loudness, higher pitch, and increased duration of the stressed vowel. While subsequent research has shown that these characteristics are not necessarily relevant to all languages and that additional characteristics are involved, these studies have confirmed two results. First, there are phonetic signals to stress. Second, while these signals can vary across languages, prominent stressed syllables are a psychological reality in the minds of speakers, and are an important aspect of the grammar to account for. This paper will be focused on this psychological aspect rather than the phonetic aspects of stress in Tondano.

In addition to the phonetic realization, stress systems also vary in the patterns of where stress is placed. This variation falls into three broad categories. In some languages, stress placement has no discernable pattern, and thus, for each word, the correct stress placement must be learned individually. Such a language might look like the hypothetical language in (1):

(1)tirésabo[2]
nádisamu
luwesí
sádoka
pinéto
ratú
méti

There are also languages where the placement of stress is exceedingly regular, In these languages, every word (or nearly every word) has stress on the same syllable position in the word (like the second-to-last syllable). An example, using the same "words" as in (1), would be (2):

(2)tiresábo
nadisámu
luwési
sadóka
pinéto
rátu
méti

Finally, there are languages such as Tondano where the placement of stress is ultimately predictable (like in (2)), but on the surface, the patterning seems nearly as irregular as (1).

### Tondano

Tondano is spoken in the northeastern part of the spider-like island of Sulawesi (formerly known as Celebes), in Indonesia, in and around the town of Tondano. Studies from around 1970 estimated the number of speakers at about seventy thousand (Sneddon 1975: 1). However, more recent estimates (such as SIL International 2000) give the number of speakers at over ninety thousand. Tondano is a part of the vast Austronesian language family, whose members occupy an impressive span of the globe. Tondano is very distantly re-

lated both to Malagasy, the language of the island of Madagascar off the coast of Africa, and Hawai'ian, thousands of miles to the east. Tondano is also related to the languages nearby in insular Southeast Asia, including Javanese (of Indonesia), Bahasa Indonesia (the official language of Indonesia) and Tagalog (the official language of the Philippines) (Campbell 1995).

The data in this paper were taken from Sneddon's (1975) grammar of Tondano, and thus, this paper retains the conventions for writing Tondano contained in that monograph. Most of these symbols follow the International Phonetic Alphabet. The most notable exception is the letter {w}, which represents a sound that varies from the *w* sound in *wind* to the *v* sound in *very*. Stress will be marked, as in (1) and (2) above, with an accent mark over the stressed vowel.

### Optimality Theory

As noted above, this analysis is drawn within the framework of Optimality Theory (abbreviated OT). OT was first introduced in Prince & Smolensky (1993), but it has been further developed by a large body of literature in the past ten years. OT is a generative theory in that it generates surface forms from abstract mental entities, known as underlying representations. However, it departs in several respects from its predecessors. First, OT is a constraint-based framework, instead of rule-based framework. The difference between rules and constraints is subtle, but one can think of it in terms of a difference in orientation. Rules are process-based (they are more focused on the path to the result), while constraints are target-based (they are more focused on the result) (Crosswhite 2000). Having constraints then feeds a second important feature of OT—the constraints can be violated. If one has the constraint DON'T EAT CHOCOLATE and the constraint DON'T EAT ICE CREAM, and the only choices are to eat chocolate ice cream, eat vanilla ice cream, or eat nothing, one of these constraints may be violated.[3] Yet, the constraints can still characterize the decision to eat vanilla ice cream or the decision to eat nothing at all.[4] The capability for violation is the source of the term optimality in Optimality Theory. The generative part of the theory creates an infinite number of possible choices then using the constraints narrows them down to one optimal choice—the one that has the fewest violations of the constraints. However, this system is not one where one can apply any constraint at any time. Instead, the constraints can be ranked so that some apply before others, although the constraints do not necessarily have to be ranked. Thus, it is the job of the analyst to determine both which constraints are involved in a particular phenomenon and how they are ranked with respect to other constraints. Finally, the constraints themselves are thought to be universal (i.e. available to every natural language), but their ranking is language-specific.

### Assumptions

For this analysis of Tondano stress, I make several assumptions about the nature of the Tondano phonological system. First, I assume (following McCarthy & Prince 1993) that Tondano has metrical structures, where the segments are dominated by morae nodes (μ), morae are dominated by syllable nodes (σ), and syllables by feet (Ft), [units, analogous to measures in music, which demarcate the rhythmic regularities of the syllables. Feet are then dominated by prosodic words (PrWd), units which are roughly equivalent to grammatical words and, in Tondano, contain just one stressed syllable.] These structural units are hierarchically arranged like the following:

PrWd
|
Ft
|
σ
|
μ
|
segments

The remainder of my assumptions rest on this idea of prosodic structure, and the next few specifically focused on the level of the mora, which will prove to be the most important level of Tondano stress. The mora is a unit of metrical time or size (Crystal 1997). Vowels are generally assumed to have a mora. I assume that long vowels in Tondano have two morae. Additionally, I assume that syllable-final consonants carry a mora, so that (C)VC syllables, like (C)VV syllables, have two morae (C stands for consonant and V for vowel). This assumption is based upon the common (but, by no means necessary) equivalence of CVC and CVV syllables in many languages. However, the analysis given in section V will make it clear that this must be the correct analysis. Fourth, I assume that the vowel "uh" (called schwa, written as ə) does not project a mora (following Crosswhite 2001). The intuition behind this assumption is that schwa is a very weak vowel and as such it just is not strong enough to project a mora. While I make this assumption outright here, over the course of the analysis it will become clear that this assumption must be correct, at least for Tondano. However, what then is the number of morae when a schwa (zero μ) is followed by a coda consonant (1 μ)? In these instances, I assume the coda consonant and the schwa share the single mora. Thus, a (C)əC syllable will count as a syllable with one mora (that is importantly vocalic), but with a nonmoraic vowel.

Finally, I assume that only one foot is formed for the purposes of stress. This foot will be graphically placed in parentheses in examples. I make this final assumption because Sneddon (1975) describes only primary stress and no secondary stress.

### Analysis of Tondano

First, let us look at simple words, given in (3) below.

(3)(wá.le) 'house'
wa.(nú.a) 'village'

Taking Sneddon's (1975: 9) claim that "[s]tress usually falls on the penultimate syllable of the word," and examples like the data set in (3), I analyze Tondano as having a stress pattern where the stress is on the first part of the metrical foot. This pattern can be captured with the following TROCHEE[5] constraint (akin to Cohn & McCarthy's [1994] FT-FORM (TROCHAIC)):

(4)TROCHEE
The stressed part of the foot must be on the left

However, words like *wanua* illustrate that TROCHEE alone is not sufficient to characterize the stress, even in these basic cases. *Wanua* has two possible footings that satisfy TROCHEE: wa(núa) and *(wánu)a.[6] To rule this latter candidate out, we also must add a type of constraint known as an alignment constraint. The intuition behind alignment constraints is that languages prefer that the boundaries of two units (such as Prosodic Word and Foot or Stem and Foot) to line

up or align on one side or the other (right or left). From wa(núa) and *(wánu)a, the correct alignment constraint looks to be ALIGN-FT-R, which is formulated in (5) below.

(5)ALIGN-FT-R (McCarthy & Prince 1993, Cohn & McCarthy 1994)
"The right edge of every foot coincides with the right edge of some prosodic word."

Finally, a third constraint must be added to the above two, since there is nothing to eliminate single syllable, single mora stresses, like *wanu(á) and *wa(lé). In these words, the stressed part is on the left because there is only one part. These, however, can be eliminated through a constraint on the size of feet, FT-MIN-2 μ.[7] , which is formulated below in (6)

(6)FT-MIN-2 μ
The foot must not have less than two morae

This constraint predicts that Tondano stress is sensitive to vowel length—a prediction that will shortly be shown as correct. We will also see, as this analysis unfolds, that the level of the morae is very central to the Tondano stress system.

With these three constraints, we now can account for the data in (3) as every attested form in (3) is optimal in that none violate any constraints whereas other possible forms do. Thus far, it seems that all the constraints are non-ranked, since there has not been any data to suggest otherwise.

Turning now to another set of words, those with the vowel schwa (ə), we see that the above three constraints alone are not sufficient. In the data set in (7), we see that the presence of ə in the penultimate syllable produces words with final-syllable stress.

| | |
|---|---|
| (7) sə.(rá)ʔ | 'fish' |
| mə.rə.rə.(dé)y | 'intends to stand' |
| rə.mə.(dé)y | 'is standing' |
| ə.(sá) | 'one' |

To deal with this pattern, we add the constraint *P/NONMORAIC (along the same lines as NON-HEAD(ə) in Cohn & McCarthy [1994] and *P/ə in Kenstowicz [1994]), given in (8).

(8)*P/NONMORAIC
Nonmoraic vowels may not bear stress.

Note that the proposed foot structures in (7) (marked with parentheses) include feet that are in violation of FT-MIN-2 μ. This sort of interaction suggests that FT-MIN 2 μ and *P/Nonmoraic might be ranked with respect to each other. However, nothing in (7) offers a test case for the ranking of FT-MIN-2 μ vs. *P/Nonmoraic. Instead, all the possible feet either have zero morae (with ə lacking a mora) or they have one mora, so all of them violate FT-MIN-2 μ, and no ranking can be ascertained from this data.[8]

Thus far, we have examined a subset of words of Tondano, all of them without long vowels and with stress either on the penultimate syllable or, in the case of a penultimate schwa, on the final syllable. However, Tondano also includes words with long vowels and with stress on the third-to-last syllable (the antepenultimate). Some examples are given in (9) below.

| | |
|---|---|
| (9)ka.ri.(máŋ.ka)ʔ | 'spider' |
| (ki.ná:ŋ).ku | 'has been eaten by me' |
| (ká:.nə).na | 'will be eaten by him' |
| wiŋ.(kó.tə.)na | 'will be asked by him' |
| ti.(kó:) | 'throat' |
| (wé:.nu) | 'will be given by you' |

To account for the words in (9), I propose using the constraint, *LAPSE (following Elenbaas & Kager 1999, ultimately from Selkirk 1984). Elenbaas & Kager's (1999) formulation of *LAPSE was in an account of a particular kind of secondary stress pattern not found in Tondano (but found in Cayuvava, Chugach Alutiiq, and Estonian, inter alia). However, as we will see below, *LAPSE helps to determine primary stress in Tondano, even without this secondary stress pattern.

The intuition behind *LAPSE is that languages do not like to have long stretches of syllables unstressed. As we saw in (9), the stress shifts from the default penultimate syllable to the left when there are long stretches of syllables to left. This intuition might shed light on why the words discussed earlier in this paper do not show any effects from *LAPSE—they are not long enough. Only words that are long enough to have a "long stretch" show the effects of *LAPSE.

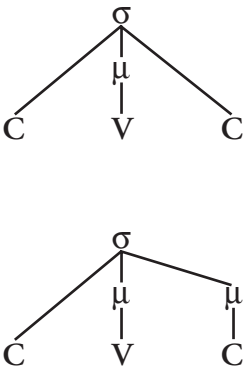
Tondano is spoken in the north-eastern part of the spider-like island of Sulawesi (indicated with an arrow), in Indonesia, in and around the town of Tondano.

However, to make *Lapse work, we must determine what exactly a "long stretch" is in Tondano. Because the prosodic structure (feet, syllables, and morae) includes units that can easily counted by the grammar, it seems like the best candidate for judging what a "long stretch" is. However, the question remains which level of prosodic structure is relevant, because *Lapse has been applied to a number of levels within this structure, including both syllables and morae (as noted for Cayuvava and Chugach Alutiiq, respectively, in Elenbaas & Kager 1999). Initially, it would seem that Tondano would be like Chugach, and be sensitive to morae. The form *(ká:)nəna* seems to support this, since stress is placed on the first syllable to avoid leaving the two-mora a: unstressed. However, a word like *wiŋ(kó*tə*)*na throws that analysis into doubt (and supports a syllabic analysis), since it has two unstressed morae in a row (the moraic vowel i and the syllable-final consonant ŋ). How should we resolve this puzzle? I propose that, instead of having *Lapse tied up with solely syllables or morae, *Lapse is relevant to moraic vowels (all those vowels except ə). This thinking also makes sense from another angle: moraic vowels would seem to be the best candidates to receive stress, since moraic consonants cannot be stressed, and there is already a constraint in Tondano to not stress nonmoraic ə. Thus, we formalize this idea of *Lapse in (10):

(10)*Lapse
PrWd must not have two adjacent non-prominent vocalic morae

This formulation of *Lapse with vocalic morae also adds further evidence for the nonmoraicity of ə. Though syllable-final consonants such as ŋ in wiŋkotəna motivated the formulation of *Lapse, the optimal candidate, *wiŋ(kó*tə*)*na, has no violations of *Lapse, with the nonmoraicityof ə. If ə was moraic, *wiŋ(kó*tə*)*na would have one violation of *Lapse, enough to eliminate it as a candidate. This analysis would, predict the correct form to be unattested **wiŋ(ko*tá*)*na. However, if ə is nonmoraic, **wiŋ(ko*tá*)*na is ruled out by its one violation of *Lapse—the lack of stress on either syllable of wiŋ.ko section of the word—thus allowing for the correct prediction.

The presence of *Lapse also helps to explain the seemingly unusual stress placement in the word ka:.(nəŋ.ku), 'will be eaten by me.' Initially, one might think that the ka: syllable would be stressed to avoid violating *Lapse. However, recall that a syllable with the sequence əC projects a single mora. Because of this, possible forms that do not stress the schwa are also in violation of *Lapse (since nəŋku part of the word also counts as two morae) and eliminated.



Examples of tree diagrams.

However, *Lapse alone does not do all the work in this form. First, ft-min-2 μ eliminates *ka:(nəŋ)ku, since there is only mora in the stressed foot. Second, the two remaining forms of ka:nəŋku, *(ká:nəŋ)ku and ka:(nəŋku), determine the critical ranking of align-ft-r over *P/Nonmoraic. Only when align-ft-r is ranked over *P/Nonmoraic is the right prediction of the stressed schwa made. Otherwise, the analysis would predict that no schwa would be stressed.

**Residual Issues**

While the above constraints of Trochee, *Lapse, ft-min-2 μ, align-ft-r, and *P/Nonmoraic can account for a large number of Tondano words, there remain a few loose ends to tie up. First is the form məŋa:ŋá:n, 'is continually eating.' The above five constraints eliminate all candidates except məŋa:(ŋá:)n and *mə(ŋá:ŋa:)n. The problem remains how to rule out this last form with a four-mora foot. To do so, I propose the following constraint, analogous to, but the opposite of ft-min-2 μ:

(11)FT-MAX-2 μ
Feet should not be more than two morae

This constraint is ranked in the "second tier" of constraints because no form critically dependent on ft-max 2 μ critically violates any of the "first tier" constraints: Trochee, *Lapse, ft-min-2 μ, and align-ft-r.

The inclusion of this last constraint may seem unparsimonious, since one constraint already references foot size (ft-min-2 μ) and another (*Lapse) indirectly limits the size of the foot (i.e. most of the possible forms that have a foot that is "too large" will be eliminated by *Lapse). Yet, fixing the problem with məŋa:ŋá:n appears to have two solutions: either add the ft-max-2μ constraint, to rule out the unattested **mə(ŋá:ŋa:)*n or stipulate this as part of the set of lexical exceptions. I choose the former on the intuition that this word acts as if it were governed by the same set of rules as the other words. However, regardless of how one goes about dealing with the problem, this troublesome part of the Tondano data suggests further investigation.

One final set of data, given below in (12), also requires explanation:

| | |
|---|---|
| (12)(tə́mpo)k | 'tip, end' |
| (rə́pə)t | 'fast' |
| (wə́ʔŋə)l | 'stupid' |

Like məŋa:ŋá:n above, the data in (12) evade the existing set of constraints. None of the vowels projects a mora[9]; thus all the syllables are in violation of ft-min-2 μ, and all the vowels are ə, so all syllables violate *P/Nonmoraic. Without these constraints, there is no mechanism to distinguish between pairs like (rə́pə)t and *rə(pə́)t. Thus, I add another constraint, given below in (13).

(13)FT-MIN 2 σ
Feet should have a minimum of two syllables

The presence of this constraint shows that Tondano assesses foot-minimality at both the level of the mora and at the level of the syllable. A critique may be raised that ft-min- 2 σ, too, is not parsimonious; a language should not evaluate foot-minimality on two levels. However, there is evidence for ft-min-2 μ across languages (certainly in Tondano, but in other languages as well) and ft-min-2 σ (cf. ft-bin for Indonesian in Cohn & McCarthy 1994), so they both should be part of the universal constraint set. As such, they could conceivably interact

within a given language, as they appear to do in Tondano.

FT-MIN-2 σ should obviously be ranked below *P/Nonmoraic, since all critical examples (as noted above in (13)) occur in words where all the vowels violate *P/Nonmoraic.

So to conclude this section on the analysis of the stress system in Tondano, given here in (14) is a summary of the constraints and their respective rankings:

(14) TROCHEE *P/Nonmoraic
*LAPSE » FT-MAX-2 μ » FT- MIN 2 σ
FT-MIN-2 μ
ALIGN-FT-R

**CONCLUSIONS**

From this study of the Tondano stress system, we can reach several conclusions. First, we can see the elegance of the Optimality Theory system. This framework can account for the rather complicated stress system with only seven small constraints. We have also seen that the elegance depends on the system of violable constraints, which provide evidence that perhaps the language would be best characterized not by invariable rules, but by complex web of constraints working with and against each other.

Second, from the analysis itself we can see that even a complex stress system such as Tondano's can be understood in a principled way. Specifically, we see that Tondano is a language that prefers penultimate stress. However, this preference is tempered by other tendencies: not leaving "large stretches" of the word unstressed, not stressing weak vowels, and having metrical feet that are just the right size—not too big or too small.

Finally, I hope to have shown that language can be very intricate, even in the area of phonology, an area of language that we are constantly in contact with whenever we speak ourselves or listen to someone else. It is these intricacies that make one realize how truly remarkable our ability to speak actually is and that motivate linguists such as myself to try to figure out how languages work. □

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# Epilepsy and Neurogenesis

## A Profile of Hippocampal Neurogenesis after Induced Temporal Lobe Epileptic Seizures

Matthew Tremblay, 2003
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**The development of epilepsy can be better understood by determining how new neurons are 'born' in this diseased state.**

Epilepsy is one of the oldest, and most well known ailments of the brain. It has afflicted many historical figures, and an enormous collection of research has been performed in an effort to understand the many facets of the disease. What causes seizures? What are the mechanisms in the process by which one becomes epileptic, known as epileptogenesis? Most of the biological processes underlying this disease have yet to be clearly understood, despite its thousand year or more history. Along the way, neuroscience evolved. A progression has taken place from the world of gross anatomy into the present molecular and cellular approaches. While the epileptic human brain is not entirely intractable, animal models have been developed out of necessity, because one cannot study the brain of a living human being unless a diseased portion is removed. The paradigm has been shifted. From the late 19th century, the time of Ramon y Cajal (the father of neuroscience), until the mid-1990's, neuroscientists believed that people are born with a fixed set of neurons. As an adult many would be lost, but nothing would replace them.[1] Contrary to this hypothesis, neuroscience is currently exploding with new findings about the birth of new neurons in ancient regions of the brain, namely the olfactory cortex and the hippocampus. Neurogenesis, as this phenomenon is called, adds yet another dimension to the epileptic brain. Another set of questions has arisen in the ever-growing complex network of molecules and cells. My work addresses whether the level of neurogenesis is altered in animal models of epilepsy, and examine possible causes of such alterations. The work is important in the quest to better understand the processes leading to the development of epilepsy.

### Brief History and Overview of Epilepsy

The victims of epilepsy included a great many famous individuals in our history including Socrates, Alexander the Great, Julius Caesar, Joan of Arc, and Dostoyevsky. In the time of Hippocrates it was believed that epilepsy, often referred to as the "falling sickness," only affected individuals that were possessed by evil spirits.[2]

John Hughlings Jackson is credited with being the first scientist to perform neurobiological analysis of epilepsy in the 1860's. Jackson was the first to describe a partial seizure, as well as the progression of seizures as they spread through the brain. For this reason the spread of partial seizures became known as the "Jacksonian march."

Treatment of epilepsy began to emerge shortly after Jackson's work. The first surgical treatment of epilepsy, involving the removal of a region of cerebral cortex (the outer surface of the brain) surrounding a skull fracture, was performed by Victor Horsley in 1886. Modern surgical treatment of epilepsy dates back to the 1950's and the work of two of the best-known neurosurgeons, Wilder Penfield and Herbert Jasper. The first pharmacological treatment of epilepsy, a drug known as Phenobarbital, dates back to a 1912 discovery by Hauptmann. In 1937, Houston Merritt and Tracey Putnam discovered one of the most common drugs in treating epilepsy, phenytoin (more commonly known as Dilantin).

Current epidemiological studies indicate that epilepsy occurs in as much as 1% of the US population. It is also estimated that 7-10% of the general population will have at least one seizure in their lifetime. A classification of types of seizures, as well as types of epilepsy, has emerged from our increased understanding of the disorder (Table 1). Seizures can be classified into two basic categories: partial and generalized. Partial seizures are those that originate in a particular region of the brain (usually an area of the cerebral cortex), but may or may not spread to other regions. Partial seizures that spread from the site of origin, or focus, are known as complex partial seizures. Interestingly, partial seizures are often preceded by a phenomenon known as an aura, during which the patient has some unusual sensations. Patients report such things as: a sense of fear, the smell of wood burning, or the taste something in their mouth. Generalized seizures are those that involve the entire brain and lead to loss of consciousness, but are not preceded by auras. Generalized seizures fall into many categories based on the symptoms of the seizure. Absence seizures, classified as nonconvulsive, are difficult to detect, because one of the only outward manifestations of these brief episodes is eye blinking. Absence seizures were known by outdated terminology as "petit mal". The visualization that most people have when they think of a seizure is a tonic-clonic, or "grand mal", seizure. Tonic-clonic seizures begin with the tightening of extremities (tonic phase), and are followed by the repetitive jerking of extremities (clonic phase) and often involve the loss of both bladder

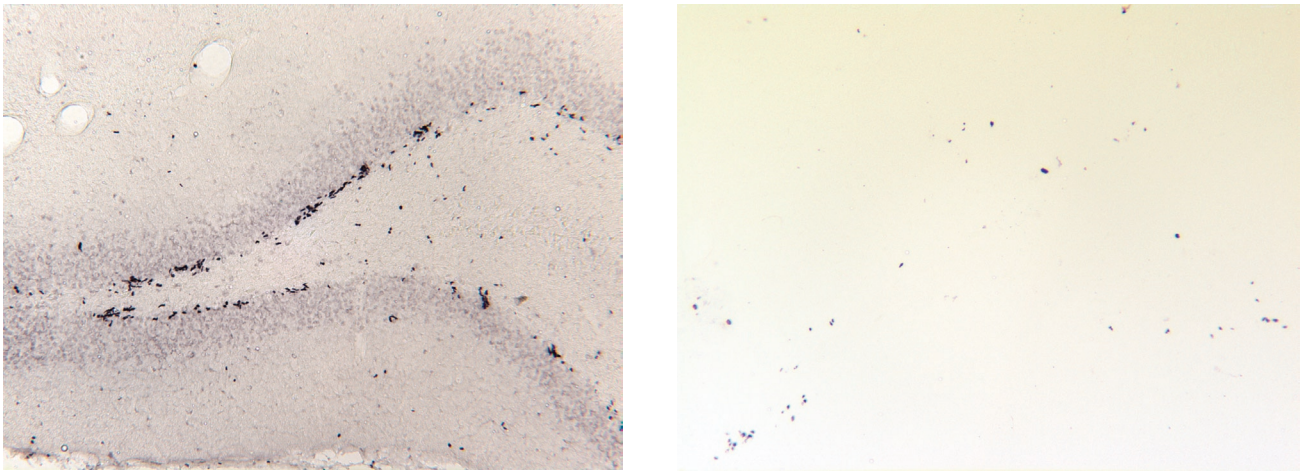

**Figure 1**. BrdU staining of detate gyrus. Left: Status epilepticus animal. Right: Control animal.

and bowel control. One final distinction in the classification of seizures is the concept of partial seizures evolving into generalized seizures. That is to say that some seizures are preceded by an aura, originate in a single focus, and spread throughout the entire brain, causing loss of consciousness.[3]

The classification of various epilepsies is not something which I intend to dwell upon. The classification of epilepsies basically breaks down to answering two questions: (1) Are the seizures partial or complex? (2) What is the etiology, or cause of the patient's epilepsy? Etiology is an interesting aspect of epilepsy, because the origins of the disorder are so disparate. Some experts think of epilepsy not as a disease itself, but rather the symptom or manifestation of some other pathology. The various problems that lead to epilepsy include but are not limited to: brain tumors, head trauma, metabolic dysfunction, infections, vascular disease (diseases involving the circulation or blood flow), and genetic predisposition. Many cases fall into the class of idiopathic origin, which is to say that the cause is unknown. For the purposes of understanding the focus of my research, it is important to understand the classification of one particularly common type of epilepsy, Temporal Lobe Epilepsy (TLE). As the name implies, TLE is characterized by partial seizures originating in the region of the temporal lobes. The major etiology of TLE is a febrile seizure, a clinical seizure in infancy or childhood resulting from fever, often induced by an infection of the central nervous system (CNS), particularly encephalitis or meningitis. The disorder often lies dormant, manifesting in adulthood with reoccurring complex partial seizures originating in an important temporal lobe structure known as the hippocampus.

### Overview of the Hippocampus

The hippocampus, meaning "seahorse" in Latin (for its unique shape in coronal slices) is a structure encased within the temporal lobes of the human brain. The hippocampus plays an important role in cognitive function as a critical structure within what is known as the limbic system. Although modern neuroscience is opposed to such gross simplification, the limbic system is most notably credited with being the seat of memory and emotion.[4]

In order to appreciate the critical role of the hippocampus in learning and memory, one has only to consider the interesting case of an epileptic patient made famous under the alias H.M. H.M. had intractable bilateral temporal lobe epilepsy, which is to say that his seizures originated in the temporal lobes on both sides of his brain, and were not treatable with standard anticonvulsant medications. In an attempt to treat the 27-year-old man, neurosurgeons removed the hippocampus on both sides of H.M.'s brain. The treatment of H.M.'s epilepsy was successful. However, without either hippocampus, H.M. was no longer able to form new long-term memories. For example, H.M. met with Dr. Brenda Milner on a regular basis for several years, yet each time they met H.M. reacted as if the two had never met and required introduction. For a Hollywood recreation of the consequences of bilateral hippocampal damage, one need only rent the film *Memento*.[5]

Before discussing my work, I must introduce the circuitry of the hippocampus in order to provide a framework for understanding the rhyme and reason of the studies. The circuitry of the hippocampus must be dealt with from both an intrinsic and extrinsic perspective. The extrinsic circuitry allows for communication between the hippocampus and the rest of the brain, while the intrinsic circuitry provides a network of communication between groups of neurons (the functional cells of the brain) within the hippocampus.

The extrinsic circuitry was first described by James Papez, and later extended by Paul MacLean. The Papez Circuit, as it is called, involves output of the hippocampus via a fiber tract known as the fornix to the mammillary bodies of the hypothalamus, which then send information to the thalamus, which sends its axons on to the cingulate gyrus, which finishes the circuit by connecting to the hippocampus.

The intrinsic circuitry as described, might better be characterized as afferent, or into the hippocampus, although neither is a perfect fit. Information from the sensory systems (e.g., vision, hearing, smell, etc) is sent back and forth to regions of the cortex known as parahippacampal and perirhinal, which in turn sends information back and forth to entorhinal cortex. The entorhinal cortex sends axons into the hippocampus starting the portion best characterized as intrinsic. Information passes from the entorhinal cortex to the dentate gyrus (via the perforant pathway) to the hippocampal CA3 region (via the mossy fiber pathway)

**Table 1.**
**TYPES OF SEIZURES**

1. Partial (focal) seizures
   - 1.1. Simple partial seizures (with motor, sensory, autonomic, or psychological symptoms)
   - 1.2. Complex partial seizures
   - 1.3 Complex partial seizures evolving to secondarily generalized seizures
2. Generalized seizures (convulsive or nonconvulsive)
   - 2.1 Absence
     - 2.1.1 Typical (petit mal)
     - 2.1.2 Atypical
   - 2.2 Myoclonic
   - 2.3 Clonic
   - 2.4 Tonic
   - 2.5 Tonic-clonic (grand mal)
   - 2.6 Atonic
3. Unclassified

**Epilepsies (abbreviated classification)**

1. Localization-related epilepsies and syndromes
   - 1.1 Idiopathic with age-related onset (eg, benign childhood epilepsy with centrotemporal spikes)
   - 1.2 Symptomatic (eg, post-traumatic epilepsy)
2. Generalized epilepsies and syndromes
   - 2.1 Idiopathic with age-related onset (eg, juvenile myoclonic epilepsy)
   - 2.2 Idiopathic and/or symptomatic (eg, Lennox-Gastaut syndrome)
   - 2.3 Symptomatic
3. Epilepsies and syndromes undetermined with respect to 1 or 2
   - 3.1 With both partial and generalized seizures (eg, neonatal seizures)
   - 3.2 Without unequivocal generalized or partial features
4. Special syndromes (eg, febrile convulsions)

to the hippocampal CA1 region (via the Schaffer-collateral pathway) and back out to the entorhinal cortex.

The primary neurotransmitter system under investigation in Temporal Lobe Epilepsy is glutamate. Throughout the circuitry described above, information is passed from one neuron to another by the release of glutamate from one neuron and binding of glutamate to receptors on the next neuron in the circuit, which induces certain changes in the cell's electrical properties. Fortunately there are very few glutamate receptors that need to be discussed. The three major types of glutamate receptors include the AMPA/Kainate receptors, NMDA receptor (both named for artificial compounds that bind to them), and the metabotropic receptors [16].

The AMPA/Kainate receptors and NMDA receptors basically allow for the flow of ions (charged particles) through a channel in the protein. AMPA/Kainate receptors primarily allow for $Na^+$ to pass through the channel, making it more positive, known as depolarizing the neuron. NMDA receptors allow both $Na^+$ and $Ca^{2+}$ to flow into the cell, thus depolarizing it. The NMDA receptor is noted for playing a crucial role in learning as well as cascades leading to neuronal death, both of which are mediated by the amount of free $Ca^{2+}$ in the cell. Metabotropic glutamate receptors are known to effect long-term changes in the properties of the cell via so-called second messengers. Metabotropic receptors have also been implicated recently as possibly affecting the brain through the other major class of cells in the brain, glia.[6]

## Overview of Neurogenesis

Most animal cells replicate as a mechanism of repair of tissues and replacement of dead cells. Neurons in the Central Nervous System (CNS) have long been considered an exception to the proliferative cycle of animal tissues, and no evidence to date suggests mitotic division of mature neurons. However, until recently, it was believed that new neurons were not produced in adulthood. Production of new neurons in the adult brain was first demonstrated just over 30 years ago.[7,8] It is only recently that the conclusions of this earlier work have become accepted as scientific truth. Research has led to the discovery of proliferative zones where new neurons are being produced in the adult brain. One area of neural progenitors is the dentate gyrus of the hippocampal formation.[9,10,11] The production of new neurons in the hippocampus occurs in mammalian species including human and non-human primates.[12,13]

Research into this new phenomenon, known as adult neurogenesis, has come to be one of the burgeoning subfields of neuroscience research. A great number of questions have arisen since the initial discovery of these neurons produced during adulthood. A subset of these questions include: From what cell lines do these adult-produced neurons come? What factors induce the division of so-called progenitor cells, the multipotent cells responsible for creating neurons? Do these new neurons help or hurt existing neurons and cognitive processes? Could these new neurons or their progenitor stem cells be useful in replacement therapy for treating diseases of the nervous system?

Neurogenesis has proven itself to be a contentious field of neuroscience research. Some members of the scientific community propose new ideas, while others tear them down for their naivety and technical differences. Elizabeth Gould recently proposed the introduction of new neurons in the cerebral cortex of primates, a finding refuted by neurogenesis aficionados Pasko Rakic and David Kornack.[14,15] Rakic has proven himself most combative to the crowd of neuroscientists pushing their way into the study of neurogenesis. In a recent review, "Adult Neurogenesis in Mammals: An Identity Crisis", Rakic delivers a deliberate argument positing a need for stringent criteria in identifying new neurons.[16] Cameron recently expressed dismay at with the technique used by many to study neurogenesis; particularly those scientists she feels are seemingly ignoring a possible role of the blood brain barrier (the brain's protective shielding from toxic molecules in the blood).[17]

Where does epilepsy fit into all of this? In 1997, a group of researchers made a discovery that there was a vast increase in the number of new neurons present in the brains (particularly the dentate gyrus) of rats that had been given pilocarpine, a drug known to elicit seizures, as well as rat given seizures by a process known as kindling.[18] This finding begs some of the questions presented early, as well as some new ones. Again, one is left wondering what causes neurogenesis, and what, in particular to seizures, would cause such an increase. Also, one is left wondering whether neurogenesis is compensating for the death of certain other neurons. Another interesting, yet erroneous, hypothesis is that a characteristic pathology of epilepsy, known as mossy fiber sprouting, is caused specifically by the improper projections of axons from the newly born neurons. This conclusion was later proven erroneous when the same research team found that killing the new neurons with radiation did not prevent mossy fiber sprouting.[19]

## Neurogenesis in the Kainic Acid Model of TLE

Increased hippocampal neurogenesis has been demonstrated in a number of animal models of TLE. Increased hippocampal neurogenesis has recently been suggested and documented in animal models of TLE paradigms including lithium-pilocarpine, flourothyl, and perforant path kindling, limbic kindling, electroconvulsive shock, and

kainic acid microinjection.[20,21,22,23,24,25,26,27] However, use of my model of TLE has yet to be used in investigating the increase in neurogenesis in the hippocampus.

The animal model of TLE used in Dr. Shirley Joseph's laboratory is the systemic kainic acid-induced status epilepticus model. Kainic acid is the drug for which kainate receptors were named. It acts by binding the kainate receptors in such a way as to allow a greater response to glutamate binding, thus it is an agonist. This increase in glutamate transmission induces seizures. The seizures spread from the hippocampus and generalize to the entire brain. In summary, kainic acid elicits a complex partial seizure with so-called secondary generalization, in which a partial seizure progresses to become generalized. It must be noted that the kainic acid model described above is distinct from the microinjection technique listed previously. The microinjection involves surgical injection of kainic acid into the desired region (in that case hippocampus CA3 region). This particular model is used more-or-less to create a lesion, or destroy a region of the brain. Our systemic injections (into the gross circulation) of kainic acid allow for smaller levels of the neurotoxin to seep into all of the brain. Status epilepticus (sometimes referred to as epileptic status) is a clinical term used to describe 30 minutes or more of generalized seizure without significant interruption. For the purposes of my research, animals are injected with kainate and allowed to reach status epilepticus for a period of no more than one hour.

How exactly does this animal model have anything to do with the development of epilepsy in human beings? As mentioned earlier, many people with TLE had some form of early insult to the brain (e.g., encephalitis, meningitis, or head injury). Occasionally, these early insults are enough to induce a period of status epilepticus, which can only be ended by use a phenobarbital or other anticonvulsants, as is also done with our animals. In essence, the use of systemic kainic acid injections to elicit seizure is analogous to the febrile seizure or early insult of children who may potentially have adult-onset epilepsy.

Birth-dating of newly born cells is a process worth discussing in order to understand the findings of subsequent studies. Bromodeoxyuridine(BrdU) is commonly used as the chemical of choice in determining the age of new cells. BrdU is able to incorporate into the DNA of cells that are in the process of dividing, known as mitosis. BrdU can later be stained for using antibodies that specifically bind to the chemical, a technique known as immunohistochemistry. Using this technique, one is able to specifically determine which cells were born at approximately the time the chemical was given.

In my earliest study, animals were given BrdU on days 5-8 following treatment with either kainic acid or saline (control group). Animals were sacrificed on day 9 in order to analyze 40um brain slices. As expected, there were vastly more cells in the dentate gyrus that stained for BrdU in animals treated with kainate in comparison to those treated with saline. However, this evidence alone neither indicates that the new cells are neurons, nor that the hippocampus is the only brain region in which the number of BrdU-stained cells were increased.

Using immunohistochemistry, it is also possible to stain for other molecules and proteins that may be present. Some of these proteins are present specifically in certain types of cells, thus allowing the investigator to stain only for the particular type of cells in which they are interested. Utilizing this technique, I was able to determine whether the same cells that had stained for BrdU were actually neurons by staining for Neuron-specific Nuclear Antigen (NeuN). Surprisingly, none of the cells stained for BrdU in the dentate gyrus of the controls nor the seizure animals were stained for NeuN. In fact, none of the BrdU cells anywhere in the brain also stained for NeuN, thus indicating that they were not mature neurons.

In a later studies animals survived 6 weeks, 8 weeks, and 6 months following the same treatments as described in the previous study. The dentate gyrus region of the hippocampus did contain neurons that stained for BrdU. There was also an apparent increase of an order of magnitude in the number of neurons born after seizure compared to control. The newly born cells in the dentate gyrus eventually differentiate into mature neurons and migrate from a region known as the subgranular zone (SGZ) into the granule cells of the dentate.

Interestingly, the number of adult-generated neurons remained relatively stable between the shortest and longest survival time following BrdU injection. The relative signal or strength of BrdU staining, however, did not remain stable. Although the number of new neurons in either case were not significantly different. There was a significant reduction in the level of staining present in the long survival time. Hypotheses for this development have been suggested, but experimental evidence lies outside of the scope of neuroscience, within genetics, chemistry, and molecular biology. The hypotheses are (in no particular order): (1) a combination of continued division of progenitor cells and death of new neurons diluted the quantity of BrdU during further replication of DNA, while offsetting the increase via the death of older BrdU-stained cells. (2) degradation of BrdU. (3) Specific excision of BrdU from DNA by enzymes during DNA repair. As a scientist, one must also concede that the possible solution may be a combination of hypotheses, or something not yet mentioned.

Curiously, other regions of the brain, all of them known for experiencing seizure-induced neuronal death, illustrated abundant increases in the quantity of BrdU stained cells. These areas include: CA3 and CA1 of the hippocampus, the subventricular zone, amygdala, piriform cortex, and nuclei of the thalamus. None of the newly born cells in these regions of the brain stained with NeuN. These newly generated cells all appear in regions involved in the Papez circuit, both intrinsic and extrinsic areas, as described earlier.

Since the majority of new cells were not neurons, the question became: What type of cells are they? A multitude of antibodies were used to stain for a vast array of cell types. Possible cell types investigated included the various types of glia and white blood cells. Our rationale for looking at white blood cells is based on a growing body of evidence indicating a crucial role of the immune system in exacerbating neuronal death. The result of our hunt for the origin of the abundant new cells throughout the Papez circuit concluded that these cells are a combination of glia and immune cells.

Microglia are phagocytic cells within the nervous system. Using an antibody named ED1, specifically created to stain macrophages (a type of phagocytic white blood cell from which microglia are derived), we were able to determine the origins of the non-neuronal BrdU-stained cells.[28] Microglia are known for their response, known as activation, during neurodegeneration. Microglial activation occurs during Parkinson's disease, Alzheimer's

disease, multiple sclerosis, AIDS-related dementia, and most notably epilepsy.[29] The phenomenon that prompted the birth of cells throughout the Papez circuit was the activation of microglia in response to excessive excitation and presumably death of neurons in the hippocampus, amygdala, and thalamus.

A topic of interest to my investigation is where these microglia came from, and what prompted their rapid proliferation. Given some recent discoveries in the field of neuroscience, there exist a number of factors that may contribute to the explosion of microglia in response to seizures. I would like to highlight three findings in particular: (1) astrocytes, a particular type of glial cell, express receptors for glutamate which influence the blood vessels to which their endfeet are attached. This glutamate-induced signaling causes an increase in the circumference of blood vessels, thus dilating them.[30] (2) a growth factor, granulocyte/monocyte-colony stimulating factor (GM-CSF) has been found to induce the rapid proliferation and maturation of microglia into their active form.[31,32] (3) GM-CSF is capable of crossing the blood brain barrier, as well as being produced in some circumstances by astrocytes.[33,34] Thus, it appears that astrocytes may play a crucial role in the process of epileptogenesis.

I propose that the implications of these three findings in concert provide the necessary environment for microglial activation in our kainic-acid model of Temporal Lobe Epilepsy. Administration of kainic acid increases excitation through AMPA/Kainate receptors, which also allow for the activation NMDA receptors and increased release of glutamate. Increases in glutamate signaling may then increase activation of signaling pathways of astrocytes including the dilation of local blood vessels and increased release of GM-CSF. The same molecules that attract microglia toward sites of neuronal death are likely to attract macrophages and other immune cells from the blood. The combination of these elements are likely to precipitate the immune response to neuronal death often described in pathologies of the brain.

One might be wondering what relationship microglial response and NMDA receptor signaling pathways play in affecting neuronal death and birth. I approached these questions in a recent study by exploiting the wonderful array of pharmacological agents at the modern scientist's disposal. The specific intent of my investigation was two-fold: (1) to examine the effects of a drug that would block the detrimental influx of calcium ions through NMDA receptors as alluded to earlier. (2) to examine the effects of suppressing the microglial immune response.

There are a great many pharmacological agents designed to specifically block the $Ca^{2+}$ channel of the NMDA receptor. Unfortunately, many of these drugs have undesirable psychotropic effects due to the very fact that they block the NMDA receptor. Such substances include phencyclidine (PCP) and ketamine. Use of psychotropic substances or doses to prevent epileptogenesis is clinically undesirable for obvious reasons. A drug known as dextromethorphan was chosen because of a moderate binding affinity to NMDA receptors and evidence of anticonvulsant and protective effects in kainic acid treated hippocampal neurons.[35] Dextromethorphan was administered as a pretreatment 30 minutes prior to administration of kainic acid.

Preliminary results of the dextromethorphan study were not as expected. Animals pretreated with dextromethorphan exhibited a greater extent of neuronal loss in the CA1 region of the hippocampus. In conjunction with this neuronal loss, the animals also exhibited a more severe microglial immune response in CA1. Surprisingly, there were no significant differences in the number of new neurons in the dentate gyrus between animals that received pretreatment and animals that received only saline prior to seizures. Thus, dextromethorphan neither increased nor decreased the effect of kainic acid-induced status epilepticus on neurogenesis, suggesting little or no involvement of NMDA-mediated signal pathways in the increased production of new neurons in the dentate gyrus.

There are surprisingly few drugs commonly used to suppress immune response. For the purposes of studying the effects of blocking microglial immune response, I chose a compound known as FK506, because it was also demonstrated to inhibit certain steps along the NMDA-mediated signaling pathways. Specifically, FK506 inhibits calcineurin, a phosphatase which is responsible for activating nitric oxide synthase (NOS), which produces nitric oxide (NO) a powerful signaling molecule known to be deleterious in large quantities.[36] Thus, the drug provided a way of inhibiting two proposed mechanisms of neuronal death simultaneously. In one sense it was preventing immune response by blocking signaling of proinflammatory cytokines, molecules that attract immune cells to damage sites. In another sense FK506 was preventing cell death by limiting NO signaling.

The preliminary results of FK506 pretreatment were encouraging. Animals treated with FK506 prior to seizure exhibited similar neuronal loss in CA1 in comparison to the dextromethorphan animals. However, there was a substantial reduction in the immune response. Far fewer ED1 stained microglia were present in the CA1 region of animals with similar seizure severity. Again, there were no significant differences in the quantity of new neurons present in the dentate gyrus when compared to animals without pretreatment. These results suggest little or no involvement of the immune response in the increased production of new neurons in the dentate gyrus.

A final group of animals was given pretreatment with both dextromethorphan and FK506. Functioning in concert, the two drugs appear to be very effective in preventing both the neuronal loss and microglial immune response in the CA1 region of the hippocampus. These animals exhibited less neuronal loss and fewer ED1 stained microglia. However, as one would come to expect, there were no significant differences in the number of new neurons in the dentate gyrus in comparison to animals without pretreatment. This once again supports the lack of an influence of NMDA-mediated signaling and immune cells in the increased production of neurons in the dentate gyrus following status epilepticus.

In essence, the results of my drug pretreatment experiments disprove two of my hypotheses: (1) that NMDA receptor-mediated signaling pathways regulate the increase in the number of adult generated neurons in rats after status epilepticus, (2) that immune response may be crucial in initiating increased neurogenesis in animals after status epilepticus. Although not discussed previously, evidence suggests that significant increases in adult generated neurons can be linked to status epilepticus and not merely to kainic acid treatment. Only approximately 60-70 percent of animals receiving kainic acid actually achieve status epilepticus. Other animals have low-grade seizures and exhibit a level of neurogenesis similar

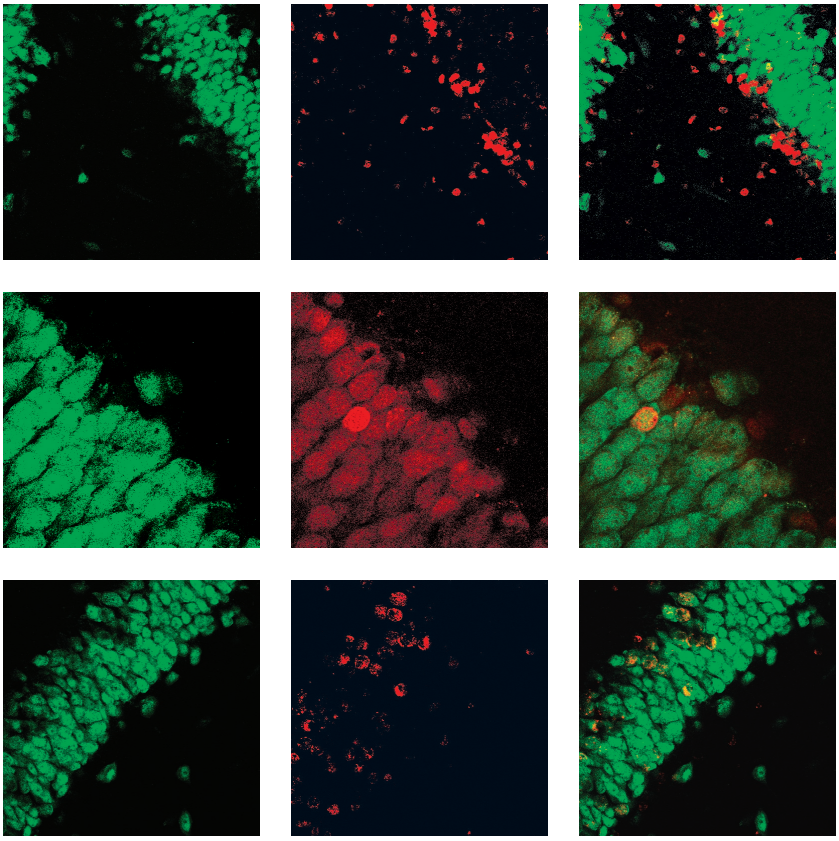

**Figure 2.** From left to right, top to bottom: **1**. NeuN (Green) SE animal (9day) dentate gyrus; **2**. BrdU (Red) SE animal (9day) dentate; **3**. Merged NeuN(Green)/ BrdU(Red) SE animal (9day) dentate; **4**. NeuN (Green) SE animal (6 weeks) dentate; **5**. BrdU (Red) SE animal (6 weeks) dentate; **6**. Merged NeuN(Green)/ BrdU(Red) SE animal (6 weeks) dentate; **7**. NeuN (Green) SE animal (6 month) dentate; **8**. BrdU (Red) SE animal (6 month) dentate; **9**. Merged NeuN(Green)/ BrdU(Red) SE animal (6 month) dentate.

to control animals. Given this information, one can only conclude that there is some trigger for neurogenesis that depends upon continuous high-grade seizures.

### Future Directions

The next phase in my studies of increased neurogenesis in epilepsy will examine the axonal projections of adult born neurons. As illustrated earlier, the correct axonal projections from the dentate gyrus will synapse in hippocampal area CA3, a projection known as the mossy fiber pathway. One of the specific changes that often takes place in an epileptic hippocampus, also demonstrated in animal models of epilepsy, is a sprouting of mossy fiber axons back onto the dentate gyrus, known as mossy fiber sprouting. There is inconsistent evidence in the literature regarding the contribution of adult generated neurons to mossy fiber sprouting.[37,38] There is recent evidence that adult generated neurons in normal animals contribute to normal mossy fiber connections between the dentate gyrus and CA3.[39,40,41] In my experiment I intend to inject a fluorescent substance into CA3 neurons that will be transported backward to the dentate gyrus and forward to CA1, known as retrograde and anterograde respectively. Exploiting BrdU for the same purpose as previously, I expect to identify a normal mossy fiber projection from adult generated neurons via fluorescent BrdU staining and the tracer substance in the same neuron.

Another project of interest is a temporal study of the level of proliferation after status epilepticus. By giving BrdU at varying times after status epilepticus it would become possible to establish a clear relationship between the timing of events. In essence, this study would be useful for determining whether the increase in proliferation in the dentate gyrus were an acute or chronic effect of status epilepticus. It would appear from the literature that the rate of neurogenesis in normal animals is indicative of a turnover in the dentate granule neuron population.[42] It may be possible that increased apoptotic death due to continuous seizures is causing an increase in the turnover rate of granule cells in the dentate gyrus, which may in fact explain why there is little discussion in epilepsy literature of neuronal loss in the dentate gyrus.[43]

Another speculative project of interest would investigate the possible role of astrocytes in propagating an increase in neurogenesis in animal models of epilepsy. As indicated earlier, evidence suggests many roles of astrocytes in affecting neurotransmission, vascular regulation, and immune response. Of particular interest are the abilities of astrocytes to release GM-CSF and modulate dilation of local blood vessels. Given the increased glutamate neurotransmission during seizures, it seems plausible that activation of metabotropic glutamate receptors on astrocytes could lead to an increase in GM-CSF via two mechanisms: (1) direct astrocytic release of the factor, (2) increased local blood flow in concert with blood brain barrier disruption. Although no evidence exists to suggest such a finding, it appears plausible that the coincidence of seizure and neurogenesis may in part be due to GM-CSF signaling in the progenitor cells from which adult generated neurons arise. Interestingly, this suggestion was also made in recent literature describing the role of GM-CSF in microglial activation.

As indicated by the many future directions of this work, many of the fundamental questions regarding the relationship between epilepsy and neurogenesis remain unanswered. There is evidence of diverse modulation of neurogenesis related to neurotransmitters, growth factors, cytokines, drugs, and experience.[44] The idea that such an effect is mediated by a single factor would appear ludicrous given the overwhelming evidence of such interactions in normal animals. However, the tight link between seizures and neurogenesis suggests that epilepsy may be an exceptional case in which the effects of multiple interactions and signaling pathways converge via a common mechanism induced by seizures.

To underscore the importance of understanding the mechanisms and effects of neurogenesis, one has only to review the literature regarding the possible roles of these adult generated neurons in learning and memory. Animal models have demonstrated a strong relationship between maze learning and hippocampal neurogenesis in rats.[45,46,47] Investigators have long known of the relationship between bird song and neurogenesis. Birds that learn song to attract a mate display neurogenesis in centers of the brain regulating song learning throughout the process of acquiring song, again implying a crucial role in learning and memory.[48,49] Studies have demonstrated an age-related decline in neurogenesis as well, although correlation with memory deficits is not necessarily related.[50,51]

Recent research into the mechanisms underlying depression and the action of antidepressants has proven relevant to our understanding of neurogenesis. Evidence for antidepressant effects on neurogenesis has been found across a vast array of drugs ranging from classical antidepressants such as selective serotonin reuptake inhibitors (SSRIs) like Prozac and Zoloft, tricyclic antidepressants, to newer drugs like tianeptine and drugs the block the neuropeptide substance P.[52,53,54] Some investigators have proposed a "neurogenic theory" of depression.[55] In conconcordance with evidence of a neurogenic effect of antidepressants is evidence that depletion of serotonin decreases neurogenesis in the dentate gyrus.[56] Recent evidence that neurogenesis is modulated by cyclic adenosine monophosphate (cAMP) and cAMP Response Element-Binding Protein (CREB) add great support to the role of neurogenesis and depression.[57] It is currently believed that these same pathways mediate the mechanism of action of antidepressants. Some scientists in the field speculate that these recent findings may lead to targeting of neurogenesis and the cAMP-CREB signaling pathway in our understanding and treatment of major depression.[58]

Given the implications that neurogenesis may have in diseases and function of the nervous system, the importance of determining the cause and effect relationship between neurogenesis and animal models of epilepsy cannot be overstated. Understanding the proliferation of neuronal progenitor cells may one day allow for brain repair. It appears possible that this may be just the purpose neurogenesis is serving in the dentate gyrus. Better understanding of the role of new neurons in replacement and plasticity can only lead to a better understanding of epileptogenesis and plasticity.[59] Although it seems unlikely at the present, the convergence of stem cell and gene therapy research may one day allow for the correction of various neurological disorders. The technology to regulate neurogenesis and modulate gene expression may afford physicians and scientists the illusion of control over the complexity of our most important organ, the brain. □

*Matthew Tremblay recieved a B.S. in Neuroscience from the University of Rochester in May 2003. The article is an abridged version of his senior honors thesis. Matthew is pursuing a M.D./Ph.D. degree in the Medical Scientist Training Program at Albert Einstein College of Medicine.*

# Digging up the Truth

## The History of the De Jongh Family Monument

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**An in-depth analysis of the historical and present trends of religious symbolism in Mount Hope Cemetery.**

There is a great deal of religious symbolism on monuments in cemeteries all over the world. The inscriptions and designs of monuments reveal the beliefs and ideals of the dead and their families. My investigation of the De Jongh family took place in the Mount Hope Cemetery in Rochester, New York. The De Jongh's monument made note of the family's ancestry, while the inscriptions and motifs captured the most important sentiments in their lives.

Mount Hope Cemetery was dedicated on October 3, 1838 as the first municipal Victorian cemetery in the United States. It was modeled after Pere Lachaise, the first major rural cemetery, located in France, and Mount Auburn Cemetery, the first major rural cemetery in the United States, founded in 1831 near Boston. Today, Mt. Hope cemetery sprawls over 196 acres of rolling hills containing over 350,000 graves.[1] Mt. Hope opened at a time when cemeteries served as public areas where people could go to experience the freshness of nature. Visitors who walk the hills and valleys of Mt. Hope will encounter large plots associated with churches and synagogues**,** and individual stones with religious symbolism.

The religious symbolism depicted in grave markers is hardly surprising, for throughout history, death has been connected to religion. Religious beliefs may give people a sense of power over death, as they offer explanations for this unknown, unavoidable, and often terrifying end to life. Quite simply, religion gives meaning to death. Thus, grave markers often have inscriptions and designs that connect the person to a god or a religious ideal, from which one can learn a great deal about the dead.

The De Jongh family monument served as an excellent research subject of an average Rochesterian living in the 19th century. My investigation began with a basic analysis of the monument. Henry De Jongh was born in Holland on February 10, 1816 and died in Rochester, NY on March 25, 1896. He shares a four-sided monument with his first wife, Johanna Lantsink and their daughter, Miss R. B. De Jongh. Nearby, in front of the monument, are three small markers, which read, "Mother," "Father," and "Reinira." The De Jongh monument is located in range 1 on Second Avenue between Elm and Evergreen Avenues. The monument was erected in 1885 after the death of Johanna on September 26, 1884. The year 1885 is cast in large print on one side of the memorial. Miss R. B. De Jongh followed her mother in death on June 27, 1890. Although there is no specific reference to Reinira De Jongh as the daughter of Henry and Johanna, she probably would not be buried with Henry and Johanna if this were not the case. If she were married, according to custom, she would have been buried with her husband's family, but the prefix "miss" indicates that she was most likely never married.

The De Jongh monument has many inscriptions that provide details of the family's past. Henry's tablet reads: "Henry De Jongh, Born in Holland, Feb. 10, 1816, DIED Mar. 25, 1896; 'My hope is built on nothing less than Jesus' blood and righteousness.'" Below this, Johanna's tablet reads:"Johanna Lantsink, wife of, Henry De Jongh, Died Sept. 26, 1884 Aged 68 years, 'Christ my only hope.'" The adjacent side has an imprint of an anchor and chain, below which is the date 1885 and a garland. The lettering at the base of this side reads: "Monumental Bronze Co. Bridgeport Connecticut." This indicates who constructed and designed the marker. The next adjacent side has a flower bouquet and DE JONGH in large letters underneath. The final side reads: "In loving memory of, Miss R. B. De Jongh, Born Holland, May 18, 1840, Died June 27, 1890." Reinira's inscription "in loving memory" shows an example of the shift that occurred during the 19th century toward a more free expression of sentiment. A century earlier, her inscription may have read, "Here lies the bones of Miss R. B. De Jongh."[2] The Mt. Hope Cemetery records show that she was buried on July 1, 1890 at the age of 50.

The dates and inscriptions indicate that the De Jongh family emigrated from Holland some time after their daughter was born in 1840. It is unclear what brought this family to Rochester, but New York has always been richly populated with Dutch. In fact, the Dutch were among the first Europeans to arrive in the Hudson River from Europe in 1609.[3] Perhaps family drew them here, or perhaps they were interested in a new industrially booming American city. Whichever the case, the De Jongh family remained in Rochester until their deaths in the late 19th century.

The Mt. Hope Cemetery records show that all three members of the De Jongh fam-

ily died of natural causes. Henry died of "Valv Disease Heart"[4], Johanna of "disease of the liver"[5], and Reinira died of asthenia or debility, an abnormal bodily weakness or feebleness accompanied by a decay of strength.[6] The De Jonghs probably all died at home without visiting a hospital, since the Rochester City Hospital's death records indicate only 8 deaths in 1896. Despite the fact that the industrial revolution caused a population boom from 60,000 to 160,000 between 1864 and 1900,[7] acceptance of public medical care was gradual, as germ theory was new, and sterilization was not practiced regularly. People therefore relied on general practitioners, physicians of various levels of training who made house calls, and their faith in God to keep them healthy.

Faith played an important part in the lives of the De Jonghs. The De Jongh family belonged to the United Presbyterian Church on Allen Street in downtown Rochester, where Henry's funeral was held on March 28, 1896.[8] The epitaphs on the De Jonghs' monument further indicate their faith in Christ. Until the 19th century, most grave stones and monuments did not have epitaphs, but in the 19th and 20th centuries it became increasingly more common for families or the deceased themselves to pick epitaphs prior to death. As evidence of their faith, both Henry's and Johanna's epitaphs refer to Christ as a savior. In fact, Henry's epitaph, "My hope is built on nothing less than Jesus' blood and righteousness" was taken from *The Solid Rock*, a hymn written by Edward Mote of England in 1834:[9]

*My hope is built on nothing less*
*Than Jesus' blood and righteousness*
*I dare not trust the sweetest frame,*
*But whole lean on Jesus' name*

*On Christ the solid rock I stand;*
*All other ground is sinking sand,*
*All other ground is sinking sand*

*When Darkness veils his lovely face,*
*I rest on his unchanging grace.*
*In every high and stormy gale,*
*My anchor holds within the veil.*

*His oath, his covenant, his blood*
*Supports me in the whelming flood.*
*When all around my soul gives way,*
*He then is all my hope and stay.*

*When He shall come with trumpet sound,*
*O may I then in him be found!*
*Dressed in his righteousness alone,*
*Faultless to stand before the throne.*

The first verse of this hymn which appears as Henry's epitaph echoes Thessalonians 4:16, "For the Lord himself, with a cry of command, with the archangel's call and with the sound of God's trumpet, will descend from heaven, and the dead in Christ will rise first." Henry's epitaph and this verse both imply transcendence to heaven through belief in Christ. The epitaph suggests that Henry hoped that he would go to heaven because Jesus served as the sacrificial lamb and died for the sins of humanity. Henry is righteous because he has faith and thus will ascend to heaven.[10] This symbolizes the idea of an immortal soul that leaves the body; death is not an ending of spirit, merely of body. Jung, a prominent Swiss psychoanalyst, defines this "psychically hopeful and enriching" belief in spiritual transcendence as a personal myth.[11] Christ serves as an immortal archetype, an "instinctual structure worthy of one's faith."[12] Henry has faith that Christ will lead him into an unknown, unimaginable place.

The Christian belief in continuity of soul after the death of the physical body gives people an explanation of the unknown and peace of mind regarding death. People create symbolic perceptions of connections with religious archetypes to transcend the idea of annihilation. Robert Jay Lifton, a professor of psychiatry who studies human actions to cope with extreme situations, refers to this phenomenon as theological or religious transcendence.[13] It is the "release of the profane burdens of life into a higher plane of existence."[14] Henry is no longer responsible for good and bad, right and wrong. Henry is beyond that and connected to heaven through Jesus; his connection to Christianity will assure him a place in with God. This ideal allows Christians such as Henry De Jongh an alternative perspective on death–they have the power to model their behavior after Jesus and follow him to heaven to see the glory of God.

Lakoff and Turner studied the metaphors human minds use to form an understanding of the world. They would apply the *life*

**Far Left**: The monument of Miss R.B. De Jongh (1840-1890), located in Mt. Hope Cemetary, Rochester, NY. **Left**: Depiction of anchor on the De Jongh monument.

**Far Left.** Depiction of a wreath on the De Jongh monument. **Left**: The stone of Mr. Henry De Jongh (d. 1896).

*is a journey* metaphor to Henry's epitaph. In the Judeo-Christian tradition, God offers us alternative paths of good and evil throughout life. Death hangs over our heads to remind us of the importance of choosing the good path.[15] Jesus serves as a guide**,** and only he can save us in the end of our physical life. Henry's epitaph is an elaboration of Johanna's which reads, "Christ my only hope." The analysis of Henry's epitaph applies to Johanna's epitaph as well. She believes in a spiritual afterlife; she will get to heaven because of Jesus' sacrifice.

Johanna's shortened epitaph and her tablet's position below Henry's is suggestive of the role women played in the late 1800s. The husband was the head of the household and the wife subservient to him. Even in death, women were buried with their husbands**,** and their inscriptions nearly always read their married names and the names of their husbands.[16] Their inscriptions were often smaller than those of their husbands and usually read "wife of" implying an identity depending on marriage.[17]

Two sides of the De Jongh monument contain symbolic images instead of words. The anchor and chain is the largest of the two and could have several meanings. It could mean that the deceased worked at sea, but Henry was a landlord and the owner of a Sash and Blind Company in Rochester, so the anchor did not necessarily relate to his profession.[18] In Christianity, however, the anchor is a symbol of hope. Given the De Jongh's epitaphs, it is more likely that the anchor was chosen for the monument for its symbolism of hope. The anchor is even mentioned in verse 11 of *The Solid Rock,* which likens the "steadfast anchor of the soul" to faith in Christ.[19] In Christian settings, the anchor is also a disguised cross and a symbol for Christ who prevents the deceased from drifting off and becoming lost.[20] An anchor with a broken chain also represents the cessation of life**,**[21] and while it is difficult to determine if the chain is broken on the De Jongh monument, the interpretations seem to match the epitaphs.

Below the anchor and chain is the date the monument was erected, 1885, beneath which is a wreath. A wreath symbolizes victory;[22] Christians are victorious in death when they reach heaven**,** following in Jesus' footsteps. The adjacent side of the monument displays a bouquet of flowers. On American tombstones, flowers often symbolize the hope for the spirit's regeneration beyond the grave.[23] The flowers can also be understood as a depiction of the *people are plants* metaphor. That is, people are the flowers or fruit of a plant that flourishes and then withers.[24] As such, flowers symbolize the frailty of life;[25] yet, they may rise again in spring, just as believers may be resurrected after death.

A geometric sun sits at the top of each side of the monument. This image is significant physically and metaphorically. The pictures on the sides of the monument reflect on death, while the sun**,** a classic symbol of American funerary art, announces a renewal of life.[26] There is a movement up from the physically decaying dead body to the ethereal soul transcending toward the sun to heaven. The soul will physically depart the body beneath the monument and rise to heaven to follow Jesus and sit with God. The crossing over occurs at the tip of the monument with a cinerary urn, whose ashes are symbolic, "The [symbolic] ashes contained within the urn signify penitence, death of the body and its return to dust in the final resting place."[27] The soul is going to heaven while the body is returning to the earth from which it was made.

The ornamental motifs on the De Jongh monument coincide with the inscriptions. In some cases**,** particularly on New England grave stones of the 17th and 18th centuries, motifs and inscriptions had no relation because the stone carver would often carve what he felt was popular at the time.[28] However, this monument was neither carved, nor made from stone, but was cast in metal by Monumental Bronze Company of Bridgeport, Connecticut whose name is printed in clear letters at the base of one side.[29] The Monumental Bronze Company originated in Connecticut and expanded throughout the United States, opening businesses in Des Moines, Ontario, and Chicago. These subsidiaries had their own names and inscribed them on the grave markers that they produced. Traveling salesmen carried catalogs with images resembling popular styles in marble and granite.[30] Companies would encourage customers such as the De Jonghs to browse their catalogs and choose exactly which designs and decorations they wanted on their memorials.

The metal used by the Monumental Bronze Company to make the markers was zinc, popularly referred to as "white

The inscriptions and designs of monuments reveal the beliefs and ideals of the dead and their families.

bronze."[31] These monuments were hollow in the middle, and turned a blue-gray color when exposed to air. White bronze was cheaper than the more common medium of granite or marble and held up better to weathering than marble, though no one knew this in the late 1800's. In addition, the tablets on the side of the monument were easily removable and names could be added when additional family members died.

It is unclear why Henry De Jongh purchased the cheaper, white bronze, material for the construction of his monument. He seemed to be fairly well off when he died, eleven years after the monument was erected. He possibly made most of his money in real estate as the city boom continued, for he died with $5,000 in personal property and an estimated $12,000 in real estate.[32] Henry may have been drawn to the large size of the marker he could purchase for a relatively small amount fee. Whatever the case, it was not common to buy such a memorial, as they were becoming increasingly unpopular. However, the removable tablets did prove to be beneficial, as Henry had to bury first his wife and then a daughter.

Henry, Johanna, and Reinira were not the only members of the De Jongh family in Rochester. Henry was survived by two sons and a daughter from his first marriage, and a second wife. There was room for all to be inscribed on the monument, but no additional family members were added. The second wife, according to custom, should have been buried in the plot with Henry, but there was a dispute between her and the De Jongh children after Henry's death.[19] The dispute over property and money left in the will resulted in Henry's second wife, Magdalena De Jongh, giving up her portion of the inheritance and moving in with her cousin in Gates, NY.[33] Magdalena was probably buried apart from Henry because of the poor relationship she had with his children. In addition, she was left with little money to make the necessary burial arrangements.

The story of the De Jongh family and their memorial is fairly typical of life in Rochester in the late 1800's. The De Jonghs emigrated from Holland at a time when business was flourishing and the population was growing during the industrial boom. The De Jonghs integrated themselves into society, belonged to a church, and owned a small business. As immigrants, they remained connected to their cultural and religious roots, while adopting the language and lifestyle of America. The De Jongh monument in Mt. Hope Cemetery stands as a symbol of their values and religious beliefs. □

*Hannah Newborn completed her degree in Religion in May 2003. Her research stemmed from an assignment in Professor Emil Homerin's Speaking Stones class. In addition to religion, Hannah has completed the Pre-Medical requirements and plans to attend medical school. She hopes to integrate her understanding of religion and its effects on people with medicine.*

# The Riemannian Metric and Curvature Tensor on a Manifold

Niles G. Johnson, 2003
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**The computation of terms in a Taylor series illustrates an interplay between key geometric notions.**

The main goal and result of our work this summer was a computation exhibiting the relationship between the curvature tensor and the Riemannian metric on a smooth Riemannian manifold. The most interesting and exciting aspects of our work, however, were the methods employed in the calculation. We will walk through an overview of these methods but leave most of the technical details in another paper. As we go, the goal for which we worked is simple and should be kept in mind. Differentiable functions can be written (in various ways) as infinite polynomials, and one of the standard ways is called the Taylor series for the function. We looked at the Taylor series for a Riemannian metric and showed that the terms in this polynomial are equal to expressions involving only the curvature tensor and its covariant derivatives.

## Groundwork

The setting for our investigation is a geometric object referred to above as a *smooth Riemannian manifold*. There are complicated technical definitions of manifolds, but the idea they describe is a place in which small regions look like (are closely approximated by) small regions in Euclidean space of a certain dimension, $m$, where $m$ can be 1 (the real line, $\mathbf{R}$), 2 (the plane, $\mathbf{R}^2$), 3 ($\mathbf{R}^3$), etc. The surface of a ball is a good example (for $m = 2$), because it shows how small pieces of a manifold could look like small pieces of a plane, while the whole manifold might not look like a plane (see Figure 1(a)). For each point on a manifold we consider a tangent space, which is a copy of flat Euclidean space affixed at each point on the manifold (see Figure 1(b)). This is a generalization of the tangent lines ($m = 1$) studied in every calculus course.

To say that a manifold is *Riemannian* is to say that it has a Riemannian metric, $G = (g_{ij})^m_{i,j=1}$, which is an inner product on each tangent space— it defines the measure of angles and distances in the tangent space. For vectors $\mathbf{x}$, $\mathbf{y}$ in some tangent space, $G(\mathbf{x}, \mathbf{y}) = \mathbf{x}G\mathbf{y}$, using matrix multiplication with $\mathbf{x}$ written as a row and $\mathbf{y}$ written as a column. If $p$ is the point whose tangent space we are considering, we may emphasize this by writing the components of $G$ as $g_{ij}|_p$ instead of just $g_{ij}$. This inner product need not be the same for each tangent space (remember there is one for every point on the manifold), but the adjective "Riemannian" means that the different inner products change smoothly. With the metric in the tangent space we produce a notion of angle and distance on the manifold itself. The 2-sphere (a mathematical model for the surface of a ball) is a Riemannian manifold. When you say that the "straight lines" are great circles and that each has length $2\pi r$ (where $r$ is the radius of the sphere), you are recognizing the notion of angle and distance which is induced by the standard inner product on the tangent spaces. The 2-sphere is in fact a *smooth* Riemannian manifold; smooth means intuitively the same thing as its English homonym, and mathematically it means that everything in sight is differentiable (even the derivatives, and the derivatives of the derivatives, and the . . .).

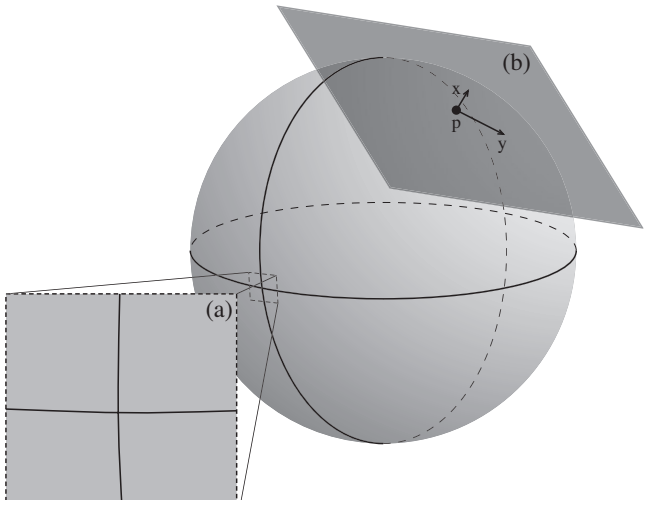


**Figure 1**. A sphere is a good example of a smooth Riemannian manifold. **a.** A small region of the sphere actually looks like a plane; **b.** At each point on a manifold, here our sphere, there is a tangent space.

Taking derivatives on a manifold is tricky business, especially differentiating a tensor. We use two kinds of derivatives, the partial derivative, $\partial$, and the covariant derivative, $\nabla$, both of which are directional derivatives. To describe how components of $G$ change along a certain direction (say $x^k$) we use $\partial_k$. One of the important facts about differentiation in Euclidean space is that partial derivatives of a smooth vector field commute; $\partial_k \partial_l = \partial_l \partial_k$ for all $l$ and $k$ between 1 and $m$. On a general manifold, this is no longer true. The *curvature tensor* is a function, $R$, which describes the difference between $\nabla_k \nabla_l$ and $\nabla_l \nabla_k$; that is, the degree to which the manifold deviates from flat Euclidean space (how `curved' it is). Whereas the metric has $m^2$ components, the curvature tensor has $m^4$ components, denoted $R_{ikjl}$ for some $i, j, k, l, = 1, 2, \ldots$. Just as with the components of the metric, each component of the curvature tensor is a real number (which may be different for different points on the manifold). For technical reasons, we have to use the covariant derivative (instead of the partial derivative) to determine how the components of $R$ change as we move across the manifold along some line. All we will need to know here is that a covariant derivative in the direction of $x^k$ is denoted $\nabla_k$.

### Game Plan

The curvature tensor is derived from the metric, and the net result of our work is a description of the opposite result— namely that the metric can be described in terms of the curvature tensor. We achieve this by considering the Taylor series for the metric, which is a natural generalization of the Taylor series studied during the first couple of semesters of calculus. One of the most interesting Taylor series is the expression for the exponential function. If $t$ is a real number and $e = 2.718\ldots$ denotes the base of the natural logarithm, then

$$e^t = 1 + t + \frac{t}{2} + \frac{t^3}{6} + \frac{t^4}{4!} + \cdots. \tag{1}$$

For a manifold, the same idea is generalized slightly to higher dimensions by taking sums over different combinations of components. If $p$ is a point on the manifold and $q$ is a point nearby whose coordinates in Euclidean space with respect to $p$ are $x^1, x^2, \ldots, x^m$ then in reasonable circumstances

$$g_{ij}\Big|_q = g_{ij}\Big|_p + \sum_{k=1}^{m} (\partial_k g_{ij})\Big|_p x^k + \frac{1}{2} \sum_{k_1,k_2=1}^{m} (\partial_{k_2}\partial_{k_1} g_{ij})\Big|_p x^{k_1} x^{k_2} + \frac{1}{6} \sum_{k_1,k_2,k_3=1}^{m} (\partial_{k_3}\partial_{k_2}\partial_{k_1} g_{ij})\Big|_p x^{k_1} x^{k_2} x^{k_3} + \cdots \tag{2}$$

The result we obtained expresses this Taylor series in terms of the curvature tensor and covariant derivatives of the curvature tensor (covariant derivatives can be thought of as partial derivatives for a tensor). The general line of reasoning we follow is:

1. Use the 'right' coordinate system and study $G$ and $R$ in that coordinate system.

2. Differentiate something called the Gauss lemma to obtain a system of equations involving appropriate partial derivatives of the $g_{ij}$.

3. Examine the summation

   $$\sum_{k_1,\ldots,k_n} \nabla_{k_n} \cdots \nabla_{k_3} R_{ik_2jk_1}\, x^{k_n} \cdots x^{k_1}$$

   Using the expression for covariant derivatives of $R$ in terms of components $g_{ij}$ (and their partial derivatives), find the highest-order derivatives of the $g_{ij}$ appearing in the summation above.

4. Use relations derived from the Gauss lemma to reduce the terrible linear combination of highest-order derivatives to a single highest-order derivative.

Inductively, the lower order derivatives of the $g_{ij}$ can be written in terms of lower order covariant derivatives of the $R_{ikjl}$, and subtraction then gives the result.

Explicitly, we will compute, for each $n$,

$$\sum_{k_1,\ldots,k_n} \partial_{k_n} \cdots \partial_{k_1} g_{ij}\Big|_p x^{k_n} \cdots x^{k_1} = \sum_{k_1,\ldots,k_n} C_n \left( \nabla_{k_n} \cdots \nabla_{k_3} R_{ik_2jk_1}\Big|_p + \begin{array}{c}\text{a polynomial in lower order}\\ \text{covariant derivatives of } R\end{array} \right) x^{k_n} \cdots x^{k_1} \tag{3}$$

for a particular constant $C_n$.

### Coordinates and the expression for $R$ in terms of G

In linear algebra one studies various choices of bases for vector spaces, and an important first step in our work was to choose a nonstandard basis more naturally suited to express the geometric relationships under consideration. We referred to this new basis as the *orthonormal frame* (sometimes called local canonical coordinates). In this orthonormal frame, we have some identities for $G$ automatically; they are

$$g_{ij}\Big|_p = \delta_{ij}$$

$$\sum_k \partial_k g_{ij}\Big|_p x^k = 0$$

The curvature tensor and its covariant derivatives are expressed as

$$\nabla_{k_n} \cdots \nabla_{k_3} R_{ik_2jk_1}\Big|_p = \frac{1}{2}(\partial_{k_n} \cdots \partial_{k_3}\partial_j \partial_{k_2} g_{k_1 i} - \partial_{k_n} \cdots \partial_{k_3}\partial_j \partial_i g_{k_1 k_2} - \partial_{k_n} \cdots \partial_{k_3}\partial_{k_1}\partial_{k_2} g_{ij} + \partial_{k_n} \cdots \partial_{k_3}\partial_{k_1}\partial_i g_{jk_2})\Big|_p + \begin{array}{c}\text{a polynomial in lower}\\ \text{order partials of } g\end{array} \tag{4}$$

for each $i, j, k_1, \ldots, k_n$. For $n = 2$ and $n = 3$, we know the relationships more explicitly (the `polynomial in lower order partials' turns out to be zero in each case).

$$R_{ik_2jk_1}\Big|_p = \frac{1}{2}(\partial_j\partial_{k_2} g_{k_1 i} - \partial_j\partial_i g_{k_1 k_2} - \partial_{k_1}\partial_{k_2} g_{ij} + \partial_{k_1}\partial_i g_{jk_2})\Big|_p \tag{5}$$

$$\nabla_{k_3} R_{ik_2jk_1}\Big|_p = \frac{1}{2}(\partial_{k_3}\partial_j\partial_{k_2} g_{k_1 i} - \partial_{k_3}\partial_j\partial_i g_{k_1 k_2} - \partial_{k_3}\partial_{k_1}\partial_{k_2} g_{ij} + \partial_{k_3}\partial_{k_1}\partial_i g_{jk_2})\Big|_p \tag{6}$$

### The Gauss Lemma

The equalities above show that one cannot simply substitute covariant derivatives of the $R$ into the Taylor series for $g_{ij}$. Instead, we use the Gauss lemma to express summations of the form found in the Taylor series (equation 2) as summations of covariant derivatives of $R$.

Geometrically, the Gauss lemma says that straight lines emanating from a point on a manifold are perpendicular to loci of constant distance from the point. Algebraically, this can be expressed as

$$x^k = \sum_{j=1}^{m} g_{kj} x^j. \tag{7}$$

### Derivatives of the Gauss Lemma

The beauty of equation 7 is that it easily allows us to derive dependencies of the $n^{th}$ order partials of $g_{ij}$ at a base point $p$ on the manifold; this base point is the origin of our coordinate system. Differentiating equation 7 by $\partial/\partial_x i$, we get

$$\delta_{ki} = \sum_{j=1}^{m} \frac{\partial g_{kj}}{\partial x^i} x^j + g_{ki}$$

Evaluating at the origin (base point), we get

$$g_{ij}(0) = \delta_{ki} \tag{8}$$

a result that is not terribly impressive, since it is a property of normal coordinates that we have already derived. Let us continue the

process by differentiating again, this time by $\partial/\partial_{x^l}$. We get

$$0 = \sum_{j=1}^{m} \frac{\partial^2 g_{kj}}{\partial x^i \partial x^l} x^j + \frac{\partial g_{kl}}{\partial x^i} + \frac{\partial g_{ki}}{\partial x^l}$$

Again, we evaluate at the base point to get

$$\left( \frac{\partial g_{kl}}{\partial x^i} + \frac{\partial g_{ki}}{\partial x^l} \right) \Big|_p = 0 \tag{9}$$

We repeat the same process for the next order to get

$$\left( \frac{\partial^2 g_{ks}}{\partial x^i \partial x^l} + \frac{\partial^2 g_{ki}}{\partial x^l \partial x^s} + \frac{\partial^2 g_{kl}}{\partial x^i \partial x^s} \right) \Big|_p = 0 \tag{10}$$

We find that if we wish to obtain equations for the $n^{th}$ order partials of $g$ at the base point, we must differentiate the Gauss Lemma $n$+1 times and then evaluate it at the origin. We introduce the useful notation

$$\frac{\partial^n g_{ij}}{\partial x^{i_1} \dots \partial x^{i_n}} \Big|_p = (i_1, \dots, i_n | i, j)$$

In our notation, equations (9) and (10) become

$$(i \mid l,k) + (l \mid i,k) = 0 \tag{11}$$

and

$$(i,l \mid s,k) + (l,s \mid i,k) + (s,i \mid l,k) = 0 \tag{12}$$

We have rearranged indices when permissible (using the facts that $g_{ij} = g_{ji}$ and that partial differentials commute in Euclidean space) in order to make the pattern apparent. In order to generate an $n^{th}$ order Gauss equation, pick $n$+2 indices. Fix one of these indices; this letter will always index an entry of $g$; that is, this entry will always stay on the right side of the horizontal bar in our tuple notation. Keep adding successive lists, cyclically permuting the other $n$+1 indices. For example, a third order Gauss equation would be

$$(a,b,c \mid d,e) + (b,c,d \mid a,e) + (c,d,a \mid b,e) + (d,a,b \mid c,e) = 0 \tag{13}$$

Or rather

$$\left( \frac{\partial^3 g_{de}}{\partial x^a \partial x^b \partial x^c} + \frac{\partial^3 g_{ae}}{\partial x^b \partial x^c \partial x^d} + \frac{\partial^3 g_{be}}{\partial x^c \partial x^d \partial x^a} + \frac{\partial^3 g_{ce}}{\partial x^d \partial x^a \partial x^b} \right) \Big|_p = 0 \tag{14}$$

An $n^{th}$ order Gauss equation is

$$\begin{aligned}(i_1, i_2, \dots, i_{n-3}, i_{n-2} | i_{n-1}, i_n) \\ + \ (i_2, i_3, \dots, i_{n-2}, i_{n-1} | i_1, i_n) + \dots \\ + (i_{n-1}, i_1, \dots, i_{n-4}, i_{n-3} | i_{n-2}, i_n) \ = \ 0\end{aligned} \tag{15}$$

### Systems of Gauss Equations

Equation (12) is not the only second order Gauss equation. We can do a simple permutation of indices to get

$$(i,l \mid s,k) + (l,s \mid i,k) + (s,i \mid l,k) = 0 \tag{16}$$

We can find the number of distinct Gauss equations that must exist for each order $n$. There is one Gauss equation for each index that we choose to be fixed in our tuple notation. We simply cycle through the other indices to get the $n$+1 tuples for an equation. Since there are $n$+2 indices that we may choose to be fixed, there are $n$+2 independent Gauss equations.

Since, for any order, there are $\binom{n+2}{n}$ distinct tuples (from the $n$+2 indices, we must choose the $n$ by which we are partially differentiating ), the number of dependent partials is

$$\binom{n+2}{n} - (n+2) = \frac{(n+2)(n-1)}{2}$$

Therefore, for any order, the Gauss Lemma will allow us to find $n$+2 linear equations involving the partial derivatives of $g$ at the origin. These equations can be used to eliminate $\frac{(n+2)(n-1)}{2}$ partials and simplify calculations involving the partial derivatives of $g$.

Permuting indices, we find systems of equations. Among the $n^{th}$-order partial derivatives of $g$ at $p$, this process produces a system of $n$+2 simple equations in $\binom{n+2}{n}$ variables (derivatives of g).

### Examining the Summation

For each $n$, we determine the coefficient of $x^{k_n} \dots x^{k_1}$ in the summation

$$\sum_{k_1, \dots, k_n = 1}^{m} \nabla_{k_n} \cdots \nabla_{k_3} R_{ik_2jk_1} \, x^{k_n} \cdots x^{k_1} \tag{17}$$

for a particular choice of $k_1, \dots, k_n$.

For $n$ = 2, we use equation (5) and find the coefficient of $x^k x^l$ in $\sum_{k,l} R_{ikjl} \Big|_p x^k x^l$ to be

$$\begin{aligned}R_{ikjl}\Big|_p + R_{iljk}\Big|_p \\ = \ -\frac{1}{2}((j,i|l,k) - (j,k|l,i)) + \frac{1}{2}((l,i|j,k) - (l,k|j,i)) \\ -\frac{1}{2}((j,i|k,l) - (j,l|k,i)) + \frac{1}{2}((k,i|j,l) - (k,l|j,i))\end{aligned}$$

which simplifies to

$$\begin{aligned}R_{kilj}\Big|_p + R_{likj}\Big|_p \ = \ -\frac{1}{2}\{2(k,l,|i,j) - ((j,k|l,i) + (j,l|k,i)) \\ -((i,k|l,j) + (i,l|k,j)) + 2(i,j|k,l)\}\end{aligned} \tag{18}$$

Likewise, for $n$ = 3, we use equation (6). The coeffecient of $x^{k_1} x^{k_2} x^{k_3}$ is

$$\begin{aligned}\nabla_{k_3} R_{ik_1jk_2} + \nabla_{k_3} R_{ik_2jk_1} + \nabla_{k_2} R_{ik_1jk_3} \\ + \nabla_{k_2} R_{ik_3jk_1} + \nabla_{k_1} R_{ik_2jk_3} + \nabla_{k_1} R_{ik_3jk_2}.\end{aligned} \tag{19}$$

Substituting, the above simplifies to

$$\begin{aligned}-\frac{1}{2}\{&6(k_3,k_2,k_1|i,j) \\ &- \ 2((k_3,k_2,j|k_1,i) + (k_3,k_1,j|k_2,i) + (k_2,k_1,j|k_3,i)) \\ &- \ 2((k_3,k_2,i|k_1,j) + (k_3,k_1,i|k_2,j) + (k_2,k_1,i|k_3,j)) \\ &+ \ 2((k_3,i,j|k_2,k_1) + (k_2,i,j|k_3,k_1) + (k_1,i,j|k_2,k_3))\}\end{aligned} \tag{20}$$

We now combine terms in equation (17) and examine the coefficient of $x^{k_n} \dots x^{k_1}$ for a particular choice of indices $k_1, \dots, k_n$. It is

$$\sum_{\sigma} \nabla_{k_{\sigma(n)}} \cdots \nabla_{k_{\sigma(3)}} R_{ik_{\sigma(2)}jk_{\sigma(1)}} \Big|_p \tag{21}$$

with the summation over all $\sigma \in S_n$ (that is, all of the $n$! distinct arrangements of the indices $k_1, \dots, k_n$). Substituting from equation (4), this coefficient becomes

$$\begin{aligned}-\frac{1}{2}\{&n!(k_n, \dots, k_1 | i,j) \\ &- \ (n-1)! \sum_{l=1}^{n} (k_n, \dots, \hat{k}_l, \dots, k_1, j | k_l, i) \\ &- \ (n-1)! \sum_{l=1}^{n} (k_n, \dots, \hat{k}_l, \dots, k_1, i | k_l, j) \\ &+ \ 2(n-2)! \sum_{l_2=1}^{n} \sum_{l_1=1}^{l_2-1} (k_n, \dots, \hat{k}_{l_1}, \dots, \hat{k}_{l_2}, \dots, k_1, j, i | k_{l_2}, k_{l_1})\} \\ &+ \ \begin{matrix}\text{a polynomial in lower} \\ \text{order partials of } g\end{matrix}\end{aligned} \tag{22}$$

where hatted indices are omitted.

### Applying the Gauss lemma

We use the $n^{th}$-order Gauss lemma to condense the $n^{th}$ order partials of equation (22) to a single multiple of $(k_n, \dots, k_1 | i,j)$. The second and third terms of equation (22) can be dealt with directly by two of the $n^{th}$ -order Gauss Lemma equations:

$$\begin{aligned}(k_n, \dots, k_3, k_2, j | k_1, i) \ &+ \ (k_{n-1} \dots, k_2, j, k_1 | k_n, i) + \dots \\ +(j, k_1, k_n, \dots, k_4, k_3 | k_2, i) \ &= \ -(k_1, k_n, \dots, k_3, k_2 | j, i)\end{aligned} \tag{23}$$

and

$$\begin{aligned}(k_n, \dots, k_3, k_2, i | k_1, j) \ &+ \ (k_{n-1} \dots, k_2, i, k_1 | k_n, j) + \dots \\ +(i, k_1, k_n, \dots, k_4, k_3 | k_2, j) \ &= \ -(k_1, k_n, \dots, k_3, k_2 | i, j).\end{aligned} \tag{24}$$

Simplification of the fourth term of equation (22) is more involved, but nonetheless straightforward. The reader can check that the sum of all $\binom{n+2}{n}$ tuples is zero. Subtracting equations (23) and (24) from this sum shows that the sum of all terms that have both $i$ and $j$ to the left of "|" is equal to the term having neither $i$ nor $j$ to the left of "|". That is,

$$\sum_{l_2=1}^{n} \sum_{l_1=1}^{l_2-1} (k_n, \dots, \hat{k}_{l_1}, \dots, \hat{k}_{l_2}, \dots, k_1, j, i | k_{l_2}, k_{l_1}) = (k_n, \dots, k_1 | i, j). \tag{25}$$

All together this shows that equation (22) is equal to

$$-\frac{1}{2}[n!+2(n-1)!+2(n-2)!](k_n,\ldots,k_1|i,j) + \begin{matrix}\text{a polynomial in lower}\\ \text{order partials of } g\end{matrix} \tag{26}$$

which is to say that

$$\sum_{k_1,\ldots,k_n=1}^{m} \nabla_{k_n}\cdots\nabla_{k_3}R_{ik_2jk_1}\,x^{k_n}\cdots x^{k_1} = \sum_{k_1,\ldots,k_n=1}^{m} -\frac{n!+2(n-1)!+2(n-2)!}{2n!}\left(\partial_{k_n}\cdots\partial_{k_1}g_{ij} + \begin{matrix}\text{a polynomial in lower}\\ \text{order partials of } g\end{matrix}\right)x^{k_n}\cdots x^{k_1}$$

By induction, this leads immediately to equation (3), with

$$C_n = \frac{-2n!}{n!+2(n-1)!+2(n-2)!}.$$

**Afterword**

After surveying some of the explicit results, one may begin to wonder if research in mathematics is just endless technical computation. This could not be more wrong. The purpose of our research was to clarify of the relationship between two qualities a manifold has— its metric and its curvature tensor. The computation is important only because it (partially) reveals this relationship.

Our work is a small part of a much larger effort to understand manifolds. In many ways manifolds are a natural generalization of Euclidean space and as such they are frequently the context for various scientific studies. General manifolds are much more complicated than Euclidean space (for Euclidean space, the curvature tensor is zero everywhere, as are all of its derivatives), and mathematicians working in differential geometry try to understand how the various properties of a manifold are related. It is known that the curvature tensor determines all of the geometry on a manifold— including the metric— but it is not fully understood *how* the curvature tensor determines these things. Indeed, although our computation is more explicit than some other results, it still does not give a closed form for this determination.

These somewhat incomplete results do, however, have an application. As one brief example, we mention the use of manifolds in the study of physics, especially in an area called string theory. In this area (as in other areas of physics, chemistry, etc.) scientists study functions defined on a manifold and try to integrate the functions with respect to the metric. Because the metric on a general manifold can be quite complicated, it is often difficult to compute this kind of integral. By considering the natural geometry of the particular manifold under discussion, the curvature tensor may be more accessible than the metric. Expanding components of the metric in terms of the curvature tensor and its covariant derivatives allows one to use knowledge of the curvature tensor to study these integrals. The moral of many mathematical stories (like this one) is that much can be gained by looking at one thing through its relationship to another. At its heart, our result is one such look at the curvature tensor. □

*Niles graduated from the University of Rochester in 2003 with a B.A. and an M.A. in Mathematics. Homer, Dave, and Niles completed their work on the Taylor expansion of a Riemannian Metric during the summer of 2002 under the guidance of Professor DaGang Yang at Tulane University. Their research was supported by an REU grant from the National Science Foundation. Niles is continuing his studies in a math Ph.D. program while his collaborators pursue careers in physics.*

# Grassroots and Government Conflict

Emily Miyamoto Faber, 2003
Advisor: Francisco Amaro Gomes, de Alencar of the Universidade Federal School for International Training

**Citizen-comprised land reform groups challenge the Brazilian government's National Plan for Agrarian Reform when it fails to complete its mission.**

Brazil is currently undertaking one of the world's largest and most ambitious land-reform programs. This is beneficial, given that 23.1% of the Brazilian labor force works in the agricultural sector.[1] During the past five years, the Brazilian government has invested US $6.5 billion to settle nearly two million people on 18 million hectares of land. Since 1995, under President Fernando Henrique Cardoso's administration, more Brazilians have been given their share of the land than in all of Brazil's 500-year history.[2]

Despite the creation and implementation of Brazil's Plano Nacional de Reforma Agrária ("National Plan for Agrarian Reform") and the existence of an official government agency responsible for administering agrarian reform, Instituto Nacional de Colonização e Reforma Agrária ("National Institute of Colonization and Agrarian Reform in Brazil" or INCRA), several outside land reform groups such as the Movimento dos Trabalhadores Rurais Sem Terra ("Landless Workers Movement" or MST) and the Comissão Pastoral da Terra ("Pastoral Land Commission" or CPT) have emerged. These grassroots organizations work side-by-side with the Brazilian farmers in land struggles.

### INCRA (National Institute for Colonization and Agrarian Reform)

INCRA is the official government agency responsible for agrarian reform in Brazil.Created in 1970 under the military regime (1970-1984), it continued to exist under President Itamar Franco's New Republic government (1985-1994) and has been much a part of current President Fernando Henrique Cardoso's agenda:

Figure 1 illustrates the numbers of families settled during these three time periods shows that the majority of families have been settled under the most recent administration.

INCRA's mission is to "Further agrarian reform with a focus on complete and sustainable local development with a base in the principals of social justice, seeking to guarantee citizenship and improve the socio-economic conditions of the rural population."[3] Consequently, INCRA's role in agrarian reform extends much beyond the expropriation of land.An INCRA official specializing in projects explains the duties of the organization:

> *Our role does not just include the expropriation of land.It includes the whole process of social organization and production of the families….It is to help the families become independent from the institution's administration.So, we work with the entire process, organization, all of the productive process—what the farmers will produce, how they will produce it, where they will produce it.*[4]

Additionally, through créditos, or money loans in the amount of 2,000 reais[a], are apportioned in order for farmers to be able to feed their families and have some start-up money for their newly-acquired farm.[5]

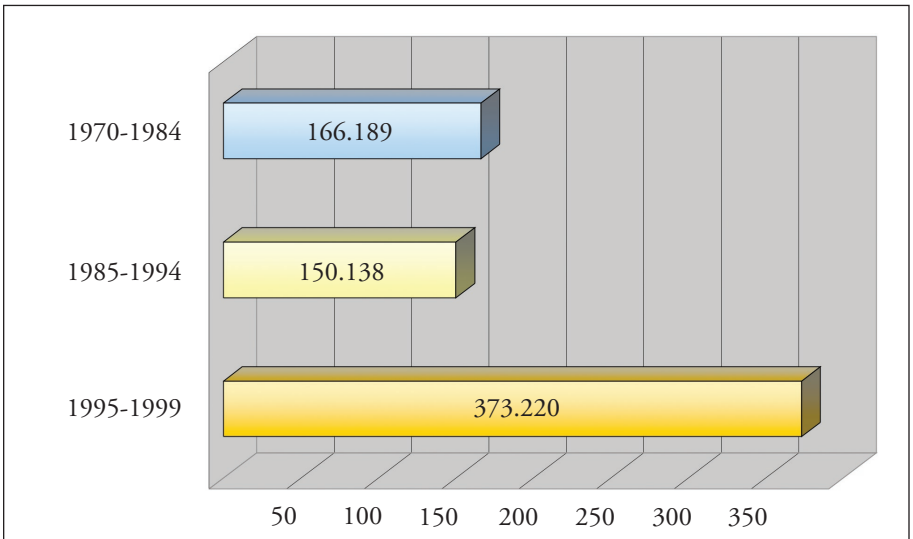


**Figure 1**. Number of families setteled 1970-1999. From INCRA website.

**Above**: Children of Assentamento Che Guevara stand by the front gates.

**CPT (Pastoral Land Commisson)**

The CPT was created in 1975 to serve the rural land workers of Brazil. It collaborates directly with the Catholic and Evangelical churches of Brazil and works as an advisor to labor unions, small producers' associations, social movements, and other popular initiatives. Between 1975 and 1984, the CPT played a fundamental role in agrarian reform by developing contacts between local and regional leaders and by creating a national dimension to land struggles.[6] Currently, the role of the CPT is to provide acompanhamento ("formal government assistance in establishing a settlement") to the men and women who work in the countryside by acting as a theological, methodological, political, and sociological advisor. The acompanhamento that the CPT provides primarily focuses on support in the production process—deciding what is produced on the settlement, how it is produced, and what is easiest to produce.This often involves working on a research project with the workers to determine what action is best.[7]

**MST (Landless Workers' Movement)**

The MST is the largest social movement in Latin America. Formed in 1984 by breaking off from the CPT, the movement was formed in response to Brazil's dire economic inequalities, principally that of land concentration. It is currently active in 23 of Brazil's 26 states. The MST pursues a variety of strategies in their fight for land, including occupations (which they consider their most important tactic).[8] The movement is the first and only grassroots pressure group in Brazil to have influenced the political agendas of the national government.[9] MST land occupations have led to the attainment of over 15 million hectares of land for between 250,000 and 300,000 families across Brazil. In addition to obtaining land and security food accessibility, MST members have created an alternative socio-economic development model, placing people before profits.[10]

If INCRA is expropriating land and effectively pursuing their mission of creating sustainable development in rural communities through aiding settled farmers by way of education and monetary assistance while simultaneously guaranteeing citizenship and improving socio-economic conditions, why do organizations and movements persist? In this paper I look at the roles and perspectives of INCRA, the Movimento Sem Terra and the Comissão Pastoral da Terra in the land reform process. Then, through interviews and document review, I will evaluate the current land reform program, combining the perspectives of the government officials, numbers of movements, and settled farmers. Finally, I will make a recommendation to the government on how to improve the implementation of Brazilian agrarian reform.

**Importance of research**

Brazil is the largest and most populous country in South America, and it is the leading economic power in all of Latin America.[11] It also continues to face the problem of severely unequal income distribution, primarily in the form of land concentration. The statistics are alarming:

- Two-thirds of Brazil's arable land is owned by less than 3% of the population.[12]
- Sixty percent (60%) of this arable land lies idle, while 25 million peasants struggle in temporary agricultural jobs.[13]
- Nearly 4.8 million families are considered landless[b], according to the 1995 Census.[14]

This inequality has led to the formation of movements and pressure groups like the MST and CPT that are critical of the government's social and economic policies. These groups are composed of members of the left wing of the Catholic Church, labor unions, and the Workers' party and have emerged in response to the mounting pressure to redistribute land and wealth.[15]

**Location of research**

My research in Brazil took place in the northeastern state of Ceará. Ceará's state population figure rests slightly above 7 million people with approximately 2.6 million people residing in the capital city, Fortaleza, where I lived while studying in Brazil. In Fortaleza, I spent a significant amount of time at the headquarters of INCRA, the MST, and the CPT. The second major piece of my research took place at assentamentos ("land settlement"), all located outside of Fortaleza. In Ceará, 13,981 families have already settled and there are land vacancies for 6,117 more families.[16] The state is divided into 184 municipalities which form the local government structure. The three assentamentos that I visited were located in different municipalities, all situated within two and one-half hours from Fortaleza. I accessed all of the settlements through a complicated combination of public transportation including mini-buses, buses, motorcycle taxis, and trucks. Settlements were selected based on their proximity to Fortaleza, the current and / or past presence of a social movement group, and the history of the settlement's formation. I chose to conduct research at the following settlements:

**Assentamento Che Guevara**, as it calls itself and is known by the MST (or **Assentamento São José**, as it is called by INCRA) is located in the Ocara municipality, approximately two hours to the south of Fortaleza. This settlement, comprised of 45 families, was formed in 1999 through a MST land occupation of a fazenda ("large-scale farm") in a neighboring municipality. The settlement follows the MST ideology, including the collective nature of production. The CPT is also very much present at Che Guevara; one representative actually lives on the settlement.[17]

**Assentamento Santa Bárbara** is located in the Caucaia municipality, on the northwest periphery of the Fortaleza metropolitan area. Santa Bárbara, home to 95 families, was created in 1996 through negotiations directly between INCRA and the former fazendeiro ("landowner of large-scale properties designed for farming"). Thus, there was no formal struggle for the land through occupation. At the end of the prolonged negotiation process, however, the MST became involved in order to pressure INCRA to keep the agrarian reform process moving forward.[18] Today, Santa Bárbara struggles to maintain the organizational structure that MST tried to implement during the early days, but the discussion groups designed for political means have lost their function as discussions of economic purposes have become predominant.[19] There is not a strong MST or CPT presence throughout this settlement.

**Assentamento Erva Moura** is located in the Pentecoste municipality, 2.5 hours to the west of Fortaleza. Like Santa Bárbara, Erva Moura was also obtained without a direct MST land occupation. INCRA negotiated directly with the former fazendeiro

While technical assistance is not explicitly spelled out as a component of INCRA's mission, it is apparent that it is a necessary factor-confirmed even by INCRA officials.

(who had expressed interest in selling the land), assembled a list of interested agricultural workers, and settled them on the land. Since its creation in 1995, 160 families are currently part of Erva Moura. During its inception, MST was a large presence on the settlement and aided in establishing its internal organization. No other MST or CPT influence is currently present.[20]

### Research methodology

My research was done during May 2002, and was conducted using two methods. Background research, as well as research regarding the official position of organizations on agrarian reform, was done through document review. Documents were accessed on the internet as well as through books, articles, and pamphlets and provided excellent sources on the missions, goals, accomplishments, challenges, and positions of these organizations.

The majority of my research was done through personal interviews at government agencies, non-governmental organizations, and land settlements. Land settlements were difficult to reach due to unreliable transportation; there were times I was not sure if I was going to make it to the settlement, or back to Fortaleza. These mishaps often turned into opportunities, as my best interviews occurred when I had the farmers' undivided attention when the buses broke down. Interviewees included agrarian reform experts in the Brazilian government, leaders of and participants in agrarian reform movements, and farmers living on settlements. Personal interviews with settled farmers involved in the struggle to obtain land and the current reality provided perspectives from local experts in these settlements. Each interview was recorded on a dictaphone, transcribed directly in Portuguese, and later translated into English. In the interest of protecting the interviewees' privacy, their names will not be revealed.

### Content of research

**Legal Provisions for Agrarian Reform**

Provisions for agrarian reform date back to the 1964 Estatuto da Terra ("land statute"), which was the first land reform law created in Brazil. It was passed under the President-Marshall Castelo Branco in response to the need to redistribute land in order to avoid social revolution, such as those in Cuba in 1959. The Estatuto da Terra introduced the concept of função social, or social function of the land. Social function is defined as land being used in a productive and adequate way with respect for the environment while still adhering to labor laws.[21]

The Plano Nacional de Reforma Agrária was created in 1985, under the direction of President José Sarney. The plan was designed to immediately implement the Estatuto da Terra and make agrarian reform a visible national priority and action.[22] It included lofty goals, such as settling 1,400,000 families within a five-year period. However, only 89,950 families actually settled on newly appropriated lands.[23]

The current Constitution, adopted on October 5, 1988 also recognizes the redistribution of land as a national issue. According to Chapter III, Title VII, Articles 184-191, lands that do not fulfill a social function, as defined in the Estatuto da Terra, must be expropriated for agrarian reform.[24] According to the Constitution, the fundamental objectives of the Brazilian government are to construct a free, just, and unified society; guarantee national development; eradicate poverty and marginalization, reduce social and regional inequality, and promote the well being of the people without regard to their origin, race, sex, color, age, or other discriminating factors.[25]

**Hypothesis of Research**

According to an article published in Newsweek during January 2002, Brazil's agrarian reform statistics are incomplete for the 4,200 some settlements. Despite not being able to reveal what the agricultures ("farmers") produce or how they fare in this process, statistics do indicate that at least one out of every four settled farmers in Brazil gives up his plot within two years.[26]

What is responsible for this breakdown in the agrarian reform program? In theory, INCRA professionals travel to the settlements, create a short-term and long-term plan for the community, and address local needs including organization and administration.[27] The problem, however, is that this acompanhamento does not reach all of the settlements in Ceará due to lack of resources, and it fails to include all necessary components.[28]

This lack of completion of the intended plan has impeded Brazil's success. It is impossible for Brazil's marginalized countryside population to become "full socioeconomic citizens" solely by having arable land; they must also be handed the education and abilities to become them. The Brazilian agrarian reform program must not only redistribute land, but also provide all settled farmers with the information, education, and training necessary to turn these former farmhands into self-sufficient agricultores.

**Problems: Grassroots Perspectives on Brazil's Agrarian Reform Program**

Based on interviews and conversations during my visits to three settlements (Che Guevara, Santa Bárbara, and Erva Moura), I have identified three persistent sentiments held by settled people regarding Brazil's agrarian reform program and the role of INCRA.

*Little Effort on the Government's Behalf*

Despite the massive program of agrarian reform that the current Cardoso administration has undertaken, settled people see the federal government's actions as acting solely as a response to social pressures, not as a true priority to attain social justice:

> *"Agrarian reform is occurring because of the movements. If it weren't for these movements, I think that the government wouldn't have done anything."* [29]

> *"Today in CearáI think we have about 200 settlements. If it weren't for the MST, it would probably be around 50.Maybe not even 50. Because the MST pressures….If we waited until INCRA expropriated on its own, if there were no pressure, it would take INCRA 10 years. Who knows if it would even do that."* [30]

The people view the government's actions as a result of social pressure, and in the absence of social movements, they believe that agrarian reform would not be a governmental priority.

*Abandonment of the Settlements*
The settled farmers also express their disappointment and disapproval of the way that INCRA was present during the encampment (if applicable) and expropriation process, but has failed to be a solid presence following the actual expropriation:

> *"INCRA was present until the expropriation of the land. When it was expropriated, INCRA left the people. We haven't had one more visit. They promised a technical presence, but they haven't come in a long time."* [31]

> *"There are some programs. But they are small that really don't help the people who have been settled.… Of the 300 settlements in Ceará, 60 benefit. So, it's a very small quantity.… The other thing in relation to empowering settled workers, the world has advanced significantly, technologically.…We don't have access to the research, we don't have access to information."* [32]

Settled farmers expressed not only their united feeling that INCRA's involvement in their settlement ended when the expropriation was finalized, but also their desire for INCRA to be present at the assentamentos, especially to be able to relay organizational and technical information.

*No Cooperation in the Process*
The residents of the land settlements not only expressed their discontent with INCRA's abandonment of the settlement, but also their frustration during their few encounters with INCRA, principally in relation to the acquisition of funds:

> *"We have been discussing a program to install electricity for over one year. INCRA said we would get it last October, but it is already May. The program has been finished for two years.…All INCRA has to do is approve the project to release the money.…INCRA is what is keeping the project from happening.…..INCRA is waiting because it is an election year. It is very political with INCRA. Very.…Someone will win the election because of this, because he can say he brought electricity to the settlement."* [33]

> *"We are always looking for more information. After getting the information we need, we look for technical assistance, which we also have to go through INCRA for. We have a hard time, there is a lack of resources, and INCRA doesn't pay. When INCRA does pay for technical support, they pay late."* [34]

Thus, when the settled people do actually come in contact with INCRA for money matters, they are frustrated with the interaction. The difficulty in accessing resources in order to be able to successfully establish their settlements seems to be the primary complaint.

**Solutions: What Land Settlements Want and Need**

Settled farmers freely articulated the primary problems their settlements experienced in the early part of the process and the problems they continue to experience. To remedy these problems, they ask for the government, through the INCRA agency, to step in and educate them on the issues that they need to address. There are three main themes on which they wish to have more information and instruction:

*Organization*
History of formation of the settlement is often determinant of its future. In some cases, such as the Che Guevara settlement, the workers had already united and formulated a sense of internal organization with priorities such as cooperative work and an established leadership program. In others, however, such as the Santa Bárbara settlement, INCRA made a list of people who were going to get land and live together in a community. Few of these people had previous experience with organizing a community:

> *"INCRA was here, four or five years ago.… It was an isolated thing. There was hardly any organization. They did the minimum amount they could. INCRA never actually worked with us. If it weren't for the three or four people here who are part of the MST, and for MST's model of organization . . . our settlement's organization is thanks to them."* [35]

> *"The government has no interest in organizing the settlements.…They know that education will enable the people to organize themselves, and to fight for more."* [36]

Not only do we see that the government failed to play a large role in organizing the community, but also that people speculate why—teaching people to organize would not be in the best interests of the government.

*Administration*
Administration and management of private property and farms is another challenge these settled farmers face. Many come directly from agricultural work, but have worked their entire lives for a boss. Thus, they have significant experience working the fields, but have never decided what to plant, where to plant it, when to plant it, and finally what to do with it:

> *"Those who come (to the settlements) were employees, worked for a boss. They worked for other people.…So, do these people know how to organize their own jobs? To produce things? To sell them? These people don't. They are totally lost."* [37]

> *"The government tells the people they don't know how to work, that they don't have the ability to manage a settlement. But we know that these people just aren't used to this role, they are used to being coordinated by a boss.…All of the production was for the owner, and now each farmer is controlling their own land.…If they don't already have the ability to manage themselves, it is hard. But the government doesn't have an interest in teaching them."* [38]

Settlements recognize that not all of the agricultores arrive with the same knowledge regarding administration, and that this disparity results in the success of some and the failure of others.

*Technical Assistance in Production*
Technical assistance, or advice and consultation in the area of production, is another area in which farmers desperately seek help. They are in dire need of learning about what crop is the best to plan on the land, when is the best season to plant these crops, how

to harvest, etc. When newly autonomous farmers are given land, they have very little experience in determining these things, especially if they have been settled far from lands that are familiar.

> *"The CPT doesn't have one land technician. We have access to some, when the workers ask for it.... We contract outside companies for technical assistance, and it should come from INCRA. But INCRA isn't providing this....they are not providing acompanhamento."* [39]

> *"The settlements are really in need of technical assistance. And the government wants to cut it. We are fighting. We are fighting to get a program of technical assistance available for the settlements....Exactly in order to contribute to the development of the settlements. For them to be able to develop economically and organize."* [40]

Thus, there exists a strong demand on behalf of the settled farmers for technical assistance. Technical assistance would include everything from learning what, where, when, and how to produce, as well as how to sell products.

## ERVA MOURA: MODEL OF A SUCCESSFUL ASSENTAMENTO

### *Life on the settlement has improved for everyone*

Through conversing with people at Erva Moura, I discovered that their satisfaction with life on the settlement was thoroughly apparent. They pointed to improvements in medical services, job opportunities, and their overall quality of life:

> *"When I first arrived here, because I lived far away, the people didn't have any awareness [of available services]. Not anymore, now they do. We have a doctor, a dentist....Living conditions got better for everyone."* [41]

> *"I consider the assentamento a success....There wasn't any electricity, houses, schools, orientations, education here before. Now we have nearly all of these things....Things improved quite a bit. There are many more opportunities for people."* [42]

**Figure 2.** Farming and horses at Assentamento Che Guevara.

After hearing mixed opinions on the successfulness of other settlements, I realized I needed to determine what differentiated Erva Moura from the other assentamentos. All ideas credited the excellent internal organization and communication of the settlement:

> *"[Internal organization] is great... Community leaders orient and teach the people what they need to do. Everyone goes to the meetings....They explain what to plant, what the land is capable of producing like beans, corn....You have to have this person to orient people."* [43]

> *"[We discuss] mostly organization. Planning....the planning of projects, agriculture, administration, and when to plant things."* [44]

Organization, education, and information were the top reasons that people believe that life conditions and opportunities have improved for the settlement. Not surprisingly, these also directly correlate to the factors that the other settled people, at the Che Guevara and São José settlements feel are lacking. Identifying these factors is helpful for determining the best course of action for the government.

*Organization/operation of the settlement*

The community's positive response to the internal organization, classes offered, and meetings begs the question, how is all of this possible? What role do the leaders in the community play?

Erva Moura is a large community of 160 families, and one of the first problems they faced was choosing a convenient meeting time for everyone. Leaders needed to organize the community, and the first step they took toward this end was dividing the settlement into three communities that have a little more than 50 families. These communities were further broken down into smaller groups of about 8 to 12 families; this strategy of organizing the community was learned from the MST. The MST, though not a permanent presence, aided the communities in the earlier days in establishing an effective internal organization. The role of the leaders of the community is to collect as much information as possible from the politicians and government. This information, dispersed through orientações ("informational meetings designed to orient, or teach the community") ranges from credit-lending programs, to health and education programs, to teaching people about selling their products, to environmental concerns, to appropriate behavior within the settlement.[45]

Finally, the internal organization that the settlement established for itself had the most beneficial impact. INCRA's presence and contribution, according to the settled people, began and ended with the expropriation of the land. Technical assistance at Erva Moura, like at the other settlements, is in high demand. The leadership of the settlement knows that if they wait for a specialist, they will not get one. This is why, they explain, they have had to assume the duty to gather as much information as possible: "We have been able have successful leadership through talking with one another, meeting together." [46]

## EVALUATION OF INCRA'S SERVICES FOR SETTLEMENTS

Drawing from Erva Moura's successful experience and the other settlements' challenges, it is important to make a recom-

mendation to the government as to how to improve their program of agrarian reform. Demonstrated from both my field research and substantiated by my document review, there are many components necessary to achieve a successful settlement. Following is an evaluation of the government's role and performance in the four fundamental aspects of the agrarian reform process:

**Expropriation of Land.** The INCRA office of Ceará reports that 290 settlements currently are home to 13,981 families in this state, while 6,117 spaces on settlements remain vacant. It is clear that during the current Cardoso administration, the rate of expropriation has increased substantially. For example, until 1994, there were only 68 settlements in Ceará measuring 190,947 hectares. During the 1995-2002 time period, 222 assentamentos were formed, measuring 484,632 hectares. While these numbers are impressive, the settled people still seem to universally hold the sentiment that without pressure from the MST, the government would be nowhere near this number.

**Credit to Construct Houses and Begin Production.** The latest INCRA statistics reveal that the Institute has paid out R$24,334,800[c] in basic aid money. This money, according to INCRA Official #1, is used to help the farmers obtain tools for working the land, a small number of livestock, etc.[47] Families are also supposed to receive money to construct their houses. Popular sentiment among settled peoples is that obtaining money for programs from INCRA is a very political and bureaucratic process, as demonstrated through the Che Guevara settlement's 2-year struggle to bring electricity to their settlement. Thus, INCRA is currently working in this area, while at the same time holding the success of the farmers back.

**Figure 3.** A man feeds fish harvested in an artificial lake at Assentamento Santa Bárbara.

***Acompanhamento* of the Settlement in Areas of Organization and Administration**. According to INCRA, this is already part of their mission and responsibility. The settlers, however, noted that INCRA is in fact not providing acompanhamento in any of these three settlements. People from all three settlements spoke of the absence of INCRA personnel. INCRA Official #2 explained this problem: INCRA has these programs established, but there are nearly 300 settlements and very few INCRA professionals.[48] This role seems to have been assumed by the MST and CPT.

**Technical Assistance.** Technical assistance, while articulated as a major need of the settlements, is not part of the INCRA mission, both INCRA officials I interviewed explained. INCRA officials interpret this mission to include providing acompanhamento, but to simultaneously exclude providing technical assistance. In spite of this, INCRA Official #2 admits that technical assistance is "fundamental" to the success of the settlements:

> *Technical assistance gives a guarantee that what is going to be produced and how it will be produced will be successful. It attends to the needs of the people….All the big companies have professionals that specialize in this area.*[49]

In sum, both INCRA and the settled peoples agree that technical assistance is an essential component of successful agrarian reform. Yet, it is not being provided by INCRA with the explanation that it does not fall within their jurisdiction. Given the importance of technical assistance, however, there is no way that INCRA's mission can be fulfilled without providing this crucial service.

## STEPS TO REMEDY THE SITUATION : INCRA's 2002/2003 Action Plan?

During my interviews with INCRA officials, they acknowledged their absence from the settlements, and explained that they had had a temporary pause in their services. But they maintain, as of March, 2002, that they have resumed their jobs.[50] INCRA launched its "Plano de Ação 2002/2003" ("2002-2003 Action Plan") in March 2002, which restarts their role of providing collecting data and implementing each settlement's systematic development plan.[51]

Nevertheless, there is reason to be skeptical. INCRA's own numbers reveal that in 2001, it was able to provide technical assistance to only 16 of 290 settlements. A few years ago, its yearly maximum peaked at 22. What further reinforces this pessimism is that even INCRA believes that it is unlikely their resources will increase.[52]

## ANALYSIS OF RESEARCH FINDINGS and RECOMMENDATION FOR ACTION

By evaluating the government's performance of the four key steps of agrarian reform, it is evident that the very agency that is supposed to carry out this massive program is actually impeding the fulfillment of its own mission. While technical assistance is not explicitly spelled out as a component of INCRA´s mission, it is apparent that it is a necessary factor—confirmed even by INCRA officials. The government has outwardly made a commitment to social change; it has already spent $6.5 billion on the agrarian reform program. But, by failing to provide sufficient funding for all components necessary to the success of the program, namely technical assistance to the settled farmers, the government makes success difficult, if not impossible.

If INCRA´s Plano de Ação 2002/2003 is indeed able to be carried out successfully in Ceará, and their acompanhamento and other services improve, this may be a start in working to consolidate the Brazilian Agrarian Reform Plan. But, cuts in funding that have decreased available resources for agrarian reform make future possibilities look bleak. In 1998, 2.2 billion Brazilian reais were budgeted for agrarian reform, and from 1999-2002 that number was cut nearly in half—only 1.3 billion Brazilian reais were allocated.[53] The Brazilian government must allot the necessary resources – both monetary and professional assistance – to their program of agrarian reform. Without it, agrarian reform will make little positive change in the socio-economic status of the Brazilian people. □

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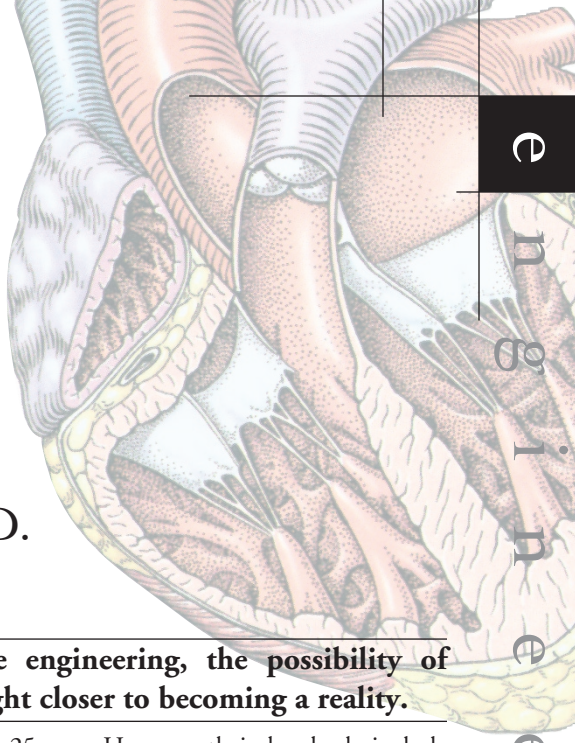

# Tissue Engineering

## Advances in Cardiac Tissue Engineering and Cardiac Tissue Replacement Modalities

Nicholas Krebs, 2002
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**With the advancements in tissue engineering, the possibility of artificial organ replacment is brought closer to becoming a reality.**

Congenital and acquired diseases of the heart such as heart valve degradation, great artery dysfunction, and coronary artery blockage are the leading causes of morbidity and mortality in the United States and other developed countries today.[1,2,3] A large fraction of the total costs that the United States spends on health care can be attributed to tissue loss or organ failure, especially with accumulating costs from heart damage and heart failure, totaling almost $100 billion yearly.[4] These costs are not only of monetary value, but more importantly, of value in human life and quality of life. Thus, there has been an urgent demand for more effective pharmaceuticals to treat heart disease, as well as new tissue engineering methods to repair and replace damaged cardiac tissue.

### Transplantation

Transplantation is a highly successful therapy for incurable end-stage heart disease, but the need for organs far outnumbers the number of donor hearts available. Consequently, 15% of the potential transplant candidates die before receiving a heart. Actual numbers show that only one to two thousand hearts are available each year for transplantation, which is far below the actual need of about 15,000 patients requiring transplantation yearly.[5] Mechanical devices such as artificial hearts, internal defibrillators, ventricular-assist devices, intra-aortic balloon pumps, and intravenous oxygenators can often lead to complications. Some risk factors include: thrombosis (blood clotting), limited durability, increased susceptibility to infections, and the need for multiple operations due to a lack of growth, requiring replacement of the device. As a result, these devices cannot completely perform all the functions of a normal organ. Therefore, these methodologies offer only temporary relief as bridging devices for patients awaiting permanent transplantation.[6]

The use of mechanical devices is not the only treatment for end-stage heart disease. Implementing xenotransplantation (i.e. transplantation from an animal source such as a pig) as a long-term therapy for tissue, vessel, or valve replacement remains speculative because of immunological barriers and high potential for biological hazards such as pig endogenous retrovirus (PERV).[7] Much remains to be accomplished in these critical areas of heart transplantation in terms of making viable cells that will organize themselves into functional myocardial tissues capable of being transplanted into patients.

Over 175,000 prosthetic cardiac valves are implanted annually throughout the world, 60,000 in the United States alone.[8,9,10,11] Presently, there are only two primary choices for cardiac valve replacement that are clinically used, either mechanical valves or xenogenic/allographic tissue valves. Xenogenic options include stented porcine aortic valves or bovine pericardial valves, both of which are preserved in gluteraldehyde, a potentially harmful chemical. Allographic valves are usually transplanted from a fresh cadaver or cryopreserved specimen to the patient.[12,13] Mechanical valves made of polymers, metals, and carbons are very durable with life expectancies of over 25 years. However, their drawbacks include the need for life-long anticoagulation therapy, which could lead to serious hemorrhagic complications, associated bleeding disorders, and flow dynamics that are significantly different from those of human heart valves. Xenogenic valves display much better flow dynamic properties and they do not require anticoagulation therapy, but their lifetime is significantly shorter (approximately 7-10 years) than mechanical valves.[14,15,16,17] This is due to calcific (calcium buildup) and non-calcific deterioration. The inability of the dead cells to maintain normally low intracellular calcium levels results in calcium-phosphate crystals building up on the valves.[18] Although these bio-prostheses mimic natural valves, their cells are nonviable, thus incapable of a normal turnover of extra-cellular matrix production. Also, the mechanical properties of the cusps of the replacement valves are much different from those of natural valves,[19] which limits their functionality. On the horizon lies the tissue-engineered valve which has the potential to be nonobstructive, nonthrombogenic, and nonhemorrhagic, enabling the valve to last the lifetime of the patient.

In addition to the large number of cardiac valve replacements required each year, there are also over 600,000 vascular grafts (i.e. bypass surgeries) required to replace damaged or blocked blood vessels.[20,21] Damage or blockage usually results in the formation of a thrombus and thus, an obstruction of the vessel to normal blood flow. Synthetic materials such as Dacron can be used in stents for large arteries like the aorta, but as the vessel diameter becomes

**Table 1. Critical Conditions for Tissue-Engineered Heart Valves**

| | |
|---|---|
| **Cells** | |
| | Tissue source, age, species |
| | Cell type, heterogeneity within cell types |
| | Phenotype |
| | Viability |
| | Extracellular matrix synthesis and remodeling |
| **Scaffold** | |
| | Chemistry |
| | Configuration/Porosity |
| | Heterogeneity (architecture, composition) |
| | Cell adherence |
| | Biocompatibility |
| | Degradation rate (in vivo, in vitro) |
| | Mechanical properties (strength, compliance) |
| | Ease of manufacture |
| **Biological Signals** | |
| | Source |
| | Pure vs. tissue extract |
| | Single vs. multiple |
| | Organ specificity |
| | Function |
| | Genetic manipulation |
| **Construct** | |
| | Bioreactor medium |
| | Static vs. flow |
| | Growth factors, morphogenetic controls |
| | Degree of maturation at implantation |
| | Functionality |
| | Biocompatibility |
| | Sterilization, storage |

significantly smaller (< 6 mm diameter), synthetic materials are no longer useful, because of the risk of thrombosis.[22,23,24,25] For vessels this size, physicians will usually use an autologous (of self) vein or arterial graft to bypass the thrombus or obstruction. However, with these procedures, there is an increased need for multiple surgeries and higher cost for the patient. Thus, there is a need for engineered small vessel prostheses, preferably naturally occurring or synthesized naturally, to reconstruct, bypass, or replace small vessels.[26]

As a result of organ shortages, sub-optimal prosthetic/bio-prosthetic materials for repair and/or replacement of damaged cardiac tissue and organs, and immunological problems associated with xenotransplantation (transplantation from animal sources), there continues to be extensive developments in the emergent field of tissue engineering, which ultimately should increase the pool of available tissues and organs available for transplantation.

### Tissue engineering

Tissue engineering is an interdisciplinary field where researchers throughout the country have worked in parallel to create a vast array of living tissue and organ replacements for therapeutic and regenerative purposes.[27] Tissue engineering aims to provide scientists, and ultimately patients, with viable tissue that has the ability to grow, repair, and remodel itself in vivo (inside the body) to improve tissue function where tissue has been lost through trauma or disease.[28] Tissue engineering avoids complications such as immunological responses (e.g. rejection), as well as viral infections through the use of autologous cells.[29]

There are three ways to approach creating new engineered tissues: the injection of isolated cells, the development of cell encapsulation systems, and the use of stem cells or biopsied cells and scaffolds (both natural and biosynthetic) to implant constructs of tissue grown in vitro (in a test tube).[30] The reabsorbable scaffold is made into the shape of the tissue being developed, such as heart valves or vascular tissue; the scaffolding materials must be biocompatible and must meet both the nutritional and biological needs for the specific cell population being grown. In the case of engineering cardiovascular tissues, myoblasts or myocytes (muscle cells) are seeded with fibroblasts onto biocompatible polymer scaffolds to grow the intended tissue.[31] (Figure 1)

Polymer scaffolds can be constructed from natural or synthetic biomaterials. Although natural biomaterials better simulate environments in vivo, they do impose limitations such as large variations in the biological tissues that have been extracted for the scaffolding, as well as restrictions with the versatility of designing devices with specific biomechanical properties. Advances in the field of polymer chemistry have allowed scientists to develop several synthetic polymers that can be precisely altered for use as scaffolding materials. The desired scaffold is first seeded with autologous, allogeneic, or xenogeneic cells, potentially derived from either biopsies or stem cells. Next, the cells are placed into a bioreactor to stimulate tissue growth in vitro before implantation. Specifically, a bioreactor is a device that produces a dynamic in vitro microenvironment for guided tissue growth. It simulates the microenvironment that would be found in the body for normal tissue formation and growth. This device allows scientists to control the flow and mixing of cell culture media, which can enhance the transfer of nutrients, wastes, and regulatory molecules to and from the growing tissue. Additionally, bioreactors provide mechanical simulations of flow conditions found in the body, which enhances the formation of tissue constructs by providing appropriate shear stresses and strains to the growing cells. For example, pulsatile flow bioreactors have been used to construct trileaflet heart valves and blood vessels. These constructs under pulsed flow conditions exhibited greater burst strengths, better suture retention, and higher collagen content than the nonpulsed constructs.[32] Currently, the use of xenogenetic and allogenetic tissues with open-celled constructs is limited because of host immune responses against the implanted tissue.[33] As a result, the majority of the tissue-engineered experiments using polymer/cell construct technology have used autologous cells.

The delivery of cells to a polymer is called seeding. Seeding may take a static form by directly applying the cells to the scaffold, or it may take a dynamic form, where agitation is used to apply the cells. Dynamic seeding is accomplished through the use of a pulsatile flow bioreactor, and it results in a higher seeding density and more uniform cell distribution. With a bioreactor mechanical forces such as stretch, pressure, and shear forces are employed, while gas and nutrient exchange is accomplished through constant changing of the culturing medium. The construct-bioreactor system allows the user to control the in vitro culture conditions such as cell population, seeding density, biochemical signals, and physical forces. The effects of these parameters on engineered tissue structure and function can be quantified and examined in the in

Tissue engineering is an interdisciplinary field where researchers throughout the country have worked in parallel to create a vast array of living tissue and organ replacements for therapeutic and regenerative purposes.

vivo environment.[34,35] The seeded cells will proliferate, organize, and produce their own extra-cellular matrix on which to organize. While in the host, the scaffold is degraded, reabsorbed, or metabolized to minimize inflammatory responses. This leaves behind a vital organ or tissue that restores, maintains, or improves tissue function.[36,37,38,39]

## Current research

Current research has been limited with the regeneration of functional cardiac muscle, because of the complex mechanisms involved with myocyte growth and proliferation.[40] However, cardiac cell-polymer constructs have been made to use as 3-dimensional models for in vitro physiological and pharmacological studies, and more importantly they will be used in vivo to repair damaged heart/vascular tissue. In addition, these tissues have been examined in various animal models to determine their effectiveness in vivo.[41,42] Cardiac cells have been seeded on 3-dimensional scaffolds and then cultured in a variety of bioreactors. Mainly, highly porous scaffolds consisting of fibrous PGA and collagen gels have been selected for the constructs based on their biocompatibility and well-characterized properties. Results have shown that the ability of the isolated cardiac myocytes to form functional tissue in vitro depends upon the developmental stage of the myocytes (embryonic or neonatal) and regeneration scaffold (fibrous PGA or collagen gel). Both high initial cell densities and utilization of dynamic pulsatile bioreactors (which provide shear stress from flow patterns) have improved structural and functional construct properties. Because these cardiac constructs have expressed proteins and structural features found in native tissue, they support the idea that engineered cardiac tissue can be used as a model of native tissue for in vivo tissue repair and eventually organ repair.[43,44]

Tissue engineered heart valves are a particularly exciting concept that is in its infantile stages of practice, development and implementation into the human heart. The method of engineering heart valves involves using an anatomically appropriate construct containing autologous myocardial cells seeded onto a biodegradable scaffold, which has been fabricated in vitro in a bioreactor and then implanted.[45,46,47,48] Prospective development of tissue-engineered valve substitutes depends upon an understanding of the fundamental and underlying principles and mechanisms that limit the durability of previous and current tissue valves. This also necessitates an understanding of the natural valve, the biology of the cells and extra-cellular matrix produced by the cells, and the changes that occur during the fabrication process.[49] Desirable characteristics of a tissue-engineered heart valve include an endothelialized blood-contacting surface, a cellular potential for extra-cellular matrix synthesis, remodeling, and repair, the potential growth inside a patient; absence of deleterious immunological and other inflammatory processes, resistance to calcification and tissue overgrowth, stable mechanical properties, resistance to infection, and easy permanent insertion.[50]

Lamb and miniature swine models are the most commonly used models by researchers to test the feasibility of tissue-engineered vessels. Various synthetic polymers have been utilized as scaffolding materials to seed autologous SMCs, endothelial cells, and fibroblast cells to create conduits and patch augmentation tissues that are then transplanted into the animal models.[51,52] Autologous aortic graphs have been successful in animal models showing biological characteristics resembling the native aorta.[53] The engineered arteries grew significantly and showed collagen and elastin content similar to that of native vessels.[54] The patch augmentation experiments have also been successful. Appearances of the augmented areas have demonstrated smooth internal surfaces with increasing

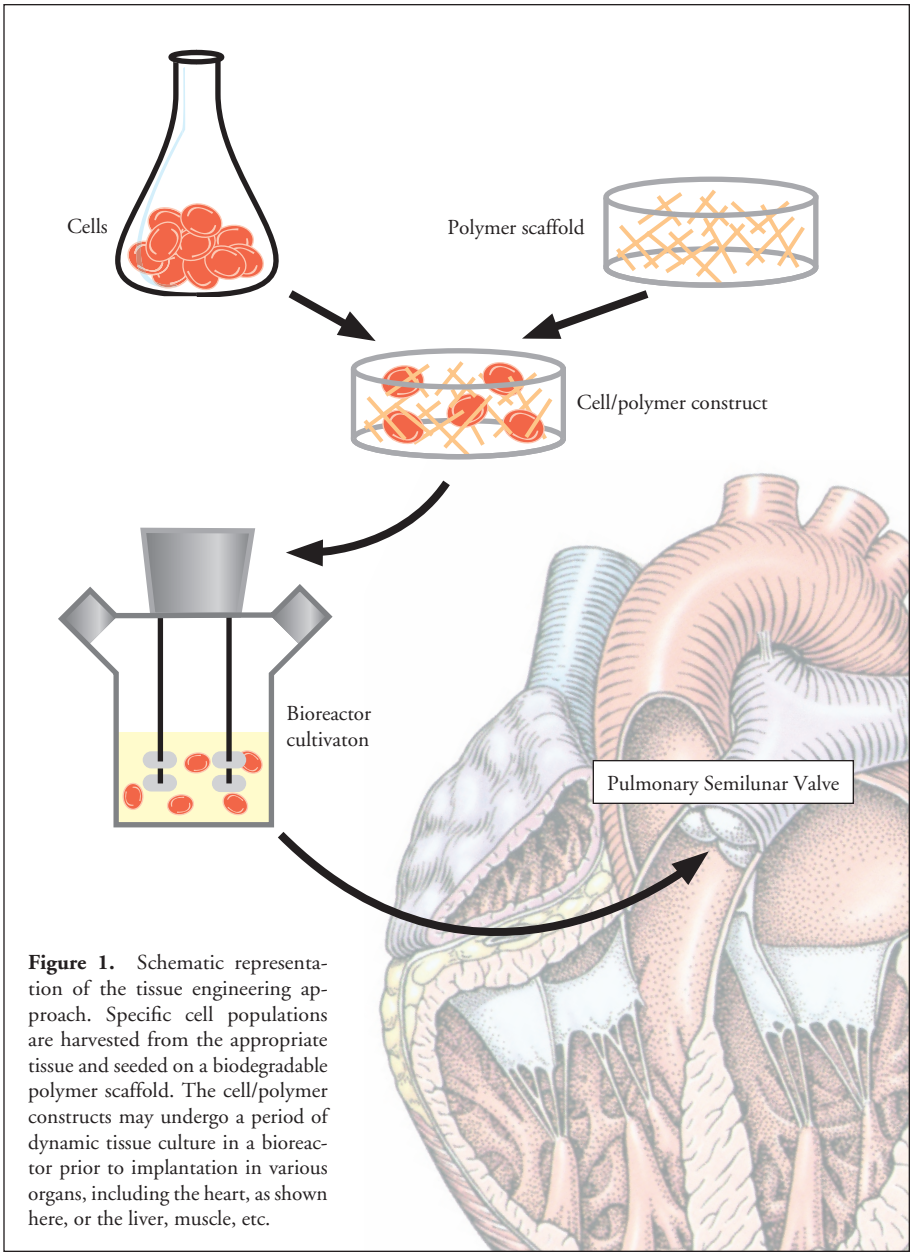


**Figure 1.** Schematic representation of the tissue engineering approach. Specific cell populations are harvested from the appropriate tissue and seeded on a biodegradable polymer scaffold. The cell/polymer constructs may undergo a period of dynamic tissue culture in a bioreactor prior to implantation in various organs, including the heart, as shown here, or the liver, muscle, etc.

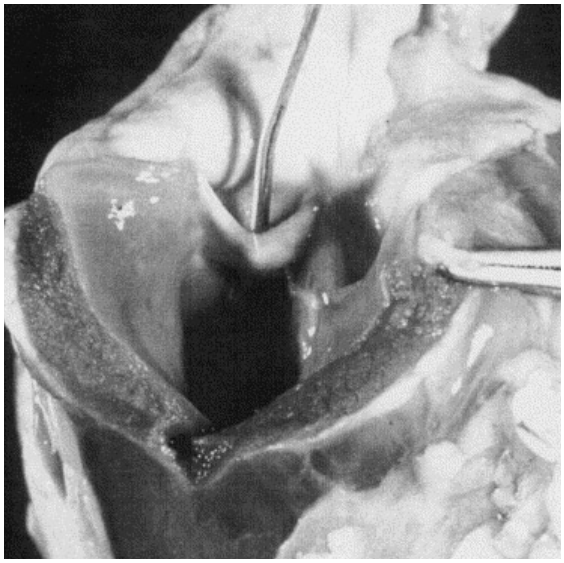

**Figure 2** Tissue engineered pulmonary valve leaflet in a lamb heart.

tissue and extra-cellular matrix formation, in addition to having near reabsorption of the polymer.[55] These models have not yet been utilized with human patients in vivo due to the need for further studies to assess the biological functioning of these vessels in both short-term and long-term implantation. However, the feasibility of culturing autologous implantable arteries has been demonstrated.[56,57] (Figure 2)

Doctor John E. Mayer M.D. at Children's Hospital and Dr. Joseph P. Vacanti M.D. at Massachusetts General Hospital in Boston have developed a pulsate bioreactor for the in vitro formation of normally functioning constructs with an architecture that mimics the naturally occurring valve environment. This system develops adequate mechanical properties (e.g. ability to withstand hemodynamic stresses) by providing physiological pressure and flow of nutrient/cytokine medium to the developing valve construct. In addition, long term in vitro developments have resulted in contamination-resistant systems.[58] This group has created tri-leaflet heart valve scaffolds from various biodegradable polymers, and has successfully implanted them into lambs for up to four months (Fig. 2). The polymers that have been tested include polyglycolic acid (PGA), poly-4-hydroxybutyrate (P4HB), and polyhydroxyalkanoate (PHA), of which the latter has proved to be the most biocompatible as a result of its synchronous opening and closing ability in the pulsate bioreactor and its ability to accommodate cell attachment and growth.[59,60] PHA has mechanical properties such as elasticity and mechanical strength that far exceed the other polymer systems mentioned above.[61,62,63] When autologous cardiac cell lines were seeded onto the PHA, they mostly viable, and formed a connective tissue matrix between the inside and the outside of the porous heart valve scaffold.[64] After being implanted into lambs at a segment of the pulmonary artery and extracted at various time intervals, no signs of thrombus formation were evident on the constructs. An acellular construct was used as a control and showed no signs of tissue growth or extracellular matrix production.[65] These early results show a promising and adequate future for the development of tissue-engineered heart valves; the goal being the development of clinically feasible valve replacements.

The use of tissue engineering techniques to create heart valve substitutes from autologous cell lines and biodegradable polymer scaffolds is growing and results in animal models have been promising thus far. Tissue engineering offers a new approach to the development of heart valves in that it allows for the creation of structures that are viable and have the capacity for self repair. This allows for greater durability without the side effects of anticoagulation therapy, thrombus formation, inflammation, immune rejection, infection, and hemorrhaging.[66] Continued progress in this area of tissue engineering with heart valves strongly depends upon: (a) understanding the fundamentals behind valve development and function, including extracellular matrix biology, valvular cell biology, response to injury and how these fundamentals relate to the progressive evolution of structure; (b) the understanding that has been developed over the past fifty years concerning failure modes of different types of valve replacement structures; (c) understanding the structural conditions of favorable valve alternatives, including the composition and organization of the collagen, elastin, and glycosaminoglycans in the engineered valve; (d) understanding the basic role/relationship between the host tissue and implanted tissue; and (e) the mechanisms of physiological repair processes.[67] Therefore, tissue-engineered valves will hopefully offer a more permanent solution to the problem.

Ideal biological grafts should possess a continuous endothelial layer and differentiated smooth muscle cells, as well as sufficient mechanical integrity and elastic properties to allow for structure retention and tolerance to arterial pressure. Shear stresses in fluid dynamics have a tremendous impact on vascular cell morphology, proliferation, orientation and both the organization and composition of extracellular matrices.[68] Thus, there have been increased interests in researching and defining shear stresses and pressures present in bioreactors for tissue conditioning and guided tissue formation, especially with tissue engineering applications involving cardiovascular structures. This is because the artery must withstand both transmural pressures acting perpendicular to the vessel wall and the shear stresses acting along the wall surfaces.[69] Shear stresses that accompany dynamic conditioning have shown positive effects on the generation of functional blood vessels. The mechanical properties of the engineered vessels are derived from the smooth muscle cells (SMCs) and extracellular matrix proteins they produce. These mechanical properties are not derived from the scaffolding material (i.e. PGA), because most biodegradable scaffolding materials utilized in repairing/replacing vessels fragment and degrade to less than 15% of their initial mass after 5 weeks in culture.[70] In addition, researchers have shown that the flow positively influences cell to cell interactions.[71]

## Future Research

An important area of future work for the generation of small diameter vessels is to repopulate tissue-engineered grafts with autologous cells in vivo instead of

...a tissue-engineered heart that would provide a viable alternative to transplantation and ultimately ease the suffering of hundreds of thousands of individuals worldwide.

in vitro. In doing so, it has been proposed that endothelial progenitor cells be seeded onto polymer scaffolds to allow for a non-thrombogenic barrier, because in previous attempts at tissue-engineered vessels, in vitro culturing of SMCs and endothelial cells has resulted in thrombosis shortly after implantation.[72] Some problems plaguing synthetic vascular graphs that must be overcome to make vascular grafts a reality are platelet adhesion and decreased compliance (ability to stretch) compared with the adjacent arterial tissue, which have both led to the ideas discussed above.[73]

Many attempts have been made to develop and produce cardiac tissue that is long lasting, biocompatible, and effective. To overcome the mechanical and biological limitations posed by synthetic implants, research has begun to focus on the development of a naturally derived biomaterial for the fabrication of cardiac tissue, heart valve tissue, and vascular grafting tissue. Further advances in tissue engineering using autologous cells are necessary before widespread applicability to multiple organ systems can become a reality. Current in vitro bioreactor environments are mere approximations to the complex biochemical and physical environments that cells are situated in during organ development and repair in vivo. Likewise, the synthetic polymers that are used as scaffolding materials for cell growth are imperfect approximations to extracellular matrices. Thus, the development of synthetic or natural scaffolding templates for cell culturing, that mimic the architecture and surface biochemistry of the target tissue will better enable the autologous cells to develop into functional and effective replacement tissues and organs. To develop and engineer complete organ systems, techniques to promote complex vascularization of capillary beds in the tissue of the organ will be a necessity to promote the mass transfer of oxygen and nutrients throughout the tissue. Stem cell biology holds an enormous potential for artificial organ and tissue development for transplantation. However, at the current time, techniques to isolate multipotent and pluripotent stem cells from adult tissues remains extremely difficult and complex.[74] Therefore, advances in molecular immunology, tissue engineering and stem cell biology will offer even better therapeutic methods for treating organ and cardiac failure in the future. Finally, interdisciplinary cooperation between engineers, scientists, and physicians will allow for discoveries and innovations to be rapidly applied for the development of clinically useful, tissue-engineered cardiovascular implants, and ultimately, a tissue-engineered heart that would provide a viable alternative to transplantation and ultimately ease the suffering of hundreds of thousands of individuals worldwide. □

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