# Proceedings of the NORTH DAKOTA Academy of Science



86th Annual Meeting

April 1994

Volume 48

# THE NORTH DAKOTA ACADEMY OF SCIENCE

P.O. Box 5567, University Station, Fargo, ND 58105

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PROCEEDINGS of the NORTH DAKOTA ACADEMY of SCIENCE is published annually. This issue contains communications (from Symposia, from Professional Contribution sessions, and from Collegiate Competition sessions) representing papers submitted and accepted for oral presentation at the April annual meeting of the ACADEMY. The PROCEEDINGS appears in April of each year.

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NORTH DAKOTA ACADEMY of SCIENCE (Official State Academy 1958 Founded December 1908)

1993 - 94

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# 86th ANNUAL MEETING

(joint with 62nd ANNUAL MEETING of the MINNESOTA ACADEMY of SCIENCE)

28 - 30 April, 199#

Fargo, North Dakota Moorhead, Minnesota

#### EDITOR'S NOTES

The PROCEEDINGS of the NORTH DAKOTA ACADEMY of SCIENCE was first published in 1948 with Volume I reporting the business and scientific papers presented for the Fortieth Annual Meeting, 2 and 3 May, 1947. Through Volume XXI the single yearly issue of the PROCEEDINGS included both Abstracts and Full Papers. Commencing with Volume XXII, the PROCEEDINGS was published in two parts. Part A, published before the meeting, contained an Abstract of each paper to be presented at the meeting. Part B, published later, contained full papers by some of the presenters.

Commencing in 1979 with Volume 33, the PROCEEDINGS changed to the present format. It is produced from camera-ready copy submitted by authors, and is issued in a single part to be distributed initially at the Annual Meeting in late April. Each presentation at the Annual Meeting is represented by a full page "Communication" which is more than an abstract, but less than a full paper. The communications contain actual results and conclusions, and permit data presentation. The communication conveys much more to the reader than did an abstract, but still provides the advantage of timeliness and ease of production.

The first section of this Volume 48 of the PROCEEDINGS contains presentations from the Symposia offered at the 86th Annual Meeting of the Academy (joint with the 62nd Annual Meeting of the Minnesota Academy of Science) held in Fargo/Moorhead, 28 - 30 April, 1993. These papers are organized by Symposia and within each are presented in the same sequence as presented at the meeting.

The second section of this volume presents the Collegiate Communications representing all those papers presented in the A. RODGER DENISON Student Research Paper Competition. Undergraduate and graduate students reported on the results of their own research activities, usually carried on under the guidance of a faculty advisor. While student competitors were required to prepare a communication similar to those prepared by their professional counterparts, these communications were not reviewed prior to publication herein. The Denison Awards Committee judges the oral presentation and the written communication in arriving at their decision for first place and runner-up awards in both the graduate and undergraduate student competitions. In this section the first papers are from the undergraduate competition (placed in alphabetical order by the last name of the author presenting the paper) and the second group of papers are from the graduate competition (arranged in similar alphabetical order).

The third section of this volume contains the communications presented in the Professional sections of the meeting. All Professional Communications were reviewed for conformity with the instructions to authors by the Editorial Committee prior to their acceptance for presentation and publication herein. The professional communications have been grouped together in order of the oral presentation at the Annual Meeting.

Readers may locate communications by looking within the major sections of these PROCEEDINGS (see the table of contents), or by referring to the author index for a page number reference to this volume.

This issue of the PROCEEDINGS also includes the Constitution and Bylaws of the ACADEMY, a list of Officers and Committee Membership for the May 1993 - April 1994 year, a list of all Academy members as of 1 March, 1994, and a copy of the most recent (1993) financial statement of the Academy.

Roy Garvey Editor

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#### The NORTH DAKOTA ACADEMY of SCIENCE

## RULES for PREPARATION of PROCEEDINGS COMMUNICATIONS

#### Submission.

- Papers presented at the Annual Meeting of the ACADEMY must be represented by single page communications in the PROCEEDINGS. This includes A Rodger Denison student research competition papers.
- 2. Only communications intended for presentation at the Annual Meeting will be considered for publication. They must present original research in a concise form. Quantitative data should be presented with statistical analysis (means with standard errors). The communication should include the purpose of the research, the methodology, results, and conclusions. Papers which merely summarize conclusions or ideas without supporting data are discouraged and will not normally be accepted.
- 3. Communications must be submitted on a single 8.5 x 11.0 inch page of white bond paper. the full surface area of the page may be used for text and figures. Send the original and four legible photo copies to the Editor, PROCEEDINGS of the North Dakota Academy of Science. The original must not be folded; a cardboard stiffener should be used to avoid damage. As a final step, the Editor will "paste" your submission to a 'blue line communications form' adding the necessary "headline and footer". The PROCEEDINGS will be published by direct photo offset of the submitted communication with a reduction to 80% of the original size to accommodate margins). No proofs will be prepared.
- 4. The authors' permission for the North Dakota Academy of Science to publish is implied by a submission. The ACADEMY does not restrict the right of authors to include data presented in a communication in full papers submitted at a later date to other publishers.

#### Manuscript.

- 5. Authors are encouraged to utilize the full space available on an 8.5 x 11.0 inch page in order to provide sufficient information to fully describe the research reported. One or two line top and bottom margins and 1 to 3 character right and left hand margins are recommended (as appropriate to your "laser Printer"). The material you submit on this page must be "camera-ready" since it will be photographed and reproduced directly in the PROCEEDINGS. Text should be presented using no smaller that "elite" (12 character per inch) fonts and single line spacing (6 lines per inch). This should allow for approximately 62 lines of 100 characters each. Unless your printer/word processor uses "micro justification", DO NOT right justify your text. Begin paragraphs with a 3 character space indentation. Use a typewriter with carbon or good quality black silk ribbon, or a "laser printer" set for the narrowest margins which will retain the printed characters on the face of an 8.5 by 11.0 inch page. Special symbols not available on the fixed character printer must be hand lettered in black ink. Dot matrix print of less than "letter quality" is not acceptable.
- 6. Text, tables and diagrams reproduced on white bond paper, and high contrast photographs may be secured to your original page of text using "Tack Note" by Dennison or with two sided mounting tape. Tape should NOT show on the top side of the bond paper or photograph being mounted. All typing, drawing and secured art or photographic materials must be within the boundaries of the single 8.5 x 11.0 inch page. Brief descriptive captions or titles must accompany each figure and table.

7. Heading: The title of the communication, typed in capitalized characters, should be centered as the first line(s). It is suggested that authors select a sufficient number of "keywords" to describe the full content of their paper, and then construct a title using as many of these as practicable. Titles normally should not exceed 140 characters in length. They should be free from unnecessary phrases such as "a preliminary investigation of" or "some notes on" which add little or nothing to their meaning. A blank line should follow immediately after the title.

The names of the authors should be centered on the line immediately following the blank line after the title of the communication. Full first names are encouraged; however, the author should use initials if he/she normally uses that form in other publications. Indicate the author to present the communication by an asterisk \* after that person's name. The business or institutional address of the author(s) should be centered on the line immediately following the line listing the name of the author. Typical entries might be:

Department of Chemistry, North Dakota State University, Fargo, ND 58105 Energy and Environmental Research Center, University of North Dakota, Grand Forks, ND 58202

USDA/ARS, Human Nutrition Research Center, Grand Forks, ND 58202 USDA/ARS, Biosciences Research Laboratory, Fargo, ND 58105 North Dakota Geological Survey, 600 East Boulevard, Bismarck, ND 58505

- 8. References: Only essential references should be cited, and each should be indicated in the text by a number enclosed in parentheses; this number should be on the same line as the rest of the text (e.g. "This topic has been discussed by Smith (5, 6)"). Note that a space is left between words and the parenthetical citation and that there is a space between numbers in multiple citations. References are to be assembled, arranged numerically in order of first appearance in the text, and placed at the end of the communication under a two inch line of \_\_\_\_\_\_. In the Literature Cited the reference numbers are followed by a period and are placed flush with the left margin; if the reference exceeds one line, the succeeding line or lines should be indented 5 spaces. The following form of citation should be used. Note that periods after abbreviations for Journal titles and spaces between initials for authors names have been omitted to conserve space.
  - 1. Neary, D., Thurston, H. and Pohl, J.E.F. (1973) Proc ND Acad Sci 40, 83.
  - Batsone, G.W., Blair, A.W. and Slater, J.M. (1971) <u>A Handbook of Pre-Natal Pediatrics</u>, pp 83-90. Medical and Technical Publishing, Lancaster.
  - 3. Farah, A.E. and Moe, G.K. (1970) in <u>Pharmacological Basis of Therapeutics</u>, 4th edition (Goodman, L.S and Gilman, A, eds), pp
  - 677-709. MacMillan, New York.

    4. Rajewsky, M.F. (1973) Abstr 2nd Meeting European Association of Cancer Research, Heilelberg, Oct 2-5, pp 164-5.
- 9. Abbreviations: Only standard abbreviations should be used, and should be written out the first time used with the abbreviation following in parentheses. The North Dakota Academy of Science (NDAS) for example.
- 10. Session Assignment: To assist the Program Committee in organizing the presentations, please indicate in a cover letter your 1st, 2nd and 3rd preferences for the topical classification of your paper.

## RULES for ORAL PRESENTATION of PAPER

- 1. All papers are limited to 18 minutes total time for presentation and discussion. It is suggested that the presentation be limited to twelve minutes with an allowance of five minutes for discussion. It is also suggested that major emphasis be placed on the significance of the results and the general principles involved rather than on the details of methods and procedures.
- 2. ACADEMY members represent a variety of scientific disciplines; therefore, speakers should avoid "jargon" and briefly explain or define specialized terminology as may be judged to be indispensable to the presentation.
- 3. Projectors for 2 x 2 inch slides and "overhead transparencies" will be available in all session rooms. Opaque projectors and video playback equipment will be made available as required if advanced notice of need is given. Only visuals which can be read easily on projection should be used. Authors who desire suggestions for preparation of slides are referred to Smith, H.W. (1957) "Presenting Information with 2 x 2 Slides", Agron J 49, 109-13.
- 4. Timed rehearsals with slides are highly recommended. There is usually time for a maximum of 6 or 7 slides for a presentation of this kind.
- Moderators are bound to remain on a strict time schedule in order that members of the audience can easily move among sessions to attend papers of special interest.

## A Symposium on

# CLINICAL PERSPECTIVES on the NEUROSCIENCES: RECENT ADVANCES in TREATMENT METHODOLOGIES

Joint Minnesota / North Dakota Academy of Science 1994 Annual Meeting

# Symposium Coordinator

## George T Gillies

Department of Biomedical Engineering, University of Virginia and Division of Neurosurgery, Medical College of Virginia

Friday, 29 April

- 8:30 INTRODUCTION to the SYMPOSIUM.

  George T Gillies, Charlottesville, Virginia, 22901
- 8:45 CLINICAL STRATEGIES for the TREATMENT of NEURODEGENERATIVE DISORDERS.
  M SEAN GRADY, M.D.
  - Neurological Surgery, Harborview Medical Center. ZA 86 University of Washington, Seattle, 98104
- 9:30 ENDOVASCULAR THERAPIES in the CEREBROVASCULATURE.
  Cyril J Schweich, Jr., M.D.
  SCIMED Life Systems, Inc., 2905 Northwest Boulevard, Suite 60
  Plymouth, Minnesota 55441
- 10:15 Informal DISCUSSION and Refreshments
- 10:30 RECENT DEVELOPMENTS in the BIOLOGY and TREATMENT of BRAIN TUMORS.
  William C Broaddus, M.D., PhD
  Division of Neurosurgery, Medical College of Virginia/Virginia
  Commonwealth University, Richmond, Virginia 23298
- 11:15 NEUROPSYCHOLOGIC ASSESSMENT in CLOSED HEAD INJURY.

  Rex A Bierley, PhD

  Stanford Alcohol and Drug Treatment Center, Stanford University
  Medical Center, Stanford, California 94305

CLINICAL STRATEGIES for the TREATMENT of NEURODEGENERATIVE DISORDERS.  $\hbox{M Sean Grady, M.D.}$ 

Neurological Surgery, Harborview Medical Center. ZA - 86 University of Washington, Seattle, 98104

Text not available at Press time.

#### ENDOVASCULAR THERAPIES IN THE CEREBROVASCULATURE

Cyril J. Schweich, Jr., M.D. SCIMED Life Systems, Inc. 2905 Northwest Boulevard, Suite 60 Plymouth, Minnesota 55441

stroke is the third leading cause of death in the United States. Each year, approximately 500,000 people suffer from a stroke, with 150,000 deaths. While many therapeutic options have been aggressively developed over the past thirty years for peripheral and coronary vascular disease, therapies aimed at treating cerebrovasculature pathology, and therefore stroke, have lagged behind.

There are two broad categories within stroke; hemorrhagic (bleeding) strokes and ischemic (occlusive) strokes. The vascular diseases that lead to these two stroke endpoints are very different from one another and therefore have varying approaches to treatment. The purpose of this talk will be to discuss the current techniques being utilized to treat cerebrovascular disease, as well as to discuss some of the less invasive therapies being researched.

#### RECENT DEVELOPMENTS IN THE BIOLOGY AND TREATMENT OF BRAIN TUMORS

William C. Broaddus, M.D., Ph.D.

Division of Neurosurgery

Box 631, MCV Station

Medical College of Virginia/Virginia Commonwealth University

Richmond, Virginia 23298

Each year over 17,000 new primary brain tumors are discovered. The majority of these are malignant, leading to an annual death rate of over 12,000. In children, brain tumors are second only to leukemia in causing cancer deaths. Even tumors which are benign by virtue of slow growth and lack of spread to surrounding brain can have malignant consequences when they develop at critical sites in the brain or skull base where removal would have unacceptable consequences. Nevertheless, survival rates after diagnosis of primary brain tumors have slowly improved over the last twenty years, and recent developments offer hopes of even greater strides in the near future.

One example of "old" technology that has had a major impact on the treatment of tumors of the skull base is the classical use of anatomical studies to derive innovative surgical approaches to tumors previously considered inaccessible. For instance, the methodical exploration of the cavernous sinus by a series of anatomists and neurosurgeons has led to a recognition that tumors involving this region of the skull base can in fact be safely resected with appropriate microsurgical techniques.

"New" technologies are also improving our ability to treat primary brain tumors. Techniques such as computerized tomography (CT) and magnetic resonance imaging (MRI) first revolutionized the diagnosis of these and other lesions of the brain. The coupling of these technologies with stereotactic localization is now revolutionizing the surgery of primary brain tumors. These systems will allow strategies for volumetric resection of tumors as shown by fused imaging techniques not only from CT and MRI, but also from functional imaging modalities such as positron emission tomography (PET), single photon emission computerized tomography (SPECT) and magnetoencephalography (MEG).

Two key factors in the malignancy of primary brain tumors are their tendency to invade surrounding brain and their relative insensitivity to the cytotoxic effects of chemotherapy and radiotherapy. Fortunately, recent advances in the molecular genetics of malignant transformation offer hopes for a variety of gene therapy strategies. Oncogenes have been identified which play key roles in the cellular proliferation common to all tumors, and more recently tumor suppressor genes have been identified which appear to be responsible for the maintenance of normal cellular function by suppressing uncontrolled proliferation.

Current approaches to gene therapy of malignant tumors include the use of genetically engineered viruses to make the tumor cells vulnerable to anti-viral therapy, or to serve as vectors for the delivery of genetic material. The genetic material may be used to replace functions, such as tumor suppressor genes, which are aberrant in tumor cells, or to suppress oncogene activities which are critical for their malignant behavior. Other delivery techniques for genetic material are also being tested, including direct implantation, intraparenchymal infusions and remotely manipulated stereotactic devices such as magnetic stereotaxis.

Despite the technological advances made by the field of medicine in the preceding 50 years, our success in treatment of malignant brain tumors has lagged behind many other areas of medicine. However, recent developments in technology and medical science suggest that more rapid progress in managing these devastating tumors is likely in the near future.

# NEUROPSYCHOLOGIC ASSESSMENT IN CLOSED HEAD INJURY

Rex A. Bierley, Ph.D.
Stanford Alcohol and Drug Treatment Center
Stanford University Medical Center
Stanford, California 94305

and

Physical Medicine and Rehabilitation Service (117)
Department of Veteran's Affairs Medical Center
3801 Miranda Avenue
Palo Alto, California 94304

Neuropsychology involves the study of brain-behavior relationships. These relationships are based on tests of specific cognitive abilities. Since it is now well established that certain brain structures have specific functions, it has been possible to develop assessment instruments that help localize cognitive deficits. In some cases, this occurs even when scanning techniques (eg., MRI or CT scans) or electrical recordings of brain activity (eg., EEG) are negative. Cognitive compromise in the absence of physical or electrical indicators most frequently occurs in cases of mild or moderate closed head injury. In such instances, actual brain damage may not be significant in terms of structural integrity, but can be significant in terms of its functional impact on common daily activities. Neuropsychologic tests are used both to aid in differential diagnosis and in helping plan the patient's cognitive, social and emotional rehabilitation. A number of brain syndromes and the instruments used to evaluate them will be discussed.

#### A Symposium on

#### NERVOUS SYSTEM RESPONSES to DISEASE and INJURY

Joint Minnesota / North Dakota Academy of Science 1994 Annual Meeting

Symposium Coordinator

#### Garl K Rieke

Anatomy and Cell Biology, UND School of Medicine, Grand Forks, 58202 9001

Friday, 29 April

- 1:30 Can the Cognitive and Memory Disturbances Characteristic of Huntington's Disease be Attributed to Damage to the Striatum? Garl K Rieke\*
- 2:15 Information Processing in Individuals with Senite Dementia of the Alzeheimer Type (SDAT): Failure to Inhibit Irrelevant Information. F Richard Ferrao\*
  Psychology, University of North Dakota, Grand Forks, 58202
  David A Balota
  Psychology, Washington University, Saint Louis, MO, 63130
- 3:00 Alzeheimer Disease: Special Abnormalities of Resting and Flash Activated EEGs.
  Alberto L Politoff\*, Richard P Stadter, Nancy Monson and Patricia Hass
  Neuropsychiatric Research Institute, Fargo, 58102, VAMC and NeuroScience, U N D School of Medicine, Grand Forks, 58202
- 3:45 Neural Development as a Model for Addressing Issues Related to Repair in the Diseased or Injured Nervous System. Kenneth G Ruit\* Anatomy and Cell Biology, U N D School of Medicine, Grand Forks
- 4:30 Panel Discussion and Questions from and Interaction with Audience.

# CAN THE COGNITIVE AND MEMORY DISTURBANCES CHARACTERISTIC OF HUNTINGTON'S DISEASE BE ATTRIBUTED TO DAMAGE TO THE STRIATUM?

Garl K. Rieke Department of Anatomy and Cell Biology School of Medicine, University of North Dakota Grand Forks, North Dakota 58202

Huntington's disease (HD) is a heritable, autosomal dominant, neurodegenerative disorder. It is characterized by psychiatric changes (cognitive/memory disturbances), and involumtary choreiform movements. A rodent model of HD can be produced following intracerebral injections of quinolinic acid (QUIN) in the rat. QUIN is a neurotoxic metabolite of tryptophan and it produces a pathophysiology that mimics the pattern of cell losses in HD. Although there is marked cell loss in the striatum (S [caudate nucleus and putamen]), the pathophysiology in HD also involves other brain structures including the medial frontal cortex (MFCx), parts of the thalamus (DM), and the substantia nigra (pars reticulata,  $SN_{pr}$ ). The cognitive circuit of interest in this study is represented in the diagram:

$$MFCx \rightarrow Med S \rightarrow SN_{pr} \rightarrow DM$$

Patients with HD have difficulty in correctly performing delayed spatial tasks, and their performance deficits, which increase with the duration of HD, serve as a measure of cognitive disabilities. Rats were trained to perform a delayed spatial orientation task, utilizing a 12-arm radial maze. The behavioral paradigm required the rats to enter six specific arms of the maze. The rat learned to enter once each one of the six baited arms. The rats were trained to criterion (>90% correct response over 4 days).

Stainless steel cannulae were bilaterally implanted into the medial striatum (Med S), or the dorsomedial thalamic nucleus (DM) in two experimental and control groups of 10 rats each. The cannulas were coupled to Alzet miniosmotic pumps filled with QUIN (3.007 mg/10 ml, pH 7.3) or saline (0.9% NaCl, pH 7.3). The pumps delivered a 0.47 uL  $\pm$  0.02 uL/hr for 14 days. All rats were returned to the maze 48 hrs after surgery and their post-implant performance records were kept for 20 days. The rats were then trained on a reversal delayed spatial orientation task.

Rats with bilateral Med S damage showed a significant (p<0.05) but transient impairment in performance of the spatial orientation task. A plot of the mean daily performance errors (± SEM) revealed a learning curve that resembled the learning curve for the saline control group. The QUIN injected Med S group reacquired the original task; however, performing at a lower criterion (75-80% correct response). Rats with bilateral damage to the DM also showed a significant (p >0.05) but transient impairment in performance. The DM rats reacquired the original task, performing at a lower criterion (80-85% correct response). Damage to the Med S or the DM had no effect on acquisition and execution of the reversal task. There was no significant difference between the control and experimental groups on performace of the reversal task. The fact that animals with damage to the striatum or the dorsomedial nucleus were able to acquire and perform the reversal spatial orientation task indicates that these nuclei may not be requisite structures for performance of spatial orientation tasks. However, functional recovery suggests the possibilities of plasticity within the Med striatum or DM, and reducdacies in connections between elements in the proposed circuit. The recovery of function may be due to the reestablishment of connections or the rerouting of circuits that permit the animal to perform the acquired task. Damage to the medial striatum is in all probablity not the basis for performance deficits in HD.

# INFORMATION PROCESSING IN INDIVIDUALS WITH SENILE DEMENTIA OF THE ALZHEIMER TYPE (SDAT): FAILURE TO INHIBIT IRRELEVANT INFORMATION

F. Richard Ferraro\*

Department of Psychology, University of North Dakota Grand Forks, North Dakota 58202

David A. Balota
Department of Psychology, Washington University
St. Louis, MO 63130

We report the results from several experiments designed to investigate the various components comprising the cognitive information processing in individuals with no, questionable (very mild), mild, or moderate SDAT. These experiments addressed topics related to (a) short-term memory retrieval, (b) visual attentional processing, (c) the distinction between implicit and explicit memory systems, (d) dual-route models of visual word namine, and (e) identity and semantic priming in lexical decision performance. Results converge on recent evidence and suggest that SDAT individuals exhibit a general failure to inhibit irrelevant information. For example, in the visual attention experiments, SDAT individuals produced equivalent levels of facilitation to cued locations compared to healthy older adults. However, inhibitory effects to invalidly cued locations in SDAT individuals were considerably reduced, as compared to older adults. Likewise, results from a work naming task revealed that SDAT individuals produced relatively larger word frequency effects than healthy older adults. However, the impact of phonological regularity did not increase in these SDAT individuals. Because one account of the phonological regularity effect (and its interactive effect with work frequency) suggests that there is competition between direct visual access routes to the lexicon and assembled phonological routes, these particular results suggest that SDAT individuals are sensitive to competition between the direct and assembled routes in word naming. Taken together, the results from the present set of experiments support the notion that a failure to inhibit irrelevant information is a useful theoretical framework with which to explore cognitive breakdowns in the SDAT information processing system.

ALZHEIMER DISEASE: SPECIAL ABNORMALITIES OF RESTING AND FLASH ACTIVATED  ${\sf EEGs.}$ 

Alberto L. Politoff\*, Richard P. Stadter, Nancy Monson and Patricia Hass Neuropsychiatric Research Institute, Fargo VAMC and Department of Neuroscience UND Medical School Fargo, North Dakota 58102

An important priority in Alzheimer diseae (AD) research is finding a reliable diagnostic marker that is detectable using a non-invasive procedure. Although AD is associated with multiple histological, electrophysiological, neurochemical and neuropsychological abnormalities, only a few histological abnormalities are accepted by some clinicians as reliable markers of AD. However, these histological abnormalities do not seem to be an absolute diagnositc standard becuase "the histological diagnosis of AD remains imperfect" (Mirra et al., 1993) due to a "lack of a specific diagnostic marker". The other abnormalities mentioned above are not recognized as markers of AD because they have not been systematically evaluated in comparative studies of dementias of different etiologies. Without a diagnostic marker, the clinical diagnosis of AD has to rely on clinical criteria derived from clinical experience, such as the ARDRA-NINCDS and DSM-III-R criteria. These criteria lead to the clinical diagnosis of AD dementia by exclusion of other common causes of dementia. However, when histological diagnosis is assumed to be the absolute diagnostic standard, the diagnostic accuracy of the clinical criteria ranges between 68 and 100%, with an average close to 80%. Therefore, an easily accessible marker would drastically change the current strategy for the diagnosis of AD and improve diagnostic accuracy. Furthermore, it would be an important clue regarding the pathogenesis and pathophysiology of AD.

Comparison of the power spectra of the occipital EEG of 13 Alzheimer disease (SD) patients and 9 age-matched control (AMC) subjects revealed that AD patients had (a) slower alpha, (b) more power at 5.7 Hz and (c) less power at 10.4 Hz in the absence of flash stimulation. In the presence of flash stimulation, the same patients had (d) smaller and (e) fewer harmonics on 2 Hz flash stimulation. Each of these differences correlated with the severity of dementia, as measured by the mini-mental state score. The envelope of the 2 Hz-induced harmonics of AD patients did not have the power peak in the 10 to 18 Hz range ("hump") that is normally found in control subjects. The 5.7 and 10.4 Hz resting abnormalities of AD patients are consistent with their known cortical deficit of cholinergic innervation. Their decreased responsiveness to flash stimulation and their lack of power hump may be due to decreased cortical connectivity.

NEURAL DEVELOPMENT AS A MODEL FOR ADDRESSING ISSUES RELATED TO REPAIR IN THE DISEASED OR INJURED NERVOUS SYSTEM

Kenneth G. Ruit, Ph.D.
Department of Anatomy and Cell Biology
University of North Dakota School of Medicine
Grand Forks, North Dakota 58202

If basic scientists and clinicians are to come to an understanding of how the nervous system repairs itself in response to disease or injury, they must understand how the nervous system comes originally to its normal structure and function. Factors involved in the natural development of the nervous system may provide clues regarding which therapeutic measures may be appropriate for enhancing the repair of damaged neural tissue. In our laboratory we have designed experiments to examine the morphological development of components of the peripheral and central nervous system with a special emphasis on the role of neurotrophic factors in this developmental process.

Nerve growth factor (NGF), the most well-characterized of the NGF family of target-derived neurotrophic factors, plays a significant role in the survival and morphological development of sympathetic ganglion neurons. By regulating the survival of sympathetic neurons, target levels of NGF dictate how much sympathetic innervation that target will receive. By the same token, NGF's regulation of sympathetic neuron morphology dictates how much preganglionic innervation that neuron receives. Therefore, neurotrophic factors play a significant role in the development of synaptic connections within peripheral targets as well as within populations of neurons that innervate those targets.

NGF is also a survival factor for a subpopulation of sensory ganglion neurons involved in nociceptive functions. Experimental deprivation of NGF in the prenatal period results in the specific loss of dorsal root ganglion neurons that innervate the superficial dorsal horn of spinal cord gray matter while other dorsal root ganglion neurons, which perform other sensory functions, remain intact. These experiments illustrate that even within a certain population of similar-type neurons, e.g., a dorsal root ganglion, the effects of target-derived neurotrophic molecules may be very selective.

Most recently we have been studying the prenatal and early postnatal development of sympathetic preganglionic neurons within the spinal cord. The neuronal migration patterns and characteristic outgrowth of dendritic processes have prompted us to ask a number of questions regarding factors that influence these developmental processes. We have concentrated our efforts on two areas: 1) Do neurotrophic factors exert transsynaptic retrograde influences on central nervous system neurons similar to their effects on peripheral nervous system neurons we have previously described? and, 2) What, if any, are the influences of developing afferent systems on the survival and morphological development of populations of central nervous system neurons?

In our view, an understanding of the temporal sequence of events in neural development coupled with a grasp of molecular cues within the environment of the developing embryo can potentially lead us to an understanding of what manipulations we must undertake to most effectively facilitate repair of diseased or injured neural tissue.

# A Symposium on

# AMPHIBIAN RESEARCH AND MANAGEMENT IN THE UPPER MIDWEST

North Dakota and Minnesota Academies of Science Joint Annual Meeting, 1994

# Symposium Coordinator Diane L. Larson

1:30 - 1:45	Amphibian Declines in the Prairie Pothole Region of Northwest Iowa: Historical Trends and Wetland Mismanagement Michael J. Lannoo*, The Muncie Center for Medical Education, Indiana University School of Medicine, Ball State University, Muncie, IN 47306
1:45 - 2:00	Auditory Frog and Toad Surveys: Wisconsin Population Trends 1984-93, and Suggestions for Regional Monitoring Michael J. Mossman*, Lisa M. Hartman, John Huff, and Robert Hay, Bureau of Research, Wisconsin Department of Natural Resources, 1350 Femrite Drive, Monona, WI 53716
2:00 - 2:15	Declines in Amphibian Populations: Real or Perceived? Douglas H. Johnson* and Diane L. Larson, National Biological Survey, Jamestown, ND 58401
2:15 - 2:30	The Management and Conservation of Amphibians and Reptiles in North Dakota Randy L. Kreil* and Michael G. McKenna, North Dakota Game and Fish Department, Bismarck, ND 58501
2:30 - 2:45	Ecological Management of the Carberry Sandhills by the Canadian Armed Forces Errol J. Bredin*, The Ernest Thompson Seton Foundation, Seton Village, Route 9, Santa Fe, NM 87505-9805
2:45 - 3:00	Hormonal Response to Acute Stress as a Biomarker for Chronic Stress in Larval Ambystoma tigrinum Diane L. Larson*, National Biological Survey, Jamestown, ND 58401, and Albert J. Fivizzani, Department of Biology, University of North Dakota, Grand Forks, ND 58202
3:00 - 3:30	BREAK
3:30 - 3:45	Contaminant Residues and Effects in Frog Populations at Floodplain Wetlands Mike Coffey, U.S. Fish and Wildlife Service, Rock Island, IL 61201
3:45 - 4:00	Biological and Molecular Studies of the Lucké Renal Adenocarcinoma of Rana pipiens and its herpesvirus Robert G. McKinnell*, Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN 55108-1095

4:00 - 4:15	Freezing Tolerance, Freezing Intolerance, and Supercooling in Overwintering Anurans in the Great Plains David L. Swanson* and Brent M. Graves, Department of Biology, University of South Dakota, Vermillion, SD 57069
4:15 - 4:30	Feeding Ecology of Juvenile Great Plains Toad (Bufo cognatus) and Woodhouse's Toad (B. woodhousei) Matthew A. Flowers* and Brent M. Graves, Department of Biology, University of South Dakota, Vermillion, SD 57069
4:30 - 4:45	Pattern Polymorphism in Leopard Frogs of Minnesota and Contiguous States David M. Hoppe*, Science Division, University of Minnesota, Morris, MN 56267, and Robert G. McKinnell, Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN 55108-1095
4:45 - 5:00	BREAK
5:00 - 6:00	Round Table Discussion: Approaches to Management and Conservation of Amphibians in the Upper Midwest Moderator: James W. Grier, Department of Zoology, North Dakota State University, Fargo, ND 58105

# AMPHIBIAN DECLINES IN THE PRAIRIE POTHOLE REGION OF NORTHWEST IOWA: HISTORICAL TRENDS AND WETLAND MISMANAGEMENT

Michael J. Lannoo\* The Muncie Center for Medical Education, Indiana University School of Medicine Ball State University, Muncie, IN 47306

Biologists have become alarmed about a worldwide decline in amphibian numbers. To properly document this decrease, changes in the distributions and abundances of species need to be quantified. The Iowa Lakeside Laboratory, a biological field station, was founded in 1909 and preserves a record of the flora and fauna of northwestern Iowa. This information provides a basis for assessing the biological changes in this region during this century. In 1920, Frank Blanchard visited the Lakeside Laboratory and conducted what may have been the earliest study of Iowa amphibian populations (1). My colleagues and I have repeated Blanchard's survey of the amphibians of Dickinson County, Iowa by sampling 34 wetlands over the course of two summers (2), and found that five species reported by Blanchard remain: the eastern tiger salamander (Ambystoma tigrinum tigrinum), the American toad (Bufo americanus), the western chorus frog (pseudacris triseriata triseriata), the grey treefrog (Hyla versicolor/ chrysoscelis), and the northern leopard frog (Rana pipiens). Two species reported by Blanchard were absent: the mudpuppy (Necturus maculosus maculosus) and Blanchard's cricket frog (Acris crepitans blanchardi). Two species not found by Blanchard were collected: the Great Plains toad (Bufo cognatus) and the bullfrog (Rana catesbeiana). We estimate that the number of amphibians has declined by at least two, and probably three orders of magnitude, probably due primarily to the loss of wetland habitat. Estimates of amphibian declines based solely on Iowa wetland losses would be about two orders of magnitude. The question remains, is this decline continuing or has it stabilized? To address this question it is necessary to consider the ecology of amphibians and their wetlands.

From the perspective of determining amphibian habitat, I suggest dividing prairie potholes into three categories. 1) Temporary wetlands, which frequently dry or go anoxic, and which may never fill during droughts. These basins are the usual breeding sites for our native amphibians. Wetlands that hold water through mid-July will support native aquatic amphibian larvae. 2) Intermediate-sized wetlands, which usually retain water throughout droughts but which go anoxic. During high water years these wetlands may support the fish populations that manage to colonize them, but during droughts will summerkill or winterkill, subsequently providing amphibian breeding habitat. 3) Lakes, which retain water and oxygen throughout droughts, and which support healthy fish populations. Preliminary results have shown that prolonged droughts provide a severe test for amphibians by eliminating temporary wetlands and reducing the amount of breeding habitat available to them. During droughts, intermediate-sized wetlands become important amphibian breeding habitat.

In northwestern Iowa, the role of intermediate-sized wetlands in providing amphibian breeding habitat has been compromised by state fisheries biologists. First, these biologists have introduced bullfrogs to the region. Originally native to southern Iowa, bullfrogs have expanded from protected sites within the county to colonize many of the intermediate sized wetlands of the region. Unlike most other native amphibians, bullfrogs are unpalatable and can coexist with predatory fish. Further, bullfrog tadpoles must overwinter; temporary wetlands will not support them. Secondly, state fisheries biologists use intermediate-sized wetlands to raise walleye (Stizostedion vitreum) and muskie (Esox masquinongy) fry to fingerling size. In the process wetlands are aerated and treated with rotenone and the herbicide aquazine. Thirdly, state biologists have connected fringing wetlands to larger lakes by constructing weirs. The decline of one wetland, Garlock Slough, from a once-productive amphibian habitat to a basin devoid of all native amphibians through the introduction of game fishes and the construction of a weir has been documented in the scientific literature (3,4). I suggest that managing wetlands for amphibians involves nothing more than leaving them alone. Wetlands tend to manage themselves through drought and anoxia, conditions native amphibians can tolerate by metamorphosis and air breathing. Once conditions become favorable for amphibians, they respond quickly, as has been shown through the rapid recolonization of restored wetlands.

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<sup>3.</sup> Bovbjerg, R.W. (1965) Proc Iowa Acad Sci 72, 412-418.

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AUDITORY FROG AND TOAD SURVEYS: WISCONSIN POPULATION TRENDS 1984-93, AND SUGGESTIONS FOR REGIONAL MONITORING

Michael J. Mossman\*, Lisa M. Hartman, John Huff, and Robert Hay Bureau of Research, Wisconsin Department of Natural Resources 1350 Femrite Drive, Monona WI 53716

This statewide survey is based on 70 permanent roadside routes, each of which consists of 10 listening stops and is run three times annually, mostly by volunteers. It has provided important data on population trends and distribution of the state's 12 anuran species. Several other states, provinces, and more local areas have initiated similar surveys based on our model. A coordinated regional approach is needed which considers stratification and timing of routes, training of field observers, and standardized data analysis and interpretation. This paper summarizes results from the Wisconsin survey, discusses problems that must be overcome if such surveys are to succeed, and makes specific recommendations for a regional survey.

#### DECLINES IN AMPHIBIAN POPULATIONS: REAL OR PERCEIVED?

Douglas H. Johnson\* and Diane L. Larson National Biological Survey, Jamestown, ND 58401

Declines of amphibian populations have been reported widely (1). Although uncertainty about the declines remains (2), their existence has been accepted by conservationists and the general public. We do not take a stand on that question here, but raise a concern that should be addressed before firm conclusions are reached. We argue that, if amphibian populations have certain characteristics of metapopulation structure (3), and if reports of population declines are based on surveys of historically favorable collecting or study sites, then there will be a bias favoring the reporting of population declines.

Suppose a population is not distributed homogeneously but consists of distinct but interconnected subpopulations. They are distinct in being spatially segregated, at least to a degree, into what we will call habitat patches. They are interconnected in that individuals on occasion move from one patch to another. If subpopulations have different dynamics, or similar dynamics that are out of phase, they will have different trends. At any time, some

subpopulations may be increasing, some decreasing, and others even extirpated.

This "twinkling lights" scenario could influence conclusions reached about the status of amphibian populations. Suppose inferences about population trends are based on surveys from which sampling sites are chosen selectively, rather than randomly or in a representative fashion. Patches with large subpopulations would more likely be noticed by researchers and studied than would patches with small or extirpated subpopulations (2). Further, subpopulations at peak numbers almost inevitably will decline. This sort of purposeful selection would not be made intentionally for a population survey, but would be appropriate for taxonomic or life-history studies.

Amphibians, more than most other taxonomic groups, seem susceptible to the difficulty we postulate. Temperate populations occur naturally as metapopulations, with reproduction confined to discrete natural water bodies (4). Limited interchange between habitat patches occurs (4, 5) and population dynamics in different patches are distinct or out of phase (5, 6, 7)

Accepting the plausibility of metapopulation dynamics, studies reporting trends in populations must be evaluated in terms of how study sites were chosen. We surveyed published studies that reported trends in amphibian populations and classified them according to method of study site selection and whether population declines were reported (Table 1). Studies involving revisits to sites of historic populations were more likely to report population declines than were those using systematic surveys (P < 0.003, Fisher's exact test). Several studies unfortunately failed to describe site selection criteria.

Table 1. Reported trends in amphibian populations as related to selection of study sites.

Selection of study sites	Declining	Not declining
Revisit historic sites	5	2
Systematic survey	0	5

Clearly the most robust conclusions about population changes stem from complete enumeration of populations, which is rarely practical. Surveys are an appropriate alternative, but careful consideration must be given to their design. Systematic or random selection of potentially suitable habitats are called for. Surveys should be made of all sites in the sample, even if the species of interest are absent.

We emphasize that we are not denying the existence of declines of many amphibian populations. We only caution that conclusions be based on information from validly drawn samples.

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# THE MANAGEMENT AND CONSERVATION OF AMPHIBIANS AND REPTILES IN NORTH DAKOTA

Randy L. Kreil\* and Michael G. McKenna North Dakota Game and Fish Department, Bismarck, ND 58501

Amphibians and reptiles are innocuous, yet important, components of North Dakota's faunal diversity. Fewer than 30 species of herptiles are known to occur in the state, many of which are at the northern-most edge of their range. The North Dakota Game and Fish Department (NDGFD) is responsible for the conservation and management of amphibians and reptiles for the use and enjoyment of North Dakota's citizens.

Threats to herptiles residing in the state are numerous and variable in their extent and visibility. Physical habitat alterations, pesticides, environmental contaminants, commercialization, and lack of biological knowledge head the list of factors which can

adversely affect amphibians, reptiles, and their management.

Current management practices and philosophies of the NDGFD are intended to eliminate or reduce adverse impacts to the state's herptile populations and habitats. Specific actions on behalf of herptiles include: consideration in the environmental review process, restriction and enforcement of regulations designed to eliminate commercialization, surveys and inventories that provide increased knowledge critical to management, and public educational efforts.

The future for amphibians and reptiles in North Dakota will be determined by a host of factors, the most critical being habitat. As with all fish and wildlife species, habitat in sufficient quantity and quality is required for herptiles to survive and flourish. The goal of the NDGFD is to ensure that important habitat is protected and properly managed for all fish and wildlife species. Amphibians and reptiles, even though they may not receive a significant amount of specific attention, will benefit from these actions.

## ECOLOGICAL MANAGEMENT OF THE CARBERRY SANDHILLS BY THE CANADIAN ARMED FORCES

Errol J. Bredin\* The Ernest Thompson Seton Foundation Seton Village, Route 9, Santa Fe, NM 87505-9805

The Carberry Sandhills of southwestern Manitoba are internationally recognized as having ecological significance. Five distinct plant communities each support rare and endangered flora and fauna. A major concern is the effective management of a large tract of native mixedgrass prairie. I have spent much of the past 25 years researching many of the unique features of the Carberry Sandhills; the majority of my work has focused on reptiles and amphibians.

The Sandhills are agriculturally inviable and thus have been spared the ravages of cultivation, yet there are serious problems. Successions, both natural and unnatural, threaten large portions of the Sandhills. Prior to settlement, the prairie and associated wetlands were managed with a natural equilibrium, prairie fires, bison grazing, etc. Since settlement that has changed and now aspen growth quickly swallows up native prairie, wetlands have been drained and several highly noxious weeds not native to this land threaten several species of reptiles and amphibians along with various rare birds, small mammals, and plants.

Until recently little has been done to address the rapid deterioration of the Carberry Sandhills. The impetus to effectively manage this resource has come from the Canadian Armed Forces - Base Shilo. Over the past 15 years the military establishment has funded many research projects, the most notable being an extensive study on the biological control of leafy spurge. All of these studies and a few other interesting factors led to a major project that has culminated in the preparation of a comprehensive Environmental Protection Plan. It is a long term project and has the potential of setting a valuable precedent. I was fortunate to have been asked to assist with the preparation of the plan and I look forward to beginning work this summer on establishing effective monitoring programs for all species of reptiles and amphibians found in the Carberry Sandhills.

It is a unique story and it will form the basis of my presentation.

# HORMONAL RESPONSE TO ACUTE STRESS AS A BIOMARKER FOR CHRONIC STRESS IN LARVAL AMBYSTOMA TIGRINUM

Diane L. Larson\*<sup>1</sup> and Albert J. Fivizzani<sup>2</sup>

Northern Prairie Wildlife Research Center, Jamestown, ND 58401

Department of Biology, University of North Dakota, Grand Forks, ND 58202

Measures of habitat suitability for a species have traditionally relied on relative population abundance across its range. Recent work shows, however, that local populations in some habitat patches are maintained primarily through immigration. Although individuals may survive in these "sink" habitats, the chronic stress of inhabiting suboptimal habitat results in reproductive rates that are too low to sustain the population. Thus, average physiological health of individuals in a population (the population's health), not simply presence of individuals, indicates suitable habitat. Population helath should be considered first when habitat is proposed for protection for the benefit of the species.

Biomarkers--defined as physiological, biochemical or histological responses in organisms to environmental stressors--hold promise as a method for assessing the health of populations. Biomarkers are commonly used in toxicological studies of response to contaminants in the laboratory, and their use under field conditions is increasing. Some biomarkers indicate exposure or reaction to specific chemicals, whereas others indicate a general stress response. General stress indicators can provide an index of a population's health, and thus the overall suitability of the habitat the population occupies. Of those biomarkers indicating a general stress response, several are potentially measurable with minimal disturbance to the population. Tests for serum glucose concentrations, various blood cell counts, and stress hormone levels, for example, require only that blood be collected from a representative sample of the population.

In this study, we examine the potential use of the stress hormone corticosterone as an indicator of chronic stress in <u>Ambystoma tigrinum</u> populations in the prairie pothole region. Objectives of the study are (1) to develop a nondestructive technique for assessing hormonal response to acute stress under field conditions (addressed in 1992); and (2) to assess the relation between acute and chronic stress responses (addressed in 1993), under the hypothesis that larvae inhabiting degraded environments would experience chronic stress and thus show a diminished response to acute stress.

We captured larvae in unbaited funnel traps left in wetlands overnight. Over a one-month period during which larvae were large enough to provide blood samples but not yet beginning metamorphosis, we obtained blood samples from 4 populations in 1992 and from 20 populations in 1993. Blood samples were taken immediately from half the captured animals removed from traps; the remaining animals were sampled after 30-min confinement in 500-mL bottles (acute stress). Larvae were marked and released at the point of capture; marked larvae were not resampled. In 1993, we also recorded composition of surrounding vegetation and water temperature, pH, alkalinity, dissolved oxygen,  $CO_2$ , chloride, and ammonia for each wetland.

Corticosterone levels were significantly lower in larvae from which blood was drawn immediately than in those subjected to acute stress. Thus, the capture technique did not obscure the acute stress response.

Of the 20 wetlands surveyed in Stutsman and Kidder counties in 1993, 9 were on the Missouri Coteau; none of these had any cropland adjacent to them. The remaining 11 wetlands were in the Drift Plain, east of the coteau, and all had at least some adjacent cropland. Response to acute stress was not consistent between larvae in these two geographically distinct groups of wetlands. For populations in the drift plain, magnitude of the acute stress response tended to be inversely related to percentage of adjacent land in crops ( $R^2$ =0.30, F=4.23, p=0.067). Populations on the coteau showed no relation between acute stress response and any measure of land use. Acute stress response was unrelated to any measure of wetland chemistry in either group.

These results, while not conclusive, are suggestive of the potential use of corticosterone levels as biomarkers for chronic stress. The geographic variability in stress response points out the importance of extensive field testing of potential biomarkers. Future work should (1) expand the number of populations sampled, (2) address year-to-year consistency in chronic stress response within populations, (3) explore the mechanisms through which corticosterone might mediate population-level response to environmental stressors, and (4) include other easily measured potential biomarkers such as serum glucose concentration and leucocyte and lymphocyte counts.

# CONTAMINANT RESIDUES AND EFFECTS IN FROG POPULATIONS AT FLOODPLAIN WETLANDS

Mike Coffey\* U.S. Fish and Wildlife Service, Rock Island, IL 61201

The U.S. Fish and Wildlife Service's Rock Island Field Office and Mark Twain National wildlife Refuge recently initiated an investigation on the effects of agricultural chemical run-off at backwater habitats along the Upper Mississippi River. For this we conducted a literature review on amphibian toxicology. In 1993, we assessed water quality and amphibian diversity among other activities at Mark Twain Refuge. An objective of this study for 1994 includes an assessment of contaminant-related impacts to amphibian communities. We plan to collect frog tissues for chemical analysis and histopathology this spring at a potentially impaired backwater lake.

The study sites are at two backwater complexes in River Pools 18 and 17 known as Keithsburg and Big Timber Divisions, respectively. Keithsburg Division has 1460 acres of backwater lakes, wetlands, and bottomland forests. Keithsburg Division is separated by a levee from the Mississippi River and receives all of its non-flood surface water from agricultural drainages at the north end of the refuge. Big Timber Division is similar in size and forest composition. Big Timber is continuous with the Mississippi River via Cooligar Slough and is protected from direct upland run-off by levees. Big Timber Division will serve as a reference site for Keithsburg Division and provide background data for the Mississippi River corridor. Geographical Information Systems (GIS) maps were created to assist in sampling design and to analyze and report the contaminants and ecological data. The null hypothesis is that there are no differences in water chemistry and tissue contaminant concentrations between the study sites.

The literature search revealed that amphibians bioaccumulate heavy metals and are relatively resistant to some organophosphate insecticides. Amphibians may bioconcentrate some insecticide compounds that are present in the water at concentrations below detection limits to measurable concentrations in the tissues. We feel amphibians are useful organisms to measure agricultural chemical availability and serve as indicators of ecological integrity.

The 1993 flood in the midwest prevented continuous water and sediment sampling. We completed frog breeding call surveys during flood stages. The seasonal wetland and forested areas at the study sites were under water up to 12 ft deep. We used boats to set up listening points in interior areas and along the perimeter. We noted frog and toad calls or choruses within a five min period at each point after dusk on warm windless evenings. Three point counts were completed at each site. We surveyed both sites in late April (water temperature 57°F), middle May (68°F) and late June (77°F). Similar diversity was noted at both sites. All expected species were represented in low numbers except for the spring peeper and gray treefrog. We did not hear any spring peepers. The gray treefrog was the dominant species at each site.

The study design for 1994 includes collecting information along four lines of evidence for amphibian populations. The lines of evidence include: 1) environmental quality, 2) tissue residues, 3) biomarkers such as hormone ratios and/or histopathology, and 4) community metrics. The amphibian data will be integrated with similar datasets for benthos, fish and aquatic plants. Environmental quality parameters include dissolved oxygen, pH, conductivity, temperature, turbidity, nutrient, metal, and herbicide concentrations. Whole tadpoles will be collected by trapping and seining and analyzed for metals and popular insecticides. Blood samples may be collected from adult frogs for endocrine chemistry. Adult frogs may be collected and infused with buffered neutral formalin for histological examination. Many pesticides and some metals have reproductive and endocrine disrupting effects. The timing of exposure to these chemicals may affect endocrine biochemistry resulting in abnormal cells in endocrine and reproductive organs. We will continue our frog and toad census and start surveys for salamanders. We invite suggestions on preferred survey techniques and recommended species for chemical analysis.

# BIOLOGICAL AND MOLECULAR STUDIES OF THE LUCKÉ RENAL ADENOCARCINOMA OF RANA PIPIENS AND ITS HERPESVIRUS

Robert G. McKinnell\*

Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN 55108-1095

Some northern leopard frogs,  $Rana\ pipiens$ , are afflicted with a renal adenocarcinoma. While the geographic distribution of  $R.\ pipiens$  is much greater, the populations known to manifest the malignancy occur only from Minnesota eastward to Vermont and into contiguous regions of Canada. The neoplasm is malignant as judged by its histology and its invasiveness, which leads to multiple metastases.

One of the reasons for early studies of the tumor was that frogs and other amphibians are viable at a diversity of body temperatures, in contrast to mice and most other mammals used in cancer research which maintain body heat near 37°C. Therefore, if temperature-controlled reactions are important to oncogenesis, it should be possible to analyze and characterize conditions essential for malignancy of this tumor by temperature treatment of the neoplasm. Indeed, many studies of the effect of temperature on the Lucké tumor have been made.

The etiological agent of the cancer is a herpesvirus (1). It has been shown that viral replication (but not tumor cell replication) occurs at the reduced temperatures found in nature in water under the ice of frozen lakes (2) as well as in the laboratory. Invasion and metastasis were inhibited at reduced temperature. In contrast, at warm temperatures (18 to 28°C), tumor cells were malignantly aggressive, i.e., they formed many metastatic colonies which grew rapidly, and virus replication was precluded. Invasion was analyzed by the coculture of normal and malignant tissue *in vitro*. That too was shown to be temperature dependent with invasion occurring at elevated temperature (3). Subsequently, a metalloproteinase that will degrade connective tissue collagen was shown to function optimally at invasion-permissive temperature but not at temperatures that preclude active invasion (4).

The molecular aspects of the tumor herpesvirus are currently being studied. The virus was isolated from tumors and purified viral DNA was prepared and sized by field inversion gel electrophoresis and summation of restriction enzyme fragments (5). A 1.2 kbp fragment was chosen for analysis by the polymerase chain reaction (PCR). All tumors taken from cold-conditioned animals tested positive for the restriction fragment. Most, but not all warm tumors tested positive for the viral DNA fragment. Of perhaps greater interest is that some (14%) kidneys of normal frogs contain the etiological agent (6).

This is the first laboratory to note that Lucké viral DNA sequences may be detected in normal frogs. The importance of this observation is that tumor *susceptible* populations can be identified with the PCR procedure, and correlations can be made concerning the vulnerability of those exposed populations to the renal cancer and the presence of putative tumor promoting cofactors. Cancer is a significant cause of death in animal and human populations, and research with the Lucké renal carcinoma may provide new epidemiological procedures useful in the study of cancer etiology. Supported by the Council for Tobacco Research-USA, Inc. Grant 2675AR1.

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# FREEZING TOLERANCE, FREEZING INTOLERANCE AND SUPERCOOLING IN OVERWINTERING ANURANS IN THE GREAT PLAINS

David L. Swanson\* and Brent M. Graves
Department of Biology, University of South Dakota, Vermillion, SD 57069

Anurans overwintering in the northern Great Plains must seek hibernacula allowing them to avoid freezing injury. In many cases, this involves locating microclimates where freezing is avoided. However, five species of terrestrially hibernating frogs, including the Boreal Chorus Frog (Pseudacris triseriata) which occurs in the Great Plains, tolerate significant freezing of hody fluids (2, 7). The freeze tolerant frogs all overwinter in shallow terrestrial hibernacula and so are presumably exposed to subfreezing temperatures at least periodically throughout the winter. Anurans that winter at the bottom of ponds or burrow in terrestrial situations are freezing intolerant, although only two Bufonids have been tested from the latter group (6). It is commonly believed that burrowing anurans avoid freezing by burrowing below the frostline, but hibernacula have been described for only a few species. In at least one species, Bufo hemiophrys, burrow depths are insufficient to completely escape subfreezing temperatures (1). Furthermore, the energetic expense associated with burrowing to great depths to avoid freezing might favor development of freezing tolerance to permit shallower hibernacula that would decrease burrowing costs. This study examines supercooling and freezing tolerance or intolerance in three terrestrially wintering anurans, Scaphiopus bombifrons, B. cognatus, and P. triseriata, in southeastern South Dakota.

In all cases, animals were captured in September and October and housed in aquaria under simulated hibernation conditions until January and February when freezing tests were conducted. For freezing tests, anurans were placed in foam-lined glass chambers and immersed in a water/ethylene glycol bath at 0 to  $-1^{\circ}$ C. Body temperature was continuously recorded with a thermocouple placed against the abdomen of the animal. Chamber temperature was reduced at approximately  $1^{\circ}$ C/h until freezing occurred (detected by the appearance of a freezing exotherm). After freezing, animals were maintained at moderate subfreezing temperatures (-2.5 to -4.5°C) for 24 h. Following this freezing exposure, anurans were allowed to thaw for 2 d prior to testing for recovery.

S. bombifrons was freezing intolerant; all animals that froze died. However, these toads exhibited the lowest supercooling point  $(-4.3 \pm 0.7^{\circ}\text{C}, n = 9)$  for any amphibian species. In addition, two S. bombifrons which supercooled to between -4.3 and -4.4°C for 24 h without freezing survived. This suggests that S. bombifrons may utilize supercooling to avoid freezing in terrestrial hibernacula, although the potential for inoculative freezing from external ice may limit the utility of this strategy (3, 4). If supercooling is effectively utilized by S. bombifrons, then burrow depths would be substantially less than those required to avoid freezing entirely. Soil temperature profiles indicate burrow depths between 50 and 100 cm would allow toads to avoid temperatures below the supercooling point, while avoiding freezing entirely requires burrow depths in excess of 1 m. This difference in burrow depth may be of significant benefit to these toads in terms of energetics and survival.

Preliminary data on *B. cognatus* also suggest freezing intolerance and low supercooling points in this species. However, at least some individuals of *P. triseriata* survive freezing of body fluids under similar freezing exposure. These data suggest that burrowing anurans in the Great Plains, like previously studied Bufonids, are freezing intolerant, but may utilize supercooling in appropriate terrestrial hibernacula as an overwintering strategy. Freezing tolerance in *P. triseriata* is in accord with previous studies on this species (5, 6) and is consistent with the conclusion that freezing tolerance is present only in anurans overwintering in shallow terrestrial hibernacula.

<sup>1.</sup> Breckenridge, W.J., and Tester, J.R. (1961) Ecology 42,637-646.

<sup>2.</sup> Costanzo, J.P., Wright, M.F. and Lee, R.E. (1992) Copeia 1992,565-569.

<sup>3.</sup> Layne, J.R. (1991) J Herpetol 25,129-130.

<sup>4.</sup> Layne, J.R., Lee, R.E., and Huang, J.L. (1990) Can J Zool 68,506-510.

<sup>5.</sup> MacArthur, D.L., and Dandy, J.W.T. (1982) Comp Physiol Biochem 72A,137-141.

<sup>6.</sup> Storey, K.B., and Storey, J.M. (1986) Comp Biochem Physiol 83A:613-617.

 $<sup>^{7}</sup>$ . Storey, K.B., and Storey, J.M. (1988) *Physiol Rev* 68,27-84.

# FEEDING ECOLOGY OF JUVENILE GREAT PLAINS TOAD (BUFO COGNATUS) AND WOODHOUSE'S TOAD (B. WOODHOUSEI)

Matthew A. Flowers\* and Brent M. Graves
Department of Biology, University of South Dakota, Vermillion, SD 57069

Although mortality is highest during early ontogeny in the genus Bufo (2), few studies have investigated the ecology of individuals at this stage in development. Those studies that have, typically investigated predation pressures (1, 4, 5). However, feeding strategies may also play an important role in juvenile anuran survival. For example, small changes in a juvenile's body size may be associated with changes in diet, and thus, prey availability and selection may be important for development. Yet, studies on the diet of Bufo which include juveniles have not looked for changes within this size class. Rather, juveniles are grouped into a broad size class and compared to adults (e.g. 3, 6). Therefore, to investigate feeding strategies within the small size class associated with early ontogeny, the diets of postmetamorphic Bufo cognatus and B. woodhousei from Clay Co., South Dakota were quantified.

Postmetamorphic juvenile B. cognatus and B. woodhousei were collected on 15 July, 1992 from Rose Lake, a prairie pothole marsh located approximately 5 km NE of Vermillion, South Dakota. The stomachs of 36 B. cognatus and 14 B. woodhousei were dissected in the laboratory. Toad weights ranged from 0.16 to 1.68 g, and all were estimated to have metamorphosed approximately one month previously (7). Despite the small size range of toads collected, diet changes reflected in different prey sizes, numbers, and types of prey were found. Regression analysis revealed a significant positive relationship between toad weight and mean prey length ( $R^2 = 0.58$ , P < 0.001). Similarly, there were linear relationships between toad weight and minimum ( $R^2 = 0.23$ , P < 0.001) and maximum ( $R^2 = 0.39$ , P < 0.001) prey lengths. The relationship between total prey number in a stomach and toad weight was also investigated using regression analysis. Some toads of all sizes had few prey in their stomachs, although toads with large numbers of prey in their stomachs were in the lower end of the size range. The linear regression was significant ( $R^2 = 0.08$ , P < 0.05). The relationship between toad weight and the porcent of stomach contents consisting of coleopterans also was significant and positive ( $R^2 = 0.52$ , P < 0.001). Significant negative relationships between toad weight and percent of stomach contents consisting of collembolans ( $R^2 = 0.27$ ; P < 0.001) and mites ( $R^2 = 0.08$ , P < 0.05) were detected.

These data indicate an ontogenetic diet change that occurs early in postmetamorphic ontogeny. Smaller toads ate significantly more prey, and these were primarily smaller species such as collembolans and mites. Larger toads consumed fewer, yet larger, prey. Coleopterans comprised a larger proportion of the diet of larger toads, compared to smaller toads. Further, since toads were collected at a single time and site, differences in diet likely reflect differences in selection of prey, rather than differences in prey availability. This appears to be confirmed by a study conducted this summer. Preliminary data from prey sampling suggest prey selection is exhibited by juvenile toads.

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<sup>2.</sup> Bragg, A.N. (1940) Am Nat 74:424-438.

<sup>3.</sup> Clarke, R.D. (1974) Am Midl Nat 91:140-147.

<sup>4.</sup> Hayes, F.E. (1989) Copeia 1989:1011-1015.

<sup>5.</sup> Heinen, J.T. (1985) *J Herpetol* 19:524-527.

Livezey, R.L. (1961) Herpetologica 17:267-268.

<sup>.</sup> Underhill, J.C. (1960) Herpetologica 16:237-242.

#### PATTERN POLYMORPHISM IN LEOPARD FROGS OF MINNESOTA AND CONTIGUOUS STATES

David M. Hoppe\* and Robert G. McKinnell<sup>2</sup> Science Division, University of Minnesota, Morris, Minnesota 56267

2Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55108

Two pigment pattern variants of the northern leopard frog, Rana pipiens, are found in Minnesota and contiguous states. One of these is burnsi which has a dorsum that is either completely devoid of spots or the dorsal spots are found in greatly reduced numbers. The other variant, kandiyohi, is characterized by a mottling of the pigment pattern between the major dorsal spots. Both burnsi and kandiyohi are inherited in simple Mendelian fashion as

independent, dominant alleles.

The frequencies of the spotless, burnsi variant have been estimated and examined for differences between populations and for temporal stability through up to 25 years of sampling. The frequency of burnsi increased significantly in one Minnesota population, from 3.1% in 1967 to a high of 21% in 1991. From 1985 to 1992 the frequency in that population remained elevated on average at 16.9%, but fluctuated significantly from year to year. The burnsi frequency varied from 0% to 16.3% among 14 frog populations sampled in 1990 through 1992. Two populations of unusually high burnsi frequency were found, both far from the area where burnsi had been previously thought to be most prevalent. One of these populations, located in Richland County, North Dakota had 13% burnsi; the other, in Otter Tail County. Minnesota had as high as 21% burnsi. Genetic drift may explain some of the temporal and microgeographic population differences, but there is also the likelihood of selection on the burnsi morph.

The kandiyohi variant is less abundant and less widely distributed than burnsi, and had

neither been reported in the literature nor seen in our collections for over 10 years. Collections in counties where kandiyohi had been recorded previously and some contiguous counties, involving examination of 2318 frogs, reveal that kandiyohi is still present in west central Minnesota and the eastern Dakotas at frequencies of 0-5%.

Recent years' collections have extended the known range of burnsi to four new counties each in Minnesota and North Dakota, and one in South Dakota. Burnsi had not been previously recorded from North Dakota in the scientific literature. The range of kandiyohi has been extended to one North Dakota and three Minnesota counties where these variants had not been recorded previously. Table 1 lists new county records of burnsi and kandiyohi from this study, compared to the most recent published map of the variants (1).

Table 1. New county records of the burnsi and kandiyohi variants.

<u>Variant</u> Burnsi	<u>State</u> Minnesota	<u>County</u> Chippewa		
		Lac Qui Parle Lincoln		
		Lyon		
	North Dakota	Dickey		
		La Moure		
		Richland		
		Sargent		
	South Dakota	Roberts		
Kandiyohi	Minnesota	Chippewa		
		Lincoln		
	Nowth Dakata	Traverse		
	North Dakota	Richland		

<sup>1.</sup> McKinnell, R.G. and Dapkus, D.C. (1973) Amer Zool 13, 81-84.

#### A Symposium on

## ELECTRICAL ENGINEERING RESEARCH I

## North Dakota / Minnesota Academies of Science 1994 Joint Annual Meeting

# Symposium Coordinators

David A Rogers and Robert M Nelson Electrical Engineering, North Dakota State University, Fargo 58105

## Thursday, 28 April

- 1:00 Power Electronics and Power Quality: The impact on the Power Engineering Curriculum.
  Paulo F Ribeiro and David A Rogers\*
  CRS Sirrine Engineers, Inc, San Jose, CA, 95113 and Electrical Engineering, North Dakota State University, Fargo, 58105
- 1:20 Prediction of Dielectric Breakdown in Underground Cables.

  Jie Deng and Donald A Smith\*

  Electrical Engineering, North Dakota State University, Fargo, 58105
- 1:40 An Improved Spice-Compatible Ferroelectric Capacitor Model.

  Douglas E Dunn and Joel D Monroe\*

  Electrical Engineering, North Dakota State University, Fargo, 58105
- 2:00 Simplifications for Numerical Impedance Matching.

  Douglas B Miron\* and Yongcheng Tu

  Electrical Engineering, South Dakota State university, Brookings, SD
- 2:20 Electromagnetic Environment of Operating Rooms.

  Robert M Nelson\* and Hualiang Ji

  Electrical Engineering, North Dakota State University, Fargo, 58105
- 2:40 Ventriculoarterial Coupling and Maximum External Work Transfer.

  Mark J Schroeder\* and Daniel L Ewert
  Electrical Engineering, North Dakota State University, Fargo, 58105
- 3:00 Informal Discussion and Refreshment Break

# POWER ELECTRONICS AND POWER QUALITY: THE IMPACT ON THE POWER ENGINEERING CURRICULUM

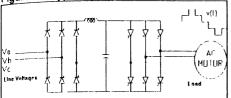
Paulo F. Ribeiro<sup>1</sup> and David A. Rogers\*<sup>2</sup>

<sup>1</sup> CRS Sirrine Engineers, Inc., San Jose, CA 95113

<sup>2</sup> Department of Electrical Engineering, North Dakota State University, Fargo, ND 58105

The new power electronics context characterized by the proliferation of sensitive electronics equipment supplied by an electrical network with very high levels of distortion, which are in part generated by the massive utilization of power electronics applications, creates an environment in which traditional circuit modeling analysis and techniques cannot be applied straightforwardly. Figure 1 illustrates the complexity of this problem by showing how ac motor loads are being commonly supplied by the power system.

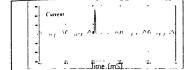
Figure 1 - Typical power electronics load



As a consequence of the new electrical environment, the currents and voltages on the electrical network substantially and randomly deviate from a sinusoidal form, and, thus, the state of the electrical system cannot be fully analyzed by traditional methods. The dynamics of distortion generation, propagation, and interaction of non-linear sources requires more powerful techniques to efficiently analyze the system performance in the presence of non-stationary distortion. High harmonic distortion, voltage notches, high-

frequency noise, etc. are among the typical situations in which sensitive electronic devices are being operated. See Figure 2 for a sample of typical voltages and currents observed in such situations.

Figure 2 - Measured current and voltage in electrical power systems

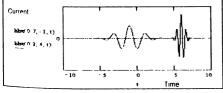




The engineering curriculum needs to be adapted to this new situation by revising the syllabus of the circuits and power system analysis courses in order to incorporate the necessary changes to emphasize the

analytical requirements to solve the real life problems. New techniques for analyzing and designing electrical and electronic systems are becoming pervasive in electrical engineering practice and the associated topics need to be taken into consideration. Among the new techniques being used one can mention: advanced signal processing analysis (such as wavelets), expert systems, fuzzy logic, genetic algorithms, and neural networks. See Figure 3 for an illustration of wavelets, an advanced alternative to analyze signals by a set of oscillatory waves which decay to zero. The wavelet transform translates the time-domain function into a representation that is localized not only in frequency but also in time. As a practical example of the situation, one can mention that in the last few years the FFT method used in signal and image processing has been replaced by the method of wavelets. In addition, hundreds of electrical, electronic, and mechanical devices are being built to operate with an artificial-intelligence control system which utilizes concepts from neural networks, expert systems, genetic algorithms, and fuzzy logic. For example, many car brakes, camcorders, toasters, etc. now utilize fuzzy logic as part of their strategy of operation.

Figure 3 - Wavelet components



Widespread acceptance of these new tools will take time, due to the computational requirements and educational barriers. However, the flexibility and adaptability of these new tools for solving many of the present practical difficulties the engineer has to deal with, seems to indicate that they need to become an integral part of the engineering undergraduate curriculum as one continues to deal with an increasingly complex electrical environment. These new emphases

should be seriously considered and urgently incorporated in the undergraduate curriculum in order to facilitate the transition from the academic world to the daily tasks of the electrical engineer.

<sup>1.</sup> Ribeiro, P.F. (1993) Future tools for power quality analysis, EPRI - Power Quality Conference, San Diego.

#### PREDICTION OF DIELECTRIC BREAKDOWN IN UNDERGROUND CABLES

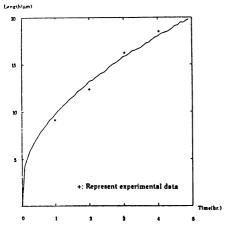
Jie Deng and Donald A. Smith\*

Department of Electrical Engineering, North Dakota State University, Fargo, ND 58105

Underground power cable exhibits electrical breakdown in the polymer insulating material after several years of service. It has been determined that a tree-shaped defect exists in the insulating material (1, 2). A tree can initiate and grow when strong electrical stress is present. When a power cable contains these trees of length comparable to the thickness of the insulating layer or even less, electrical breakdown has a good chance of occurring (3), causing a cable failure. Before this cable breakdown phenomenon was discovered, installers of these polymer insulated cables thought that these cables would probably last for at least forty years. Later it was found that the cables could have a much shorter life span when a tree defect is present. It is important, from an engineering point of view, to predict the real life span of these cables. A large number of research papers have been published about this topic since the discovery of this degradation of polymer by water and electric stress in 1969.

The tree growth law is very important in predicting a cable's life span. If the growth law could be related to the physical variables, it could also reveal the true mechanism of tree growth. This kind of law is not readily available now. Only empirical equations have been established. The main objective of this paper is to establish a mathematical model which describes the relationship of the real physical variables that affect tree growth. The mathematical model has been selected based on solid state diffusion theory.

The model used is intended to introduce a mathematical approach which has a more obvious physical meaning in the hope that this model could also be helpful in revealing the real mechanisms of tree growth. An example of the solution of the diffusion equation under the influence of an electric field is shown on Figure 1 compared with experimental data (4) which is available for only a limited time span. By using the solution of the diffusion equation over a longer time span, a prediction of cable breakdown may be obtained as shown in Figure 2.





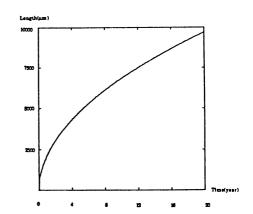


Figure 2. Predicted tree growth to breakdown.

Compared to most empirical types of tree growth law, this tree growth law better fits the experimental data. This model has the same order of accuracy as Dissado's (5).

To predict the cable breakdown time, the curve obtained from experimental data for a shorter period of time is extrapolated to a longer period of time. Using this tree growth law to predict the cable breakdown time is a new approach. The accuracy of the prediction is influenced by many factors. Refined work must be done before this prediction method has real engineering value.

<sup>1.</sup> Shaw, M.T. and Shaw, S.H. (1984) IEEE Trans. on Electrical Insulation, EI-19, p 419.

Garton, A., Bamji, S., Bulinski, A. and Densley, J. (1987) <u>IEEE Trans. on Electrical Insulation</u>, EI-22, p 405.

<sup>3.</sup> Steennis, E.F. and Krueger, F.H. (1990) IEEE Trans. on Electrical Insulation, EI-25, p 989.

<sup>4.</sup> Yoshimura, N. and Noto, F. (1977) IEEE Trans. on Electrical Insulation, EI-12, p 411.

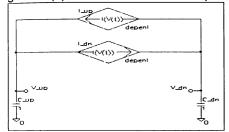
Dissado, L.A., Wolfe, S.V., Filippini, J.C., Meyer, C.T. and Fothergill, J.C. (1988) <u>IEEE</u> <u>Trans. on Electrical Insulation</u>, EI-23, p 345.

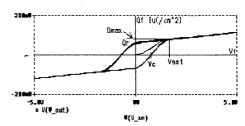
## AN IMPROVED SPICE-COMPATIBLE FERROELECTRIC CAPACITOR MODEL

Douglas E. Dunn and Joel D. Monroe\*
Department of Electrical Engineering, North Dakota State University, Fargo, ND 58105

The first SPICE compatible model for the ferroelectric capacitor (fe-cap) was recently proposed (1). The disadvantage of that model is the requirement that all dipoles in the dielectric are switched at the same applied voltage (Vcoercive). Therefore this model does not simulate partial switching transient effects or a non-saturated hysteresis loop. An improved model is developed to take into account the above problems. The equivalent circuit (Figure 1a) is based on forcing charge (in the form of current) to move between two capacitors, C\_up and C\_dn, at a rate controlled by the voltage across the fe-cap and its time derivative. The charges stored on C\_up and C\_dn represent dipoles in the fe-cap polarized "up" and "down" respectively. The total amount of charge available is constant and the difference of the two charge components gives the net charge storage. Additional circuitry surrounds this basic idea to control the direction, amount of, and time the current flows.

Figure 1. (a) Basic circuit for new fcap model and (b) the circuit's typical output





The functional definition of the current sources, I\_up and I\_dn, allows the polarization to switch over a range of applied voltages, thereby improving the previous model. The function lcap must meet certain requirements: (1) the shape must be controllable (for curve fitting purposes), (2) the integral must be finite and relatively simple (3) the function must be easily implemented in PSPICE. A variation of the hyperbolic secant function was found to meet these needs.

The function is defined as follows,  $I_{cap} = \kappa C(V_f) \partial V_f / \partial t$ , where,  $C(V_f) = \sec h(\alpha(V_c - V_f)) - \beta$ . As illustrated in Figure 1b, Vf is the voltage across the fe-cap, Vsat is the input voltage when the hysteresis loop reaches saturation and Vc is the coercive voltage ( defined to be one-half of Vsat). Using the LIMIT function in PSPICE the function Icap is limited to zero except when Vf and dVf/dt are greater than zero Alpha controls the width of the function. Beta is defined such that Icap will go to zero at Vf equal zero and Vf equal Vsat by using the equation,  $\beta = \sec h(\alpha V_{sat}/2)$ . The gain factor, kappa, is defined as,  $\kappa = \int_{Q_{sat}}^{Q_{max}} \partial Q / \int_{V_{sat}}^{V_{sat}} C(V_f) \partial V_f$ , where Qon and Von are the charge and voltage across the fe-cap when Icap is greater than zero. Kappa is recalculated whenever either current source turns on, such that all available charge on C\_up or C\_dn will be transferred to the opposite capacitor by the time Vf reaches Vsat. This ensures that complete polarization reversal is modeled

Comparing the new model to data shows how reasonable fits may be obtained to both saturated and non saturated hysteresis loops. Small linear capacitors placed in series with the model account for linear charge storage in the fe-cap, producing the complete hysteresis loop in Figure 1b.

as V<sub>f</sub> sweeps past Vsat.

<sup>1.</sup> Dunn, D.E., 1994, IEEE Trans. on UCCF.

# SIMPLIFICATIONS FOR NUMERICAL IMPEDANCE MATCHING

Douglas B. Miron\* and Yongcheng Tu Electrical Engineering Department, South Dakota State University, Box 2220, Brookings, SD 57007

In 1977, Carlin (1) published the first paper on using purely numerical methods to match a resistive generator to a complex-impedance load which is represented only by measured or calculated data. The system envisioned consists of the source and its resistance, a two-port lossless network for matching, and the load. The power transferred across the junction from the network to the load depends only on the impedances looking both ways from the junction, that is, the load impedance and the impedance looking back into the source-resistance terminated network. Carlin modeled the real part,  $R_q(\omega)$ , of the network impedance as a piecewise-linar function. Assuming a minimum-reactance network, the imaginary part,  $X_q(\omega)$ , is related to  $R_q$  by an integral (2). Carlin used the form  $R_q = \sum_{k=0}^N r_k a_k(\omega)$ , where  $a_k(\omega)$  is 0 for  $\omega \leq \omega_{k-1}$ ,  $(\omega - \omega_{k-1})/(\omega_k - \omega_{k-1})$  for  $\omega_{k-1} \leq \omega \leq \omega_k$ , and 1 for  $\omega_k \leq \omega$ . That is,  $a_k(\omega)$  is a ramp from 0 to 1 between the corner frequencies, and  $r_k$  is a multiplier which is the actual change in resistance between the corner frequencies.  $r_0$  is the dc resistance. Correspondingly,  $X_q = \sum_{k=0}^N r_k b_k(\omega)$ , where  $b_k$  is a sum of xln(x) terms related to  $a_k$  by the afore-mentioned integral. The  $\omega_k$  are the set of corner frequencies in the piecewise-linear function, chosen by the designer. The matching process has two major steps.

1. Find the  $r_k$  that minimizes an error function between the actual gain and a target gain function. 2. Find a network with a finite number of elements which gives a good approximation to the resistance function found in step 1.

To be realizable by a passive network,  $R_q \ge 0$ . This implies that all partial sums of the  $r_k$  in Carlin's formulation must be nonnegative. This condition is easy enough to check during an optimization process, but difficult to correct when it fails. Thus the optimization of the  $r_k$  requires a complex algorithm. We have made two simple changes. First, instead of the ramp basis function, we use a triangle basis function,  $t_k = a_k - a_{k-1}$ . This makes  $r_k$  the triangle amplitude and the value of  $R_q$  at the point of the triangle. The realizability constraint becomes only that  $r_k \ge 0$ . Next, we define  $r_k = e^{u_k}$  and optimize on the  $u_k$ . Now the optimization is unconstrained.

Major step 2 is also an approximation problem. Classical methods for network synthesis take one from a given polynomial ratio for  $Z_q(s)$  to the network elements, and ladder network development terminating in a resistor is the approach for this problem, once such a  $Z_q$  is found. Until recently, this also was either a chancy unconstrained optimization or a complex constrained optimization problem. We have formulated  $R_q$  for the lowpass case in terms of the products of its symmetric four-pole groups (3). Each group is represented by two basic values, a real part and an imaginary part, either of which can have either sign without affecting the value of  $R_q$  because they appear only in squares. More recently, we have improved the procedure for optimally placing the poles by partitioning the frequency range between the poles' imaginary parts and minimizing the error function over the frequencies from 0 to a point above the current pole, with respect to all the poles in the current frequency range. This is done because the effect on the resistance function of low and high frequency poles is not the same. A pole has diminishing effect on  $R_q$  for frequencies below it, but pulls  $R_q$  down as the fourth power (because there are four poles to a group) at frequencies above it. Thus, if the fit to the target curve is good at high frequencies, it locks in the low frequency poles, even if the fit is not good at low frequencies. Therefore, we cycle through, optimizing from low to high frequencies.

As a potentially practical example, we used software (4) to design a series-resonant small dipole antenna for 15 MHz. It is a center-fed post-and -plate structure, with two arms 1 cm in diameter, 80 cm long, each arm holds a plate 2 m in diameter and 20 cm thick. This antenna has an impedance of about 7  $\Omega$  at 15 MHz and an unloaded Q of 24.4. If driven by a 7  $\Omega$  source, it would have a bandwidth of 1.2 MHz. We calculated its impedance in 0.1 MHz steps from 10 to 20 MHz. We found by trial with various target gain values, corner frequency sets, and error criteria, that the best we could do for bandwidth is a normalized power transfer equal to or slightly greater than 0.5 from 13 to 17 MHz. To match the piecewise-linear  $R_q$  obtained, we used the pole-placement method described above and found a 9-element lowpass ladder that gives half-power from 13.15 to 16.9 MHz, a 3.75 MHz or 25 % bandwidth.

<sup>1.</sup> Carlin, H.J. (1977) IEEE Trans. on Circuits and Systems, CAS-24, pp 170-175.

<sup>2.</sup> Tuttle, D.F. (1956) Network Synthesis, Wiley, p. 390.

<sup>3.</sup> Miron, D.B. (1993) Proc. 36th Midwest Symposium on Circuits and Systems, IEEE, New York.

<sup>4.</sup> Miron, D.B. (1991) J. Applied Computational Electromagnetics Society, 6, pp 99-132.

### ELECTROMAGNETIC ENVIRONMENT OF OPERATING ROOMS

Robert M. Nelson\* and Hualiang Ji Department of Electrical Engineering, North Dakota State University, Fargo, ND 58105

The term "electromagnetic environment" has been defined as "the time-variant totality of electromagnetic phenomena existing at a given location" (1). In other words, to know what the electromagnetic environment is at a certain location, one must know what electromagnetic fields exist at that point. Although the effect that the electromagnetic environment has on human beings is a question being currently debated, it is well known that the electromagnetic environment can have an adverse effect on electronic devices. Electromagnetic interference (EMI) is the term generally used to describe the situation where the performance of an electronic device is affected by the presence of electric and/or magnetic fields (i.e. "noise") created either by the device itself or by some other electronic device.

One of the places where it is critical to avoid EMI in electronic devices is in the operating room of a hospital. It is not difficult to imagine scenarios where equipment malfunction could have disastrous effects. Unfortunately, the electromagnetic environment in a typical operating room can be very harsh. The electric and magnetic fields in a typical operating room are created both by sources outside the room (such as  $\Lambda M$ , FM and television broadcast transmissions and radio transmissions from various radios) as well as sources inside the room. Of the sources inside the operating room, the electrosurgical unit (ESU) is one of the devices known to create fields which can cause EMI in other medical devices (2). One of the methods of minimizing EMI in medical devices is to ensure that the devices themselves are immune to EMI. This can be accomplished by applying appropriate mitigation techniques so that the medical devices are "EMI-hardened". However, in order to accomplish this task in a cost-effective manner, one must know what the "electromagnetic environment" is i.e. what levels of electric and magnetic fields will the medical device be subjected to? The most reliable means of answering this question is to measure the fields in health care facilities.

Electric field strength measurements were taken in operating rooms at the Veterans Administration Hospital in Fargo, North Dakota. Of particular interest were the fields created by electrosurgical units. Measurements were performed 1 meter from both the ESU and specimen being operated on. All three components of the electric field were measured. The measurement frequency range was either 150 kHz to 200 MHz or 10 kHz to 30 MHz, depending on the mode of operation of the ESU. A plot of the electric field versus frequency for one mode of operation in shown in Figure 1. The maximum field strength was 152.7 dB $\mu$ V/m (43.5 V/m). Interference was observed on some of the electronic medical equipment when the ESU was being used. These measurement results can be used by both the manufacturers of medical equipment as well as appropriate standards organizations when evaluating how to minimize the severity of electromagnetic interference in operating rooms.

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  Prentice Hall International (UK), Hertfordshire, UK.
- 2. Silberberg, J.L. (1993)

  <u>Compliance Engineering, X, pp 25-39.</u>

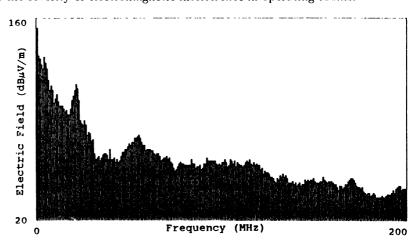


Figure 1. Electric field for CUT mode with maximum power.

### VENTRICULOARTERIAL COUPLING AND MAXIMUM EXTERNAL WORK TRANSFER

Mark J. Schroeder\* and Daniel L. Ewert
Cardiovascular Engineering Laboratory
Department of Electrical Engineering, North Dakota State University, Fargo, ND 58105

A theory exists which states that the left ventricle of the heart is coupled to the arterial system in such a way as to provide maximum external work (1). It has been shown that in order to achieve maximum external work, the "effective" arterial elastance must equal the end-systolic elastance of the left ventricle (2). We have done work to provide further insight into two areas: 1) defining a "physiological" arterial elastance as opposed to an "effective" arterial elastance and 2) testing whether the left heart and arterial system are coupled to provide maximum external work. Attention must be given to the actual definition of coupling which can be defined during systole as concerning "steady flow of blood through the body's capillaries" and during diastole as concerning "perfusion of the heart as an organ" (3).

An electrical analog circuit was designed to represent the cardiovascular system. The arterial system was represented by a resistor and capacitor in parallel, representing total peripheral resistance (TPR) and arterial compliance (inverse of arterial elastance), respectively. The left ventricle and aortic valve were represented by a series voltage source and a diode, respectively. The governing nodal equation was adapted to solve for values of the resistance and capacitance simultaneously. Results of both values compared favorably to values shown in literature. This method was recently tested with other current methods for obtaining capacitance by a leading researcher and our method faired quite well (4).

External work was defined as the area within the pressure-volume loop of the left ventricle, approximately equal to the mean ejecting pressure times the ejected volume. With a good method for obtaining TPR and arterial elastance in hand, an explicit equation was written to determine the arterial elastance that would provide maximal external work transfer. Refined by the presence of a "physiological" arterial elastance, this equation proved to have essentially the same result as the one we were trying to improve upon. Namely, the optimum arterial elastance always resulted in the ejection of one half of the effective volume of the left ventricle in order to achieve maximum external work transfer. It was then that we realized the importance of the time-course of an ejecting beat. In other words, the left ventricular pressure did not remain constant during ejection and should not be treated as such. This result led us to depend on a computer simulation to advance our research.

Early stages of experimenting with a computer simulation showed poor results using the current model. Changes were made until we eventually settled on a four element model for the arterial system, three element model for the coupling, and a two element model for the left ventricle. Estimations for each of the model parameters were determined using physiological data from a given beat. Then, using left ventricular elastance determined from physiological data as the input to our model, we were able to simulate left ventricular pressure, aortic pressure and aortic flow. The simulated data compared very well to the physiological data so we moved on to the next step.

Parameters of the arterial system model were altered, namely TPR and compliance, to provide us with a new simulated beat. For each new beat, the external work was calculated and compared to that of the original beat. The definition of coupling, above, influenced us to set constraints on our results in the areas of cardiac output and mean arterial pressure. Adhering to these constraints, preliminary results have shown that relatively large changes in arterial elastance and/or TPR have little effect on the external work of the left ventricle. Intuitively, the cardiovascular system seems to rest at a sort of equilibrium with respect to external work. For instance, if the afterload were to change in such a way as to increase arterial pressure, stroke volume would then decrease, everything else remaining constant. However, the area within the pressure-volume loop, or external work, may remain quite constant. Controlling mechanisms would then intervene to maintain proper cardiac output and mean arterial pressure. Therefore, ventriculoarterial coupling seems to submit more to life supporting features than it does to providing maximum external work transfer.

<sup>1.</sup> Elzinga, G., Westerhof, N. (1980) Cardiovascular Research, 14, pp 81-92.

<sup>2.</sup> Sagawa, K., Maughan, L., Suga, H., Sunagawa, K. (1988) <u>Cardiac Contraction and the Pressure-Volume Relationship</u>, pp 277-280, Oxford, New York.

<sup>3.</sup> Yin, Frank C. P. (1987) Ventricular/Vascular Coupling, p 5, Springer-Verlag, New York.

<sup>4.</sup> Westerhof, N. (1993) personal communication.

### A Symposium on

### ELECTRICAL ENGINEERING RESEARCH II

North Dakota / Minnesota Academies of Science 1994 Joint Annual Meeting

### Symposium Coordinators

David A Rogers and Robert M Nelson
Electrical Engineering, North Dakota State University, Fargo 58105

Thursday, 28 April

- 3:20 Control of Robots.

  Jacob S Glower\*

  Electrical Engineering, North Dakota State University, Fargo 58105
- 3:40 Some Current and Recent Robust Control Research.

  Jenny L Rawson\*

  Electrical Engineering, North Dakota State University, Fargo 58105
- 4:00 On Linear Skewing for Parallel Array Access.

  Rajendra Katti and Longfei Hu\*

  Electrical Engineering, North Dakota State University, Fargo 58105
- 4:20 Experiments with Generalizers and Artificial Neural Networks.

  Douglas B Miron\*

  Electrical Engineering, South Dakota State University, Brookings, SD
- 5:00 Multiresolution / Decision Tree Pattern Recognition.
  Floyd M Patterson\*
  Electrical Engineering, North Dakota State University, Fargo 58105
- 5:20 MOS Controlled Thyristors for Power Conversion.

  Danny Quek\* and S Yuvarajan

  Electrical Engineering, North Dakota State University, Fargo 58105

### CONTROL OF ROBOTS

Jacob S. Glower\*

Department of Electrical Engineering, North Dakota State University, Fargo, ND 58105

Robots have become common items in several workplaces -- most notably in automobile manufacturing, chip placement on electric circuit boards, and similar operations. In other areas, however, there has been very little success using robotics. One such area is in assembly tasks.

Assembly operations are most commonly conducted by human operators at present. In order for robots to be used in this area, several capabilities are required -- capabilities that are presently not available and are likewise current areas of research. A few of these areas related to control systems are being investigated at NDSU. A brief description of these follows.

1) Active Control of Vibrations for Lightweight Robots: To increase the usefulness of robots for assembly tasks, faster and lighter robots are required. Faster operation makes the robot competitive with human workers in the USA and elsewhere. Light weight allows for greater precision in applying and controlling contact forces inherent in assembly operations. These requirements conflict, however, by potentially bringing vibrational modes within the bandwidth of the controller. Active damping of these vibrational modes is then required.

A great deal of research has been conducted towards the damping of vibrations. As early as 1928, Ormondroyd analyzed the vibration of buildings and beams and developed a mass-damper to reduce the resonance's of such structures. In the 1960's, modern control approaches started to dominate research in the control community, and likewise, research in active damping. Unfortunately, both of these approaches have had minimal success when applied to systems such as lightweight robotic arms for several reasons. Because of this, a third approach based upon scattering theory was proposed by von Flotow in 1986.

Under the paradigm of scattering theory, resonances are a result of energy waves reflecting off discontinuities. When these reflections add in phase, a resonance is produced. The purpose of the controller, therefore, is to minimize these reflections. Von Flotow and the PI has demonstrated that this paradigm results in simpler and more robust controllers for beams and vibrating strings respectively. Future work will attempt to show the connections among these three approaches, allowing the wealth of knowledge in these three fields to be brought together. This should allow for simpler, more robust, better performing controllers for systems such as lightweight robotic arms to be designed in the future.

2) Adaptive Control of Robots Under Repetitive Tasks: A second problem encountered when controlling robots relates to the load being unknown or uncertain. This uncertainty can make it difficult to keep the tip of the robot at its desired position -- especially if lightweight compliant motion is desired. This problem has led to the investigation of using an adaptive control law for the control of robot manipulators.

Most assembly operations are repetitive in nature. One may wonder, therefore, if the repetitive nature of this activity could be used in the design of the controller to minimize the error in the tip position in spite of uncertainty in the load.. The research being conducted at NDSU looks at this problem. Here, adaptive control routines which tune the feedback gains for the specific periodic motion are being investigated. These routines modify present adaptive control routines in a similar way that servo-compensators modify fixed-gain feedback control laws. Such modifications allow the adaptive control law to track the repetitive nature of the robot's motion, increasing the accuracy of the robot's motions. This likewise increases the feasibility of using lightweight robotic manipulators.

### SOME CURRENT AND RECENT ROBUST CONTROL RESEARCH

Jenny L. Rawson\* Department of Electrical Engineering, North Dakota State University, Fargo, ND 58105

The goal of robust control is to design a system which will be stable and meet performance specifications over ranges of operating conditions and system modelling errors. For example, the aerodynamics of an aircraft must be modelled differently depending on the airspeed and altitude. A variable parameter controller can be designed to stabilize the aircraft at each of several combinations of conditions. However, rapid changes in airspeed and altitude will necessitate frequent changes in the control parameters with resulting chattering or instability. These problems can be prevented by having a single set of parameters in effect throughout a certain maneuver; this may be possible with a robust controller.

One approach to robust design is the  ${\rm H}_{\infty}$  method (1). It is based on a generalization of the old techniques of frequency response shaping from classical control and the Small Gain Theorem from nonlinear control (a generalization of the Popov and circle stability criteria). space model is devised for the system to be controlled; performance objectives and parameter variations due to changes in operating conditions are included in the model. Then, two Riccati equations, similar to those used in the linear quadratic regulator or linear quadratic Gaussian methods, may be solved to obtain a robust controller. H $_{\infty}$  design has been applied in many situations to obtain controllers which are robust over a wide range of operating conditions. Unfortunately, these controllers are of relatively high order; implementation may be difficult, so a lower-order controller achieving the same level of robustness is desired. The author has developed a low-order controller design method wherein robustness is maximized in a search process that includes solving one or two parameterized Riccati equations at each iteration.

It was discovered that there can be numerical problems in the solution of the Riccati equations associated with this new method. First, for certain systems, numerical instabilities can yield a "no solution" response from the best Riccati equation solvers (based on the Schur decomposition (2)) when solutions can be found via ad hoc methods. This problem is avoided by using the QZ decomposition and eliminating the matrix inversions required in the Schur method. A second problem is that with the many iterations needed in the minimization process, obtaining a controller for a large-scale system would be fairly time consuming. A new project, with Dr. Rajendra Katti, is to develop a parallel Riccati equation solver which will be numerically stable. Currently, work is proceeding on a new algorithm for solving Lyapunov equations, which can be used as a step in solving Riccati equations, and also has some immediate applications in other robust control design methods.

Doyle, J.C., Glover, K., Khargonekar, P.P., and Francis, B.A. (1989) <u>IEEE</u> <u>Trans Automatic Control</u> 34, pp 831-847.

<sup>2.</sup> Pappas, T., Laub, A.J., and Sandell, N.R. (1980) IEEE Trans Automatic Control 25, pp 121-135.

### ON LINEAR SKEWING FOR PARALLEL ARRAY ACCESS

Rajendra Katti and Longfei Hu\*
Department of Electrical Engineering, North Dakota State University, Fargo, ND 58105

Many large computers have a large number of memory banks that can be accessed independently in parallel. The speed and efficiency of such computers depends on their ability to access parts of a two-dimensional array in one memory cycle. Any storage scheme that maps elements of a two-dimensional array to memory banks for the conflict-free access of parts of the array, is called a skewing scheme. By parts of an array we mean any row, column, diagonal, anti-diagonal or sub-array. Let M denote the number of memory banks in a computer system. M is usually fixed during the design phase of the computer. We are interested in mapping the elements of an  $N \times N$  array to the M memory modules such that any row, column, diagonal, anti-diagonal or any  $Y \times Y$   $(Y \leq \lfloor M^{1/2} \rfloor)$  or smaller sub-array of the array can be accessed in one memory cycle. We shall restrict ourselves to  $N \times N$  arrays where  $N \leq M$ . This can be done without loss of generality as an array with N > M can always be broken down into a set of  $L \times L$  sub-arrays with  $L \leq M$ . The most used skewing schemes are the linear skewing schemes (1), defined by,

$$s(i,j) = a \cdot i + b \cdot j \pmod{M}; \quad 0 \le i, j \le N - 1.$$

The above equation states that element (i,j) of the array is mapped to module s(i,j). The mapping is obtained by a (a,b) skew. In this paper we give a technique to find 'a' and 'b' so that any row, column, diagonal, anti-diagonal or any  $Y \times Y$  ( $Y \leq \lfloor M^{1/2} \rfloor$ ) or smaller sub-array of the  $N \times N$  array can be accessed in one memory cycle. Previous works (2,3) deal exclusively with rows, columns or diagonals or exclusively with sub-arrays only. The results obtained can be summarized as follows:

- 1. The optimal size of sub-arrays that can be accessed without conflict is  $\lfloor M^{1/2} \rfloor \times \lfloor M^{1/2} \rfloor$ .
- 2. If N is divisible by 2 or 3 then there is no skew that results in conflict free access for rows, columns, diagonals and sub-arrays, with N modules.
- 3. In a (1,b) skew, b must be as close to  $\lfloor M^{1/2} \rfloor$  as possible for optimal sub-array access. If b is too large or too small then the sub-array size that can be accessed without conflicts becomes smaller than  $\lfloor M^{1/2} \rfloor$ .
- 4. If there is a prime number, P, between N and M, then conflict free access for rows, columns, diagonals and  $|P^{1/2}| \times |P^{1/2}|$  sub-arrays is possible.
- 5. If there is no prime number between N and M, and there exists a number, R, between N and M that is not divisible by 2 and 3, then conflict free access for rows, columns, diagonals and  $Y \times Y$  sub-arrays is possible, where  $Y \leq |R^{1/2}|$ .
- 6. If there is no prime number between N and M, and there does not exist any number between N and M that is not divisible by 2 and 3, then conflict free access for rows, columns, diagonals and sub-arrays is not possible.

<sup>1.</sup> Budnik, P. and Kuck, D. J. (1971) IEEE Trans. Comput., C-20, pp 1566-1569.

<sup>2.</sup> Wijshoff, H. A. G. and Van Leeuwen, J.(1987) IEEE Trans. Comput., C-36, pp 233-239.

<sup>3.</sup> Lawrie, D. H.(1975) IEEE Trans. Comput., C-17, pp 1145-1155.

### EXPERIMENTS WITH GENERALIZERS AND ARTIFICIAL NEURAL NETWORKS

Douglas B. Miron\*

Electrical Engineering Department, South Dakota State University, Box 2220, Brookings, SD 57007

Wolpert has developed a formal mathematical theory of generalization (1), in an attempt to find criteria that would limit the choice of possible functions and lead to a best generalizer. While he has not succeeded in this objective, he has proposed a couple of heuristic all-purpose generalizers, and written some severe criticism of "back-propagated" artificial neural network, ANNs (2). In (3) he tries to imitate the external behavior of a human who, given a training set of data, must guess an answer to a question in the input space. Taking a geometric point of view, if the input data is one or two-dimensional and the question is within the limits of the input data (an interpolation problem), the human is likely to visually fit a line or plane to the points nearest the question, and find the guess on that surface. This concept generalizes to fitting a surface with a local hyjperplane. That is, if m is the number of components of the input vector  $\overline{q}$ , then  $g = a_0 + \sum_{i=1}^m a_i q_i$ . The  $a_i$  are found by solving this equation for the m+1 nearest given data points. I will refer to this generalizer as SURFIT below. In (4) Wolpert used another method, in which the given output values are inversely weighted by the distance of the corresponding given input values from the question.  $g = [\sum y_k/d(\bar{x}_k, \bar{q})]/[\sum 1/d(\bar{x}_k, \bar{q})]$ . d(, ) is any distance function. I will refer to this generalizer as POLES in the following discussion. Both of these generalizers have the properties that if the question is in the training set, the answer is exactly correct (zero error), and accuracy monotonically improves as the density of the training set. They also require no training, as ANNs do. On the other hand, one could say that the training is done for every question, since all the distances have to be computed, and for SURFIT sorted, for each question. Both are very computationally intense for large training sets. If SURFIT had the human ability to "see" in a visual presentation which were the nearest points, much less computation would be needed.

ANN needs to be trained. Since the functions passing values from one layer to the next are continuous if the sigmoid neuron is used, it is possible to write an expression for the gradient of the error with respect to all the weights and offsets. Beigi has adopted a quasi-Newton method of Davidon (5) and devised a method for growing a two-layer network (6) which tends to a global error minimum. These methods are also many orders of magnitude faster than "back propagation".

Consider a simple problem, with the parent function  $y = (x_1^2 + x_2^2)/1000$ , with  $0 \le x_i \le 1000$ . The range of y is 0-2000. The question could be asked, "Given a pair of numbers in the domain, what is y?" Both SURFIT and POLES will do very well on this question. If we want even 0.1 binary-coded output layer and a network of two or more layers would be needed. On the other hand, if we divide the range of y into a few large intervals and ask, "For a given pair of numbers, which interval is the output in?", this is a problem more suited to a simple ANN, and the heuristic generalizers will still do well. A pseudo-randomly selected set of 500 pairs of input numbers and corresponding y values was used as the training set for all that follows, and another such set was used as the testing set. The range of y was divided into three parts,  $0 \le y < 300$ ,  $300 \le y < 700$ , and y > 700. To make a one-high target function, the basic parabolic function was passed through these tests to produce a three-component output, 1 0 0, 0 1 0, or 0 0 1, depending on the region. This requires three scalar generalizers, one for each component, and an ANN with three output neurons. Since, in all cases, the basic outputs are continuous functions, I converted them to binary values by finding the maximum of the three outputs and then testing each output for equality with this maximum. The result will always be two zeros and a one. Since the output is really a three-element vector, a guess is counted right if the  $\sum_{j=1}^{3} |y_j - g_j| = 0$ . Each nonzero result is counted as an error in the test set. For the particular training and test sets used, SUFIT had 17 errors. The POLES generalizer, with the metric being the sum of squares of the component differences, produced 13 errors. Using Beigi's method, I grew several networks from random intial values for the weights, and zero offsets. Originally, I was too cautious in limiting the range of values so that the neuron outputs were between 0.49-0.51. This led to ANNS with as many as 7 neurons in the first layer, training errors in the range of 15-40, and testing errors in the range of 15-60. After I modified the initial-weight generation routine to give neuron outputs between 0.2-0.8, training and growing process was much improved. I was able to get down to 1 training error with only two first-layer neurons, and 4 testing errors. Converting next to a Gray code in wich the three regions are represented by outputs of 0 0, 10, and 11, I modified the basic generalizers and ANN outputs by rounding their outputs to the nearest integer. For the same 500-case testing set, SURFIT had 26 errors and POLES had 13 errors. I could produce a single pair of neurons that gave about 70 errors on both training and testing sets. On two trials with Beigi's method, I found networks with 4 first-layer neurons. The first had 1 training error and 6 testing errors, the second had 0 training errors and 5 testing errors.

These simple experiments show several things. First, while Wolpert's heuristic generalizers can serve as benchmarks for ANN performance, they are definitely not limits. Secondly, Beigi's method is much superior to previous approaches in both training time and final accuracy. Finally, as always, the way a problem is structured or a question asked has a strong effect on outcomes.

<sup>1.</sup> Wolpert, D.H. (1990) Complex Systems, 4, pp 151-249.

<sup>2.</sup> Fukuda, T. and Shibata, T. (1992) IEEE Trans. Industrial Electronics, 39, pp 472-489.

Wolpert, D.H. (1989) Biological Cybernetics, 61, pp 303-313.

<sup>4.</sup> Wolpert, D.H. (1990) Neural Networks, 3, pp 445-452.

<sup>5.</sup> Davidon, W.C. (1975) Mathematical Programming, 9, pp 1-30.

<sup>6.</sup> Beigi, H.S.M. (1993) Proc. 36th Midwest Symposium on Circuits and Systems, IEEE, New York.

### MULTIRESOLUTION/DECISION TREE PATTERN RECOGNITION

Floyd M. Patterson\* Department of Electrical Engineering, North Dakota State University, Fargo, ND 58105

This research employs a two-step process for pattern recognition. It adapts multiscale/multiresolution analysis coupled with hierarchical classification using decision trees. This approach is advantageous for those signals which are believed to contain both shortterm and long-term correlation properties in which stationarity assumptions are valid over limited regions of support. Advantages, approaches and procedures, and pitfalls and problems are presented.

Multirate system analysis (1) is now becoming a major area of study in signal processing for this leads to an alogorithm that interrogates the signal for properties which exist in identical ways except for shifts and scale changes. These methods employ polyphase and quadrature mirror filters which maintain lossless processing algorithms that can be executed at high speed. The data signal is simultaneously processed by two compatible operators(two parallel filters) and the outputs from these are decimated(downsampled) by 2 in the usual case. The output of one downsampled filter then contains the high resolution detail part of the signal while the output from the second downsampled filter contains signal characteristics which appear to be of lower frequency and exist over greater regions of support. This lower detail signal can then be processed using the same algorithm as that on the original signal, resulting in an iterative process of extracting fine to coarse features of the signal. The coarse outputs characterize the signal over broad regions of support and give initial partitioning information on the classification process, while the finer outputs characterize more subtle features to be used in subsequent classification.

Decision trees (2,3) are very useful whenever data is of high dimensionality, when probability distribution functions of the classes of data are not known, and when classification must be fast. A decision tree is a structural representation of an algorithm which attempts to properly classify feature data, the attributes, as having originated from one member in a set of objects. Identification occurs by moving from the root of the tree to a node while performing conditional tests on the attributes at each branch so as to navigate to a terminal node. One or more classes are represented at each terminal node and the identified class is assumed to be that one with the greatest percentage representation. Binary trees generate two branches from each internal node and thus the conditional tests are of the if-then type and can be executed extremely quickly. This research uses the outputs of the above filter banks as attributes on which to perform if-then tests.

In addition, a significant extension to processing binary data with the above procedure is presented. It is shown that constrained filters can be found for the filter banks which maintain the desired lossless property of filter banks for non-binary data. The explicitly desired decorrelation of filter outputs cannot be predicted and determined however, and the meaning of orthogonality of data is unclear since binary processing algorithms are not operating in a Hilbert space. Decorrelation of the elements in the feature space via probability density functions is given. Applications, examples, and results along with diagrams of filter banks and decision trees is presented.

**<sup>1</sup>**. Vaidyanathan, P.P. (1993) Multirate Systems and Filter Banks, Prentice-Hall Book Co.,

Quinlan, R. (1990) IEEE Trans. on Systems, Man, and Cyb., vol. 20, pp 339-346. 2.

<sup>3.</sup> Safavian, S. and Landgrebe, D. (1991) IEEE Trans, on Systems, Man and Cyb., vol. 21, pp 660-674.

### MOS CONTROLLED THYRISTORS FOR POWER CONVERSION

Danny Quek\* and S. Yuvarajan
Department of Electrical Engineering, North Dakota State University, Fargo, ND 58105

Electric power is converted from one form to another using power converters which use an array of power semiconductor devices. The thyristor is the power semiconductor device used in many large-capacity power conversion applications. Other devices like Power MOSFET and Insulated Gate Bipolar Transistor (IGBT) are also used to build power converters. The search for a perfect power switch has led to the development of the MOS Controlled Thyristor (MCT) which combines the advantages of several devices (1, 2). It has the high current density characteristics of a thyristor and the high-speed response of a MOSFET. It is a voltage controlled device and it can be turned on and off using narrow voltage-pulses of proper polarity. The MCT is presently available as an engineering sample and it will be commercially available soon.

The switching characteristics of the MCT has to be obtained before it can be used as a power switch. The MCT has cathode, anode, and gate terminals like a thyristor. It is turned on by applying a negative pulse to the gate with respect to the anode, and turned off by applying a positive pulse. The pulse width is kept small in order to reduce the switching loss. The gate pulses to the MCT are generated using a control circuit with operational amplifiers. The control circuit must be synchronized with the power circuit. In a controlled rectifier, the gate pulses are to be synchronized with the ac voltage waveform so that the MCT is triggered at a chosen firing angle. In a dc chopper, the gate signal is generated using an astable multivibrator. The gate triggering circuit generates a square waveform which defines the conduction period of the MCT. The square wave is then passed through a pair of monostable multivibrators (positive and negative edge triggered) which generate gate pulses of desired width.

Like any MOS gated device, the MCT has an input capacitance. The gate pulses are to be passed through a push-pull amplifier to give the current amplification needed to drive the capacitive gate of the MCT. The resulting waveforms of the gate voltage have small rise and fall times. The transient responses of the MCT during turn-on and turn-off are recorded using a digital storage oscilloscope. The turn-on and turn-off times of the MCT are accurately measured and they are of the order of 1 to 2 µs. Thus it is possible to operate the MCT at switching frequencies as high as 100 kHz. A high switching frequency is desirable in switching regulators and choppers since it reduces the size of the filter components. The conduction drop of the MCT is small compared to that of a thyristor (3).

The performance of the MCT is studied by operating it as a phase controlled rectifier. The fact that the MCT can be turned on and off using gate pulses makes it possible to operate the rectifier with a leading or unity power factor, which are difficult to obtain with a conventional thyristor. The rectifier can supply an R-L load or a motor load. It is also possible to operate the circuit as an ac voltage controller with very little modification in the control circuit. In both the controlled rectifier and the ac voltage controller, it is possible to vary the output voltage over a wide range. The waveforms of voltage and current obtained on the experimental converter are presented.

The MCT has a number of advantages compared to other power devices. It is the power semiconductor device of the future. It can be used in a variety of applications. The experimental characteristics and wavefoms demonstrate the efficient and smooth operation of the MCT converter.

- 1. Temple, V.A.K. (1986) IEEE Trans. Electron Devices, 33, pp 1609-1618.
- 2. Jahns, T.M. (1991) IEEE Trans. Industry Applications, 27, pp 589-597.
- 3. <u>User's Guide to MCTs.</u> (1989) Harris Semiconductor Corporation.

### A Symposium on

TRENDS in NORTH DAKOTA'S SOCIAL, DEMOGRAPHIC, and ECONOMIC DEVELOPMENT During the 1980s and their Implications for the 1990s

North Dakota / Minnesota Academies of Science 1994 Joint Annual Meeting

Symposium Coordinator

Mohammad Hemmasi Geography, University of North Dakota, Grand Forks, 58202

Thursday, 28 April

- 2:00 The Collapse of Main Street in Rural America.

  Lowell Goodman\*

  Geography, University of North Dakota, Grand Forks. 58202
- 2:20 Bankruptcy and other Indicators of Community Stress in North Dakota 1985 1993.

  Paul Meartz\*

  Mayville State University, Mayville 5827 1299
- 2:40 Gender and Development: Evidence from North Dakota Data.

  Devon Hansen
  Metropolitan Planning Organization, Grand Forks, 58206
- 3:00 Informal Discussion and Refreshments.
- 3:20 Population Trends in North Dakota.

  Richard W Rathge
  North Dakota State Census Data Center, Fargo, 58105
- 4:00 Discussion and Conclusion.

### A SYMPOSIUM ON DEVELOPMENT

TRENDS IN NORTH DAKOTA'S SOCIAL, DEMOGRAPHIC, AND ECONOMIC DEVELOPMENT DURING THE 1980s AND THEIR IMPLICATIONS FOR THE 1990s

Symposium Organizer: Mohammad Hemmasi Geography Department, University of North Dakota, Grand Forks, ND 58202

The 1980s is considered the "Lost Decade" of development because the gap between rich and poor populations and regions widened everywhere. During the decade, North Dakota declined in real per capita income (-8.2%), adjusted housing values (-27.0%), and population size (-2.1%). Out-migration exceeded in-migration by over fifty thousand between 1985 to 1990. Lack of high paying employment opportunities is the main reason for the departure of most young and educated North Dakotans. Politicians and planners are concerned about these trends and search for policies to reverse them.

Currently, the policy makers debate about the future direction of socioeconomic development in the state. Which sector of the economy can provide more decent jobs and how? Who are the potential outside investors and what effect will industrial investment have on the environment? Could better utilization of local resources be a viable alternative source? Since agricultural yields are already high and production volume is impressive, farm related value adding activities seem an attractive alternative. activities include farm product processing, livestock raising, and energy generation.

The participants in the symposium examine the obstacles to development during the 1980s and discuss their implications for development planning in the 1990s. Experts of different backgrounds and varied perspectives exchange ideas and ponder about a common objective: how to achieve a better quality of life for all the state's residents.

Goodman's paper traces "The Collapse of Main Street in Rural America". He mentions a host of factors, such as lower labor input in the farming sector (due to mechanization of agriculture), introduction of Conservation Reserve Program (CRP), and the universal trend in urbanization as causes of decline in the population of "central places" and their rural trade areas. analyzes weekly data on "Bankruptcy and Community Stress" which plagued the local economy during the 1980s. He depicted the geographic patterns of bankruptcy rates and socioeconomic indicators on maps to show their interrelationships.

Hansen's paper is concerned with women's role in development during recent She documents women's achievement in narrowing the gender gap in higher educational attainment and labor force participation. She also reports on the persisting disparities in income between men and women which still remain to be eliminated. Rathge investigates the dynamics of demographic change in the state and its effects on development efforts. He identifies migration and aging as the two leading causes of demographic change in North Dakota.

focuses on Quality of Life (QOL) as the ultimate goal "development". Principal components of quality of life showed some notable changes during the 1980s. Although the state's major cities prospered, the rural areas, especially the Native American settlements, suffered the most. Future development projects should seriously consider the challenge of closing the inequality gap between ethnic groups, gender, and regions of the state.  $\bar{\mathbf{I}}$ hope this symposium not only helps us to understand the state's problems of development, but also identify practical solutions to them.

### THE COLLAPSE OF MAIN STREET IN RURAL AMERICA

By Lowell Goodman
Department of Geography, University of North Dakota, Grand Forks, ND 58202

The 1980's represent the visible decline of rural communities throughout the central regions of the United States and Canada. Prior to the 80s and beginning around 1960, rural communities underwent an invisible decline. This invisible decline was subtle and unnoticed for the most part.

### The Invisible Decline

The invisible decline began in 1936 in ND and elsewhere in the Great Plains. The time will change slightly in other geographic areas but the process is the same. 1936 represents a time of economic depression both here and abroad, as well as political upheaval and instability globally. Further, it was clear that farms of one or two quarters in size, were insufficient for survival let alone success. Therefore the stability and organization or rural areas began to change. Between 1936 and 1946 large scale population migration and movement took place. The war and war related employment made major changes in rural economic patterns and created a dispersement of families and community members not seen before.

Following WWII, 1945, the depression was history, crops were good and the economy was strong. Rural communities settled back and enjoyed the good life. The war had curtailed spending on both durable and nondurable goods and the pent up consumer demand broke loose creating a tremendous growth in retail expenditures even in small towns. This growth created the infamous saturday enights in rural main streets across the country. This economic surge tied to a sense of community ended the major migration flows out of state and once again focused on the community and its trade area. Farms were still getting larger and there were more and more sidewalk farmers. These excess farmers and sidewalk farmers moved into the local trade center and built or bought homes. Jobs were available because each community had at least one implement dealer and at least one auto dealership with a garage. These were major employers with a good implement dealer hiring ten to twelve employees and an auto dealership hiring from 4 to 8 employees. In fact many small communities of 500 to 700 had as many as four implement dealers and as many auto dealers.

With the pent up consumer demand and a settling of the population, most rural areas were not aware of what was really happening. In reality, the over all trade area was losing population. The trade center was modestly growing at the expense of the trade area, consumer demand was high, and agricultural equipment sales were very high because there had been a constant lack of equipment until following the war. Finally, nearly everyone needed a new car or two.

By the mid sixties both the trade area and trade centers were loosing population. Farm implement dealers were going out of business, auto dealers were closing there doors and businesses on main street were closing. Each of these closings ended the employment of locals who as you might expect, moved away reducing further the trade area population which again negatively impacted main street.

### The Visible Decline

This negative spiral continued into the 80s when the rural communities were finally hit with the ultimate stunning blow of CRP (Conservation Reserve Program). This program paid farmers not to farm. The Great Plains bought into this program in a major way. As farmers stopped farming there was a reduced demand for agricultural equipment, seed, chemicals, and cars and trucks. This was the visible decline of rural main street. The invisible would have accomplished the same thing, it simply would have taken longer.

To counter this demise is, on the whole not possible or rational. However selected locations can be turned around through industrial development. Most rural communities attempt to rebuild by assisting store start ups on main street, like it used to be. But that takes people and there aren't any. Rural communities must work toward job creation in the manufacturing sector to create pay checks that can be spent on main street.

Community, multi community, or regional development activity is the only way to survival. People represent the base commodity needed and industrial jobs are the principle way to attract people.

### BANKRUPTCY AND OTHER INDICATORS OF COMMUNITY STRESS IN NORTH DAKOTA 1985-1993

Paul D. Meartz Mayville State University, Mayville, ND 58257-1299

While bankruptcy rates declined nationally and in North Dakota during 1993, they have increased over most years since 1979 (1). Rising numbers of bankruptcies signal the existence of economic and personal crisises for those filing and impacted by such filings. Filing rates have been found to be significantly related to other ecological variables that measure stress within the social and economic fabric of society (2).

Average bankruptcy rates per county per year over the 1985-1993 period were determined using weekly filing information of record in the <u>Fargo Forum</u>. Lacking survey data that would identify the level at which rates would be held by local persons in any county to be significant, counties having bankruptcy rates one standard

Figure 1. Average yearly bankruptcy rates per 100 population (1985-1993)

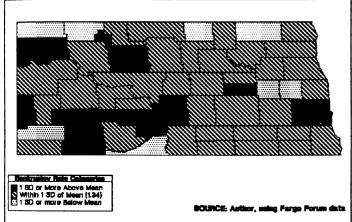
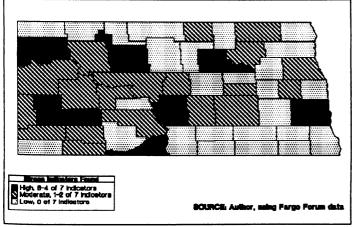


Figure 2. Count of stress indicators found at one standard deviation or more above the mean



deviation or more above the mean of the county rates were labeled as possessing "high" rates. Analysis of the distribution pattern of these rates shows a tendency for high rates in select western and central counties, and in the eastern counties of Cass and Foster. (see Figure 1).

A larger level of community stress in the western and central counties, and in a larger selection of the eastern counties, can be illustrated by expanding the indicators of stress to non-bankruptcy measures. Using seven variables that measure six dimensions (economic souring, general economic conditions, lack of opportunities, family troubles, youth troubles, and social violence), the same one standard deviation above the mean benchmark was employed to identify counties with high rates of these problems (3). The western and central areas of North Dakota have numerous counties recording one or more of the variables at a high level (Figure 2).

- Associated Press. (1994) Fargo Forum Jan 8, B5; Bodovitz, Kathy. (1991) American Demographics July: 48.
- Meartz, P. (1989) <u>Bulletin ANDG</u> 38, 113-126.
- 3. Data on crime rates, percentage below the poverty level, income change, and out migration from North Dakota Census Data Center (1992, 1991)

  Population Bulletin; divorce and teenage pregnancy rates from Mayer, D. (1993) ND Resident Vital Event Summary Data 1978-1992. ND State Department of Health and Consolidated Laboratories.

#### GENDER AND DEVELOPMENT: EVIDENCE FROM NORTH DAKOTA DATA

#### Devon Hansen Grand Forks/East Grand Forks Metropolitan Planning Organization Grand Forks, North Dakota 58206

Human development is defined in the United Nation's 1990 Human Development Report as "a process of enlarging people's choices" (1). Indicators of the well-being of individuals and of a society may be measured in terms of higher levels of education and income, access to better housing and health care, and greater social and political participation. A fundamental purpose of development is to improve the quality of life for an individual. Yet, the concept of human development remains largely unfulfilled unless all members of a society benefit. An examination of North Dakota data reveals that disparities still exist between women and men, especially with regard to income and poverty status. The purpose of this study is to investigate the spatial and temporal variations relating to gender and development among North Dakota counties over the last three decades.

For this study, three variables--labor force participation, educational attainment, and median income -- were selected to evaluate how women have fared in development within the state. Greater female labor force participation should be related to higher levels of education and income, which are often used as measures of social and economic development. Descriptive statistics, including mean, standard deviation, and the coefficient of variation, are calculated to summarize the spatial and temporal changes in these variables (Table 1). The coefficient of variation, a relative index of dispersion, indicates change over time in spatial patterns.

At the state level, female labor force participation increased from 35.4 percent to 57.3 percent between 1970 and 1990. Likewise, the number of females earning a bachelor degree or higher rose from 6.6 percent in 1970 to 16.7 percent in 1990. Labor force participation and higher levels of educational achievement of women are becoming increasingly widespread among the counties, as indicated by the decrease in the coefficient of variation over the decades (Table 1).

The gender gap with regard to income is still evident across North Dakota. In 1990, the median income for women was approximately 46 percent of men's at the state level. This can be partly attributed to women holding a majority of the part-time jobs. Furthermore, more women are employed in the service and retail related occupations, which are generally the lowest paying sectors. The level of income is not evenly distributed throughout the counties, as shown by an increase in the coefficient of variation over the last two decades. These income level differences within the state may be explained, in part, by accessibility to urban job opportunities. It should be noted, however, that the median income for women was about 65 percent of men's at the state level, when only full-time workers were considered.

Findings of this study indicate that North Dakota women improved their status over the last three decades, evident by their increasing numbers in the labor force and by their educational achievements. However, greater female labor force participation does not necessarily imply higher incomes, largely because of women's traditional occupational roles. Because of family responsibilities, many women lack continuous employment records. Many women work only part-time, and therefore, do not receive the benefits generally offered by full-time employment. Furthermore, women are often paid less than men working identical jobs. One implication is that attitudes toward women's traditional roles in the workplace and in the household need to be changed to

facilitate social policy improvements (i.e., work-family policies).

Women's integration in development efforts will require more active roles for them in all aspects of the economic, social, and political life of the state. One encouraging note is that women in North Dakota are advancing their education. This should serve to raise their employment opportunities and potential earning power.

Table 1. Var	iations in	Gender-related	Development	Variables,	1970-1990
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<u>Female</u>					Male				
		standard	coefficient		standard	coefficient			
	mean	deviation	of variation	<u>mean</u>	deviation	of variation			
Percent	of lab	or force par	ticipation (16	years and	over):				
1970	30.0	6.4	21.5	71.5	6.1	8.6			
1980	40.2	7.5	18.7	72.5	6.7	9.3			
1990	49.6	6.5	13.2	69.5	7.0	10.0			
Percent	with a	bachelor de	egree or higher	(25 years	and over):				
1970	4.7	2.3	48.2	6.9	3.6	52.6			
1980	9.8	2.8	28.9	12.1	4.8	39.4			
1990	12.7	3.4	27.1	13.5	4.9	36.1			
Median	income	in dollars (	15 years and o	ver)*:					
1980	3,655		15.9	10,328	1,915	18.5			
1990	6,505	1,228	18.9	15,681	2,743	17.5			
* * *		3-4	ound for modian	1	1000				

No comparable data was found for median income in 1970.

<sup>1. 1990</sup> Human Development Report. (1990) New York: United Nations Development Programme.

### POPULATION TRENDS IN NORTH DAKOTA

Richard W. Rathge
North Dakota State Census Data Center
North Dakota State University, Fargo, North Dakota 58105

The population shifts in North Dakota during the past decade center around four major trends. The first is a continuation of movement of people from rural areas to urban centers. Rural is defined as a place of less than 2,500 people. At the turn of the century, more than 90 percent of the state's residents lived in rural areas. Today, North Dakota is an urban state because the majority of people live in the state's 17 urban centers. The consolidation of North Dakota's population into its larger cities has accelerated to the point that Fargo, Grand Forks, Bismarck, and Minot now hold over two-thirds of the state's urban population. In contrast, the vast majority of communities in North Dakota have very few residents. In fact, half of the state's 366 incorporated places have fewer than 200 residents.

A second major trend in the state has been one of selective migration. A disproportionate number of residents who move tend to be young adults and those in their early career stages. Between 1980 and 1990, the outmigration of those in their twenties exceeded 50 percent in more than half of the state's 53 counties. As a result of this selective pool of migrants, the birth rate in the state has fallen to its lowest rate since 1920. In fact, 35 counties now have fewer births than deaths, thus creating a situation of natural decline.

Growth in the proportion of elderly is a third trend which characterizes population shifts in North Dakota. In 1990, 14.3 percent of the state's residents were above the age of 64. This rate is well above the national average of 12.6 percent and two percentage points higher then the previous decade. Among the state's elderly, nearly one in three live alone.

A final demographic trend which is noteworthy is the increase in women's labor force participation. The downturn in the state's economy paralleled an unprecedented increase in employed women. The proportion of women in North Dakota's labor force with children under the age of 6 increased from 47.3 percent in 1980 to 69.1 percent in 1990. This is 10 percentage points higher than the national average. Similarly, 79.4 percent of women with children between the ages of 6 and 17 were in the labor force in 1990 compared with 59.1 percent in 1980.

These trends in the state's population continue to create important challenges for planners and policy makers. The viability of small communities intensifies as the economies of larger cities continues to pull residents and employment. The delivery of services becomes increasingly more difficult and expensive as the state becomes more sparsely populated. Many of the social institutions (e.g., education, religion, government, health care) in the state are jeopardized by shifting residential movement. The challenge for policy makers is to interpret these trends and plan North Dakota's future.

### A MULTIVARIATE ANALYSIS OF QUALITY OF LIFE IN NORTH DAKOTA

Mohammad Hemmasi Department of Geography, University of North Dakota, Grand Forks, ND 58202

The 1980s was considered the 'Lost Decade' of development because almost everywhere the disparities between poor and rich population groups and regions increased significantly. During the decade, North Dakota recorded a notable decline in its real per capita income (-8.2%). The state continued losing population (-2.1%), and added over eight thousand to the people who lived below the poverty line (14.4%). Despite these general losses, a few cities prospered economically and grew in population size. This study examines the spatial variations in the quality of life (QOL) among North Dakota counties for 1980 and 1990. Quality of life research helps to monitor public policy and locational decisions. The choice of variables was conditioned by two main considerations: appropriateness of the variables for a Midwestern state, and availability of data. Nineteen comparable variables were selected to measure income, employment, health, education, and social and family issues.

health, education, and social and family issues.

An overall composite index of quality of life was calculated using all the variables. The "best" score in each variable is made equal to 100, the "worst" to 0, and intermediate values are determined by the following formula:

 $S = ((R - R \text{ worst}) / (R \text{ best } - R \text{ worst}))*100; \text{ Overall QOL Index } = \Sigma S / N$ 

If a county has the best performance on every variable, it should have a score of 100 and the one with the worst record on all the variables 0. In 1990, Cass County had the highest QOL index (84) and Sioux the lowest QOL score (9.8). During the 1980s, 22 counties (42%) declined in their quality of life indexes. Counties with the three largest cities maintained their highest rank in the system, but the counties where the Native Americans reside became the greatest losers.

For each period, a principal components analysis was also performed on the same nineteen variables. The principal components analysis transforms the original set of variables into a smaller set of linear combinations that account for most of the variance of the original set. The principal components or factors are extracted so that the first principal component accounts for the largest amount of the total variation in the data. The analyses produced three components: Affluence, human Suffering, and Demographic. However, there are notable differences between the two periods in the order and amount of variance accounted for by each factor. For the 1980 data, the order of emerging dimensions and their respective power of representing the original variables were: Affluence (35%), Suffering (34%), and Fertility (6%), accounting for 75% of the total variance. In contrast, the 1990 data matrix generated Suffering (41%), Affluence (34%), and Mortality (7%), capturing 82% of the total variance. Thus, by the end of 1980s, human Suffering was the most significant dimension of the state's QOL and Affluence a distant second. Furthermore, mortality and old age replaced fertility and youthful population as the third factor.

Results of a quintal optimum classification of counties based on scores of the overall QOL, Affluence, and Suffering reveals the following spatial patterns. First, most counties along the main highways enjoy "very high" or "high" ratings. Second, counties which are in the "shadow" of a larger city or off the main highways often show "low" ratings. Third, on the Affluence factor, Benson, Rolette, and Sioux Counties always rank "very low", while Cass, Burleigh, and Grand Forks Counties invariably rank "very high". Plight of the state's minority residents during the "Lost Decade" of development is also reflected in the strong positive correlation coefficients between percent non-white and the human Suffering factor scores, as well as negative coefficients with the QOL indexes (Table 1). Fourth, the reported east-west regional differences in political preference hardly emerge in the quality of life patterns. Significant correlations exist between QOL scores and county in-migration rates (r=0.628 and 0.772), as well as population change during 1980 to 1990.

Public policy makers in North Dakota should recognize these persistent spatial disparities in the quality of life and seriously consider them in their regional development policies. This study identifies the locations where development efforts are needed most.

Table 1. Correlation between QOL Indicators and % Non-White (NW), In-Migration Rate (MIG), and % Population Change (PC) in 1980-90

		1980		1990			
	NW	MIG	PC	NW	MIG	PC	
Overall QOL	-0.594*	0.652*	0.507*	-0.765	0.507*	0.206	
Affluence	-0.138	0.628*	0.591*	-0.149	0.772*	0.705*	
Suffering	0.907*	-0.145	0.044	0.952*	0.058	0.432*	

<sup>\*</sup> Statistically significant at  $p \leq 0.001$ .

STATISTICAL METHODS APPLIED in a VARIETY of SCIENTIFIC DISCIPLINES

North Dakota / Minnesota Academies of Science 1994 Joint Annual Meeting

Symposium Coordinators

Madhusudan Bhandary and Nuwan Nanayakkara

Statistics, North Dakota State University, Fargo 58105

Thursday, 28 April

8:30 Using Item Response Theory (IRT) Models to Identify Unusual Response Patterns on the Strong Interest Inventory.

George A Henly\*

Counseling, University of North Dakota, Grand Forks, 58202

9:00 Diagonal Copulas and Dependence Plots.

Engin A Sungur\* and Matthew Diersen

Science and Mathematics, University of Minnesota, Morris, 56267

9:30 Analyzing Mixture Distributions to Evaluate the Prevalence of Hereditary Iron Overload in the United States.

Christine E McLaren\*

Mathematics, Moorhead State University, Moorhead 56560 Victor R Gordeuk

Hematology and Oncology, The George Washington University Medical Center, Washington, D C 20037 Anne C Looker

Health Examination Statistics, National Center for Health Statistics, Hyattsville, MD 20782

10:00 Informal Discussion and Refreshments

10:30 ANOVA Power Estimation Formulae.

David R McCormack\*

Mathematics and Computer Science, Minot State University, Minot 58707 Dale G Shaw

Education, Research and Development, University of Northern Colorado, Greeley, CO,  $\,\,$ 80639

11:00 A Comparison of Two Estimators of InterClass Correlation using Monte Carlo Simulation.

Kirk Scott\*

CIS, The College of Saint Scholastica, Diluth, 55811 Nuwan Nanayakkara and M B Rao

Statistics, North Dakota State University, Fargo, 58105

11:30 A Comparison of Tests for the K-sample, Nondecreasing Alternative.

Joanne M Mahrer and Rhonda C Magel\*

Statistics, North Dakota State University, Fargo, 58105

### USING ITEM RESPONSE THEORY (IRT) MODELS TO IDENTIFY UNUSUAL RESPONSE PATTERNS ON THE STRONG INTEREST INVENTORY

George A. Henly Department of Counseling, University of North Dakota, Grand Forks, ND 58202

Several families of logistic IRT models have been proposed to describe the responses of individuals to psychological tests. Briefly, these models provide a probabilistic account of the interaction of an individual with items on a unidimensional measure as a function of a person parameter (latent trait score  $\theta$ ) and one or more parameters for each item (e.g., location, discrimination). Although initial models were developed for dichotomous ability items, more elaborate models for polytomous items, such as those on attitude measures, have been formulated. One advantage of use of these models is that when a suitable model has been identified for a measure, then it is possible to identify individuals whose responses are anomalous under the model. These may be persons whose interactions with the test are very different in nature from those of typical respondents; consequently, customary interpretations of their test results would appear unwise.

The Strong Interest Inventory (SII) is one of the oldest and most successful psychological tests. The 325 items of the SII were selected empirically, based on their discrimination of individuals in different occupations. The majority of the items require the respondent to indicate their degree of liking ("Like", "Indifferent," "Dislike") for various occupations, school subjects, leisure activities, and types of persons. This research investigated the applicability of IRT models to responses to the SII, and their use in identifying unusual response patterns.

SII item responses from 6567 males and 9917 females, ages 18-22, were captured from the stream of answer sheets sent to the test publisher's scoring center. In order to identify a limited number of strongly unidimensional item sets, male and female responses to SII items were subjected separately to multiple rounds of exploratory factor analysis. For males, three item sets were identified, ranging from 14 to 21 items in length. For females, four item sets were established, ranging from 13 to 16 items in size.

Bock's (1) Nominal model, the most flexible logistic model for polytomous responses, was chosen to describe item responses within each set. Under the nominal model, the probability of selecting response k to an item with m options may be expressed as

$$P(x-k) = \frac{\exp\left[a_k\theta + c_k\right]}{\sum_{i=1}^{n} \exp\left[a_i\theta + c_i\right]}$$
[1]

where a and c are parameters associated with each response category, and  $\theta$  is the individual's trait level. Item parameters were estimated using the program MULTILOG (2). Maximum likelihood estimates of theta were obtained for each respondent on each scale. Two indices of appropriateness (model fit) were computed for each individual's responses on each item set.

Results indicated modest consistency in the identification of unusual respondents across item sets, and some convergence of IRT appropriateness indices with traditional infrequency scales on the SII. Several cases are presented for detailed inspection. Remaining questions about model fit, definitions of appropriateness, and interpretation of response anomaly are addressed.

Bock, R.D. (1972) Estimating item parameters and latent ability when responses are scored in two or more nominal categories. *Psychometrika*, 48, 129-141.

<sup>2.</sup> Thissen, D. (1991) MULTILOG 6.0. Chicago, IL: Scientific Software.

#### DIAGONAL COPULAS AND DEPENDENCE PLOTS

Engin A. Sungur and Matthew Diersen Division of Science and Mathematics, University of Minnesota, Morris, MN 56267

The graphical representation of dependence between the random variables is a challenging and difficult task. Especially in large data set applications, ordinary scatter plots may be heavily overplotted yielding uninterpretable displays. In this paper, we suggest a type of dependence plot which is easy to interpret and uncovers some of the interesting features of the dependence relations in the data. In order to explain the construction of such dependence plots, we introduce the notion of diagonal copulas and develop their basic properties. The results are generalized into higher dimensions by considering truncation invariant class of copulas. Also, possible applications of diagonal copulas to nonparametric and parametric estimation, and test of hypotheses of perfect dependence are briefly discussed.

In this paper we use the copula representation of multivariate distribution function (see. Schweizer (1)). Copulas link multivariate distributions to their one dimensional marginals. By eliminating the effect of the marginals, they provide a clear picture of the dependence structure. Thus, if F is an n-dimensional distribution function (d.f.) with one-dimensional margins  $F_1, \ldots, F_n$ then there exists an n-dimensional copula C (which is unique when  $F_1, \ldots, F_n$  are continuous) s.t.  $F(x_1,...,x_n)=C(F_1(x_1),...,F_n(x_n))$ . The diagonal copula is defined by D(u)=C(u,...,u). and it corresponds to the d.f. of the  $\max\{F_1(X_1), \dots F_n(X_n)\}$ . Some properties of the diagonal copulas are given in the paper. Although the diagonal copula reveals some of the interesting features of the dependence structure, it has a restriction. It explains only the dependence structure at  $F_1(x_1) = \cdots = F_n(x_n)$ . On the other hand, within the Archimedean class of copulas (see, Frank (2)) diagonal copula uniquely determines the copula, thus it provides all the information hidden in the copula. To simplify our notation we will concentrate on the bivariate copulas and represent them as C(u, v).

Archimedean class of copulas satisfy the following associativity equation

$$C(C(u, v), w) = C(u, C(v, w))$$
 for all  $u, v, w \in [0, 1]$ ,

and can be represented as C(u,v) = f(g(u) + g(v)), where g is a strictly decreasing convex function with g(1) = 0, and f is the pseudo-inverse of g. The main results are:

- (i) For any copula D(u) = u if and only if  $C(u, v) = \min\{u, v\}$  (perfect positive dependence).
- (ii) For the Archimedean class  $g(u) = \lim_{n \to \infty} 2^n (1 D^{-n}u)$  where  $D^{-n} = D^{-1} \circ \cdots \circ D^{-1}$ . Therefore, any Archimedean copula can be uniquely determined by its diagonal copula.

Implications of these results are the following. For any copula the hypothesis that X and Y are perfectly positive dependent is equivalent to the hypothesis that  $\max\{F_1(X), F_2(Y)\}$  has a uniform distribution on [0,1]. For the Archimedean class goodness-of-fir test (including test of independence) can be easily carried out by using the corresponding one-dimensional diagonal copula and the empirical d.f. of  $\max\{F_1(X), F_2(Y)\}$ .

Theoretical diagonal dependence plots can be constructed by plotting diagonal copula or 
$$\delta(u;D) = \begin{cases} [D(u) - D^0(u)] / [D^+(u) - D^0(u)] & \text{if } D(u) \geq u^2, \\ [D(u) - D^0(u)] / [D^0(u) - D^-(u)] & \text{if } D(u) < u^2. \end{cases}$$

diagonal copula to the independence  $(D^-, D^0, D^+)$  are the diagonal copulas corresponding to the perfect negative dependence, independence and perfect positive dependence, respectively). Theoretical diagonal dependence plots of various copulas are given in the paper.

For model selection and model checking purposes empirical diagonal dependence plots are constructed by using the empirical d.f. of the  $M = \max\{F_1(X), F_2(Y)\}$  and the estimate of the  $\delta$ . Since checking the structure of the dependence has been reduced to one-dimension by the diagonal copula, any one-dimensional graphical tool, such as histograms, quantile plots, quantile-theoretical quantile plots, boxplots etc., can be used to understand the dependence structure. Also, plots for quadrant dependence, left (right) tail dependence can be easily constructed. Various examples of such plots by using real-world-data sets are provided in the paper.

<sup>1.</sup> Schweizer, B. (1991) in Advances in Probability Distributions with Given Marginals. (Dall'Aglio, G., Kotz, S., and Salinetti, G., eds) pp 13-50. Kluwer, Dordrecht.

<sup>2.</sup> Frank, M. (1975) Aequationes Mathematicae 12: pp 121-144

### ANALYZING MIXTURE DISTRIBUTIONS TO EVALUATE THE PREVALENCE OF HEREDITARY IRON OVERLOAD IN THE U.S.

Christine E. McLaren\*
Department of Mathematics, Moorhead State University, Moorhead, MN 58103

Victor R. Gordeuk

Division of Hematology and Oncology, The George Washington Univ. Medical Center, Washington, D.C. 20037 and

Anne C. Looker

Division of Health Examination Statistics, National Center for Health Statistics, Hyattsville, MD 20782

Individuals with an inherited condition known as hereditary hemochromatosis absorb excessive amounts of iron from a diet of normal iron content. Once considered to be a rare disorder, hereditary hemochromatosis is now recognized to be one of the most common autosomal recessive disorders in white populations but estimates for the frequency of the condition vary greatly. Surveys to determine the gene frequency of hereditary hemochromatosis have been based predominantly on the principle of identifying homozygotes in the population. A recent large study of predominantly white blood donors in Utah estimated that the prevalence of homozygotes was 4.5 per 1000, corresponding to the hemochromatosis gene frequency of 0.067 and to a proportion of 125 per 1000 for heterozygotes (1). If the results from Utah are applicable nationwide, more than one million Americans are hemochromatosis homozygotes who are at risk for major iron overload and more than 25 million are heterozygotes. Such a common condition might have important implications for disease screening and for policies regarding the fortification of food with iron.

Since the discovery that the gene for hemochromatosis is linked to the HLA locus, it has been possible through HLA typing of affected families to assign the homozygous affected, heterozygous, or homozygous normal genotype to family members. Transferrin saturation is regarded as the best single screening test for hemochromatosis and family studies indicate that herozygotes have a higher mean transferrin saturation than unaffected individuals. We postulated that the distribution of transferrin saturation in the United States reflects several populations based on individual genotype for hemochromatosis, and that maximum-likelihood methods could be applied to separate finite mixtures of distributions and quantify these groups. To determine if the second National Health and Nutrition Examination Survey reflects the presence of hemochromatosis heterozygotes, we examined the distribution of transferrin saturation values for 1325 white males and 1547 females. After truncating to remove values for possible homozygotes, we used maximum-likelihood methods to fit finite mixtures to the distribution of transferrin saturations for each gender (2, 3).

Since we had found that transferrin saturation is normally distributed in normal homozygotes, the physiologic models we considered were a single normal distribution and a mixture of two normal distributions. The expectation-maximization algorithm was applied to the distributions of transferrin saturation values for parameter estimation. The statistical test used to determine the best fitting model was based upon the likelihood ratio statistic. For each observed distribution, the maximized log-likelihood function for a mixture of normal distributions was evaluated (Log  $L_1$ ) and compared with the maximized log-likelihood function for a single normal distribution (Log  $L_0$ ). Significance of the likelihood ratio statistic, -2Log( $L_0/L_1$ ), was assessed using a resampling technique. The chi-square statistic was then used to test goodness of fit of each observed distribution to the best fitting model.

Two populations based on transferrin saturation were present (p < 0.01 for each gender) and the fit to a mixture of two normal distributions was good (p=0.813 for males; p=.177 for females). When weighted to reflect the United States adult white males as a whole, an estimated proportion of 850 per thousand men (95% confidence interval of 0.81, 0.89) were included in a population with a lower mean saturation of 29.7% (95% confidence interval of 29.1%, 30.3%), while 150 per thousand (0.11, 0.19) comprised a population with a higher mean saturation of 47.0% (45.1%, 49.0%). Similar results were obtained for females. Among United States whites, two populations are present based on transferrin saturation. The population with the higher mean saturation may include predominantly heterozygotes for hemochromatosis and the population with the lower mean saturation mainly unaffected individuals. Our results confirm that the gene for hemochromatosis is common.

<sup>1.</sup> Edwards C.Q., Griffen, L.M., Goldgar D., et al. (1988) Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. N Engl J Med 318, 1355-62.

<sup>2.</sup> McLaren CE, Wagstaff M, Brittenham GM, Jacobs, A. (1991) Detection of two component mixtures of lognormal distributions in grouped doubly-truncated data. <u>Biometrics</u> 47(2), 607-622.

<sup>3.</sup> Dempster AP, Laird NM, Rubin DB. (1977) Maximum likelihood from incomplete data via the EM algorithm. J R Stat Soc. Series B 38, 1-22.

### ANOVA POWER ESTIMATION FORMULAE

David R. McCormack\*
Department of Math and Computer Science, Minot State University, Minot, ND 58707

### Dale G. Shaw

Department of Education, Research, and Development, University of Northern Colorado, Greeley, CO 80639

Multiple regression may be used to examine the relationship between a single dependent variable and a set of several independent variables. McCormack (1) and Shaw treated Cohen's (2) 66 page ANOVA power tables as such a data set to produce formulae which estimate ANOVA power. The following formulae allow estimation of power of an ANOVA design without need for Cohen's table book.

(1) 
$$p = -.034\eta^{3} + (.240 - .720\sqrt{\alpha})\eta^{2} + (2.178\sqrt{\alpha} + .043u)\eta - (.192f + .268)$$
 where:  
(2)  $p = .058 + .149 \ln(\alpha) + (.355 + .045u)\eta + .197 \ln(n)\sqrt{f}$ . where:  
(3)  $p = \frac{1}{1 + 2.81\alpha^{-.72}u^{(.31 - .27\eta)\eta}e^{[.91f - (2.31 + .17u)\eta]}}$ . where:  
 $\alpha = \text{significance level}$   $u = k-1 \text{ for k group ANOVA}$   $n = \text{per group sample size}$   $f = \text{Cohen's effect size}$ ,  $(\text{small} = .10, \text{ medium} = .25, \text{ large} = .40)$   $n = f\sqrt{n}$ 

### Linear Formulae

The first two formulae were developed by regressing the power on linear combinations of predictors which were products of the basic four predictors. Because a linear model cannot accurately fit the asymptotic tails of a power curve, the data set was reduced to include only those points with power in the interval [.25, .90]. This was justified by noting that precise estimates may not be needed for extreme power values. Formulae 1 and 2 will sometimes give negative power estimates because the formulae were developed with the restricted data set. Such estimates should simply be interpreted as very low power, a design lacking credibility. Similarly, when the formulae yield power estimates greater than 1.00, this result should be interpreted by recognizing the design is of high power.

### Non-Linear Formulae

Because power data is not linear, many non-linear models were also attempted. The logistic model, based on a sigmoidal curve with the equation  $P = 1/(1 + e^{p'})$  provided the best results. Although P is sigmoidal,  $p' = \ln((1 - P)/P)$  is linear. Using regression, coefficients for p' were obtained and the inverse transformation produced Formula 3. This formula is especially accurate in the low and high power regions without sacrificing accuracy in the mid regions.

### Accuracy of the Estimates

Perhaps the greatest concern of potential formula users is accuracy. The table below shows the residuals are tightly contained. They have low standard deviation, but a few extreme residuals exist. (Actual power = predicted power + residual.) Assuming normality of the residuals, most errors under Formula 3 are less than  $\pm 2.5\%$ . The extreme estimates are infrequent, and are probably tolerable to those lacking Cohen's tables.

u	n	Residuals of Linear Formula 1, p in [.25, .90]			Residuals of Linear Formula 2, p in [.25, .90]			Residuals of Formula 3, p in [.01, .99]		
Values	Values	St Dev	Min	Max	St Dev	Min	Max	St Dev	Min	Max
[1, 8]	n ≥ 5	.0313	1466	.0617	.0338	1221	.1080	.0126	0984	.0320
[1, 8]	n ≥ 10	.0306	1414	.0608	.0335	1221	.1080	.0115	0516	.0295
[1, 4]	n ≥ 5	.0242	0979	.0617	.0247	0876	.1080	.0125	0984	.0320
[1, 4]	n ≥ 10	.0235	0897	.0608	.0243	0876	.1080	.0113	0516	.0295

<sup>1.</sup> McCormack, D. R. (1993). Formulae for estimating the power of one-factor ANOVA designs. (Doctoral dissertation, University of Northern Colorado, Greeley).

Cohen, J. (1988). <u>Statistical power analysis for the behavioral sciences</u>, 2nd edition, pp 289-354. Hilldale, NJ: Lawrence Erlbaum Associates.

## A COMPARISON OF TWO ESTIMATORS OF INTRACLASS CORRELATION USING MONTE CARLO SIMULATION

# Kirk Scott\* CIS Department, The College of St. Scholastica, Duluth, MN 55811 Nuwan Nanayakkara and M. B. Rao

Department of Statistics, North Dakota State University, Fargo, ND 58105

This research is concerned with the problem of testing whether or not there is a correlation between parents and offspring (interclass correlation) for a particular quantitative characteristic. A test statistic is derived for the hypothesis that the interclass correlations between mother and offspring and father and offspring are both zero. The calculation of the test statistic involves the correlation between the offspring (intraclass correlation). Since the maximum likelihood estimator of intraclass correlation is not easily computable, this research examines two estimators of the intraclass correlation which are more easily computable, one proposed by Srivastava (1) and one proposed by Fisher (2).

The research consisted of the following parts: A linear model approach was used to develop the hypothesis concerning interclass correlations and the test statistic for it. It was shown that if the intraclass correlation were known the test statistic would have an exact F-distribution with known parameters. A Monte Carlo simulation was done in which multivariate normal familial data were generated, the estimators of the intraclass correlation were calculated, and these were substituted into the formula for the test statistic. The Kolmogorov-Smirnov test was then used to determine whether the test statistic agreed with the theoretical F-distribution which the test statistic should have for known intraclass correlation.

The number of rejections obtained from the Kolmogorov-Smirnov test allows some conclusions to be drawn about the usefulness of the estimators over a range of possible intraclass correlation values. The ordinate of the graphs shown below represents the number of rejections out of 1,000 trials when using the Kolmogorov-Smirnov test to compare the observed distributions with the theoretical F-distributions for the 2 estimators, Srivastava's (S) and Fisher's (F). The abscissa gives the actual intraclass correlation used in generating the familial data. The different graphs correspond to different sample sizes of familial data.

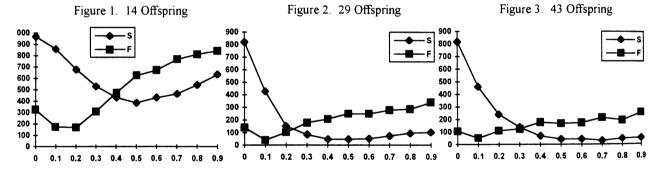
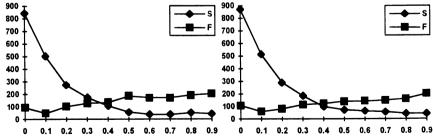


Figure 4. 57 Offspring

Figure 5. 72 Offspring



- 1. Srivastava, M. S. "Estimation of Interclass Correlations in Familial Data." Biometrika, Vol. 71, No. 1, 1984, pp. 177-185.
- 2. Snedecor, George W. and William B. Cochran. Statistical Methods. Ames, IA: Iowa State University Press, c. 1980.

# A COMPARISON OF TESTS FOR THE K-SAMPLE, NONDECREASING ALTERNATIVE Joanne M. Mahrer and Rhonda C. Magel Department of Statistics, North Dakota State University, Fargo, ND 58105

Department of Statistics, North Dakota State University, Fargo, ND 58105

A comparison between three nonparametric tests to test for a nondecreasing trend in the location parameters of several populations was conducted using Monte Carlo simulations. It was assumed that the underlying distributions were unknown. The hypotheses for this comparison were:  $H_0: \mu_1 = \mu_2 = ... = \mu_k$ ,  $H_1: \mu_1 \leq \mu_2 \leq ... \leq \mu_k$ , where  $\mu_i$  is used to designate a general location parameter for the ith population, not necessarily the mean.

Three nonparametric tests, found in the literature, designed to test the above set of hypotheses are, the Jonckheere-Tempstra test (1,2), the Cuzick (3) test, and the Chap T. Le (4) test. It should be noted that other tests, such as the Chacko test (5), exist that could also be used. These tests were selected for the comparison, because of their ease in calculation.

The power of each test for a variety of location parameter shifts was estimated by simulating 10,000 different sets of samples from populations having specified distributions. The distributions considered were the uniform, normal, exponential, and Cauchy. The power for each test, and given alternative was estimated for significant levels of 0.10, 0.05, and 0.01.

The underlying distribution did not appear to make a difference in the relative power comparisons of the tests. Sample sizes did not make a difference in the relative power comparisons.

The powers turned out to be so close among the three tests that it is recommended that the researcher use whichever test hershe is most comfortable with. The following table gives the uniform power results for five samples when the sample sizes are all either 10 or 30.

Table:	Uniform	Power	Results	For	5	Samples
--------	---------	-------	---------	-----	---	---------

$\mathbf{n} = 10$		<u> J-T</u>		2	uzick		_	Le	
location shifts	. 10	. 05	.01	. 10	.05	.01	. 10	. 05	. 01
0.0,0.0, 0.0,0.0,0.1	.2622,	.1590,	.0470	.2617,	. 1577,	.0386	.2688,	. 1582,	.0416
0.0,0.0,0.0,0.1,0.1	.3809,	.2427,	.0381	.3932,	.2616,	.0823	.3915.	. 2552.	.0796
0.0,0.0,0.1,0.1,0.1	.3908,	.2563,	.0865	.3838,	.2553,	.0835	.3911,	.2585,	.0777
0.0,0.1,0.1,0.1,0.1	.2613,	.1502,	.0432	.2619,	.1542,	.0428	.2694,	. 1556,	.0396
0.0,0.0,0.0,0.1,0.2	.6305,	.4718,	.2171	.6297,	.4793,	.2144	.6268,	.4771,	.2116
0.0,0.0, 0.1,0.2,0.3	.8991,	.8131,	.5667	.9021,	.8165,	.5585	.9001,	. 8168,	.5520
0.0,0.1,0.2,0.3,0.4	.9766,	.9386,	.7952	.9737,	.9326,	.7739	.9734,	.9415,	.7829
0.0,0.0,0.0,0.05,0.3	.7641,	.6319,	.3430	.7670,	.6335,	.3382	.7802,	.6469,	.3432
0.0,0.0,0.1,0.1,0.3	.8297,	.7135,	.4370	.8255,	.7133,	.4244	.8266,	.7095,	.4146
0.0,0.05,0.1,0.1,0.3	.7853,	.6559,	.3691	.7838,	.6586,	.3616	.7887,	.6596,	.3639

.4419,.3072,.1146	.4549,.3109,.1157	.4364,.3044,.1093
.6673,.5224,.2589	.6778,.5317,.2612	.666952052623
.6666,.5259,.2631	.6687,.5203,.2613	.6707,.5277,.2558
.4408,.2947,.1071	.4504,.3103,.1149	.4546,.3114,.1135
.5671,.4164,.1771	.5579,.4143,.1813	.5648,.4170,.1799
.8431,.7429,.4845	.8393,.7379,.4653	.8424,.7348,.4679
.9415,.8844,.6948	.9421,.8829,.6871	.9363,.8789,.6883
.8943,.8087,.5695	.8921,.8076,.5658	.8885,.8040,.5657
.6307,.4908,.2370	.6270,.4770,.2289	.6270, .4874, .2350
.5624,.4233,.1898	.5723,.4268,.1895	.5668,.4196,.1831
	.6673,.5224,.2589 .6666,.5259,.2631 .4408,.2947,.1071 .5671,.4164,.1771 .8431,.7429,.4845 .9415,.8844,.6948 .8943,.8087,.5695 .6307,.4908,.2370	.4419,.3072,.1146 .4549,.3109,.1157 .6673,.5224,.2589 .6778,.5317,.2612 .6666,.5259,.2631 .6687,.5203,.2613 .4408,.2947,.1071 .4504,.3103,.1149 .5671,.4164,.1771 .5579,.4143,.1813 .8431,.7429,.4845 .8393,.7379,.4653 .9415,.8844,.6948 .9421,.8829,.6871 .8943,.8087,.5695 .8921,.8076,.5658 .6307,.4908,.2370 .6270,.4770,.2289 .5624,.4233,.1898 .5723,.4268,.1895

<sup>1.</sup> Jonckheere, A. R. 'A Distribution-Free K-Sample Test Against Ordered Alternatives', Biometrika, 41, 133-145 (1954).

Terpstra, T. J. 'The Asymptotic Normality and Consistency of Kendall's Test Against Trend When Ties Are Present in One Ranking', Indag. Math., 14, 327-333 (1952).

<sup>3.</sup> Cuzick, Jack. 'A Wilcoxon-Type Test For Trend', Statistics in Medicine, 4, 87-90 (1985).

<sup>4.</sup> Le, Chap T. 'A New Rank Test Against Ordered Alternatives in K-Sampled Problems', Biom. J., 30, 87-92 (1988).

<sup>5.</sup> Chacko, V. J. 'Testing Homogeneity Against Ordered Alternatives', Ann. Math. Statist., 34, 945-956 (1963).

### A Symposium on

### GEOGRAPHY INFORMATION SYSTEMS

North Dakota / Minnesota Academies of Science 1994 Joint Annual Meeting

Symposium Coordinator

Chandra S Balachandran Geosciences, North Dakota State University, Fargo 58105

Friday, 29 April

1:30 G I S Progress in North Dakota.

Mark R Luther

North Dakota Geological Survey, Bismarck, 58505 0840

2:00 NDDOT - State-Wide GIS-T Development Through the Proposal Process.

Tim Horner
North Dakota Department of Transportation, Bismarck, 58505 0700

2:30 Geotechnical Applications of Ground Penetrating Radar.
Richard E Faflak
GeoArc Consulting, Moorhead, MN, 56560

### GIS Progress in North Dakota

Mark R. Luther Geologist/GIS Manager North Dakota Geological Survey 600 East Boulevard Avenue Bismarck, ND 58505-0840

Several state agencies in North Dakota, including the North Dakota Geological Survey (NDGS), have made significant progress toward implementation of a fully-functioning Geographic Information System (GIS) over the past two years. In addition, public and agency awareness of, and support for, GIS has increased dramatically during that same time period. The efforts of the state GIS Technical Advisory Committee, which has sponsored two GIS Symposiums, are in large part responsible for the increased awareness of GIS potential and applications in the state.

A GIS is composed of computer hardware, software, personnel, and digital spatial data (DSD); of these, the greatest long-term investment is in DSD. It is established fact that it is less expensive to buy existing high-quality DSD, if available, than to develop it locally. In that regard, we are very fortunate in that there is more high-quality, US Geological Survey produced, DSD available for the state of North Dakota than for nearly any other state. Some of the DSD currently available for North Dakota includes: 1:24K public land survey and boundaries (81% complete), 1:24K digital elevation models (19% complete), 3-Arc second digital elevation models (100% complete), 1:100K transportation (100% complete), and 1:100K hydrography (100% complete). These basic coverages are the foundation on which additional thematic layers can be added and then used for purposes of automated-map production, or actual GIS modelling.

Since the relative cost of DSD is so high, it is imperative that duplication be minimized. To that end, the NDGS has been operating a DSD clearinghouse, the purpose of which is to keep track of which GIS groups are producing what types of DSD, if the DSD is available for distribution, and who to contact. This service will become increasingly useful as the GIS community grows.

To keep GIS implementation costs to a minimum, state agencies and universities have relied on a mix of co-operative ventures, federal grants/funds, and industry grants/incentives. This has proven adequate during the initial, implementation phase. However, increasing demands on our GIS resources, coupled with potential decreases in federal support, will require greater support or reallocation of resources at the state and local level in order to meet the demands that will be placed on the system. That, and the need for locally-trained GIS personnel are perhaps our greatest challenges in the near future.

### NDDOT — STATE-WIDE GIS-T DEVELOPMENT THROUGH THE PROPOSAL PROCESS

Tim Horner
Planning Division
North Dakota Department of Transportation
Bismarck, ND 58505-0700

The North Dakota Department of Transportation [NDDOT] is in the process of developing a statewide GIS for Transportation [GIS-T]. The NDDOT is still in the Pilot phase of development and is doing this development through the request for proposal [RFP] process to acquire GIS Advisement services, hardware and software services and data conversion services. The development of GIS through this process is slower than typical direct acquisition and implementation practices but the end product can be a strong institutionally documented and supported process. The steps taken by NDDOT to date are acquisition of a GIS consultant, a hardware and software platform, and data conversion services. This presentation will also include roadblocks, stepping stones and other milestones encountered by the NDDOT to date.

### Geotechnical Applications of Ground Penetrating Radar

Richard E. Faflak\*
GeoArc Consulting, Moorhead, MN 56560

Ground penetrating radar (GPR) is a remote sensing technology which has tremendous potential for data acquisition in many geotechnical fields. It is perhaps one of the most underused, non-destructive remote sensing technologies available today. Typical applications include stratigraphic mapping, void and sinkhole detection, water table depth determination, geoarchaeology, hazardous waste mapping, storage tank detection, utilities detection, runway integretity, pavement thickness, and ice thickness. GPR wavelenghts are normally between 80 to 1000 megahertz (MHz) which penetrate nearly all materials, except metals. Penetration depths at these wavelengths range from about 70 m. at 80MHz, to about 1m. at 1000 MHz. Surveys can be conducted through materials as diverse as concrete, frozen ground, snow, ice, or water.

The operating principle behind GPR is based upon the ability of long radar wavelengths to reflect from interfaces between materials possessing different dielectric constant (Er) values. Er is a dimensionless measure of the capacity of a material to store charge when an electric field is applied. A large, rapid change of Er over a short distance will produce a good interface reflection. Conversely, when the Er changes gradually with depth, reflections from the boundary interface are weak or non-existent. Typical Er values encountered in GPR operation range from 1 for air to 81 for water (Table 1).

Electrical conductivity (mho/m) of subsurface materials determines the depth of penetration of radar signals. Conductivity is a material's ability to conduct electrical current. Conductivity is governed primarily by the amount of salts in solution, and to a lesser extent the water content, density, and temperature of subsurface materials. The conductivity value of a dielectric material causes the transmitted radar pulse to lose energy in the form of heat. The lower the conductivity, the greater the depth of radar signal penetration, and conversely the higher the conductivity, the greater the signal attenuation over a given distance.

GPR equipment consists of (1) a control unit with graphic recorder; (2) a micro processor; (3) a video monitor; (4) a digital audio tape (DAT) recorder; and (5) an antenna. The size of the antenna determines the wavelength of the propagating energy. Reflected signals are processed and displayed on the video monitor in real time, as well as being recorded on the graphic recorder and DAT. The DAT record can be stored for future image processing if more extensive interpretation of the data is required.

Radar data is a time-based record. The vertical axis on the graphic display represents travel time, or depth, to a subsurface interface. The horizontal axis represents distance along the surface. Normally, the horizontal scale is compressed 20 times or more in relation to the vertical scale on the graphic chart.

Interpretation of radar data is dependent upon (1) the composition of subsurface materials, (2) the "shape" of the reflected profile, and (3) image processing skill. The transmitted radar signal extends downward from the antenna in a cone shaped fashion. Normally the reflected signal is received perpendicular to the reflecting object. There is one notable exception to this rule. If a subsurface object is round, the forward and rearward extending cones will be reflected back to the receiver causing the initial and final appearance of the object to appear deeper than its true depth. The locus of these point readings is a hyperbola. Thus, round point objects appear in the data as a "vertical comet". Similarly, an excavation dug into the ground disturbs well defined strata. When the material is used to backfill the trench, the fill material disrupts the local stratigraphy, creating what is called a "trenching effect". Also, metal objects are 100 percent reflectors, which reveals the object well, but shadows anything beneath it. Image processing algorithms based on slight shifts in frequency, phase, or amplitude can be used to develop signatures for identification of earth materials.

Table 1. Dielectric constants (Er) for typical earth materials at radar wavelengths.

Material	Er	Material	Er	Material	Er
air	1.0	dolomite	4.85.6	dry sandy land	6.2
quartz	4.2	limestone	5.2	sand (saturated)	11.0
granite	4.4	clay (saturated)	5.2	water	81.0
dry sand	4.04.8	silt (saturated)	6.2		

### A Symposium on

### COMPUTER (and Pre-computer) BASED SPATIAL INFORMATION TECHNOLOGIES in K-12 CURRICULA

North Dakota / Minnesota Academies of Science 1994 Joint Annual Meeting
Symposium Coordinator

Chandra S Balachandran Geosciences, North Dakota State University, Fargo 58105

Friday, 29 April

3:00 J PAUL GOODE at the UNIVERSITY of MINNESOTA and MOORHEAD STATE NORMAL SCHOOL, 1884 - 1898
Warren D Kress

Geosciences, North Dakota State University, Fargo 58105 5517

3:30 COMPUTER-BASED SPATIAL INFORMATION TECHNOLOGIES in K-12 CURRICULA:
Needs and Prospects.
Chandra S Balachandran
Geosciences, North Dakota State University, Fargo 58105 5517

4:00 SOUTH and CENTRAL AMERICAN DEFORESTATION: Incorporating a Geographic Information System in the K-12 Curriculum. Eric Pauly

Ben Franklin Junior High School, Fargo, 58102

4:30 An INTERACTIVE MULTI-MEDIA TUTORIAL for HIGH SCHOOL STUDENTS on the SPATIAL DYNAMICS of A I D S Shawn Johnson

Geosciences, North Dakota State University, Fargo 58105 5517

### J. PAUL GOODE AT THE UNIVERSITY OF MINNESOTA AND MOORHEAD STATE

NORMAL SCHOOL, 1884-1898.

Warren D. Kress Department of Geosciences North Dakota State University Fargo, ND 58105-5517

The most outstanding American cartographer of his time, John Paul Goode, was born (1862) and raised in the farming area south of Rochester, Minnesota. He attended rural school in District No. 4 and later took further study in Rochester at the Rochester English and Classical School, from which he acknowledged the beneficial influence of the founder, Sanford Niles, and Priscilla Niles; and at Rochester Seminary and Normal School, from which he acknowledged the influence of the teacher of music, H. Brotherick. (1)

He entered the University of Minnesota in Fall Term, 1884, assigned to the Sub-Freshman Year in order to complete college entrance requirements. He turned 22 years of age during that term (November 21). In Fall Term, 1885, he enrolled in the Scientific Course in the College of Science, Literature, and the Arts, and during his four years as a member of the Class of 1889, he achieved what we would recognize today as a sound liberal education. Professors who were particularly influential upon his work at the University, and for some, in later years, were Christopher Webber Hall, geology, mineralogy and biology; Henry F. Nachtrieb, animal biology; Henry Pratt Judson, history; and Maria L. Sanford, rhetoric and elocution. During his senior year, he also enrolled in the School of Design, Free Hand Drawing and Wood Carving, especially for the work in drawing and design. Three classmates of interest with reference to the Red River Valley were Earle J. Babcock, Walter Lincoln Stockwell, and Henry Johnson. In the extra-curriculum, he was an active participant in athletics (Football Varsity Eleven all four years), musical organizations (especially men's quartette), student publications (both student newspaper and yearbook), and oratory and debate. Also, he was president of his sophomore class, Captain, Company C, University Cadet Battalion; and active member of Delta Tau Delta Fraternity.

In Fall Term, 1889, he joined the faculty at Moorhead State Normal School as teacher of natural science (geology, chemistry, physics, anatomy and physiology, and botany) and geography (in which he stressed the free-hand drawing of maps on the chalkboard). He was active in the extra-curriculum especially in music and in promotion of athletic field days. He also organized the first successful alumni meetings. He did graduate work in geology at Harvard and Chicago and served as summer instructor in geology at Minnesota and in physiography and meteorology at Chicago while on the Moorhead faculty.

Around the turn of the century, he published articles which, in effect, drew upon his study and teaching over the period 1866-1898 and which gave indication of possible future outstanding contribution to the field of geography in general and the specialty of cartography in particular. (2)

The content of this paper drew mainly on the archives of the Olmsted County Historical Society, Rochester; the University of Minnesota Archives, Minneapolis; the Moorhead State University Archives and the Clay County Historical Society Archives, Moorhead. Inspection of Goode's academic record at the University of Minnesota was made possible by the kind permission of his granddaughter.

- 1. <u>Hist. of the Rochester Old School Boys and Girls Assn</u> p 18. Published by the Association, Rochester (1927).
- Goode, J. Paul (1900). <u>A Hand-book to Accompany the Rand-McNally Physical Wall Maps</u>. Rand-McNally & Company, Chicago.
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  - Goode, J. Paul (1901). "Geography in America" Bull. Am. Bureau of Geogr.p 301-312.
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### COMPUTER-BASED SPATIAL INFORMATION TECHNOLOGIES IN K-12 CURRICULA:

### **NEEDS AND PROSPECTS**

Chandra S. Balachandran
Department of Geosciences
North Dakota State University
Fargo, ND 58105-5517
E-mail: balachan@plains.nodak.edu

Spatial information, or geographic information, is any information which is tied to location. It is used in practically in every type of activity which affects our environment, economic interconnections among places, where we choose to live, etc. As the modern information age progresses and we are confronted with a great volume of information, it is important for us to not only be able to meaningfully handle vast amounts of information, but also to do it efficiently, cheaply, and rapidly.

Computer-based spatial information technologies, including a genre of software called Geographic Information Systems [GIS], continue to gain wide application in a variety of fields. These technologies enable us to visualize and use information in ways hitherto not possible. The major effect of these technologies is to globalize life on an unprecedented scale.

To ensure that we, and our students who will be future citizens of the "global village", can live successfully in such an information-rich world, we need to equip ourselves with the necessary "vehicles" to traverse the "information superhighway." Towards this, the Department of Geosciences at NDSU, the North Dakota Geographic Alliance [NDGA], and Environmental Systems Research Institute, Inc. [ESRI] have formed a partnership to address a variety of needs in bringing these technologies to the K-12 curricula and classrooms across North Dakota. In addition, work by several students at the Department of Geosciences, NDSU is progressing towards developing lesson plans and tutorials for K-12 students on issues of current interest including the geography of the AIDS pandemic.

This presentation examines:

- 1. The hardware, software, and infrastructural needs of K-12 schools in preparing teachers and students for the information superhighway at an early stage,
- 2. the case for the use of GIS in K-12 curricula,
- 3. work underway in addressing these needs, and
- 4. the future directions envisaged under the triumvirate partnership mentioned above.

Plans include summer workshops [with credit] for in-service teachers in learning the use of GIS, in-service presentations, development of user networks, and a regional database of lesson plans. Courses are also currently available for students training to be teachers.

## SOUTH AND CENTRAL AMERICAN DEFORESTATION: INCORPORATING A GEOGRAPHIC INFORMATION SYSTEM IN THE K-12 CURRICULUM

Eric Pauly
Ben Franklin Junior High School, Fargo, ND 58102

This exercise will compare information on South and Central American countries utilizing a Geographic Information System [G.I.S.]. The G.I.S. is a combination of computer hardware, software and data which together provide rapid analysis of specific data. The type of analysis is dependent upon the variables selected by the user. The G.I.S. used in this exercise is ArcView, a product of Environmental Systems Research Institute, Inc. which is appropriate for use in the K-12 setting.

Through the process of using G.I.S., students in the K-12 environment have the ability to observe the relationships that exist between individual factors and the contributing nature these factors have on environmental issues, in this case the deforestation issue. This creates an appreciation and understanding of the inter-connectedness of human and environmental issues and fosters a desire for further inquiry on the part of the students.

In an effort to attain a more complete and comprehensive understanding of the deforestation issue as it pertains to South and Central America, a number of factors may be considered. These factors can be displayed spatially in the form of maps and compared in rapid succession using the G.I.S. When viewed in this manner, a greater understanding of the complex nature of the deforestation issue can be observed and further questions can be formed.

Additionally, it is possible to view maps of the South and Central American region with multiple variables of data represented simultaneously. It is in this realm that the true power of G.I.S. is realized. The computer creates maps that are layered, with each layer representing a chosen variable. This allows selective analysis of the chosen data on a single map. If the user chooses to alter the variables, it is accomplished easily and quickly. In this manner, it is possible to see how individual factors affect the deforestation issue.

### AN INTERACTIVE MULTI-MEDIA TUTORIAL FOR HIGH SCHOOL STUDENTS ON

### THE SPATIAL DYNAMICS OF AIDS

Shawn Johnson Department of Geosciences North Dakota State University Fargo, ND 58105-5517

With recent developments in information technologies, new ways of teaching and learning are emerging. Students often prefer learning through computers because they can work individually and at their own pace. Computers are increasingly used in classrooms, leading to interactive learning. This tutorial teaches the geography of AIDS in an interactive multi-media environment using animation, maps, and text. The tutorial contains the following modules:

- 1. General information concerning AIDS
- 2. The possible origins of AIDS
- 3. The geography of AIDS
- 4. The future of AIDS
- 5. How to protect oneself from contracting AIDS.

In the beginning of the tutorial, students have the choice to run the entire tutorial or just particular modules through using several buttons. Each module has a variety of screens showing text, maps, animations, and questions. With each screen, the student clicks a button to continue onto the next screen. At various points, the student will be asked questions which he/she must answer correctly. If the student answers the question incorrectly, the screen pertinent to the question will reappear. Each answer has a button associated with it. The student clicks on a button, thus selecting an answer. Of the four possible buttons that they can click, the three incorrect choices have a pathway back to the previous screen, with the correct choice allowing the student to move to the next screen. This questioning style assists students in concentrating on what they have read before continuing with the tutorial. It also provides feedback on their comprehension. Since this is a computer-based tutorial, students could have access to this program both at school and home. The multi-tiered presentation is usable in a variety of classes, such as geography, sociology, history, and health education.

As student involvement with computers is increasing rapidly, so is the technology of the applications they are operating. This interactive multi-media tutorial is the first step of many in achieving inter-disciplinary learning through computers. With several perspectives on AIDS included, this same tutorial can be meaningful for a variety of classes. The instructor can emphasize the tutorial's information within his/her specific discipline when the tutorial is implemented, and also sensitize the students to the other disciplines involved. Thus, some of the benefits of a team-taught multi-disciplinary course approach can be realized using this tutorial.

### Symposium Coordinator

# David A Watson Veterinary and Microbiological Sciences North Dakota State University, Fargo 58105 5406

Friday, 29 April

### PATHOGENESIS of STREPTOCOCCAL INFECTIONS

- 8:00 Streptococcal Toxic Shock Syndrome: Clinical Characteristics and Pathogenic Mechanisms.

  Dennis Stevens\*, Sean P Hackett and Amy Bryant, Infectious Disease Section, Veterans Affairs Medical Center, Boise, 83702
- 8:50 Virulence Factors of Encapsulated Bacteria: What the
  Pneumococcus has Taught Us.
  David A Watson\*, Veterinary and Microbiological Sciences, and
  Daniel M Musher, Infectious Disease Section, Veterans Affairs
  Medical Center, Houston, TX 77030
- 9:40 Informal Discussion and Refreshment Break

### PATHOGENESIS of GRAM-NEGATIVE BACTERIAL INFECTIONS

- 10:10 Detection of <u>Salmonella</u> in Cattle Using P C R.
  Patricia P Rosen\*, Biology, Moorhead State University,
  Moorhead, MN 56562 and
  Lisa Noland, Veterinary and Microbiological Sciences, N D S U,
  Fargo 58105 5406
- 11:00 The Molecular Biology of Neisserial Pilin Proteins. Ellen Aho\*, Amy Bohling, Paul Jones and David Pipho Biology, Concordia College, Moorhead, MN 56562

### STREPTOCOCCAL TOXIC SHOCK SYNDROME: CLINICAL CHARACTERISTICS AND PATHOGENIC MECHANISMS

Dennis L. Stevens\*, Sean P. Hackett, and Amy Bryant, Infectious Disease Section, Veterans Affairs Medical Center, Boise, ID 83702

Severe invasive group A streptococcal infections of humans have been reported with increasing frequency from North America, Europe and Australia. Such cases have occurred largely since 1989 and have involved peoples of all races, ages and religions. The greatest age-specific increase in attack rates has been in those individuals from 20-50 years of age. The clinical manifestations have included malaise, localized pain, chills and myalgias. Patients may have confusion, which sometimes delays their presentation to their physician. If the symptomatology is ambiguous early in the course of the infection, the outcome is dramatic and frequently devastating. The majority of such patients have shock, acute respiratory distress syndrome, renal failure and extensive tissue deterioration.

Although some 83 M protein serotypes of group A streptococci are known, strains isolated from patients with the streptococcal toxic shock syndrome (strep TSS) have been shown to be primarily of M types 1 and 3, both of which produce pyrogenic exotoxin A and possibly an additional, novel toxin. Such toxins interact with human T-lymphocytes in a unique way which results in massive lymphocyte proliferation. These toxins are referred to as superantigens, since they stimulate multiple clones of T-lymphocytes, in contrast to the usual mechanism where only a single T-cell clone expands in response to antigenic stimulation. The consequences of this intimate interaction between the human host and streptococcal toxins determine the clinical characteristics of strep TSS.

In vitro clonal proliferation of T-lymphocytes by streptococcal superantigens is maximal at 72 hours. We have shown that this corresponds to a switch in cytokine synthesis from the monokine  $TNF\alpha$  to the lymphokine  $TNF\beta$ . Thus, unlike other forms of septic shock, strep TSS is largely the consequence of toxin-induced lymphocyte activation. Strategies designed to control this largely host-induced, and often injurious inflammatory response via modulation of cytokine responses to the presence of these toxins may therefore prove valuable.

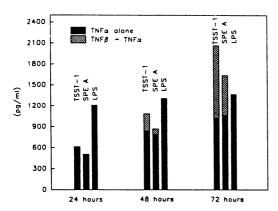


Figure 1. Comparison of TNF $\alpha$  and TNF $\beta$  synthesis by mononuclear cells stimulated with either streptococcal pyrogenic exotoxin A (SPE A: 10  $\mu$ g/mL), toxic shock syndrome toxin 1 (TSST-1; 10  $\mu$ g/mL), or lipopolysaccharide (LPS; 100  $\mu$ g/mL). Data are from three normal donors studied in duplicate and are means.

### Additional Reading:

Stevens DL, Tanner MH, Winship J, et al. 1989. Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. N. Engl. J. Med. 321:1-8.

Stevens DL. 1992. Invasive group A Streptococcus infections. Clin. Infect. Dis. 14:2-13.

The Working Group on Severe Streptococcal Infections. 1993. Defining the group A Streptococcal toxic shock syndrome: Rationale and Consensus Definition. J. Amer. Medical Assoc. 269:390-391.

Hackett SP, and Stevens DL. 1993. Superantigens associated with staphylococcal and streptococcal toxic shock syndrome are potent inducers of tumor necrosis factor-β synthesis. J. Infect. Dis. 168:232-235.

### VIRULENCE FACTORS OF ENCAPSULATED BACTERIA: WHAT THE PNEUMOCOCCUS HAS TAUGHT US.

David A. Watson<sup>1\*</sup>, and Daniel M. Musher<sup>2</sup>. <sup>1</sup>North Dakota State University, Dept. of Veterinary and Microbiological Sciences, Fargo, ND, 58105, and <sup>2</sup>Veterans Affairs Medical Center, Infectious Disease Section, Houston, TX, 77030.

Practically by definition, the principal virulence determinant of almost any encapsulated bacterial pathogen is its extracellular capsule. With few exceptions, this covering is composed of heterogeneous mixtures of five or six carbon monosaccharides linked into long linear or branched polysaccharide polymers. The most extensively studied of these are the capsules of *Haemophilus influenzae* and *Streptococcus pneumoniae*, respectively. This discussion will be limited to aspects of the biology of the *S. pneumoniae* capsule, and to the putative roles played by accessory virulence factors of this pathogen.

First, even though the interruption of genes encoding accessory, usually proteinaceous, factors have been shown to attenuate virulence to some degree, the removal of the pneumococcal capsule or the interruption of encapsulation genes completely abolishes virulence in mice. Similarly, loss of capsule expression interferes with virulence in other streptococci, including S. agalactiae and S. pyogenes. The presence of the capsule is thought to interfere with efficient complement-mediated opsonophagocytosis of pneumococci in the absence of specific anticapsular antibodies. Lack of efficient clearance by the host results in proliferation of the infecting bacterium. The most attractive current hypothesis is that following a period of rapid increase in bacterial numbers autolysis occurs and the pneumococci release cell wall polysaccharide (a teichoic acid common to all pneumococci), peptidoglycan fragments, and pneumolysin (a nonsecreted cytolytic toxin), stimulating the host to produce a massive inflammatory response. The role of the capsule in pathogenesis is not completely clear, however, since it is not known whether this structure is important in colonization, the obligatory first step in the process.

Second, as mentioned above, a number of proteins have been implicated as possible accessory virulence factors. These include both pneumolysin and autolysin (the muramidase responsible for the breakdown of the pneumococcal cell wall), neuraminidase, an IgA1 protease, and two surface proteins, pspA (a putative capsule-stabilizing surface protein), and psaA (a putative adhesin). While interruptions of some of these proteins have been shown to attenuate virulence (others have yet to be examined), it has not proven possible to abolish virulence completely by knocking out of these accessory factors. It is intriguing to speculate that a pneumococcal strain attenuated for multiple accessory virulence factors might be nearly avirulent; that is, the effect of each accessory factor might be additive with respect to virulence. The capsule would then be considered necessary, yet not sufficient for full virulence.

Third, proteinaceous accessory virulence factors may prove important to the development of second generation pneumococcal vaccines. Both pneumolysin and surface protein A are currently being evaluated as carrier proteins conjugated to pneumococcal polysaccharides in attempts to improve the immunogenicity of polysaccharide vaccines, primarily in small children. Of perhaps even greater promise would be the use of the putative adhesin psaA as a vaccine, either alone to prevent adherence of pneumococci to the nasopharynx, or conjugated to pneumococcal polysaccharides to assist in the stimulation of anticapsular antibody responses. Contrary to recent speculation, a serologically invariant pneumococcal surface protein such as psaA may be a better vaccine candidate than the serologically variable surface protein pspA, since its lack of variablility may be due to positive selection for a single virulence-associated composition or conformation.

For Additional Information:

Watson DA, and Musher DM. 1990. Interruption of capsule production in *Streptococcus pneumoniae* serotype 3 by insertion of transposon Tn916. *Infect. Immun.* 58:3135-3138.

Watson DA, Musher DM, Jacobsen JW, and Verhoef J. 1993. A brief history of the pneumococcus in biomedical research: A panoply of scientific discovery. Clin. Infect. Dis. 17:913-24.

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Paton JC, Andrew PW, Boulnois GJ, and Mitchell TJ. 1993. Molecular analysis of the pathogenicity of *Streptococcus pneumoniae*: The role of pneumococcal proteins. *Annu. Rev. Microbiol.* 47:89-115.

### DETECTION OF SALMONELLA IN CATTLE USING PCR

Patricia P. Rosen\* and Lisa K. Nolan Department of Biology, Moorhead State University, Moorhead, MN 56562 Department of Veterinary and Microbiological Sciences, North Dakota State University, Fargo, ND 58105

Salmonella species are intracellular pathogens that colonize and invade the intestinal epithelium; they are also capable of invading and multiplying in phagocytic cells. These organisms can cause significant morbidity and mortality in animals. Bovine salmonellosis is a growing problem for United States' cattle producers. Since 10 to 13% of the livestock in the United States are thought to be infected with Salmonella, many of the losses due to scours (severe diarrheal disease), the number one killer of newborn cattle in this country, are probably due to salmonellosis (1). Further, the largest known outbreaks of human salmonellosis have originated from contaminated foods of cattle origin. Therefore, bovine salmonellosis is an expensive problem for the cattle industry due to cattle losses and tragic events of public health significance.

Bovine Salmonella control programs are designed around identification and elimination of diseased animals and carriers, but many carriers go undetected due to failure of the current diagnostic techniques. Improved detection methods, based on polymerase chain reaction (PCR) techniques, should soon be available.

In a study performed here in North Dakota, detection of carriers in a herd of dairy cattle using PCR and routine culture methods was attempted. Buffy coats of blood samples and fecal samples from 102 animals were inoculated into tetrathionate broth (TB) for enrichment and selection. The samples were incubated at 37 C overnight and plated onto brilliant green agar with novobiocin (BGAN). Broth cultures were held at room temperature for 5 days and these cultures used to inoculate fresh TB. The fresh TB was incubated at 37 C overnight and plated onto (BGAN). Suspect colonies were further tested and identified to Salmonella serotype. Twenty-two of the 36 calves sampled harbored S. typhimurium in their feces, but none of the samples from the 66 cows were shown to contain Salmonella. Extended enrichment of the fecal cultures in TB did result in a significant increase in Salmonella isolations. No Salmonella were isolated by culture from the buffy coats following initial or extended enrichment periods.

The buffy coats were further examined with PCR. PCR is a rapid in vitro procedure for enzymatic amplification of specific DNA sequences which increases the number of copies of the target sequence. It is a sensitive procedure that can detect viable and nonviable cells as long as the DNA is intact. PCR has been used successfully to identify Salmonella in blood, in food, and in environmental samples. In our studies, PCR was used to detect Salmonella in the buffy coats of cattle with the use of primers specific for the *invA* and *pagC* genes (2,3). The *invA* gene is necessary for invasion of cultured epithelial cells by S. typhimurium. The *pagC* gene is essential for virulence and survival of S. typhimurium. PCR analysis of the buffy coats revealed 3 positive samples. Since no Salmonella were cultured from the buffy coats, PCR, in this case, detected nonviable organisms. Methods to aid interpretation of PCR-positives of culture-negative samples will be discussed.

<sup>1.</sup> Blood, D.C., Radostits, O.M., and Henderson, J.A. (1983) Veterinary Medicine, 6th ed., pp.576-581,585. Bailliere Tindall, London.

<sup>2.</sup> Galan, J.E. and Curtiss, R. III. (1989) Proc Natl Acad Sci USA 86,6383-6387.

<sup>3.</sup> Miller, V.L., Beer, K.B., Loomis, W.P., Olson, J.A., and Miller, S.I. (1992) Infect Immun 60,3763-3770.

#### THE MOLECULAR BIOLOGY OF NEISSERIAL PILIN PROTEINS

Ellen Aho\*, Amy Bohling, Paul Jones and David Pipho Department of Biology, Concordia College, Moorhead, MN 56562

The genus Neisseria contains the human pathogens N. gonorrhoeae and N. meningitidis, which are responsible for gonorrhea and meningitis respectively. The specific mechanisms by which these organisms cause disease are not totally clear; however, several traits have been associated with neisserial pathogenesis. Among these virulence-associated components are pili, which are filamentous surface appendages composed of the protein pilin. Pili are involved in the attachment of both gonococci and meningococci to mucosal surfaces.

Neisserial pili exhibit a great deal of diversity. Antigenic heterogeneity is observed among different strains within a neisserial species. Intrastrain pilin variation is also present. A single strain of N. meningitidis or N. gonorrhoeae can turn the synthesis of pilin on and off (phase variation) or switch between different antigenic versions of pilin (antigenic variation). These processes may play a role in immune evasion, and functional differences have been observed among different pilin variants in studies examining the ability of Neisseria to attach to eukaryotic cells in vitro.

Neisserial pilin variation is regulated by a number of interesting genetic events. Pilin regulation has been most clearly elucidated in *N. gonorrhoeae*. All gonococcal strains that have been examined contain one or two complete pilin expression loci (pilE) and several silent loci (pilS). All pilE loci share homologous 5' coding sequences as well as regions of internal homology that are interspersed by variable sequence information. The incomplete pilS gene copies share regions of internal homology with the pilE loci, but lack 5' coding sequences. Antigenically distinct pilin proteins are expressed by a gonococcus when a pilS locus donates new, variable sequence information to a pilE locus via a process of non-reciprocal recombination. Additional genetic mechanisms that result in changes in pilE loci are involved in pilin phase variation. Although pilin variation is a complex process in the gonococcus, all gonococci possess pilE genes with similar 5' sequences and all gonococcal pilin proteins share certain conserved epitopes (1).

Pilin expression is less well understood in N. meningitidis. Some strains of N. meningitidis demonstrate a pattern of pilin expression that is essentially the same as that of the gonococcus. These strains contain pilE and pilS loci that possess conserved sequences homologous to those of gonococcal pilin genes (2). The pili expressed by these meningococcal strains are antigenically related to gonococcal pili and have been designated class I pili. A second group of meningococcal strains express class II pili, which are composed of slightly smaller pilin proteins that lack the conserved epitopes shared by all gonococcal and class I meningococcal pilin proteins. The genes responsible for class II pilin expression have not been described.

We describe preliminary studies addressing the genetic basis for differences between class I and class II pilin in N. meningitidis. We have purified class II pilin from N. meningitidis strain FAM18. N-terminal amino acid sequence analysis indicates a high degree of similarity to class I pilin. Paradoxically, Southern blot analyses using class I pilE probes fail to detect homologous expression loci in class II pilin-producing meningococci, a finding also reported by others (3, 4). We have used immunologic probes to clone regions of DNA from N. meningitidis FAM18 that are putatively involved in class II pilin expression. Ongoing analyses seek to discern the character of meningococcal class II pilin expression loci and the possible consequences of this additional level of diversity among neisserial pili.

<sup>1.</sup> Meyer, T.F. (1988) Trends in Genetics 3, 319-24.

<sup>2.</sup> Potts, W.J. and Saunders, J.R. (1988) Mol Microbiol 2, 647-53.

<sup>3.</sup> Perry, A.C.F., Nicolson, I.J., Saunders, J.R. (1988) J Bacteriol 170, 1691-7.

<sup>4.</sup> Aho, E.L. and Cannon, J.G. (1988) Microbial Pathogenesis 5, 391-8.

#### THE TWIN CITIES METROPOLITAN AREA URBAN HEAT ISLAND: ANALOG FOR GLOBAL WARMING?

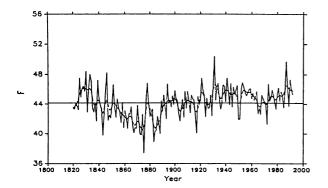
#### Paul Todhunter\*

Department of Geography, University of North Dakota, Grand Forks, ND 58202-9020

Climate impact studies of the possible consequences of global climate change share numerous methodological limitations, including uncertainty regarding the rate and magnitude of climate change, omission of linkages between related environmental systems, neglect of the direct effects of CO<sub>2</sub> fertilization, lack of consideration of human adaptation and technological change, and inadequate local-scale climate change information. S.A. Changnon (1) recently argued that the study of inadvertent climate modification in urban areas over the past century can serve as a useful (though imperfect) analog for the possible climate impacts of global warming.

The urban heat island exhibits several features which make it a suitable analog for climate impact studies. These include: (1) the rate and magnitude of urban climate change approximate those being simulated by current global climate models; (2) the pattern of urban warming, being concentrated at night and during winter in mid-latitude continental locations, is similar to the pattern of historical warming over the past century (2); (3) urban areas provide a natural model of how environmental systems respond to climate change; (4) urban ecosystems have adjusted to the direct effects of a 25% increase in the  $CO_2$  concentration over the past century; and (5) urban areas provide an example of how climate change may be mitigated by human and technological adjustment.

The Twin Cities Metropolitan Area (TCMA) is examined as a possible analog for the climate impacts of global warming. Historical mean annual air temperatures for the Minneapolis Weather Service Forecast Office are shown in Figure 1 for the period 1820-1992 (N=173 years). The solid line (with high frequency noise) is the annual time series, the dashed line (with lower frequency noise) gives the annual time series smoothed by a nine-term binomial filter, and the horizontal solid line shows the long-term mean (44.2°F). Air temperatures have warmed at a rate of 1.5°F per century over the past 100 years (significant at the 0.05 level). Figure 2 shows the spatial pattern of the 1989 TCMA mean annual urban heat island for an area of 30 miles radius centered at downtown Minneapolis (the Minneapolis WSFO AP is shown with a '+' sign). The isolines in Figure 2 were interpolated from a grid derived from a 26 station network of mean monthly air temperatures which had been adjusted for data errors, missing data, and the effects of time-of-observation bias, and daylight savings time. Table 1 presents a summary of various environmental indices for 1989 derived from the primary monthly air temperature data. The results in Table 1 demonstrate that the environmental effects of historical warming have been quite pronounced, and that the effects have not been uniform among the environmental indices examined.



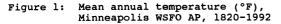




Figure 2: Mean annual temperature (°F), TCMA, 1989

Table 1: Summary of TCMA urban heat island environmental impacts, 1989

Variable	maximum	minimum	range
Mean annual temperature (°F)	43.9	39.3	4.6°F
Melting degree-days (>32°F)	411	276	49 %
Growing degree-days (>32°F)	6572	5261	25 %
Growing degree-days (>50°F)	2908	1832	59 %
Cooling degree-days (>65°F)	929	312	198 %
Heating degree-days (<65°F)	9614	8378	15 %
Freezing degree-days (<32°F)	2528	2027	25 %
Freeze change-days (<32°F)	238	114	109 %
Frost-free season length	151	135	16 days

Changnon, S.A. (1992) <u>Bull Amer Meteor Soc</u> 73, 619-627.

Balling, R.C. (1992) The Heated Debate: Greenhouse Predictions Versus Climate Reality, 199 pp, Pacific Research Institute, San Francisco.

Thursday, 28 April

1:40 .. Warning Barks and Alarm Behaviors of Gunnison's Prairie Dogs (Cynomys Gunnisoni) in Colorado.

Michael D Aho\*, Donna M Bruns Stockrahm, Stacy L Adolf, Beth L Steffan

Biology, Moorhead State University, MN, 56563 Tyson H Harty and Thomas M Workman The School for Field Studies, Beverly, MA, 01915

- 2:00 .. Effects of Dutch Elm Disease on the Vegetation of the Upper Mississippi River Floodplain Forests. Michael G Hubbard\* and Wallace J Wanek Bemidji State University, Bemidji, MN, 56601
- 2:20 .. Effects of Putrescine, Indole-3-acetic acid and Inhibitors of Putrescine Biosynthesis on Organogenesis in Euphorbia Esula L. David G Davis\* and Prudence A Olson USDA-ARS Biosciences Research Laboratory, Fargo, ND, 58105
- 2:40 .. Infection and Mortality of the Sugarbeet Root Maggot (Diptera:Otitidae) Following Application of Entomopathogenic Nematodes.

C A Wozniak\*, G A Smith, L G Campbell USDA-ARS Northern Crop Science Laboratory, Fargo, ND, 58105

3:00 .. Evaluation of Potato Sprout Suppression with Natural Compounds. Martin T Glynn\*, Paul H Orr, Edward C Lulai USDA-ARS Red River Valley Potato Research Laboratory, East Grand Fork

### WARNING BARKS AND ALARM BEHAVIORS OF GUNNISON'S PRAIRIE DOGS (CYNOMYS GUNNISONI) IN COLORADO

Michael D. Aho\*, Donna M. Bruns Stockrahm, Stacy L. Adolf, and Beth L. Steffan
Department of Biology, Moorhead State University
Moorhead, MN 56563 and
Tyson H. Harty and Thomas M. Workman
The School for Field Studies, Beverly, MA 01915

Prairie dogs received their name because the sound of their repetitive "warning bark" resembles the bark of a small dog. Various aspects of the warning bark and associated alarm/alert behaviors have been studied on different prairie dog species (1, 2, 3). Hoogland (4) found that as ward size and/or prairie dog density increased, both black-tailed (Cynomys ludovicianus) and white-tailed prairie dogs (C. leucurus) spent less time in alert behaviors and that black-tailed adults living on the ward periphery were more cautious than centrally-located adults. The purpose of our study was to document various aspects of the warning barks and associated behaviors of Gunnison's prairie dogs (C. qunnisoni). Furthermore, even though our study methods differed from those of Hoogland (4), we hoped to determine if the trends he noted were evident in Gunnison's prairie dogs.

Our study was conducted in Archuleta County northwest of Pagosa Springs, CO (T36N, R3W, S13). Observations were made throughout the summers of 1991-1993, primarily in August 1993. For data collection, observers walked with binoculars through the colony and located prairie dogs. Each dog was then approached directly at a slow, constant rate; a marker was dropped on the ground at the point when any of the following behaviors by the dog occurred: initial sight of observer, initial bark, run to burrow, crouch in burrow opening, and entrance into burrow. The distance was then measured from each marker to the entered burrow which was then classified as either "central" or "peripheral" based on its relative position in that cluster of burrows. We used the point-quarter method to find the average distance between the entered burrow and its 4 nearest surrounding burrows, assuming that less distance between burrows was associated with denser prairie dog populations. For some analyses, the data were divided into 2 approximately equal-sized groups, i.e., mean distance <7.74m versus >7.84m.

A total of 72 prairie dogs were observed and approached. Only 18 responded by barking (25.0%); of these, only 5 (27.8%) were from peripheral burrows. Of the nonbarking animals, 64.8% (n = 54) crouched and remained alert in the burrow entrance before entering the burrow. Barking dogs were usually at a burrow entrance before they initiated barking and were in an upright rather than a crouched position. Twenty-three dogs (31.9%) ran to a burrow before barking, crouching, or entering a burrow (with the exception of 1 dog which barked before it ran). This indicated that prairie dogs were usually in the relative safety of a burrow entrance before drawing attention to themselves with warning barks. The mean distance at which prairie dogs sighted the approaching human observer was not significantly different between areas of dense (<7.74m) and less dense (>7.84m) burrows (<7.74m:  $\overline{X} = 27.7m$ , S.D. = 5.22m, n = 21; >7.84:  $\dot{X}$  = 25.00m, s.D. = 7.01m, n = 31; t = 1.5012, d.f. = 50, P > 0.05) nor was the mean distance of the approaching human at which the dog entered its burrow (<7.74m:  $\bar{X} = 14.96m$ , S.D. = 8.95m, n = 31; >7.84m:  $\bar{X} = 12.86m$ , S.D. = 8.38m, n = 37; t = 0.9997, d.f. = 66, P > 0.05). Nearly identical results were obtained for the above parameters when central burrows were compared with peripheral burrows because most central burrows were in the <7.74m group and, likewise, most peripheral burrows were in the >7.84m group. Other comparisons were difficult due to small sample sizes.

We found few obvious differences in the behaviors studied between central/peripheral dogs and between those from dense/less dense burrow areas suggesting that the warning system of the Gunnison's prairie dog differs from that of other prairie dog species. However, "central/peripheral" designations might be less distinct or even irrelevant for Gunnison's prairie dogs because clusters are often small with varying distances between clusters. Further studies could include the influence of inter-cluster distance on behavior and could use actual prairie dog densities rather than distances between burrows as indices of density.

<sup>1.</sup> Waring, G.H. (1970) Amer Midl Nat 83, 167-185.

<sup>2.</sup> Slobodchikoff, C.N., J. Kiriazis, C. Fischer, and E. Creef. (1991) Anim Behav 42, 713-719.

<sup>3.</sup> Motiff, J.P. (1980) Psychol Rep 46, 1164-1166.

<sup>4.</sup> Hoogland, J.L. (1979) Anim Behav 27, 394-407.

THE EFFECTS OF DUTCH ELM DISEASE ON THE VEGETATION OF THE UPPER MISSISSIPPI RIVER FLOODPLAIN FORESTS

Michael G. Hubbard\* and Wallace J. Wanek Bemidji State University, Bemidji, Minnesota 56601

Lowland forest communities in northern Minnesota have undergone tremendous change as a result of Dutch elm disease. The disease fungus, Ceratocystis ulmi Buisman, arrived in the United States in the late 1920's in a load of infected elm logs from Europe (1). It was first reported in Minnesota in 1961 (2) and by 1970 was found from the east coast states west to Idaho, and south into Texas (3). At this time the disease was present in most of the southern counties of Minnesota (2). Expansion into Beltrami County was within the first few years of the 1980's.

In the summer of 1970, a field study characterized the vegetation (trees, saplings, shrubs, and herbs) of the floodplain forests using twenty sites along the upper Mississippi River within Beltrami County (4). American elm, Ulmus americana L., was present on all twenty sites and was found to be a dominant species in the community (4). This field study was used for baseline data. The present research was undertaken to record the vegetative changes in the upper Mississippi River floodplain forest from 1970-1993, and relate these changes to the loss of American elm. The sites used in the earlier study were reinventoried. Ten sites lie upstream of a power dam constructed in 1909 and are still subject to flooding; ten lie downstream of the dam and no longer flood regularly. At each site quantitative data were collected for trees, saplings and shrubs using the point-centered quarter method (5). Herbaceous data were collected using a frame which encloses an area 25% of a square meter at each of the points. Changes in plant species composition and community structure were determined from these surveys.

The most notable changes to the floodplain forests came as a result of death of American elm trees in the canopy. Decrease was shown using the importance values for American elm tested with an ANOVA ( $\underline{F}(1,18)=140.87$ ,  $\underline{p}<.0005$ ). One result of this loss was a significant change in the number of trees of all species per acre ( $\underline{t}(19)=8.78$ ,  $\underline{p}<.0005$ ). In 1970, before the onset of Dutch elm disease, the mean number of trees per acre was 386. In 1993, approximately ten years after the arrival of the disease, the mean number of trees per acre was 230. This opening of the canopy resulted in a significant increase in numbers for both saplings ( $\underline{t}(19)=4.06$ ,  $\underline{p}<.001$ ) and shrubs ( $\underline{t}(19)=2.25$ ,  $\underline{p}<.05$ ). Replacement of American elm across all twenty sites was by Quercus macrocarpa Michx. ( $\underline{F}(1,18)=8.55$ ,  $\underline{p}<.01$ ) and by Fraxinus spp. (Fraxinus nigra Marsh. and Fraxinus pensylvanica var. subintegerrima (Vahl) Fern.) ( $\underline{F}(1,18)=6.92$   $\underline{p}<.05$ ). With sites divided into two categories, above or below the dam, there was also replacement of American elm below the dam by Populus tremuloides Michx. ( $\underline{F}(1,18)=26.09$ ,  $\underline{p}<.0005$ ), and Abies balsamea (L.) Mill. ( $\underline{F}(1,18)=6.38$ ,  $\underline{p}<.05$ ).

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# EFFECTS OF PUTRESCINE, INDOLE-3-ACETIC ACID AND INHIBITORS OF PUTRESCINE BIOSYNTHESIS ON ORGANOGENESIS IN EUPHORBIA ESULA L.

David G. Davis\* and Prudence A. Olson USDA, Agricultural Research Service, Biosciences Research Laboratory, Fargo, North Dakota 58105

DL- $\alpha$ -Difluoromethylornithine (DFMO) and DL- $\alpha$ -difluoromethylarginine (DFMA) are respective inhibitors of the ornithine decarboxylase and arginine decarboxylase pathways of putrescine biosynthesis. DFMO and DFMA also inhibit root and shoot formation in aseptically-isolated hypocotyl segments of leafy spurge (Euphorbia esula L.) grown on full strength B5 medium (Table 1). Exogenous putrescine in full strength medium had little or no effect on root or shoot formation. Putrescine did not overcome the inhibitory effects of DFMO on root or shoot formation. nor of shoot formation by DFMO (Table 1). Inhibition of root formation by DFMA was overcome by putrescine (Table 1). Beyond eight days of culture, no consistent relationship appeared to exist between organogenesis and the cellular levels of putrescine and spermidine in leafy spurge hypocotyl segments (1). This may indicate that putrescine is not physiologically relevant in shoot formation or is important only in the early (cell division) stages. DFMA strongly inhibited organogenesis in medium diluted 10-fold (Table 2). Unexpectedly, agmatine (an intermediate between arginine and putrescine) did not reverse this inhibition, but was inhibitory by itself (Table 2). In the absence of DFMO and DFMA, exogenous auxin, indole-3-acetic acid, induced an increase in the cellular levels of putrescine and spermidine, but not of spermine (the aminopropyl adduct of spermidine metabolism) (1). This increase was only observed during the first few days. Perhaps residual putrescine or spermidine already present at the time of excision may have been involved in the early stages of root formation, even in the tissues in which putrescine biosynthesis levels were declining.

Table 1. Effects of putrescine ± DFMO or DFMA on organ formation in isolated leafy spurge hypocotyl segments.

Co	oncentration (r	Number per s	egment ± SE	
DFMO	DFMA	Putrescine	Shoots	Roots
0	0	0	$1.5 \pm 0.2$	$0.4 \pm 0.1$
0	0	1	$1.8 \pm 0.2$	$0.5 \pm 0.1$
0.5	0	0	$0.9 \pm 0.2^{a}$	$0^{\mathbf{a}}$
0.5	0	1	$0.7 \pm 0.1^{a}$	0 <sup>a</sup>
0	0.5	0	$1.1 \pm 0.1^{a}$	$0.2 \pm 0.1^{a}$
0	0.5	0.5	$1.0 \pm 0.2^{a}$	$0.6 \pm 0.1^{b}$

Table 2. Effects of DFMA ± agmatine on organ formation in isolated leafy spurge hypocotyl segments grown on 10-fold diluted medium.

Concentration (mM)		Number per s	egment ± SE
DFMA	Agmatine	Shoots	Roots
0	0	$1.40 \pm 0.12$	$0.44 \pm 0.05$
0.5	0	$0.28 \pm 0.06^{a}$	$0.15 \pm 0.03^{2}$
0	1.0	$0.17 \pm 0.05^{a}$	$0.14 \pm 0.04^{2}$
0.5	1.0	$0.03 \pm 0.02^{a}$	$0.06 \pm 0.02^{2}$

<sup>&</sup>lt;sup>a</sup>Differs from control (p < 0.05).

<sup>&</sup>lt;sup>b</sup>Differs from DFMA alone (p < 0.01).

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# INFECTION AND MORTALITY OF THE SUGARBEET ROOT MAGGOT (DIPTERA:OTITIDAE) $_{\rm FOLLOWING}$ APPLICATION OF ENTOMOPATHOGENIC NEMATODES

C. A. Wozniak, G. A. Smith, and L. G. Campbell USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58105

Entomopathogenic nematodes, Steinernema carpocapsae, S. feltiae, S. glaseri and Heterorhabditis bacteriophora, were all determined to infect, reproduce and successfully exit third instar sugarbeet root maggot (SBRM) larvae (Tetanops myopaeformis Röder) during in vitro bioassays. Approximately 270 infective juveniles (IJ) were applied to 5 g of sterile, coarse sand in a multi-well dish containing 3 SBRM larvae/well. This approximated a field rate of 7.5 billion IJ/hectare. After 3 days of incubation at 24°C, larvae were rinsed and moved to plaster mounts. Infectivity percentages ranged from 1.3 to 8.6%, varying with species and strain of nematode used, as calculated from observations of exiting IJ at 14 and 21 days. It was noted in some bioassays that infected SBRM larvae (as evidenced by presence of subcuticular IJ) failed to demonstrate reproduction of nematodes. Studies on the endogenous flora of SBRM indicated that bacterial species vary with source of larvae, and it is hypothesized that antagonistic SBRM microflora may have precluded septicemic establishment by the nematode symbiont, Xenorhabdus spp., in some instances.

During laboratory challenges of fully diapaused third instar SBRM, an enhanced rate of pupation also was observed with challenges of all six strains of *Steinernema* tested. Sclerotization of SBRM larvae was usually initiated within 24 to 48 h post-exposure to IJ. Mature pupae were never observed to be infested with nematodes and many (31 to 60%) gave rise to imagos within 12 to 18 days. On average, 24.5% of the flies emerging from pupae were aberrant; saline controls did not yield any aberrants. Morphological aberrations included: vestigial or absent wings, misshappened head capsules, unretracted ptilina, reduced external genitalia, reduced body size, poor body segmentation, and reduced cuticular sclerotization. This is the first demonstration of a sublethal effect influencing development of dipteran larvae following nematode challenge. The tactile or chemical stimuli between IJ and SBRM larvae that are responsible for this induced aberration are unknown. Aberrant adults arising from these challenges were functionally sterile. Mating attempts were noted during co-incubation with normal flies; however, no eggs were produced. Approximately equal numbers of male and female aberrants were produced; external genitalia were too distorted to discern sex in some instances.

Field application of steinernematid nematodes to a non-irrigated sugarbeet crop resulted in infected SBRM larvae with all six strains of the three species examined. Untreated controls were not, however, significantly (p=.05) different from treated plots with respect to % sucrose, tons/hectare, damage rating, and sucrose purity.

Adult SBRM were evaluated in vitro by exposure to IJ for 2 to 36 h. Co-incubation of lab reared or field collected flies and S. glaseri '326' nematodes (4100 IJ per 9 cm Petri dish) upon moistened filter paper discs approximated a field rate of 7.5 billion IJ/hectare. At various times after co-incubation, flies were removed and observed in multi-well dishes on coarse sand. Percent nematode infection was 42, 50, 70, 75 and 100 after 2, 4, 6, 18 and 36 h co-incubation, respectively. Flies were determined to be infected by microscopic examination of cadavers. Infected individuals typically succumbed to septicemia within 12 to 24 h of co-incubation. Egress of IJ was noted at 4 to 6 days post-infection. All six strains of Steinernema evaluated were found highly virulent on adult SBRM (relative to larvae); however, S. glaseri '326' and S. feltiae 'SN' were the most virulent strains with short co-incubation times. The high motility of these species may be responsible for the observed rapid infection rate.

The susceptibility of flies in vitro prompted a field assessment of nematodes for control of adult SBRM. S. feltiae 'SN' IJ were applied to moist sand:polyacrylamide (12:1, v/v) mixture, overlaying 2% (w/v) Gelrite, within a milk carton trap positioned adjacent to a sugarbeet field. Although adult emergence patterns precluded accurate assessment of % infection, infected adult SBRM were collected from field traps. Nematode survival was evaluated within the traps via addition of susceptible Galleria mellonella larvae. IJ of S. feltiae '27' were viable for at least 7 days under field conditions within the sand:polyacrylamide mix. Further evaluation of trap design, chemical attractants and nematode strain evaluation are planned for 1994 to assess the impact of fly reduction on larval populations.

#### EVALUATION OF POTATO SPROUT SUPPRESSION WITH NATURAL COMPOUNDS

Martin T. Glynn\*, Paul H. Orr, and Edward C. Lulai

Sprout control is an essential component of quality maintenance in stored potatoes. However, there is only one sprout inhibiting compound currently licensed for application to stored potatoes. This compound (CIPC) is a chlorinated carbamate developed in the 1950's. The potato industry requires a modern, natural means to control sprouting of stored potatoes.

We developed a multi-vessel, mid-scale (201b potatoes/vessel) storage system

to test naturally occurring organic compounds for their efficacy as potato sprout suppressants. Our system is designed to mimic a typical commercial potato storage environment, while allowing us to evaluate several natural compounds for sprout suppression properties (1).

Over the past three years, we have evaluated 16 compounds at three different concentrations using three different methods of application. The concentrations were selected to represent a high, a medium and a low application rate based on our knowledge of the materials and research by others (2,3). Application methods were: 1) fogging, compound was administered as a thermo mist into storage; 2) dipping, tubers were rotated in a tray containing the organic compound; and 3) volatilized, incoming ventilating air passed over a wick submerged in a solution of the compound. Example data from one of the application methods and some of the compounds are shown in Table 1; other results will be presented orally.

Table 1. Weeks before sprouting occurred following dipping treatments of high, medium or low concentration at harvest time.

<u>Treatment</u>		1991			1992	
	<u>High</u>	Medium	Low	High	Medium	Low
Untreated (control)	3	3	3	6	6	6
CIPC (reference)	9	9	9	15	15	15
1,8,Cineole (patented)	6	6	7	7	6	6
Menthol (patented)	6	6	7	10	10	10
Benzaldehyde (patented)	9	10	11	16	14	14
Salicylaldehyde (patented)	9	10	11	11	12	12
Cinnamaldehyde (patented)	5	5	4	-	_	-
Diisopropylnaphathlene	4	4	4	-	_	-
1,4, Dimethylnaphathlene	5	4	4	_	_	-
PRL -7 (patent pending)	7	10	12	13	15	16
PRL -14 (considering patent)	-	-	-	14	13	11
PRL -21 (considering patent)	-	-	-	12	12	10
PRL -11 (considering patent)	-	-	_	12	10	6
PRL -17 (considering patent)	-	-	-	9	7	6
PRL -23 (considering patent)	-	-	-	15	12	9

"CIPC was applied at 1% as a thermo mist into storage (per label).

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<sup>\*</sup>Martin T. Glynn, Fargo, 58105, Paul H. Orr and Edward C. Lulai, USDA-ARS, Red River Valley Potato Research Laboratory, East Grand Forks, 56721.

Thursday, 28 April

- 1:00 .. Sandstone Petrography as a Tool in Mapping Cenozoic Rock Units in Southwestern North Dakota.
  - Nels F Forsman\*, Edward C Murphy, and John W Hoganson Geology/Geological Engineering, U N D, Grand Forks 58202 and North Dakota Geologic Survey, Bismarck, ND, 58505
- 1:20 .. Preliminary Assessment of Zeolite Occurrences in North America. Nels F Forsman\* Geology/Geological Engineering, U N D, Grand Forks 58202
- 1:40 .. Progress Report: Fingerprinting and Correlation of Tuffs in North Dakota. Nels F Forsman\* and Richard D LeFever Geology/Geological Engineering, U N D, Grand Forks 58202 and North Dakota Geologic Survey, Bismarck, ND, 58505
- 2:00 .. North American Landforms Explained by Multiple-Step Deglaciation. Eric N Clausen\* Midcontinent Institute, Minot State University, ND, 58707
- 2:20 .. Ice Thickness in the Lake Agassiz Basin During the Wisconsinan Eric C Brevik\* and John R Reid Geology/Geological Engineering, U N D, Grand Forks 58202
- 2:40 .. Ice-Thrust Origin of Cooperstown Hill, North Dakota Paul A Brown and John R Reid\* Geology/Geological Engineering, U N D, Grand Forks 58202
- 3:00 .. Meltwater Origin for North Dakota's Most Recent Ice Sheets. Eric N Clausen\* Midcontinent Institute, Minot State University, ND, 58707

SANDSTONE PETROGRAPHY AS A TOOL IN MAPPING CENOZOIC ROCK UNITS IN SOUTHWESTERN NORTH DAKOTA

Nels F. Forsman<sup>\*1</sup>, Edward C. Murphy<sup>2</sup>, and John W. Hoganson<sup>2</sup>
<sup>1</sup>Department of Geology and Geological Engineering University of North Dakota, Grand Forks, ND 58202 <sup>2</sup>North Dakota Geological Survey, 600 E. Boulevard, Bismarck, ND 58505

An examination of sandstone petrographic character was conducted as part of a multi-year effort to distinguish and map middle Cenozoic rock units in North Dakota. The study was conducted as part of the Cooperative Geologic Mapping Program (COGEOMAP) between the North Dakota Geological Survey and the United States Geological Survey (1).

A major consideration in the mapping project was the desire to determine if petrographic characteristics of lithified sandstone units in the buttes of southwestern North Dakota could be used either to distinguish geologic formations and members or to serve as aids in determining stratigraphic level within the strata being considered. It was hypothesized that changes in sediment source areas, either geographic or climatologic, might be reflected in the petrographic (mineralogic) character of sandstones. Thus, sandstone petrographic data were gathered, with the prospect of using the information in support of or as a test of other evidence suggesting formal stratigraphic units.

Thin sections of sandstone samples from fifteen buttes were examined and point counted using standard petrographic techniques. Considerable differences in the effects of diagenesis were observable between many samples. All samples were described, but point count data were collected only from those samples with clear discernability between matrix and framework grains, and between

primary and secondary constituents.

The sandstones have been divided into two petrographic groups (Figure 1) using three criteria. Particularly noticeable in each thin section is the proportion of volcanic rock fragments; thin

sections from a given sandstone unit generally have either many or few such rock fragments. The two sandstone groups based on this criterion were also found to differ in the ratio of quartz to total feldspar, and in the type of mineral cement. Differences in these characteristics are attributable to changes in provenance, and to changes in burial setting and climate conditions insofar as these affect groundwater chemistry. It is reasonable to expect such changes with the passage of geologic time, and the two sandstone petrographic groups do appear to correspond to vertical separation of the sandstones in the geologic column. That is, one group occurs within what is interpreted to be Arikaree, Brule, or Chadron strata, and the other group occurs within more problematic rocks known to occur lower in the section, i.e., Golden Valley or Sentinel Butte Formation strata. Sandstones of the former group have volcanic rock Feldspars fragment contents averaging 26%, and quartz to total feldspar ratios averaging 1.6. Nearly all the sandstones of this group are cemented by forms of

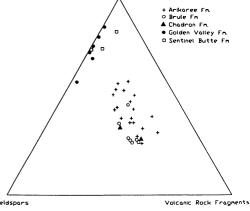


Figure 1. Ternary diagram depicting two sandstone

quartz, ranging from opal to fibrous or mosaic chalcedony to microcrystalline quartz. Sandstones mapped as occurring within the Sentinel Butte or Golden Valley Formations normally contain a very low proportion of volcanic rock fragments, and have a quartz to total feldspar ratio averaging These samples are normally cemented by zeolites, and occasionally by kaolinite: silica 3.84. (quartz) cement is absent.

In conclusion: Petrographic data was of use in distinguishing and mapping Cenozoic rock units in western North Dakota. In a few cases, the petrographic data did call into question and lead to correction of certain preliminary mapping interpretations.

Murphy, E.C., Hoganson, J.W., Forsman, N.F. (1993) NDGS Rept. of Invest. 96, 144 p.

#### PRELIMINARY ASSESSMENT OF ZEOLITE OCCURRENCES IN NORTH DAKOTA

Nels F. Forsman Department of Geology and Geological Engineering University of North Dakota, Grand Forks, ND 58202

7e0lites are minerals of important industrial use. And in sedimentary rocks, zeolites, as secondary products, reflect the composition of pore waters and depths of burial. Thus they are of potential value toward interpretations of paleoenvironmental conditions. summarizes results of preliminary study of several zeolite occurrences in North Dakota.

Zeolites have been detected in variable amounts in sandstones of the Sentinel Butte Formation (1, 2). In some samples, zeolites are abundant enough to be easily located in thin section, but they must be searched for in other samples. Many zeolites in the Sentinel Butte Formation occur as pore-filling subhedral and euhedral crystals ranging in size from <10  $\mu$ m to 200  $\mu$ m in maximum dimension. Growth of these crystals post-dated the development of pore-lining clay cement, clearly indicating that the zeolites formed as chemical precipitates from pore fluids. Thuse nore-filling zeolites are interpreted to be members of the heulandite structural group, based on crystal morphology and composition: crystals were not present in amounts suitable for x-ray analysis. Analcime was also detected in some Sentinel Butte sandstones. In some samples, this zeolite cement was found by examining sand-size aggregates to see what enabled them to survive ultrasonic treatment. In other samples, analcime appears as the dominant cement in some fairly well indurated sandstones. On Sentinel Butte itself, massive concentrations of sand-size analcime crystals occur in multiple thin layers associated with a lignite seam. Still another zeolite, chabazite, occurs as the major cement in Sentinel Butte Formation sandstone capping Black Butte, in Slope County.

Sandstone of the Golden Valley Formation, near the top of Bullion Butte, in Billings County, contains a significant amount of analcime cement in the form of crystal clusters. Additional study of zeolites in the Golden Valley Formation, including a comparison with zeolite species in the underlying Sentinel Butte Formation, is needed.

Large amounts of the zeolite species erionite are present in tuffaceous units of the Arikaree Formation, in the Killdeer Mountains of Dunn County (3). The sequence of diagenesis in those units involved partial dissolution of volcanic glass shards, followed by precipitation of porelining montmorillonite, in turn followed by the growth of elongate erionite crystals which project outward into remaining pore spaces, using the montmorillonite coatings as a substrate. It is not clear whether these Killdeer Mountain zeolites formed strictly as a result of groundwater interactions with glass shards following burial, or as a result of a more closedsystem process involving reaction of glass shards with water trapped during initial sedimentation of the ash in a saline-alkaline lake.

A few additional zeolite deposits occur in North Dakota, but have not been adequately evaluated for inclusion in this report. The first commercial deposit of zeolites in the United States was not reported until 1957. Maps of zeolite occurrences in the U.S. still do not include the deposits in North Dakota. This is an oversight not only with regard to a general inventory of zeolite resources, but also with regard to recognizing the scientific value of North Dakota's rocks.

<sup>1.</sup> Forsman, N.F. (1985) Ph.D. Diss, Univ North Dakota, Grand Forks, 222 p.

Murphy, E.C., Hoganson, J.W. and Forsman, N.F. (1993) NDGS Rept of Invest 96, 144 p.
 Forsman, N.F. (1986) NDGS Rept of Invest 87, 13 p.

#### PROGRESS REPORT: FINGERPRINTING AND CORRELATION OF TUFFS IN NORTH DAKOTA

Nels F. Forsman\* and Richard D. LeFever Department of Geology and Geological Engineering University of North Dakota, Grand Forks, ND 58202

At least five stratigraphically distinct tuff units occur in North Dakota. Because tuffs are isochronographic, i.e, were deposited during an "instant" of geologic time, they have excellent potential as stratigraphic marker beds. And if fingerprints of tuffs can be determined, they can be of use toward regional, rather than simply local, correlations. Thus, the tuffs in North Dakota are of potential use to paleontologists, to sedimentologists and stratigraphers, and to other geologists whose interests and use of tuffs might lead to paleogeographic and paleoecologic reconstructions and interpretations of tectonic history.

Tuffs in North Dakota include: 1) tuff or tuffs associated with the "burrowed marker unit" in the Killdeer Mountains, Dunn County (1, 2, 3); 2) the Antelope Creek tuff in Stark County (2, 3); 3) the Sentinel Butte tuff in McKenzie County (2, 4, 5); 4) the Marmarth tuff in Slope County (3, 4, 5); 5) the Linton tuff in Emmons County (2, 4); and 6) an unnamed tuff in Sloux County (2). This report describes the progress made in attempts to fingerprint and correlate several of these tuffs.

The Sentinel Butte tuff occurs over an area of nearly 1500 square miles (2). It can be traced visually for many miles in the Little Missouri Badlands where it typically occurs above a prominent clay covered bench. It is sporadically exposed within the prairie immediately north of the North Unit of Theodore Roosevelt National Park and is seen as far north as the southern wall of the Missouri River Valley along the northern border of McKenzie County. The most detailed description of this tuff is found in Forsman (5). Whole rock major and trace element data have been collected (5, 6), but an unequivocal fingerprint has not yet been determined. The primary value in obtaining a fingerprint for this tuff would be to examine potential correlation with upper Paleocene tuffs from other basins, should they be discovered. Additionally, the lower portion of this tuff has nearly everywhere altered to bentonite, which raises a perhaps unparalleled opportunity for testing various hypotheses about the alteration of glass to clay. For example, do tuffs that have altered in terrestrial settings preserve a decipherable fingerprint within the secondary clay products, or do variations in diagenetic conditions along the course of terrestrial tuffs make such bentonite fingerprints unlikely? A current collaborative study with G.H. Shaw of Union College involves a comparison of the rare-earth composition of apatite phenocrysts from the Sentinel Butte tuff and its associated bentonite. This will address the question of whether apatite grains are significantly affected by the diagenesis of tuff, and thereby provide a check on the utility of using apatite rare-earth compositions as fingerprints for bentonites.

A comparison of several samples of tuff from the burrowed marker unit in the Killdeer Mountains and tuff from two localities in Stark County has recently been completed (3). The statistical comparison of rare-earth composition of glass separates was aimed at testing the correlation of each of these units. Results reveal that the two Stark County tuff deposits represent a single tuff which has been given the name Antelope Creek tuff (3). Samples from the Killdeer Mountains are apparently not correlative with the Antelope Creek tuff. The burrowed marker unit in the Killdeer Mountains consists of normally eleven friable sandy tuff beds separated by indurated layers of calcite-replaced tuff. More detailed study is needed to answer the question of how many ash accumulation events are recorded within this unit. It remains possible that materials from multiple eruptions have become undecipherably mixed together on the floor of the lake interpreted

to exist at the time and site of deposition of the burrowed marker unit.

In conclusion: Progress has been made toward correlating and fingerprinting tuffs in North Dakota but additional opportunities for study exist.

Forsman, N.F. (1992) NDGS Misc Series 76, pp 267-272. 2.

<sup>1.</sup> Forsman, N.F. (1986) NDGS Rept of Invest 87, 13 p.

Murphy, E.C., Hoganson, J.W., Forsman, N.F. (1993) NDGS Rept of Invest 96, 144 p. 3.

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<sup>6.</sup> Larsen, R.A. (1988) M.S. Thesis, Univ. North Dakota, Grand Forks, 163 p.

#### NORTH AMERICAN LANDFORMS EXPLAINED BY MULTIPLE-STEP DEGLACIATION

Eric N. Clausen\*
Midcontinent Institute, Minot State University, Minot, ND 58707

Drainage network analysis shows most North American landforms developed in response to a thick "late Tertiary" ice sheet with meltwater radiating outward to the Pacific, Gulf of Mexico, and Atlantic. The ice sheet collapsed, probably during "Pliocene" time, with inward-flowing meltwater first funneled through the Mississippi Valley (or a Hudson-St. Lawrence outlet); second, north to Hudson Bay; and third, trapped on the former ice sheet floor—where meltwater repeatedly froze to produce a series of short-lived thin ice sheets. The thick ice sheet is documented by the presence of a large "hole," which formed due to deep glacial erosion as described by White (1) and crustal downwarping caused by ice sheet weight. The "hole" perimeter is identified by drainage divides which separate outward-oriented drainage, which flowed directly to adjacent oceans, from inward-oriented drainage, which flowed to "hole" outlets—the Mississippi Valley and Mohawk-Hudson-Lake Champlain-St. Lawrence valley complex, and later "preglacial" north-oriented valleys (e.g. Red River Valley). The "hole" perimeter is today the east-west continental divide and the Missouri-Arkansas, Ohio-Gulf of Mexico, Ohio-Atlantic, and Lake Ontario-Atlantic drainage divides.

Several lines of evidence suggest outward-oriented drainage networks formed in response to large volumes of water radiating out from the "hole" perimeter. First, drainage networks themselves document large outward-oriented flow events. These drainage systems include the St. Lawrence, Connecticut, Hudson-Mohawk, Susquehanna, Potomac, James, Roanoke, Peedee-Yadkin, Santee, Savannah, Apalachicola-Chattahoochee, Mobile (Alabama and Tombigbee), Mississippi, Brazos, Rio Grande, Colorado, Columbia (Snake, Salmon, Clark's Fork, and Kootenai), and Fraser river systems. Second, large erosional escarpments generally face outward and formed in response to outward-oriented flow events. Examples include the Catskill and Pocono escarpments, Allegheny Front, Blue Ridge Escarpment, Caprock Escarpment, and Mogollon Rim. Third, deep erosion of outward-oriented drainage basins is documented by numerous outward-oriented water gaps, wind gaps, and deep canyons. Fourth, rapid erosion and transport of alluvium during outward-oriented flow events is documented by alluvial fans and fan-like deposits of coarse-grained alluvium found at mouths of outward-oriented valleys and canyons. For example, Campbell has described a large alluvial fan deposited by the Potomac River (2).

Headward erosion along what began as a Mobile-Alabama-Coosa-Upper Tennessee drainage route and later a Mississippi-Tennessee drainage route, diverted large volumes of what had been Atlantic-oriented flow to the Gulf of Mexico. Likewise, headward erosion by the Rio Grande and by what began as a direct Wyoming-Gulf of Mexico route (later progressively captured by headward erosion of the Red, Arkansas, and Platte drainage networks) also diverted what had been Pacific-oriented flow to the Gulf of Mexico. Capture of Atlantic- and Pacific-oriented flow by headward erosion along Gulf of Mexico-oriented drainage routes, similar to captures which can be observed north of the present-day Vatnajökull Ice Cap, increased Gulf Stream strength, which in turn transferred heat to northern latitudes and increased rates of ice sheet melting. Ameliorating "Pliocene" climates, fueled by increasing meltwater flow to the Gulf of Mexico, progressively increased ice sheet wastage rates, as in a chain reaction, and led to thick ice sheet collapse. This interpretation is consistent with observations that a slight "Pliocene" cooling occurred in subtropical and tropical regions while North Atlantic water temperatures significantly increased (3).

Ice sheet collapse opened up drainage routes to Hudson Bay and the Arctic Ocean and, once initiated, headward erosion by north-oriented drainage systems rapidly captured south-oriented flow. Evidence for this drainage reversal includes numerous sequences of abandoned headcuts and of stream captures which demonstrate that large volumes of outward-flowing water reversed direction to flow into the "hole" and then north. A continent-wide drainage reversal of this magnitude weakened the Gulf Stream, by reducing flow to the Gulf of Mexico, and significantly strengthened the Labrador Current, by increasing flow to the Arctic. The resulting climate deterioration then froze north-oriented meltwater to form a thin ice sheet, with thick ice sheet remnants embedded within it, and forced another drainage reversal—this time diverting flow back to the Gulf of Mexico. Subsequent weakening of the Labrador Current and strengthening of the Gulf Stream again triggered climate amelioration, which ultimately led to thin ice sheet melting and collapse, initiating a new climatic cycle. Climatic conditions continued to oscillate, like a yo-yo, until sufficient meltwater had left the continent that south-oriented meltwater flow was no longer possible.

A "Pliocene" or "early Pleistocene" age for thick ice sheet melting is consistent with published interpretations of sediments and geomorphic features ascribed to both outward- and inward-flowing meltwater (4) and to climatic conditions which triggered thick ice sheet collapse (3) while a "Pleistocene" age is consistent with published reports of an episode of oscillating climatic conditions (5). However, the length of time required to establish climatic equilibrium, by melting of thick ice sheet remnants and drainage of all meltwater from the North American continent, may have been much shorter than published age dates suggest.

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#### ICE THICKNESS IN THE LAKE AGASSIZ BASIN DURING THE WISCONSINAN

Eric C. Brevik\* and John R. Reid Department of Geology and Geological Engineering University of North Dakota, Grand Forks, ND 58202

Post-glacial rebound has had a profound influence on those living in the Lake Agassiz Basin. Because rebound has been greater in the northern parts of the basin and rivers in the basin flow north, the decreasing gradient of the rivers has resulted in changes such as increased flooding within the basin(1). The key to understanding the character of this problem and to making well-informed predictions as to what to expect in the future lies in understanding the processes which led to these changes. To determine how much the crust was depressed and, therefore, how much rebound to expect, we must determine how thick the former ice mass was.

The most direct method of measuring rebound is from the strandlines left by glacial Lake Agassiz. Because the entire Herman strandline presumably rebounded, with the northern end rebounding more because the ice had melted from that end later, the difference in elevation of the two ends of the strandline represents absolute minimum rebound. The 54.5 m elevation difference of the Herman reflects a minimum ice thickness of 160 m, assuming an ice density of  $0.90~\text{g/cm}^3$  and a crust density of  $2.67~\text{g/cm}^3$ . But, because as much as 73% of rebound is restrained, i.e., occurs as the ice is thinning(2), the ice may have been up to 660 m thick. This would cause a depression of as much as 200 m. But, the restrained rebound may have been retarded as ice was replaced by Lake Agassiz water (density 1.00 g/cm³) and sediments (density 2.00 g/cm³). The average depth of Lake Agassiz at Grand Forks was as much as 100 m, and the sediments eventually accumulated to an average thickness of as much as 46 m(4). These masses would cause crustal depressions of 38 m and 40 m, respectively.

These values can be checked in other ways. Mathews proposed a method for determining ice thickness based on change in the elevation of lateral moraines(3). Using his values, former ice thicknesses for the Grand Forks area range from 245 to 625 m.

It is fairly certain that ice thickness exceeded the minimum of 160 m calculated from the Herman. Mathews' method gives values that indicate between 23.6% and 71.6% of the rebound in the Grand Forks area was restrained. In addition, several beach and scarp remnants have been found as much as 30 m above the Herman strandline(5), indicating that the Herman does not represent the very earliest stages of Lake Agassiz. On the other hand, the water and sediments of Lake Agassiz presumably slowed rebound. With all factors considered, ice thickness was most likely about 450 m, only.

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#### ICE-THRUST ORIGIN OF COOPERSTOWN HILL, ND

Paul A. Brown and John R. Reid Department of Geology and Geological Engineering University of North Dakota, Grand Forks, ND 58202

Cooperstown Hill is an elongate ridge (1.6km by 400m) north of Cooperstown, ND. It trends east-west, with its steeper side facing east. It has been most recently mapped as a till-veneered thrust mass (1). Recent excavation of the ridge has exposed a complex of sediment units, allowing for a better interpretation of its origin. The three main units are: sand and gravel, diamicton, and shale masses (Fig. 1). The sand and gravel beds are well sorted to poorly sorted. The well-sorted beds contain the most abundant reverse faults, but these are not limited to such beds. This unit is interpreted to be of glaciofluvial origin.

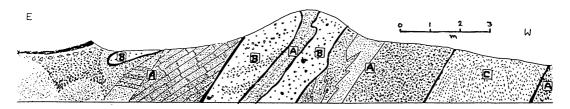


Figure 1. Map of vertical exposure at Cooperstown Hill (Unit A= sand and gravel; B= diamicton; C= shale mass)

The diamicton beds are silty, matrix-supported deposits, and contain many large clasts of locally-derived Pierre shale. Because of their position in the exposure and their relatively thin character it is likely that they are not orthotills, but some type of flow deposit, e.g., a debris flow.

The third unit is from the Cretaceous Pierre Formation, which is at or near the surface in this area (2). Where present in the pit, the shale is highly deformed.

The morphology of the hill indicates that it could be a drumlin, an overridden esker, a segment of an end moraine, or a thrust mass. The bedding structures are similar to those formed by flow into a subglacial cavity, like the Velva "drumlins" (3). However, such bedding structures would tend to be oriented normal to the elongation which would be parallel to ice flow (4). A drumlin origin is therefore rejected. Although the orientation is parallel to other eskers in the vicinity, the composition and form leads to rejection of that origin, too. The fact that the ridge is oriented parallel to eskers and normal to the Cooperstown "end moraine" (thrust ridge) (1) also leads to its rejection as an end moraine segment, leaving a thrust ridge interpretation as the most likely one.

The internal structures of Cooperstown Hill indicate shear stresses from the eastnortheast. In addition, the elongation is parallel to eskers, to fluting ridges between 7 and 8km to the northwest (5), and normal to Cooperstown "end moraine." It must therefore be a longitudinal thrust mass. The identification of a depression of similar size 1.3km to the north-northeast allows for an interpretation as a hill-hole pair (6). The thrust mass was formed during an advance from the east-northeast, probably immediately prior to the Luverne drift phase.

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#### MELTWATER ORIGIN FOR NORTH DAKOTA'S MOST RECENT ICE SHEETS

Eric N. Clausen\*
Midcontinent Institute, Minot State University, Minot, ND 58707

This paper reinterprets North Dakota landforms in the context of a "late Tertiary" ice sheet and a glacially produced North American "hole" (1). North Dakota's northeast-trending slope is located on the "hole's" southwest wall. Deep glacial erosion removed from northeastern North Dakota more than 1000 meters of Cretaceous and Paleocene strata. These strata can be observed in southwestern North Dakota. North Dakota's northeast-trending drainage network developed when what had been south- and west-flowing meltwater reversed direction and flowed north into and through the "hole." Study of alluvium scattered across southwestern North Dakota and topographic map analysis permitted reconstruction of at least three distinct meltwater episodes. The first episode occurred during "Oligocene" time while the final episode ended in "late Pleistocene" time.

The "Oligocene" meltwater episode cut several deep narrow valleys which were subsequently back filled with White River Group and overlying sediments. Evidence for high energy streams includes numerous cobbles and small boulders contained within thick White River Group conglomerates (2). Later, additional high energy streams cut another sequence of deep valleys in northwest South Dakota (3). North Dakota's limited sequence of "Oligocene" and "Miocene" sediments can be explained by a small number of such flow events.

The second meltwater flood episode occurred when the thick ice sheet collapsed inward and is documented by asymmetric drainage divides, abandoned headcuts, high-level divide crossings, stream captures, and alluvial deposits (4, 5, 6). Meltwater, which had been flowing south, reversed direction and flowed north into and through the "hole." Isolated buttes document the significant bedrock thicknesses which were stripped from much of southwest North Dakota and adjacent regions. North Dakota's northeast-trending drainage networks were formed as part of a continuous sequence of headcuts and drainage basins. The sequence began with southoriented flow (e.g. Trinity and Sabine rivers) and then changed to southeast-oriented flow (Red and Arkansas rivers), east-oriented flow (Kansas and Platte rivers), northeast-oriented flow (Cheyenne River), and finally north-oriented flow (Little Missouri, Powder, Bighorn, Yellowstone, Musselshell, and Upper Missouri rivers). The headcuts; including the Red Hills escarpments of south central Kansas, the Goshen Hole escarpments of Wyoming and Nebraska, as well as the Bates Hole and Beaver Divide escarpments of Wyoming; document both the rapidity of the erosion and the immense volumes of water involved. Once inside the "hole" meltwater first flowed south to the Mississippi outlet and later north, through and across the wasting ice sheet, to reach Hudson Bay. The Missouri Coteau, Turtle Mountains, and Prairie Coteau, characterized by hummocky collapsed glacial topography (often referred to as "dead-ice moraine"), were locations of thick ice sheet remnants. The Missouri and Pembina escarpments and escarpments surrounding the Prairie Coteau and Turtle Mountains formed when meltwater flow carved broad lowlands between ice sheet remnants. Valleys, which cut the Missouri Coteau (e.g. north of Williston), were originally formed when meltwater broke through what had been the southwest ice sheet wall. These breaches explain the previously observed sequence of Northern Great Plains drainage basins (6)

The third flood episode, which immediately followed the second, occurred when north-flowing meltwater masses became trapped in the North American "hole," surrounded thick ice sheet remnants, and froze to produce a series of thin glacial lobes. These ice lobes blocked north-oriented drainage, forcing another drainage reversal which cut the Missouri Valley. This drainage reversal triggered climate amelioration and sufficient melting to restore north-oriented drainage. North-flowing drainage once again initiated freezing and the process continued until sufficient meltwater had drained from the continent that south-oriented meltwater flow was no longer possible. Multiple freeze-thaw cycles produced a series of frozen meltwater lobes—the James, Des Moines, Souris, and Leeds lobes in North Dakota—which record the gradual shrinkage of the trapped meltwater mass. Also in North Dakota, evidence for ice thrust masses (7), subglacially molded surfaces (8), and related landforms document the presence of wet-based, dynamic ice lobes, covering lowland regions, while thick "dead-ice moraines" document remnants of the thick ice sheet and earlier ice lobes. Lack of evidence for deep glacial erosion of the northeast-trending drainage network documents both the meltwater origin and the short-lived nature of the dynamic ice lobes.

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Friday, 29 April

1:40 .. Magnesium Deprivation Affects Macromineral Metabolism in Postmenopausal Women.

Forrest II Nielsen\*

USDA-ARS, Grand Forks Human Nutrition Research Center, 58202

- 2:00 .. Whole Body and Heel Retention of <sup>47</sup>Ca in Obese Women.

  Berislav Momcilovic\*, Henry C Lukaski, Glenn I Lykken and
  William A Siders
  USDA-ARS, Grand Forks Human Nutrition Research Center, 58202
- 2:20 .. Effects of Chromium Supplementation on Changes in Strength and Body Composition of Young Men During Strength Training. Henry Lukaski\*, William W Bolonchuk, William A Siders, David B Milne USDA-ARS, Grand Forks Human Nutrition Research Center, 58202 Health, Physical Education and Recreation, UND, Grand Forks, 58202
- 2:40 .. Body Composition, Somatotype and Nutritional Status Predictors of Strength Gains.
  W A Siders\* and H C Lukaski
  USDA-ARS. Grand Forks Human Nutrition Research Center, 58202
- 3:20 .. Mitochondrial Energy States and Ultrastructural Changes
  Associated with Copper Deficiency in Platelets.
  Samuel M Newman, Jr\* and W Thomas Johnson
  USDA-ARS, Grand Forks Human Nutrition Research Center, 58202
- 3:40 .. Library Least Square (LLSQ) Method for Analysis of <sup>65</sup>Zn and <sup>40</sup>K in Human Urine Samples.

  Liqiang Tao\*, Glenn I Lykken, Berislav Momcilovic
  Physics, University of North Dakota and USDA-ARS, Grand Forks Human
  Nutrition Research Center, 58202
- 4:00 .. Measurement of Polonium 210 in Hair by alpha particle Counting.

  Hassaan A Alkhatib\* and Glenn I Lykken

  Physics, University of North Dakota, Grand Forks, 58202

## MAGNESIUM DEPRIVATION AFFECTS MACROMINERAL METABOLISM IN POSTMENOPAUSAL WOMEN

Forrest H. Nielsen\*
USDA, ARS, Grand Forks Human Nutrition Research Center
Grand Forks, ND 58202

Magnesium (Mg) catalyzes or activates more than 300 enzymes in the body. Among the enzyme systems in which Mg has a role are those involved in the hydrolysis and transfer of phosphate groups, and in calcium (Ca) ion transport and utilization. Thus, it is not surprising that the induction of Mg deficiency through dietary restriction can be done with relative ease in young experimental animals. What is surprising is that no consequences of Mg deprivation have been described for humans in which only the dietary intake of Mg is restricted. Described cases of clinical Mg deficiency have virtually always been conditioned deficiencies. Thus, an experiment was performed with 13 postmenopausal women housed in a metabolic unit to ascertain whether the dietary intake of Mg [and boron (B)] affected macromineral metabolism. All women participated in four dietary periods of 42 days in which Mg supplements of 0 and 200 mg/day, and B supplemented at 0 and 3 mg/day, were varied in a Latin-square design. At an intake of 2000 kcal, the three-day menu rotation diet provided about 115 mg Mg and 0.23 mg B. Plasma and urine variables were determined by our usual methods (1,2).

Table 1. Effect of Dietary Magnesium on Calcium, Magnesium and Phosphorus Balance and Excretion, and Serum 25-OH-Cholecalciferol

Dietary Tre	atment	Calc	ium*	Magn	esium*	Phosp	horus*	Serum 25-OH-
Mg mg/d	B mg/d	Urine mg/d	Balance mg/d	Urine mg/d	Balance mg/d	Urine mg/d	Balance mg/d	Cholecalciferol** ng/ml
115	0.23	165	35	77	-24	578	42	24.8
115	3.23	168	30	76	-13	586	44	29.0
315	0.23	173	16	138	12	554	53	23.3
315	3.23	177	4	136	8	549	42	22.7
			Analy	sis of Varia	nce - P Valu	ies		
Boron effect		0.48	0.27	0.82	0.90	0.89	0.57	0.32
Magnesium ef	fect	0.02	0.04	0.0001	0.002	0.002	0.77	0.02
Boron x magn	esium	0.89	0.69	0.78	0.52	0.47	0.17	0.13
Root mean sq		0.013	0.038	0.0075	0.029	0.033	0.031	5.74

<sup>\*</sup>Average per day over the complete 42-day period.

The reduction in urinary excretion of Mg and negative Mg balance (Table 1) indicate that the dietary changes produced the desired effects on Mg status. Unexpectedly, however, Mg repletion at an amount near the recommended dietary allowance after Mg deprivation produced effects often considered undesirable in some variables associated with Ca metabolism. Mg repletion increased Ca excretion in the urine and decreased Ca balance. Additionally, it decreased serum 25-OH-cholecalciferol. Because of concerns about osteoporosis, regimens that decrease urinary Ca and increase Ca balance and serum 25-OH-cholecalciferol have been suggested as desirable for postmenopausal women. However, Mg deprivation in experimental animals results in soft tissue calcification, especially in the kidney (3). Perhaps the changes in Ca excretion and balance reflect changes in soft tissue, not skeletal, Ca accumulation or loss. Thus, whether the changes shown above are beneficial or detrimental remains to be determined. Reduced urinary phosphorus (Table 1) and no significant changes in urinary cyclic AMP or hydroxyproline (data not presented) suggest that the Mg supplementation was not markedly affecting bone. In summary, findings have been obtained that show Mg deprivation affects macromineral metabolism in postmenopausal women; the functional consequences of these effects remain to be determined.

<sup>\*\*</sup>Values from the last 21 days of each dietary period.

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<sup>3.</sup> Koh, E.T., Reiser, S. and Fields, M. (1989) J Nutr 119, 1173-1178.

#### WHOLE BODY AND HEEL RETENTION OF <sup>47</sup>Ca IN OBESE WOMEN

Berislav Momčilović,\* Henry C. Lukaski, Glenn I. Lykken and William A. Siders USDA, ARS, Grand Forks Human Nutrition Research Center Grand Forks, ND 58202

The determination of whole body (WB) retention of radiocalcium by using external gamma ray detectors is a standard method for assessment of calcium absorption of humans. However, the presence of the non-absorbed radiomarker renders it impossible to use WB counting as a reliable indicator of early gastrointestinal calcium absorption before the non-absorbed radiomarker is excreted. The aim of this study was to investigate the possibility that heels, which are distantly placed to gastrointestinal tract, can be used for an assessment of early <sup>47</sup>Ca absorption.

Twelve obese (Body Mass Index 30-46 kg/cm<sup>2</sup>) women aged 25-30 yr consumed a standard western diet balanced for essential nutrients. After an overnight fast, each woman received 2 µCi <sup>47</sup>Ca (Amersham, England) in 200 ml of apple juice. The isotope was allowed to internally equilibrate for 0.5 h before the <sup>47</sup>Ca WB radioactivity was assessed in a large WB counter for 10 min and at various time intervals thereafter (Table 1). The WB counter consists of two 16-detector (10x10x40cm) NaI(Tl) (Bicron Corp., Newbury, OH@) planar arrays each which provides 2.0x0.4 m of crystal surface. The detector outputs were connected to a computer-based pulse-height analyzer (VAX Station 4000 VLC, Canberra, Schaumburg, IL). To minimize the impact of individual variability in stature, the nosition of subjects was standardized by centering the umbilicus at the point 37.5% (golden ratio) of the bed length, measured from the cranial side. The heel (H) regional <sup>47</sup>Ca retention was assessed simultaneously with WB measurement by using a portable cylindrical 5x5 cm NaI(Tl) scintillation detector (see @). A lead-shielded top, to prevent cranio-caudal cross-radiation, was located between the heel bones below the medial tibial malleoli. WB and H retention data were matched to that of a cylindrical phantom with an approximate elliptical cross-section, and the lower limbs of a humanoid phantom (Alderson, Stamford, CT), respectively. The WB and H activity were assessed by using the Library Least Square and Window method, respectively. Total bone and heel bone mineral content were estimated by dual x-ray absorptiometry (QDR 2000, Hologenic Inc., Waltman, MA) and were found to be 2441.3 (304.5) and 22.6 (4.21) g, respectively [Mean (SD)]. <sup>47</sup>Ca activity in WB and H was expressed in percentage of administered dose.

Table 1. Kinetics of <sup>47</sup>Ca Retention in Whole Body and Heel Bone of Adult Obese Women (Mean ± SD)

und Treer B		Women (Wear 2 8D)
Time (h)	Whole body	Heels
	<sup>47</sup> Ca (%	dose)
	100.0 ± 0.04	10.0 ± 1.76
1.5		13.9 ± 1.81 15.2 ± 1.86
I -	$95.2 \pm 1.69$	
6		$16.6 \pm 1.84$
48 120	$68.8 \pm 8.23$	
192	$52.1 \pm 4.63$ $46.5 \pm 3.49$	$13.7 \pm 1.87$ $11.6 \pm 1.69$
	Total bone	Calcaneus
	<sup>47</sup> Ca (% do	se/g bone)
48	$2.84 \pm 0.48$	$76.2 \pm 18.8$
120	$2.16 \pm 0.30$	$63.3 \pm 16.7$
192	$1.93 \pm 0.27$	$53.5 \pm 13.5$

The activity in WB decreased by less than 8% over the first 6 h after the <sup>47</sup>Ca ingestion. An initial, rapid <sup>47</sup>Ca H uptake approached its saturation peak which indicated that <sup>47</sup>Ca "primary" absorption might have occurred within the first 3 h. The later small increase may be a consequence of enteral <sup>47</sup>Ca re-excretion and re-absorption cycles. At 48, 120 and 192 h, the ratio of <sup>47</sup>Ca disappearance from the WB and H was constant; this indicates that H adequately represents overall calcium metabolism in the skeleton. However, the amount of <sup>47</sup>Ca retained in H greatly exceeded its small share in total bone mass. Indeed, the specific activity of <sup>47</sup>Ca in H revealed that approximately 1% of total bone mass retained more than 10% of administered dose. These data strongly favor the possibility that absorbed 47Ca is unequally distributed within the skeleton and that the weight-bearing bones take up the highest proportion of <sup>47</sup>Ca. Perhaps the high <sup>47</sup>Ca uptake in weight-bearing bones reflects increased bone remodeling in response to kinematic-stress induced micro-structural damage. We may only speculate on how such presumable load-induced differences in <sup>47</sup>Ca bone distribution may be affected in the states of prolonged bed-rest, inactivity or weightlessness.

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## EFFECTS OF CHROMIUM SUPPLEMENTATION ON CHANGES IN STRENGTH AND BODY COMPOSITION OF YOUNG MEN DURING STRENGTH TRAINING

Henry C. Lukaski,\* William W. Bolonchuk, William A. Siders and David B. Milne USDA, ARS, Grand Forks Human Nutrition Research Center and Department of Health, Physical Education and Recreation University of North Dakota, Grand Forks, ND 58202

Chromium (Cr) is a mineral element that is purported to exert androgenic effects when it is consumed as a supplement to the diet, particularly in conjunction with a vigorous program of strength training (1). These beneficial effects of Cr have been ascribed to the compound Cr picolinate (CrPic) which has been suggested to enhance the bioavailability of Cr, as compared to Cr chloride (CrCl). We examined whether Cr supplementation exerts a beneficial effect on strength development and changes in body composition of men participating in strength training.

Thirty-six healthy men aged 18-30 yr were selected to participate on the basis of somatotype to enhance the probability of maximizing strength gain. One man did not complete the study. The men were assigned to groups based on somatotype and body composition; there were no differences in these variables by group. Each group received one of three supplements; CrPic, CrCl or placebo (starch). The supplements contained 176 and 173  $\mu$ g Cr in the CrPic and CrCl, respectively. There was no detectable Cr in the placebo.

The men participated in strength training for 50 min/d, five consecutive d/wk for eight wk. Each training session consisted of bench press, latissimus pull down (lat pull), leg press and curl. The training intensity was 1 repetition maximum after a warm up in the specific exercise. Maximal performance for each exercise was assessed every two wk; training intensity was changed accordingly.

Body composition was assessed by using anthropometry and dual x-ray absorptiometry before and during the last week of the training.

Weight training resulted in significant increases (p<0.0001) in the amount of weight lifted in each exercise in all treatment groups. There was no significant effect of Cr supplementation on relative changes [100% x (post - pre)/pre] in body weight, body composition or strength (Table 1).

Table 1. Percent Changes in Body Composition and Strength After Resistance Training

	CrCl n=12	CrPic n=12	Placebo n=11	F	p
Weight	$2.0 \pm 0.7*$	$2.4 \pm 0.6$	$0.9 \pm 0.6$	1.59	0.22
Fat	$2.0 \pm 3.1$	$0.3 \pm 2.6$	$-5.2 \pm 3.4$	1.48	0.24
FFMF**	$3.0 \pm 0.9$	$3.1 \pm 0.8$	$2.3 \pm 0.8$	0.28	0.76
Leg press	$52.9 \pm 6.5$	$47.7 \pm 4.6$	$41.3 \pm 3.2$	1.3	0.29
Leg curl	$27.0 \pm 5.8$	$25.2 \pm 6.2$	$14.7 \pm 2.8$	1.57	0.22
Bench press	$19.1 \pm 3.1$	$16.6 \pm 3.5$	$16.1 \pm 2.6$	0.27	0.77
Lat pull	$22.0 \pm 3.2$	$17.1 \pm 2.3$	$20.4 \pm 3.5$	0.70	0.50
Legs <sup>A</sup>	$45.8 \pm 6.0$	$41.1 \pm 4.3$	$33.7 \pm 2.7$	1.72	0.20
Upper body <sup>B</sup>	$20.4 \pm 2.8$	$16.3 \pm 2.3$	$18.2 \pm 2.9$	0.58	0.56
Total body <sup>C</sup>	$35.6 \pm 4.4$	$31.2 \pm 3.1$	$27.8 \pm 2.4$	1.28	0.29

<sup>\*</sup>Values are means ± SEM

These findings indicate that Cr supplementation, either in the form of CrCl or CrPic, has no beneficial effect on body composition or strength gain of young men seeking to maximize strength development.

ASum of leg press and curl

<sup>\*\*</sup>Fat-free, mineral-free mass

BSum of bench press and lat pull CSum of legs and upper body

<sup>1.</sup> Evans, G.W. (1989) Int J Bios Med Res 11, 163-180.

#### BODY COMPOSITION, SOMATOTYPE AND NUTRITIONAL STATUS PREDICTORS OF STRENGTH GAINS

W.A. Siders\* and H.C. Lukaski USDA, ARS, Grand Forks Human Nutrition Research Center Grand Forks, ND 58202

When screening volunteers for studies of strength resistance training, a concern is to select for optimum strength gaining potential. This seemingly implies that researchers select for good nutritional status, and a mesomorphic somatotype (1). The purpose of this study was to relate pre-training body composition, somatotype, and nutritional indices to resistance

training strength gains.

Thirty-five men, aged 19 to 30 years, were recruited to participate in resistance training (five days per week for eight weeks). Each workout consisted of three sets each of four exercises (leg press, leg curl, latissimus pull down and bench press) with the third set being a one repetition maximum (1 RM). Subjects underwent pre-training anthropometric measurements for somatotyping, dual x-ray absorptiometry for body composition assessment and a venipuncture for blood biochemical indices of nutritional status. The 1 RM resistance at the third workout was taken as the baseline strength index and compared to 1 RM resistance at the final workout to determine percent change in strength.

Table 1 lists the means and ranges of selected pre-training measurements and baseline 1 RM resistances. Also, Pearson correlation coefficients relating percent change in the sum of 1 RM resistances for the four exercises to anthropometric, compositional and biochemical measures are shown. Table 2 presents the multiple regression results of a maximum R² improvement calculation to predict percent change in the sum of 1 RM resistances.

The greatest strength gains were made by subjects with low baseline strength indices and low copper status indices. The best pre-training predictors of strength gain were indices of fat arms, lean trunk and low iron status. Notably, no other compositional variables nor somatotype indices correlated with strength gains. These findings suggest that pre-training body composition along copper nutritional status were significant predictors of strength gain.

Table 1. Descriptive Characteristics of Lifters <u>Mean</u> Range Height, cm 162 - 188 -0.118178 Weight, kg 80.8 66.0 - 111.5-0.096Sum of four skinfolds, mm 42.4 23.5 - 77.4 0.072 Endomorphy 3.7 2.0 - 6.00.041 4.4 2.0 - 7.5-0.274Mesomorphy 1.8 0 - 4.00.029 Ectomorphy Total body FFMF<sup>B</sup>, kg BMC<sup>c</sup>, g 63.4 54.5 - 87.0 -0.1362.92 2.15 - 4.25-0.261Fat, kg 14.3 4.1 - 30.1-0.049Fat, % 17.4 6.2 - 32.2-0.020Plasma Cu,  $\mu$ mol/L 97 70 - 131 -0.563\* 21.6 13.0 - 32.0-0.189Fe, μmol/L 1.98 1.70 - 2.220.036 Mg,  $\mu$ mol/L Zn,  $\mu$ mol/L 11.6 - 16.8 -0.29814.0 Platelet, 10°/L 255 149 - 341 0.112 Iron Status Hematocrit, % 45.7 42.0 - 48.00.081 140 - 167 Hemoglobin, g/L 157 0.105 24 - 267 Ferritin,  $\mu$ g/L 84 0.113 Copper Status Cp-ENZ<sup>E</sup>, mg/L 29.7 - 57.7 -0.413\* 41.6 Baseline 1 RM resistances, kg leg press 122 - 311192 -0.504\* leg curl 72 45 - 118 -0.603\*48 - 118 lat pull down 78 -0.370\* 86 41 - 134 -0.407\* bench press <sup>A</sup>Bicep, suprailiac, tricep and subscapular. BFFMF = fat-free, mineral free mass. <sup>c</sup>BMC = bone mineral content.  $^{\mathrm{D}}\mathrm{Cp-ENZ}$  = enzymatic activity of ceruloplasmin.

\*p < 0.05.

Table 2. Prediction of Strength Change variable parameter F probability estimate 0.44 0.522 Intercept -9.01 Bicep skinfold 12.46 49.4 0.0001 Subscapular skinfold -4.1331.19 0.0003 Arm fat 15.72 7.43 0.023 Plasma Fe -0.3519.43 0.002 Platelet count 0.19 18.3 0.002

<sup>1.</sup> Bolonchuk, W.W., Lukaski, H.C. and Siders, W.A. (1990) Med. Sci. Sports and Exercise, 22(2), S129.

#### MITOCHONDRIAL ENERGY STATES AND ULTRASTRUCTURAL CHANGES ASSOCIATED WITH COPPER DEFICIENCY IN PLATELETS

Samuel M. Newman, Jr.\* and W. Thomas Johnson USDA, ARS, Grand Forks Human Nutrition Research Center Grand Forks, ND 58202

Cytochrome c oxidase (CCO) is a mitochondrial enzyme that contains copper and is responsible for catalyzing the terminal reaction in the electron transport chain. Severe copper deficiency causes a 95% reduction in CCO activity in rat platelets (1). It may be expected that such a drastic reduction in CCO activity would impair electron transport and affect mitochondrial ATP production. However, total platelet ATP is reduced only 23% by copper deficiency (2). Thus, the biological consequences of reduced CCO activity caused by copper deficiency are not clear. Because mitochondrial CCO is profoundly affected by copper deficiency, the purpose of the present study was to investigate the possibility that copper deficiency produces ultrastructural modifications in mitochondria that could reflect changes in their energy state.

Male, weanling Sprague-Dawley rats were fed diets that contained either <1 µg Cu/g (CuD) or 6 μg Cu/g (CuA). After 35 days, platelet-rich plasma was obtained by low-speed centrifugation (160 x g for 20 min) of whole blood. Platelets were stabilized by adding 0.25 mL of 0.1% glutaraldehyde in 0.2 M sodium cacodylate (pH 7.4) to 0.25 mL of platelet-rich plasma. Following centrifugation (900 x g for 10 min), the platelet pellet was fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate, treated with 0.75% osmium tetroxide, dehydrated with ethanol and embedded in EMbed-812. Sections were stained with lead and uranium.

Table 1. Effects of Dietary Copper on Platelet Mitochondria (Mw)

DIET	Number Mw Examined	Number Mw with Dense Bodies	Number Mw in Condensed Configuration
CuD	109	57ª	71 <sup>b</sup>
CuA	75	24	16

<sup>\*</sup>Significant diet effect: Chi-square = 7.4, degree freedom = 1, p<0.01.

As shown in Table 1, the number of platelet mitochondria containing dense bodies and the number in the condensed configuration are elevated in rats fed CuD. Mitochondria in the condensed configuration exhibit dense matrix and swollen cristae. The condensed configuration is characteristic of mitochondria engaged in oxidative phosphorylation (OP)(3). An increase in dense bodies (DB) is also consistent with OP, as Ca2+ influx during OP would increase the size and number of DB (4). These findings suggest that the number of mitochondria performing oxidative phosphorylation increases during copper deficiency. An increase in the number of mitochondria engaged in oxidative phosphorylation may compensate for decreased CCO activity and may, therefore, partially explain why platelet ATP concentrations are only modestly affected by copper deficiency.

bSignificant diet effect: Chi-square = 34.02, degree freedom = 1, p<0.0001.

<sup>1.</sup> Johnson, W.T., Dufault, S.N. and Thomas, A.C. (1993) Nutr Res 13, 1153.

Dufault, S.N. and Johnson, W.T. (1993) Proc ND Acad Sci 47, 60. 2.

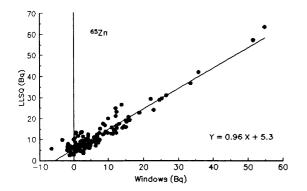
<sup>3.</sup> Hackenbrock, C.R. et al. (1971) J Cell Biol 51, 123.

Peachy, L.D. (1964) J Cell Biol 20, 95. 4.

## THE LIBRARY LEAST SQUARE (LLSQ) METHOD FOR ANALYSIS OF <sup>65</sup>Zn AND <sup>40</sup>K IN HUMAN URINE SAMPLES

Liqiang Tao,\* Glenn I. Lykken and Berislav Momčilović University of North Dakota Physics Department and USDA, ARS, Grand Forks Human Nutrition Research Center Grand Forks, ND 58202

The window method (W) and the library least-squares method (LLSQ) are used for elemental spectra analysis of data obtained with a multichannel pulse-height analyzer (MCA). The W method is based on evaluating contributions of radioisotope gamma ray emissions to selected channels of the MCA data after correction for background in each channel. The activities of radioisotopes are found by solving linear equations (matrices) corresponding to the spectral photopeak area(s) associated with each radioisotope. The LLSQ method is based on the fundamental assumption that the sum of the contributions of individual library components to each point (channel count) in a spectrum must equal that of the composite spectrum to be analyzed. The contribution of each individual library component is expressed by a mathematical formula and a matrix algebra technique is used to solve these equations (1). Although the high sensitivity and specificity of LLSQ for analysis of low level gamma ray activity is recognized, it has not been widely adopted because, until recently, only large computers were capable of processing the amount of data required for LLSQ.



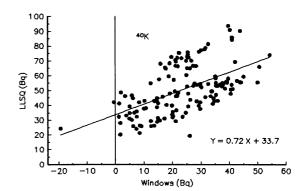


Fig.1a. Assessment of <sup>65</sup>Zn in human urine by LLSQ and W methods

Fig.1b. Assessment of <sup>40</sup>K in human urine by LLSQ and W methods

The purpose of this research was to use a personal computer to compare the results of the LLSQ method with those obtained with an advanced W method for the analysis of  $^{65}$ Zn in the presence of  $^{40}$ K in human urine samples. A total of 151 daily, bi-daily or tri-daily urine samples were collected in 4 L plastic containers that fully matched the cylindrical geometry of a counter with a 21 cm diameter x 4 cm NaI(TI) detector (Harshaw, USA). By plotting the  $^{65}$ Zn activity of samples assessed by LLSQ (Y-axis) against those assessed by W (X-axis), a linear relationship for  $^{65}$ Zn was found Y = 0.96 X + 5.3 with the 95% confidence interval at the intercept (Y) between 4.8% and 5.8% (Fig. 1a). Similarly, the relationship for  $^{40}$ K was found to be Y = 0.72 X + 33.7 with the 95% confidence interval at the intercept (Y) between 29.0% and 38.4% for  $^{40}$ K (Fig. 1b).

The detection limits of W approached zero at 5.3 Bq <sup>65</sup>Zn/L and 33.7 Bq <sup>40</sup>K/L above those for LLSQ for <sup>65</sup>Zn and <sup>40</sup>K, respectively (Fig. 1a and 1b). This result demonstrates a high specificity and greater sensitivity of the LLSQ method when compared to the W method for the analysis of <sup>65</sup>Zn in the presence of <sup>40</sup>K in human urine samples. Also, the high specificity and high sensitivity of the LLSQ method render it superior to the W method in simultaneous assessment of several gamma emitters at a low level of radioactivity. These calculations are within the reach of the computing capacity of standard personal computers.

<sup>1.</sup> Gardner, R.P., Wielopolski, L. and Verghese, K. (1977) Atom Energy Rew 15, 701-754.

#### MEASUREMENT OF POLONIUM-210 IN HAIR BY ALPHA-PARTICLE COUNTING®

Hassaan A. Alkhatib\* and Glenn I. Lykken Department of Physics, University of ND, Grand Forks, ND 58202-7129

The radiotoxicological risk of occupational exposure to 222Rn (radon) and its decay products (progeny) due to  $\alpha$ -particle emissions is well documented (1). Tobacco smoke has been linked to lung cancer (2). More recently however. documented environmental exposure to radon at home and school have become a concern of public health (3). Until recently it has not been possible to accurately determine radioactive radon progeny in biological samples directly (i.e. without radiochemistry). Semiconductor technology has been developed to the point where it is possible to purchase high purity, solid state alphaparticle detectors with ultra-low background counts (<0.5 counts/24 hour). These detectors have been used to measure  $\alpha$ -particle emissions from inorganic substances (4). The aim of this study was to determine if 210Po activity in hair from cigarette smokers (CS) and non-smokers (NS) could be accurately measured with solid state detectors (ULTRA, EG&G ORTEC, Oak Ridge, TN) and if hair 210 Po activity reflects ambient bedroom 222Rn concentration. Hair samples were collected from 14 persons including 7 CS, (4 men, 3 wowem) and 7 NS, (4 men, 3 women) whose bedroom <sup>222</sup>Rn concentrations had been measured with a standard charcoal canister method (5). Preparation and analysis of hair samples have been reported elsewhere (6). Measurable 210 Po activity was detected in all hair samples; results are given in the table below. The hair 210 Po activity was significantly correlated with ambient bedroom <sup>222</sup>Rn concentration, with Spearman correlation coefficient of r=0.60 and p=0.02. The measured (hair <sup>210</sup>Po)/(bedroom <sup>222</sup>Rn) ratio was greater in the CS group indicating that cigarettes may have been a contributor to hair <sup>210</sup>Po activity. The practical advantage of this method is the ability to plate polonium isotopes directly on to silver disks without the necessity of performing radiochemical separations(6). Finally, ultra-low background count rates allow a precision with respect to minimum detectable activity unobtainable with other common measurements (4).

Bedro	oom	<sup>222</sup> Rn c	oncentr <b>a</b>	tion and	hair <sup>210</sup> Po act	ivity in	cigar	ette smok	ers and n	on-smokers.
			CIGARE	TTE SMOK	ERS		No	ON-SMOKERS	3	
Subje	ect	Age	<sup>222</sup> Rn	<sup>210</sup> Po	<sup>210</sup> Po/ <sup>222</sup> Rn	Subject	Age	<sup>222</sup> Rn	<sup>210</sup> Po	<sup>210</sup> Po/ <sup>222</sup> Rn
(ID)		(y)	(kBq/m³)	(mBq/g)	(liter/kg)	(ID)	(y)	(kBq/m³)	(mBq/g)	(liter/kg)
WOMEN	N A	53	0.13	1.6	12.3	H	73	3.40	4.6	1.4
	B	36	1.80	4.0	2.2	I	52	0.06	0.8	13.3
	C	40	0.13	1.2	9.2	J	55	0.06	1.2	20.0
MEN	D	41	1.80	3.0	1.7	K	76	3.40	14.0	4.1
	E	33	0.13	2.0	15.4	L	28	0.56	1.4	2.5
	F	28	0.13	3.5	29.9	M	63	0.06	1.8	30.0
	G	16	0.13	12.0	92.3	N	54	0.13	5.2	40.0
Mean	(SD)	35(12	) 0.61(0	.8) 3.9(	3.7) 23(32)		57(1	6) 1.1(1.6	6) 4.1(4.	70) 16(15)

<sup>1.</sup> Morgan, M.V. and Samet, J.M. (1985) Health Physics 50: 656-662.

<sup>2.</sup> Martell, E.A. (1975) Amer. Sci. 63, 404-412.

<sup>3.</sup> Lees, R.E.M., Steele, R. and Roberts, J.H. (1987) Int. J. Epid. 16:7-12.

<sup>4.</sup> Harvey, B.R., Lovett, M.B. and Blowers, P. (1993) Appl. Radiat. Isot. 44: 957-966.

<sup>5.</sup> George, A.C. (1984) Health Physics 46:867-872.

<sup>6.</sup> Lykken, G.I. and Alkhatib, H.A. (1993) FASEB Journal 7:A676.

<sup>&</sup>lt;sup>a</sup> This work was supported by U.S. Environmental Protection Agency through the State Indoor Radon Grants Program and the North Dakota Department of Health and Consolidated Laboratories. under Contract 92-257.

Thursday, 28 April

3:40 .. Evolution of Naticid Gastropod Prey Selectivity in the North American Costal Plain.

Patricia Kelley, Thor A Hansen, Robert N Sickler\* Geology and Geological Engineering, U N D, Grand Forks, 58202 and Geology, Western Washington University, Bellingham, WA, 98225

4:00 .. Relationship of two Populations of Dallarca from the Saint Marys Formation (Maryland, Miocene).

Vicky D Andrews\* and Patricia H Kelley Geology and Geological Engineering, U N D, Grand Forks, 58202

4:20 .. Evidences of Paleopathology in a Series of Prehistoric Sites from South Dakota.

John A Williams Anthropology, U N D, Grand Forks, 58202

4:40 .. Stratigraphy and Paleontology of the Cretaceous Hell Creek Formation, Stumpf Site, Morton County, North Dakota. John W Hoganson\*, Johnathan M Campbell, and Edward C Murphy North Dakota Geologic Survey, Bismarck, ND, 58505

## EVOLUTION OF NATICID GASTROPOD PREY SELECTIVITY IN THE NORTH AMERICAN COASTAL PLAIN

Patricia Kelley<sup>1</sup>, Thor A. Hansen<sup>2</sup>, Robert N. Sickler\*<sup>1</sup>

<sup>1</sup>Dept. of Geology and Geological Engineering,
University of North Dakota, Grand Forks, ND 58202

<sup>2</sup>Dept. of Geology, Western Washington University,

Bellingham, WA 98225

Naticid gastropods have been important predators of molluscs since the Cretaceous. Naticids drill parabolic-shaped holes in the shells of their victims, producing a record of predation attempts that is amenable to study by paleontologists. This record of predation can be used to test hypotheses about the evolution of predator-prey systems, including Vermeij's (1) controversial "hypothesis of escalation." This hypothesis states that, during the Phanerozoic, biologic hazards have increased, as have adaptations to those hazards. The hypothesis predicts that the hazard of naticid predation increased through time, and that prey responded with antipredatory adaptations (such as increased armor).

To test this hypothesis, we have conducted a comprehensive survey of naticid predation on 44,000 mollusc specimens from the Cretaceous and Paleogene of the North American Coastal Plain. The survey includes a study of the history of naticid prey selectivity through time. Extant naticids select prey based on their cost-benefit ratios; cost is determined by drilling time (controlled by shell thickness) and benefit is based on biomass (dependent on internal volume of the shell). Because cost-benefit ratios generally decrease as prey size increases, naticids normally select the largest prey item with the thinnest shell. Previous work indicates that such selectivity occurred in the Neogene also.

We predicted that, if escalation occurred, prey selectivity by naticids may have developed through time, so that naticids were less selective of prey early in their history. To test this hypothesis, cost-benefit analyses were conducted for key prey species within several Paleogene Coastal Plain assemblages. Predator preferences predicted by cost-benefit analysis were compared with actual drilling frequencies for the Bashi, Cook Mountain, and Moodys Branch Formations.

Prey selectivity occurred in the lower Eocene Bashi Formation. Cost-benefit analysis predicted predator preferences for different size classes of three bivalve species, <u>Venericardia horatiana</u>, <u>Corbula subengonata</u>, and <u>Vokesula aldrichi</u>. As predicted, larger size classes of the three species were preferred (35-64% mortality) over smaller classes (16-31%), with differences significant at p<0.05.

Results for the other formations are more ambiguous. In the upper Eocene Moodys Branch Formation, drilling of all size ranges of <u>Spisula jacksonensis</u> was anomalously low (7%), while that of medium-sized <u>Lucina curta</u> was greater than expected (42%). In the Cook Mountain Formation of Virginia (middle Eocene), drilling on several size classes of the bivalves <u>Callista perovata</u> and <u>Lucina pomilia</u> were compared. All prey items were drilled at about the same intensity (16-23%), regardless of cost-benefit ratio. These results suggest that naticid drilling may have been less well developed in the Paleogene than in the Neogene or at present.

<sup>1.</sup> Vermeij, G.J. (1987) Evolution and Escalation: An Ecologic History of Life. Princeton University Press, Princeton.

#### THE RELATIONSHIP OF TWO POPULATIONS OF <u>DALLARCA</u> FROM THE ST. MARYS FORMATION (MARYLAND, MIOCENE)

Vicky D. Andrews\* and Patricia H. Kelley Department of Geology and Geological Engineering University of North Dakota, Grand Forks, ND 58202

A problem that arises frequently in paleontology is the evaluation of the relationship between two fossil populations. If the populations are extinct there is no readily available method to assess their genetic relationship. In such cases a statistical study of morphology is needed to determine the relationship of the populations.

Such a problem exists for two populations of <u>Dallarca</u> from the Miocene St. Marys Formation of Maryland. It has been questioned whether the populations are the same species or subspecies (1) or maybe different species. Although Ward (1) assigned both populations to <u>Dallarca idonea</u>, Ward and Blackwelder (2) had earlier suggested the older population was a separate species, informally referred to as <u>"chesapeakensa."</u> In general, <u>D. "chesapeakensa"</u> specimens are smaller in size and tend to resemble the juvenile forms of <u>D. idonea</u>. This could be due to the fact that <u>D. "chesapeakensa"</u> are juvenile <u>D. idonea</u>; alternatively, the small size could be a function of the environment (1). Neither of these may be the case; the populations may be subspecies of a single species, or different species altogether.

Kelley (3) collected samples from both populations: D. "chesapeakensa" from Little Cove Point on the western shore of Chesapeake Bay, and slightly younger D. idonea from the St. Marys River. Ten characteristics, including shell length, height, and width, muscle scar size and position, hinge area dimensions, and rib number, were measured for each specimen. A preliminary multivariate discriminant analysis was run to help determine the relationship of the two populations. A comparison between the calculated F value of 7.91 and the tabled F value of 1.88 (9 and 130 degrees of freedom, 95% confidence level) show that the specimens of the two populations are distinguishable from each other. Nevertheless, a graph of specimen scores on the discriminant axis indicated significant overlap between the two populations, indicating a possible subspecific relationship.

The discrimination of the two groups is in part based on size; anterior-posterior shell length was one of the variables that contributed highly to the discrimination (the others were valve width, cardinal area length, and muscle scar length). This suggests that the populations may have been differentiated primarily because different size ranges were represented. To test this hypothesis, additional discriminant analyses were performed that compared equivalent size classes of the two populations. For the 30-40 mm and 40-50 mm size classes, the two populations were still distinguishable statistically (95% level).

Ongoing research explores the growth patterns within each group in order to determine whether the two populations are indeed subspecies or could represent different growth stages of a single species.

<sup>1.</sup> Ward, L.W. (1992) Molluscan Biostratigraphy of the Miocene, Middle Atlantic Coastal Plain of North America. Virginia Museum of Natural History Memoir 2.

<sup>2.</sup> Blackwelder, B.W. (1977) person communication.

<sup>3.</sup> Kelley, P.H. (1983) Jour of Paleontology 57, 581-598.

#### EVIDENCES OF PALEOPATHOLOGY IN A SERIES OF PREHISTORIC SITES FROM SOUTH DAKOTA

John A. Williams\*
Department of Anthropology, University of North Dakota, Grand Forks, ND 58202

The human skeletal remains described here represent an assemblage from 10 different prehistoric sites (1). Recovery dates span a large portion of this century, from 1917 through 1992. Excluding the remains lacking provenience, all of the recovery locations lie east of the Missouri River. These are distributed in six counties approximately marking the perimeter limits of eastern South Dakota. Six locations involve recorded sites.

A total of 19 discrete individuals was identified from the human skeletal remains recovered from these 10 locations in South Dakota. Eleven individuals were adults, six males, four females, and one of undetermined sex. Eight were juveniles (<16 years of age), four of which were subadults. Among the latter one was sexed as male, and two as female.

The identified anomalies and pathological conditions fall into six descriptive categories (Table 1). These were tallied using the differential diagnosis on a case by case basis, rather than by individual. This permitted the recording of cases that occurred among commingled remains where individual associations were impossible. Variations of the same anomalous/pathological conditions when present in the same individual were treated as a single occurrence instead of being recorded separately. Twenty-eight anomalous/pathological conditions were identified. Trauma was the most frequently recorded. Osteoarthritis which is normally the most commonly observed pathological condition was slightly less than a third as frequent as trauma (2). Tumors were relatively infrequent. One case could not be clearly assigned to any specific category.

Table 1. The distribution of anomalous and pathological states.

Category	No. of Cases	Percentage
Trauma	9	31.0%
Osteoarthritis	6	20.7%
Inflammatory	5	17.2%
Metabolic	3	10.3%
Developmental Anomalies	3	10.3%
Tumors	1	3.4%
Unassigned	1	3.4%

Although these individuals are not part of a unified skeletal sample the identification of anomalous and pathological states can still provide a partial understanding of the health parameters of the populations from which they are derived. At least nine <u>individuals</u> displayed one or more anomalous or pathological states. These ranged in severity from asymptomatic developmental anomalies such as sacralization to severe arthritis and specific infection. Most of the identified pathological conditions were non life threatening and were at worst debilitating.

<sup>1.</sup> Williams, J.A. (1993) <u>Analysis of Miscellaneous Human Osteological Remains Recovered from Multi-County Areas of South Dakota</u>. Report submitted to the South Dakota Archaeological Research Center, Rapid City.

Williams, J.A. (1994) Health Profiles of Pre-Horticultural Peoples of the Northeastern and Middle Missouri Plains. In: D.W. Owsley and R.J. Jantz (eds) <u>Skeletal Biology in the Great Plains: A Multidisciplinary View</u>. Smithsonian Institution Press, Washington, D.C.

# STRATIGRAPHY AND PALEONTOLOGY OF THE CRETACEOUS HELL CREEK FORMATION, STUMPF SITE, MORTON COUNTY, NORTH DAKOTA

John W. Hoganson\*, Johnathan M. Campbell, and Edward C. Murphy North Dakota Geological Survey, 600 East Blvd., Bismarck, ND 58505

One of the thickest and best exposed outcrops of the Late Cretaceous Hell Creek Formation in the Missouri River trench area of North Dakota is located at the Stumpf site, 9 kilometers southeast of Huff, Morton County. Approximately 45 meters of Hell Creek strata and 9 meters of overlying Paleocene Ludlow Formation are exposed at this site (Figure 1). The palynologically defined Cretaceous/Tertiary (K/T) boundary occurs near the Hell Creek/Ludlow formational contact in this area (1). Lithologies of the Hell Creek Fm. consist primarily of interbedded fluvial and lacustrine sandstones, siltstones, and mudstones. Sandstone is the dominant rock type exposed at the site (Figure 1). It is from these fluvial sandstones that the most diverse Hell Creek fauna in the Missouri River trench area has been recovered. Five meters above the base of the site is a 1-meter-thick, fossilbearing marine sandstone (Figure 1, level C), the Breien Member of the Hell Creek Fm. The Breien sandstone documents the last Cretaceous marine incursion into the upper mid-continent.

At least 34 taxa of vertebrates and 9 taxa of invertebrates have been identified from the several hundred fossils recovered from the Hell Creek Fm. at the Stumpf site (Table 1). The vertebrate fossils occurred as disarticulated skeletal elements and the invertebrate fossils were found mostly as steinkerns. The nonmarine fossil assemblages (levels A and B) are dominated by dinosaur remains, particularly hadrosaurs. These assemblages, the farthest east Hell Creek fossil assemblages so far described, are similar to, but seemingly less diverse, than those being recovered from similar-age Hell Creek fossil sites in southwestern North Dakota. Less diversity in the Stumpf site assemblages may, however, be a sampling artifact. No unequivocal dinosaur fossils were found above level A, about 32 meters below the K/T boundary. Plant fossils, leaf impressions and seeds, were also collected but have not yet been studied.

Shark and ratfish fossils are the most common remains found in the shoreline sandstones of the Breien Member (level C). These fossils provide insight to the types of organisms that inhabited the last Cretaceous sea to cover south-central North Dakota. The Breien fauna is currently being studied by Hoganson.

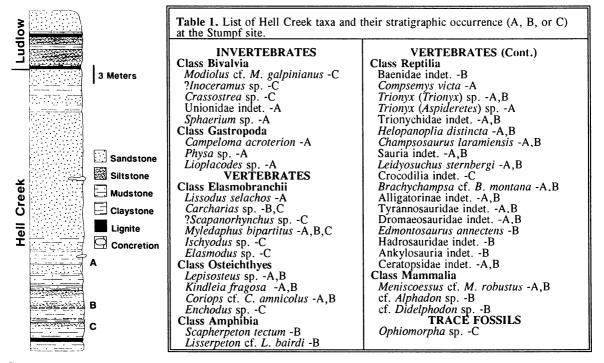


Figure 1. Geologic section at the Stumpf site and stratigraphic position of fossil assemblages.

<sup>1.</sup> Murphy, E. C., Hoganson, J. W. Nichols, D. J. and Forsman, N. F. (1993) Geol Soc Am Abs: 25(6):A-113.

Thursday, 28 April

8:40 .. Cloning, Expression, and Functional Characterization of Two New Mutant Human Polymorphic N-acetyltransferase (NAT2) Alleles. Ronald J Ferguson\*, Mark A Doll, Timothy D Rustan, Kevin Gray, David W Hein Pharmacology/Toxicology, UND School of Medicine, Grand Forks, 58202

9:00 .. Construction of Syrian Hamster Lines Cogenic at the Polymorphic Acetyltransferase Locus: NAT2-Dependent Metabolic Activation and Deactivation of Arylamine Carcinogens. David W Hein\*, Mark A Doll, Timothy D Rustan, Kevin Gray, Ronald J Ferguson, Yi Feng, Erik J Furman Pharmacology/Toxicology, UND School of Medicine, Grand Forks, 58202

9:20 .. Metabolic Activation of Aromatic and Heterocyclic N-hydroxyarylamine Carcinogens by Recombinant Human NAT1 and Wild-type and Mutant Recombinant Human NAT2 N-acetyltransferases. Mark A Doll\*, Timothy D Rustan, Ronald J Ferguson, Kevin Gray, David W Hein Pharmacology/Toxicology, UND School of Medicine, Grand Forks, 58202

9:40 .. Nuclear Translocation of Prolactin Requires Activation of Tyrosine Kinase and Protein Kinase C. Yi-ping Rao\*, Donna J Buckley, Mark D Olson, Arthur R Buckley Pharmacology / Toxicology and Anatomy / Cell Biology, UND School of Medicine, Grand Forks, 58202

10:20 .. Depletion of Cellular Glutathione Enhances Fumonisin B1 Toxicity in Pig Kidney (LLC-PK1) Cells. Y James Kang\*

Pharmacology/Toxicology, UND School of Medicine, Grand Forks, 58202

10:40 .. A Medium for the Selective Isolation of Corynebacterium Species from Mixed Flora Samples. Hua Tu\* and James R Waller Microbiology/Immunology, University of North Dakota, Grand Forks

11:00 .. Characteristics of Pyrazinamidase in Corynebacterium Species. James R Waller and Brian D Hass\* Microbiology/Immunology, University of North Dakota, Grand Forks

11:20 .. Use of Transformation/Insertion Mutagenesis to Isolate Cadmium Sensitive Mutants in Chalmydomonas reinhardtii. Joyce McHugh\* and Jonathan G Spanier Microbiology/Immunology, University of North Dakota, Grand Forks

## CLONING, EXPRESSION, AND FUNCTIONAL CHARACTERIZATION OF TWO NEW MUTANT HUMAN POLYMORPHIC N-ACETYLTRANSFERASE (NAT2) ALLELES

Ronald J. Ferguson\*, Mark A. Doll, Timothy D. Rustan, Kevin Gray, and David W. Hein
Department of Pharmacology and Toxicology,
University of North Dakota School of Medicine, Grand Forks, ND 58202

Acetyl coenzyme A (AcCoA)-dependent N-acetyltransferases (E.C.2.3.1.5) catalyze the N-acetylation of various therapeutic arylamine and hydrazine drugs and carcinogens from environmental, industrial, and dietary sources. N-acetylation phenotype may be a predisposing factor in a variety of drug-induced toxicities and arylamine carcinogenesis. Although N-oxidation via P4501A2 is the primary activation event, acetylation reactions are central metabolic deactivation (N-acetylation) and/or activation (O- and N,O-acetylation) steps in the biotransformation of arylamines and hydrazines to highly reactive electrophiles which can bind to nucleophilic sites of DNA and proteins. This binding is considered as the putative mechanism responsible for the underlying toxicity or initiation of carcinogenesis associated with these compounds. Humans possess two NAT loci (NAT1 and NAT2) which encode N-acetyltransferase isozymes that differ in substrate specificity. The NAT1 and NAT2 structural genes contain 870 bp open reading frames encoding 290 amino acid proteins. NAT2 is the "classical polymornhic" isozyme and inheritance via autosomal codominance at the NAT2 locus results in homozygous rapid, heterozygous intermediate and homozygous slow acetylators. In a previous study, we uncovered discrepancies between apparent NAT2 acetylator genotype based on polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP), in vitro colon arylamine N-acetyltransferase activity, and expected frequency of slow acetylator phenotype in African Americans, which suggested the presence of not yet defined mutant NAT2 alleles. PCR primers were synthesized using NAT2 sequence data with engineered EcoRI and SphI restriction endonuclease sites to facilitate cloning. Primer sequences were 5'-TTAG (GAATTC) ATG GAC ATT GAA GCA TAT TTT GAA AGA ATT-3' and 5'-AGT GAG TTG GGT GAT (GCATGC) ACA AGG G-3'. A 929 bp PCR product, containing the complete 870 bp coding region, was amplified via genomic DNA templates from African Americans with undefined NAT2 genotype. These NAT2 PCR products were cloned into pUC19 and sequenced by the dideoxy chain-termination method. Two novel NAT2 alleles were discovered. One allele (NAT2<sup>191</sup>) contained a point mutation at nucleotide 191 [G  $\rightarrow$  A (Arg  $\rightarrow$  Gln)], while the other allele (NAT2<sup>341/803</sup>) contained two point mutations [341T → C (Ile → Thr); 803A → G (Lys → Arg)]. To characterize the expressed enzyme from these NAT2 alleles, we developed a prokaryotic-expression system. The NAT2<sup>191</sup>, NAT2<sup>341/803</sup>, and NAT2<sup>wt</sup> alleles were subcloned into the tac promoter-based plasmid vector pKK223-3 for over-production of recombinant human NAT2 in E. coli strain JM105. Upon induction, all three NAT2 alleles expressed functional N-acetyltransferases capable of catalyzing both arylamine N-acetylation and the metabolic activation (via O-acetylation) of N-hydroxy-2aminofluorene (Tables 1,2). However, the NAT2<sup>191</sup> and NAT2<sup>341/803</sup> each exhibited significantly lower N- and Oacetylation capacity and were intrinsically less stable than NAT2wt. Partially supported by USPHS grant CA-34627.

TABLE 1. Recombinant Human NAT2 N-Acetyltransferase Michaelis-Menten Vmax

Apparent Maxin	num Velocity for N-Acc	etylation		
NAT2 Allele	2-Aminofluorene	4-Aminobiphenyl	3,2'-Dimethyl-4-aminobiphenyl	Glu-P-2
		(pmoles/	min/U)	
wt	$2304 \pm 59$	$2078 \pm 38$	$1793 \pm 83$	$1359 \pm 82$
191	$447 \pm 38^{h}$	$337 \pm 24^{h}$	$340 \pm 25^{h}$	$283 \pm 14^{h}$
341/803	212 ± 20 <sup>h</sup>	$173 \pm 16^{h}$	187 ± 14 <sup>h</sup>	179 ± 9 <sup>h</sup>

Table values represent Mean  $\pm$  S.E. for 4-5 individual determinations. Apparent V<sub>max</sub> for NAT2<sup>91</sup> and NAT2<sup>91/803</sup> are significantly lower than NAT2<sup>91</sup> following one-way analysis of variance with Bonferroni correction:  $^4p$ <0.01;  $^4p$ <0.001.

TABLE 2. Metabolic Activation of N-hydroxy-2-aminofluorene by recombinant human NAT2

NAT2 Allele	N-hydroxy-2-aminofluorene O-acetyltransferase activity
	(pmoles/min/mg DNA/U)
wt	197 ± 34
191	$104 \pm 12^{a}$
341/803	31 ± 8 <sup>h</sup>

Table values represent Mean  $\pm$  S.E. for five determinations. Activities for NAT2<sup>91</sup> and NAT2<sup>91</sup> are significantly lower than NAT2<sup>91</sup> following one-way analysis of variance with Bonferroni correction:  $^4p = 0.05$ ;  $^8p < 0.01$ .

# CONSTRUCTION OF SYRIAN HAMSTER LINES CONGENIC AT THE POLYMORPHIC ACETYLTRANSFERASE LOCUS: *NAT*2-DEPENDENT METABOLIC ACTIVATION AND DEACTIVATION OF ARYLAMINE CARCINOGENS

David W. Hein\*, Mark A. Doll, Timothy D. Rustan, Kevin Gray, Ronald J. Ferguson, Yi Feng, and Erik J. Furman Department of Pharmacology & Toxicology, University of North Dakota School of Medicine

Grand Forks, North Dakota 58202-9037

Human epidemiological studies have associated rapid acetylator phenotype with colorectal cancer incidence, suggesting metabolic activation of arylamines via polymorphic N-acetyltransferase (NAT2). Our laboratory constructed two separate sets (Bio. 1.5/H and Bio. 82.73/H) of Syrian hamster lines congenic at the NAT2 gene locus. In both sets of congenic lines, N-acetylation capacity was NAT2-dependent in vivo and in vitro, with highest levels in homozygous rapid acetylators (NAT2'), intermediate levels in heterozygous acetylators (NAT2'/NAT2') and lowest levels in homozygous slow acetylators (NAT2s). Genomic DNA from both sets of congenic hamster lines was enzymatically digested with different restriction endonucleases and subjected to restriction fragment length polymorphism analysis. The DNA fingerprints were indistinguishable between NAT2<sup>r</sup> and NAT2<sup>s</sup> acetylators of each congenic line. NAT1 and NAT2 acetyltransferase isozymes isolated from liver and colon cytosols of the congenic lines were tested for capacity to activate N-hydroxy-2-aminofluorene (N-OH-AF) to DNA adducts. NAT2-catalyzed metabolic activation of N-OH-AF was highest in NAT2', intermediate in NAT2', And lowest in NAT2's acetylator congenic hamsters. NAT1 also catalyzed metabolic activation of N-OH-AF, but at levels independent of acetylator genotype. NATI and NAT2 from rapid and slow acetylator congenic hamsters were cloned, sequenced, and expressed in a prokaryotic expression system. As shown in TABLE I, hamster NAT1 and NAT2 from rapid acetylator congenic hamsters exhibited high nucleotide and deduced amino acid sequence homologies with NAT1 and NAT2 from other species. As shown in TABLE II, recombinant NAT1 from rapid and slow acetylators activated N-OH-AF at equivalent rates, but recombinant NAT2' activated N-OH-AF at rates over 750-fold higher than recombinant NAT2'. These results provide clear evidence for metabolic activation of arylamine carcinogens by polymorphic Nacetyltransferase and provide mechanistic support for NAT2 acetylator genotype as a risk factor in arylamine-related cancers. Partially supported by USPHS grant CA-34627.

TABLE I. Nucleotide and deduced amino acid sequence homologies of congenic hamster  $NATI^{r}$  and  $NAT2^{r}$  with acetyltransferase genes from other species

Species	<u>Gene</u>	<u>Hamste</u>	r NATI¹	Hamster NAT2 <sup>1</sup>			
			Deduced		Deduced		
		Nucleotide	Amino Acid	Nucleotide	Amino Acid		
			Identity(%)				
Mouse	NAT1	86.3	83.8	83.8	82.0		
Human	NAT1	77.7	71.4	83.6	81.7		
Rabbit	NAT1	76.6	69.7	78.7	73.4		
Mouse	NAT2 <sup>r</sup>	82.1	78.6	90.5	93.1		
Human	NAT2 <sup>r</sup>	76.6	68.6	80.4	74.8		
Rabbit	NAT2 <sup>r</sup>	76.5	70.3	78.7	74.1		

TABLE II. Metabolic activation of N-hydroxy-2-aminofluorene by recombinant rapid and slow acetylator Syrian hamster NAT1 and NAT2 expressed in E. coli

v		<b>_</b>
Allozyme	Phenotype	N-OH-AF O-acetyltransferase activity <sup>a</sup>
NAT1 <sup>r</sup> NAT1 <sup>s</sup>	Rapid Slow	$3.75 \pm 0.40$ $3.52 \pm 0.33$
NAT2 <sup>r</sup> NAT2 <sup>s</sup>	Rapid Slow	$\begin{array}{c} 15.1  \pm  1.7 \\ 0.020  \pm  0.016 \end{array}$

a nmol/min/mg DNA/mg protein.  $NAT2^{S}$  significantly less than  $NAT2^{T}$  (p=0.0001).

# METABOLIC ACTIVATION OF AROMATIC AND HETEROCYCLIC N-HYDROXY-ARYLAMINE CARCINOGENS BY RECOMBINANT HUMAN NAT1 AND WILD-TYPE AND MUTANT RECOMBINANT HUMAN NAT2 N-ACETYLTRANSFERASES

Mark A. Doll\*, Timothy D. Rustan, Ronald J. Ferguson, Kevin Gray, and David W. Hein Department of Pharmacology and Toxicology, University of North Dakota School of Medicine, Grand Forks, ND 58202.

Aromatic and heterocyclic arylamines found in food, cigarette smoke and other sources, have been shown to cause cancer in humans. N-acetyltransferases (NAT) catalyze the acetylation of these arylamines. Humans exhibit a genetic polymorphism in hepatic N-acetylation capacity yielding rapid, intermediate, and slow acetylator individuals. Since biotransformation of these arylamines is necessary for their mutagenicity and their ability to cause cancer, it has been suggested that this polymorphism in acetylation capacity can predispose certain individuals to cancers related to arylamine exposure. Recombinant human NAT1 and polymorphic NAT2, wild-type and mutants (encoded by NAT2) alleles with mutations at 282/857, 191, 282/590, 341/803, 341/481/803, and 341/481) were expressed in Escherichia coli strains XA90 and/or JM105 to test their capacity to catalyze the metabolic activation (O-acetylation) of aromatic and heterocyclic N-hydroxyarylamines. Human NAT1 and NAT2 were amplified from genomic DNA templates using the polymerase chain reaction (PCR). The amplified products were cloned, sequenced, and expressed in a prokaryotic expression system. As shown below, both NAT1 and NAT2s (wild-type and mutants) are capable of catalyzing the metabolic activation of aromatic and heterocyclic hydroxyarylamines to DNA adducts. However, the various mutants of NAT2 showed a reduced capacity to catalyze the metabolic activation. To assess the relative importance of the NAT2 polymorphism, we looked at the NAT1/NAT2 activity ratios for various substrates. Ratios varied substantially with N-hydroxyarylamine substrate ranging from 13.9 for N-OH-ABP to 4.8 for N-OH-AF, to 1.9 for N-OH-IQ, to 0.09 for N-OH-PhIP and 0.06 for N-OH-MeIQx. Our findings suggest that a decrease in contribution by NAT2 in the metabolism of N-hydroxyarylamines, especially N-OH-PhIP and N-OH-MeIQx, could significantly alter the NAT1/NAT2 ratio and the total metabolism of that compound, thus making an individual more or less susceptible to certain cancers. Partially supported by USPHS grant CA-34627.

#### Metabolic activation of N-hydroxyarylamines by recombinant human N-acetyltransferases

					Metabolic Activation <sup>2</sup>		
Host	<u>Gene</u>	<u>Allele</u>	N-OH-AF	N-OH-ABP	N-OH-IO	N-OH-MeIOx	<u>N-ОН-РЫР</u>
				(	(pmoles/min/mg DNA/U)		
XA90	<i>NAT1</i>	WT	$946 \pm 343(5)$	$917 \pm 510(2)$	$28.4 \pm 11.3(2)$	3.1	3.5
XA90	NAT2	WT	$201 \pm 76(4)$	$66 \pm 35(2)$	$17.7 \pm 2.6(2)$	61.1	47.4
JM105	NAT2	WT	$197 \pm 34(5)$		$12.2 \pm 2.4(2)$	38.0	32.5
JM105	NAT2	191	$104 \pm 12(5)$		16.8	28.8	28.6
			(p = 0.0037)				
JM105	NAT2	282/857(M3)	$73.6 \pm 14.2(5)$		5.5	17.6	91.9
			(p = 0.0007)				
<b>JM</b> 105	NAT2	282/590(M2)	$31.4 \pm 13.6(5)$		$10.5 \pm 1.2(2)$	21.6	13.2
			(p < 0.0001)				
<b>JM</b> 105	NAT2	341/803	$30.8 \pm 8.2(5)$		8.2	14.5	15.8
			(p < 0.0001)				
<b>JM</b> 105	NAT2	341/481/803	$22.5 \pm 8.5(5)$		8.2	22.2	29.4
			(p < 0.0001)				
<b>JM</b> 105	NAT2	341/481(M1)	$6.4 \pm 5.0(5)$		$10.4 \pm 2.2(2)$	17.6	21.5
			(p < 0.0001)				

<sup>&</sup>lt;sup>a</sup> Values represent Mean ± S.E.M. The number of determinations are indicated in parentheses. Where no value is given, a single determination was carried out due to shortage of N-hydroxyarylamine substrate.

b NAT2 mutant activities tested for significant differences by one-way analysis of variance. Significant differences from JM105/NAT2<sup>wt</sup> are indicated in parentheses.

#### NUCLEAR TRANSLOCATION OF PROLACTIN REQUIRES ACTIVATION OF TYROSINE KINASE AND PROTEIN KINASE C

Yi-ping Rao\*, Donna J. Buckley, Mark D. Olson, and Arthur R. Buckley Departments of Pharmacology and Toxicology and Anatomy and Cell Biology University of North Dakota School of Medicine Grand Forks, ND

Prolactin (PRL) is an adenohypophyseal polypeptide hormone which exhibits growth factor properties in several experimental systems. PRL-induced mitogenesis reflects activation of multiple nuclear processes most notably transcription of growth-related genes. The initial step which ultimately leads to these nuclear effects is the binding of PRL to its plasma membrane receptor and consequent stimulation of signal transduction mechanisms involving tyrosine and serine/threonine kinases.

Recent evidence from our laboratories demonstrated that specific high affinity PRL receptors are constituitively expressed in the nuclei of hepatocytes (1) and in PRL-dependent rat Nb2 lymphoma cells (2). PRL binding to its membrane receptor stimulated a rapid ATP-dependent process of hormone internalization and subsequent nuclear translocation (2). These results suggest that the mitogenic action of PRL may reflect its direct interaction with components of nucleus. However, the mechanism(s) governing PRL endocytosis and nuclear accumulation remain unknown.

To delineate the mechanism(s) underlying PRL trafficing in Nb2 cells, we assessed the effect of several pharmacological inhibitors of tyrosine kinases and protein kinase C (PKC) on endocytosis and intracellular targeting of PRL by radioligand binding and immunofluorescent microscopy. Consistent with our previous observations (3,4), each of the selected inhibitors blocked PRL-stimulated cell proliferation of Nb2 cells in a concentration-dependent manner. The IC50s for genistein and tyrphostin, specific tyrosine kinase antagonists, were 4.2 µg/ml and 23.6 µM, respectively. In other experiments, staurosporine and calphostin C, specific inhibitors of PKC, similarly blocked PRL-stimulated cell proliferation with IC50s of 0.85 nM and 101 nM, respectively. Results obtained from radioligand binding studies indicated that at least 30% of internalized and 5% of the total cell associated <sup>125</sup>I-rPRL could be recovered within nuclei obtained from Nb2 cells which had been preincubated with the radiolabel for 3 hr at 37°C. In addition, protein fractionation by SDS-PAGE showed that cytosolic and nuclear PRL was present as the intact hormone. Genistein and tyrphostin exerted similar effects on PRL internalization and nuclear translocation. Each of these agents significantly (p<0.01) reduced the level of cell surface binding of <sup>125</sup>I-PRL as well as inhibited hormone internalization. As a result, these inhibitors abolished its nuclear translocation. In contrast, neither the level of cell-associated nor internalized 125I-rPRL differed between cells treated with the PKC antagonists and vehicle-control cultures. Instead, PKC inhibition significantly (p<0.01) reduced translocation of PRL to the Nb2 cell nucleus. Results from indirect-immunofluorescence analysis of PRL internalization and nuclear translocation confirmed these results obtained by radioligand binding.

We conclude that collaboration of a PRL-stimulated tyrosine kinase(s) and PKC is required for hormone internalization and transport to the Nb2 cell nucleus. Tyrosine kinase activation may play a role either in PRL receptor binding or hormone internalization. Activation of PKC appears to be necessary for nuclear targeting of PRL. Since inhibition of tyrosine kinase and PKC activity blocks cell proliferation and hormone trafficking to the nucleus, we suggest that an important component of PRLstimulated mitogenesis is its direct interaction within the cell nucleus.

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## DEPLETION OF CELLULAR GLUTATHIONE ENHANCES FUMONISIN B<sub>1</sub> TOXICITY IN PIG KIDNEY (LLC-PK<sub>1</sub>) CELLS

Y. James Kang\*
Department of Pharmacology and Toxicology
University of North Dakota School of Medicine
Grand Forks, ND 58202-9037

Fumonisin B<sub>1</sub> (FB<sub>1</sub>) is produced by Fusarium moniliforme, which is a prevalent fungal contaminant of corn, other grains, and agricultural commodities throughout the world. FB1 has been shown to be a causative agent of equine leukoencephalomalacia, porcine pulmonary edema, and rat liver tumors and chronic nephritis. In addition, the incidence of Fusarium moniliforme infection of home-grown corn is correlated with the high incidence of human esophageal cancer in China and in southern Africa. Recent surveys indicate that high levels of FB<sub>1</sub> are present in the United States feeds, which have been associated with field cases of the animal diseases. At present, the mechanism of action of FB<sub>1</sub> is unknown. Studies with a pig kidney cell line (LLC-PK<sub>1</sub>) (1) showed that FB<sub>1</sub> is a potent and specific inhibitor of sphingosine and sphinganine N-acyltransferase, a key enzyme in the pathways for de novo sphingolipid biosynthesis and turnover. As a result, free sphinganine accumulates intracellularly and this elevation correlates with the FB<sub>1</sub>-induced inhibition of proliferation and cell death. However, a mechanistic relationship between the sphinganine accumulation and the FB<sub>1</sub> cytotoxic effect has not been demonstrated. Under normal aerobic metabolic conditions noxious free radicals are continuously generated. Maintenance of cell integrity depends on the balance between the free radical generation and the free radical defense mechanisms. Imbalance occurs when increased free radical generation overwhelms the defense mechanisms. As a result of the metabolic effects of FB<sub>1</sub>, accumulation of sphinganine and its metabolites would result in enhanced free radical generation, potentially leading to enhanced oxidative stress and ultimately producing cell injury. The present study was undertaken to examine the effect of depletion of cellular glutathione (GSH), a key antioxidant, on the FB<sub>1</sub> toxicity in LLC-PK<sub>1</sub> cells. The hypothesis to be tested is that depression of the antioxidant system makes the cells more sensitive to FB<sub>1</sub> toxicity.

LLC-PK<sub>1</sub> cells were cultured in DMEM/F-12 medium supplemented with 5% fetal calf serum. Cellular GSH levels were measured using a high-performance liquid chromatography (HPLC) method (2). The FB<sub>1</sub> cytotoxicity was determined by an MTT and a long-term survival assay, and the value for lethal concentration for 50% of the cells (LC<sub>50</sub>) was estimated as described previously (3). Treatment of LLC-PK<sub>1</sub> cells for 12 hr with 0.1 mM buthionine sulfoximine (BSO), a selective inhibitor of the enzyme  $\gamma$ -glutamylcysteine synthetase that catalyzes the rate-limiting reaction in *de novo* GSH synthesis, markedly decreased cellular GSH levels. The remaining GSH content in these cells was about 20% of that found in the non-BSO treated cells. This BSO treatment, however, did not affect the cell viability. The cells pretreated with 0.1 mM BSO for 12 hr and controls treated with the same volume of saline were then exposed to varying concentrations of FB<sub>1</sub>. Both MTT and long-term survival assays revealed that the LC<sub>50</sub> value of FB<sub>1</sub> for the BSO-treated cells was significantly lowered (p<0.01). Therefore, depletion of cellular GSH sensitizes the cells to FB<sub>1</sub> toxicity. Because GSH is an important antioxidant participating in cytoprotective responses to oxidative injuries (4), the results obtained suggest that generation of toxic free radicals may be a mechanism by which FB<sub>1</sub> induces cell injury. Supported in part by USDA grant 93-37208-9277.

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### A MEDIUM FOR THE SELECTIVE ISOLATION OF CORYNEBACTERIUM SPECIES FROM MIXED-FLORA SAMPLES

Hua Tu\* and James R. Waller
Department of Microbiology and Immunology, University of North Dakota, Grand Forks, ND 58202

Increasing numbers of Corynebacteria other than <u>C. diphtheriae</u> are being reported as opportunistic pathogens in immunecompromised individuals. Isolating these organisms from polymicrobic clinical specimens is problematic since there is no selective medium that will isolate both <u>C. diphtheriae</u> and the normally nonpathogenic diphtheroids from a mixed culture. Tellurite containing media such as Tinsdale (0.03% tellurite) are not suitable for isolating corynebacteria other than <u>C. diphtheriae</u> because tellurite suppresses the growth of most other Corynebacterium species even at very low concentrations (0.005%).

Table 1. Organisms recovered from throat or nasal samples and amount of growth on different Fosfomycin media.

	Fosphomycin Containing Media						
	Blood Agar	$100~\mu \mathrm{g/ml}$	$200 \mu g/ml$	300 μg/ml			
Streptococcus	+++	++	+	-			
Staphylococcus	++	+	-	-			
Neisseria	+++	++	-	-			
Corynebacteriu	m -/+	++	++	++			

A selective medium containing ticarcillin, nystatin and  $100 \mu g/ml$  fosfomycin has been useful for isolating JK strains of Corynebacteria (1,2). This medium is inhibitory to many Corynebacterium species other than JK. Most bacteria are sensitive to fosfomycin, but Corynebacteria are very resistant (MIC equal to or greater than  $1000 \mu g/ml$ ). The proper concentration of fosfomycin will suppress the growth of nearly all bacteria other than Corynebacteria (Table 1). At  $100 \mu g/ml$  Staphylococcus, Streptococcus

and Neisseria isolates grew and at 200 µg/ml some streptococci grew; only Corynebacteria grew at 300 µg/ml.

Attempts were made to reduce the amount of fosfomycin required by including in the medium a second antibiotic with low activity against Corynebacteria. Addition of penicillin, colistin, or ticarcillin caused the inhibition of many Corynebacteria as well as <u>Streptococcus</u> and <u>Neisseria</u>. Sulfadiazine (SD) showed promise, but also proved inhibitory to some Corynebacteria during clinical trials (Table 2).

To further evaluate Fosfomycin medium and compare it with a medium commonly used to isolate <u>C. diphtheriae</u>, 41 throat swabs were taken from student volunteers and inoculated onto Fosfomycin medium (F), Fosfomycin-Sulfadiazine medium (F-SD) and Tinsdale medium (T). Plates were incubated at 35°C for 48 hours. 40 Corynebacterium-type isolates (gram positive, catalase positive, motility negative, acid-fast negative, esculin negative rods) from 30 plates were recovered from the 41 samples (Table 2). Recovery of Corynebacteria from F medium was much higher than from either F-SD or T media. Only Corynebacterium-type/bacteria grew on F medium. A few Yeast colonies did grow up on F medium, but they were easily distinguished by colony morphology and gram stain. Most isolates growing on F-SD and T media were <u>Streptococcus</u> or <u>Neisseria</u> species (Table 2).

Fosfomycin medium containing 300  $\mu$ g/ml Fosfomycin efficiently suppressed the growth of all the bacteria, other than Corynebacteria, commonly found in our clinical specimens. Yeast growth could be eliminated with Nystatin.

Our Fosfomycin medium is composed of Tindale base (Difco) with bovine serum (1%), L-cystine (240  $\mu$ g/ml), glucose-6-phosphate (50  $\mu$ g/ml) and Fosfomycin (300  $\mu$ g/ml).

Table 2. Growth of bacterial and yeast isolates from 41 throat swabs on Fosfomycin (F), Fosfomycin - Sulfadiazine (F-SD) and Tinsdale (T) agar media.

	Corynebacterium		Streptococcus		Neisseria		Yeast	
Medium	# Isolates	Recovery (%)*	# Isolates	Recovery (%)	# Isolates	Recovery (%)	# Isolates	Recovery (%)
F**	31	75.6	0	0	0	0	4	9.8
F+SD**	6	14.6	32	78	2	4.9	4	9.8
Т	4	9.8	41	100	0	0	4	9.8

<sup>\* = %</sup> of the 41 samples yielding the indicated bacteria or yeast.

<sup>\*\*</sup> F = 300  $\mu$ g/ml; F+SD = 100  $\mu$ g/ml + 100  $\mu$ g/ml; T= 0.03% tellurite

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#### CHARACTERISTICS OF PYRAZINAMIDASE IN CORYNEBACTERIUM SPECIFS

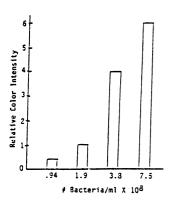
James R. Waller and Brian D. Hass\*
Department of Microbiology and Immunology
University of North Dakota, Grand Forks, ND 58202

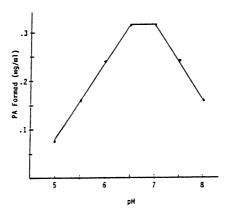
Pyrazinamidase (PZAse) hydrolyzes pyrazinamide (PZA) to pyrazinoic acid (PA) which forms a reddish-brown complex with FeSO<sub>4</sub> or FeNH<sub>4</sub>SO<sub>4</sub> used as a developer.

PZAse activity is a marker useful for defining catalase positive Gram positive rods as members of the genus Corynebacterium (1). A number of procedures are used to detect PZAse activity, but little is known about the conditions that affect this enzyme reaction in bacteria. The only report concerning conditions affecting PZA metabolism was published in 1973 and dealt with PZAse activity in animal tissues (2). Mouse liver PZAse exhibited an optimum pH of 7.3 - 7.8 and required many hours incubation to produce detectable enzyme activity. Current methods applied to bacteria require incubation for several hours to overnight at pH values from pH 6.0 to 7.0 or in aqueous solutions in which pH is not controlled.

Corynebacteria are relatively slow growing and not very metabolically active, so long reaction times and/or dense cell concentrations are required to produce detectable enzyme activity.

Our laboratory has demonstrated significant PZAse activity in <u>Corynebacterium xerosis</u> within 30 minutes by using a dense suspension of the organism (408 X 10<sup>8</sup> bacteria/ml) (Fig 1). Use of dense suspensions allowed us to study the properties of Corynebacterium PZAse quite easily. The optimum pH for PZAse activity was between 6.5 and 7.0 (Fig 2).





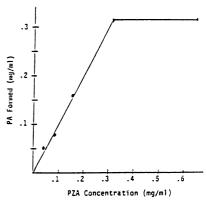


Figure 1. Cell concentration

Figure 2. Effect of pH

Figure 3. Substrate concentration

A substrate concentration curve (Fig 3) showed that the enzyme system was saturated at 0.32 mg PZA/ml (2.6 X 10<sup>-3</sup>M). The Michaelis constant (K<sub>m</sub>) and maximum velocity (V<sub>max</sub>) values were 0.125 mg/ml (1 X 10<sup>-3</sup>) and 0.0052 mg (4.2 X 10<sup>-5</sup> moles)/min, respectively. PZAse activity seems to be energy independent since glucose did not stimulate PZA hydrolysis.

The information from these studies was applied to development of a disc test for detection of PZAse activity. A bacterial mass equivalent to a 2-3 mm colony is rubbed into the surface of a blank one half inch paper disc loaded with 2 drops of 1 mg/ml PZA at pH 6.5. The inoculated disc is incubated for 30 minutes at 37C in a humidified petri dish. Pyrazinoic acid is detected by adding one drop of 2% FeSO<sub>4</sub> or FeNH<sub>4</sub>SO<sub>4</sub> to the disc and observing a rusty-brown color. A negative test shows no significant color development.

<sup>1.</sup> Sulea, I.T., Pollice, M.C. and Barksdale, L. (1980) Int. J. Syst. Bacteriol. 30, 466-472.

<sup>2.</sup> Toida, I. (1973) Amer. Rev. Resp. Dis. 107, 630-637.

# THE USE OF TRANSFORMATION/INSERTIONAL MUTAGENESIS TO ISOLATE CADMIUM SENSITIVE MUTANTS IN CHLAMYDOMONAS REINHARDTII

Joyce McHugh\* and Jonathan G. Spanier Department of Microbiology and Immunology University of North Dakota, Grand Forks, ND 58202

An arg7, cw15, mt+ strain of *Chlamydomonas reinhardtii* (CC1618) was transformed with pARG7.8, a plasmid containing the wild-type ARG7 gene. Over 2300 Arg+ transformants were selected on TAP media. Upon subsequent analysis on TAP plus cadmium plates, five of the transformants failed to grow at a level of 400uM cadmium and were designated as cadmium sensitive(CdS) mutants (Figure 1). Hybridization data indicated that vector(pBR329) sequences were present in these five mutants, but not in the untransformed parental strain. To date two of the mutants have been back crossed to an arg7, cw15, Cd+, mt<sup>-</sup> strain (CC425) and found to have progeny which always cosegregate the Arg<sup>+</sup> and Cd<sup>S</sup> phenotypes. This suggests that the Cd<sup>S</sup> phenotype in these two mutants results from the insertion of the plasmid pARG7.8 into a gene involving cadmium detoxification. Plasmid marker rescue is underway to clone the gene(s) responsible for the Cds phenotype in these two strains.

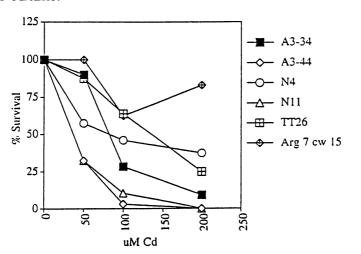


Figure 1. Percentage Survival of the Parental arg7, cw15 Strain and Five  $Arg^+$ ,  $Cd^S$  Transformants.

Friday, 29 April

8:20 .. Intrinsic Stability of Recombinant Human Wild-Type, Mutant, and Chimeric NAT2.

Kevin Gray\*, Mark A Doll, Timothy D Rustan, Ronald J Ferguson, David W Hein

Pharmacology/Toxicology, UND School of Medicine, Grand Forks, 58202

8:40 .. Modeling Ground Water Flow Patterns Using Topographic Maps and Soil Survey Data Sets.

Luke T Rutten\*

Geosciences, North Dakota State University, Fargo 58105 5517

- 9:00 .. Alternative Strategies for a Football Pool.

  Brenda Krogen\*, Nuwan Nanayakkara and M B Rao
  Statistics, North Dakota State University, Fargo 58105
- 9:20 .. Determination of Picogram Levels of Mercury in Environmental Air Samples Using Atomic Fluorescence.

  Charlene R Crocker\* and David J Hassett
  Energy & Environment Research Center, U N D, Grand Forks, 58202 9018
- 9:40 .. Origin of Iron Concretions in the Golden Valley Formation.
  Asuka Tsuru\*
  Geosciences, North Dakota State University, Fargo, 58105
- 10:20 .. Copper Deficiency Alters the Microsomal Mixed Function Oxidase System in Rat Intestine.

  Theresa Smith\*, University of North Dakota, and
  W Thomas Johnson, USDA-ARS Human Nutrition Research Center,
  Grand Forks, 58202
- 10:40 .. Low-Noise Design in Digital Audio Systems.

  John M Dahl\*

  Electrical Engineering, University of North Dakota, Grand Forks

### INTRINSIC STABILITY OF RECOMBINANT HUMAN WILD-TYPE, MUTANT, **AND CHIMERIC NAT2**

Kevin Gray\*, Mark A. Doll, Timothy D. Rustan, Ronald J. Ferguson, and David W. Hein

Department of Pharmacology and Toxicology University of North Dakota School of Medicine, Grand Forks, ND 58202

The importance of biological acetylation and person to person differences in the capacity for acetylation exert significant effects on individual responses to drugs, and other environmental arylamines and hydrazines.

Acetylation reactions in humans are controlled by two 870 base pair introlless genes (NAT1 and NAT2) located on chromosome number eight. The NAT2 gene codes for the polymorphic NAT2 acetyltransferase, which contributes to both the activation and deactivation of drugs and carcinogens.

The acetylation polymorphism is due to mutations in the NAT2 gene and is responsible for individuals separating into rapid, intermediate and slow acetylator phenotypes. Mutations have been identified at nucleotide positions 191 [G→A(Arg→Gln)], 282 [C→T(silent)], 341  $[T\rightarrow C(Ile\rightarrow Thr)]$ , 481  $[C\rightarrow T(silent)]$ , 590  $[G\rightarrow A(Arg\rightarrow G(Ir))]$ , 803  $[A\rightarrow G(Lys\rightarrow Arg)]$ , and 857 [G-A(Gly-Glu)]. Thus, some of the point mutations result in amino acid changes whereas others are silent.

Slow acetylators are often more prone than rapid acetylators to drug-induced toxicities such as isoniazid-induced peripheral nerve damage. Rapid acetylators, on the other hand, are more likely to show reduced levels of therapeutic drug effect. Slow acetylator phenotypes have been associated with increased incidence of urinary bladder cancers, whereas rapid acetylators have been associated with increased incidences of colorectal cancers.

Our goal was to examine the relative intrinsic stability of the wild-type and fourteen different mutant and chimeric NAT2 enzymes to determine whether differences in intrinsic stability may be wholly or partially responsible for the NAT2 polymorphic acetyltransferase activity differences that separate individuals into rapid, intermediate, or slow acetylator

The wild-type, mutant, and chimeric NAT2 proteins were tested for intrinsic stabilities at 37°C and 50°C. When we compared the inactivation rate constants of the NAT2 allozymes, some mutant NAT2 proteins were less stable than the wild-type. Point mutations in NAT2 at nucleotides 191, 590, and 857 or combinations thereof resulted in the expression of NAT2 proteins that were intrinsically less stable than wild-type NAT2. In particular, the 191 [G→A (Arg→Gln)] and the 857 [G→A (Gly→Glu)] mutations produced NAT2 that were much less stable.

These results suggest that NAT2 intrinsic stability may contribute to activity differences observed between rapid and slow acetylator phenotypes. Studies are in progress to further delineate molecular differences in the NAT2 allozymes responsible for rapid, intermediate, and slow acetylator phenotypes. This research was partially supported by USPHS grant CA-34627 and the University of North Dakota Ah'jo'gun program.

#### MODELING GROUNDWATER FLOW PATTERNS USING TOPOGRAPHIC MAPS AND SOIL SURVEY DATA SETS

Luke T. Rutten, Geosciences Dept., North Dakota State University, Fargo, ND 58105-5517

Determination of groundwater flow patterns in a glacial landscape by traditional methods is expensive and time-consuming. Such studies include laborious field methods and years of data collection.

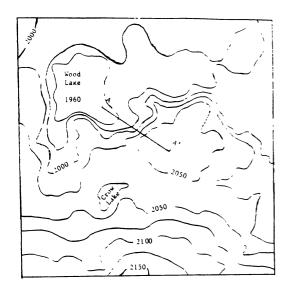
The purpose of this study is to test a method for making quick and inexpensive determinations of groundwater flow patterns. Estimations are made through analysis of topography, soil type, and hydrology. Using this information, a groundwater topographic map is constructed. Flow patterns can be checked and confirmed through construction of flownets for various transects in the study region.

A 67 km² area of closed-till topography in Dickey Co., North Dakota, was selected for ady. The following steps were taken to produce a groundwater topographic map. study.

1. Delineation of drainage basins onto a 7.5' quadrangle.

- 2. Delineation of wetland boundaries using USDA-SCS Soil Survey maps.
- 3. Classification of Tonka and Parnell Series soils as recharge wetlands.
- 4. Classification of Southam and Vallers Series soils as flow-through wetlands.
- 5. Classification of open water and Lallie Series soils as discharge wetlands.
- 6. Measurement of drainage basin area, relief, wetland area, and wetland elevation. 7. Assumption that wetland elevations represent water table elevations.
- 8. Assumption that the water table follows the subhumid form outlined by Lissey (1).
- 9. Connection of equal elevation points to produce a water table topographic map.
- 10. Construction of flownets for various transects using FLOWNET software (2).

Following these procedures, groundwater flow patterns for this region were modeled. Examples of the water table topographic map (Fig. 1) and a flownet (Fig. 2) resulting from the study are presented below.



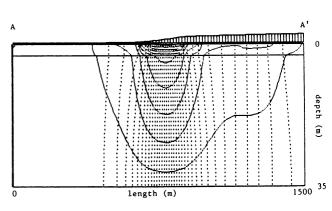


Fig. 1. Water table topographic map of 23 km² portion of study area.

Fig. 2. Flownet of transect A-A' (Fig.1) showing flow lines and equipotential lines.

Lissey, A., (1971) Geol Assoc Can Spec Pap, 9: 333-341.
 VanElburg, H., Engelen, G.B. and Hemeker, C.J., (1986) Flownet Software. Inst. Earth Sciences, Free University, Amsterdam.

#### ALTERNATIVE STRATEGIES FOR A FOOTBALL POOL

Brenda Krogen\*, Nuwan Nanayakkara and M. B. Rao Department Statistics, North Dakota State University, Fargo, ND 58105.

I and Miranda want to wager on the outcome of a game between two teams. Each team has the same probability of winning the game. We put a dollar each into the kitty and write down the winner of the game on a piece of paper. After the game is over, whoever guesses the winner correctly gets the pot. Otherwise, the pot is shared. For this game the expected value of my winnings is 0. The game is fair to both of us. I introduce a variation. I tell Miranda that my brother wants to participate in the betting game. There are three dollars in the kitty now. I and my brother form a coalition and fill the pieces of paper with opposite teams. Then the expected value of our winnings is \$0.25. This strategy of filling the forms in exactly opposite manner in the general case of n games involving m+2 players (which include both of us) in the betting game is advantageous to our coalition. This is simply a prototype of playing a football pool of n matches by (m + 2) players. See DeStefano, Doyle, and Snell (1993).

In this paper we consider other strategies for coalition and evaluate the expected values of our joint winnings under each strategy. The strategies and the expected values are given below.

 $S_1$ : Fill the forms exactly opposite.

- $S_2$ : Same strategy. I and my brother submit identical forms.  $S_3$ : Shift down one strategy. My choices moved down by one position are my brother's choices.
- S<sub>4</sub>: Flip-flop strategy. That is, my choices flipped (upside down) are my brother's choices.
- S<sub>5</sub>: Flip-flop-opposite strategy. This is a combination of the strategies S<sub>4</sub> and S<sub>1</sub>. That is,
- my brother's choices are the opposite of my choices flipped.

  Solit down once and opposite strategy. This is a combination of the strategies Solit and Solit That is, first, shift down my choices by one position and then choose the opposites for my brother.

For different strategies our expected winnings are calculated using a computer program written in FORTRAN language. Due to obvious reasons, we had to restrict ourselves to consider (m, n) configurations for which m = 1, 2, 3 and n = 1, 2, 3, 4 and 5. We present the results in the table below (N/A : not available at present):

		n=1 `	′ n=2	n=3	n=4	n=5
	$S_1$	.2500	.1875	.2500	.2148	.2500
	$S_2$	2500	3125	3438	3633	3769
	$S_3$	2500	0625	0391	0273	0211
m=1	$ S_4 $	2500	0625	1406	0429	0923
	S <sub>5</sub>	.2500	0625	.1094	0429	.0669
	$S_6$	.2500	0625	.0391	0273	.0211
	$S_1$	.3333	. 2499	.3333	.2864	.3333
	$ S_2 $	3333	4167	4583	4844	5026
1	$ S_3 $	3333	0833	0521	0365	0282
m=2	S <sub>4</sub>	3333	0833	1875	0573	0892
	S <sub>5</sub>	. 3333	0833	.1458	0573	.0892
	$S_6$	. 3333	0833	.0521	0365	.0282
	S <sub>1</sub>	.3437	.2637	.3437	.2992	.3437
1	$S_2$	3438	4629	5100	5408	N/A
	$S_3$	3438	0996	0554	0402	N/A
m=3	S <sub>4</sub>	3438	0996	2069	0671	N/A
	S <sub>5</sub>	.3437	0996	.1515	0671	N/A
	S <sub>6</sub>	.3437	0996	.0554	0402	N/A

DeStefano, J., Doyle, P., and Snell, J. L. (1993) The evil strategy for a football pool. American Mathematical Monthly. April: pp 341-343.

# THE DETERMINATION OF PICOGRAM LEVELS OF MERCURY IN ENVIRONMENTAL AIR SAMPLES USING ATOMIC FLUORESCENCE CHARLENE R. CROCKER\* AND DAVID J. HASSETT

The presence of mercury (Hg) as an environmental contaminant primarily from anthropogenic sources has been examined in greater detail as analytical techniques have improved. With the advancement of cold vapor generation by Hatch and Ott (1) in the mid 1960s, detection limits and ease of measurement allowed routine Hg determinations to be made at the part per billion level with relative ease. Recent revelations concerning contamination and erroneous values near cold vapor absorbance detection limits have led to the more common use of atomic fluorescence for low-level Hg determination. This has resulted in refinements in sample handling techniques. This paper describes a technique for Hg measurement at the picogram (pg) level. The technique has been used for surveying air in laboratories and areas where low-level Hg control technologies are being evaluated. Combustion research at the Energy & Environmental Research Center (EERC) involving flue-gas cleanup for Hg and other contaminants requires that Hg be determined at levels below regulatory requirements. These levels of detection are necessary to evaluate Hg removal and for accurate mass balances in process equipment. Additionally, other combustion equipment using small sample sizes of coal (50 g or less) for combustion testing require total Hg released to be on the order of one nanogram (ng).

Samples of air are drawn through tubes containing carbon (C) treated with potassium iodide for 3-5 hours. Hg trapped in this manner is subsequently leached with strong acid and quantitated using a commercial atomic fluorescence detector. The technique involves reduction of Hg in solution to elemental Hg using stannous ion as the reducing agent. Hg is quantitatively transferred to a quartz tube containing gold (Au)-coated sand. Hg amalgamated on this trap is thermally desorbed into the detector.

The precision and accuracy study for this method using a 500-pg Hg standard resulted in a peak area with a 1.26% relative deviation and a peak height with a 2.25% relative deviation. A calibration curve for Hg at the 50-500-pg range is shown in Figure 1. The correlation coefficients for peak area and peak height were 0.9816 and 0.9802, respectively. Results of a C collection tube leaching experiment are listed in Table 1. Results from preliminary air sampling of laboratory and office space at the EERC are compiled in Table 2.

The precision and accuracy for this method are excellent. It has been found that integrating the peak area from the desorption results yields more precise data. One major variable in the Au-amalgamation technique is believed to be the variable amalgamation depth in the Au layer resulting in different desorption curve geometry from sample to sample. Integrating peak area also allows the substitution of Au traps without extensive recalibration. Therefore, the atomic fluorescence technique is a precise and accurate method for quantitatively measuring airborne Hg at the pg level.

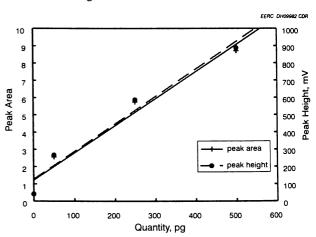
Table 1 Carbon Tube Leaching Study

F	eak Height	
Sample	(mV)	Peak Area
500-pg Hg Std	452	3.86E7
Carbon Tube 1	402	3.81 <b>E</b> 7
Carbon Tube 2	388	3.54 <b>E</b> 7
Carbon Tube 3	398	3.53E7
Acid blank	30	2.06E6
C Tube avg:	396 (1.52%RSD)	3.63E7 (3.58%RSD)
% Recovery	87.5	93.9

Table 2 Preliminary Air Survey Sample ng Hg/m<sup>3</sup>

Sample _	ng Hg/m³
New Office Space	14
Laboratory Bench	70
Laboratory Floor	63
Pilot Plant	85
Water Laboratory	43

Figure 1 Calibration Curve



<sup>1.</sup> Hatch, W.R. and Ott, W.L. (1968) Anal Chem 40, 2085

#### THE ORIGIN OF IRON CONCRETIONS IN THE GOLDEN VALLEY FORMATION

Asuka Tsuru Geosciences Department, North Dakota State University, Fargo, ND 58105

The Golden Valley Formation extensively outcrops in the Little Badlands, Stark County, North Dakota. Its Eocene age was determined through its fossil content, particularly that of floating fern, *Salvina Pheauriculata* (1). Common within the Golden Valley Formation are orange-colored iron concretions which have distinctive cone shapes (Figure 1).

These iron concretions are typically 5 to 10 cm in length and 4 to 6 cm in maximum diameter. Each specimen collected during the study has a narrow circular cavity at the center of the base, represents opening of a tube passing towards the apex. The surface of each concretion is rugged

and somewhat spiral in morphology.

The origin of these concretions has not been well studied; however, I believe that these were formed by the interaction between plant roots and microorganisms. This relationship of plants and microorganisms is well-established (2). As plant roots through growth are forced into deeper soil, hard mineral grains will rupture the root cells. Through these ruptured cells, plants release organic matter which supports the growth of microorganisms around the roots, leading to the formation of rhizospheres. A rhizosphere is a thin layer around a plant root which has very high concentration of microorganisms. For their growth, plants require such nutrient ions as calcium and sodium. These ions may be locked within mineral grains within the soil, and plants only can absorb ions when they are in solution. Microorganisms within the rhizosphere facilitate chemical reaction in which ions are released. Different species of microorganisms initiate various types of chemical reactions. *Thiobacillus ferrooxidanes* and *Gallionella ferruginea* are among bacteria which oxidize iron in soil (3).

Hickey (4) described the Golden Valley Formation as having distinctive orange color,

attributable to its high ferric iron content. At the contact between the Golden Valley Formation and the overlying Chadron Formation, an unconformity exists represented by a paleosol and implying a long period of non-deposition and soil development. In my model, around plant roots, bacteria were actively oxidizing iron ions which were at high concentrations. This process altered the chemistry of the soils around plant roots, forming an iron concretion within the surrounding soil. Later, when the plants decayed, the roots left small circular cavities at the centers of the concretions.

For future study, the orientation of concretions within the outcrop needs to be examined. Also, cross-sectional views of concretions should be analyzed to better describe the morphology of the cavities.

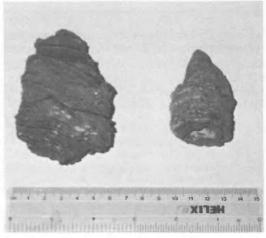


Figure 1. Iron oxide concretions.

<sup>1.</sup> Jepsen, G.L. (1963) Geological Society of America Bulletin. 74, 673-684.

<sup>2.</sup> Jungk, A.O. (1991) in Plant Roots. (Y.A. Waisel, eds.) Marcel Dekker Inc., New York. 455-482

<sup>3.</sup> Widdel, F. et al. (1993) Nature. 362, 834-836.

<sup>4.</sup> Hickey, L.J. (1977) Geological Society of America Memoir. 150, 183.

#### COPPER DEFICIENCY ALTERS THE MICROSOMAL MIXED FUNCTION OXIDASE SYSTEM IN RAT INTESTINE

Theresa Smith1\* and W. Thomas Johnson2 <sup>1</sup>University of North Dakota and <sup>2</sup>USDA, ARS, Grand Forks Human Nutrition Research Center Grand Forks, ND 58202

In the last 30 years it has become clear that diet strongly influences the therapeutic effects of drugs, and the toxicity or carcinogenicity of environmental chemicals. Intestinal xenobiotic metabolism is a primary defense against potentially harmful ingested chemicals. Severe copper deficiency impairs heme synthesis and results in the development of microcytic, hypochromic anemia characteristic of defective hemoglobin synthesis. The defect in heme synthesis could also influence the cytochromes P450, which are hemoproteins and components of the mixed function oxidase system that is important for the biotransformation of ingested xenobiotics.

Male Sprague-Dawley rats were fed copper-deficient (0.40 ppm Cu, 44.9 ppm Fe) and control (5.5 ppm Cu, 44.5 ppm Fe) casein-based diets for 5 weeks. Sixteen hours before sacrifice 5 animals in each group received an oral dose (80 mg/kg body weight) of 5,6-benzoflavone. Rats fed copper-deficient diet had significantly (P<0.05, ttest) lower hematocrit, hemoglobin concentration, plasma ceruloplasmin activity and liver copper concentration than control rats. The small intestine was excised from each animal and microsomes were prepared from the mucosal lining. Protein concentration of the microsomal samples was determined and cytochrome-P450 (cyt-P450) content was measured by difference spectra analysis. Ethoxyresorufin-O-deethylase activity was assayed by monitoring the fluorescence of resorufin formed during the O-dealkylation of ethoxyresorufin. NADPH-cyt-P450 reductase activity was measured by cytochrome C reduction. Table 1 illustrates the values for each intestinal parameter tested and their corresponding statistical significance.

Table 1. The Effect of Copper Deficiency and 5,6-Benzoflavone (BF) Treatment on Intestinal Copper and Iron Content, 7-Ethoxyresorufin-o-deethylase (EROD) and Cytochrome P-450 Reductase Activities, and Cytochrome P-450 (Cyt-P450) Content

Group:	μg Cu/g dry intestine	μg Fe/g dry intestine	EROD pmol resorufin formed/ (min • mg protein)	Reductase nmol cyt c reduced/ (min•mg protein)	Cyt-P450 (pmol/ mg protein)
CuDef CuDef + BF Control Control+ BF	$1.8 \pm 0.1$ $1.8 \pm 0.3$ $5.1 \pm 0.8$ $5.3 \pm 0.8$	$28.7 \pm 4.6$ $29.6 \pm 3.0$ $32.7 \pm 2.5$ $33.8 \pm 1.8$	$6 \pm 14^{a}$ $390 \pm 270^{b}$ not detectable $42 \pm 2^{c}$	40 ± 7 46 ± 4 20 ± 3 32 ± 5	58.0 ± 20.3* 177.2 ± 16.7* 88.2 ± 35.1 203.2 ± 35.1
ANOVA Cu BF Cu×BF	P 0.0001 0.85 0.82	P 0.02 0.51 0.95	P 0.02 0.006 0.02	P 0.0001 0.0009 0.21	

CuDef = Copper-deficient. Values are means ± SD. abcEROD activities with superscripts are significantly different (P<0.05, Tukey's Test). \*No difference between CuDef and controls, P>0.05, t-test.

The presence of anemia in the CuDef animals indicates that the deficiency was sufficient to impair iron utilization for heme synthesis. Copper deficiency had no effect on cyt-P450 content, as indicated by difference spectra analysis, nor on cyt-P450 1A1 induction by 5,6-benzoflavone. Because 5,6-benzoflavone specifically induces cyt-P450 1A1 in the intestine, it is unlikely that the difference in EROD activity between CuDef and control rats is due to a variation in the cyt-P450 isoform induced. The higher EROD activity may be a result of an effect of copper deficiency on NADPH-cyt-P450 reductase activity. Cyt-P450 reductase transfers electrons from NADPH to cyt-P450 which is the terminal oxidase in the catalytic process. Our results show that intestinal cyt-P450 reductase activity in CuDef rats is double that of control rats. The elevated intestinal EROD activity may result from an increase in the rate at which electrons are transferred in the oxidation-reduction cycle that cyt-P450 undergoes during the O-deethylation of ethoxyresorufin. The upregulation in cyt-P450 reductase activity may be in response to metabolic problems associated with severe copper deficiency.

#### LOW-NOISE DESIGN IN DIGITAL AUDIO SYSTEMS John M. Dahl Department of Electrical Engineering, University of North Dakota, Grand Forks

As current digital signal converter technology allows resolution of 20 bits and higher, proper low-noise circuit design becomes critical; even 20-bit converters have a least-significant bit value of over half a million times less than the full scale value. In 1994, a prototype converter was assembled to minimize noise and interference to prevent masking of least-significant-bit information. Although often overlooked, passive component selection, buffer amplifier design, and printed circuit board design are all critical when designing with a goal of absolute minimal system noise. These aspects of current technology are briefly examined here.

The most prevalent noise source at frequencies under 100kHz is called resistor excess noise and is due to inhomogeneities of the resistive element. Johnson, or thermal noise, is a secondary consideration(1). Thermal resistance change and inductance are not of primary concern due to the nature of the audio filters, but are also minimized in this design. Vishay VTA55 resistors, based on a bulk metal channel trimmed to .1% tolerance, were utilized. Their low nonuniformity and high performance is due to the bulk resistive element as opposed to the metal film element found in most high-performance resistors. Their average thermal coefficient over operating temperatures is 1.5ppm/Celsius degree, and the current noise is not measurable but is guaranteed less than 25nV/V $^{(2)}$ . The resistive element pattern also guarantees lower inductance than metal film and a .0025%/year maximum value drift under normal use.

Capacitor choice is another critical step in reducing audible imperfections in current hi-fi audio reproduction. Polystyrene capacitors are exceeded in quality for audio filtering applications only by teflon, which is minimally better in some areas and several times as costly. The dissipation factor of polystyrene (a factor of Equivalent Series Resistance+Equivlent Series Inductance) which measures percent of received energy lost by the capacitor is less than .1%. They also boast very low dielectric absorption, with values typically less than .01% in the audio frequency ranges (lowest among all types)(3). These superior properties of the dielectric also result in a selfdischarge time constant on the order of one million megohm-microfarads. These values make this a superior choice to teflon based on cost, and superior to polypropylene, polyester, and other more common resistors due to superior performance.

Also, typical low-noise op-amp DAC buffers have recently been improved upon by Analog Device's AD797, which is a single-stage differential amplifier. Sporting noise of less than .9nV/Sqrt(Hz) at 1kHz and -120dB THD at 20kHz, it is ideal for audio applications. It also features a slew rate of 20V/microsecond and a 110MHz gain-bandwidth product, certainly making it capable as a buffer amplifier for single-digit audio voltages. These specifications are possible due to a unique amplifier structure which takes advantage of the similarity between paired transistors etched on the same chip (PNP and PNP or NPN and NPN) instead of focusing on beta and early voltage values between devices(4).

Finally, care must be used in building a low-noise printed circuit board. Adequate shielding, grounding, and power supply filtering are essential to minimize outside interference. Short and fat ground traces should be used with liberal bypass capacitors to ensure low power supply impedance and guard against interference, and all traces should be heavy plated copper on a fiberglass board which is defluxed and coated after construction (however, care must be taken not to destroy polystyrene capacitors with defluxer.) All board space which is not used for other conductors should also be gound plane, to ensure a low-impedance ground and guard devices from surface leakage. Contaminants on a printed circuit board, especially in humid conditions, can cause currents of several nanoAmeres to leak from op-amp supply rails though a resistance of several Megohms. This surface leakage can result in a voltage error of as much as a tenth of a volt on a poor board. Capacitance between traces and dielectric absorption in the board material are also common problems which can be avoided if care is exercised in board layout. Teflon cables and teflon printed circuit boards are available for cases where high-frequency low-noise performance is critical, but are likely overkill for most audio applications. As a final test of construction quality, the measured noise of the system should not greatly exceed that of the noise contributions of the individual components in the circuit.

<sup>1,3.</sup> Analog Dialogue, Vol 17-2, Avoiding Passive Component Pitfalls, 1983, 1991

<sup>2.</sup> Vishay Data Sheet VTA55, 1992

<sup>4.</sup> Analog Devices AD797 Data Sheet, 1992

Friday, 29 April

8:20 .. 2,3,7,8-Tetrachlorodiphenyl-p-Dioxin (TCDD)-Induced Apoptosis in Growth Factor-Dependent and Resistance in Autonomous Rat Nb2 Lymphoma Cells.

Matthew A Leff\*, Donna J Buckley, John T McCormack, Natthew Friederichs and Arthur R Buckley Pharmacology / Toxicology and Anatomy / Cell Biology, UND School of Medicine, Grand Forks, ND, 58202

- 8:40 .. Glucocorticoid-Prolactin Interactions in T Lymphocytes.

  Han-Qian Liang\*, Donna J Buckley, and Arthur R Buckley
  Pharmacology/Toxicology, UND School of Medicine, Grand Forks, 58202
- 9:00 .. N-Acetylation in American Indians and other People with Diabetes Mellitus.

Erik J Furman\*, Ronald J Ferguson, Mark A Doll, Larry Bull, David W Hein

Pharmacology/Toxicology, UND School of Medicine, Grand Forks, 58202

9:20 .. DNA Adduct Formation in Liver, Urinary Bladder, Heart, Colon, and Prostate in Rapid and Slow Acetylator Syrian Hamsters Cogenic at the NAT2 Locus Administered 2-Aminofluorene.

Yi Feng\*, Timothy D Rustan, and David W Hein Pharmacology / Toxicology, UND School of Medicine, Grand Forks, 58202

9:40 .. Antioxidant Capacity and Copper Deficiency-Induced Damage in the Heart of Rats.

Yan Chen\* and Y James Kang, Pharmacology / Toxicology, UND School of Medicine, and Jack T Saari, USDA Human Nutrition Center, Grand Forks 58202

10:00 .. Characterization of Plant Pathogenicity and Strain Variation of Stenotrophomonas Maltophilia. S E Hinz\* and C A Wozniak

USDA ARS Northern Crop Science Laboratory, Fargo, 58105

10:20 .. Generation, Characterization and Reactivity of Transition Metal-Fulvene Cationic Complexes in the Gas Phase.

Dawn J Kardash\*, Kami Poland, Reza Bakhtiar, Denley B Jacobson Department of Chemistry, North Dakota State University, Fargo, 58105

### 2,3,7,8-TETRACHLORODIPHENYL-P-DIOXIN (TCDD)-INDUCED APOPTOSIS IN GROWTH FACTOR-DEPENDENT AND RESISTANCE IN AUTONOMOUS RAT Nb2 LYMPHOMA CELLS

Matthew A. Leff, Donna J. Buckley, John T. McCormack, Matthew Friederichs, and Arthur R. Buckley Departments of Pharmacology and Toxicology and Anatomy and Cell Biology University of North Dakota School of Medicine Grand Forks, ND

The ubiquitous environmental toxin, TCDD, infamous as a contaminant in the Vietnam era defoliant, Agent Orange, has been extensively investigated as a potential human carcinogen and teratogen. Its carcinogenic actions not withstanding, recent evidence has suggested that the immunotoxic actions of TCDD may pose an even greater human health risk (1). An exceedingly potent prototype of other halogenated aromatic hydrocarbons, TCDD-provoked immunotoxicity is manifested by premature thymic involution in rats and the induction of cytolysis in rodent and human T-lymphocytes. Importantly, recent evidence has suggested that its cytotoxicity in immune cells is due to the capacity of TCDD and related compounds to activate the physiological process of programmed cell death (apoptosis).

Two rat Nb2 node lymphoma cell lines which resemble T lymphocytes at an intermediate stage of differentiation, prolactin (PRL)-dependent Nb2-11 cells and an autonomous subline (Nb2-SFJCD1), were utilized as a model system for the investigation of TCDD-induced toxicity in T-lineage immune cells. Initial experiments revealed PRL-dependent Nb2-11 cells to be exquisitely sensitive to TCDD-mediated cytotoxicity; significant (p < 0.05) cytolysis was detected at TCDD concentrations of 10 nM. Notably, mitogenic stimulation by PRL or the T cell growth factor, interleukin-2, reversed TCDD-induced cytotoxicity even in the presence of high concentrations of the toxin (500 nM). In contradistinction, PRL-independent Nb2-SFJCD1 cells were completely resistant to the cytotoxic actions of TCDD at all concentrations examined. Gel electrophoresis of DNA obtained from Nb2-11 cells 12 hrs after the addition of TCDD showed significant DNA fragmentation, a hallmark of apoptosis presumably reflecting chromatin hydrolysis by a toxin-activated Ca<sup>2+</sup>-dependent endonuclease. Addition of PRL to TCDD-treated cultures inhibited the consequent DNA fragmentation. In contrast, Nb2-SFJCD1 cells did not undergo TCDD-induced DNA cleavage. To further characterize toxinstimulated apoptosis, 3'-end labeling of DNA in situ was performed in TCDD-treated Nb2-11 cells. The results indicated that the kinetics of DNA fragmentation subsequent to TCDD are extremely rapid; enhanced fragmentation of DNA was detected within 15-30 min.

Many of the toxic properties of TCDD have been linked to its initial interaction with the arylhydrocarbon (Ah) receptor (2), a cytosolic protein which is activated by TCDD binding and in turn, alters gene transcription. To assess whether the TCDD-resistance identified in the Nb2-SFJCD1 line was due to impaired expression of the Ah receptor in these cells, immunoblot analysis was performed on lysates prepared from each of the cell lines. The results confirmed the presence of the well-characterized 90-95 kDa Ah receptor in each line. Importantly, an additional 78 kDa immunoreactive protein was also detected in Nb2-11 cell lysates, but not in Nb2-SFJCD1 cell preparations.

We conclude that: (1) TCDD induces apoptosis characterized by rapid DNA fragmentation in PRLdependent Nb2-11 cells; (2) mitogenic stimulation ameliorates TCDD-induced apoptosis in the Nb2-11 line; (3) progression from hormone-dependence to autonomy in Nb2 lymphoma cells confers resistance to TCDD immunotoxicity; and (4) a differential pattern of Ah receptor expression between the two cell lines may be responsible for the variations in TCDD sensitivities observed. We suggest that the PRL-dependent Nb2 lymphoma and its sublines represent an ideal paradigm in which to investigate toxin-provoked immunotoxicity leading to apoptosis. Supported in part by DK44439 from the National Institutes of Health.

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<sup>2.</sup> Durrin, L.K., Jones, P.B.C., Fisher, J.M., Galeazzi, D.R., Whitlock, L.P. (1987) J. Cell. Biochem. 35: 153-160.

#### GLUCOCORTICOID-PROLACTIN INTERACTIONS IN T LYMPHOCYTES

Han-Qian Liang, Donna J. Buckley, and Arthur R. Buckley Department of Pharmacology and Toxicology University of North Dakota School of Medicine Grand Forks, ND

Glucocorticosteroids are widely employed clinically for their antiinflammatory and immunosuppressive qualities. Utilizing a transformed in vitro T lymphocyte model system, prolactin (PRL)-dependent Nb2-11 lymphoma cells and an autonomous subline (Nb2-SFJCD1), the mechanism(s) by which dexamethasone (DEX), a potent synthetic glucocorticoid, causes immunosuppression was investigated.

In initial experiments, the effect of DEX on cell proliferation, assessed by <sup>3</sup>H-thymidine incorporation, in PRLstimulated Nb2-11 and Nb2-SFJCD1 cultures was determined. Addition of DEX significantly (p < 0.05) reduced proliferation determined at 72 hrs in each of the cell lines in a concentration-dependent manner. To investigate whether the inhibitory effect of DEX on mitogenesis reflected an interaction early in cell cycle, synchronized Nb2-11 cells. growth arrested in the early G<sub>1</sub> phase, and similarly treated Nb2-SFJCD1 cells were incubated in the presence of the steroid with and without the addition of PRL. The cells were subsequently harvested at various time intervals and the effect of DEX was determined on the growth-dependent expression of the heat shock 70 (HSP70) gene by northern blot analysis. As previously demonstrated (1), PRL-induced expression of HSP70 mRNA peaked within 6 hrs, a time corresponding to the late G<sub>1</sub> phase of cell cycle. PRL-stimulated HSP70 expression was attenuated by DEX (100 nM) in lactogen-dependent Nb2-11 cells but was uneffected in the autonomous line.

Previous studies by others have linked the immunosuppressive actions of DEX in rodent lymphocytes to its capacity to provoke physiological cell death mechanisms (apoptosis), characterized by endonuclease-mediated DNA fragmentation. Moreover, DEX has been recently reported to exert a similar effect in Nb2 cells (2). Therefore, the effect of DEX (100 nM) on DNA fragmentation in Nb2-11 and Nb2-SFJCD1 cells at 12 hrs was assessed by agarosegel electrophoresis and by DNA quantitation using the diphenylamine procedure. Fragmentation of DNA was significantly (p < 0.05) induced by DEX in Nb2-11 cells, an effect abrogated by PRL or interleukin-2 stimulated mitogenesis. In contrast, Nb2-SFJCD1 cells were entirely resistent to DEX-stimulated apoptosis.

## Immunosuppressive Effects of Dexamethasone on Nb2 Lymphoma Cells

Treatn	nent <sup>a</sup>	<sup>3</sup> H-Thymidine Inc	orporation cpm/well	HSP70 Gene	e Expression <sup>b</sup>	DNA fragmentation (% of total cellular DNA)		
DEX	PRL	Nb2-11	Nb2-SFJCD1	Nb2-11	Nb2-SFJCD1	Nb2-11		
_	_	$337 \pm 45.1$	46689 ± 5402	100 ± 0	100 ± 0	15.7 ± 1.1		
-	+	$42713 \pm 138^{\circ}$	$59005 \pm 5981$	$253.7 \pm 89.7$	$^{\circ}$ 111.5 $\pm$ 3.5			
+	-	$76.5 \pm 18$	$35799 \pm 2333^{\circ}$	$24 \pm 4.87^{\circ}$	$92.2 \pm 0.85$	$40.9 \pm 1.1^{\circ}$		
+	+	$21830 \pm 639^{\circ}$	$51891 \pm 3519$	$194.7 \pm 75.9$	$118.3 \pm 1.3$	$10.2 \pm 4.7$		

Values represent the mean  $\pm$  SD. a: 100 nm DEX; 10 ng/ml PRL b: arbitrary density units c: p<0.05

It is concluded that the immunosuppressive actions of DEX in T lymphocytes are the result of its activation of endogenous programmed cell death mechanisms coupled to an antiproliferative activity expressed early following mitogenic stimulation of cell cycle progression. Furthermore, addition of mitogen nullifies DEX-induced apoptosis but does not reverse the growth inhibition caused by this agent. Finally, the oncogenic progression from hormonedependence to hormone-independence, a process demonstrated to occur in certain endocrine tumors, appears to confer resistance to glucocorticosteroid-induced cytolysis produced by apoptosis. This work was supported in part by DK44439 from the National Institutes of Health.

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# N-ACETYLATION IN AMERICAN INDIANS AND OTHER PEOPLE WITH DIABETES MELLITUS

Erik J. Furman\*, Ronald J. Ferguson, Mark A. Doll, Larry Bull, and David W. Hein
Department of Pharmacology and Toxicology
University of North Dakota School of Medicine
Grand Forks, North Dakota 58202-9037

Research was conducted to determine whether the polymorphic N-acetyltransferase (NAT2) phenotype could be used as a genetic marker for Type I and Type II diabetes mellitus in Caucasians and American Indians, as well as to phenotype American Indians as this has yet to be adequately done. The NAT2 gene is located on chromosome 8, pterq11. It is coded by one rapid allele and at least ten slow alleles. These alleles are codominant and thus divide Nacetylation phenotype into three functional categories: rapid, intermediate, and slow acetylators. Acetylator phenotype was determined by comparing the urinary excretion ratio of two caffeine metabolites; 5-acetyl-6-amino-3-methyluracil (AAMU) and 1-methyl-xanthine (1X). Participants were asked to ingest at least 1-1.5 mg caffeine/kilogram body weight found naturally in coffee, tea, or caffeinated soft drink. A urine specimen was taken 2-6 hrs after consumption and then analyzed by size exclusion high performance liquid chromatography for the AAMU/1X ratio. Genotyping NAT2 for the four most common alleles was conducted using the polymerase chain reaction, followed by digestion with restriction enzymes that distinguish common NAT2 mutations (rapid allele shows an absence of mutations). The Caucasian population in this study had a very diverse European background. The American Indians in the study were principally the Chippewa and Cree of the Turtle Mountain Reservation and the Dakota of the Sisseton-Wapheton and Devils Lake Reservations. As shown in the Table below, a significant association was found between rapid acetylator phenotype and Type II diabetes mellitus among American Indians. Higher frequencies of rapid acetylator phenotype also were found among Caucasians with Type I and Type II diabetes mellitus than among Caucasians without diabetes mellitus, although the association was not significant. However, a compilation of the data of this study with other studies done in European and Arab populations demonstrates an extremely significant association between rapid acetylator phenotype and Type I and Type II diabetes mellitus. Partially supported by USPHS grant CA-34627.

# Relationship Between Acetylator Phenotype and Type I and Type II Diabetes Mellitus in American Indians and Caucasians

		Nondiabet	<u>ics</u>		Type I Di		Type II Diabetics		
	Nu: <u>Slow</u>	mber <u>Rapid</u>	Percent Rapid	Nun <u>Slow</u>	nber <u>Rapid</u>	Percent Rapid	Nur <u>Slow</u>	mber <u>Rapid</u>	Percent Rapid
American Indians	22	14	39%				16	30	65 % a
Caucasians	78	75	49%	25	25	50% <sup>b</sup>	23	30	57%°

<sup>&</sup>lt;sup>a</sup> The association (Odds ratio 2.946) between rapid acetylator phenotype and Type II diabetes mellitus among American Indians is highly significant (p=0.02554).

<sup>&</sup>lt;sup>b</sup> The association (Odds ratio 1.040) between rapid acetylator phenotype and Type I diabetes mellitus in Caucasians was not significant (p=0.99991).

The association (Odds ratio 1.357) between rapid acetylator phenotype and Type II diabetes mellitus in Caucasians was not significant (p=0.42256).

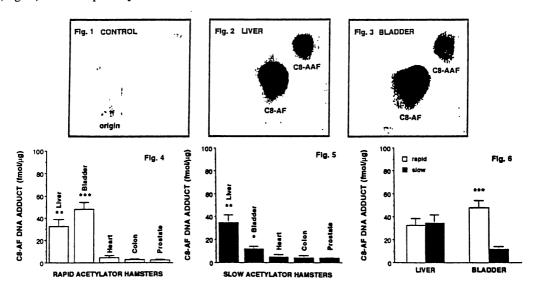
# DNA ADDUCT FORMATION IN LIVER, URINARY BLADDER, HEART, COLON, AND PROSTATE IN RAPID AND SLOW ACETYLATOR SYRIAN HAMSTERS CONGENIC AT THE NAT2 LOCUS ADMINISTERED 2-AMINOFLUORENE

Yi Feng\*, Timothy D. Rustan, and David W. Hein Department of Pharmacology and Toxicology, University of North Dakota School of Medicine, Grand Forks, ND 58202

2-Aminofluorene is one of the major carcinogenic arylamines produced in the synthetic fuels industry. It is a procarcinogen and is bioactivated to a proximate carcinogen, N-hydroxy-2-aminofluorene, by hepatic cytochrome P-4501A2. N-hydroxy-2-aminofluorene is further activated in different organs to an electrophilic arylnitrenium ion which binds DNA covalently and causes mutagenic and carcinogenic lesions. A polymorphic N-acetyltransferase (NAT2) encoded at the *NAT2* gene locus is involved in metabolic activation and/or deactivation of arylamines. Human epidemiological studies suggest that rapid and slow acetylators are at higher risk for colorectal cancer and urinary bladder cancer, respectively. In this study, rapid and slow acetylator Syrian hamsters congenic at the *NAT2* locus were administered 2-aminofluorene to measure DNA adduct levels in target and non-target organs and to assess the role of *NAT2* acetylator genotype on 2-aminofluorene-DNA adduct formation.

A single dose of 60 mg/kg 2-aminofluorene was administered i.p. to rapid (Bio. 82.73/H-Pat) and slow (Bio. 82.73/H-Pat) acetylator hamsters congenic at the NAT2 locus. Livers, urinary bladders, hearts, colons, and prostates were collected at 6, 18, 24, and 36 hr post-injection (5 animals in each group). DNA from these organs was isolated by phenol/chloroform extraction and the arylamine-DNA adduct levels were measured by <sup>32</sup>P-postlabeling analysis.

No DNA adducts were detected in control animals administered vehicle alone (Fig. 1). Two DNA adducts. N-(deoxyguanosin-8-yl)-2-aminofluorene (C8-AF, major adduct) and N-(deoxyguanosin-8-yl)-N-acetyl-2-aminofluorene (C8-AAF, minor adduct), were detected in animals injected with 2-aminofluorene (Figs. 2 and 3). DNA adducts achieved peak levels at 18 hr post-injection in liver, heart, colon, and prostate. Urinary bladder DNA adduct levels increased up to 36 hr post-injection. DNA adduct levels in liver and urinary bladder (target organs) were significantly higher than in heart, colon, and prostate (non-target organs, Figs. 4 and 5). There were no significant differences in DNA adduct levels between rapid and slow acetylators in liver, heart, colon, and prostate. However. DNA adduct (C8-AF and C8-AAF) levels in urinary bladder were significantly higher in rapid versus slow acetylators after 24 (Fig. 6) or 36 hr post-injection.



These results suggest that 2-aminofluorene causes significantly higher levels of DNA adducts in target organs than in non-target organs and that *NAT2* acetylator genotype plays a significant and specific role in formation of 2-aminofluorene-urinary bladder DNA adducts. Partially supported by USPHS grant CA-34627.

# ANTIOXIDANT CAPACITY AND COPPER DEFICIENCY-INDUCED DAMAGE IN THE HEART OF RATS

Yan Chen,<sup>1\*</sup> Jack T. Saari,<sup>2</sup> and Y. James Kang<sup>1</sup>
<sup>1</sup>Department of Pharmacology and Toxicology, University of North Dakota School of Medicine and <sup>2</sup>USDA, Grand Forks Human Nutrition Center, Grand Forks, ND 58202

Development of selective severe damage to the heart resulting from dietary copper deficiency has long been recognized (1). Although oxidant stress has been implicated in copper deficiency-induced pathogenesis (2), the mechanism for the selective cardiotoxicity has not been demonstrated. Therefore, we determined the effect of copper-deficiency on lipid peroxidation in the heart, in relation to the antioxidant capacity, to test the hypothesis that weak antioxidant defenses make the heart more vulnerable to the copper deficiency-induced oxidative damage.

Weanling rats were fed a purified diet deficient in copper  $(0.4 \mu g/g \text{ diet})$  or one containing adequate copper  $(6.0 \mu g/g \text{ diet})$  for 4 weeks. Copper deficiency induced a two-fold increase in lipid peroxidation in the heart (\*=p<0.01), but caused no significant change in the liver (Fig. 1). Copper-deficiency significantly depressed the antioxidant enzyme activities of Cu,Zn-superoxide dismutase (Cu,Zn-SOD, Fig. 2) and glutathione peroxidase (GSH-Px, Fig. 3), but not catalase (Fig. 4) in the heart. When these enzyme activities were compared between the two organs, antioxidant capacity in the heart was found to be significantly lower than that in the liver (\*=p<0.01, Table 1). Glutathione reductase (GR), which provides the reductant glutathione to support GSH-Px function, was also lower in the heart than in the liver (Table 1).

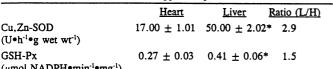
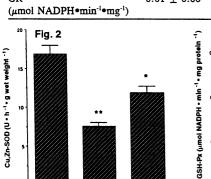


Table 1. Comparison of antioxidant enzyme activities in the liver and the heart of normal copper-adequate rats

GSH-Px  $(\mu \text{mol NADPH} \bullet \text{min}^{-1} \bullet \text{mg}^{-1})$  0.27 ± 0.03 0.41 ± 0.06\* 1.5  $(\mu \text{mol NADPH} \bullet \text{min}^{-1} \bullet \text{mg}^{-1})$  Catalase 0.32 ± 0.05 15.97 ± 4.14\* 50.4  $(k \bullet \text{min}^{-1} \bullet \text{mg}^{-1})$  GR 0.01 ± 0.00 0.05 ± 0.01\* 4.3



Cu DF

Cu PF

Cu AD

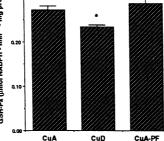
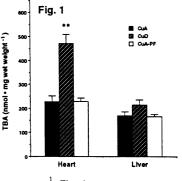
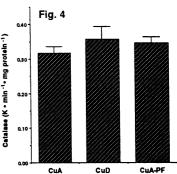


Fig. 3





The results obtained revealed that the heart has a less efficient antioxidant system than other tissues such as the liver. Although copper deficiency reduces the antioxidant activity in both organs (3), the pre-existing weak defenses most likely represent a mechanism for the copper deficiency-induced selective oxidative damage to the heart. The information generated from this study is not only valuable for the future study on the mechanism for copper deficiency-induced pathogenesis, but also useful for other investigations to peroxidant and antioxidant responses.

<sup>1.</sup> Kopp, S.T., Klevay, L.M. and Feliksik, J.M. (1983). Am. J. Physiol., 245:4855-4866.

<sup>2.</sup> Johnson, M.A., Fischer, J.G. and Kays, S.E. (1992). Crit. Rev. Food Sci. Nutr., 32:1-31.

<sup>3.</sup> Chen, Y., Saari, J.T. and Kang, Y.J. (1994). Free Radical Biol. Med., in press.

## CHARACTERIZATION OF PLANT PATHOGENICITY AND STRAIN VARIATION OF STENOTROPHOMONAS MALTOPHILIA

S. E. Hinz and C. A. Wozniak USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58105

The species Pseudomonas maltophilia was designated by Hugh and Ryschenkow in 1960 following extensive phenotypic characterization. In 1984, this taxon was renamed Xanthomonas maltophilia (Swings et al.) and established as a taxon following analysis of rDNA homologies within the genera Xanthomonas and Pseudomonas by Swings et al. (1981). In an article published in the International Journal of Systematic Bacteriology (Palleroni and Bradbury 1993) it was proposed that X. maltophilia (Xm) should be transferred to a newly established genus and renamed Stenotrophomonas maltophilia (Hugh 1980; Swings et al. 1983) Palleroni and Bradbury 1993, with the former Xm being the type strain (ATCC 13637). Their analysis illustrated the differences between S. maltophilia (Sm) and Xanthomonas spp. In the generic definition of Xanthomonas (Bergey's Manual First Edition, pp.199-210, 1984) plant pathogenicity, a single polar flagellum and the presence of xanthomonadins (brominated aryl polyene pigments), are fundamental characteristics for inclusion in Xanthomonas. As of yet, no significant research has been done to explore the plant pathogenicity of Sm (Xm). Our TEM photos of ATCC 13637 and an environmental isolate of Sm show the presence of more than one polar flagellum. There have also been discrepancies in the previous taxonomic work performed by workers in the same lab on identical strains with regard to the melting temperature of the hybrid nucleic acid molecules (Palleroni and Bradbury 1993).

We have isolated over 500 bacteria that fit the definition of the Sm taxon. The bacteria were identified through BIOLOG, API and standard biochemical tests. Sm was ubiquitously encountered in larvae of the sugarbeet root maggot (Diptera:Otitidae) from four geographically isolated populations and was also found to be a common commensal of the sugarbeet rhizoplane. Some of these isolates have been evaluated for their ability to produce disease symptoms or hypersensitive reaction on cultivars of Beta vulgaris, Beta 1745 and Hilleshog 5135, and Nicotiana tabacum, Xanthi(NN). The evaluation of pathogenicity was done by inoculating plants with a variety of isolates from the geographical locations, ATCC type strains, and positive and negative controls. The isolates were grown in nutrient broth in 10ml cultures at 30°C overnight and 100  $\mu$ l of the turbid cultures were then transferred to fresh broth. The fresh broth cultures were grown overnight. The broth cultures were pelleted, washed and diluted with 0.85% saline. The inoculation procedure involved the use of syringes fitted with Tygon tubing. The syringes were filled with the diluted bacterial solution and 200 µl of the solution, which contained a range of 2.0 x 108 in some of the type strains to 6.6 x 1010 cfu/ml in the environmental isolates, was forced through the stomatal openings on the underside of the leaf. To date, no pathogenic or hypersensitive responses (HR) have been attributable to Sm isolates from the sugarbeet rhizoplane, endogenous microflora of the sugarbeet root maggot or ATCC type strains. The tobacco and sugarbeet cultivars have exhibited chlorosis and water-soaking symptoms after inoculation with some Sm isolates, but no true disease symptoms or hypersensitive reactions occurred. Positive controls (Clavibacter michiganense and Pseudomonas syringae) gave appropriate HR under our conditions. Negative controls (Micrococcus luteus and Escherichia coli JM109) did not induce HR. Buffer and saline controls were negative as well.

SDS-PAGE analysis of cytoplasmic and outer membrane proteins indicated substantial differences between isolates from the sugarbeet root maggots and those from nosocomial origins. Further analysis of *Sm* will include the use of Repetitive Extragenic Palindromic (REP) and Enterobacterial Repetitive Intergenic Consensus (ERIC) sequences to evaluate the variation between the environmental isolates, the ATCC type strains and isolates of nosocomial origin. These highly repetitive sequences are common among many bacteria and often have conserved sequence motifs. They therefore provide a method of estimating variation in strains from diverse origins with little selective evolutionary pressure on these highly abundant, repetitive sequences.

#### GENERATION, CHARACTERIZATION, AND REACTIVITY OF TRANSITION METAL-FULVENE CATIONIC COMPLEXES IN THE GAS PHASE

Dawn J. Kardash\*, Kami Poland, Ray Bakhtiar, and Denley B. Jacobson Department of Chemistry, North Dakota State University, Fargo, ND 58105

Fulvene analogs (C<sub>5</sub>H<sub>4</sub>(=CH<sub>2</sub>)) have been a focus of interest as prototypes of cyclic, cross-conjugated molecules because of their unique properties which include novel conjugated structures and a small HOMO-LUMO gap. <sup>1,2</sup> Due to these unique properties, transition metal-fulvene complexes have been a target for study, however, it has proven difficult to synthesize these species in solution. Here, we describe a general route for the synthesis of transition metal-fulvene complexes in the gas phase by using Fourier transform mass spectrometry (FTMS). The metal-fulvene complexes were generated by dehydrogenation of methylenecyclopentane by atomic metal cations (Fe<sup>+</sup>, Co<sup>+</sup>, Ni<sup>+</sup>), reaction 1. The resulting MC<sub>6</sub>H<sub>6</sub><sup>+</sup> ions were structurally

$$M^+ + C_5H_8(=CH_2) \longrightarrow MC_6H_6^+ + 2H_2$$
 (1)

characterized by reaction with acetylene, propyne, allene, benzene- $d_6$ , and propene- $d_6$ . These results were then compared with those for authentic M(benzene)<sup>+</sup> ions generated by dehydrogenation of cyclohexene, reaction 2. The results of the structural studies clearly indicate

$$M^+ + C_6H_{10} \longrightarrow MC_6H_6^+ + 2H_2$$
 (2)

that fulvene complexes were generated in reaction 1 and that the metal does not induce skeletal rearrangement to the more stable benzene speices.

The M(fulvene)+ complexes undergo a novel cycloaddition reaction with acetylene to yield a M(pentalene)+ complexes. In addition, allene and propene both yield formation of metal-indene cations. Both Fe(fulvene)+ and Ni(fulvene)+ complexes undergo H/D exchange with benzene-d<sub>6</sub> indicating activation of aromatic C-H bonds. The corresponding M(benzene)+ complexes do not activate aromatic C-H bonds and do not activate acetylenes or allene. Finally, the bond dissociation energy of these metal-fulvene complexes exceeds that of the corresponding metal-benzene species. These results illustrate that M(fulvene)+ are easily generated in the gas phase and exhibit a rich and varied chemistry compared to the related M(benzene)+ complexes.

<sup>1.</sup> Bergmann, E. D. (1968) Chem. Rev. 68, 41-84.

<sup>2.</sup> Yates, P. (1968) Adv. Alicycl. Chem. 2, 59-184.

#### C O N S T I T U T I O N of the NORTH DAKOTA ACADEMY of SCIENCE

( Founded 1908, Official State Academy 1958 )

#### ARTICLE I - Name and Purpose

- 1. This association shall be called the NORTH DAKOTA ACADEMY of SCIENCE ( NDAS ).
- The purpose of this association shall be to promote and conduct scientific research and to diffuse scientific knowledge.

#### ARTICLE II - Membership

- 1. Membership in the NDAS shall be composed of persons active or interested in some field of scientific endeavor. Candidates for membership may be proposed by any active member of the NDAS by submitting the candidate's name to the chairman of the Membership Committee for approval. Specific categories of membership shall be defined in the bylaws of the NDAS.
- 2. Annual dues for the various categories of membership shall be determined by the members present at the Annual Meeting.

#### ARTICLE III - Officers

- 1. The Officers of the NDAS shall be a President, President-Elect, and the Secretary-Treasurer who shall perform the duties usually pertaining to these offices. The President-Elect shall be chosen by ballot at the Annual Meeting and will hold the office for one year and then assume the office of President for one year. The Secretary-Treasurer shall be appointed for a three-year term by the Executive Committee.
- 2. The Executive Committee, consisting of the above-named officers, the retiring President, and three members-at-large, shall have charge of the ordinary executive duties. The members-at-large shall be elected for a three-year term on a rotating basis.

#### ARTICLE IV - Meetings

- 1. There shall be an Annual Meeting each year held at such time and place as the Executive Committee may determine.
- 2. Special meetings shall be called by the President upon the request of ten percent of the active members. Only matters specified in the call can be transacted at a special meeting.
- Ten percent of the active members shall constitute a quorum at the Annual Meeting.
   Special meetings require twenty percent of the active members for a quorum.

#### ARTICLE V - Miscellaneous

- 1. In the event of dissolution of the NDAS, any remaining assets shall be distributed to organizations organized and operated exclusively for educational and scientific purposes as shall at the time qualify as exempt organizations under Section 501(c) (3) of the Internal Revenue Code of 1954.
- 2. No substantial part of the activities of the NDAS shall be the carrying on of propaganda, or otherwise attempting to influence legislation, and the Academy shall not participate in or intervene in, any political campaign on behalf of any candidate for public office.
- 3. No part of any net earnings shall inure to the benefit of, or be distributable to, NDAS members or officers, or other private persons, except that the academy may authorize the payment of reasonable compensation for services rendered.

#### ARTICLE VI - Amendments

- This Constitution may be amended at any Annual Meeting of the NDAS by a two-thirds vote. Proposed amendments shall be submitted in writing to the Secretary who shall send them to the members at least two weeks before the meeting at which such amendments are to be considered.
- 2. Bylaws may be adopted or repealed at any regular meeting by a two-thirds vote.

- 1. The NDAS official guide for parliamentary procedure shall be the "Standard Code of Parliamentary Procedure" by Alice F. Sturgis. (1965 Revision)
- 2. The annual dues shall be determined by a two-thirds vote at an Annual Meeting. These dues are payable January 1 of each year. (1965 Revision)
- 3. Members shall be dropped from the active list on 31 December following the nonpayment of dues during the membership year commencing the previous 1 January. A member may return to the active list by paying the current year dues and a membership renewal charge of \$5.00.
  ( 1975 Revision )
- 4. Every member in good standing shall receive a copy of the annual Proceedings of the North Dakota Academy of Science. ( 1965 Revision )
- 5. Special offices such as Historian may be created by the unanimous vote of the members at the Annual Meeting. (1965 Revision)
- 6. The Executive Committee shall annually appoint an Academy representative to the National Association of Academies of Science and to Section X (General) of the American Association for the Advancement of Science. (1979 Revision)
- 7. The Committee structure of the NDAS shall be as follows, the President appointing the members and chairpersons for all except the Executive Committee:
  - a. Executive Committee.

Membership: Past-President, President, President-Elect, Secretary-Treasurer, three members-at-large. Three-year terms.

Duties: The Executive Committee shall be the governing board of the NDAS, responsible only to the membership. It shall arrange for programs, approve committee appointments, be responsible for the fiscal affairs of the Academy, and transact such business as necessary and desirable for function and growth of the NDAS.

b. Editorial Committee.

Membership: Three members.

Three-year terms.

Duties: The Editorial Committee shall develop and recommend the NDAS publication program and policies to the Executive Committee. It will assist the Editor in reviewing manuscripts for the Proceedings.

c. Education Committee.

Membership: Seven members, two shall be high school teachers. Five-year terms.

Duties: The Education Committee shall work with high school students and teachers in the state, in visitation programs, Science Talent Search programs, and other programs to stimulate an interest in science by the youth of the state. It shall operate the Junior Academy of Science program and administer the AAAS high school research program.

d. Denison Awards Committee.

Membership: Six members.

Three-year terms.

Duties: The Denison Awards Committee shall have as its prime duty the judging of student research and paper competitions, both undergraduate and graduate, and any other similar competitions. The committee shall also maintain the criteria to be used in the judging and selection of papers, such criteria to be circulated to prospective competitors. (1985 Revision)

e. Necrology Committee.

Membership: Three members.

Three-year terms.

Duties: The Necrology Committee shall report to the annual meeting on those departed during the preceding year. Obituaries may be included in the minutes of the annual meeting and/or published in the Proceedings.

f. Nominating Committee.

Membership: The five most recent past-presidents.

Duties: The Nominating Committee shall propose a slate of at least two nominees for each of the offices as needed. The committee report shall be submitted to the President prior to the annual meeting as well as reported to the membership at the appropriate time for action.

g. Resolution Committee.

Membership: Three members.

Three-year terms.

Duties: The Committee on Resolutions shall prepare such resolutions of recognition and thanks as appropriate for the annual meeting. Further, the Committee shall receive suggested resolutions for the membership and transmit such resolutions and the Committee recommendation to the membership.

h. Membership Committee.

Membership: Unlimited number.

Appointed annually.

Duties: The Membership Committee shall promote membership in the NDAS. It shall conduct an annual canvass of the Institutions of Higher Education, Government Agencies, and other related organizations for the purpose of providing opportunity for prospective members to join the NDAS. Further, this Committee shall make recommendations to the Executive Committee of potential candidates for emeritus and honorary memberships.

- 8. The Nominating Committed shall be responsible for all nominations to elective office and shall be required to advance at least two names for each open position. Academy members shall have been encouraged to suggest nominees to the committee prior to the Committee submitting its report. A ballot, incorporating brief biographical information, shall be distributed by the Secretary-Treasurer to all members prior to the Annual Meeting. Those ballots may be returned by mail, or in person at the Annual Meeting, until the announced deadlines. The results of the election shall be announced at the Annual Meeting.
- 9. Categories of Membership:
  - a. Active members -- shall be persons interested or actively participating in some scientific endeavor. Active members may participate in all activities of the NDAS.
  - b. Student members -- shall be graduate or undergraduate College students in some field of science. Student members may participate in all activities of the NDAS, with the exception of holding office.
  - c. Sustaining members are persons or organizations interested in the activities of the NDAS. Sustaining members may participate in all activities of the NDAS, with the exception of voting or holding office. Sustaining members may be of three types: Individual, Corporate, or Institutional. (1965 Revision) This bylaw is implemented by the following action of the Executive Committee (10-25-85):

There shall be two categories of Corporate Sustaining Membership, Patron members and Sponsor members. The annual membership fee shall be \$100 for Patron members and \$50 for Sponsoring members. Benefits accruing to Corporate Sustaining Members include:

- 1. Positive public relations through support of science and technology in North Dakota
- 2. Preference in mounting commercial displays at the annual meetings of the NDAS.
- 3. Early access to research results and early awareness of research programs through first hand association with scientists and engineers.

- Improved commercial opportunities through association with members, institutions, and other sustaining members.
- 5. Improved future commercial opportunities through exposure to students contemplating careers in science or technology.

Until action is taken otherwise, the  $\bar{\text{C}}$ orporate Sustaining Membership fees shall be placed in the North Dakota Science Research Foundation for the support of scientific research.

- d. Emeritus Membership. Any member in good standing upon formal retirement is eligible for emeritus membership. Nominations may be forwarded to the Membership Committee by any member, and it shall be the responsibility of the membership committee to review the membership list for possible candidates. The Executive Committee shall approve nominations. Emeritus members shall retain all rights of active members but will be exempt from payment of dues. (1973 Revision)
- e. Honorary Membership. The Academy may recognize, by awarding honorary membership, any person (nonmember or member) who has in any way made an outstanding contribution to science. It shall be the responsibility of the Membership Committee to be aware of individuals whom it would be fitting for the NDAS to honor in this fashion. Any member may submit nominations along with supporting data to the Membership Committee. Approval of nominations shall be by a two-thirds majority of those attending the annual meeting.

  ( 1973 Revision )
- 10. The President, with the approval of the Executive Committee, shall appoint members to serve on ad hoc committees. Reports of ad hoc committees shall be presented to the Executive Committee or to the annual meeting. Ad hoc committees serve only during the tenure of the president who appointed them. (1965 Revision)
- 11. The Executive Committee shall appoint an Editor who shall edit the PROCEEDINGS. The Editor shall be appointed for a three-year term. The salary of the Editor shall be set by the Executive Committee. (1975 Revision)
- 12. The annual dues shall be \$12.00 per year for professional members, with \$2.00 designated for the North Dakota Science Research Foundation, and \$5.00 per year for student members.

  ( 1985 Revision )
- 13. The Executive Committee is empowered to charge a publication fee of authors of up to \$10.00 per page. (1965 Revision)
- 14. All student research participants shall receive a properly inscribed certificate and be invited to the dinner as the guests of the NDAS. (1965 Revision)
- 15. All activities of the Academy, including grant applications, are to be handled through the Academy Offices from now on. (1966 Revision)
- 16. The Executive Committee of the NDAS is instructed to establish a J Donald Henderson Memorial Fund and to administer this fund so that the proceeds will be used to promote science in North Dakota.

  (1967 Revision)
- 17. The fiscal year of the North Dakota Academy of Science, for the purpose of financial business, shall be 1 January to 31 December. ( 1973 Revision )
- 18. The NDAS establishes the North Dakota Academy of Science Achievement Award to be given periodically to a NDAS member in recognition of excellence in one or more of the following:
  - a. Nationally recognized scientific research.
  - b. Science education.
  - c. Service to the NDAS in advancing its goals.

The Nominating Committee will administer the selection process, will develop a separate funding source for a monetary award, and will develop, for Executive Committee approval, the criteria for the award. (1988 Revision)

The North Dakota Science Research Foundation is established as an operating arm of the 19. NDAS. The purposes of the Foundation are: (1) to receive funds from grants, gifts, bequests, and contributions from organizations and individuals, and (2) to use the income solely for the making of grants in support of scientific research in the State of North Dakota. Not less than 50% of the eligible monies received shall be placed in an endowment from which only the accrued interest shall be granted.

The Foundation shall be responsible for soliciting the funds for the purposes described. The Foundation funds shall be in the custody of the Secretary-Treasurer of the NDAS and shall be separately accounted for annually.

The Foundation Board of Directors shall be comprised of five members of the NDAS, representing different disciplines. Members shall be appointed by the President for staggered five year terms. The chairperson of the Board shall be appointed annually by the President. The Board shall be responsible for developing operating procedures, guidelines for proposals, evaluation criteria, granting policies, monitoring procedures, and reporting requirements, all of which shall be submitted to the Executive Committee for ratification before implementation.

The Foundation shall present a written and oral annual report to the membership of the NDAS at each annual meeting, and the Secretary-Treasurer shall present an accompanying financial report. (1989 Revision)

Last Revised, May 1989

## EXECUTIVE COMMITTEE

Glen Statler, President Department of Plant Pathology North Dakota State University Targo, ND 58105 237-		John Brauner, Department of 2932 Legend Grand Forks,	Lane	-94
Carolyn Godfread, President el 216 West Avenue F Bism	ect -96 marck, ND	58501	223–2546	
Roy Garvey, Secretary-Treasure Department of Chemistry North Dakota State University Fargo, ND 58105 237 NUO25304@NDS	, '-86 <b>9</b> 7	Division of Minot State		
Patricia Kelly, Member at Larg Department of Geology/Geo Eng University of North Dakota Grand Forks, ND 58202 777	ge -96 ginr 7-2811	Department o	State University	
EDITORIAL COMMITTEE		RESOLUT1	ONS COMMITTEE	
John Hammen -9	16	John Reid		<b>-</b> 95
University of North Dakota  James Lindley -9	-	University David Hein	of North Dakota	<b>-</b> 96
North Dakota State Universit Robert Seabloom, Chairman -9 University of North Dakota		A William Joh	of North Dakota nnson, Chairman of North Dakota	-94
NOMINAT	'ING	сомміл	ТЕЕ	
David Davis -9 USDA Biosciences Research La Clark Markel, Chair -9 Minot State University	a b	Bonnie Heidel	Nutrition Research	-94 Center -93
EDUCAT	I O N	COMMIT	T E E	
Om Madhok -9 Minot State University State Science Fair	)5	Dennis Disrud Minot State	University	<b>-9</b> 6
Mike Burton -9 Agassiz Jr High School, Fargo State Science Olympiad		Marcia Steinwa Robinson Hig		-94
Jerome Knoblick -9 Jamestown College AAAS Mini-Grant Coordinator	95	Robert Biek North Dakota	a Geologic Survey	<b>-</b> 96
Junior Academy Liason Marla Behm -9 Bismarck, ND	97	Harold Fish Watford City	ı	<b>-97</b>

OFFICEDO	4	COMMITTEES	,
OFFICERS	ana	COMMITTEES	١

#### May 1993 - April 1994

#### NECROLOGY COMMITTEE

-94 Michael Thompson, Chairman -95 Duane Erickson Minot State University North Dakota State University AWARDS DENISON COMMITTEE -96 -96 Lyle Prunty Daniel Mott North Dakota State University Dickinson State University Hans Goettler -94 Carl R Steffan -94 North Dakota State University Jamestown College Dorothy Johansen -95 Eileen Starr -95 Mayville State University Valley City State University NORTH DAKOTA SCIENCE RESEARCH FOUNDATION BOARD of DIRECTORS Om Madhok -95 David Berryhill **-9**6 Minot State University North Dakota State University -97 -94 Jim Walla Larry Campbell, Chairman University of North Dakota North Dakota State University Ray Taylor -98 North Dakota State University MEMBERSHIP COMMITTEE Gary Clambey Vernon Feil North Dakota State University USDA- Bioscience Research Laboratory Myron Freeman Carolyn Godfread Dickinson State University Bismark Janet Hunt Richard Baltisberger Human Nutrition Research Center University of North Dakota

Incomb Chickler

Joseph Stickler Valley City State University

Dorothy Johansen
Mayville State University

LOCAL ARRANGEMENTS

C O M M I T T E E -- Fargo/Moorhead

North Dakota State University

Michael Thompson

David Berryhill

Minot State University

Facilities: Dwain Meyer A V: Jim Tilton
Housing/Parking: Rodney Utter Banquet: Judy Strong (MSU)
Lunch/Refrehments: Dave Davis Tours: Jack Rassmussen
Keynote Speakers: Gary Clambey Social Hours: Marty Draper
Funding: Larry Campbell Public Relations: Don Galitz
Registration: Roy Garvey Coordinator: Glen Statler

## and Location of the Annual Meeting of the NORTH DAKOTA ACADEMY of SCIENCE

			<b>.</b>	1050		
1909	M A Brannon	Grand	Forks	1953	Wilson Laird	Grand Forks
1910	M A Brannon	Fargo		1954	C O Clagett	Fargo
1911	C B Waldron	Grand	Forks	1955	G A Abbott	Grand Forks
1912	L B McMullen	Fargo		1956	H B Hart	Jamestown
1913	Louis VanEs	Grand	Forks	1957	W E Cornatzer	Grand Forks
1914	A G Leonard	Fargo		1958	W C Whitman	Fargo
1915	W B Bell	Grand	Forks	1959	Arthur W Koth	Minot
1916	Lura Perrine	Fargo		<b>19</b> 60	H J Klosterman	Fargo
1917	A H Taylor	Grand	Forks	1961	Vera Fac <b>e</b> y	Grand Forks
1918	R C Doneghue	Fargo		1962	J F Cassel	Fargo
1919	H E French	Grand	Forks	1963	C A Wardner	Grand Forks
1920	J W Ince	Fargo		1964	Fred H Sands	Fargo
1921	L R Waldron	Grand	Forks	1965	P B Kannowski	Grand Forks
1922	Daniel Freeman	Fargo		1966	Paul C Sandal	Fargo
1923	Norma Preifer	Grand	Forks	1967	F D Holland, Jr	Grand Forks
1924	O A Stevens	Fargo		1968	W E Dinusson	Fargo
1925	David R Jenkins	Grand	Forks	1969	Paul D Leiby	Minot
1926	E S Reynolds	Fargo		1970	Roland G Severson	Grand Forks
1927	Karl H Fussler	Grand	Forks	1971	Robert L Burgess	Fargo
1928	H L Walster	Fargo		1972	John C Thompson	Dickinson
1929	G A Talbert	Grand	Forks	1973	John R Reid	Grand Forks
1930	R M Dolve	Fargo		1974	Richard L Kiesling	Fargo
1931	H E Simpson	Grand	Forks	1975	Arthur W DaFoe	Valley City
1932	A D Wheedon	Fargo		1976	Donald R Scoby	Fargo
1933	G C Wheeler	Grand	Forks	1977	Om P Madhok	Minot
1934	C I Nelson	Fargo		1978	James A Stewart	Grand Forks
1935	E A Baird	Grand	Forks	1979	Jerome M Knoblich	Aberdeen, SD
1936	L R Waldron	Fargo		1980	Duane O Erickson	Fargo
1937	J L Hundley	Grand	Forks	1981	Robert G Todd	Dickinson
1938	P J Olson	Fargo		1 <b>9</b> 82	Eric N Clausen	Bismark
1939	E D Coon	Grand	Forks	1983	Virgil I Stenberg	Grand Forks
1940	J R Dice	Fargo		1984	Gary Clambey	Fargo
1941	F C Foley		Forks	1985	Michael Thompson	Minot
1942	F W Christensen	Fargo		1986	Elliot Shubert	Grand Forks
1943	Neal Weber	_	Forks	1987	William Barker	Fargo
1944	E A Helgeson	Fargo		1988	Bonnie Heidel	Bismark
1945	W H Moran	_	Forks	1989	Forrest Nielsen	Grand Forks
1946	J A Longwell	Fargo		1990	David Davis	Fargo
1947	A M Cooley		Forks	1991	Clark Markell	Minot
1948	R H Harris	Fargo		1992	John Brauner(elect	
1949	R B Witmer		Forks	1993	John Brauner	Jamestown
1950	R E Dunbar	Fargo		1994	Glen Statler	Fargo/Moorhead
1951	A K Saiki	_	Forks	- / / -	J_011	- 3- 60/ .1002
1952	Glenn Smith	Fargo				
1956	OTCINI DINTEN	rargo				

End of Fiscal Year STATEMENT of FINANCIAL STATUS				BAL	ANCE	SHEE	T > 1		
Fiscal Year	1988	1989	1990	1991	1992	1993	1994	1995	1996
ASSETS	31483.43	30902.44	33655.5	34486.88	38027.91	36264.02	34688.72	34688.72	34688.72
Operating Accounts Checking Savings / Certificates	2579.2 7634.09		1741.74 6232.93	3381.24 2000.00	3205.93	1575.30			
Trust Accounts Scholarship Principal Research Foundation		16505.83 7159.60		19536.06 9569.58	23953.30 10868.68	23358.98 11329.74	23358.98 11329.74	23358.98 11329.74	
LIABILITIES	26106.64	26958.71	28165.83	30865.64	37261.98	35388.72	34688.72	34688.72	34688.72
Advanced Dues Payments Restricted Purpose Funds	1585.00	1285.00	585.00	760.00	1540.00	700.00			
Scholarship Principal AAAS Grant	15361.13 900.00	16505.83	17166.26 1900.00	19536.06 1000.00	23953.30 900.00	23358.98	23358.98	23358.98	23358.98
Research Foundation Cash	5909.01 2351.50	7159.60 2008.28	8514.57	9569.58	10868.68	11329.74	11329.74	11329.74	11329.74
ACCUMULATED SURPLUS	5376.79	3943.73	5489.67	3621 • 24	765.93	875.30			
CHANGE in SURPLUS		-1433.06	1545.94	-1868.43	-2855.31	109.37	-875.30		
=======================================	======	======	= ======	=======		=======	=======	=======	=
OPERATING CASH	FLOI	И							
CASH on HAND 31 December	10135.49	10292.90	8415.30	8027.02	5381.24	3205.93	1575.30	1575.30	1575.30
RECEIPTS for Year RESOURCES Available DISBURSEMENTS	24394.41	20041.33	11173.58 19588.88 11561.86	9021.40 17048.42 11667.18	8144.73 13525.97 10320.04	6329.45 9535.38 7960.08	1575.30	1575.30	1575.30
CASH BALANCE 31 December	10292.90	8415.30	8027.02	5381.24	3205.93	1575.30	1575.30	1575.30	1575.30
Increase over Year	157.41	-1877.60	-388.28	-2645.78	-2175.31	-1630.63			
=======================================	======	= ======	= ======	=======		=======		******	=
MEMBERSHIP									
Emeritus Students Professional Deliquent Dropped Other	60 43 283	58 53 290 58	59 61 290	54 104 312 86	56 41 210 115 86	57 47 211 163 55 13			
TOTALS	386	459	410	556	508	546	=====	*****	

End of Fiscal Year STA	TEMENT of	FINANCI	AL STATUS	6	OPEF	RATING	INCO	) M E > 2	2
Fiscal Year	1988	1989	1990	1991	1992	1993	1994	1995	1996
DUES	3617.00	2992.00	2680.00	2755.00	3320.00	1806.00			
Reinstatements	67.00	50.00	90.00	20.00	90.00	50.00			
Current year	1965.00	1657.00	2005.00	1975.00	1690.00	980.00			
Future years Sponsor/Patron	1585.00	1285.00	585.00	760.00	1540.00	700.00 76.00			
INSTITUTIONS	1950.00	2200.00	2200.00	1200.00	200.00	200.00			
UND	1000.00	1000.00	1000.00						
NDSU	750.00	1000.00	1000.00	1000.00					
Minot State Jamestown College	200.00	200.00	200.00	200.00	200.00	200.00			
INDUSTRY				200.00					
Basin Electric				100.00					
Red River Sugarbeet Grow				100.00					
ASSOCIATES									
ANNUAL MEETING	6398.20	3460.00	3613.04	2286.00	2252.00	2998.00			
Projetration Food	1800.00	2810.00	2191.00	1377.00	1729.00	1970.00			
Registration Fees Banquet Ticket Sales	1965.00	2010.00	2131.00	809.00	423.00	350.00			
Assocn ND Geographers	1000100	50.00		333,03	50.00	50.00			
Sigma Xi UND	50.00	50.00	50.00	50.00	50.00				
Sigma Xi Minot		50.00		50.00					
Sigma Xi NDSU		100.00	150.00						
SD Academy	233.20		400.00						
ND Geol Society	50.00 2000.00	100.00	100.00			20.00			
Subsidy RRV Amer Chem Sco	300.00	300.00	350.00			28.00			
NDSU Engineering	300.00	300.00	772.04						
Jamestown College						600.00			
AWARDS PROGRAMS	1647.50	481.78	2375.20	2355.65	2226.40	1473.51			
AAAS Sec Schl Research	900.00		1900.00	1000.00	900.00				
Scholarship Dividends	747.50	481.78	475.20	612.15	372.40	708.45			
ND Research Foundation				743.50	954.00	765.06			
PUBLICATION SALES	167.00	123.00	102.00	52.00	106.00	154.00			
INTEREST on SAVINGS	479.22	491.65	203.34	172.75	40.33				
	=======	======	=======	======	=======	======	======	======	
TOTAL INCOME	14258.92	9748.43	11173.58	9021.40	8144.73	6631.51			

End of Fiscal Year STA	TEMENT of	FINANCI	AL STATUS	i	OPER	ATING	EXPEN	SES	> 3
Fiscal Year	1988	1989	1990	1991	1992	1993	1994	1995	1996
ANNUAL MEETING	6708.68	3564.03	3928.22	3007.59	2915.66	2563.36			
Speakers Expenses	2651.40	973.83	1122.07	514.00	918.16	513.80			
Meals/Refreshments	2734.36	1856.30	1929.39	1903.95	1656.30	838.50			
Printing				589.64	320.20	448.30			
General Expenses	1322.92	733.90	876.76		21.00	762.76			
AWARDS PROGRAMS	1360.00	1725.00	1100.00	1975.00	1750.00	1600.00	850.00	850.00	850.00
AAAS Sec Schl Research ND Science Olympiad	710.00	900.00	700.00	1200.00 100.00	900.00	800.00			
ND Science/Engineer Fair	25.00			50.00	50.00	50.00	50.00	50.00	50.00
Denison Awards	500.00	450.00	400.00	300.00	400.00	300.00	400.00	400.00	400.00
ND Jr Academy Awards				325.00	400.00	450.00	400.00	400.00	400.00
Dunbar Award	75.00	175.00							
Henderson Award	50.00								
Abbott Scholarship		200.00							
PUBLICATIONS	2353.84	2836.65	3883.37	2883.28	2704.00	2406.00			
Proceedings	2103.84	2586.65	3133.37	2633.28	2704.00	2406.00			
Editor Fees	250.00	250.00	750.00						
Dakota Science Teacher				250.00					
PROGRAM OPERATIONS	475.60	55.80	471.55	132.76	255.19	41.75			
Junior Academy			350.00	132,76					
Exec Committee	475.60	55.80	121.55		255.19	41.75			
OFFICE EXPENSES	2171.92	2376.25	1199.49	1857.55	1648.59	1103.97	49.00	49.00	49.00
Postage	762.72	403.16	550.56	1194.95	924.08	702.54			
Post Office Box Rental	39.00	39.00	39.00	39.00	49.00	49.00	49.00	49.00	49.00
Duplicating	218.68	215.08	208.42	324.95	392.26	300.56			
Supplies	414.02	349.01	259.01	228.65	98.25	51.87			
Clerical Assistance	137.50	170.00	92.50	70.00	185.00				
Sec Treas Fee	600.00	1200.00	50.00						
MISCELL ANEOUS	1031.47	1068.30	979.23	1811.00	1046.60	245.00	86.00	86.00	86.00
Fidelity Bond	26.00	26.00	26.00	26.00	26.00	26.00	26.00	26.00	26.00
AAAS Delegate Expenses		1000.00	911.73	1000.00					
NAAS Dues	44.90	42.30	41.50	41.50	66.60	60.00	60.00	60.00	60.00
Funds Transfers				743.50	954.00	159.00			
		======	******	******	======	======		======	
TOTAL DISBURSEMENTS	14101.51	11626.03	11561.86	11667.18	10320.04	7960.08	985.00	985.00	985.00

End of Fiscal Year STA	TEMENT of	FINANCI	AL STATU	5	TRUST	FUN	DS A	C C O U	NTS
Fiscal Year	1988	1989	1990	1991	1992	1993	1994	1995	1996
	SCIEN	CE R	ESEA	RCH	FOUN	DATIO	N		
CASH INCOME									
Donations from Members	279.00	296.50	270.00	261.50	303.00	159.00			
Allocations from Dues	678.00	544.00	438.00	482.00	651.00	304.00			
Intrest Accrued	250.57	310.09	396.97	311.51	345.10	302.06			
Sponsors / Patrons Other Sources	300.00	100.00	250.00						
TOTAL	1507.57	1250.59	1354.97	1055.01	1299.10	765.06			
CASH EXPENSE									
Grants									
Awards									
Interest Compounding	250.57	310.09	396.97	311.51	345.10	302.06			
Other Disbursements	1257.00	940.50	958.00	743.50	954.00	159.00			
TOTAL	1507.57	1250.59	1354.97	1055.01	1299.10	461.06			
in checking NET CHANGE						304.00			
400570									
ASSETS	44O1 44	5000 01	7150 60	851/ 57	9569.58	11320 7/	11320 7/	11320 7/	11320 7/
Pass Book Savings 31 Dec Investment 1	4401•44	3303.01	1133.00	0314.31	3303.30	11323.14	11323.14	11323414	11323.14
Unit valuation									
Book Value									
BOOK VALGE									
Investment Value TOTAL	5909.01	5909.01	8514.57	9569.58	10868.68	11329.74	11329.74	11329.74	11329.74
CHANGE	1507.57	1507.57	1250.59	1354.97	1055.01	461.06			
	SCHOL	ARSH	IP F	UND					
CASH INCOME									
S D G E Dividends	257.50	267.50	270.00	205.00	70.00	407.00			
S D G E Dividends I E S Industries					302.40	407.00 407.20			
S D G E Dividends I E S Industries CD Interest	257 <b>.</b> 50 490 <b>.</b> 00	267.50	270.00 205.20	205.00 216.00	302.40 13.59				
S D G E Dividends I E S Industries	257.50	267.50	270.00	205.00	302.40				
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research	257.50 490.00 900.00	267.50 214.28	270.00 205.20 1900.00	205.00 216.00 1000.00	302.40 13.59 900.00	407.20			
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research TOTAL	257 <b>.</b> 50 490 <b>.</b> 00	267.50	270.00 205.20	205.00 216.00 1000.00	302.40 13.59				
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research	257.50 490.00 900.00	267.50 214.28	270.00 205.20 1900.00	205.00 216.00 1000.00	302.40 13.59 900.00	407.20	400.00	400.00	400•00
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research TOTAL	257.50 490.00 900.00 1647.50	267.50 214.28 481.78	270.00 205.20 1900.00 2375.20	205.00 216.00 1000.00	302.40 13.59 900.00 1285.99	407 <b>.</b> 20	400 <b>.</b> 00	400 <b>.</b> 00 400 <b>.</b> 00	400.00 400.00
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL CASH EXPENSE Denison Awards	257.50 490.00 900.00 1647.50	267.50 214.28 481.78	270.00 205.20 1900.00 2375.20 400.00	205.00 216.00 1000.00 1421.00 300.00	302.40 13.59 900.00 1285.99 400.00	407.20 814.20 300.00			
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL CASH EXPENSE Denison Awards Junior Academy Awards	257.50 490.00 900.00 1647.50 500.00	267.50 214.28 481.78 450.00	270.00 205.20 1900.00 2375.20 400.00	205.00 216.00 1000.00 1421.00 300.00 325.00	302.40 13.59 900.00 1285.99 400.00 400.00	407.20 814.20 300.00 500.00			
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL CASH EXPENSE Denison Awards Junior Academy Awards AAAS Mini Grant	257.50 490.00 900.00 1647.50 500.00 710.00	267.50 214.28 481.78 450.00	270.00 205.20 1900.00 2375.20 400.00	205.00 216.00 1000.00 1421.00 300.00 325.00 1200.00	302.40 13.59 900.00 1285.99 400.00 400.00 900.00	407.20 814.20 300.00 500.00 800.00	400.00	400.00	400.00
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL  CASH EXPENSE Denison Awards Junior Academy Awards AAAS Mini Grant ND Science/Engineer Fair Dunbar Award Henderson Award	257.50 490.00 900.00 1647.50 500.00 710.00 25.00	267.50 214.28 481.78 450.00 900.00	270.00 205.20 1900.00 2375.20 400.00	205.00 216.00 1000.00 1421.00 300.00 325.00 1200.00	302.40 13.59 900.00 1285.99 400.00 400.00 900.00	407.20 814.20 300.00 500.00 800.00	400.00	400.00	400.00
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL  CASH EXPENSE Denison Awards Junior Academy Awards AAAS Mini Grant ND Science/Engineer Fair Dunbar Award	257.50 490.00 900.00 1647.50 500.00 710.00 25.00 75.00	267.50 214.28 481.78 450.00 900.00	270.00 205.20 1900.00 2375.20 400.00	205.00 216.00 1000.00 1421.00 300.00 325.00 1200.00	302.40 13.59 900.00 1285.99 400.00 400.00 900.00	407.20 814.20 300.00 500.00 800.00	400.00	400.00	400.00
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL  CASH EXPENSE Denison Awards Junior Academy Awards AAAS Mini Grant ND Science/Engineer Fair Dunbar Award Henderson Award	257.50 490.00 900.00 1647.50 500.00 710.00 25.00 75.00 50.00	267.50 214.28 481.78 450.00 900.00 175.00	270.00 205.20 1900.00 2375.20 400.00 700.00	205.00 216.00 1000.00 1421.00 300.00 325.00 1200.00 50.00	302.40 13.59 900.00 1285.99 400.00 400.00 900.00	407.20 814.20 300.00 500.00 800.00 50.00	400.00	400.00	400.00
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL  CASH EXPENSE Denison Awards Junior Academy Awards AAAS Mini Grant ND Science/Engineer Fair Dunbar Award Henderson Award Abbott Scholarship	257.50 490.00 900.00 1647.50 500.00 710.00 25.00 75.00 50.00	267.50 214.28 481.78 450.00 900.00 175.00	270.00 205.20 1900.00 2375.20 400.00 700.00	205.00 216.00 1000.00 1421.00 300.00 325.00 1200.00 50.00	302.40 13.59 900.00 1285.99 400.00 400.00 900.00 50.00	407.20 814.20 300.00 500.00 800.00 50.00	400.00 50.00	400.00 50.00	400.00
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL  CASH EXPENSE Denison Awards Junior Academy Awards AAAS Mini Grant ND Science/Engineer Fair Dunbar Award Henderson Award Abbott Scholarship	257.50 490.00 900.00 1647.50 500.00 710.00 25.00 75.00 50.00	267.50 214.28 481.78 450.00 900.00 175.00 200.00	270.00 205.20 1900.00 2375.20 400.00 700.00	205.00 216.00 1000.00 1421.00 300.00 325.00 1200.00 50.00	302.40 13.59 900.00 1285.99 400.00 400.00 900.00 50.00	407.20 814.20 300.00 500.00 50.00	400.00 50.00 850.00	400.00 50.00	400.00
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL  CASH EXPENSE Denison Awards Junior Academy Awards AAAS Mini Grant ND Science/Engineer Fair Dunbar Award Henderson Award Abbott Scholarship  TOTAL  NET CHANGE ASSETS	257.50 490.00 900.00 1647.50 500.00 710.00 25.00 75.00 50.00	267.50 214.28 481.78 450.00 900.00 175.00 200.00 1725.00 -1243.22	270.00 205.20 1900.00 2375.20 400.00 700.00	205.00 216.00 1000.00 1421.00 300.00 325.00 1200.00 50.00	302.40 13.59 900.00 1285.99 400.00 900.00 50.00	407.20 814.20 300.00 500.00 50.00 1650.00	400.00 50.00 850.00 -850.00	400.00 50.00 850.00 -850.00	400.00 50.00
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL  CASH EXPENSE Denison Awards Junior Academy Awards AAAS Mini Grant ND Science/Engineer Fair Dunbar Award Henderson Award Abbott Scholarship  TOTAL  NET CHANGE	257.50 490.00 900.00 1647.50 500.00 710.00 25.00 75.00 50.00 1360.00 287.50	267.50 214.28 481.78 450.00 900.00 175.00 200.00 1725.00 -1243.22 289.48	270.00 205.20 1900.00 2375.20 400.00 700.00	205.00 216.00 1000.00 1421.00 300.00 325.00 1200.00 50.00 1875.00 -454.00 315.18	302.40 13.59 900.00 1285.99 400.00 900.00 50.00 1750.00 -464.01 657.40	407.20 814.20 300.00 500.00 800.00 50.00 1650.00 -835.80 694.36	400.00 50.00 850.00 -850.00 694.36	400.00 50.00 850.00	400.00 50.00 694.359
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL  CASH EXPENSE Denison Awards Junior Academy Awards AAAS Mini Grant ND Science/Engineer Fair Dunbar Award Henderson Award Abbott Scholarship  TOTAL  NET CHANGE  ASSETS SDGE Shares (1983) 250 Price 18.50	257.50 490.00 900.00 1647.50 500.00 710.00 25.00 75.00 50.00 1360.00 287.50 277.06 38.25	267.50 214.28 481.78 450.00 900.00 175.00 200.00 1725.00 -1243.22 289.48 45.13	270.00 205.20 1900.00 2375.20 400.00 700.00 1100.00 1275.20 302.00 43.63	205.00 216.00 1000.00 1421.00 300.00 325.00 1200.00 50.00 1875.00 -454.00 315.18 45.00	302.40 13.59 900.00 1285.99 400.00 900.00 50.00 1750.00 -464.01 657.40 29.50	407.20 814.20 300.00 500.00 800.00 50.00 1650.00 -835.80 694.36 25.00	400.00 50.00 850.00 -850.00 694.36 25.00	400.00 50.00 850.00 -850.00 694.36 24.38	400.00 50.00 694.359 24.38
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL  CASH EXPENSE Denison Awards Junior Academy Awards AAAS Mini Grant ND Science/Engineer Fair Dunbar Award Henderson Award Abbott Scholarship  TOTAL  NET CHANGE  ASSETS SDGE Shares (1983) 250	257.50 490.00 900.00 1647.50 500.00 710.00 25.00 75.00 50.00 1360.00 287.50 277.06 38.25	267.50 214.28 481.78 450.00 900.00 175.00 200.00 1725.00 -1243.22 289.48 45.13	270.00 205.20 1900.00 2375.20 400.00 700.00 1100.00 1275.20 302.00 43.63	205.00 216.00 1000.00 1421.00 300.00 325.00 1200.00 50.00 1875.00 -454.00 315.18 45.00	302.40 13.59 900.00 1285.99 400.00 900.00 50.00 1750.00 -464.01 657.40	407.20 814.20 300.00 500.00 800.00 50.00 1650.00 -835.80 694.36 25.00	400.00 50.00 850.00 -850.00 694.36 25.00	400.00 50.00 850.00 -850.00 694.36 24.38	400.00 50.00 694.359 24.38
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL  CASH EXPENSE Denison Awards Junior Academy Awards AAAS Mini Grant ND Science/Engineer Fair Dunbar Award Henderson Award Abbott Scholarship  TOTAL  NET CHANGE  ASSETS SDGE Shares (1983) 250 Price 18.50 Value 4625.00	257.50 490.00 900.00 1647.50 500.00 710.00 25.00 75.00 50.00 1360.00 287.50 277.06 38.25	267.50 214.28 481.78 450.00 900.00 175.00 200.00 1725.00 -1243.22 289.48 45.13	270.00 205.20 1900.00 2375.20 400.00 700.00 1100.00 1275.20 302.00 43.63 13176.26	205.00 216.00 1000.00 1421.00 300.00 325.00 1200.00 50.00 1875.00 -454.00 315.18 45.00 14183.10	302.40 13.59 900.00 1285.99 400.00 900.00 50.00 1750.00 -464.01 657.40 29.50 19393.30	407.20 814.20 300.00 500.00 800.00 50.00 1650.00 -835.80 694.36 25.00 17358.98	400.00 50.00 850.00 -850.00 694.36 25.00 17358.98	400.00 50.00 850.00 -850.00 694.36 24.38 16925.00	400.00 50.00 694.359 24.38 16925.00
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL  CASH EXPENSE Denison Awards Junior Academy Awards AAAS Mini Grant ND Science/Engineer Fair Dunbar Award Henderson Award Abbott Scholarship  TOTAL  NET CHANGE  ASSETS SDGE Shares (1983) 250 Price 18.50 Value 4625.00  IES Industries (1990)	257.50 490.00 900.00 1647.50 500.00 710.00 25.00 75.00 50.00 1360.00 287.50 277.06 38.25	267.50 214.28 481.78 450.00 900.00 175.00 200.00 1725.00 -1243.22 289.48 45.13	270.00 205.20 1900.00 2375.20 400.00 700.00 1100.00 1275.20 302.00 43.63 13176.26	205.00 216.00 1000.00 1421.00 300.00 325.00 1200.00 50.00 1875.00 -454.00 315.18 45.00 14183.10	302.40 13.59 900.00 1285.99 400.00 900.00 50.00 1750.00 -464.01 657.40 29.50 19393.30	407.20 814.20 300.00 500.00 800.00 50.00 1650.00 -835.80 694.36 25.00 17358.98 192.00	400.00 50.00 850.00 -850.00 694.36 25.00 17358.98	400.00 50.00 850.00 -850.00 694.36 24.38 16925.00	694.359 24.38 16925.00
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL  CASH EXPENSE Denison Awards Junior Academy Awards AAAS Mini Grant ND Science/Engineer Fair Dunbar Award Henderson Award Abbott Scholarship  TOTAL  NET CHANGE  ASSETS SDGE Shares (1983) 250 Price 18.50 Value 4625.00  IES Industries (1990) Price 120 @ 31.63	257.50 490.00 900.00 1647.50 500.00 710.00 25.00 75.00 50.00 1360.00 287.50 277.06 38.25	267.50 214.28 481.78 450.00 900.00 175.00 200.00 1725.00 -1243.22 289.48 45.13	270.00 205.20 1900.00 2375.20 400.00 700.00 1100.00 1275.20 302.00 43.63 13176.26 120.00 33.25	205.00 216.00 1000.00 1421.00 300.00 325.00 1200.00 50.00 1875.00 -454.00 315.18 45.00 14183.10 192.00 27.88	302.40 13.59 900.00 1285.99 400.00 400.00 900.00 50.00 1750.00 -464.01 657.40 29.50 19393.30 192.00 23.75	407.20 814.20 300.00 500.00 800.00 50.00 1650.00 -835.80 694.36 25.00 17358.98 192.00 31.25	400.00 50.00 850.00 -850.00 694.36 25.00 17358.98 192.00 31.25	400.00 50.00 850.00 -850.00 694.36 24.38 16925.00 192.00 30.13	694.359 24.38 16925.00 192.00 30.13
S D G E Dividends I E S Industries CD Interest AAAS Sec Schl Research  TOTAL  CASH EXPENSE Denison Awards Junior Academy Awards AAAS Mini Grant ND Science/Engineer Fair Dunbar Award Henderson Award Abbott Scholarship  TOTAL  NET CHANGE  ASSETS SDGE Shares (1983) 250 Price 18.50 Value 4625.00  IES Industries (1990)	257.50 490.00 900.00 1647.50 500.00 710.00 25.00 75.00 50.00 1360.00 287.50 277.06 38.25 10597.55	267.50 214.28 481.78 450.00 900.00 175.00 200.00 1725.00 -1243.22 289.48 45.13 13064.23	270.00 205.20 1900.00 2375.20 400.00 700.00 1100.00 1275.20 302.00 43.63 13176.26 120.00 33.25 3990.00	205.00 216.00 1000.00 1421.00 300.00 325.00 1200.00 50.00 1875.00 -454.00 315.18 45.00 14183.10 192.00 27.88 5352.96	302.40 13.59 900.00 1285.99 400.00 900.00 50.00 1750.00 -464.01 657.40 29.50 19393.30	407.20 814.20 300.00 500.00 800.00 50.00 1650.00 -835.80 694.36 25.00 17358.98 192.00 31.25 6000.00	400.00 50.00 850.00 -850.00 694.36 25.00 17358.98 192.00 31.25 6000.00	400.00 50.00 850.00 -850.00 694.36 24.38 16925.00 192.00 30.13 5784.00	694.359 24.38 16925.00 192.00 30.13 5784.00

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#### EMERITUS Members

ALESSI	Jos <b>e</b> ph	1210 Eleventh Street South	FARGO	ND	58103	701	293-1405
ANDERSON	Edwin M	1151 Twelveth Avenue West	DICKINSON	ND	58601		
AUYONG	Theodore	3614 Eleventh Avenue North	GRAND FORKS	ND	58201	701	772 <b>–</b> 31 <b>6</b> 6
BARNEY	William G	1525 Cottonwood	GRAND FORKS	ND	58201		
BELINSKEY	Carol R	Minot State University	MINOT	ND	58702		
BLISS	Harald N	Post Office Box 522	MAYVILLE	ND	58257		
BOLIN	F M	1505 Sixth Street South	FARGO	ND	58103	701	235-9528
BROPHY	John A	702 South Drive	FARGO	ND	58103	701	235-2772
BROWN	Ralph C	Box 89	STONEHAM	ME	4331		
CALLENBACH	John A	North Dakota State University	HULTZ HALL	ND	58105	701	237-7582
CARLSON	Kenneth T	515 East Thirteenth Street	CASPER	WY	82601	, , ,	
CARMICHAEL		1013 North Anderson Street	BISMARCK	ND	58501		
CARTER	Jack F	1345 Eleventh Street North	FARGO	ND	58102	701	232-0482
	J Frank	83 West Boulder Street	COLORADO SPRNG		80903	701	232-0402
CASSEL		2022 Nameh Washington Change					
CORNATZER	William E	2033 North Washington Street	BISMARCK	ND	58501	701	0/5 3/30
DAFOE	Arthur W	551 Third Street North East	VALLEY CITY	ND	58072		845-2439
DEBOER	Benjamin	312 Alpha Avenue	GRAND FORKS	ND	58203	701	775–4354
DINGA	Gustav P	Concordia College	MOORHEAD	MN	56560		
EDGERLY	Charles G M	1317 Eighth Avenue South	FARGO	ND	58103	701	235-5105
FISK	Allen L	1122 Avenue B West	BISMARCK	ND	58501	701	223–7447
FOSSUM	Guilford O	1828 Cottonwood Street	GRAND FORKS	ND	58201	701	775–7842
FRANK	Richard E	1010 Boyd Drive	GRAND FORKS	ND	58203	701	775-8593
HOEPPNER	Jerome J	2518 Nineth Avenue North	GRAND FORKS	ND	58203		
HOFFMAN	Charles A	Minot State University	MINOT	ND	58702		
HOLLAND	F D Jr	University of North Dakota	GRAND FORKS	ND	58202	701	777-2531
	Jean H	4686 Belmont Road	GRAND FORKS	ND	58201		775-0995
HOLLAND					58201		
JACOBS	Francis A	1525 Robertson Court	GRAND FORKS	ND			772–2447 772–4184
KANNOWSKI	Paul B	1800 Lewis Boulevard	GRAND FORKS	ND	58203	701	//2-4104
KIESLING	Richard	Post Office Box 204	FARGO	ND	58107	701	000 11/1
KLOSTERMAN	Harold J	1437 12 Street North	FARGO	ND	58102		232-1141
KOLSTOE	Ralph H	2108 Seventh Avenue North	GRAND FORKS	ND	58203	701	772–3972
LAIRD	Wilson M	101 Spanish Oak Lane	KERRVILLE	TX	78028		
LOW	Frank N	2511 Saint Charles Avenue	NEW ORLEANS	LA	70130		
MARWIN	Richard M	1519 Chestnut Street	GRAND FORKS	ND	58201	701	775–9728
MELDRUM	Alan	512 Columbia Road North	GRAND FORKS	ND	58203	701	772–1166
MINETTE	Ray	209 Fourth Street Wouth West	RUGBY	ND	58368	701	776–6484
MITCHELL	Earl N	220 Glenhill Lane	CHAPEL HILL	NC	27514		
MCMAHON	Kenneth J	North Dakota State University	VANES HALL	ND	58105		
NELSO <b>N</b>	CN	North Dakota State University	BOTTINEAU	ND	58318		
OWEN	John B	1118 Reeves Drive	GRAND FORKS	ND	58201	701	775-8089
ROGLER	George A	Box 459	MANDAN	ND	58554		.,.
RUDESILL	James T	1318 Twelveth Street North	FARGO	ND	58102	701	235-4629
SCHMIDT	Claude H	1827 North Third Street	FARGO	ND	58102		293-0365
SCOBY	Donald R	North Dakota State University	STEVENS HALL	ND	58105	,01	273-0303
SEVERSON	Roland	2682 Catalina Drive	GRAND JUNCTN	CO	81506		
SLEEPER	Bayard P	Post Office Box 2236	PAULSBO	WA	98370	701	005 (705
SMITH	Glenn S	3140 North Tenth Street	FARGO	ND	58102	701	235–6785
SNOOK	Theodore	343 Sheridan Road	RACINE	WI	53403		
SOUBY	Armand M	103 Nichols	SAN MARCOS	TX	78666		
STARCHER	George W	700 John Ringling Boulvd # 908		FL	34236		
STEWART	James A	Pembroke K8A 1X2	ONTARIO	CAN	ADA		
SUGIHARA	James M	1001 Southwood Drive	FARGO	ND	58103	701	235-8266
SUMMERS	Lawrence	1019 Potter Avenue # 121	BISMARCK	ND	58501		
WALSH	Robert G	Rural Route 6 Box 124 CC Acres	MINOT	ND	58701		
WEISSER	Wilber O	55 Parkview Circle	GRAND FORKS	ND	58201	701	772-4013
WHITMAN	Warren C	North Dakota State University	STEVENS HALL	ND	58105		

#### S T U D E N T Members

ABELL	Pau1	715 North 43rd Street # 205	GRAND FORKS	58203	701	780-0874
ADOLF	Stacy L	Moorhead State University	MOORHEAD	56563		
AHO	Michael	Moorhead State University	MOORHEAD	56563		
ANDERSEN	Susan R	HCR 1 Box 226	BATTLEVIEW	58773	701	464-5770
BALLIET	Jason	Dickinson State University	DICKINSON	58601		101 3770
BANYAI	Shawn	576 Seventh Avenue South	DICKINSON	58601		
BENGEN	Jonas	283 F Court University Village		58102		
BOND	Joyce M	3219 Maple Street	FARGO		<b>7</b> ∩1	232-5660
BREVIK	Eric C	University of North Dakota	GRAND FORKS	58202		746–4353
		•			701	740-4333
BRUNEAU	Theresa	114-1 Tangley Road	MINOT A F B	58704	701	920 2010
CERVINSKI		817 Tenth Avenue North West	MINOT	58701	701	83 <b>9–</b> 3910
DESHAW	Lawrence D	P 0 Box 793	MINOT	58702	701	020 0260
FALLIS	Mary A	1200 Robert Street	MINOT	58701		838-8362
FEIST	Susan A	P 0 Box 251	MINOT	58702	701	83 <b>9</b> –7225
FILKOWSKI	Liane	462 Eighth Street South East	DICKINSON	58601		
FLAGG	Edward	147-2 Delta Drive	MINOT A F B	58704		0.0
FOX	Sheri	Box 185	PARSHALL	58770		862–3465
FREELAND	John A	1410 Seventh Street North	FARGO	58102	701	280–1816
FURMAN	Erik	3725 University Avenue #301	GRAND FORKS	58203		
GRAY	Kevin	3810 Berkeley Drive Apt 4	GRAND FORKS	58203	701	777–8031
GRISSO	Cara	UND School of Medicine	GRAND FORKS	58202		
HANSEN	Jana M	North Dakota State University	WALSTER HALL	58105	701	235–4713
HEINE	Rose	2561 Villa Drive SW #304	FARGO	58103		
HERMAN	Gregory S	1016 Second Street North East	MINOT	58701	701	839 <b>-88</b> 13
HERMAN	Jeffery A	1016 Second Street North East	MINOT	58701	701	839-8813
HILL	Lynn	836 Fifth Avenue West	DICKINSON	58601		
HINZ	Sarah E	1310 Twenty Eighth Avenue S 10	7MOORHEAD	56560	218	233-9278
HUBBARD	Mike	2510 Irvine Avenue North West	BEMIDJI	56601		
JOHNSON	Lori K	825 Forty Second Street SW #209		58103	701	281-0278
JOHNSON	Paul M	Jamestown College	JAMESTOWN	58401		
JOHNSON	Shawn	2501 North 10th Street	FARGO	58102		
JOHNSTON	Kristi S	1537 Cottage Grove Drive	DICKINSON	58601		
JORDAN	Carla	Dickinson State University	DICKINSON	58601		
KALIKIVAYA	Sudhakara	3904 Univeristy Avenue # 212	GRAND FORKS	58203	701	780-9241
KARDASH	Dawn	North Dakota State University	LADD HALL	58105	,01	700 )241
KOPCHYNSKI	David M	Apartment 432	GRAND FORKS	58201	701	772-0970
KOTTOM	Theodore J	North Dakota State University	VANES HALL	58105	701	112-0910
KROEGER	Timothy J	University of North Dakota	GRAND FORKS	58202	701	777-2811
LARSON	Jeffrey P		SALT LAKE CITY			1 595-0958
LIANG	· •	University of North Dakota	GRAND FORKS	58202		780–9214
MAHONEY	Hanqian	University Village				
	Douglas W	Dickinson State University	FARGO	58102	701	239–0350
MILLER	Kenneth		DICKINSON	58601		
MUILENBURG	Scott	Dickinson State University	DICKINSON	58601		
OSMUNDSON	Carl S	322 Third Avenue South	GRAND FORKS	58201		
PERKINS	Kevin L	527 22nd Avenue N W, Aptm 26	MINOT	58701	-01	770 (500
PERRYMAN	Wendy C	1118 Twenty Eight Avenue So 11				772–6593
REZVANI	Ahmad B	University of North Dakota	GRAND FORKS	58202		777–2741
ROCKWELL	Curtis	207 State Street # 306	GRAND FORKS	58203		795–0233
RUTTEN	Luke T	1104 13th Avenue North #4	FARGO	58102	701	232–4481
SAILER	Frances	UND School of Medicine	GRAND FORKS	58202		
SCHMIT	Mike L	1130 Sixteenth Avenue S W	MINOT	58701	<b></b> -	
SPENCER	Kathleen M	University of North Dakota	GRAND FORKS	58202		777–2408
VOLKER	K Warren	University of North Dakota	GRAND FORKS	58202		777–3915
WALKER	Suzann M	University of North Dakota	GRAND FORKS	58202		777–8943
WELLS	Robert C	743 Eleventh Street NE #12	JAMESTOWN	58401	701	392-8855

Members

	le Heather	Human Nutrition Research Center		58202		
ALEXANDER	Bonnie J	Valley City State University	VALLEY CITY	58072		
ANDERSON	O <b>rdea</b> n S	Rural Route 1 Box 269	NEW PRAGUE	56071	612 364–8744	
ANGEL	Kathleen	7500 University Drive	BISMARCK	58504		
ARMSTRONG	Kathy	604 East Boulevard Avenue	BISMARCK	58505	701 224–2525	
ASCHBACHER	Peter W	97 Woodland Drive North East	FARGO	58102	701 237–5923	
AULT	Charles R	Jamestown College	JAMESTOWN	58405	701 252-4346 24	48
AULT	Cynthia L	Jamestown College	JAMESTOWN	58405	701 252-3467 24	48
BALACHANDRAN	Chandra S	North Dakota State University	STEVENS HALL	58105	701 237-7115	
BALTISBERGER	Richard	University of North Dakota	GRAND FORKS	58202	701 777–3941	
BARKER	William T	North Dakota State University	HULTZ HALL	58105	701 237-7222	
BARNHART	Michael P	2704 Tenth Avenue North West	MANDAN	58554	701 663-4980	
BEHM	Marla	516 North Nineteenth Street	BISMARCK	58501	701 258~7451	
BERKEY	Gordon B	Minot State University	MINOT	58702		
BERRYHILL	David L	North Dakota State University	VANES HALL	58105	701 237-7694	
BIEK	Robert F	600 East Boulevard Avenue	BISMARCK	58505	701 224-4109	
BITZAN	Edward F	2200 University Avenue	GRAND FORKS	58203		
BLEIER	Willaim J	North Dakota State University	STEVENS HALL	58105	701 237-8421	
BLUEMLE	John P	600 East Boulevard Avenue	BISMARCK	58505	701 258-4981	
BOLONCHUK	William W	University of North Dakota	GRAND FORKS	58202	701 777-4347	
BRAUNER	John F	2932 Legend Lane	GRAND FORKS	58201		
BRAUNER	Carolyn R	2932 Legend Lane	GRAND FORKS	58201	701 795–1924	
BREKKE	David W	University of North Dakota	GRAND FORKS	58202	701 777-5154	
BRISKE-ANDER		1504 CottonWood	GRAND FORKS	58202	701 777–9134	
BUCKLEY	Arthur R	University of North Dakota	GRAND FORKS	58203	701 777-4294	
BUTLER	Malcolm G	North Dakota State University	STEVENS HALL	58105	701 777-4294	
CAMPBELL	Larry G	Northern Crop Science Laboratory		58105	701 237-7398	
CLAMBEY	Gary K	North Dakota State University	STEVENS HALL	58105	701 237-8404	
CLAUSEN	Eric N	Minot State University	MINOT	58702	701 237-0404	
CONNELL	Marvin D	2606 Fifth Avenue North	GRAND FORKS	58203	701 772–7658	
	Robert L				701 772-7036	
CRACKEL		Minot State University	MINOT	58701	701 250 6027	
CUNNINGHAM DAHLEEN	Richard	Rural Route 2 Box 224	BISMARCK	58504	701 258–6937 701 239–1384	
DAVIS	Lynn Dowid C	USDA Northern Crops Institute Bioscience Research Laboratory	FARGO	58105 58105		
	David G	413 Hillcrest Drive	FARGO		701 239–1247	
DISRUD	Dennis T		MINOT	58701	701 839–3784	
DOGGER	James R	P 0 Box 208	GORE	22637	701 007 705/	
DRAPER	Martin A	North Dakota State University	WALSTER HALL	58105	701 237–7854	
EARNHARDT	Todd S	Rural Route 1 Box 165	FOXHOLM	58738	701 468–5624	
EIDE	John D	Northern Crop Science Laborator		58105	701 239–1354	
ELHARDT	Dale G	801 25th Street North West	MINOT	58701	701 839–7449	
ELRICK	Donald L	Mayville State University	MAYVILLE	58257		
EPSTEIN	Paul N	U N D School of Medicine	GRAND FORKS	58203	701 777–2293	
ERICKSON	J Mark	St Lawrence University	CANTON	13617		
FAFLAK	Richard E	1307 Thirteen 1/2 Street North		56560	218 233–6183	
FARNUM	Bruce W	Bldg 41-2 Box 33131	SAINT PAUL	55133	612 458–2268	
FEIL	Vernon J	Biosciences Research Laboratory		58105	701 239–1236	
FILLIPI	Gordon M	1005 South Twentieth Street	GRAND FORKS	58201	701 772–4593	
FINLEY	John W	Human Nutrition Research Center		58202	701 795–8366	
FISH	Harold F	Box 338	WATFORD CITY	58854	701 842-2304	
FIVIZZANI	Albert J	University of North Dakota	GRAND FORKS	58202	701 777–4671	
FORSMAN	N <b>e</b> 1s	University of North Dakota	GRAND FORKS	58202	701 777–4349	
FUNKE	Berdell R	North Dakota State University	VANES HALL	58105	701 237–7846	
GARVEY	Roy	North Dakota State University	DUNBAR HALL	58105	701 237–8697	
GERLA	Philip J	University of North Dakota	GRAND FORKS	58202	701 777–2811	
GILLIES	George T	4310 Beaver Creek Road	EARLYSVILLE	22936	804 924-3781	
GODFREAD	Carolyn	216 West Avenue F	BISMARCK	58501	701 223-2546	
GOETTLER	Hans J	North Dakota State University	DOLVE HALL	58105	701 237-8836	
GOODMA::	Lowell	University of North Dakota	GRAND FORKS	58202	701 777-4246	
GROTH	Larry	U N D Lake Region	DEVILS LAKE	58301	701 662-1550	
HADLEY	Mary	North Dakota State University	HOME ECONOMIC	58105	701 237-7476	
		•				

HALVORSON	Gary A	Box 459	MANDAN	58554	701	667-3002	
HAMMEN	John L	Univeristy of North Dakota	GRAND FORKS	58202	701	777-4589	
HANSEN	Devon	2917 South 17th Street #201	GRAND FORKS	58201			
HARMONING	Arlen	1708 North Fourth Street	BISMARCK	58501			
HARTMAN	Joseph H	University of North Dakota	GRAND FORKS	58202	701	777-2551	
HASSETT	David J	Energy/Environment Research Cent		58202		777-5192	
HASTINGS	Michael	Dickinson State University	DICKINSON	58601		02,2	
HEIDEL	Bonnie	1575 Sixth Avenue	HELENA	59620	406	444-3019	
HEILMANN	Larry J	USDA Bioscience Research Laborat		58105		239-1301	
HEIN	David W	U N D School of Medicine	GRAND FORKS	58202		777-4293	
HEMMASI	Mohammad	University of North Dakota	GRAND FORKS	58202		777-4587	
HOFF	Donald L	402 East First Street	VELVA	58790			
HOGANSON	John W	600 East Boulevard	BISMARCK	58505	701	222-4939	
HOWELL	Francis L	University of North Dakota	GRAND FORKS	58202		777-3516	
HUNG	Yung Tse	Cleveland State University	CLEVELAND	44115			
HUNT	Curtiss D	Human Nutrition Research Center		58202	701	795-8423	
TUUH	Janet R	Human Nutrition Research Center		58202		795-8328	
HUNT	Mary Anne	1118 Forty nineth Avenue South		58104		239-1634	
IDSO	Laura A	USDA Human Nutrition Research	GRAND FORKS	58202			
JACOBSON	Arlen L	415 Fraine Barracks Road	BISMARCK	58504			
JOHANSEN	Dorothy	Mayville State University	MAYVILLE	58257			
JOHNSON	A William	University of North Dakota	GRAND FORKS	58202	701	777-2742	
JOHNSON	Douglas H	Route 1, Box 96C	JAMESTOWN	58401			
JOHNSON	Lester E	Post Office Box 224	BOTTINEAU	58318			
JOHNSON	Phyllis E	800 Buchanan Street	ALBANY	94701			
JOHNSON	W Thomas	Human Nutrition Research Center		58202	701	795-8411	
JORDE	Dennis G	U S Fish and WildLife Service	LAUREL	20708			
JENSEN	Bruce R	Jamestown College	JAMESTOWN	58405			
KANG	Y James	University of North Dakota	GRAND FORKS	58202	701	777-4293	
KANTRUD	Harold A	Route 7 Box 52	JAMESTOWN	58401		252-5639	
KELLEHER	James J	School of Medicine UND	GRAND FORKS	58202		777-2214	
KELLEY	Patricia H	University of North Dakota	GRAND FORKS	58202		777-2811	
KEYS	Ross D	1836 Billings Drive	BISMARCK	58504	701	255-4211	
KIHM	Allen J	Minot State University	MINOT	58702			
KILLINGBECK	James	ND State Health Department	BISMARCK	58504	701	221-5188	
KIRBY	Don	North Dakota State University	HULTZ HALL	58105	701	237-8386	
KONTZ	Bradley	1116 1/2 South Dakota Avenue	SOUIX FALLS	57105			
KNOBLICH	Jerome	Jamestown College	JAMESTOWN	<b>584</b> 05	701	252-3467	248
KNULL	Harvey	University of North Dakota	GRAND FORKS	58202	701	777-2786	
KONTZ	Brad	1919 Seventh Avenue North	GRAND FORKS	58203			
KOTASKA	Су	Box 223	SAWYER	58781			
KRAFT	Donald J	Bemidji State University	BEMIDJI	56601	218	755-2795	
KRAFT	Kathy	Jamestown College	JAMESTOWN	58401	701	252-3467	248
KRESS	Warren D	North Dakota State University	STEVENS HALL	58105	701	237-7145	
KRUGER	Robert M	Mayville State University	MAYVILLE	58257			
KRUPINSKY	Joseph M	Agriculture Research Service	MANDAN	58554			
KUIPERS	Gilbert	Valley City State University	VALLEY CITY	5 <b>8</b> 072			
LAMBETH	David O	University of North Dakota	GRAND FORKS	58202	701	777–2759	
LANKOW	John	Mayville State University	MAYVILLE	58257			
LARSON	Linda	University of North Dakota	GRAND FORKS	58202		777–2648	
LARSON	Omer R	University of North Dakota	GRAND FORKS	58202		777–4674	
LEHR	Eugene R	Box 724	LINTON	58552	701	254–5471	
LINCOLN	Terry	P 0 Box 711	BISMARCK	58502			
LINDLEY	James A	North Dakota State University	AG ENGR	58105		237-7273	
LINZ	George M	North Dakota State University	STEVENS HALL	58105		237-7054	
LONG	William M	University of North Dakota	GRAND FORKS	58202		777–3956	
LORENZ	Russell J	1924 North Grandview Lane	BISMARCK	58501		223-3421	
LU	Ruey Pyng	North Dakota State University	WALDRON HALL	58105		237-7492	
LUKASKI LURA	Henry Charles L	Human Nutrition Research Center North Dakota State University		58202	/01	795–8464	
LYKKEN	Glenn I	•	BOTTINEAU	58318	701	777 2510	
LYTTON	Barbara	University of North Dakota Box 58	GRAND FORKS	58202 58781	/01	777–3519	
TITION	שומומ	DOV DO	SAWYER	587 <b>8</b> 1			

MADHOK	Om P	Minot State University	MINOT	58702	701 857-3067
MARKELL	Clark	Minot State University	MINOT	58702	<b>7</b> 01 857-3160
MARTIN	<b>DeWay</b> ne C	2104 Seventh Avenue North West	MINOT	58701	701 852-4726
MARTIN	Paula J	Dickinson State University	DICKINSON	58601	
MARTSOLF	John T	U N D School of Medicine	GRAND FORKS	58203	701 777-4242
MASON	Harry	Post Office Box 1116	JAMESTOWN	58401	<b>70</b> 1 25 <b>2</b> –1247
MEARTZ	Paul D	Mayville State University	MAYVILLE	58257	
MEYER	Dwain W	North Dakota State University	LOFTSGARD HA	58105	701 237-8154
MIESS	Robert	Mayville State University	MAYVILLE	58257	
MILLER	James E	3807 Michael Lane	GLENVIEW	60025	
MORGAN	Rose M	823 Sixth Street South West	MINOT	58701	
MOTT	Daniel J	Dickinson State University	DICKINSON	58601	<b>7</b> 01 22 <b>7–2</b> 111
MUESSIG	Karen D	1204 Shyview Drive	BELLEVUE	68005	402 291-2687
MUNSKI	Douglas	University of North Dakota	GRAND FORKS	58202	<b>7</b> 01 777– <b>4</b> 591
MUNSKI	Laura	University of North Dakota	GRAND FORKS	58202	701 772–8207
MACCARTHY	Ronald F	University of North Dakota	GRAND FORKS	58202	<b>7</b> 01 777– <b>4</b> 424
MCCOLLOR	Donald P	University of North Dakota	GRAND FORKS	58202	701 777-5121
MCDONALD	Clarence E	North Dakota State University	HARRIS HALL	<b>5</b> 8105	701 237–7738
NALEWAJA	John D	North Dakota State University	LOFTSGARD HAL	58105	<b>70</b> 1 237–81 <b>5</b> 8
NELSON	Berlin D	North Dakota State University	WALSTER HALL	58105	701 237–7057
NELSON	Robert M	North Dakota State University	EEE	58105	<b>7</b> 01 237–7619
NELSON	Mary L	1156 Twenty First Street West	DICKINSON	58601	701 225–4384
NIELSEN	Forrest H	Human Nutrition Research Center	GRAND FORKS	58202	<b>7</b> 01 795–8455
NOLAN	Lisa K	North Dakota State University	VANES HALL	58105	<b>7</b> 01 237–8530
NORDLIE	Robert C	University of North Dakota	GRAND FORKS	58202	701 777–2751
OCONNELL	James W	535 Eighth Avenue South West	VALLEY CITY	58072	
OWENS	Thomas C	University of North Dakota	GRAND FORKS	<b>5</b> 8202	<b>7</b> 01 777–4244
PEARSON	Dean	Box 589	BOWMAN	58623	
PECK	Wesley D	University of North Dakota	GRAND FORKS		777–5000
PENLAND	James G	Human Nutrition Research Center	GRAND FORKS	<b>5</b> 8202	<b>7</b> 01 <b>7</b> 95–8471
PERKINS	Dexter	University of North Dakota	GRAND FORKS	58202	701 777–2811
PFISTER	Philip C	North Dakota State University	DOLVE HALL	58105	
PFLUGHOEFT-H	ASSET Debra	F University of North Dakota	GRAND FORKS	<b>5</b> 8202	701 777–5261
PHILION	Richard	Box 162	ELGIN	58533	701 584–3721
POELLOT		Human Nutrition Research Center		58202	701 795–8406
PRUNTY	Lyle D	North Dakota State University	WALSTER HALL	58105	701 237–8580
RAO	•	North Dakota State University	WALDRON HALL	58105	701 237–8178
RAWAT	Banmali	University of Nevada Reno	RENO	89557	<b>7</b> 02 746–1438
RAY	Paul D	University of North Dakota	GRAND FORKS	58202	701 777–3937
REEVES	Philip G	Human Nutrition Research Center		58202	701 795–8497
REID	John R	University of North Dakota	GRAND FORKS	58202	701 777-2248
REINITZ	David M	UND School of Medicine	GRAND FORKS	58202	701 777-2214
RICHARDSON	Jim	North Dakota State University	WASLTER HALL	58105	701 237-8573
RIES	Ronald E	908 Second Avenue North West	MANDAN	58554	701 663–7335
RIGLEY	Louis	515 North Washington Street	BISMARCK	58501	701 255–6575
ROGERS	David A	North Dakota State University	FARGO	58105	701 237-7216
ROYER	Ron	Box 88	BURLINGTON	58722	701 838–7648
SAARI	Jack T	Human Nutrition Research Center		58202	<b>7</b> 01 <b>7</b> 95–8499
SCHEIBE	Paul 0	3 Still Creek Road	WOODSIDE	94062	701 705 0/00
SCHELKOPH	Gwen M	Human Nutrition Research Center		58202	701 795–8498
SCHMID	Thomas	109 Durango Drive	BURLINGTON	58722	701 838-6032
SCHWERT	Donald P	North Dakota State University	STEVENS HALL	58105	701 237–7496
SEABLOOM	Robert W	University of North Dakota	GRAND FORKS	58202	701 777-4676
SEABORN	Carol D	Human Nutrition Research Center 2205 Fourth Street North East		58202	701 795–8353 040
SHAFFER SHULER	Terry Terrence R	2974 Columbine Court	JAMESTOWN	58401 58201	701 251–2399 701 772–7919
SIDERS	William A	1105 South Twenty Second Street	GRAND FORKS	58201	701 772-7919 701 746-8921
SILVERMAN	Louis B	2524 Olson Drive	GRAND FORKS	58201	101 140-0321
SILVERMAN	Roger L	5050 Fifth Avenue North	GRAND FORKS	58203	
SMITH	Donald A	North Dakota State University	FARGO	58105	701 237-7401
SPANIER	Jon G	U N D School of Medicine	GRAND FORKS	58202	701 777-2750
STACK	Robert W	North Dakota State University	WALSTER HALL	58105	701 237-7077
JINOK	WODELL M	north bandla plate university	AMITOTIME (WITH	20102	101 201-1011

STARR	Eileen M	Valley City State University	VALLEY CITY	58072	
STATLER	Glen D	North Dakota State University	WALSTER HALL	58105	<b>7</b> 01 <b>237–70</b> 58
STEFFAN	Carl R	Jamestown College	JAMESTOWN	58405	701 252-3467 248
STICKLER	Joseph C	Valley City State University	VALLEY CITY	58072	
STINNETT	Henry O	U N D School of Medicine	GRAND FORKS	58202	<b>701 777–3</b> 955
STITH	Jeffrey L	University of North Dakota	GRAND FORKS	<b>582</b> 02	<b>7</b> 01 <b>777–3</b> 178
STOAKS	Ralph	5888 Our Way	CITRUS HEIGHT	95610	<b>9</b> 16 965 <b>–4</b> 045
STOCKRAHM	Donna M Bru	ns Moorhead State University	MOORHEAD	56563	218 236-2576
SUKALSKI	Katherine	University of North Dakota	GRAND FORKS	58202	<b>701 777–4</b> 049
SWANSON	Richard	507 Third Street Court	WEST FARGO	58078	701 282-0577
TAYLOR	Raymond	North Dakota State University	WALSTER HALL	58105	<b>7</b> 01 <b>237–80</b> 51
THOMPSON	Michael B	2208 Crescent Drive	MINOT	58701	<b>7</b> 01 839–6305
TIMIAN	Roland G	North Dakota State University	WALSTER HALL	58105	701 237-7853
TODD	Robert G	221 Seventh Avenue West	DICKINSON	58601	<b>7</b> 01 8 <b>73</b> –6440
TOMANEK	Debora	North Dakota State University	STEVENS HALL	58105	701 237-7336
ULMER	Michael G	202 East Divide	BISMARCK	58501	701 258-6454
URLACHER	Kenneth	Route 2 Box 25	NEW ENGLAND	58647	<b>7</b> 01 579 <b>–4</b> 414
UTHUS	Eric O	Human Nutrition Research Center	GRAND FORKS	58202	701 795–8382
UTTER	Rodney A	North Dakota State University	WALSTER HALL	<b>5</b> 8105	701 237-7561
VANALSTINE	James B	University of Minnesota	MORRIS	56267	612 589-2535
VANDERPOOL	Richard A	Human Nutrition Research Center	GRAND FORKS	58202	701 795-8436
VENETTE	James R	North Dakota State University	WALSTER HALL	<b>5</b> 8105	701 237-7073
WALLA	James A	North Dakota State University	WALSTER HALL	58105	701 237-7069
WALLER	James R	University of North Dakota	GRAND FORKS	58202	701 777-2615
WALSH	Naney J	Post Office Box 430	DEVILS LAKE	58301	<b>701 662–4</b> 629
WANEK	Wallace J	16901 Irvine Avenue North West	BEMIDJI	56601	218 243-2245
WATSON	David A	North Dakota State University	VANES HALL	58105	701 237-7692
WILLIAMS	John A	University of North Dakota	GRAND FORKS	58202	701 777-4617
WILLMAN	Clyde A	620 Tenth Street South	FARGO	58103	701 237-0376
WINCZEWSKI	Laramie M	12614 Vindon Drive	HOUSTON	77024	
WOOD	Charles A	University of North Dakota	GRAND FORKS	58202	701 777-3167
WOZNIAK	Chris A	Northern Crop Science Laborator	yFARGO	58105	<b>7</b> 01 239-1358
WRENN	William J	P O Box 9019	GRAND FORKS	58202	701 777-4597
WRETLING	Diane	Rural Route 1 Box 14	GARRISON	58540	
ZIEMAN	Dale M	1128 Ocala Road Apt A-2	TALLAHASSEE	32304	904 575-8704
ZOELLNER	Robert	Northern Arizona University	FLAGSTAFF	86011	602 523-3008

ADOLF, Stacey L	70	HEMMASI, Mohammad	* 39 * 44
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RAO, Yi-ping	*101	STEFFAN, Beth L	<b>7</b> 0
RATHGE, Richard W	* 43	STEVENS, Dennis L	<b>*</b> 63
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ROGERS, DAVID A	<b>*</b> 25	TODHUNTER, Paul	<b>* 6</b> 8
ROSEN, Patricia P	<b>*</b> 65	TSURU, Asuka	*111
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