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85th Annual Meeting

April 1993 Volume 47

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The PROCEEDINGS contains communications (from three Symposia, from Professional Contributed Paper, 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.

Subscriptions: All members of the ACADEMY receive the PROCEEDINGS either at the Annual Meeting or subsequently by mail at no charge. Annual dues for ACADEMY membership are $12.00 for regular membership, $5.00 for student membership, and Corporate Sustaining memberships for patrons at $100.00 and for sponsors at $50.00. Copies of the PROCEEDINGS may be purchased separately for $5.00 per copy, prepaid at the meeting or for $5.00 plus a postage and handling fee of $2.50 by mail. Correspondence concerning subscriptions (standing orders), as well as instructions for authors and other related matters, should be directed to:

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P R O C E E D I N G S
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Volume 4 7 April 1 9 9 3

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

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85th ANNUAL MEETING

29 - 30 April, 1993

Jamestown, North Dakota

E D I T O R's N O T E S

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 I, published before the meeting, contained an Abstract of each paper to be presented at the meeting. Part II, published later, contained full papers by some of the presenters.

Commencing in 1979 with Volume 33, the PROCEEDINGS changed to the present format, is produced from camera-ready copy submitted by authors, and is issued in a single part prior to the Annual Meeting to be distributed initially at the 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 47 of the PROCEEDINGS contains 31 presentations in the three Symposia offered at the 85th Annual Meeting of the Academy held in Jamestown, 29 - 30 April, 1993. These papers are organized by Symposia and are presented in the same sequence as presented at the meeting.

The second section of this volume presents the 7 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 paper is from the undergraduate competition (placed in alphabetical order by the last name of the author presenting the paper) and the second group of 7 papers are from the graduate competition (arranged in similar alphabetical order).

The third section of this volume contains the 25 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 April 1992 - March 1993 year, a list of all Academy members as of 1 March, 1993, and a copy of the most recent (1992) 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.

1. 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
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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.
2. Batsone, G.W., Blair, A.W. and Slater, J.M. (1971) A Handbook of Pre-Natal Pediatrics, pp 83-90. Medical and Technical Publishing, Lancaster.
3. Farah, A.E. and Moe, G.K. (1970) in Pharmacological Basis of Therapeutics, 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 15 minutes total time for presentation and discussion. It is suggested that the presentation be limited to ten 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.

5. 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

GLOBAL CLIMATE CHANGE RESEARCH IN THE NORTHERN GREAT PLAINS

North Dakota Academy of Science 1993 Annual Meeting
Jamestown, North Dakota

Symposium Coordinator
Douglas H. Johnson

Symposium Editors
Douglas H. Johnson, Diane L. Larson, and Jeff T. Price

Thursday, 29 April

8:00 - 8:15	*Introduction to the Symposium and Announcements* Douglas H. Johnson*, U.S. Fish and Wildlife Service, Jamestown, ND 58401
8:15 - 8:35	*Relations Between Upper-Air Flow Patterns, Climate, and Hydrologic Variability in the Red River of the North Basin, United States and Canada* Gregg J. Wiche* and John L. Knox, U.S. Geological Survey, Bismarck, ND 58501
8:40 - 9:00	*Historical Temperature and Precipitation Trends at Jamestown, North Dakota* Paul Todhunter*, Department of Geography, UND, Grand Forks, ND 58202-9020
9:05 - 9:20	*Sulfur Dioxide and Global Cooling* Abdul J. Alkezweeny*, Department of Atmospheric Sciences, UND, Grand Forks, ND 58202
9:25 - 9:45	*Nested Modeling of Regional and Local-scale Climate in North Dakota* David A. Matthews*, U.S. Bureau of Reclamation, Denver, CO 80225-0007
9:50 - 10:10	*Comparison of Environmental Parameter Estimates from a Regional-scale Model with North Dakota Wetland Observations* Jonnie G. Medina*, U.S. Bureau of Reclamation, Denver, CO 80225
10:15 - 10:30	BREAK
10:30 - 10:50	*Effect of Climate on Water Availability in Prairie Wetlands of North and South Dakota* Diane L. Larson*, U.S. Fish and Wildlife Service, Jamestown, ND 58401
10:55 - 11:15	*Climate Change and Prairie Wetlands: Model Simulations of Hydrology and Vegetation* W. Carter Johnson*, Department of Horticulture, Forestry, Landscape and Parks, South Dakota State University, Brookings, SD 57007, and Karen A. Poiani, Center for the Environment, Cornell University, Ithaca, NY 14853
11:20 - 11:35	*Data Base Development for Climate Change Research* Donald L. Phillips*, U.S. EPA Environmental Research Laboratory, Corvallis, OR 97333; John J. Kineman, NOAA National Geophysical Data Center, Boulder, CO 80303; and Jeffrey S. Kern, ManTech Environmental Technology, Inc., Corvallis, OR 97333
11:40 - 11:55	*Research Management Strategies for Fostering Interdisciplinary Team Research* Bruce P. Van Haveren*, Bureau of Land Management, P.O. Box 25047, Lakewood, CO 80225-0047

A Symposium on

GLOBAL CLIMATE CHANGE RESEARCH IN THE NORTHERN GREAT PLAINS

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Friday, 30 April

8:00 - 8:05 *Announcements*

8:05 - 8:25 *Projecting the Vegetation Response to Climatic Change in the North American Central Grasslands Region*
James Lenihan* and Ronald Neilson, U.S. EPA Environmental Research Laboratory, Corvallis, OR 97333; Timothy Kittel, University Corporation for Atmospheric Research, Boulder, CO 80307; Roger Pielke, Department of Atmospheric Science, Colorado State University, Ft. Collins, CO 80523; William Reiners and Bruce Embury, Department of Botany, University of Wyoming, Laramie, WY 82071; and Filippo Giorgi, National Center for Atmospheric Research, Boulder, CO 80307

8:30 - 9:00 *The Palliser Triangle Global Change Project: Examining the Link between Climate and Landscape Processes in the Southern Canadian Grassland*
Donald S. Lemmen* and Robert E. Vance*, Terrain Sciences Division, Geological Survey of Canada, Calgary, Alberta T2L 2A7

9:05 - 9:25 *A Landscape-scale Model of Dispersal: Applying GIS to Investigate Species Range Shifts*
James M. Dyer*, Department of Geography, UND, Grand Forks, ND 58202-9020

9:30 - 9:50 *Calculating the Climate Envelope for North American Grassland Birds*
Jeff Price*, U.S. Fish and Wildlife Service, Jamestown, ND 58401

9:55 - 10:10 BREAK

10:10 - 10:30 *Ozone Depletion and UVB Radiation: Implications for the Northern Prairie*
Edward E. Little* and David L. Fabacher, National Fisheries Contaminant Research Center, Columbia, MO 65201

10:35 - 10:50 *Marine Seismic Technique as a Tool for Paleoclimatic Research, Devils Lake, North Dakota*
Steve W. Cates* and Robert M. Lent, U.S. Geological Survey, Water Resources Division, Bismarck, ND 58501

10:55 - 11:10 *Lake Levels, Groundwater Paleohydrology, and Paleoclimate in West-central Minnesota at 7,000 yr B.P.*
James E. Almendinger*, Limnological Research Center, University of Minnesota, Minneapolis, MN 55455

11:15 - 11:35 *Paleolimnological View of Climate History in the Northern Great Plains*
Dan Engstrom, Sherilyn Fritz, and Kathleen Laird*, Limnological Research Center, University of Minnesota, Minneapolis, MN 55455

11:40 - 12:00 *Paleoclimatic Conditions and Timing of Recharge to Buried-valley Aquifers in North Dakota--Evidence from $\delta^{18}O$, δD, and Tritium Data*
Robert M. Lent*, U.S. Geological Survey, Water Resources Division, Bismarck, ND 58501

GLOBAL CLIMATE CHANGE RESEARCH IN THE NORTHERN PLAINS: INTRODUCTION TO THE SYMPOSIUM

Douglas H. Johnson*
U.S. Fish and Wildlife Service, Jamestown, North Dakota 58401

Global climate change is a contentious issue among policymakers, with the general public, and even in the scientific community. Several reasons for this divisiveness can be identified. First, the weather is always changing--what's so different now? How extreme the current climate seems to be depends on how long a view one takes of the historical record. Second, it is hard to predict weather for tomorrow, let alone years ahead. How can we trust predictions made decades into the future? Third, earlier predictions of major climatic shifts failed to materialize. What became of the global-warming scare in the 1930's, or the 1970's predictions of a new ice age? Fourth, the lack of agreement among scientists leaves policymakers and the public confused and in a quandary as to who to believe. And finally, as frightening as some predictions are, it is comforting to ignore them.

Some facts are clear. Man has changed the atmosphere in ways that can influence climate. Climate is a very complicated physical system of enormous spatial scale; changes in input can have diffuse, time-delayed, and unpredictable consequences in output. Climatic patterns have changed throughout Earth's time. Our accurate measurements and our conception of what is "normal" cover only a geologic eye blink. Nonetheless, the rate of change of some climatic variables is unprecedented in recorded history. Although models have been constructed to simulate climate, they require extensive amounts of supercomputer time, their spatial resolution is coarse, and models developed by different scientists often disagree markedly. Influences of some major components of the system, such as the oceans and cloud cover, are too poorly understood to model with confidence. Fortunately, perhaps, most of the models agree about the prospects for the geographical area of our attention, the northern Great Plains.

Why concern ourselves? With a clearer understanding of how the system works, we may be able to reduce our influence on climate or mitigate those influences. Also, by knowing the likely consequences of global change, we can better prepare for any effects it may have. By our actions, humans are conducting a massive experiment on the Earth. The responses to the treatments we apply, in combination with the variation supplied by nature, are unknown. The more we learn about the system, how it functions naturally, and how we are altering it, the better we will be prepared to cope with what the future brings.

The issues involve science, economics, and politics. They cross national boundaries, although each nation will have its own way of managing them. Within the United States, no single agency has primacy for dealing with the issues. Indeed, the broad spectrum of federal agencies represented here testifies to the breadth of response needed.

The purpose of this symposium is to bring together scientists and others involved with the issue of global climate change as it relates to the northern Great Plains. We will discuss what we know, what we are trying to learn, and what projects we plan to undertake in the future. By exchanging ideas, perhaps we can find areas of collaboration, share data of mutual interest, avoid overlap in our efforts, and in general provide the greatest return on our investment.

The papers in this symposium cover a wide range of topics within our scope. Disciplines include physics, geology, hydrology, meteorology, botany, zoology, and sociology. There is much history, for we can best understand the present and the future by knowing about the past. Techniques used range from slogging through sloughs to advanced computer simulation. There should be something to stimulate and interest everyone concerned about the climate and its effects on our future. Whatever your views on climate change, the ongoing science is exciting. I hope this symposium imparts a taste of that excitement.

RELATIONS BETWEEN UPPER-AIR FLOW PATTERNS, CLIMATE, AND HYDROLOGIC VARIABILITY IN THE RED RIVER OF THE NORTH BASIN, UNITED STATES AND CANADA

Gregg J. Wiche* and John L. Knox
U.S. Geological Survey, Water Resources Division,
821 East Interstate Avenue, Bismarck, ND 58501

In 1990, the North Dakota District of the U.S. Geological Survey and the Hydroclimatological Section, Canadian Climate Centre, Atmospheric Environment Service, began a cooperative study to investigate the relations between large-scale atmospheric circulation anomalies in the lower troposphere and hydrologic variability in the Red River of the North basin. Large-scale anomalies in wind speed and direction, mean temperature, and pressure heights in the lower troposphere are related to hydrologic variability of the Red River of the North basin. Anomalous winds at both the 100- and 50-kilopascal (kPa) pressure surface heights and the mean temperature for the thickness of the air column from the 50- to 100-kPa height are related to monthly precipitation within the basin for wet and dry study years. In addition, teleconnection indices at the 50-kPa height are related to high and low streamflow years. The purpose of this presentation is to describe these relations.

The Red River of the North basin is located at the eastern margin of the northern Great Plains. The Red River of the North drains about 290,000 square kilometers in parts of Minnesota, South Dakota, North Dakota, Manitoba, and Saskatchewan. Upper-air flow data are available for 1946-89. A 200-year flood history is available from documents of fur traders, explorers, and missionaries, as well as from gaging station records. Several major floods occurred during the 1800's that were larger than major floods that have occurred since 1946. Although the upper-air flow data can be used to describe relations between precipitation and streamflow, the relations may not represent the most extreme climatic conditions that have occurred during the last 200 years.

The Pacific-North American (PNA) teleconnection index has the largest amplitude and the most consistent change in sign relative to bimonthly precipitation for the wet and dry study years. The sign of the PNA index is negative for most bimonthly periods for the wet study years and positive for most bimonthly periods for the dry study years. The location, amplitude, and sign of the 100- and 50-kPa pressure surface height anomalies, based on bimonthly precipitation, are most strongly related to the large-scale atmospheric circulation that supports the precipitation regime.

A strong relation exists between the direction of the anomalous wind at both the 100- and 50-kPa pressure surface heights and bimonthly precipitation (wet or dry study years). For wet study years in the Red River of the North subbasins east of the river at the 100-kPa height, the anomalous wind direction is east-southeast and the anomalous wind speed is 0.5 m/s. At the 50-kPa height, the anomalous wind direction is south and the anomalous wind speed is 2.4 m/s. For dry study years, the sign of the anomaly is reversed, indicating a prevailing anticyclonic flow over the Red River of the North basin. An anomalous west-northwesterly wind speed of 0.6 m/s occurs at the 100-kPa height, and an anomalous northerly wind speed of 1.0 m/s occurs at the 50-kPa height.

The thickness of the air column from the 100- to the 50-kPa pressure surface height was used to compute the mean temperature of the air column. Temperatures for October-November, December-January, and February-March wet study years are about 1°C less than the mean (1946-89) for the Red River of the North basin, and temperatures for the dry study years are about 0.8°C greater than the mean. However, the temperature anomaly is not uniform.

The PNA teleconnection index was computed on the basis of the mean October-May 50-kPa height data for low-flow and high-flow years for the Red River of the North basin. Even for this 8-month mean, the PNA index for low-flow years is positive. For the low-flow years, an elongated north-south oriented central cell whose axis intersects latitude 50° north at about longitude 120° west exists. A negative PNA index occurs during high-flow years, and the central cell of the teleconnection is located at about latitude 50° north longitude 130° west along the west coast of North America.

The Baffin Island-West Atlantic teleconnection index is positive for the high-flow years. The positive index often is caused by greater-than-normal retrograde blocking over the North Atlantic.

HISTORICAL TEMPERATURE AND PRECIPITATION TRENDS AT JAMESTOWN, NORTH DAKOTA

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

Recent research has identified possible negative consequences of global warming upon the number and quality of northern prairie wetlands (1). The purpose of this study is to examine historical annual and seasonal air temperature and precipitation trends at Jamestown, North Dakota, located in the Southern Drift Plain.

Monthly temperature and precipitation data for Jamestown State Hospital (JSH) were obtained from the Historical Climatology Network (HCN). The HCN JSH air temperature and precipitation time series began in 1907 and 1891, respectively, and ended in 1987. Archival sources were used to extend both time series through 1990 to include critical drought years. Simple linear regression analyses were applied to the air temperature and precipitation data to test for climatological trends over time.

Historical annual air temperature (N = 84 years) and precipitation (N = 100 years) time series are presented in Figures 1 and 2. A 9-term binomial filtered time series and the long-term mean are also shown. Statistical summaries of annual and seasonal variables are given in Table 1. Annual air temperature warmed at a rate of 2.4F over the past century. Seasonal temperatures also warmed between 0.7 to 3.7F, although only the summer trend is significant. Most of the annual warming (>70%) is due to an increase in minimum (night) temperatures. The annual maximum (day) temperature warming trend is not significant. Seasonal maximum and minimum temperature trends show a much larger minimum than maximum temperature warming, except for summer when the two are of comparable magnitude. Only fall lacks a significant minimum temperature warming, and only summer shows a significant maximum temperature warming. As a result, the annual temperature range has decreased at a rate of 2.2F per 100 years. Annual precipitation has decreased at a rate of 4.2 inches per century. Although drying trends of 0.1-1.9 inches occurred for all seasons, only the spring and summer trends are significant. The ratio of winter to summer precipitation has decreased over time.

Most of the historical warming occurred at night and in winter, probably because of a trend toward increased cloudiness (2), possibly linked to an increase in sulfur dioxide emissions. Although precipitation in the United States has increased over the past century (3), the Northern Great Plains has experienced a drying trend. The summer maximum temperature increase is probably a consequence of changes in the partitioning of surface net radiation resulting from the spring and summer drying trends. Both similarities and differences are found between model-predicted and historical climate trends. Important aspects of regional climate change may be overlooked by focusing upon annual trends rather than seasonal and maximum/minimum temperature patterns.

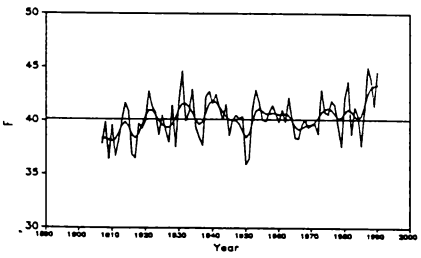

Figure 1: Annual air temperature

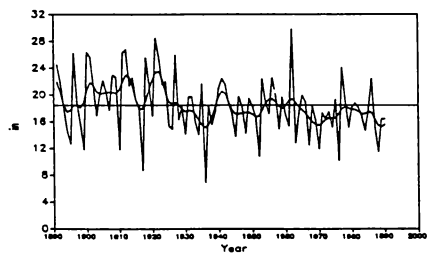

Figure 2: Annual precipitation

Table 1: Linear Regression Coefficients (b)

Variable	b	Variable	b	Variable	b	Variable	b
Tanmax	0.013	Tspmax	0.009	Tfmax	-0.004	Pw	-0.012[3]
Tan	0.024[3]	Tsp	0.023[1]	Tf	0.007	Psp	-0.019[3]
Tanmin	0.034[3]	Tspmin	0.039[3]	Tfmin	0.018	Psu	-0.010
Twmax	0.011	Tsumax	0.036[3]	Trange	-0.022[3]	Pf	-0.001
Tw	0.029	Tsu	0.037[3]	Pan	-0.042[3]		
Twmin	0.045[2]	Tsumin	0.035[3]	Pratio	-0.006[2]		

Level of significance: [1] = 0.10, [2] = 0.05, [3] = 0.01

1. Poiani, K.A. and Johnson, W.C. (1991) *Bioscience* 41, 611-618.
2. Plantico, M.S., Karl, T.R., Kukla, G. and Gavin, J. (1990) *J Geophys Res* 95, 16617-16637.
3. Karl, T.R., Heim Jr., R.R. and Quayle, R.G. (1991) *Science* 251, 1058-1061.

SULFUR DIOXIDE AND GLOBAL COOLING

Abdul J. Alkezweeny*
Department of Atmospheric Sciences, University of North Dakota, Grand Forks, ND 58202

Global climate models use the observed and extrapolated upward trends in the concentrations of CO_2 and other greenhouse gases to estimate current and future global warming. Their results qualitatively agree with the observed averaged temperature from many land and ocean monitoring stations for past 100 years. The models' estimates for the global warming is about 1.5°C, which is nearly double the observed value.

There are several factors that influence the surface temperature such as volcanic emissions, solar insolation variations, increase in atmospheric aerosol concentration, changes in surface albedo due to deforestation, and natural climate changes. Recently, considerable attention has been given to the effect of increase aerosol concentration (resulting from increased SO_2 emissions) on the cloud albedo, sometimes called Twomey's effect.

Associated with the upward trend in the emissions of the greenhouse gases are the emissions of other gases such as SO_2. SO_2 undergoes chemical reactions in the atmosphere during transport leading to the formation of sulfate aerosols. Sulfate concentrations in ice samples from different depths at a site in southern Greenland show an upward trend since 1890, which qualitatively agrees with the reported SO_2 emission trend. Sulfate aerosols are good cloud condensation nuclei and thus participate in cloud formation; any changes in their concentration are reflected in changes in the cloud droplet concentration. For the most common types of clouds, albedo is a function of the cloud droplet-size distribution.

The change in cloud albedo since 1890 was estimated from data on sulfate from the ice cores. It is shown that this change in cloud albedo can account for the discrepancy between the models' calculations and the observed surface temperature. The cooling effect due to increases in the SO_2 emissions may offset or partially offset the greenhouse effect and, therefore, Twomey's effect should be included in any global climate models.

NESTED MODELING OF REGIONAL AND LOCAL-SCALE CLIMATE IN NORTH DAKOTA

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Reclamation (U.S. Bureau of Reclamation) has adopted a nested-modeling approach for global climate change studies. This approach explicitly simulates the physical mechanisms that control evaporation and precipitation on the global, regional, and watershed scales. This modeling system simulates the spatial and temporal evolution of precipitation. This paper reports on the initial stages of research that will apply the modeling system to help determine the impact of climate change on the functions and values of prairie wetlands of North Dakota. Medina (these proceedings) describes some details of this project. This study is part of Reclamation's research in its GCCRP (Global Climate Change Response Program), which includes collaborative efforts in modeling described by Matthews et al. (1), and Medina (2). This collaboration involves scientists from the NCAR (National Center for Atmospheric Research) and the USGS (U.S. Geological Survey). A component of the GCCRP is designed to determine the effects of climate change on water resources in selected western drainage basins. Since 1989, Reclamation and the USGS have been jointly examining the precipitation and hydrologic characteristics of the Gunnison River Basin. The objective of this study is to determine streamflow characteristics and reservoir management needs in present and future climates. This paper briefly outlines the nested-modeling approach and its application to North Dakota wetlands studies.

Reclamation's GCCRP modeling approach involves two phases. Phase 1 evaluates the nested regional/local-scale modeling method in current climate to determine its capability to accurately simulate existing conditions in well-documented cases. In phase 1, Giorgi et al. (3) initialized the regional model with observed data sets and ran a climate simulation for 40 months from January 1982 to December 1983, and from January 1988 to April 1989. Medina (these proceedings) describes the evaluation of the regional-scale model environmental variables for North Dakota. As confidence in the large-scale, general circulation model with its spatial resolution of 300 to 500 km improves, phase 2 will apply the nested-modeling approach to future climate simulations that include effects from doubled CO_2. These simulations will nest the latest versions of the NCAR CCM (Community Climate Model) and the MM4 (Pennsylvania State University/NCAR Mesoscale Model Version:4) regional model, which will nest to local scales. In phase 2, the general circulation model will provide synoptic-scale information that initializes the regional model, which simulates the regional evolution of storm structure. The regional information with a resolution of 60 km will then initialize a local-scale nested domain that simulates local-scale precipitation with high resolutions of about 5 to 10 km. Giorgi et al. (4) used GENESIS, an advanced version of the CCM, for regional climate change simulations. This model incorporates 1) a 50-meter mixed-layer ocean model that includes corrections for meridional heat transport, dynamical sea ice; and 2) an advanced land vegetation-atmosphere interaction scheme. The MM4 model includes a sophisticated surface physics/soil hydrology package that is consistent with the CCM. This package includes the effects of solar radiation and boundary layer processes on evapotranspiration, soil moisture and temperature, and precipitation.

Results from the nested MM4 and local-scale model simulations for the Gunnison Basin indicate that, while the regional MM4 model provides useful information for regional-scale climate simulations, local-scale nested domains are required for more accurate simulations of watershed precipitation. Similar results are expected in the prairie wetlands region of North Dakota.

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COMPARISON OF ENVIRONMENTAL PARAMETER ESTIMATES FROM A REGIONAL-SCALE MODEL WITH NORTH DAKOTA WETLAND OBSERVATIONS

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Estimates of daily values of maximum and minimum temperatures, precipitation, and total evaporation, developed by a RSM (regional-scale model; here, 60-km grid-point spacing), were compared with corresponding historical measurements from the wetlands areas of North Dakota. The modeling is a component of the Bureau of Reclamation's Global Climate Change Response Program project entitled, "The Impact of Global Climate Change on the Functions and Values of Prairie Wetlands." The results reported here were derived from the ongoing atmospheric modeling effort that will produce multiyear simulations of a future climate under doubled-CO_2. The simulations will yield estimates of environmental parameters important to prairie wetland invertebrate communities and ecological functions.

Regional-scale model simulations of hourly values of environmental parameters were obtained from Filippo Giorgi and Gary Bates of the NCAR (National Center for Atmospheric Research). Reclamation and NCAR scientists are cooperatively using modeling to study future global climate change. Giorgi et al. (1) discussed the RSM simulations that produced the estimates used in this study. The RSM used was the Pennsylvania State University/NCAR model, known as MM4, modified by Giorgi and Bates for climate modeling. The MM4 is a three-dimensional, time-dependent model. It contains a surface physics/soil hydrology module known as BATS (biosphere atmosphere transfer scheme) for modeling soil hydrologic budgets. The BATS produces hourly estimates for 31 environmental parameters. Matthews (these proceedings) discusses the MM4 model and the nesting of an RSM, a general circulation model, and a local-scale model to simulate future climate.

Giorgi and Bates produced MM4 estimates covering the entire Western U. S., including the Northern Plains. The MM4 was initialized at 12-hr intervals from the large-scale analysis generated by the European Center for Medium Range Weather Forecast model for the periods 1982 through 1983, and 1988 through March 1989. Results presented here are for March through May of 1982 and 1983, time periods of the year crucial to wetland habitat. Model parameter hourly values were used to develop daily values for 20 grid points, located over a 180-km long by 240-km wide rectangle in central and eastern North Dakota. Gauge measurements were obtained for the same time period for 15 stations located mostly in the eastern part of the state (7 in the Jamestown vicinity).

Simulation-measurement comparisons were made from correlation tables. Of the three environmental parameters studied, the model estimates were most highly correlated with maximum and minimum temperatures. Each gauge's temperatures were correlated with values from each of the 20 model grid points. For maximum temperatures, correlation values ranged from a low of 0.81 to a high of 0.93, with a typical sample size of 167 days. The corresponding minimum temperatures yielded correlations of 0.84 to 0.92, all significant at the 0.0001 level or better. Temperature data from Jamestown produced the highest correlations while those from Valley City yielded the lowest values. Correlations generally increased as the distance between model grid points and gauge sites decreased. These values suggest the MM4 model can well estimate daily temperature extremes.

Correlations were developed with daily precipitation measurements from nine gauge sites and the 20 model grid points. As expected, the correlations were considerably lower than with temperatures, reflecting the more complex problem of precipitation simulation. Correlations ranged from slightly negative values to 0.57. The median correlation was 0.32. Least correlated with gauge measurements were grid points located along far eastern North Dakota. Results for those grid points probably reflect the model's coarse resolution of 60 km, which cannot account for effects from the numerous small lakes located upwind and near that area.

Evaporation data were available for only five sites, all with incomplete records. Consequently, correlations obtained may not reflect model capability. The Mandan site contained the better record (N = 76 days). It yielded correlations ranging from 0.07 to 0.42, depending on the model grid point. Williston data produced a maximum correlation of 0.50 (N = 74 days).

Correlation values suggest the model is well simulating temperatures but needs improved physics and spatial resolution in order to better estimate daily precipitation. More evaporation measurements are needed for comparisons. Other environmental parameters estimated by the model, such as soil moisture and temperatures, need to be compared with historical measurements. Simulations with improved model physics and resolution will take place in the next couple of years.

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EFFECT OF CLIMATE ON WATER AVAILABILITY IN PRAIRIE WETLANDS OF NORTH AND SOUTH DAKOTA

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The amount of water held in individual wetland basins depends not only on local climate patterns but also on groundwater flow regime, soil type, vegetation, and surrounding land use. Most wetland basins in the northern prairies alternate between being wet (i.e., holding water) and dry. To assess the potential effect of climate change on the number of prairie wetlands holding water in a given year, one must first determine how much of the variability in number of wet basins is accounted for by climatic variables. In this paper I discuss the results of a modelling effort to assess the effect of climate on the number of wet basins in the prairie pothole region from 1972 through 1987.

Data used to construct the model were from two sources. The Historic Climatology Network provided monthly temperature and precipitation data from carefully screened weather stations throughout the United States. I used data from 50 stations in the eastern Dakotas to produce monthly grids for each variable for each year, using the kriging technique for geographically interpolating data. The Office of Migratory Bird Management, U.S. Fish and Wildlife Service, provided pond count data collected during May waterfowl surveys. The number of wet basins were counted from low-flying aircraft that followed established east-west transects across the eastern Dakotas. Data from temperature and precipitation grids were averaged over ten random points along each transect to obtain a mean value for each month of each year on each transect.

I used multiple linear regression to examine the relationship between climate variables and percentage of wet ponds. Independent variables included monthly, seasonal, and annual temperature and precipitation. The effects of one-year lags were examined for the seasonal and annual data. Two indices were calculated, the Thornthwaite Moisture Index (1) and a Conserved Soil Moisture Index (2). All calculations were based on a year defined to be June-to-May.

I developed two alternative models, one that maximizes predictive power and one that provides more insight into the relative importance of the explanatory variables. The most predictive model explained 67% of the variation in May wet basins. However, multicollinearity among the explanatory variables rendered the coefficients uninterpretable. Nonetheless, it is important to note that climate does, indeed, account for a considerable proportion of the variation in wet basins.

A second, simpler model is readily interpretable and explains 56% of the variation in May wet basins:

$$\%\ \text{wet basins} = 129.97 + 0.051(\text{YPC}) - 0.0089(\text{FT}) - 0.015(\text{LAGMAYT}) + 0.031(\text{LAGFPC}),$$

where YPC = total precipitation for the year; FT = mean monthly minimum temperature during the fall, defined as September through November; LAGMAYT = mean maximum temperature the previous May; and LAGFPC = total fall precipitation the previous year. All terms in the model are significant at $P = 0.0001$ ($t = 17.256$, 7.166, 6.205 and 6.685, respectively, $df = 304$), as is the model itself ($F = 100.093$, $df = 4, 304$).

This model indicates that water in prairie wetlands depends largely on the amount of precipitation received during the preceding year as well as during the previous fall. High temperatures during the previous May or during the fall tend to reduce the number of wet basins in May. Modification of any of these variables brought about by anthropogenic climate change is likely to have substantial effects on wetland habitat in the prairie pothole region.

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CLIMATE CHANGE AND PRAIRIE WETLANDS: MODEL SIMULATIONS OF HYDROLOGY AND VEGETATION

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A spatially defined, rule-based simulation model was developed to assess the potential effects of climate change on the hydrology and vegetation of a semi-permanent prairie wetland. The hydrologic submodel estimated seasonal and yearly water levels based on rainfall, evaporation, transpiration, and runoff. The vegetation response to hydrologic change was based on seed bank composition, seedling recruitment and establishment, and plant survivorship. The amount and distribution of emergent cover and open water were simulated in time and space by a geographic information system. Data for parameterization, calibration, and testing of the model were from the U.S. Fish and Wildlife Service's Cottonwood Lake study site near Jamestown, North Dakota. Climate change scenarios were from the Goddard Institute for Space Studies global circulation model interpolated to the Jamestown, North Dakota weather station.

Model simulations suggested that climate change would reduce water depths in semipermanent wetlands and increase the frequency of dry basins. Simulations also suggested significant changes in vegetation; balanced emergent cover-open water ratios in normal climate simulations shifted to a strongly unbalanced condition with no open water areas in the greenhouse simulations. These possible changes in wetland hydrology and vegetation indicate a significant decline in habitat quality for breeding birds, particularly waterfowl, in prairie wetlands. Uncertainty analyses showed the model to be particularly sensitive to spring precipitation. Rainfall would need to increase by about 10 percent to compensate for the greater evaporative demand in the greenhouse climate.

We are presently expanding the modeling effort through EPA's Global Change Research Program to simulate changes in a wetland complex, including wetlands of other permanence classes, such as temporary and seasonal. Knowledge of the condition of these wetlands in spring, together with the condition of semipermanent wetlands in summer, is crucial in making comprehensive assessments of the effects of climate change on waterfowl populations. Assessment of the effects of climate change on wetlands and waterfowl will be made in the context of other stressors such as drainage, pesticides, and cropping systems. The project also calls for the development of methods to detect early stages of climate change in prairie wetlands using remote sensing.

DATA BASE DEVELOPMENT FOR CLIMATE CHANGE RESEARCH

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Studies under the Global Change Research Program at EPA's Environmental Research Laboratory (ERL)-Corvallis focus on (1) the effects of global climate change on ecosystems; (2) ecosystem feedbacks to climate through water, energy, and carbon fluxes; and (3) assessment of biosphere management options to conserve and sequester carbon. This type of research, whether focusing on regions such as the Northern Plains or large continental to global areas, requires the development of spatial data bases on numerous aspects of climate, terrain, soils, and vegetation.

In order to help fill these needs, EPA and NOAA have established an Interagency Agreement to produce an integrated, quality-controlled data base for spatially distributed modelling of global change at continental to global scales (1). The Global Ecosystems Database Project focuses on data sets with spatial resolutions of 30 seconds to 2.5 degrees, which is intermediate between site-specific process model scales and coarser general circulation model scales. This should provide the means to extrapolate process information to global distributions and to apply generalized predictions to more local scales. The temporal resolution focuses on monthly time series and averages. The data base has been produced on a CD-ROM and contains multi-thematic raster and vector files, registered to a common reference system (lat/lon). The files are GIS-compatible with Idrisi/DOS (portions are provided with the data), GRASS/UNIX, and other GIS's. The data base currently contains two Global Vegetation Index products; two climatic data bases; four vegetation/land cover/land use data sets; four soils data sets; two elevation and terrain data sets; and vector boundary files. Most of the files are raster data files in compatible grids between 10 minutes and 1 degree lat/lon, with some experimental data at 2 and 5 minutes lat/lon. Documentation includes information about the source, contributors, variables, document references for the original data and georeferencing, geographic coverage and sampling, time period and temporal sampling for the original and integrated data sets. Individual data sets have undergone intensive review by disciplinary experts, and a beta test version of the entire CD-ROM was peer-reviewed by 38 scientists worldwide. A revised version is now being publicly distributed at cost, and user feedback will provide a continuous review process. Yearly updates are planned, which will incorporate additional data sets as they are developed.

Among the spatial data bases relating to climate change research that have recently been developed at ERL-Corvallis are soil water holding capacity (WHC) and soil organic carbon (SOC) for the contiguous U.S. The spatial patterns of soil WHC are important for studying the response of vegetation and hydrologic systems to climate change. SOC represents a major biospheric pool of carbon and is important in the cycling of carbon dioxide and methane, two major greenhouse gases. Six regression models of WHC as a function of soil texture, bulk density, and organic carbon were validated using the National Soil Survey Laboratory's pedon data base. Soil WHC was aggregated and mapped to Major Land Resource Areas (MLRA's) using the Soil Conservation Service's NATSGO data base, and to soil map units using FAO's Soil Map of the World (2). SOC was also determined by three different methods (3). The calculated SOC values were aggregated and mapped using ecosystem complexes, soil taxonomic groups and MLRA's, and Soil Map of the World map units.

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RESEARCH MANAGEMENT STRATEGIES FOR FOSTERING INTERDISCIPLINARY TEAM RESEARCH

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Global climate change research requires new approaches to conducting and managing research. Effective collaboration between research organizations and between scientific disciplines is essential. Interdisciplinary research is the synthesis of two or more scientific disciplines and the integration of their contributions in examining a particular problem. Interdisciplinary research involves synthesizing concepts and data to achieve a synergistic result, collaboration in planning and executing a research project, and joint production of a product characterized by an integrated theory and coherent vocabulary. Although it is recognized that global change research must involve multiple disciplines, not enough thought or attention has been given to the practical implications of organizing for interdisciplinary research (1). Such research requires appropriate institutional structures that favor teamwork. Of particular importance to global change research is the integration of the natural and social sciences (1, 2). Rayner argued for interdisciplinary approaches in global environmental change research and suggested that such research avoid reductionistic methods, but rather employ a constructionist or synthesis approach (3).

The benchmark studies of interdisciplinary team research were conducted by Birnbaum (4) and Rossini and Porter (5) in the mid to late 1970's. Kline (6) and Epton et al. (7) more recently published on the subject of interdisciplinarity and interdisciplinary research management. The research management premises and strategies presented in this paper are derived from the literature, from my observations in the U. S. and Europe, and from personal experience as a leader and participant-observer on research teams.

Three key conditions must be satisfied to ensure successful interdisciplinary research activity:

1. The research effort must be defined by common, mutually agreed-upon goals and objectives and a unifying theory. The research problem should be defined collaboratively by the team. It helps to agree first on who the clients or research users are. Working on a problem analysis together leads to a unifying theory, clarity of purpose, and congruence in terms of a group goal. Disagreements over research approaches are actually beneficial for heterogeneous research teams as long as the discussions are healthy and constructive (4).

2. Team members must invest the time and energy necessary to understand research methodologies used by other disciplines. Successful interdisciplinary teams have members with positive attitudes toward the project and who are committed to the group goals. Team members must invest a great deal of energy and time to achieve congruence of purpose and goals, to openly discuss paradigmatic and methodological differences, and to develop integrating mechanisms. Very heterogeneous research groups, for example those composed of natural and social scientists, are characterized by a variety of inquiring systems and analytical techniques unique to the particular disciplines represented.

3. Team interactions must be characterized by a balance between the task and social dimensions of group activity. Team members must get along both intellectually and socially. Team interactions are enhanced by the right team size and composition. Optimum size for interdisciplinary research teams appears to be four to seven members, including the team leader. The team must be large enough to have all needed disciplines represented, but small enough to allow for effective communications. Team members must be competent in their fields, but it is equally important that members are chosen for their ability to work effectively on a team. Team leaders should adopt a democratic, participatory style of leadership and concentrate their efforts on gathering resources, facilitating a common group goal, and promoting open communications among team members.

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PROJECTING THE VEGETATION RESPONSE TO CLIMATIC CHANGE IN THE NORTH AMERICAN CENTRAL GRASSLANDS REGION

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Anticipated changes in global climate may seriously impact the North American Central Grasslands Region in complicated ways. The U.S. National Park Service has land units within this region for which projections of change are needed to make management policies. To provide a basis for such projections, a coordinated modeling effort is underway in which high-resolution climate change projections generated by the Regional Atmospheric Modeling System (RAMS) will be used to drive the Mapped Atmosphere-Plant-Soil System (MAPSS) to simulate regional and landscape scale changes in vegetation.

RAMS is a highly versatile atmospheric model consisting of equations of motion, heat, and moisture in a terrain-following coordinate system of flexible vertical and horizontal resolution. Two-way interactive grid nesting in RAMS allows local fine-mesh grids to resolve compact atmospheric systems such as convectional storms, while simultaneously modeling the large-scale environment of the systems on a coarser grid. A linkage of General Circulation Model (GCM) output and RAMS simulations will be executed using a series of nested grids that range from a subset of GCM grid points (covering most of the conterminous United States) to a high-resolution (40 km) grid for the Central Grasslands domain, to a finer 10-km grid covering regions around park units. The climate simulations will be driven by a suite of different GCM climate change scenarios.

MAPSS is an integrated vegetation and hydrologic model that calculates a complete site water balance and predicts potential leaf area (LAI) within the constraints of the abiotic climate and seasonal soil moisture patterns. Vegetation is linked to hydrology in the model through root distribution, LAI, and stomatal conductance, which is modulated by soil water potential and potential evapotranspiration. MAPSS iteratively solves for the leaf area of both woody and grass lifeforms in full competition for both light and water, while maintaining a site water balance consistent with observed runoff. A constraint on woody LAI by fire is also simulated as a function of soil water potential and lifeform LAI. The LAI values and critical climatic thresholds are processed by the model rule-base to simulate the distribution of closed conifer and broad-leaved forest, chaparral, tree and shrub savannas, grasslands, and deserts.

Initial estimates of the potential impact of climatic change on the Central Grasslands region, using coarse-scale output from GCM doubled-CO_2 experiments and a coarse resolution of life forms in MAPSS, include significant changes in grass leaf-area throughout the region. But the magnitude and even the sign of the change varies with the climate scenario and with assumptions concerning wind speed and plant water-use efficiency.

Future enhancements of MAPSS for research in the Central Grassland Region will include a higher resolution of life-forms and communities, more specific calibration of stomatal response functions for incorporation of the direct effects of CO_2, provisions for the influence of the interannual variability of weather, and the incorporation of spatially distributed soil data. A structured database containing ecological, physiological, and distributional data for Central Grassland species and functional life-forms is currently being compiled to support the enhancement and calibration of the vegetation model.

The research described in this paper is being funded by the U.S. National Park Service, the U.S. Forest Service, and the U.S. Environmental Protection Agency.

THE PALLISER TRIANGLE GLOBAL CHANGE PROJECT: EXAMINING THE LINK BETWEEN CLIMATE AND LANDSCAPE PROCESSES IN THE SOUTHERN CANADIAN GRASSLAND

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The Palliser Triangle (map) marks the northernmost extent of the mixed-grass prairie. As elsewhere on the Great Plains, severe drought is a recurrent feature of the regional climate. Owing to a strong dependence on agriculture, the potential socioeconomic impact of future global changes is as great in this region as anywhere in Canada. For this reason, the Geological Survey of Canada established the Palliser Triangle Integrated Research and Monitoring Area (IRMA) in 1991 to enhance our understanding of the relationships between climate and geomorphic processes in this region. The project employs a co-operative, multidisciplinary approach involving both government and university researchers. Its goal is to provide land-use planners with a greater appreciation of landscape changes that are likely to accompany global change.

The initial objective of the project is to produce a high resolution reconstruction of Holocene climate change, with emphasis on the last 2000 years, providing a long-term perspective on drought intensity and periodicity. The sparse paleoclimatic database for the area will be supplemented by paleolimnological investigation of several lakes and dried lake basins (see below). Analyses will include development of diatom, ostracode, plant macrofossil and pollen stratigraphies in addition to detailing the physical, chemical and mineral characteristics of the sediments. The potential for obtaining high-resolution paleoclimatic records from saline lakes, which are ubiquitous across the region, is exemplified by a recently completed study of Chappice Lake, southeastern Alberta (1). This small, shallow (< 1m), hypersaline lake has experienced significant water-level fluctuations in the past 7.5 ka, evidenced by both physical and biological indicators. Chronologic control, provided by AMS ages of upland plant seeds, shows broad correlation with established hemispheric climatic events. The deposition of carbonate-rich laminae during periods of extremely low water stands provides potential for annual, and perhaps finer, resolution during severe drought periods.

The second objective involves the correlation of geomorphic process rates with climate. This work will use ^{137}Cs analysis to determine rates of recent soil erosion and general landscape sensitivity, monitoring of contemporary processes, and stratigraphic investigations of former process activity. Process studies are centred on the semi-arid heart of the Triangle (sites B-D, below), which is both the most climatically sensitive area and the most appropriate analog for adjacent terrain in the event of future climatic warming. Focus will be on eolian and fluvial erosion, as well as colluvial processes in upland areas. A 30-year record of dune activity from the Great Sand Hills provides a base for expanding work. Climatic correlation of paleo-process studies will be based on paleolimnological reconstructions. Spatial data will be compiled in a GIS, permitting regional extrapolation of site-specific studies and identification of landscape units most sensitive to climate change. Final maps as well as complete data bases will be available to clients upon completion of the project.

PRINCIPAL STUDY SITES. Lakes where cores have been collected, or future monitoring work is planned: 1 - Chappice Lake (1); 2 - Harris Lake (2); 3 - Elkwater Lake; 4 - Antelope, Freefight, North Ingebright, Vincent and Chain lakes in the Great Sand Hills; 5 - Clearwater Lake; 6 - Hand Hills and Little Fish lakes; 7 - Old Wives Lake basin; 8 - Kenosee Lake; and 9 - Killarney and Pelican lakes. Studies of geomorphic processes are centred upon: A - Cypress Hills; B - Maple Creek basin; C - Great Sand Hills; and D - Dinosaur Provincial Park.

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A LANDSCAPE-SCALE MODEL OF DISPERSAL: APPLYING GIS TO INVESTIGATE SPECIES RANGE SHIFTS

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Global temperatures have been projected to rise considerably next century due to greenhouse gas emissions; the predicted rate of warming is unprecedented. Biotic ranges undoubtedly would shift in response to changing climate, and there is concern that the extent of these range shifts will exceed the dispersal capabilities of many plants, especially in light of extensive habitat fragmentation.

Several studies have investigated potential range shifts driven by global warming; most of these have focused on the eastern forests of North America. A common approach is to determine the value of key climatic variables that are coincident with the current range limit of one or several species, which are assumed to be in equilibrium with modern climate. Once climate correlates are assigned, the consequences of a climatic warming on species distributions are assessed by delimiting geographic displacement of the presumed climate control under one or more general circulation model scenarios. This method is admittedly simplistic, especially in grasslands which likely have more complex species-environment relationships than forests. Nevertheless, this approach can give general indications of potential vegetation reorganization.

Although previous work has addressed the potential rate and magnitude of range adjustments in response to future warming, no studies have explicitly sought to assess the ability of species to migrate under current landscape conditions. The purpose of this research was to develop models of migration for two common means of dispersal (by wind and bird) and to apply these models in present-day landscapes in the eastern United States. The models examined range expansion resulting from the cumulative effect of many dispersal events over many years. The goal was to assess the potential impact of different land-use patterns and means of dispersal on migration success. Applications of the model can be extended to investigate migration in grassland areas in the central United States.

Three study areas were selected in the eastern United States, each consisting of two 1:250,000 USGS land use land cover (LULC) quadrangles. The areas were selected to reflect the diverse environments migrating species might encounter, from areas with extensive cover of the natural vegetation to areas dominated by agriculture. In order to simplify the migration modeling process, LULC categories were reclassified into three groups (Impermeable, Semipermeable, and Permeable) according to the likelihood of their successful colonization by migrating species.

Initially, migration was modeled on contiguous 10 x 10 km grids extracted from the study areas. Various indices of landscape configuration were computed for each grid. The objective in developing the models was to determine the number of generations required for species to migrate from the bottom through to the top of the grids. Simulations were performed using the Idrisi geographic information system; dispersal parameters for both wind-dispersed and bird-dispersed species were derived from the literature. A second phase in the analysis examined potential migration patterns at a larger scale. Information obtained from analysis at the smaller scale was aggregated to assess potential migration rates for the original study areas.

Correlation analysis revealed a strong negative relationship between average wind-dispersed migration rates and permeable land use ($r = -0.81$) for the 10 x 10 km grids, which substantiated the intuitive expectation that as the proportion of "favorable" cover increased, the number of generations required for through-migration would decrease. Grids with the slowest wind-dispersed migration rates were highly fragmented and tended to have a mixture of land use permeability classes. Permeable cover existed in isolated tracts, which did not provide good "targets" for wind-dispersed seed. In direct contrast, fastest average bird-dispersed migration rates occurred in grids with widely spaced permeable land cover, which facilitated rapid "jump dispersal" through the grids; slowest average bird-dispersed migration rates occurred in grids with continuous permeable cover. The relationship between bird-dispersed migration and land use configuration was sensitive to slight differences in landscape geometry, and thus its quantification was not possible. Aggregating these results revealed that both the wind- and bird-dispersed species met with greatest migration success in the least-disturbed study area. However, if significant global warming occurs next century, these migration rates would fall short of projected range shifts by at least an order of magnitude.

Results of this modeling exercise indicate that some species may have difficulty migrating in response to climatic warming, when only the means of dispersal and land use variables are considered. If species are unable to migrate to their new ranges in conjunction with climatic warming, the vegetation cover is likely to be in widespread climatic disequilibrium for several centuries, or perhaps millennia.

CALCULATING THE CLIMATE ENVELOPE FOR NORTH AMERICAN GRASSLAND BIRDS

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Climate in the Great Plains is among the most variable in North America. Fretwell (1) has suggested that avian species breeding in this part of the continent have evolved mechanisms, such as plasticity in their breeding sites, to deal with yearly climatic fluctuations. If a given area is unsuitable one year, the species in question may move, if possible, to an area that is more suitable. A species that is plastic in its responses to climate is much more likely to survive if the climate changes. The *climate envelope* of a species is defined as the attributes of a set of climates in which a species can persist if all other nonclimatic requirements are met (2). The climate envelope is used to model a theoretical distribution (i.e., all locations in which the climate needs of the species are met regardless of whether the species is found there) for each species in my study. I use Breeding Bird Survey (BBS) data to demonstrate annual variation in the distribution of North American grassland birds for the period 1985 through 1989. These distributions are compared with the theoretical distributions from models based on the climate envelopes.

The Breeding Bird Survey is annually performed to estimate the population trends of many of the species that nest in the United States and Canada. Initiated in 1965, it currently consists of about 2000 routes. To examine changes in the distribution patterns of grassland birds, I used the geostatistical technique of kriging to interpolate a grid of regularly spaced points based upon the data collected along BBS routes. Maps showing the annual and five-year-average distributions of grassland species for 1985-1989 were created using standard contouring algorithms. This time period was chosen to minimize potential effects from large-scale habitat changes and includes a mix of average, wet, and drought years.

Climate data were obtained for all of the United States and Canada for 1970-1989. The data for 1985-1989 were extracted and kriging was used to create a climatic surface for each variable in each month in each year. These surfaces were then sampled to determine the monthly climate variables for each BBS route (using the latitude and longitude of the starting point of the route) for each year of the study. Monthly means were then calculated for each of the variables.

I plotted the mean number of birds per BBS route against each climate variable to determine the minimum and maximum values of each climate variable associated with each species distribution. These values, taken together, form the climate envelope. The values from the climate envelopes are then used, in conjunction with the previously calculated climate surfaces, to develop a theoretical distribution for each species. Finally, the theoretical distribution is compared with the actual species distribution maps.

For example, data from a total of 894 BBS routes are incorporated in my analysis. Of these, observers on 56 routes reported Lark Buntings in at least one of the five years. I calculated a climate envelope for this species based upon annual total precipitation, average summer precipitation, average annual maximum temperature, and average summer maximum temperature. This relatively simple climate envelope excluded 89% of the BBS routes that had never detected Lark Buntings. Logistic regression is used to further refine these models.

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OZONE DEPLETION AND UVB RADIATION: IMPLICATIONS FOR THE NORTHERN PRAIRIE

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Recent reports have called attention to alarming global decreases in stratospheric ozone with corresponding increases in ultraviolet radiation (UVB) at the Earth's surface. Although decreases have been greatest in Antarctica, significant ozone thinning is anticipated in the northern hemisphere (1). Three depletion scenarios (2) need to be considered in the assessment of UVB radiation hazards to ecosystems of the northern prairie. The first concerns the gradual and long-term thinning of the ozone layer over the temperate regions, where ozone is predicted to decline by 2% at the 30° N latitude to more than 15% at the 60° N latitude. A second scenario is that Arctic ozone holes would enhance UVB radiation. Depletions are not projected to be as severe or as long-lasting as in Antarctica, but could reach 40% depletion and result in an 80% increase in UVB. In addition, polar and midlatitude mixing can yield masses of ozone-deficient air, which will drift across the northern plains. A third scenario is that chemical reactions of volcanic sulfur dioxide with chlorinated compounds would cause localized ozone depletions similar to those at the polar vortices. These depletions could occur anywhere, but certain regions may be particularly vulnerable. High-altitude habitats would be at greater risk; there is a 4% increase in UVB with each 300-m increase in altitude as filtering and reflecting properties decline.

Increases in UVB radiation could damage freshwater and terrestrial ecosystems in temperate North America, including the prairie pothole region. Much of the research on enhanced UVB effects in temperate areas has emphasized impacts on human health and on agricultural crops. Little study has been made of the impact of ozone depletion on temperate ecosystems. Many species exist at their limits of tolerance for UVB, so any increase may prove harmful. More severe depletions are predicted for early spring, subjecting emerging plant and animal life, such as amphibians and zooplankton developing in shallow temporary ponds, to midsummer-like radiation intensities before they develop protective mechanisms.

Ozone depletion will have direct biological effects ranging from cellular responses to population changes (3). Cellular impacts of UVB radiation may include in animals the formation of DNA pyrimidine dimers, synthesis of stress proteins, and changes in pigmentation; and in plants, the accumulation of flavenoids, altered chromophores, and impaired electron transport. Marine studies indicate organism-level effects such as increased mortality, developmental abnormalities, reduced growth, behavioral dysfunction, lesions, cataracts, and immune system dysfunction. Many of these effects appear to be induced at present UVB levels. Population-level responses may include reduced species diversity as more sensitive species are lost. Biomass and yield also will be reduced by UVB exposure as the number of organisms and their growth are impaired. Diminished reproductive success, reduced survival of offspring, and decreased seed yield are documented effects of solar UVB exposure.

Potential indirect impacts of ozone depletion are many, including a diminished forage base (3). Limited invertebrate productivity in prairie wetlands, for example, may result in degraded nutrition for reproducing waterfowl. Reduced nitrogen binding in plants may result in less nutritive forage. Another indirect impact is enhanced toxicity of contaminants (4) due to greater intensity of UVB irradiance as well as greater depth of UVB penetration in the water column. Finally, UVB-exposed plants may have an impaired capacity to bind atmospheric carbon dioxide (5).

We need a broader understanding of the biological impacts of UVB exposure to aquatic and terrestrial populations. This will require a determination of thresholds for adverse biological effects in controlled laboratory tests as well as an assessment of the risk of exposure for organisms in the field. An assessment of the risk should consider the probability that such exposures would occur relative to projected ozone depletion, atmospheric conditions, UVB-filtering characteristics of the habitat, and time of appearance of the most sensitive life stages. Once these impacts are determined, we may be able to adopt limited mitigative measures such as imposing harvest limits for populations that have received significant UVB exposure or controlling the release of contaminants that might undergo photoenhanced toxicity.

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MARINE SEISMIC TECHNIQUE AS A TOOL FOR PALEOCLIMATIC RESEARCH, DEVILS LAKE, NORTH DAKOTA

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Studies of saline, closed-basin lakes have shown that the effects of temperature, salinity, and lake level are preserved in lacustrine sediments in the lakes. Sediment-core data from saline lakes can be used to construct climatic histories for a drainage basin. However, paleoclimatic studies based strictly on sediment data are limited by the length of the sediment core obtainable, the general lack of information concerning sediment hiatuses, the lack of spatial information to define core-to-core correlations, and the expense of sediment-core collection and geochemical analyses. Multidisciplinary approaches can be used to circumvent these limitations and to provide a more definitive paleoclimatic interpretation. Techniques from a variety of disciplines, including limnology, geochemistry, and geophysics, can be applied to develop a unified depositional and climatic history for a drainage basin. During 1992, a study was conducted to test the usefulness of the marine seismic technique for paleoclimatic investigations in Devils Lake, North Dakota.

Three seismic transects were completed in Main Bay and Creel Bay of Devils Lake, one north to south across the center of Main Bay perpendicular to the shores, one west to east in Creel Bay perpendicular to the shores, and one west to east in Main Bay parallel to the south shore. The two perpendicular transects indicate the complex nature of the antecedent glacial topography beneath the lacustrine sediments and the areas of maximum sediment thickness--information that is critical for optimum location of sediment cores designed to sample the complete history of deposition. These two transects also indicate that sediment deposition in Devils Lake can be characterized by nearly parallel beds that are continuous across much of the lake. The parallel beds in the center of the lake are possibly the result of wind-blown sediment or of organic matter produced in-situ, or both, and not the result of detrital sediments from a single tributary source. Near-shore areas appear to have received little or no sediment.

The transect parallel to the south shore of Main Bay indicates a more complex sediment geometry, including a number of erosional and depositional sequences that probably relate to lake-level fluctuations. On the basis of these sequences, important reflectors can be identified and traced across Main Bay. These reflectors may represent depositional hiatuses in Main Bay.

Preliminary evaluation of geophysical data from Devils Lake indicates that the marine seismic technique can provide valuable geologic information about the geometry of the lacustrine sediments and antecedent glacial topography and the locations of maximum sediment thickness. The marine seismic technique also can provide important paleoclimatic information about variations in the paleodepositional environments, depositional hiatuses, and long-term accumulation rates.

LAKE LEVELS, GROUNDWATER PALEOHYDROLOGY, AND PALEOCLIMATE IN WEST-CENTRAL MINNESOTA AT 7,000 YR B.P.

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The levels of closed-basin lakes are commonly sensitive to climate. The climatic variable "effective moisture" is defined as both net precipitation over the lake surface (precipitation minus evaporation, P - E) and net precipitation over the land surface (precipitation minus evapotranspiration, P - ET). For a given lake, high lake levels correspond to times of high effective moisture, and low lake levels correspond to times of low effective moisture. Effective moisture affects lake levels at two scales. First, at the scale of the lake catchment, lake levels respond to changes in the lake surface-water budget, which includes runoff from the catchment (a function of P - ET) plus P - E over the lake itself. Second, at the scale of the regional water table, lake levels track changes in water-table altitude, which responds to groundwater recharge (another function of P - ET). A change in groundwater recharge changes the water-table altitude most in the middle of interfluves and least near rivers, which are hinge points for the water table.

Lake-sediment stratigraphy can reveal past lake levels, which provide evidence of past effective moisture. Nine lakes and wetlands from the Parkers Prairie sandplain in west-central Minnesota were studied. In three of the lakes, transects of cores were taken in shallow water to obtain evidence of lower water levels with the Digerfeldt method by correlating strata of sand, macrofossils, and pollen (1). Chronological control was provided by radiocarbon dating. The three lakes had their lowest levels at about 7,000 years before present (yr B.P.), about 500 yr after the vegetation changed from a pine forest to prairie. Lake levels ranged from 2.8 m to 6.2 m below modern levels. The greatest lake-level change occurred far from the river that drains the sandplain, and the least lake-level changes occurred close to the river. This pattern indicates that the lake-level lowerings were caused, at least in part, by a lowering of the regional water table resulting from reduced groundwater recharge, compared to present values (2).

A steady-state analytic-element groundwater model of the Parkers Prairie sandplain related lake levels to water-table altitude, groundwater recharge, and lake surface-water budgets. Because overland flow on a sandplain is limited, groundwater recharge was presumed to equal P - ET, and the surface-water budget of each lake was presumed to equal P - E. Both P - ET and P - E were manipulated to match the model water table with the past lake levels. Model results indicated that lake levels at 7,000 yr B.P. could have been caused by a reduction in groundwater recharge to about 50 to 60 mm yr^{-1} (40-50 percent of the present value), coupled with a lake P - E value of about -100 to -400 mm yr^{-1} (100-400 percent of the present value) (3).

The low lake levels at Parkers Prairie are consistent with the regional time-transgressive pattern of the time of maximum aridity (least effective moisture) across the Upper Midwest. Maximum aridity occurred earlier in the northern part of the area and later in the southern part. This pattern may have been caused in part by changes in seasonal insolation during the post-glacial times. The early Holocene was a time of increased seasonality in the northern hemisphere with presumably warmer summers and colder winters than at present. The increased summer temperatures of the early Holocene could increase the intensity of continental low-pressure systems and allow more frequent and deeper incursions of moist air masses from the Gulf of Mexico into the Upper Midwest. As seasonality decreased over the Holocene, the depth of moist air penetration lessened and shifted progressively southward, leaving arid conditions and low lake levels in the wake (3, 4).

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PALEOLIMNOLOGICAL VIEW OF CLIMATE HISTORY IN THE NORTHERN GREAT PLAINS

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The Great Plains region is particularly sensitive to changes in moisture because of its present semi-arid climate and the low recharge rate of aquifers. Future water availability in this region is of concern, particularly the extent to which climate change will alter the distribution and frequency of precipitation patterns and increase the likelihood and severity of droughts. Paleoecological data are useful for defining natural climatic variability, providing past analogs of future climate change, and in testing simulations of past climate from general circulation models. However, our understanding of past precipitation patterns in the northern Great Plains is still quite tentative. This is a result in part of the paucity of pollen studies and the difficulty of interpreting pollen records in climatic terms because of the low taxonomic resolution of grassland pollen and the distance of most sites from climatically sensitive ecotones. A far more direct and sensitive record of past precipitation in the northern Great Plains may be obtained by paleolimnological methods.

Closed-basin lakes in arid and semi-arid regions potentially act as paleoclimatic recorders as a result of climatic control of water budgets. In such systems fluctuations in the balance between precipitation and evaporation result in changes in lake level and the concentration or dilution of dissolved salts. Fluctuations in salinity are recorded in a variety of paleolimnological indicators, including the community composition of diatoms and ostracode shells, and in the trace-metal composition of ostracode shells. We have developed transfer functions to reconstruct paleosalinity from diatom assemblages and the trace-metal composition of ostracode shells. These methods have been applied to Holocene stratigraphic records in the northern Great Plains to quantitatively reconstruct paleosalinity, which is used as a proxy for paleoclimate.

The relationship of salinity to climate is complex and varies from lake to lake depending on many factors. Thus, to use the paleolimnological record for climatic reconstruction, the relationship between salinity fluctuation and climate must be explicitly determined; that is each lake must be calibrated to climate. We have taken meteorological data from climate stations near our lake sites, with continuous instrumental records for monthly temperature and precipitation extending back about 100 years, and have converted these data to estimates of mean annual precipitation minus evaporation (P-E). The paleosalinity reconstructions from both ostracodes and diatoms for the period of instrumental record are compared with these estimates of effective moisture to determine the extent to which salinity reflects changes in P-E and the extent to which it is controlled by factors related to local hydrology.

Quantitative reconstruction of past lake-water chemistry from diatom assemblages involves modelling modern diatom distributions with respect to environmental parameters such as salinity, and calibration, where the modelled responses are used to infer salinity from stratigraphic diatom assemblages. To determine the relationships between diatom distributions and measured environmental gradients in the northern Great Plains, surface-sample diatom data collected from 57 lakes, spanning a salinity gradient from 1 to > 100 o/oo, were ordinated with canonical correspondence analysis (CCA). The transfer function derived with CCA was tested by comparing historical salinity measurements from Devils Lake, North Dakota, with the diatom-inferred salinity from a short core representing the last 100 years of sediment accumulation. The diatom-inferred salinity closely approximates the measured salinity in the range of freshwater and low salinity (< 10 o/oo) and can clearly differentiate modern and high salinity (> 15 o/oo) from low salinity or freshwater intervals.

Ostracode shells can be analyzed for isotopic and trace-metal composition to provide a geochemical account of the lake water in which the ostracodes lived. The partitioning of Mg and Sr in the carbonate shell of a given species is defined by the molar distribution coefficient, K_D, which can be determined from laboratory experiments in which ostracodes are cultured under controlled temperatures and salinities. We determined the distribution coefficients for *Candona rawsoni*, a widespread Great Plains ostracode, and tested these against field collections of live ostracodes from lakes in North and South Dakota. The K_D-inferred values for Mg/Ca and Sr/Ca ratios are in close accord with the same ratios measured in the lakes. Past changes in lake-water chemistry can be inferred from fossil ostracodes by measuring trace-element concentrations in their shells and using the modern relationship established between water chemistry and shell chemistry. A comparison of ostracode-reconstructed and historically measured salinites for Devils Lake, North Dakota, shows good agreement within the salinity range preferred by *C. rawsoni* (1-10 o/oo).

We are now analyzing long cores from four sites spanning the late glacial and Holocene, for which the correlation between effective moisture and salinity is strong. Paleosalinity will be calculated from the diatom and ostracode transfer functions, and the calibration derived for each lake will be used to reconstruct changes in effective moisture at 100-year intervals throughout the stratigraphic record.

PALEOCLIMATIC CONDITIONS AND TIMING OF RECHARGE TO BURIED-VALLEY AQUIFERS IN NORTH DAKOTA--EVIDENCE FROM $\delta^{18}O$, δD, AND TRITIUM DATA

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Buried-valley aquifers are important sources of water for many areas of North Dakota. Previous investigations indicate that active recharge to buried-valley aquifers may have occurred during cooler, wetter climatic conditions and that little or no recharge is occurring at present. To investigate the effects of long-term climate change on recharge to buried-valley aquifers, $\delta^{18}O$, δD, and tritium data were compiled from ground-water samples collected from wells completed in the New Town, New Rockford, and Spiritwood buried-valley aquifers and from associated near-surface bedrock aquifers and glacial tills. The purpose of this investigation was to evaluate the paleoclimatic conditions and the timing of recharge to buried-valley aquifers in North Dakota.

The $\delta^{18}O$ composition of ground water in the three buried-valley aquifers is distinct from that of ground water in near-surface bedrock aquifers and glacial tills associated with the buried-valley aquifers. In each of the three buried-valley aquifers, $\delta^{18}O$ composition of ground water had a smaller range and a more negative mean value than $\delta^{18}O$ composition of ground water from either near-surface bedrock aquifers or glacial tills. Near New Town, ground water in the New Town aquifer ranged from -19.9 to -17.4 per mil [$\bar{x}$ = -18.6 per mil, number of samples (n) = 6] and ground water in near-surface bedrock aquifers ranged from -19.5 to -12.4 per mil ($\bar{x}$ = -16.3 per mil, n = 6). Near New Rockford, ground water in the New Rockford aquifer ranged from -17.2 to -15.2 per mil ($\bar{x}$ = -16.1 per mil, n = 6) and ground water in glacial tills ranged from -17.7 to -13.4 per mil ($\bar{x}$ = -15.5 per mil, n = 15). Near Devils Lake, ground water in the Spiritwood aquifer ranged from -15.5 to -13.1 per mil ($\bar{x}$ = -15.1 per mil, n = 9) and ground water in glacial tills ranged from -16.2 to -11.5 per mil ($\bar{x}$ = -13.4 per mil, n = 5).

The stable-isotopic composition of ground-water samples from buried-valley aquifers is similar to that of modern winter precipitation and plots on the meteoric water line defined by the $\delta^{18}O$ and δD composition of modern precipitation in North Dakota. Therefore, evaporation effects are not apparent. In contrast, ground-water samples from near-surface bedrock aquifers and glacial tills are enriched in ^{18}O relative to modern precipitation and plot to the right of the meteoric water line defined by the $\delta^{18}O$ and δD composition of modern precipitation in North Dakota. Therefore, the water has experienced evaporation.

Ground-water samples from buried-valley aquifers did not contain detectable tritium concentrations, with the exception of one sample from the New Town aquifer. The general lack of detectable tritium indicates that the buried-valley aquifers do not contain significant amounts of modern recharge. In contrast, ground water in near-surface bedrock aquifers and glacial tills routinely contains elevated tritium concentrations; these elevated tritium concentrations indicate that they are receiving modern recharge.

It is unlikely that glacial tills (or near-surface bedrock aquifers) are significant sources of recharge to underlying buried-valley aquifers. If glacial tills were significant sources of recharge, then the effects of evaporation would be apparent. Instead, the stable-isotopic composition of the ground water indicates that most of the recharge to buried-valley aquifers occurred during cooler climatic conditions when evaporation was less than at present. Cooler, wetter climates occurred in the mid- to late-1800's, for 100- to 500-year periods during the past 7,000 years, and for a 3,000- to 4,000-year period subsequent to the last retreat of the Laurentide ice sheet. The timing of recharge to buried-valley aquifers cannot be determined on the basis of existing age data, which are restricted to tritium analyses. Tritium analyses are useful for dating recharge only back to the 1950's.

A S Y M P O S I U M o n

Molecular, Biochemical, and Behavioral Aspects of Endocrinology

Symposium Coordinator: Arthur R Buckley
Department of Pharmacology and Toxicology
University of North Dakota School of Medicine
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Thursday

1:00 pm Nuclear Site of Action for Polypeptide Hormones and Growth Factors
Arthur R Buckley* and Donna J Buckley, UND School of Medicine, Grand Forks 58202

1:30 pm Molecular Changes in the Striatal Dopamine D2 Receptor During Aging
Eugene R Mesco*, Moorhead State University, Moorhead 56563

2:00 pm Hormonal and Neural Basis of Behavior in Sex Role Reversed Shorebirds
Albert J Fivizzani*, UND, Grand Forks 58202

2:30 pm * * * Refreshment / Discussion BREAK * * *

3:00 pm Endocrine and Autonomic Regulation of Cellular Stress Response Genes in Mammalian Tissues
Michael J Blake*, Kathleen P Wikel, Donna J Buckley, Tiffany Bartlett and Arthur R Buckley, UND School of Medicine, Grand Forks 58202

3:30 pm Hexokinase Regulation of Pancreatic Beta Cell Function
Mildred Voss-McCowan and Paul N Epstein*, UND School of Medicine, Grand Forks 58202

4:00 pm Selective Cell Targeting with Novel Cytotoxin Conjugates
W K Samson*, UND School of Medicine, Grand Forks 58202

NUCLEAR SITE OF ACTION FOR POLYPEPTIDE HORMONES AND GROWTH FACTORS

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Polypeptide hormones and growth factors are generally believed to provoke cellular responses via stimulation of second messenger pathways coupled to integral, plasma membrane receptors. Indeed, an initial interaction at the level of the plasma membrane appears to be required for of all such factors studied to date. However, evidence has accumulated to suggest that membrane receptor binding of several polypeptide hormones and growth factors stimulates an alternative pathway characterized by hormone-receptor endocytosis and activation of intercellular processes which target the complexes to the nucleus. At the nucleus, these factors may activate nuclear receptor-coupled second messenger systems ultimately leading to the desired biological response.

Prolactin (PRL) is an adenohypophyseal polypeptide hormone with growth factor properties which is known to be a hepatic mitogen and internalized by receptor-mediated endocytosis. Therefore, we hypothesized that certain of its molecular effects may reflect a direct interaction at the hepatocyte nucleus. In our initial studies we observed that PRL caused rapid and concentration-dependent activation of nuclear protein kinase C (PKC, 1), a second messenger system commonly coupled to growth factor-stimulation of proliferation. The effect of PRL to activate nuclear PKC was found to be specific for lactogens, inhibited by PKC antagonists, and blocked by antisera to PRL. Importantly, highly specific monoclonal antibodies to the PRL receptor completely nullified PKC stimulation by PRL suggesting a nuclear receptor-mediated process.

Based on these results we sought the putative nuclear PRL binding site by employing radioligand binding techniques. Binding of [^{125}I]-PRL was found to be specific, time-dependent, and saturable. Nuclear binding appeared to reflect a single population of high-affinity (Kd=0.5 nM) receptors with a density of ca. 3600 sites per nucleus. Immunogold electron microscopy of nuclei incubated with anti-PRL receptor monoclonal antibodies revealed PRL binding sites clustered within heterochromatin, the most active site for transcription. Interestingly, PRL receptors did not appear to be associated with the nuclear envelope. In other studies, fluorescence-activated cell sorting analysis of nuclei initially incubated with antisera to PRL and then treated with a fluorescein-conjugated secondary antibody, demonstrated the presence of endogenous PRL presumably bound to its nuclear receptor (2).

The nuclear PRL receptor was subsequently characterized employing immunoblot and biochemical analysis. The receptor from detergent-extracted nuclei was found to be present in two forms, a 177 kDa and 42 kDa species, under nonreducing conditions. With reduction, the larger receptor resolved into an additional 56 kDa band. Affinity crosslinking studies served to confirm the prodominance of the 56 and 42 kDa variants. Further studies indicated that the nuclear receptor, similar to its membrane counterpart, is phosphorylated on tyrosine residues. However, in contrast to the membrane binding site, the nuclear receptor is not glycosylated.

In aggregate, these results suggest an important site for the mitogenic effects of PRL in liver may be at the level of the hepatocyte nucleus. PRL once internalized, perhaps targeted by glycosylation/deglycosylation, binds to specific nuclear receptors within chromatin. Receptor occupation, in turn, may stimulate intranuclear PKC activation subsequently leading to transcription of specific growth-related genes. Thus, growth regulation coupled to the action of PRL and other growth factors may reflect sequential plasma membrane receptor-mediated internalization followed by nuclear ligand-receptor interactions. Supported in part by NIH grant DK4443.

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MOLECULAR CHANGES IN THE STRIATAL DOPAMINE D2 RECEPTOR DURING AGING

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Loss of motor control due to a decrease in striatal D2 dopamine receptors is one of the most consistent and best characterized manifestations of central nervous system senescence in mammalian species. Some D2 receptor-containing neurons are lost and decreased rates of receptor synthesis occur in the surviving neurons. The steady state level of D2 receptor mRNA, like those for several other genes, has been reported to change during aging. To our knowledge, the possible transcriptional mechanisms responsible for such alterations have not been elucidated. Therefore, it became necessary to examine the rates of D2 receptor mRNA synthesis in striatal nuclei from rats of different ages. We discuss here our findings which indicate a 50% decrease in the synthesis of the D2 receptor mRNA during aging. The deficit appears to be specific, since no significant differences were noted for the synthetic rates of actin and tubulin mRNA species.

HORMONAL AND NEURAL BASIS OF BEHAVIOR IN SEX ROLE REVERSED SHOREBIRDS

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In birds, intense intrasexual competition for mates is usually characteristic of the male, whereas primary parental care (incubation and brooding) is most often provided by the female. The reversal of these typical roles where the female competes aggressively for a mate and the male is the predominant incubator and brooder of young is relatively rare. The spotted sandpiper, Actitis macularia and Wilson's phalarope, Phalaropus tricolor, are two sex role reversed species that provide an ideal model system to study the hormonal and neural basis of female aggression and male parental care. Initial studies centered on collecting blood samples from wild birds at different stages of their reproductive cycle, analysis of hormones associated with aggression and parental behavior and establishing correlations with the behaviors. These studies indicated that male incubation is correlated with higher levels of prolactin in incubating males than in non-incubating males or females. Incubation behavior is facilitated by a drastic decline in testosterone, which if artificially maintained, will interfere with normal incubation. The presence of eggs in the nest appears to maintain elevated prolactin levels (1).

In contrast to the correlation of reversal of incubation behavior and prolactin levels, female aggression is not correlated with a reversal in typical sex steroid ratios. Prior to incubation, male sandpipers and phalaropes have 6 to 8-fold greater levels of testosterone than females and females have greater levels of estradiol and progesterone than males. Intense female aggression is also not based upon atypical steroid hormone metabolism in neural tissue. The levels of the steroid hormone activating enzymes aromatase and reductase were greater in the male brain and correlated with circulating levels of testosterone, a characteristic typical for most vertebrates (2).

If intense intrasexual competition for males in shorebird females is not based upon atypical adult hormone levels, unusual hormone levels during embryonic development may account for female aggression. In chickens and Japanese quail, the development of the female brain is dependent upon exposure of the embryo to an elevated level of estradiol which serves to demasculinize the brain. Our sampling of phalarope embryo blood during the final third of development and subsequent steroid analysis has not revealed differences in estrogens and androgens between males and females. The absence of elevated embryonic estradiol levels may be the basis for the aggressive intrasexual behavior of females in this species.

Current research is being conducted to test new hypotheses concerning dimorphic regulation of transcription in neural tissue during differentiation. In situ hybridization and Northern blot analysis will be utilized to document sexual differences in transcription of key mRNA's which have been reported during mammalian dimorphic neural differentiation.

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ENDOCRINE AND AUTONOMIC REGULATION OF CELLULAR STRESS RESPONSE GENES IN MAMMALIAN TISSUES.

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The induction of heat shock proteins (HSPs) by cellular stress and the activation of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS) by physiologic stress, are biological responses that aid in the maintenance of cellular and organismal homeostasis, respectively. Based on previous studies, we have hypothesized that HSPs play a functional role in neural and endocrine stress response mechanisms in mammalian organisms. In previous reports we have established that stress-induced hormones can selectively activate expression of heat shock protein-70 (HSP70) in the aorta and adrenal gland. Specifically, it was shown that adrenocorticotropic hormone (ACTH) induced expression in the adrenal while adrenergic receptor stimulation caused expression in the aorta. Continuing investigations in our laboratory focus on determining the physiologic regulation of this response and the intracellular mechanisms responsible for coupling this response to activation of the HSP70 gene. Results from these studies provide substantial evidence that endocrine and autonomic stress-response mechanisms regulate the expression of cellular stress-response genes in mammalian tissues.

To delineate endocrine and/or neural components regulating stress-induced HSP70 expression *in vivo*, we have employed the long acting, synthetic propylergoline dopamine agonist, CQP 201-403 (CQP). We report the novel observation that CQP mimics the effect of restraint stress to induce HSP70 expression in both adrenal gland and aorta of the rat. The presence of CQP-induced HSP70 mRNA and protein was preceded by the activation of a protein factor capable of binding to the heat shock transcriptional control element. CQP-induced HSP70 expression in the adrenal gland was restricted to the cortex as previously observed in restraint-stressed animals. However, the distribution of expression between the three cortical layers was distinct. Hypophysectomy virtually eliminated the effects of CQP on the adrenal gland while also markedly reducing HSP70 induction in the aorta. These results provide evidence that, in addition to adrenergic receptor activity, dopaminergic systems contribute to the physiologic regulation of HSP70 expression in adrenal gland and aorta directly through actions on receptors in responsive tissues and/or indirectly through the release of pituitary hormones.

The previous investigations indicated that, in addition to ACTH, other pituitary hormones may be involved with the regulation of HSP70 expression *in vivo*. Vasopressin is a posterior pituitary hormone known to have potent vasoconstrictor properties and was a likely candidate to initiate HSP70 expression especially in the aorta. To test this possibility, we administered vasopressin, to restrained and unrestrained rats. Both vasopressin and restraint were found to activate heat shock transcriptional regulatory factors responsible for HSP70 gene transcription and induce a delayed increase in levels of functional HSP70 protein. In contrast, administration of the vasodilating agent, aminophylline, markedly attenuated restraint-induced HSP70 expression in the aorta. These data, combined with our previous results suggests that any compound capable of producing vasoconstriction will cause a concomitant increase in vascular HSP70 expression. Activation of both adrenergic α_1 receptors and vasopressin v_1 receptors in the blood vessels result in increased intracellular Ca^+ following the formation of inositol triphosphate and resulting in smooth muscle contraction. Aminophylline increases intracellular cAMP through inhibition of phosphodiesterase resulting in reduced intracellular Ca^+ and smooth muscle relaxation. Thus, we propose that changes in intracellular Ca^+ alter the activity of heat shock transcriptional regulatory factors that ultimately leads to the activation of the HSP70 gene. Presumably, hormonal regulation of HSP70 expression is an important component of vascular smooth muscle homeostasis.

HEXOKINASE REGULATION OF PANCREATIC BETA CELL FUNCTION

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It has been proposed that endogenous hexokinases of the pancreatic beta cell control the rate of glucose-stimulated insulin secretion and that genetic defects which reduce beta cell hexokinase activity may lead to diabetes. To test these hypotheses we have produced transgenic mice that have a two-fold increase in hexokinase activity specific to the pancreatic beta cell. As we have previously reported (1), this increase was sufficient to significantly augment glucose-stimulated insulin secretion of isolated pancreatic islets, increase serum insulin levels *in vivo* and lower the blood glucose levels of transgenic mice by 20 to 50 percent below control levels.

In addition to regulating insulin secretion, glucose also regulates several other specific functions of the pancreatic beta cell including insulin gene transcription, insulin messenger RNA translation and maintenance of the capacity to secrete insulin. To determine whether hexokinase activity of the pancreatic beta cell also mediates control over these glucose regulated processes we compared them in normal and transgenic islets. For this purpose islets from normal and transgenic mice were cultured at several glucose concentrations for 48 hours. At the end of this period they were assayed for insulin secretion, insulin protein content and insulin messenger RNA content. Secretion assays of normal islets cultured at 5 and 10 mM glucose demonstrated that secretory capacity was reduced by approximately 80 percent by culture in 5 mM glucose. In contrast, transgenic islets cultured under the same conditions demonstrated only a 40 percent reduction in secretory capacity. When cultured at lower glucose concentrations the hexokinase transgene did not appear to protect the capacity of the islet to secrete insulin. Insulin content of normal islets declines markedly at glucose concentrations less than 10 mM, dropping by 30 percent at 5 mM glucose and 60 percent at 3 mM glucose. Transgenic islets, however, showed no decline in insulin content at 5 or 3 mM glucose, only at 1 mM glucose was insulin content significantly reduced. The above results indicate that maintenance of insulin content and secretory capacity are both determined by the level of hexokinase activity of the beta cell. Since hexokinase activity is believed to be rate limiting for glucose metabolism in the beta cell these results imply that the rate of glucose metabolism regulates both of these processes. We also compared insulin messenger RNA levels in normal and transgenic islets cultured at 3 and 10 mM glucose. In both normal and transgenic islets insulin messenger RNA levels were reduced by approximately 50 percent by culture in 3 mM glucose. Therefore insulin gene transcription and/or insulin messenger RNA stability are not controlled by hexokinase activity. Our results suggest that there are at least two mechanisms by which glucose controls pancreatic beta cell function.

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SELECTIVE CELL TARGETING WITH NOVEL CYTOTOXIN CONJUGATES

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In order to establish the physiological relevance of an endogenous neuropeptide in central nervous system function, one must demonstrate its pharmacologic effects, establish its site of action, and ultimately demonstrate the consequences of its absence in functional or actual terms. For some neuropeptides, most noticeably vasopressin and luteinizing hormone releasing hormone, genetic mutations have occurred naturally creating neuropeptide deficient animal strains which serve as models for studying the physiologic consequences of absence (or reapplication) of those peptides. For the myriad other CNS active neuropeptides mutant strains do not exist. Investigators have turned then to the use of neutralizing antibodies or structural homologs with antagonist activity to examine physiological relevance. Even when antagonists or selective antibodies are available, technical problems limit their usefulness. For this reason we created neuropeptide conjugates which target for destruction cells within the CNS which respond uniquely to that peptide. In this manner we create functionally deleted models which, while they still produce a given neuropeptide, cannot respond to it. Peptides are conjugated by conventional techniques to the cytotoxic A chain of the plant lectin Ricin. The A chain of Ricin acts at the ribosome to uncouple protein synthesis, resulting in cellular starvation and eventual compromise. The A chain by itself cannot enter cells, instead when conjugated to a neuropeptide is carried into a cell when surface receptors for that peptide are internalized. In this manner, selective cell targeting can occur. Control peptide conjugates are easily constructed which confer specificity. We have employed this technology to examine the physiologic relevance of several endogenous neuropeptides, particularly the family of natriuretic peptides for which there are no antagonists.

Pharmacologic application of the natriuretic peptides into the CNS results in well characterized effects on cardiovascular function, appetitive behaviors, and neuroendocrine events. Autoradiographic mapping studies have intimated the general region of action of these peptides; however, the exact cellular site of action (i.e. the neurotransmitter content or cellular identity of the responsive element) and the physiologic relevance of those pharmacologic actions has not been established. A-type natriuretic peptide (ANP) inhibits dehydration-induced water intake and basal LH and PRL secretion when applied pharmacologically *in vivo*. Animals pretreated with Ricin A chain conjugated to ANP respond to dehydration challenge with exaggerated drinking suggesting a physiological role for endogenous peptide in the control of fluid intake. Basal LH and PRL secretion are not altered in these animals but cyclic changes in hormone secretion are, indicating a potential roles for endogenous peptide in the rhythmic control of LH and PRL secretion. The exact cellular site of action of the endogenous peptide can be inferred by pharmacologic challenge and ascertained directly by anatomic means. In the case of the water drinking responses, we have identified a circumventricular site of action, while the neuroendocrine events are hypothalamic and due to interactions in the case of LH secretion with cells bearing clearance receptors for the natriuretic peptides. These appear to be glial elements. With regard to PRL secretion the effects appear to be via as of yet unidentified interneurons which control the activity of the tuberoinfundibular dopaminergic neurons in the arcuate nucleus. We have validated the efficacy of this novel methodology in additional physiologic paradigms and the use of cytotoxic neuropeptide targeting promises a novel technology for the neurosciences.

A SYMPOSIUM on

STATISTICS Theory and Applications

Symposium Coordinator: Ruey-Pyng Lu
Department of Statistics
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Friday

1:30 pm ANOVA as a Tool in Observational Studies:
A Practitioner's Perspective
Terry L Shaffer*, USDI Fish and Wildlife Service,
Jamestown 58401

2:00 pm Reference Ranges for Clinical Laboratory Data
LuAnn K Johnson* and Sandra K Gallagher, USDA/ARS
Human Nutrition Research Center, Grand Forks 58202

2:30 pm A Comparison of Exponential Smoothing and Artificial Neural Network Forcasting of Stock-Market
Douglas W Mahoney* and Ruey-Pyng Lu, NDSU, Fargo 58105
and Shaun-inn Wu, California State University, San Marcos, California 92069

3:00 pm * * * Refreshment / Discussion BREAK * * *

3:30 pm Effect Size: the Forgotten Element in Hypothesis Testing
Wesley E Newton*, USDI Fish and Wildlife Service,
Jamestown 58401

4:00 pm Heteroskedasticity Robust Estimation of a Population Total
Mary Nelson*, DSU, Dickinson 58601 and Nuwan Nanayakkara,
NDSU, Fargo 58105

4:30 pm Federal Budget Deficits and Exchange Rates: Testing for Causality with Standard Granger Tests and Error-correction Models
Golam M Farooque*, Moorhead State University, Moorhead 56563

ANOVA AS A TOOL IN OBSERVATIONAL STUDIES: A PRACTITIONER'S PERSPECTIVE

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Many interesting questions in ecology do not lend themselves to an experimental approach; direct manipulation of the environment is not often feasible. For example, a biologist studying the effect of wetland conditions on the behavior of breeding ducks must accept what nature provides in the way of varying wetland conditions. Studies of this type are oftentimes referred to as "observational studies." Sample surveys, in which the objective is generally estimation of one or more parameters in a real population, fall into this category (1). Observational studies are also widely used to investigate and test hypotheses about some parameter of interest. Analysis of variance (ANOVA) is often the statistical tool of choice for testing hypotheses (2). Most students of statistics learn ANOVA in the context of designed experiments, however. While the same basic principles apply for using ANOVA in an observational study as in a designed experiment, observational studies typically present more complications for the data analyst (3). I discuss some of these complications and suggest ways of avoiding or dealing with them. Similar complications arise in analyses involving designed experiments, but are much more prevalent when dealing with observational data. The examples I give are based on real problems encountered by my colleagues or me at Northern Prairie Wildlife Research Center.

One frequent complication involves proper identification of the experimental unit, a step essential for determining the correct ANOVA model. Often, in studies involving multiple "treatments," more than one size of experimental unit is involved (4). Studies involving repeated measures of the same individual fall into this category. If unrecognized, this situation can lead to pseudoreplication and erroneous conclusions (5).

Lack of independence among observations can also be a problem. Unlike designed experiments, in which treatments can be randomly assigned to experimental units, in observational studies "treatments" are assigned by nature or some other, presumably non-random, force. Moreover, acquiring a random sample of mobile or secretive animals is seldom possible, and sampled animals may not be independent. These features give rise to misleading results from statistical tests.

Another problem that frequently plagues analyses involving multiple treatments is incompleteness in factorial type models. For example, a study examining the effects of season and sex on body weight of sandhill cranes (Grus canadensis) may fail to obtain data for both sexes in all seasons. In that situation, the usual tests for main effects and interactions are no longer valid and the investigator must specify alternate hypotheses to test (4). A similar but much less troublesome problem occurs when sample sizes within each group are unequal, but nonzero.

A related concern is the confounding of effects. If, for instance, in the example above, female cranes migrate earlier than male cranes, then a sample of female cranes during the fall may be largely from collections made in September and a sample of male cranes from collections in October. This situation could result in confounding of seasonal effects with those of sex. This problem can be mitigated if the seasonal effect is recognized and samples of each sex in each time period are adequate.

Insufficient range in the observed values of the treatment variable may result in no measurable effect on the response. For example, a multi-year study concerned with the effects of wetland conditions on behavior of breeding ducks would have little value if all years had similar wetland conditions. One remedy for this problem, which may not always be feasible, is to ensure that studies are of sufficient duration to reflect a wide range in the treatment variable.

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A Comparison of Exponential Smoothing and Artificial Neural Network Forecasting of Stock-Market

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Let $y_1, y_2, ..., y_n$ denote time series data. Given these observed values, a prediction of future values, y_{n+t} t=1,2,... is desired. Within a statistical framework, one might employ regression analysis, exponential smoothing techniques, or Box-Jenkins Methodology to make these predictions. These above techniques do have their drawbacks in that they can be rather labor intensive. Possibly, a more convenient and potentially more powerful method of using the past observations to predict future values is to convert them into weights of an artificial neural network of a given structure.

Currently, an artificial neural network (ANN) has been trained to forecast Standard and Poor 500 composite indexes by using daily data from the past twenty years with some surprisingly accurate results. An ANN is an algorithmic attempt to mimic the biological nervous system in the sense that it tries to "learn" a specific task much like human beings learn. An ANN structure consists of nodes, adjustable weights, connections, and layers. Disjoint nodes are usually grouped together to form layers, and each layer performs a specific task.Each node witnin a layer is connected to other nodes within other layers and with each connections there is a corresponding weight assigned. In order for the learning process to begin, the ANN was introduced to the previous day's closing index and a forecast of the next day's closing index was produced. When discrepancies have occurred, the weights of the ANN are adjusted in such a way as to minimize error. The method employed to accomplish this was Backward Propagation, in which errors are feed back through the ANN, layer by layer. After experimentation with the number of nodes and layers, the ANN used for discussion in this paper will by compared to the exponential smoothing technique.

A forecasting technique which seems to parallel the ANN learning algorithm is that of exponential smoothing. If it can be assumed that y_t fluctuates about a gradually changing mean level then a reasonable model for y_t is $y_t=\beta_t+\varepsilon_t$ where β_t indicates that the mean level of y_t is time dependent and ε_t are uncorrelated errors. An estimate for β_t is $\hat{\beta}_t=\bar{y}$. When it comes to an on going forecasting system, however, applying equal importance to past observations may not be reasonable. It might by more advantageous to apply heavier weights to current observations and declining weights to observations far into the past. Exponential smoothing is a forecasting technique which assigns unequal weights to the observed data to make estimations of the model parameter β_t. Let $\hat{\beta}_{t-1}$ be the estimate of β_t at time t-1, t=1,2,... where $\hat{\beta}_0=y_1$. Now, at time period t, $\hat{\beta}_{t-1}$ is updated to $\hat{\beta}_t$ by using the smoothing equation $\hat{\beta}_t=\alpha y_t+(1-\alpha)\hat{\beta}_{t-1}$ where $0<\alpha<1$ and is chosen in such a way as to minimize sum of squares forecast errors. If $\hat{\beta}_t$ is the estimate at time t for β_t then a point forecast for y_{t+1} is $\hat{\beta}_t$. Thus, much like the ANN algorithm, exponential smoothing seeks an optimal weight to minimize forecast errors and is an iterative forecasting process.

The S&P 500 index does conform to the assumptions needed in order to apply the exponential smoothing technique. It was found that a value of $0.80\leq\alpha\leq0.95$ provided minimal sum of squares forecast errors and a value of $\alpha=0.95$ was chosen. A comparison of exponential smoothing and ANN forecasting where conducted by comparing their respective Mean Absolute Deviation (MAD) and their ability to track market movement. For exponential smoothing, $MAD_{smoothing}=1.6497$ with standard error of 0.0444 and for ANN forecasting, $MAD_{ANN}=4.2$ with standard error of 0.4165 . Using a threshold value of 0.1, the ability to track market movement is given in the following tables:

		Movement of S&P index		
		up	same	down
Movement	up	150	15	122
Forecasted	same	861	97	793
by Smoothing	down	145	14	202

		Movement of S&P index		
		up	same	down
Movement	up	440	48	379
Forecasted	same	223	22	184
by ANN	down	550	56	479

Exponential smoothing is superior in the sense that it provides a tighter fit, as represented by their respective MADs. It is important to note that the ANN produced several wild forecasts which inflated its respective MAD. Also, as indicated by the tables, each method has considerable difficulty in tracking the movement of the S&P 500 index, but under stable market conditions the ANN actually out performs that of the smoothing technique.

REFERENCE RANGES FOR CLINICAL LABORATORY DATA

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Interpreting clinical laboratory data is a vital part of medical diagnosis, prognosis and research. Physicians and scientists need reliable criteria for deciding when the result of a single clinical test or a panel of tests is indicative of a disease state or of an abnormal condition. Traditionally, the outcome for each laboratory test is compared to its corresponding population-based reference range. The reference range is assumed to be calculated from a random sample of a population representative of the subject of interest. In practice, reference subjects are seldom selected at random, but are selected from some definable population.

The methods discussed here will assume that each of the clinical variables being measured is normally distributed with mean μ and variance σ^2. Some variables will need to be transformed to achieve a normal distribution. Outliers and aberrant data should be eliminated (1). The presence of a single outlier may be tested by using any of several statistical techniques available (2). Alternatively, a robust estimate of the sample mean and standard deviation may be obtained by trimming or Winsorizing a predefined percentage of observations from each end of the observed distribution.

The purpose of a reference range is to predict the interval into which a single measured value would occur if the subject being tested was typical of the reference population (3). Thus, a prediction reference range for a single value must account for the uncertainty in a single observation, as well as the variability in the population reference sample. We estimate the true mean and variance of the clinical variable by calculating the mean ($\bar{x}$) and variance (s^2) of the reference sample. The univariate reference predictive range is given by:

$$\bar{x} \pm (1+1/n)^{1/2}\, t(\alpha/2; n-1)\cdot s \qquad [1]$$

where n is the sample size and t is the Student's t for a given significance level $\alpha/2$ and n-1 degrees of freedom. This interval will include at least 100(1 - α)% of the reference population with 50% confidence.

The concept of a predictive reference range can be extended to the multivariate case in which the outcomes from a panel of k clinical tests must be assessed and interpreted. Assuming that the k variables are from a multivariate normal distribution, a multivariate reference region can be calculated by using:

$$(\mathbf{x}_0-\bar{\mathbf{x}})^T\, \mathbf{S}^{-1}\, (\mathbf{x}_0-\bar{\mathbf{x}}) \;\le\; \frac{k(n^2-1)F(1-\alpha;k,n-k)}{n(n-k)} \qquad [2]$$

In standard matrix notation, $\mathbf{x}_0$ represents the (k x 1) vector of observed values on the subject of interest, $\bar{\mathbf{x}}$ the (k x 1) vector of observed means and **S** the (k x k) matrix of variances and covariances of the variables in the reference population. F(1-α;k,n-k) denotes the (1-α) percentile of the F distribution with k and n-k degrees of freedom. If the "distance" of the observed profile ($\mathbf{x}_0$) from the mean ($\bar{\mathbf{x}}$) of the reference population (the left-hand side of [2]) exceeds the critical value (the right-hand side of [2]), then the profile should be considered abnormal. Using multiple univariate reference ranges to interpret a profile of clinical tests instead of a multivariate reference region can result in too many profiles being considered abnormal.

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EFFECT SIZE: THE FORGOTTEN ELEMENT IN HYPOTHESIS TESTING

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This past decade has seen excellent articles discussing the need for valid scientific practices in ecological research. Topics include collecting reliable information (1), executing sound experiments with valid replication (2, 3), and conducting statistical power analyses (4). One neglected area, however, has been that biologists fail to specify the effect size for power analyses and sample size calculation. Effect size is the magnitude of the difference between population parameters (e.g. means) that is meaningful to detect (5). The purpose of my talk is to discuss the importance of specifying the effect size for power analyses and sample size calculations.

Scientists often seek statistical advice on needed sample sizes prior to conducting research when there are specific hypotheses to be tested. Effect size is one of four parameters required to determine appropriate sample sizes and conduct power analyses. The other three parameters are Type I and Type II error rates, and the population variance. Type I error rate is the probability of rejecting a true null hypothesis and Type II error rate is the probability of failing to reject a false null hypothesis. Statistical power is the probability of rejecting a false null hypothesis, or one minus the Type II error rate. Although the actual variance of a population is rarely known, reasonable estimates can often be obtained from prior research. Once the above information has been specified, required sample sizes can be calculated. Alternatively, statistical power can be determined for various combinations of effect size, Type I error rate, variance, and sample size.

Researchers often do not give much thought to what a meaningful effect size should be for their study. Instead, they seem content to find a "significant" effect, and then subjectively evaluate whether the effect is meaningful. However, to conduct a power analysis or estimate sample sizes, the effect size must be specified a priori. I find it fruitless to dictate the effect size in terms of increases or decreases in units of standard deviations as suggested in some statistical texts (6), since most researchers do not think in terms of differences between means in units of standard deviations. Determining a range of sample sizes that are required for various effect sizes (e.g., 10, 20, etc. percent increase or decrease in mean difference) and then subjectively choosing the sample size that meets one's budget detracts from objectivity in the study.

If the desired effect size is unknown, it should be determined prior to conducting the experiment. Methods for determining the effect size can include experimentation, prior research, or theoretical modelling. Prior consideration of the effect size will allow studies to be more objective and give better estimates of required sample sizes. The sample size should be large enough to detect the effect size of interest, but small enough so as not to reveal "significant," but meaningless effects.

As an example, a biologist desires to know if the introduction of fish into wetlands causes a reduction in the production of plant biomass. The fish potentially increase water turbidity which in turn affects plant growth. Before sample sizes or power can be determined, the biologist needs to know what constitutes a meaningful reduction in plant biomass.

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HETEROSKEDASTICITY ROBUST ESTIMATION OF A POPULATION TOTAL

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Suppose that associated with each unit of a population consisting of a finite number of units is a 1×2 vector of variables (Y, X) where Y is the variable of interest and X is an auxiliary variable. More specifically, let (y_{ij}, x_i) where $i = 1, \ldots, k$, and $j = 1, \ldots, N_i$ denote the values of the vector of variables (Y, X) associated with each unit of the population. Let $N = \sum N_i$ be the total number of units. It is assumed that the value of the X-variable for each unit in the population is known apriori. It is our desire to estimate the population total $T = \sum y_{ij}$, based on a sample s, taken from this population. Let p be a sampling design, S be the collection of all possible samples under design p. For each sample s taken from the population of interest, the design p assigns a probability of selection $p(s) \geq 0$ such that $\sum p(s) = 1$ where the summation is taken over S.

It is commonly believed that a good sampling strategy should be guided by the prior knowledge one has about the population structure. A well known strategy is to incorporate one's prior knowledge or beliefs into a so-called super-population or prediction model [1]. In this approach, the numbers y_{ij}'s are treated as realizations of random variables Y_{ij}'s and that the random variable Y_{ij} is related to the known auxiliary variable X_i according to a Model ξ:

$$Y_{ij} = X_i\beta + \epsilon_{ij},\ E_\xi(\epsilon_{ij}) = 0,\ \mathrm{var}_\xi(\epsilon_{ij}) = \sigma_i^2,\ \mathrm{cov}_\xi(\epsilon_{ij}, \epsilon_{kl}) = 0 \text{ for all i, j, k, and l,}$$

where β is an unknown parameter. Here we have a Model ξ relating Y to the auxiliary variable X and a sampling design p. It is assumed that the sampling design p is independent of the prediction model ξ. We say that an estimator $\hat{T}$ of the population total is p-ξ-unbiased or design-model unbiased if:

$$E(\hat{T} - T) = E_p[E_\xi\{(\hat{T} - T) \mid S\}] = 0,$$

where S denote the chosen sample. In the literature [2, 3] it is assumed that $\sigma_i^2 = X_i\sigma^2$. Naturally, we should ask, "What if such a detailed modeling of the variance function σ_i^2 is not feasible?" Here, our goal is to relax the assumption $\sigma_i^2 = X_i\sigma^2$. Suppose we stratify the units of the population according to the value of the variable X which is assumed to be known. Consider the sampling design p that selects a single unit from each stratum. Without loss of generality assume that (Y_{i1}, X_i) $i = 1, \ldots, k$ denote the sample being selected. Let $\hat{T}_M = \sum Y_{i1} + \left(\sum (N_i - 1)x_i\right)(\sum x_i Y_{i1})/S_X$, and $V_M = A\{\sum (N_i - 1)x_i/S_X\}^2 + B$, where $A = \{1 + \sum X_i^4/S_X(S_X - 2X_i^2)\}^{-1}\{\sum S_X X_i^2 e_i^2/(S_X - 2X_i^2)\}$, $B = \{\sum (N_i - 1)(S_X^2 e_i^2 - AX_i^2)/(S_X^2 - 2X_i^2)$, $S_X = \sum X_i^2$, $e_i = (Y_{i1} - \hat{\beta}X_i)$ and $\hat{\beta} = (\sum X_i Y_{i1})/S_X$. Where appropriate all the summations are taken over $i = 1$ to k. It can be shown that $\hat{T}_M$ is a p-ξ-unbiased estimator of the population total T, and V_M is an unbiased estimator of its error variance irrespective of the structure of σ_i^2. Thus the interval:

$$(\hat{T}_M - 2\sqrt{V_M},\ \hat{T}_M + 2\sqrt{V_M})$$

is a heteroskedasticity robust approximate 95% interval estimator for the population total T.

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Federal Budget Deficits and Exchange Rates: Testing for Causality with Standard Granger Tests and Error-correction Models.

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There has been much debate centered on the view that the large and persistent U.S. federal budget deficits have caused the value of dollar to rise at its record high in 1985 and, subsequently, have increased U.S. trade deficits. According to conventional view, the large U.S. budget deficit increased the U.S. real interest rate relative to the rest of the world, inducing a large capital inflows into the U.S. The capital inflows appreciated the real exchange value of dollar. The alternative of this view suggests that budget deficits do not cause interest rates to rise and hence budget deficits do not cause the dollar value to rise. Evidence on the causal relationship between the federal deficit and the dollar is mixed.

The main purpose of this paper is to reexamine which of these views is correct using data covering the periods 1972:I-1989:4 and 1961-1989 with testing procedures: standard Granger causality tests and error-correction models. I examine, simultaneously, the causal links connecting budget deficits with the trade-weighted exchange rate, the interest rate, the inflation rate, real money, real output, real federal government expenditures, trade deficits, and the after-tax profits. The advantage of error-correction models over Granger causality tests is that the former incorporates information from the cointegrated properties of time series variables. The presence of causality among the variables is investigated using a multivariate vector autoregressive model.

The findings reveal that there is a direct causal relationship between the budget deficit and the exchange rate, however the causality is from exchange rates to budget deficits. Inclusion of error correction terms has not changed the direction of causality of budget deficits with either exchange rates or any other variables substantially, although error correction terms are highly significant. We also find that budget deficits do not cause interest rates, money supply and inflation rates. Instead exchange rates cause money supply, inflation and real output, and interest rates cause exchange rates. Using annual data we do not find any significant change in the direction of causality between budget deficits and exchange rates. In this case, interest rates cause budget deficits and trade deficits directly. Interest rates also cause exchange rates indireclty by effecting the trade deficit.

Based on our results, we reject the conventional view that budget deficits cause interest rates, which, subsequently, cause trade deficits by affecting the value of dollar. Our evidence support the ''Ricardian Equivalence'' proposition in that that the budget deficit does not cause the interest rate and hence the dollar. The study also finds that the causal link or the direction of causality does not differ greatly due to methodological difference or data difference.

A RODGER DENISON

Student Research Competition

Friday

Undergraduate

9:00 am Dietary Zinc and Endurance Exercise Training Affect Sketal Muscle and Bone Mineral Concentrations of Rats
Erin M Thorsgard*, Mary E Sleeper, Kim G Michelsen, Clinton B Hall and Henry C Lukaski, USDA/ARS Human Nutrition Research Center, Grand Forks 58202

Graduate

9:20 am Activity of Gluconeogenic Enzymes in Rabbit Enterocytes
Sherry A Wuensch* and Paul D Ray, UND School of Medicine, Grand Forks 58202

9:40 am Characterization of the Sinu-Nasal-Bronchovascular Reflex
Penny R Kuhn*, Pamela M Brule and William M Long, UND School of Medicine, Grand Forks 58202

10:00 am The Use of In-line Filtration to Remove Fish Pathogens from Garrison Diversion Water
David M Kopchynski*, UND, Grand Forks 58202

10:20 am Concordance of Human Acetylator Phenotyping and Genotyping Assays
Erik Furman*, Ronald Ferguson, Mark Doll, David Hein, UND School of Medicine, Grand Forks 58202

10:40 am 2-Aminofluorene-Hemoglobin Adduct Formation in Rapid and Slow Acetylator Syrian Hamsters Congenic at the NAT2 locus
Yi Feng* and David W Hein, UND School of Medicine, Grand Forks 58202

11:00 am Catastrophic Debris Flow in Southern Manitoba
Eric C Brevik* and John R Reid, UND, Grand Forks 58202

DIETARY ZINC AND ENDURANCE EXERCISE TRAINING AFFECT SKELETAL MUSCLE AND BONE MINERAL CONCENTRATIONS OF RATS

Erin M. Thorsgard,* Mary E. Sleeper, Kim G. Michelsen,
Clinton B. Hall, and Henry C. Lukaski
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Surveys of physically active adults suggest that zinc (Zn) status may decline with exercise training. Interpretation of these observations is complicated by the fact that blood biochemical indices of Zn status may not reflect tissue Zn concentrations. Thus, it is unclear if marginal Zn intake, exercise training, singly or in combination, affect trace element homeostasis. To address this question, we examined the effects of reduced dietary Zn and exercise training on plasma and tissue Zn, copper (Cu), and calcium (Ca) concentrations of rats.

Male, weanling Sprague-Dawley rats were matched by weight into six groups (n=10) in a 2 x 3 experimental design. Thirty rats were trained to run on a motorized treadmill (30 m/min at 15% incline) up to 60 min/d, 5 d/wk, for 8 wk. Thirty other rats did not run on the treadmill. The rats were fed a semipurified diet containing all essential nutrients but variable Zn concentration. Within each exercise treatment, one group (n=10) of rats was fed *ad lib* a Zn-adequate (20 μg/g) diet, another was fed *ad lib* a marginal Zn (5 μg/g) diet, and the third group was pair-fed (PF) the Zn-adequate diet in amounts equal to that consumed by the matched animal fed the marginal Zn diet. The animals were killed 24 h after the last exercise bout, and selected organs and tissues were obtained.

Table 1. Body Weight (g), Plasma Zn (μmol/L), Muscle and Bone Zn and Cu (μg/g) and Ca (mg/g) Concentrations

	Body	Plasma	Soleus			Gastrocnemius			Tibia		
	Weight	[Zn]	[Zn]	[Cu]	[Ca]	[Zn]	[Cu]	[Ca]	[Zn]	[Cu]	[Ca]
Zn Adeq											
Ex	339	1.26	208	6.2	332	59	3.9	255	244	3.0	358
NonEx	370	1.71	228	3.6	193	37	2.4	174	223	3.0	259
Zn Marg											
Ex	334	1.33	202	5.9	265	46	3.8	170	137	3.2	229
NonEx	351	1.36	205	3.6	240	38	2.7	155	98	3.0	265
Pair-Fed											
Ex	324	1.36	209	5.7	267	54	3.5	247	235	3.2	326
NonEx	350	1.43	216	3.2	232	45	2.9	179	220	3.0	261
			Analysis of Variance - P values								
Diet	0.07	0.37	0.34	0.76	0.42	0.02	0.91	0.002	0.01	0.05	0.001
Ex	0.001	0.03	0.23	0.01	0.03	0.001	0.001	0.001	0.01	0.01	0.001
Diet x Ex	0.64	0.08	0.71	0.97	0.09	0.03	0.15	0.09	0.08	0.42	0.001

Daily food intake was similar among all groups of rats. Endurance exercise reduced body weight (Table 1). Plasma Zn concentrations decreased after exercise training but were unaffected by diet. Neither dietary Zn nor exercise influenced soleus Zn concentration; Cu and Ca concentrations, however, were increased by exercise training. Endurance exercise increased gastrocnemius Cu concentration. As compared to marginal Zn, adequate Zn, PF and exercise increased gastrocnemius Zn and Ca concentrations. Adequate Zn, PF and exercise increased tibial Zn and Cu concentrations. Marginal Zn blunted the exercise-induced increase in tibial Ca. These findings indicate that dietary Zn affects Zn concentration of bone and specific muscles. Endurance exercise training decreases circulating Zn in the plasma while it increases the concentrations of Zn, Cu and Ca in muscle and bone. Adequate dietary Zn apparently promotes Zn storage in specific types of muscle types (slow twitch vs fast twitch) in the form of Zn-dependent enzymes involved in energy production (e.g., lactate dehydrogenase and carbonic anhydrase) along with Zn and Ca stores in bone as an adaptation to endurance exercise training.

ACTIVITY OF GLUCONEOGENIC ENZYMES IN RABBIT ENTEROCYTES

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Of major interest to us is the metabolic role of phosphoenolpyruvate carboxykinase (PEPCK) and we recently reported the presence of a large amount of PEPCK activity in the mucosal lining of small intestine from fed and 24 hr fasted rabbits(1). The only proven role for PEPCK activity is in gluconeogenesis and small intestine has not been shown to be a significant site for this process in adult animals.

To explore the role of PEPCK in this tissue, we have used enterocytes isolated from the upper small intestine of 24 hr fasted rabbits by a procedure which basically employs chelation of calcium with citrate and EDTA (2). Isolated cells were suspended in a solution of KCl, EDTA and TrisCl (pH 7.4), pelleted, resuspended in KCl, TrisCl (pH 7.4) plus 0.1% collagenase type I at room temp for 10 min at which time DNAse I was added to prevent cell aggregation. Cells were pelleted, resuspended in KCl, EDTA and TrisCl (pH 7.4) and oxygenated mildly until use. Cell viability was about 85% based on exclusion of 0.2% trypan blue. Some cells were fractionated using 0.078% digitonin which removes the cytoplasmic membrane and leaves mitochondria intact thereby allowing for preparation, by differential centrifugation, of cytosolic and mitochondrial fractions which were analyzed for PEPCK activities. Preparations from other cells solubilized with Triton-X100 were analyzed for total PEPCK, pyruvate carboxylase (PC), glycerol kinase (GK), fructose 1,6 bisphosphatase (FBPase), and glucose 6-phosphatase (G6Pase) activities.

The total activities determined for PEPCK, PC, GK, FBPase, and G6Pase were 375 $\pm$ 67.9, 5.79 $\pm$ 5.79, 9.37 $\pm$ 6.16, 1150 $\pm$ 374 and 2150 $\pm$ 292 nmol/min/10^8 cells (means $\pm$ 1 S.E., n = 10 to 11). At least 90% of the PEPCK activity was mitochondrial.

Results suggest that gluconeogenesis from lactate or glycerol is unlikely given the low activities of PC and GK. Given the primarily mitochondrial location of PEPCK, gluconeogenesis from amino acids feeding into the Krebs cycle is possible only if cytosolic reducing equivalents can be provided as for example from β-oxidation of fatty acids. Intestinal cells can synthesize lipids including sterols and intestinal mitochondrial PEPCK may be participating in this process (3). (Supported by NIH R01 DK 41631)

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CHARACTERIZATION OF THE SINU-NASAL-BRONCHOVASCULAR REFLEX

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Sinusitis or upper airway inflammation often occurs concurrently with bronchial hyper-responsiveness or asthma. Histamine is a mediator from mast cells frequently released in upper airway inflammation. We have reported that histamine applied to the nasal or frontal sinus mucosa causes an increase in bronchial artery blood flow (Qbr). The purpose of this study was to determine how histamine applied to the nasal or frontal sinus mucosa elicits bronchovascular responses in the lower airways of pentobarbital anesthetized sheep. Open-chest sheep were ventilated mechanically using a piston respirator with air and supplemental oxygen delivered via a nasotracheal tube and 5 cm H2O PEEP. Qbr was measured with an electromagnetic flow probe after a left thoracotomy. Qbr was measured because the bronchial artery is richly innervated and blood flow through this artery is believed to be a sensitive index of mediator involvement in the bronchi. The frontal sinus was trephined and sinus drainage was blocked with a balloon catheter. Histamine (5% weight /volume in 0.1 ml PBS) applied to the nasal or frontal sinus mucosa caused an increase in Qbr of similar magnitude and duration (290 $\pm$ 90% of base line (22 $\pm$ 3 ml/min), mean $\pm$ standard error, n=5). There was no evidence that tachyphylaxis to histamine occurred upon repeated application. Resection of the opthalmic and maxillary branches of the trigeminal nerve attenuated the peak histamine-induced effect on Qbr by 71 $\pm$ 14%. However, application of histamine to the contralateral frontal sinus with an intact trigeminal nerve caused an increase in Qbr equivalent to the response elicited before nerve was cut. The histamine-induced effect on the contralateral side was attenuated by 72 $\pm$ 13% after resection of the right and left vagus nerves. The residual histamine-induced increase in Qbr was blocked by pretreatment with intravenous chlorpheniramine, a histamine H1 antagonist(2 mg/kg), suggesting that some histamine was also absorbed across the sinus mucosa after application. In 3 other anesthetized sheep with intact nerves, the bronchovascular effect of histamine applied to the sinus or nasal mucosa was attenuated by 60 $\pm$ 10% after pretreatment with intravenous atropine (0.2 mg/kg). Atropine had no effect on base-line Qbr. These results indicate that histamine applied to either the sinus or nasal mucosa can evoke an increase in Qbr that is mediated in part by an atropine sensitive vagal reflex pathway,and in part by systemic absorption of histamine across the upper respiratory mucosa. This study shows that an inflammatory mediator applied to the sinus mucosa can elicit effects in the lower airways and explains in part how inflammation of the sinuses can elicit effects in the lower airways. (Supported in part by American Heart Association Dakota Affiliate and NIH BRSG RR-05407)

THE USE OF IN-LINE FILTRATION TO REMOVE FISH PATHOGENS FROM GARRISON DIVERSION WATER

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PURPOSE

The purpose of this research was to determine seasonal design parameters for an in-line filtration system to remove fish pathogens from Garrison Diversion Unit (GDU) Water. These parameters will be utilized to develop cost and design information for a proposed, full scale in-line filtration plant. Design parameters determined in this research were filter run length, bacterial removal, coagulant dosages and turbidity removal. Support for this research was provided by the North Dakota Water Resources Research Institute.

METHODOLOGY

Bench-scale in-line filtration runs were performed at the Snake Creek Pumping Plant near Cole Harbor, North Dakota. Four filter runs were performed during the period of July 28 to 31, 1992 (Runs 1S-4S) and during the period of October 5 to 9, 1992 (Runs 1F-4F). Runs 1S-4S used a rapid mix step with a velocity gradient of 500 reciprocal seconds ($G=500\ sec^{-1}$). Runs 1F-4F did not include a rapid mixing step and the coagulants were added directly to the filter. Run 1S-4S, 3F and 4F used a dual-media bed with 15-18 inches of 1.3-1.5 mm e.s. anthracite coal on top of 6-8 inches of 0.45 mm effective size (e.s.) silica sand. Runs 1F and 2F consisted of a mono-media filter bed with 5 feet of 1.7-1.9 mm e.s. anthracite coal. Dual-media and mono-media beds were loaded at the rates of 10 gallons per minute per square foot (gpm/ft^2) and 13.5 gpm/ft^2 respectively. All runs utilized either a nonionic polymer with aluminum sulfate (Alum) as coagulants or a cationic polymer as the sole coagulant.

Raw and filtered turbidities were monitored at half-hour intervals and were recorded in nephelometric turbidity units (ntu) with a laboratory turbidimeter. Raw and filtered water samples were taken daily to determine bacterial removal in each filter run. Bacterial counts were determined by the Pour Plate method described in Standard Methods (1). Also, filter heads were measured at half-hour intervals with piezometers placed at various depths in the filter bed. Filter run lengths were predicted by interpolating the filter head data.

RESULTS

Design parameters derived from Snake Creek filter run data are presented in Table 1.

TABLE 1
Snake Creek Pumping Plant Filter Run Results

Run No.	Predicted Run Length (hours)	Average Turbidity Influent (ntu)	Average Turbidity Effluent (ntu)	Average Turbidity Removal (%)	Alum Dosage (mg/L)	Polymer Dosage (mg/l)	Bacterial Removal (%)
1S	9.8	2.52	0.91	64	N/A	0.22	91
2S	5.6	2.69	0.40	85	N/A	0.66	92
3S	13.0	3.54	0.71	80	3.2	0.03	94
4S	7.6	3.03	0.16	95	7.0	0.08	82
1F	4.4	8.00	1.55	81	N/A	1.11	NR
2F	8.0	6.14	0.82	87	10.2	0.22	NR
3F	8.0	5.28	0.52	90	N/A	0.55	NR
4F	8.7	4.46	0.44	90	7.2	0.20	NR

Key: N/A- coagulant not applied; NR- No reduction reported

CONCLUSIONS

Filter runs performed at the Snake Creek Pumping Plant possessed projected run times of 4 to 13 hours with average bacterial and turbidity removals in the ranges of 0-94% and 64-95% respectively. Runs without a rapid mix step reported no bacterial reduction and required three to seven times the polymer dosages used in identically configured runs with rapid mix.

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CONCORDANCE OF HUMAN ACETYLATOR PHENOTYPING AND GENOTYPING ASSAYS

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Genetic factors control every aspect of pharmacodynamic and pharmacokinetic properties of drugs. Genetic factors are responsible for many unanticipated adverse drug reactions and inadequate drug responses. The understanding and identification of these genetic factors are crucial to achieve the most efficacious drug therapy possible. Determination of these genetic factors can be of great benefit to the patient in many instances. A polymorphic N-acetyltransferase isozyme (NAT2) is one of the best characterized of these genetic factors. The NAT2 isozyme catalyzes the N-acetylation of a number of environmentally and occupationally encountered arylamine chemicals as well as arylamine and hydrazine drugs. Classifications of N-acetylation phenotype have been shown to be associated with a number of diseases, such as increased incidence of bladder cancer among slow acetylators exposed to arylamine carcinogens, as well as adverse reactions to certain clinical drugs and inadequate drug effects. Classifications of N-acetyltransferase activity can be divided into three phenotypes: rapid, intermediate, or slow acetylators.

Acetylator phenotype was determined by comparing the urinary excretion ratio of two caffeine metabolites: 5-acetylamino-6-amino-3-methyluracil (AAMU) and 1-methyl-xanthine (1X). They are both thought to be formed from an unstable metabolic intermediate (possibly ring-opened). NAT2 is the enzyme that catalyzes formation of AAMU from this intermediate. Thus, acetylator phenotype determines the AAMU/1X ratio. Because of this, the AAMU/1X can be used as a good means of phenotyping people in regards to acetylator status. The molar ratio of AAMU to 1X is determined by size exclusion high performance liquid chromatography. Although this method of phenotyping is not very invasive (caffeine equivalent of a cup of coffee and spot urine collection), a method that did not involve a test drug would be better, especially if phenotyping required other drugs with more dangerous side effects. Therefore, we tested the concordance of acetylator phenotype determinations by AAMU/1X ratios in urine specimens with acetylator genotyping assays. Acetylator (*NAT2*) genotype was accomplished using Restriction Fragment Length Polymorphism. The genomic DNA was isolated from the hair follicles and amplified using Polymerase Chain Reaction. The DNA was enzymatically digested and the fragments were separated by electrophoresis. Acetylator genotypes were determined by the pattern of restriction fragments after the gel was stained. Based upon acetylator genotype, we observed a distribution of 10 slow acetylators, 7 intermediate acetylators, and 3 rapid acetylators among the 20 people tested. We found a concordance between acetylator genotype and phenotype using the two methods (Table 1).

Table 1. *NAT2* Genotype and Phenotype

NAT2 Acetylator Genotype	N	Expected Acetylator Phenotype	AAMU/1X Excretion ratio
M1M1	4	slow	3.52 ± 0.97
M1M2	5	slow	2.99 ± 0.45
M1M3	1	slow	2.81
Total	10	slow	3.19 ± 0.42*
WTM1	4	Intermediate	6.66 ± 1.02
WTM2	3	Intermediate	6.69 ± 1.29
Total	7	Intermediate	6.67 ± 0.73*
WTWT	3	Rapid	7.78 ± 1.04*

*AAMU/1X ratios differ significantly with expected acetylator phenotype based on determination of acetylator genotype ($p=0.0002$).

2-AMINOFLUORENE-HEMOGLOBIN ADDUCT FORMATION IN RAPID AND SLOW ACETYLATOR SYRIAN HAMSTERS CONGENIC AT THE *NAT2* LOCUS

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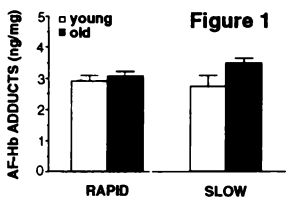
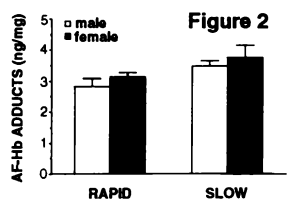
Humans and hamsters segregate into rapid, intermediate, or slow acetylator phenotypes according to a genetic polymorphism at the *NAT2* gene locus encoding for an acetyltransferase isozyme (NAT2). Human epidemiological studies have suggested that rapid acetylators are associated with colorectal cancer and slow acetylators are at higher risk for urinary bladder cancer, particularly in individuals exposed to high levels of carcinogenic arylamines. Since these conclusions are compromised by uncontrolled environmental and genetic factors, our Syrian hamster model congenic at the *NAT2* locus may provide controlled exposure to arylamine carcinogens and virtual elimination of genetic variability.

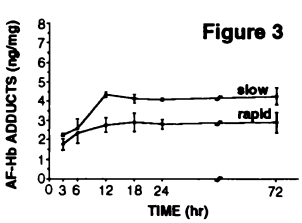
2-aminofluorene is an environmental carcinogen produced during refining synthetic fuels from coal, and has been intensively studied in different animal models. Arylamines such as 2-aminofluorene undergo metabolic activation by hepatic cytochrome P-450IA2 to form N-hydroxyarylamine derivatives that bind covalently to proteins and DNA. N-hydroxylamine derivatives bind to DNA leading to carcinogenesis in the target organ and to β-93 cysteine residue of hemoglobin. The arylamine-hemoglobin adducts formed serve as a valuable dosimeter for assessing arylamine exposures and carcinogenic risk. In this study, we investigated the characteristics of 2-aminofluorene-hemoglobin adducts in our acetylator congenic Syrian hamsters to assess the role of acetylator genotype on 2-aminofluorene activation and hemoglobin-adduct formation.

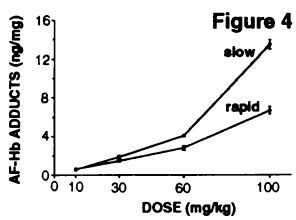
Rapid (Bio. 82.73/H-*Pat*^{r}) and slow (Bio. 82.73/H-*Pat*^{s}) acetylator hamsters congenic at the *NAT2* locus were administered a single dose of [^{3}H]2-aminofluorene by i.p. injection (60 mg/kg, about 100μCi/hamster) to measure the effects of age, sex and acetylator genotype at 24 hr post-injection, and to determine the time course at 3, 6, 12, 18, 24 and 72 hr post-injection. Hamsters were also administered 10, 30, 60, or 100 mg/kg to determine the effect of dose at 24 hr post-injection. Hemoglobin was isolated and decolorized. The levels of covalently bound [^{3}H] arylamine-hemoglobin adduct were determined by liquid scintillation counting and converted into ng 2-aminofluorene bound per mg hemoglobin by calculation.

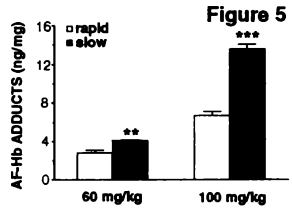
2-aminofluorene-hemoglobin (AF-Hb) adduct levels did not differ significantly between young (5-6 week) and old (32-49 week) hamsters (Figure 1), nor between male and female hamsters within either acetylator genotype (Figure 2). As shown in Figure 3, peak adduct levels were achieved at 12-18 hr and retained a plateau up to 72 hr post-injection in rapid and slow acetylator congenic hamsters. Adduct levels appeared to increase in a dose-dependent manner (Figure 4), and were consistently higher in slow versus rapid acetylator congenic hamsters in both studies of time course and dose effect (Figures 3 and 4). The magnitude of the difference was a function of dose; adduct levels were about 2-fold higher in slow acetylator congenic hamsters at 100 mg/kg ($p < 0.0001$) but 1.5-fold higher at 60 mg/kg ($p = 0.0013$, Figure 5).











The above results show a specific and significant role for *NAT2* acetylator genotype in formation of arylamine-hemoglobin adducts, which may reflect the relationship of acetylator genotype to the incidence of different cancers related to arylamine exposures. Partially supported by USPHS grant CA-34627.

EVIDENCE FOR CATASTROPHIC DEBRIS FLOWS IN PLEISTOCENE DEPOSITS, SOUTHEAST MANITOBA

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The lower-most unit exposed in the Gull Lake gravel pit, located about 85 kilometres northeast of Winnipeg, Manitoba, was examined to determine the process responsible for its deposition. The study also sought to discover the source material for the sediments. Very little work has been done on the surficial geology of this area. Hopefully, this is one small step towards developing a better understanding of southeast Manitoba's surficial deposits.

The unit was studied in the field to assess the clast sizes, matrix type, bedding characteristics, and any other items of perceived importance. Grain size was determined using the modified Udden-Wentworth scale (1). Samples were then gathered for laboratory analysis. In the lab, a gravel count was performed and the matrix was checked for carbonate content with hydrochloric acid. Shape was calculated for the gravel clasts using Zingg's method (2).

The most striking feature of the lower-most unit is that it is matrix-supported. The unit contains a wide range of clast sizes, from clay to boulders. One boulder measured in this unit was 2.3 by 2.4 by 0.8 metres, meaning it has a mass of over 11,700 kg. Boulders of this size are found throughout the unit, "floating" within it. Rough bedding is exhibited as zones of concentration of gravel or fine material. The clasts are well rounded and do not show any preferred orientation. The unit is poorly sorted and poorly consolidated. Lab results show that the clasts are predominantly oblate shaped. The unit contains 72% crystalline and 28% carbonate clasts, and the matrix effervesces violently when tested with hydrochloric acid.

Two glacial drifts are present in this area (3), and represent probable sources for the sediments in the lower-most unit. The older one is known as the Belair Drift. It is composed of crystalline rock fragments transported from the Canadian Shield. The younger drift, known as the Libau Drift, is composed mainly of carbonate fragments transported to this area from the northwest. When the ice that deposited the Libau Drift advanced into the area, it over-rode a portion of the Belair Drift and incorporated it. Therefore, the Libau Drift contains an increasing amount of crystalline fragments as it approaches the Belair contact.

This study concluded that: 1) the matrix-supported unit represents a debris flow deposit, 2) the source material for this deposit is the Libau Drift, and 3) the sediment's source was close to the Belair-Libau contact. This deposit is believed to be related to glacial activity in the area within the last 11,500 years BP.

1. Prothero, D.R., (1990) Interpreting the Stratigraphic Record, W.H. Freeman and Co, New York, p 26.

2. Blatt, H., Middleton, G., and Murray, R., (1980) Origin of Sedimentary Rocks, Prentice Hall Inc, Englewood Cliffs, NJ, p 80.

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EARTH SCIENCES

Thursday, 29 April

1:00 pm Provisional Palynological Recognition of the Fern Spike at the Cretaceous Tertiary Boundary, Makoshika State park, Dawson County, Montana
Timothy J Kroeger*, UND, Joseph H Hartman and Wesley D Peck, EERC, Grand Forks 58202

1:20 pm Paleocene Stratigraphy of the Nesson Anticline: Placement of the Bullion Creek-Sentinel Butte Formational Contact, Williams and McKenzie Counties, North Dakota
Joseph H Hartman, Wesley D Peck*, EERC, Grand Forks 58202, and Allen J Kihm, MinSU, Minot 58701

1:40 pm A Diverse Assemblage of Paleocene Nonmarine Mollusks and Mammals from the Sentinel Bute Formation of North Dakota
Joseph H Hartman*, EERC, Grand Forks 58202, Barry Roth, Museum of Paleontology, University of California, Berkeley, 94720 and Allen J Kihm, MinSU, Minot 58701

2:00 pm Evolution of Drainage Networks: Western United States
Eric Clausen*, MinSU, Minot 58701

SOCIAL SCIENCES

Thursday, 29 April

3:00 pm The Influence of Spatial Structure of Geographic Divisions on Migration Rates
Mohammad Hemmasi*, UND, Grand Forks 58202

3:20 pm Spatial and Temporal Consistency in the Determinants of North Dakota's In-migration Rates
Mohammad Hemmasi and Devon Hansen*, UND, Grand Forks 58202

PROVISIONAL PALYNOLOGICAL RECOGNITION OF THE FERN SPIKE AT THE CRETACEOUS-TERTIARY BOUNDARY, MAKOSHIKA STATE PARK, DAWSON COUNTY, MONTANA

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The palynological Cretaceous-Tertiary (K/T) boundary has been located at two sites in Makoshika State Park near Glendive, Montana. At both sites, the stratigraphic level of the boundary approximates the contact between the Hell Creek and Tullock Formations.

Several palynomorph taxa, diagnostic of the Upper Cretaceous, undergo extinction at or below the K/T boundary. None of these taxa are common in the uppermost Cretaceous strata, as their sum does not exceed 3 ± 1% of the identified palynomorph total for samples lying near the K/T boundary. Diagnostic angiosperm taxa include several species of *Aquilapollenites* (*sensu* Tschudy and Leopold [1]), including *A. quadricretaeus*, *A. amplus*, *A. delicatus* var. *collaris*, *A. reductus*, and *A.* n. sp. (2). Other angiosperm taxa suffering extinction include *Liliacidites complexus*, *Tricolpites microreticulatus*, *Proteacidites* spp., *Cranwellia rumseyensis*, *Sindorapollis pilatus*, and *Libopollis jarzenii*. The fungal thallus *Trichopeltinites* sp. and the spores *Foraminisporis undulosus* and *?Concavissimisporites* cf. *?C. variverrucatus* survive the Cretaceous, but undergo severe reduction or extinction within basal Tertiary strata.

One of the sites (M4770) was sampled across the boundary in 5-cm sampling intervals. The basal Tertiary sample from this site bears an anomalous abundance of fern spores, totaling 94 ± 2% of the palynomorphs (excluding megaspores and fungal and algal taxa) (Figure 1). The fern spore assemblage has relatively low diversity and is dominated by *Laevigatosporites* spp. (52 ± 3%) and *Reticuloidosporites dentatus* (29 ± 3%). At least seven other fern spore species are present in addition to algal and fungal spores and fragments of *Azolla* spp. megaspores. All of the fern spore taxa are also present in samples from the underlying Cretaceous rocks. The sum of angiosperm and gymnosperm pollen is 6 ± 2% of the identified grains, an unusually low incidence. The fern spore spike is not coincident with a lithologic change as the two uppermost Cretaceous samples and the fern spike sample occur within a dark brown mudstone containing no obvious lithologic breaks (Figure 1, Unit 3). The "boundary claystone" (3) is apparently not present in the section. An abundance of algal palynomorphs and *Azolla* spp. suggest an aquatic setting for deposition of the mudstone.

In the three Tertiary samples collected within 15 cm above the fern spore spike, palynomorph assemblages begin to regain diversity, although relatively few taxa tend to dominate the assemblages. Dominant taxa in these assemblages include spores of the Sphagnaceae (*Stereisporites* spp.), pollen of the Taxodiaceae-Cupressaceae-Taxaceae complex, and the angiosperm pollen *?Rutihesperipites* sp., *Rhoipites* cf. *R. globosus*, and *Retitricolpites crassus*. Change to these palynomorph assemblages is coincident with a lithologic change from mudstone to coal (Figure 1, Unit 4), and may in part represent an ecologic change to a swamp-forest plant community. Although detailed systematic studies of the palynomorphs have not been completed, no taxa have yet been discovered that were introduced in basal Tertiary strata.

Similar fern spore spikes immediately above the K/T boundary have been reported from nonmarine rocks in the Western Interior (4). The boundary fern spike described here is considered provisional in that these sediment samples have yet to be analyzed for iridium. The iridium anomaly has been used elsewhere to confirm continuous deposition across the K/T boundary. The sharp increase in fern spores has been attributed to the rapid recolonization of the region by ferns following a severe ecological disruption caused by a bolide impact (3). The Makoshika occurrence in easternmost Montana is well within the known distribution of fern spore anomalies delineated by Nichols and Fleming (4), who speculated that the known localities could be the result of a bolide impact near Manson, Iowa.

This research is part of molluscan and vertebrate studies supported by the National Science Foundation (JHH), U.S. Department of Energy (JHH), U.S. Bureau of Mines (JHH), and the Beta Zeta Chapter of Sigma Gamma Epsilon (TJK). These studies are in collaboration with David W. Krause of the State University of New York at Stony Brook and the cooperation of the Montana Department of Fish, Wildlife & Parks.

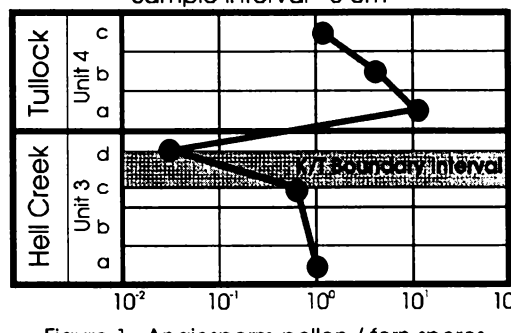

Figure 1. Angiosperm pollen / fern spores

1. Tschudy, B.D. and Leopold, E.B. (1971) Geol Soc Am Spec Paper 127, 113-167. 2. Nichols, D.J. (1993) written comm. 3. Tschudy, R.H., Pillmore, C.L., Orth, C.J., Gilmore, J.S. and Knight, J.D (1984) Science 225, 1030-1032. 4. Nichols, D.J. and Fleming, R.F. (1990) Geol Soc Am Spec Paper 247, 245-455.

PALEOCENE STRATIGRAPHY OF THE NESSON ANTICLINE: PLACEMENT OF THE BULLION CREEK-SENTINEL BUTTE FORMATIONAL CONTACT, WILLIAMS AND McKENZIE COUNTIES, NORTH DAKOTA

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Since the Fort Union Group was designated by Meek and Hayden (1), which strata should be included within the group, how it can be subdivided, and what the appropriate nomenclature for these designations should be have remained controversial (2-5). The lithostratigraphy of the outcrops of the Fort Union Group along the bluffs of the Missouri River are of particular concern from a paleontological perspective, because they contain the type localities of a relatively large number of nonmarine mollusks described by Meek and Hayden (6, 7). The placement of the 195 known molluscan localities within a well-founded stratigraphic context is critical to a meaningful biostratigraphy and temporal interpretation of the late Paleocene in North Dakota (8). This communication presents recent stratigraphic and paleontologic research bearing on the placement of this contact relative to named coal beds exposed about the Nesson Anticline in T. 153-154 N., R. 95 W., of southeastern Williams and northeastern McKenzie Counties. This work is part of ongoing stratigraphic and paleontologic studies concerning the greater Fort Union Historic Site area (9).

The placement of the contact between the Bullion Creek (or Tongue River of some workers) and Sentinel Butte Formations in the upper part of the Fort Union Group has been problematic in North Dakota outside of its best delimited area in portions of Billings, Golden Valley, and southern McKenzie Counties. Relevant Paleocene stratigraphic studies in the area of the Nesson Anticline include the naming of the feature and correlation of coal beds by Collier (10), coal studies and/or the placement of the contact (without descriptive explanation) by Nevin (11), Freers (12), Spencer (13), and Clayton (14), and descriptive consideration to the placement of the contact by Royse (3) and Carlson (5). Royse (3) documented the criteria needed to recognize the Bullion Creek-Sentinel Butte contact throughout the Little Missouri River drainage in western North Dakota. The results of his observations on the Missouri River formed, in part, the basis for Clayton's (14) interpretation of the contact as portrayed on the North Dakota State Geologic Map. The contact along the Missouri River was only generally defined by maps of small scale by the works of Royse and Clayton, but the levels appear to be defined at about the horizon of the Williston coal bed (Royse) or higher (Clayton) as recognized in this area. Royse (3) also noted that, on the basis of his criteria, the contact chosen by Nevin (11, horizon L) was "at least 200 ft [61 m]" too high in the section about the Keene Dome, an observation with which the authors concur. The placement of the contact by Freers (12) is consistent with that of the Williston coal and associated clinker on the north side of the Missouri River. Spencer (13) differed from other studies by placing the contact at the top of the Pittsley coal bed, about 61 m (200 ft) below the Williston bed.

Recent stratigraphic studies by the authors (unpublished observations of 1987, 1990-1992) in the area of the Nesson Anticline suggest that the contact may be most consistently placed at about the horizon of an unnamed lignite 11 to 13 m (35 to 44 ft) below the Pittsley coal bed. The unnamed bed is part of a sequence of three coals that may represent the uppermost part of the Bullion Creek Formation in this general area. This decision is based on the interpretation of geologic sections measured through this portion of the Fort Union Group on both the north and south sides of the Missouri River, observations in the Fort Union area, and subsurface correlations. The occurrence of Sentinel Butte-like lithologies and colors interpreted by others (e.g., Carlson) to be within the Bullion Creek, are, by our definition, believed to be part of the Sentinel Butte Formation. The proposed placement of the contact, along with the collection of mollusks from a number of localities in this area, suggest that a few important Meek and Hayden molluscan type localities, previously of dubious stratigraphic position, may occur in the Sentinel Butte Formation rather than the Bullion Creek Formation as generally thought (6). The stratigraphic range of other taxa may be modified by these studies and enhance the resolution of nonmarine molluscan biostratigraphy in the greater type area of the Fort Union Group. This research has been supported by the National Science Foundation, the U.S. Department of Energy, and the U.S. Bureau of Mines.

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A DIVERSE ASSEMBLAGE OF PALEOCENE NONMARINE MOLLUSKS AND MAMMALS FROM THE SENTINEL BUTTE FORMATION OF NORTH DAKOTA

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Diverse nonmarine mollusk assemblages are uncommon in strata of Paleocene age. A local fauna with more than eight taxa (the average is about four) is usually due to the admixture of freshwater and terrestrial taxa. This communication is the first report of an unusually diverse assemblage of at least 26 taxa of both freshwater and terrestrial mollusks from a Paleocene locality in North Dakota. This assemblage, known as the Riverdale Locality, is in the Sentinel Butte Formation and is middle late Paleocene age. Land mammal age abbreviations used herein are La = Lancian (latest Cretaceous), To and Ti = Torrejonian and Tiffanian (middle and late Paleocene), and Wa = Wasatchian (Early Eocene).

The Riverdale Locality (L1) was discovered on the shores of Lake Sakakawea near Riverdale, McLean County, in the early 1960s by the Vinje family of Hazen (1). Although known primarily for its mammalian fossils (2), a few interesting terrestrial mollusks were part of the original collections (3). The Riverdale Locality of the Vinjes was destroyed by the then rising waters of the reservoir, but lowering lake levels of the late '80s exhumed a comparable lithology in the same (quite specific) location. From available data (2, 4), the Riverdale sites discovered over the last few years (e.g., L5507 and L6200) are stratigraphically, at the most, from 5.8 to 7.9 m above the Vinje's Riverdale Locality, respectively.

The Riverdale molluscan assemblage consists notably of a diverse assemblage of snails (see table; * = land snails). Many, if not most, of the taxa, are undescribed. The named taxa, *Viviparus leai*, *V. retusus*, *Grangerella mcleodensis* (see 5), and New Genus T *planoconvexa* indicate a late Paleocene age. In addition, the terrestrial assemblage is remarkable in that it shares no taxa in common with the Ti3-age Judson local fauna (L6-L8, L25) from near the Slope-Bullion Creek formational contact in Morton County. The record of *G. mcleodensis*, known previously from Paleocene formations of Alberta, is its first report in the United States. The record of New Genus T *planoconvexa* is its first report in North Dakota since it was first described in 1857 from the Sentinel Butte Formation near Fort Berthold on the Missouri River (L4279) (6). The mollusks indicate a moist tropical or subtropical forest near a shallow lacustrine environment associated with a major fluvial system.

The Riverdale Locality (L1) was previously reported as belonging to mammalian biochron Ti4? (early late Tiffanian) (1). A reassessment of the mammalian local fauna identifies the following taxa: Order (O.) Multituberculata — *Ptilodus kummae* (Ti3-Ti4), O. Proteutheria — *Propalaeosinopa* sp. (To3-Ti5), O. Condylarthra — *Phenacodus magnus* (Ti4-Ti5), an indeterminate arctocyonid (La-Wa), and O. Pantodonta — *Titanoides primaevus* (?Ti3-Ti5?). Although a Ti4 biochron is indicated by this fauna, the identification of *P. magnus* at certain localities does not rule out the possibility of a Ti3 age for the Riverdale Locality. Note that this small local fauna does not share any species in common with the Ti3-age Judson and Brisbane (L5385, Slope Formation in Grant County) Localities. This research has been supported by the National Science Foundation, the U.S. Department of Energy, and the U.S. Bureau of Mines.

RIVERDALE MOLLUSKS

- Class Bivalvia
  - Subclass Heteroconchia
  - Order Unionoida
  - Family Unionidae
    - Gen. & sp. undet.
  - Order Veneroida
  - Family Pisidiidae
    - Gen. & sp. undet.
- Class Gastropoda
  - Subclass Prosobranchia
  - Order Diotocardia
  - Family Grangerellidae
    - *Grangerella mcleodensis**
  - Order Mesogastropoda
  - Family Viviparidae
    - *Viviparus leai*
    - *Viviparus retusus*
  - Family Hydrobiidae
    - *Hydrobia* spp.
  - Family Pleuroceridae
    - *Lioplacodes nebrascensis*
    - *Lioplacodes* sp. B
  - Order & Family *incertae sedis*
    - New Gen. A *limneaformis*
  - Subclass Pulmonata
  - Order Archaeopulmonata
  - Family Ellobiidae
    - *Pleurolimnaea tenuicosta*
  - Order Basommatophora
  - Family Acroloxidae
    - Gen. & sp. undet.
  - Family Physidae
    - *Physa* cf. *P. canadensis*
  - Family Planorbidae
    - Gen. & sp. undet.
  - Order Stylommatophora
  - Family undet.
    - New Gen. T *planoconvexa**
  - Family Discidae
    - *Discus* cf. *D. sandersoni**
    - *Discus* cf. *D. marmorensis**
  - Family Oreohelicidae
    - *Radiocentrum* sp. A*
    - *Gen. undet.* sp. A*
    - *Gen. undet.* sp. B*
  - Family Zonitidae
    - cf. *Mesomphix* sp. A*
    - cf. *Vitrea sp. B**
  - Stylommatophora *incertae sedis*
    - "Big tree snail" (*?Glypterpes*)*
    - "Carinate helicoid"*
    - "Conic helicoid"*
    - "Bulbous helicoid"*
    - "The Ribmeister"*

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EVOLUTION OF DRAINAGE NETWORKS: WESTERN UNITED STATES

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Introduction: *Quaternary Nonglacial Geology: Conterminous U. S.*, volume K-2 of the Geological Society of America's Decade of North American Geology Series (1), outlines commonly accepted interpretations of drainage evolution in the western United States. Numerous age dates are cited to support the hypothesis that drainage routes south and west of the Missouri evolved prior to Laurentide glaciation. While summarizing work of hundreds of investigators, this hypothesis is flawed because: 1.) it does not provide routes for Laurentide meltwater (i.e. landscapes and sediments interpreted as "old" block all possible meltwater routes), 2.) supporting age dates fail to describe sequences of stream captures which can be determined from topographic map evidence, and 3.) the hypothesis fails to explain numerous landforms including escarpments, barbed tributaries, asymmetric drainage divides, unusual bends in river valleys, major canyons, and sedimentary deposits. Most inexplicable evidence is ignored, however paradoxes and unresolved problems discussed in the volume include: 1.) no adequate explanation for the Nebraska Sandhills region (pp. 467-8), 2.) no explanation for canyons cut across the Laramie Range or for drainage patterns on the floor of the Laramie Basin (p. 426), 3.) circumstances of the capture of the Snake River by the Columbia (p. 253), 4.) the location of the Colorado River prior to erosion of the Grand Canyon (p. 377), and 5.) controversies concerning events in the Bighorn Basin (p. 413) and in the Lake Bonneville basin (p. 301).

Constructing an Alternate Hypothesis: An alternate hypothesis can be constructed by treating major escarpments (the Russian Spring Escarpment in North Dakota, the Pine Ridge/Hat Creek Breaks and Goshen Hole Escarpments in South Dakota, Nebraska, and Wyoming, the Bates Hole and Beaver Rim Escarpments in Wyoming, and the Red Hills Escarpment in Kansas) as headcuts formed by immense rivers of Laurentide meltwater. This hypothesis permits reconstruction of Laurentide meltwater drainage routes and provides a mechanism which can be used to explain most, if not all, drainage networks and related landforms in the western United States.

Laurentide Meltwater Routes: The initial, identifiable, Laurentide meltwater drainage route was south from central Montana, through the Bighorn Basin, to the Great Divide Basin (Wyoming) where major meltwater routes diverged: 1.) west into the Great Basin and to the Pacific; 2.) south across the Colorado Plateau; and 3.) southeast across the Laramie Basin and Range to the Gulf of Mexico. At one time or another immense volumes of meltwater flowed along each route. Water flowing west filled tectonic basins in Utah, Nevada, and elsewhere, and overflowed to the Pacific and may have been responsible for drainage networks and related landforms in northern California, Oregon, and southern Idaho and Washington. Prior to erosion of the Grand Canyon, meltwater flowing south into the Colorado Plateau region flowed east along the route of the Little Colorado River to emerge on the Great Plains, just south of the Sangre de Cristo Mountains, where it may have been responsible for formation of the Canadian, Red (of the South), Colorado (of Texas), Pecos, and Rio Grande drainage basins, among others. Meltwater, flowing southeast from the Laramie Range, moved southeast to join water from the Colorado Plateau route and may have contributed to formation of the Brazos, Trinity, and Sabine drainage basins. This flow was progressively diverted east and then northeast to form: 1.) the Red River (of the south) , 2.) the Arkansas River, 3.) the Kansas River and tributaries, 4.) the Platte and Niobrara Rivers, 5.) the White, Cheyenne, Moreau, Grand, Cannonball, Heart, and Knife River drainage basins in progressive sequence; 6.) the Little Missouri, 7.) the Powder River, 8.) the Bighorn River (formed when flow through the Bighorn Basin reversed), 9.) the Yellowstone River, and 10.) the Missouri River. Prior to formation of the Missouri Valley, immense volumes of flow crossed the Missouri Coteau to reach large headcut complexes which were developing in the decaying ice sheet (the Souris and James River lowlands parallel one of these headcut complexes). The Missouri River and continental divide in North Dakota formed when ice readvanced into the headcut complexes, blocked earlier flow routes, and diverted the main meltwater drainage route to the region just south and west of the Missouri Coteau.

Explanation For Meltwater Routes Described: Meltwater flow across Wyoming divides, 2000 meters higher than present drainage routes, and the progressive shift in meltwater routes can be explained by: 1.) the presence of Laurentide ice in regions previously thought to be unglaciated (ice, and evidence of that ice, was removed by deep erosion of the ice and underlying bedrock), 2.) deep erosion of the North American continent by Laurentide ice and meltwater, and 3.) crustal warping caused by rapid removal of large volumes of sediment from the Rocky Mountain and Colorado Plateau regions and its subsequent deposition in the Gulf of Mexico and elsewhere.

Testing of the Hypothesis and Conclusions: The new hypothesis has been tested by study of drainage divides, sequences of stream captures, and related features (as observed on detailed topographic maps) to determine consistency with meltwater flow routes and the progressive shift of meltwater flow routes as predicted by positions of major escarpments. Initial testing, using map data from portions of the states of Wyoming, Colorado, Kansas, Oklahoma, Nebraska, South Dakota, North Dakota, and Montana, suggests the new hypothesis can explain most topographic map evidence, including numerous drainage features which can not be explained by the earlier hypothesis. However, the new hypothesis conflicts with most interpretations of Cenozoic history of the western United States, as determined by previous investigators, and with numerous age dates which have been calculated using paleontologic and diverse other methods. How these conflicts can be resolved has not yet been determined.

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1. Morrison, R.B., editor, (1991) *Quaternary Nonglacial Geology: Conterminous U. S.*, Geological Society of America, Boulder, Colorado, 672 pp.

THE INFLUENCE OF SPATIAL STRUCTURE OF GEOGRAPHIC DIVISIONS ON MIGRATION RATES

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Population mobility is one of the major components of spatial interaction models in social science research. This mobility includes both residential mobility and migration. Residential change involves local moves over a short distance, often within the same community, i.e. from one house/apartment to another. Migration takes place over a longer distance, although this study only considers crossings of county or state boundaries. Conventionally, these changes are both defined in terms of moving from one discrete geographic unit to another. However, the national subdivisions and census geographic units of most countries are neither "natural" nor "economic," but a result of political decisions and administrative considerations. Furthermore, their populations are unevenly distributed, with a disproportionate concentration in major cities. This paper demonstrates the influence of spatial structure of migration-defining areas on migration rates. The geometric properties of a spatial unit which have particular relevance to the study of intercounty/interstate migration are size, shape, distribution of population, and boundary form. This is best shown by a simple theoretical example.

Figure 1 portrays the mobility status of eight persons moving out from a center covering the same distance and directions. Varied is the spatial structure of the units (e.g., b = 2a in size). All the movers from a would be recorded as migrants and none from b. Thus, the larger the migration-defining geographic unit, the smaller the out-migration rate would be. This is a well-documented concept in spatial interaction research based on the gravity model. Size of an area, along with other social, economic, and demographic variables, often is used as a predictor of migration in multivariate statistical analysis (1).

Yet, size should be considered in relation to shape. Here, rectangle c is the same size as circle b. In this case, six of the movers cross the boundary and qualify for migration status. Generally, the closer a given unit approaches a circle, the smaller the chance that a move of random direction, shorter than the maximum dimension of the area, would cross a boundary line. Thus, ceteris paribus, an "elongated" spatial unit is expected to have a higher migration rate than a "compact" unit. A simple method of measuring shape is by calculating Form Ratio, C (2).

$$\text{Form Ratio} = C1 = 4A / \pi L^2 \approx 1.27 (A / L^2)$$

where A = area of the migration-defining spatial unit, L = length of the line joining the area's two most distance points, and $\pi = 3.14$. The value of the Form Ratio varies between 1.0 for a circular shape to nearly 0 for a long narrow shape.

Distribution of population within the area also influences the migration rates. Comparing models b and d shows that concentration of population along a boundary results in greater intercounty migration than from a point in the center or from an even distribution. This concept can be quantified by measuring a coefficient of Eccentricity, E. Eccentricity of a point P, the locus of population in an irregular shape, may be summarized by the formula E = D / R. R is the radius of the circumcircle of the area, D is the distance between the center of the circle and point P. Values of E have no unit and vary between $0 \leq E \leq 1$ (provided the point is within or on the edge of the circle). A highly irregular boundary also influences the migration rate of an area. In general, the longer the boundary in relation to the size of an area, the greater the chance of a migrant crossing it. The effect of a boundary may be measured by dividing the length of the boundary by the area of the unit: C2 = B / A.

Census-based mobility data is being used for assessing housing demand, labor supply, and economic analysis, as well as urban/regional planning. Although the possible flaws in the area-based data have been discussed by a few scholars, in practice they have rarely been taken into account (3). But, an awareness of distortions introduced by the geometry of migration-defining units and ability to measure them reduces the risk of misrepresentation and erroneous comparisons. The advent of computers and advances in cartographic/GIS modeling provide unprecedented opportunities to detect and easily incorporate these spatial structural properties into research design. Combining the Census Bureau's versatile geographic database (TIGER files) and capabilities of a powerful GIS system significantly facilitate the process.

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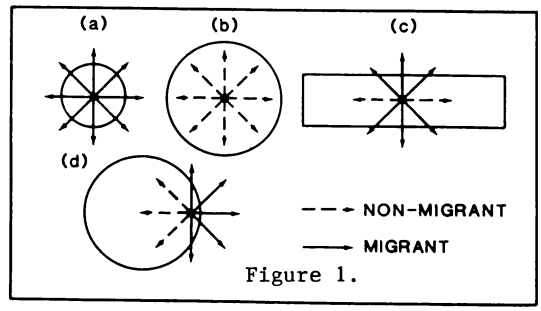

Figure 1. Relationship between an area's shape, size, and population distribution and out-migration.

SPATIAL AND TEMPORAL CONSISTENCY IN THE DETERMINANTS OF NORTH DAKOTA'S IN-MIGRATION RATES

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Migration is often a response to economic opportunities at the destination or disutility of the origin--and has a significant impact on the demographic, economic, and social structure of participating communities. North Dakota's general population redistribution trends include moves from rural and small towns to major urban centers and to a few counties with non-farm employment opportunities (i.e. Mercer). Since the 1940s, the state's economy has gone through a number of changes. Employment-generating activities, such as construction of Garrison Dam and establishment of the Grand Forks and Minot Air Force bases, have contributed to local population increases. In the 1970s, the impact of development in the energy related sectors stimulated population growth in the western counties. However, national and international economic conditions in the 1980s reduced the employment demand in these energy sectors, causing stagnation or even a declining trend in some. This paper investigates the determinants of county in-migration rates over the last three decades (Table 1).

The comparable dependent variables used in the multiple regression equations are the in-migration rates for 1965-70, 1975-80, and 1985-90 (INMIG). The three independent variables included are the previous decade's in-migration rates (PASTMIG), the percentage change in the number of civilian employed persons during the intercensal period (EMPLOY), and a dummy variable assigning 1 to the counties with a technical college or university and 0 to the others (COLLEGE). The latter variable captures the "pull" of amenities offered by a higher educational institution and the demographic force of the student residents.

Each model was quite successful in accounting for the overall variations in county in-migration rates, ranging between 70 to 77 percent (See R^2 in Table 1.) All of the regression coefficients have the expected signs and are significant, with the exception of the COLLEGE variable in the 1975-1980 equation not being significant. Between 1970 and 1980, the energy producing counties located in the western part of the state experienced dramatic increases in employment. The in-migration rate of these counties was significantly higher for the 1970s than for either 1960s or 1980s. Apparently, the combined influence of prior migration streams and a substantial rise in employment reduced the explanatory power of the college variable.

The relative contribution of the individual independent variables can be determined by examining the absolute value of the beta coefficients (Table 1). The rank order of all the variables is remarkably consistent throughout the decades--past migration trend emerging as the best predictor, followed by change in employment, and finally, presence or absence of a higher educational institution.

Findings of this study reiterate the need for policies and innovative strategies which create employment opportunities in the state. Some counties have shown a consistent population growth and need further strengthening of their employment base. Loss of population from small towns and some of the consolidated farms seems inevitable, and should not be considered a harmful process. Intra-state population redistributions are mainly responses to the changing agriculture economy and/or the perceived opportunities in other economic sectors and in the selected localities.

If the goal is to retain the state's population size, potential migrants must have alternative opportunities. In order to foster employment and population growth, this study indicates the need to stimulate the economic growth potential of selected localities through their existing resource bases. The emergence of educational institutions as an influential variable reinforces their vital position in the economy of the local communities and the surrounding areas. One policy implication of this finding is that they must be further developed by eliminating duplications and promoting areas of their academic strengths. Furthermore, utilizing existing infrastructures, cities containing educational institutions could also be used as sites for future economic development investments. As regional "growth poles," they are highly accessible and spread throughout the state.

TABLE 1. MULTIPLE REGRESSION MODEL EXPLAINING IN-MIGRATION RATES

Predictor Variable	Regression Coefficient (b)	Beta Coefficient	Probability (t-test)	Adjusted R^2
County in-migration rate model for 1965-1970: N = 53				
PASTMIG	0.605	0.521	0.0001	
EMPLOY	0.118	0.283	0.0030	0.766
COLLEGE	2.670	0.207	0.0231	
County in-migration rate model for 1975-1980: N = 53				
PASTMIG	0.601	0.517	0.0001	
EMPLOY	0.148	0.486	0.0001	0.698
COLLEGE	0.098	0.006	0.9527	
County in-migration rate model for 1985-1990: N = 53				
PASTMIG	0.316	0.407	0.0002	
EMPLOY	0.155	0.320	0.0015	0.714
COLLEGE	3.490	0.292	0.0023	

PROFESSIONAL COMMUNICATIONS

DIETARY ELEMENTS

Friday, 30 April

8:00 am Dietary Cystine Affects Signs of Arsenic Deprivation in Rats
Eric O Uthus*, USDA/ARS Human Nutrition Research Center, Grand Forks 58202

8:20 am Biochemical Responses to Dietary Boron over a 20-fold Physiological Range in Male Rats
Gayle H Aasen* and Curtiss D Hunt, USDA/ARS Human Nutrition Research Center, Grand Forks 58202

8:40 am Effects of Dietary Boron Depletion on Boron and Molybdenum Balance and Blood Mineral Concentrations in Postmenopausal Women
Curtis D Hunt* and Jo Layne Herbel, USDA/ARS Human Nutrition Research Center, Grand Forks 58202

9:00 am The Effects of Dietary Boron on Food Consumption and Weight of Male Long-Evans Rats
Gloria J Krank*, James G Penland and Tonya C Murphy, USDA/ARS Human Nutrition Research Center, Grand Forks 58202

9:20 am Platelet Nucleotide Concentrations as Measured by Reverse-Phase Ion-Paired Liquid Chromatography are Affected by Copper Deficiency.
Steven N Dufault* and W Thomas Johnson, USDA/ARS Human Nutrition Research Center, Grand Forks 58202

9:40 am * * * Refreshment / DISCUSSION Break * * *

10:00 am Nickel Deprivation Affects the Response of Rats to Manganese Deprivation
Forrest H Nielsen*, Rhonda A Poellot and Eric O Uthus, USDA/ARS Human Nutrition Research Center, Grand Forks 58202

10:20 am Dietary Silicon and Germanium Affect the Iron Concentration and Density of Bone
Carol D Seaborn* and Forrest H Nielsen, USDA/ARS Human Nutrition Research Center, Grand Forks 58202

10:40 am Dietary Selenium Affects Locomotor Activity and Startle Responses in Rats
James G Penland* and Paula C Cultice, USDA/ARS Human Nutrition Research Center, Grand Forks 58202

11:00 am Use of Stable Isotopic ^{67}Zn and Radioactive ^{65}Zn to Simultaneously Determine the Absorption of Zn from Different Pools in the Prefused Rat Intestine.
Richard A VanderPool* and John Finley, USDA/ARS Human Nutrition Research Center, Grand Forks 58202

11:20 am Use of a Colon Carcinoma Cell Line to Study the Influence of Bile Pancreatic Fluid on Absorption of Zinc.
John Finley*, Mary Briske-Anderson and Philip Reeves, USDA/ARS Human Nutrition Research Center, Grand Forks 58202

DIETARY CYSTINE AFFECTS SIGNS OF ARSENIC DEPRIVATION IN RATS

Eric O. Uthus*
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Recent studies indicate that arsenic has a physiologic role affecting methionine metabolism (1, 2). Methionine metabolism can be categorized into two main areas, methionine cycling and transsulfuration. Methionine cycling involves the donation of a methyl group via S-adenosylmethionine, and the reactions that regenerate methionine by methylation of homocysteine through utilizing the methyl sources choline and methyltetrahydrofolate. Transsulfuration involves the irreversible catabolism of methionine through homocysteine and cystathionine ß-synthase. Transsulfuration results in the formation of the methionine metabolites cysteine and taurine. The general site (recycling or transsulfuration) at which arsenic physiologically affects methionine metabolism is unknown. The purpose of this experiment was to determine if alteration of the transsulfuration pathway, through manipulation of dietary cystine, could affect the response to arsenic deprivation.

In a 2 x 2 factorially arranged experiment, male weanling Sprague-Dawley rats were assigned to groups of six and fed a 14% amino acid, 76% ground-corn based diet. Dietary supplements were arsenic as As_2O_3, 0 or 1 μg/g, and L-cystine, 0 or 3 g/kg. Rats had free access to food and water. After 8 weeks the rats were fasted for 16 hours, weighed and decapitated subsequent to ether anesthesia and cardiac exsanguination with a heparin-coated needle and syringe.

Table 1. Effects of Dietary Arsenic, Cystine and Their Interaction on Body Weight, Liver Glutathione, Blood Arsenic, Specific Activity of Liver Malic Enzyme, Plasma Copper and Molybdenum, and Femur Calcium

Treatment[a]		Body	Liver	Blood	Malic	Plasma		Femur
Arsenic	Cystine	Weight	Glutathione	As	Enzyme	Cu	Mo	Ca
μg/g	g/kg	g	μmol/g	μg/ml	units[b]	μg/ml	μg/ml	mg/g
0	0	215	4.50	0.08	0.110	0.77	0.029	220
1	0	226	3.81	14.0	0.069	0.67	0.017	212
0	3	235	5.34	0.10	0.125	0.77	0.035	227
1	3	236	5.87	16.1	0.121	0.79	0.019	216
			Analysis of Variance - P Values					
Arsenic		NS	NS	0.0001	0.001	NS	0.005	0.04
Cystine		0.02	0.01	0.001	0.0001	0.05	NS	NS
Arsenic x Cystine		NS	NS	0.001	0.004	0.03	NS	NS
Error Mean Square		191.1	1.426	0.403	0.0002	0.004	0.0001	107.1

[a] Amounts of arsenic and cystine supplemented to a basal diet containing approximately 10 ng As/g (analyzed), 0.14% cystine and 0.54% methionine (calculated).
[b] μmol NADPH formed/min/mg total protein.

Cystine deprivation decreased body weight and liver glutathione (GSH and GSSG) concentration. The concentration of arsenic in whole blood, the specific activity of liver malic enzyme (EC 1.1.1.40) and the concentration of copper in plasma were all affected by an interaction between arsenic and cystine. Generally, in the arsenic-supplemented rats, these variables were markedly decreased by cystine deprivation; there were only slight or no effects of dietary cystine in the arsenic-deprived groups. Plasma molybdenum and femur calcium were increased by arsenic deprivation. Glutathione, a cysteine-containing tripeptide, is thought to be a reservoir of cyst(e)ine (3). Malic enzyme can supply NADPH, a coenzyme needed for the formation of reduced glutathione from oxidized glutathione.

Signs of arsenic deprivation include depressed specific activities of S-adenosylmethionine decarboxylase, ornithine decarboxylase, and cystathionase (4). Also, the concentration of taurine in rat and hamster plasma was decreased by arsenic deprivation (4). In another study that used a casein rather than an amino-acid based diet, kidney calcium was elevated in female rats; supplementation of arsenic, however, tended to moderate this elevation (5). Rats, especially females, are prone to nephrocalcinosis when fed semi-purified diets containing concentrations of calcium, phosphorus, and magnesium similar to the present study. Addition of L-cystine to the diet can alleviate accumulation of calcium in kidney (P.G. Reeves, personal communication). These effects of arsenic and cystine on kidney calcium may perhaps occur because female rats metabolize methionine differently than males.

The past and present findings indicate that there is an interaction between dietary cystine and arsenic. Arsenic has been suggested to have a physiologic role in methionine metabolism. It is still unclear whether arsenic acts physiologically in methionine recycling or in transsulfuration. It is known, however, that arsenic deprivation signs can be altered either by methionine or cystine deprivation.

1. Uthus, E.O. and Poellot, R. (1990) Proc ND Acad Sci 44, 90.
2. Uthus, E.O. and Poellot, R. (1992) J Trace Elem Exper Med 5, 153-164.
3. Tateishi, N. et al. (1977) J Nutr 107, 51-60.
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BIOCHEMICAL RESPONSES TO DIETARY BORON OVER A 20-FOLD PHYSIOLOGICAL RANGE IN MALE RATS

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Previous research indicated that certain indices of normal physiology respond to boron supplementation in vitamin D-deprived rats. For example, dietary boron supplementation decreased plasma aspartate transaminase activity (AST), increase triglyceride concentrations and had no effect on plasma pH or body weight (1). Thus, an experiment was designed to test the hypothesis that general indicators of physiological status respond over a range of physiological concentrations of dietary boron.

Weanling male Sprague-Dawley rats (9 per group) were fed a ground corn-casein-corn oil basal diet which contained adequate amounts of vitamin D (1000 IU/kg) and 0.08, 0.22, 0.39, 0.78, 1.18, 1.93 and 3.60 mg boron (as orthoboric acid)/kg. After 58 days, the animals were fasted for 16 hours, weighed and sedated with IM Ketamin-Rompun anesthesia prior to cardiac exsanguination and decapitation. Biochemical measures were determined by established methods (1). Selected findings are shown in Table 1.

Table 1. Effects of Dietary Boron on Selected Physiological Indices

Treatment Boron mg/kg	Body Weight g	Serum pH	Serum Triglycerides mg/dL	Serum Aspartate transaminase U	Serum Alanine transaminase U
0.08	288	7.46	45.0	113	37.0
0.22	278	7.47	40.4	103	30.9
0.39	306	7.47	31.1	126	34.7
0.78	296	7.47	49.1	95	29.6
1.18	276	7.50	45.1	96	29.0
1.93	294	7.48	65.1	107	34.8
3.60	284	7.43	41.9	102	39.8
		Analysis of variance - P values			
Boron	NS	0.04	0.01	0.05	0.03
(Mean Square Error)$^{1/2}$	28	0.04	17.1	20.3	7.1

The lowest and highest amount of dietary boron supplementation were comparable to the amounts of boron found in human diets low or high in fruit and vegetable content, respectively. Intermediate boron supplementation depressed serum alanine transaminase activity. In a previous study with vitamin D-deprived rats (1), dietary boron (0.06 versus 2.46 mg B/kg) increased plasma triglycerides (45 versus 64 mg/dL), an effect remarkably similar to that found in serum at comparable boron intakes (0.08 and 1.93 mg/kg) in the present study. The same concentrations of dietary boron did not affect mixed venous plasma or serum pH in either study. However, increments in boron intake increased, then decreased, serum pH in the present study, a phenomenon that may not be apparent with fewer dietary boron treatments. These findings suggest that physiological amounts of dietary boron affect lipid metabolism and may be important in the control of serum H+ balance.

1. Hunt, C.D. and Herbel, J.L. (in press) Mg Tr Elem.

EFFECTS OF DIETARY BORON DEPLETION ON BORON AND MOLYBDENUM BALANCE AND BLOOD MINERAL CONCENTRATIONS IN POSTMENOPAUSAL WOMEN

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In vitamin D_3-deficient chicks, increasing dietary boron increased plasma boron concentrations. An interaction between dietary boron and molybdenum modulated plasma molybdenum concentrations. Finally, an interaction between dietary magnesium and molybdenum affected tibial marrow sprout width (1). These findings suggest that interactions among boron, molybdenum and magnesium are important in mineral metabolism. Accordingly, mineral metabolism data collected from a human boron metabolic study were examined. As described in detail elsewhere (2), 13 postmenopausal Caucasian women aged 48 to 82 years were housed in a metabolic unit and fed a conventional diet that supplied about 0.239 mg boron, 105 mg magnesium, 0.231 mg molybdenum and <0.10 mg aluminum/2000 kcal. All women participated in the first four dietary periods of 24 days each: 1) basal diet only, 2) +1000 mg aluminum/day, 3) +200 mg (adequate) magnesium/day, 4) +1000 mg aluminum + 200 mg (adequate) magnesium/day. After this experimental phase, 12 women participated in two dietary periods (3 mg supplemental boron/day). Six women were fed: 1) the boron basal diet only and 2) +1000 mg aluminum/day. The other six women were fed: 1) +200 mg magnesium and 2) +200 magnesium + 1000 mg aluminum. Two of the women were on estrogen therapy throughout the study. Selected findings are shown in Table 1.

Table 1. Effect of Boron, Aluminum and Their Interaction on Plasma, Red Blood Cell Mineral Concentrations and Boron and Molybdenum Balance

Dietary treatment mg/day		Plasma μg/ml		Red blood cell μg/g			Balance mg/6 d	
B	Al	B	Mo	B	Mo	Fe	B	Mo
				Postmenopausal women fed a Mg-low diet				
0.24	0	0.051	0.059	0.236	0.258	2640	−0.03	0.74
0.24	1000	0.048	0.057	0.215	0.202	2740	−0.25	−1.98
3.23	0	0.109	0.179	0.260	0.233	2770	2.21	0.41
3.23	1000	0.068	0.050	0.313	0.263	3360	1.86	−1.52
				Analysis of variance - P values				
Boron		0.002	NS	NS	NS	NS	0.0003	NS
Aluminum		NS	NS	NS	NS	NS	NS	0.002
B x Al		NS	NS	NS	NS	NS	NS	NS
Root MSE		0.026	0.191	0.096	0.169	687	1.24	1.61
				Postmenopausal women fed a Mg-adequate diet				
0.24	0	0.077	0.033	0.253	0.238	2720	−0.51	1.01
0.24	1000	0.071	0.035	0.301	0.340	2690	−0.50	−0.55
3.23	0	0.107	0.027	0.413	0.537	2830	0.99	1.15
3.23	1000	0.091	0.023	0.273	0.358	2860	1.27	0.07
				Analysis of variance - P values				
Boron		0.03	0.04	NS	NS	0.02	0.06	NS
Aluminum		NS	NS	NS	NS	NS	NS	0.02
B x Al		NS	NS	NS	NS	NS	NS	NS
Root MSE		0.023	0.007	0.197	0.318	111	1.76	0.97

Supplemental boron did not increase boron retention in the red blood cell (RBC). Thus, an appropriate indicator of dietary boron intake may be plasma boron concentrations. Supplemental boron increased RBC iron concentrations during adequate magnesium nutriture. The boron balance data suggest that adequate magnesium intake reduces boron retention. Independent of boron intake, high dietary aluminum reduced body stores of molybdenum. Thus, it is possible that molybdenum status should be monitored in individuals consuming high amounts of aluminum.

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THE EFFECTS OF DIETARY BORON ON FOOD CONSUMPTION AND WEIGHT OF MALE LONG-EVANS RATS

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Previous research suggests that boron (B) is an essential nutrient for developing animals and, in the presence of other nutritional stressors (e.g., magnesium deficiency), that dietary B is related to several growth indices including body weight (1). Because absolute B intake is partly a function of total food consumed and because consumption may be influenced by the concentration of B in the diet, the effect of dietary B on food consumption was examined in five studies of male Long-Evans rats (see Table 1 for details of each study). A corn (acid-washed), casein and corn oil based diet containing <0.25 μg B/g diet was used in all studies and supplemented with orthoboric acid to achieve higher B concentrations. The diet and demineralized water were available ad libitum from plastic food cups and water bottles. Animals were housed in stainless steel cages under a reversed light-dark cycle. Within each study, initial (arrival) weights of animals assigned to different dietary treatments were equivalent. Daily throughout each study, between 8 and 9 a.m., food remaining and spilled were subtracted from food given the previous day. Body weight was determined weekly.

Table 1. Overview of Boron Studies Assessing Food Consumption and Body Weight

Study	N/Age (d)	Boron (μg/g)	Other (μg/g)	Weeks[a]	Assessments[b]	Procedures[c]
1	96/100	0,3,15	1,7.5 Cu	9	E,A	H,B,S
2	72/70	0,3,15	5,50 Fe	9	E,A	H,B,T,S
3	72/23	0,3,12,42,144,500	-	6	O	H,B,S
4	60/100	0,3,15	-	10	S,H	H,B
5	80/100	0,3	500,5000 Ca	8	E	H,B,S

[a] Weeks = number of weeks on experimental diet
[b] E=Electrocorticogram, A=Activity, C=Conditioning, S=Startle Response, H=Heat Algesiometry
[c] H=Handling, B=Habituation, S=Surgery, T=Tail blood draws

Average daily intake and body weight as a percentage of initial weight (i.e., weight gain) were calculated for each week. Analysis of variance found that the concentration of B in the diet significantly ($p<0.05$) affected food consumption in 4 of 5 studies (Table 2), but found no evidence of reliable effects on either body weight or weight gain in any study. In factorial studies involving other nutrients, there were no significant interactions among the three measures.

Table 2. Weeks Showing Effects ($p<0.05$) of Dietary Boron Concentration on Food Consumption

Study	$B^- > B^+$	$B^+ > B^-$	ND
1	1 - 5	NONE	6 - 8
2	NONE	2 - 5, 8	1, 6
3	NONE	NONE	1 - 5
4	NONE	1, 7	2 - 6, 8 - 9
5	5	NONE	1 - 4, 6 - 7

B^- = boron deficient diet (< 0.25 μg B/g)
B^+ = boron supplemented diet (≥ 3 μg B/g)
ND = weeks on experimental diet in which there were no significant effects

As shown in Table 2, the effect of dietary B concentration on consumption was consistent within but not across studies. Studies 1 and 2 showed effects for 5 of 9 weeks, the first found greater intake of the basal diet whereas the second found greater intake of the B-supplemented diets. A similar inconsistency was found between studies 4 and 5, where significant effects occurred less frequently. Study 4, which did not show an effect, was conducted on weanling animals, in contrast to the mature animals used in all other studies. Although the consistency of effects within studies argues against chance findings, the inconsistency across studies suggests the presence of intervening variables as yet unidentified. Diet freshness consistency, and looseness of packing in cups, animal handling, and other procedural details were examined but could not account for the differences between studies. Consequently, future research must be alert to the complexity of this relationship. Notwithstanding, the demonstration of an effect of dietary B concentration on food consumption, particularly in the absence of a parallel effect on body weight or gain, would provide important evidence that B can affect metabolic rate in animals.

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PLATELET NUCLEOTIDE CONCENTRATIONS AS MEASURED BY REVERSE-PHASE ION-PAIRED LIQUID CHROMATOGRAPHY ARE AFFECTED BY COPPER DEFICIENCY

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High-performance liquid chromatography (HPLC) is an accepted standard method for determining the nucleotide content of biological samples. Recently, improved C-18 columns have provided increased resolution of nucleotides under gradient, reverse-phase, ion-pair conditions. Using this system, with a step gradient, we investigated the influence of copper and iron deficiencies on the concentrations of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and guanosine triphosphate (GTP) in platelets obtained from copper- and iron-deficient rats.

Male, weanling Sprague-Dawley rats were divided into three groups and assigned diets containing either <1 ppm copper and 42 ppm iron (CuDFeA), 5 ppm Cu and 12 ppm Fe (CuAFeD) or 5 ppm Cu and 42 ppm Fe (CuAFeA). After 35 days, blood was withdrawn from these rats and platelet-rich plasma (PRP) was obtained by low-speed centrifugation (160 x g) for 20 minutes. Platelet pellets, obtained by centrifuging PRP, were extracted by using perchloric acid and potassium hydroxide. The extracts were stored at −20°C for no more than three days before HPLC analysis. The extracts were applied to a 7 μm Nucleotide/Nucleoside (C-18) HPLC column (250 mm x 2.6 mm i.d., Alltech, Deerfield, IL) and the nucleotides were eluted with a step gradient generated by using 60 mM ammonium dihydrogen phosphate, 5 mM tetrabutylammonium phosphate, pH 5.0 (buffer A) and 100% methanol containing 5 mM tetrabutylammonium phosphate (buffer B). The eluted nucleotides were detected by absorbance at 254 nM. The retention times for AMP, ADP, GTP and ATP were 17 min., 31 min., 35 min. and 53 min., respectively. Nucleotide peaks were identified by coelution of standards and quantified by integration of their areas.

Table 1. Effects of Dietary Copper and Iron Deficiencies on Platelet Nucleotide Content (pmole/10^6 platelets).

DIET	N	AMP	ADP	GTP	ATP
CuAFeA	6	$1.7^{ab}\pm1.3$	16.0±7.3	$5.3^{a}\pm1.2$	$34.2^{a}\pm7.3$
CuAFeD	5	$1.2^{a}\pm0.7$	14.3±3.3	$5.0^{a}\pm0.7$	$35.7^{a}\pm3.5$
CuDFeA	8	$2.8^{b}\pm1.1$	16.1±6.9	$3.0^{b}\pm0.7$	$26.4^{b}\pm4.6$

Values are means±SD. Means within a column not sharing a common superscript are significantly different ($p<0.05$, Tukey's contrasts).

As shown in Table 1, platelet AMP content tended to be elevated by copper deficiency, but it was not significantly higher than the AMP content of platelets from rats fed adequate copper and iron. Platelet ADP content was not affected by either copper or iron deficiency. However, compared to platelets from rats fed adequate copper and iron, platelet GTP and ATP were both significantly reduced by copper deficiency but not iron deficiency.

These results indicate that copper deficiency has a more pronounced effect on platelet nucleotide content than does iron deficiency. In response to physiologic agonists, platelets require ATP to secrete the contents of their secretory granules and to change shape. Copper deficiency may impair ATP dependent functions by reducing platelet ATP content. Reduction of GTP content also may have a negative influence on platelet function because GTP is critical for the transduction of extracellular signals into platelet responses. Thus, reduced ATP and GTP content may help explain why copper deficiency alters cytoskeletal remodeling, dense granule secretion and protein kinase c activation in thrombin-stimulated platelets (1, 2).

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NICKEL DEPRIVATION AFFECTS THE RESPONSE OF RATS TO MANGANESE DEPRIVATION

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There is circumstantial evidence that the biochemical functions of nickel (Ni) and manganese (Mn) are interrelated. For example, both Mn and Ni activate the calmodulin-stimulated phosphatase calcineurin (1) and liver arginase (2); both Ni and Mn deprivation affect pancreatic amylase (3, 4), but in an opposite manner, and dietary Ni affects the biliary excretion and tissue distribution of Mn (5). Thus, it was thought possible that depriving an animal of Mn would alter its response to Ni deprivation, which might give a clue to the unidentified function of Ni in higher animals. Therefore, male weanling Sprague-Dawley rats were assigned to groups of six in a two-way 2x2 factorially arranged experiment. Supplemented to the basal diet, based on acid-washed ground corn and skim milk (containing between 0.1 and 0.3 μg Mn and about 30 ng Ni per g), were Ni (as $NiCl_2$) at 0 and 1 μg/g, and Mn (as $Mn\text{-}SO_4$) at 0 and 20 μg/g. The rats were fed ad lib their respective diets for 11 weeks, fasted overnight, weighed and decapitated subsequent to ether anesthesia and cardiac exsanguination with a heparin-coated syringe and needle. Plasma cholesterol, vitamin B_{12}, glucose and glucagon, and red blood cell (RBC) folate were determined by using commercially available kits. Pancreatic amylase (4) and liver arginase (2) were determined by published methods.

Table 1. Effect of Ni, Mn and Their Interaction on Body Weight and Selected Biochemical Variables.

Treatment		Body	Plasma				RBC	Pancreatic	Liver Arginase	
Ni	Mn	wt	cholesterol	vit. B_{12}	glucose	glucagon	folate	amylase	Mn-act.	Ni-act.
μg/g	μg/g	g	mg/dL	pg/mL	mg/dL	pg/mL	ng/mL	U/mg protein	U/g protein	
0	0	288	48	535	114	92	1366	64	616	394
0	20	323	52	821	115	80	1399	48	807	428
1	0	304	45	128*	137	75	1077	92	755	504
1	20	316	57	614	124	95	1445	50	769	397
				Analysis of Variance - P Values						
Ni		0.75	0.85	0.0007	0.04	0.89	0.17	0.13	0.46	0.40
Mn		0.12	0.04	0.0001	0.38	0.53	0.03	0.007	0.14	0.43
Ni x Mn		0.41	0.32	0.20	0.36	0.02	0.06	0.19	0.20	0.14

*Does not include two animals in which vitamin B_{12} was too low to measure.

Table 1 shows that growth was not significantly affected by the dietary treatments. As expected, Mn deprivation depressed plasma cholesterol and elevated pancreatic amylase concentrations; in both cases, the Mn effect seemed to be less marked in the Ni-deprived rats. Manganese deprivation also depressed plasma vitamin B_{12} and RBC folate concentrations (Mn deprivation effects not previously described); both depressions seemed less pronounced in the Ni-deprived rats. Nickel deprivation depressed plasma glucose; Mn deprivation seemed to enhance the effect. Plasma insulin (data not shown) was not affected by dietary Mn and Ni; however, Ni deprivation elevated plasma glucagon in Mn-deprived but depressed glucagon in Mn-supplemented rats. No marked effects on Mn-stimulated or Ni-stimulated arginase occurred. The findings indicate that Ni and Mn functions are interrelated. Moreover, because of the marked effects on plasma vitamin B_{12} and RBC folate, the findings support the hypothesis that Ni affects methyl group metabolism involving these vitamins, and suggest that a Ni function utilizes some form of vitamin B_{12} or folate, which is not replenished when Mn is low.

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DIETARY SILICON AND GERMANIUM AFFECT THE IRON CONCENTRATION AND DENSITY OF BONE

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Germanium and silicon have similar outer shells and ionic radii. These properties explain why various studies have shown that an interaction between germanium and silicon affects several biochemical systems in lower forms of life. The purpose of this experiment was to determine if germanium would affect the utilization of silicon, a nutrient believed to be essential for rats, and also whether germanium would act like silicon by affecting bone composition.

Male weanling Sprague-Dawley rats were assigned to groups of six in a 2 x 2 factorially arranged experiment. Supplemented to a ground corn/casein basal diet, containing 5 g arginine and 1.2 mg Si/kg, were silicon as sodium meta-silicate at 0 or 50 μg/g, and germanium as sodium germanate at 0 or 5 μg/g. The rats were fed ad libitum their respective diets for nine weeks, fasted overnight, weighed and decapitated subsequent to ether anesthesia and cardiac exsanguination. The femur and skull were removed and frozen for analysis. After ashing, mineral concentrations were determined by inductively coupled argon plasma atomic emission spectrometry. Tibia density was determined by underwater weighing using Archimedes principle.

Table 1. Effects of Silicon and Germanium on Hematocrit (Hct), Hemoglobin (Hgb) and Bone Minerals and Tibia Density.

Treatment Si μg/g	Ge μg/g	Hct %	Hgb g/100 ml	Skull Ca mg/g	Skull Mg μg/g	Skull K μg/g	Skull Fe μg/g	Femur Fe μg/g	Tibia Bone Density g/cm³
0	0	46	14.4	185	3288	744	26	57	1.43
0	5	46	14.7	173	3134	751	24	54	1.47
50	0	45	14.1	194	3493	611	20	50	1.43
50	5	44	14.0	196	3494	651	21	60	1.44
				Analysis of Variance - P Values					
Silicon		.0006	.01	.04	.05	.01	.002	NS	NS
Germanium		NS	NS	NS	NS	NS	NS	NS	.03
Silicon x Germanium		NS	NS	NS	NS	NS	NS	.02	.06

There were no significant differences in body weight, organ weight/body weight ratios or femur ash weights (data not shown). Hematocrit and hemoglobin, and the iron and potassium concentrations of the skull were decreased, and concentrations of calcium and magnesium of the skull were increased when 50 μg Si/g diet were fed. Femur iron concentrations were affected by an interaction between silicon and germanium. When animals were fed 50 μg Si/g diet, the animals fed 5 μg Ge/g had greater iron concentrations in the femur than animals fed 0 Ge. Tibia bone density was increased in those animals fed 5 μg Ge/g diet.

Germanium did not seem to inhibit silicon utilization or to alter mineralization of skulls of rats fed 1.2 or 50 μg Si/g diet. As the decreased hematocrit, hemoglobin and iron concentration in bone are within the expected values, silicon probably does not directly affect iron metabolism. However, the small changes may reflect an effect of silicon on bone composition and maturation. Iron is essential for collagen formation; decreased collagen in bone has been found in silicon-deficient animals. The elevation in femur iron by 5 μg Ge/g diet in animals fed 50 μg Si/g diet suggests bone compositional changes associated with the organic matrix. The increased tibia bone density that occurred when Ge was added to the rat diet suggests that Ge may alter bone composition, especially when dietary silicon is low. The findings confirm that silicon deprivation affects bone composition, and that a germanium supplement of 5 μg/g diet did not enhance those effects; thus, this amount of germanium apparently does not increase the requirement for silicon. On the other hand, the bone iron and density findings suggest that silicon status affects the response to germanium supplementation, and that further studies of the effect of dietary germanium on bone composition are warranted.

DIETARY SELENIUM AFFECTS LOCOMOTOR ACTIVITY AND STARTLE RESPONSES IN RATS

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Selenium (Se) deficiency has been associated with muscular dystrophy and cardiac myopathy in livestock and humans (1,2). Biochemically, Se functions *in vivo* as an antioxidant, and recent research has found that Se concentration in the rat brain varies with the amount of Se in the diet (3). To determine whether Se may thus play a role in central neurologic and behavioral function, the present study assessed locomotor activity and startle responses in rats fed varying amounts of Se.

Forty-five male Sprague-Dawley rats, aged 100 days, were assigned randomly on the basis of arrival weight to a group receiving either 0.01 (low), 0.10 (adequate) or 1.00 (high) μg Se/g diet. The basal diet was based on Torula yeast, contained 0.01 μg Se/g, and adequate in Vitamin E; it was supplemented with sodium selenate to achieve higher Se concentrations. Animals were handled individually for approximately five minutes once each week throughout the study. Following seven weeks on the diet, 30 minutes of locomotor activity were assessed by a Digiscan Activity Monitor, which uses photo cells to detect movements of 1 inch or greater. The first 10 minutes were recorded under quiet conditions, the next 10 minutes under noisy conditions (broadband, 100 dBA), and the final 10 minutes under quiet conditions. During that same week, all animals were habituated for five minutes to the startle response recording chamber (no stimuli presented). One week later, startle responses to 60 pure tone stimuli (1000 Hz, 100 dBA, 20 ms duration), with an average interstimulus interval of 30 seconds, were measured by a Coulbourn Startle Response System. This system uses a strain gauge to convert response force into an electrical signal suitable for waveform analysis. Data were edited off-line to exclude trials with no response, an elevated baseline or a saturated signal, and grouped into three blocks of 20 trials each, corresponding to the early, middle and late trials. All handling and testing were done under red light conditions during the dark phase of the light-dark cycle. Activity and startle response variables were analyzed by using a Diet X Noise Condition and Diet X Block (respectively) mixed-effects analysis of variance model; *post hoc* analyses were done using Bonferroni's *t* test.

Although activity variables showed no significant ($p<0.05$) main effect of diet, there were numerous significant interactions between diet and noise condition. Differences between dietary groups in horizontal activity were limited to the post-noise condition, whereas differences in stereotypy and rotational behavior were found only in the pre-noise condition. Horizontal activity, indexed by number of photo beam interruptions, number of discrete movements and movement time, was significantly greater in the group fed high Se than in the group fed adequate Se; these groups did not differ from the low Se group. Prior to noise onset, the low Se group made more stereotypic movements than the high Se group and more clockwise rotations than the adequate Se group. Measures of horizontal movement speed and distance, vertical movement and location of activity showed no significant effects of diet or its interaction with noise condition.

Startle waveform variables also showed no significant main effect of diet, but there were numerous significant interactions between diet and trial block. In all cases, differences between dietary groups were limited to the middle block of 20 trials. Waveform threshold and peak amplitudes (g), slope (g/ms), area (g-ms) and center of gravity (g) were all significantly greater for the group fed adequate Se than for either of the other two groups, which did not differ from each other. Thus, the groups fed inadequate or excess Se were less responsive than the Se-adequate group. Latency of the startle response did not differ significantly among the three diet groups.

Findings from analysis of locomotor activity indicate that dietary Se may influence only specific aspects of motor behavior, e.g., horizontal activity and stereotypy. However, Se effects on the former were found only after the animals had experienced a secondary stressor (noise). Findings from analysis of startle responses indicate that consuming a diet either low or high in Se may reduce central nervous system reactivity to external stimuli. The finding that this effect was significant only during the middle of a long series of trials was likely because of the high variability during both early and late trials. Collectively, the data suggest that Se may play a role in central nervous system function and behavior and indicate the need for further study.

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USE OF STABLE ISOTOPIC ^{67}Zn AND RADIOACTIVE ^{65}Zn TO SIMULTANEOUSLY DETERMINE THE ABSORPTION OF Zn FROM DIFFERENT POOLS IN THE PERFUSED RAT INTESTINE

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The major control of the amount of zinc (Zn) that remains in an animal is apparently the fraction of Zn returned to the gut after absorption; i.e. endogenously secreted Zn (1). Large amounts of bile and pancreatic fluid flow into the gut lumen on a daily basis; these secretions contain large amounts of Zn. If the Zn in these secretions is absorbed to a different degree than Zn that is associated with a meal, then this may be a way in which endogenously secreted Zn controls the amount of Zn in the body. The objective of this study was to determine simultaneously, in the same animal, the absorption of Zn associated with a meal and the absorption of Zn associated with bile-pancreatic fluid.

This study was conducted with perfused intestines of adult rats fed diets containing either 10 or 300 ppm Zn. The absorption of Zn from bile-pancreatic fluid was determined by labelling previously collected bile-pancreatic fluid with ^{65}Zn. The absorption of Zn from a dietary source was determined by labelling diet with ^{67}Zn followed by digestion in HCl and pepsin to simulate gastric digestion. Diet and bile-pancreatic fluid then were infused into the gut for 3 h, after which time animals were killed and the gut contents and tissue analyzed for ^{65}Zn (gamma counting) and ^{67}Zn (inductively coupled plasma mass spectrometry or ICP-MS).

ICP-MS samples for isotope ratio analysis were digested three times in 16 M subboiling distilled HNO_3 and evaporated to dryness to remove chlorides. Samples and bovine liver (BL) standards were diluted in 1% HNO_3 for an average zinc concentration of 87 ppb, as calculated from atomic absorption analysis. Ion intensities were obtained for ^{66}Zn, ^{67}Zn and ^{68}Zn by using an ICP-MS. Samples were analyzed concurrently with 75 ng/mL natural abundance Zn standard and BL standards. From each sample and standard, an observed natural abundance isotope ratio, ${}^{NAObs}R_{66/68}$, was obtained. Bias corrected ratios, ${}^{Corr}R_{67/68}$, were calculated from observed sample ratios, ${}^{Obs}R_{67/68}$, by ${}^{Corr}R_{67/68} = {}^{Obs}R_{67/68} * K$, where $K = {}^{NALit}R_{66/68}/{}^{NAObs}R_{66/68}$ and ${}^{NALit}R_{66/68} = 1.488$ (2).

Interferences for 68 m/z (mass per charge) include Ni, S_2, SO_2 and Ba^{2+}. Therefore, interferences with the ^{68}Zn signal were monitored by comparing the calculated versus literature isotope ratios in the 75 ng/mL Zn standards and BL standards. Corrected $R_{66/68}$ ratios in the 75 ng/mL standards were found to be low by 3%, and the BL standards low by 5%, which indicated a potential 2% interference. However, calculated ^{67}Zn in excess of natural abundance (Table 1) was high in the lumen and gut mucosa (duodenum, jejunum, ileum) samples and was a greater fraction of the total zinc pool (%Pool) than was measured in the natural abundance BL standard.

Rats fed 300 ppm Zn removed less ($p < 0.01$) Zn from the lumen and incorporated less Zn into the gut mucosa than did rats fed 10 ppm Zn, regardless of the source of the Zn ($p < 0.01$) (Table 2). A smaller ($p < 0.01$) percentage of the total ^{65}Zn from bile-pancreatic fluid was removed from the lumen of the gut than was ^{67}Zn from dietary sources. Mucosal uptake, however, was greater ($p < 0.01$) for Zn associated with bile-pancreatic fluid than Zn from the diet. These data indicate that a substance , yet undefined, in bile-pancreatic fluid influences the absorption of Zn from the gut. Whether or not this differential absorption is a mechanism of maintaining Zn homeostasis is a question for continuing research.

Table 1. ^{67}Zn in Excess of Natural Abundance for Unlabeled Bovine Liver Standards and Labeled Gut Samples.

Sample	ng	%Pool
BLS*	50	0.16
Lumen	45448	34
Duodenum	994	4.9
Jejunum	4672	16
Ileum	9365	5.9

*Bovine liver standard

Table 2. Percent of Infused ^{65}Zn or ^{67}Zn Removed from the Lumen or Incorporated into the Gut Mucosa of Perfused Rats. Values are Means ± Standard Deviation.

Dietary Zn ppm	^{67}Zn 10	^{67}Zn 300	^{65}Zn 10	^{65}Zn 300
Lumen†	76 ± 6	50 ± 26	53 ± 12	35 ± 10
Gut mucosa†	31 ± 4	14 ± 10	40 ± 3	27 ± 9

†Significant effect of dietary Zn ($p<0.01$) and form of Zn ($p<0.01$).

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USE OF A COLON CARCINOMA CELL LINE TO STUDY THE INFLUENCE OF BILE-PANCREATIC FLUID ON ABSORPTION OF ZINC

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Large amounts of zinc (Zn) associated with bile-pancreatic (B/P) fluid are secreted daily into the lumen of the small intestine (1). It is possible that a component of such secretions may influence the resorption of Zn (2). A series of investigations from this laboratory, utilizing rats as a model, have indicated that B/P fluid may decrease the absorption of Zn associated with such secretions. Absorption studies utilizing animals, however, are prone to relatively large amounts of experimental error. To define more precisely the events occurring during Zn absorption, we have utilized a carcinoma cell line derived from the human colon (CACO). This cell line mimics many of the characteristics of the mature intestinal enterocyte and has been used by many investigators as an in vitro model of absorption (3). The objective of this study was to examine apical to basolateral transport of Zn by CACO cells in the presence or absence of B/P fluid.

CACO cells were grown on semipermeable membrane filters for 21 days. The semipermeable filters were placed in wells on tissue culture plates. Confluent cells then divided the growth environment into an apical domain (the area inside the filter; medium was adjacent to the apical membrane) and a basolateral domain (the area outside the filter or in the well; medium was adjacent to the basolateral domain). At the start of the experiment, growth medium was added to both the apical and basolateral domains. Radioactive Zn was then added to the apical domain; additionally, freshly collected rat B/P fluid was added to the apical domain of one-half of the inserts. Plates were allowed to incubate for set amounts of time, then the basolateral medium was removed and analyzed for ^{65}Zn. Cells were scraped from the inserts and fractionated into nuclear, mitochondrial, microsomal and cytosolic fractions.

Table 1. Effect of Presence or Absence of Biliary-Pancreatic Fluid on the Uptake, Transport and Subcellular Distribution of ^{65}Zn by CACO Cells. Values are Mean ± Standard Deviation. Values with Different Superscripts are Significantly Different ($p<0.05$).

Item	5% B/P fluid	No B/P fluid
Transport (pmols/hr/cm²)	71 ± 6[a]	104 ± 21[b]
Uptake (pmols/hr/cm²)	66 ± 7[a]	72 ± 6[a]
Total removal from apical domain (pmols/hr/cm²)	137 ± 12[a]	176 ± 21[b]
% in nucleus	36 ± 4.7[a]	32 ± 3.6[b]
% in mitochondria	10 ± 1.0[a]	11 ± 0.5[a]
% in microsomes	7 ± 3.6[a]	10 ± 1.6[a]
% in cytosol	47 ± 3.5[a]	47 ± 3.5[a]

Although the presence of B/P fluid did not significantly alter the uptake of ^{65}Zn by CACO cells, it decreased the rate of ^{65}Zn transported by approximately 30% ($p<0.0001$). As a result, less ^{65}Zn was removed from the apical media when B/P fluid was present than when it was not included in the media. The presence of B/P fluid also significantly changed the subcellular distribution of Zn. More ^{65}Zn was found in the nuclear fraction when B/P fluid was present, whereas more tended to accumulate in the microsomal compartment when B/P fluid was not present. Overall, these data show that a substance in B/P fluid alters the uptake and transport of Zn by CACO cells, which may be a consequence of a redistribution of Zn inside the cell. How this may affect the regulation of body Zn stores is being studied.

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PROFESSIONAL COMMUNICATIONS

RELATIONSHIPS

Friday, 30 April

1:30 pm Body Composition, Nutritional Status and Swim Performance
W A Siders* and H C Lukaski, USDA/ARS Human Nutrition Research Center, Grand Forks 58202

1:50 pm Inhibition of Prolactin-Dependent and -Independent Nb2 Lymphoma Cell Proliferation by Genistein, A Tyrosine Kinase Antagonist
Hanqian Liang*, Donna J Buckley, Arthur R Buckley, UND School of Medicine, Grand Forks 58202

2:10 pm Congenic Syrian Hamster Monomorphic N-Acetyltransferase (NAT1): Cloning, Sequencing, and Expression in E Coli
Ronald J Ferguson*, Mark A Doll, Timothy D Rustan, Barbara R Baumstark, David W Hein, UND School of Medicine, Grand Forks 58202 and Georgia State University, Atlanta 30303

2:30 pm Metabolic Activation and Deactivation of Arylamine Carcinogens by Recombinant Human NAT1 and Polymorphic NAT2 Acetyltransferases
Kevin Gray*, Mark A Doll, Timothy D Rustan, Yi Feng, Ronald J Ferguson and David W Hein, UND School of Medicine, Grand Forks 58202

2:50 pm Relationship of Human Acetylator Genotype to Incidence of Colorectal Cancer
Mark A Doll*, Jose W Rodriguez, Ward G Kirlin, Ronald J Ferguson, Kevin Gray, Timothy D Rustan, Mark E Lee, Paul Urso and David W Hein, UND School of Medicine, Grand Forks 58202 and Morehouse School of Medicine, Atlanta 30310

3:10 pm 3,2'-Dimethyl-4-aminobiphenyl N-Acetylation and DNA Adduct Formation in a Congenic Hamster Model of Urinary Bladder Carcinogenesis
David W Hein*, Yi Feng, Timothy D Rustan, Mark A Doll, Letha H Couch, Thomas J Flammang, UND School of Medicine, Grand Forks 58202

BODY COMPOSITION, NUTRITIONAL STATUS AND SWIM PERFORMANCE

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We have previously shown that body composition variables (1) and iron nutritional status are related to 100-yd swim times of collegiate women and men. Because competitive events at longer distances have different anaerobic and aerobic requirements, we sought to determine body composition and nutritional status correlates of 100-yd and 200-yd performance in collegiate female and male swimmers, aged 18-21 years.

Six women and seven men from the University of North Dakota varsity swim teams were studied at the end of their competitive season. They underwent anthropometric measurements for the determination of somatotype, or physique, and dual x-ray absorptiometry for the determination of regional and whole body composition. Fasting venous blood samples also were obtained. Performance was actual times during swimming competitions at the last meet of the season in the 100-yd and 200-yd freestyle events.

The women were shorter, weighed less, had less fat-free, mineral-free and bone mineral mass, but more body fat than the men (Table 1). Women had similar times as the men in the 100-yd races, but were slower in the 200-yd events. In women, copper status, indicated by plasma copper concentration and enzymatic ceruloplasmin activity, was increased whereas iron status (hematocrit and hemoglobin) was decreased. Biochemical indices of nutritional status were within normal ranges.

Table 1. Descriptive Characteristics

	Women (n=6)		Men (n=7)	
	Mean	SD[A]	Mean	SD
Height, cm	165	5.0	180[B]	4.3
Weight, kg	63.1	4.5	76.0[B]	4.4
FFMF[C], kg	45.0	2.3	64.6[B]	3.8
BMC[D], kg	2.18	0.33	2.66[B]	0.24
Fat, kg	15.9	2.8	8.6[B]	3.2
Fat, %	25.1	3.1	11.3[B]	3.9
Plasma				
Cu, μmol/L	114	29	78.9[B]	6.6
Fe, μmol/L	19.0	2.2	19.6	2.6
Mg, μmol/L	0.8	0.1	0.8	0.1
Red Blood Cell				
Cu, μmol/g Hgb[E]	0.4	0.1	0.4	0.1
Mg, μmol/g Hgb	59.4	8.3	62.6	5.6
Iron Status				
Hematocrit, %	39.5	0.8	44.4[B]	1.7
Hemoglobin, g/L	132	3.0	151[B]	7.0
Ferritin, μg/L	14.7	5.2	45.0[B]	10.9
TIBC[F], μmol/L	72.0	12.4	66.0	7.5
Copper Status				
Cp-ENZ[G], mg/L	51.5	9.9	37.8[B]	5.7
Time				
100-yd, s	54.1	3.6	53.3	3.6
200-yd, s	124.9	4.0	112.3[B]	5.0

[A]SD = standard deviation.
[B]Mean is statistically different from mean for women, $p < 0.05$.
[C]FFMF = fat free, mineral free mass.
[D]BMC = bone mineral content.
[E]Hgb = hemoglobin.
[F]TIBC = total iron-binding capacity.
[G]Cp-ENZ = enzymatic activity of ceruloplasmin.

Correlations were calculated between swim times, compositional and blood biochemical indices of nutritional status by sex (Table 2). Faster swim times in the 100-yd event were related to low plasma copper, low ceruloplasmin enzymatic activity and high red blood cell magnesium in women. Faster 200-yd swim times were related to high ferritin in women and to low plasma iron, low red blood cell copper and high ferritin in men.

Table 2. Correlations Between Body Composition and Nutritional Indices and Swimming Performance.

	100-yd		200-yd	
	Women	Men	Women	Men
FFMF, kg	−.140	−.302	.648	−.356
BMC, g	−.194	−.561	.449	−.278
Fat, %	.242	.035	−.274	.152
Plasma				
Cu, μmol/L	.964*	.419	−.223	−.045
Fe, μmol/L	.520	.576	−.200	.881*
Mg, μmol/L	.106	−.277	−.632	−.254
Red Blood Cell				
Cu, μmol/g Hgb	−.202	−.335	−.154	.834*
Mg, μmol/g Hgb	−.926*	−.394	.075	.287
Iron Status				
Hematocrit, %	.052	−.149	−.231	−.504
Hemoglobin, g/L	.499	−.267	.335	−.614
Ferritin, μg/L	.523	−.362	−.631	−.869*
TIBC, μmol/L	.434	−.009	.160	.185
Copper Status				
Cp-ENZ, mg/L	.981*	.333	−.285	−.170

*p<0.05

Body composition variables did not account for large portions of the variability in 100-yd and 200-yd swimming performance. However, blood biochemical indices of iron, copper and magnesium were related to enhanced performance for women and men. These results support the hypothesis that performance in different athletic events is related to different trace element status variables for women and men.

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INHIBITION OF PROLACTIN-DEPENDENT AND -INDEPENDENT Nb2 LYMPHOMA CELL PROLIFERATION BY GENISTEIN, A TYROSINE KINASE ANTAGONIST

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The prolactin (PRL)-dependent Nb2 node lymphoma cell line is a widely studied model for investigation of molecular mechanisms coupled to the mitogenic action of PRL. A subline, Nb2-SFJCD1, obtained by lactogen starvation and subsequent cloning, is entirely independent of PRL for stimulation of proliferation although it retains partial sensitivity to PRL stimulation. Despite numerous investigations, the transmembrane signalling mechanism coupled to PRL-induced mitogenesis remains unknown. Recent evidence has suggested that PRL may stimulate enhanced tyrosine kinase (TK) activity as an early consequence of receptor activation (1,2). Therefore, in the present study, we employed genistein, a selective TK inhibitor, to determine whether PRL stimulation of proliferation in PRL-dependent and -independent Nb2 lymphoma cells reflects a requirement for TK activation.

The addition of PRL to quiescent G_1-arrested Nb2 cells obtained by prior incubation for 18-24h in lactogen-free medium supplemented with 10% gelding serum, stimulated resumption of cell cycle progression. The data presented in the table below demonstrate that genistein addition to PRL-treated Nb2 cultures significantly reduced cell proliferation assessed by [^{3}H]-thymidine (TdR) incorporation as well as cell density. Genistein, at a concentration of 10 ug/ml, completely inhibited PRL-stimulated mitogenesis in the PRL-dependent cells. Proliferation of the PRL-independent SFJCD1 subline was likewise profoundly inhibited by genistein. Genistein consistently blocked Nb2-SFJCD1 cell growth irrespective of whether PRL was present in the medium. Time course experiments in PRL-dependent cells revealed that genistein added at the time of PRL treatment reduced DNA synthesis to a significantly greater extent than when it was added 0.5-10 hrs after PRL-stimulation.

Effect of Genistein on Lymphoma Cell Growth

	Cell Line			
	Nb2 (PRL-Dependent)		Nb2-SFJCO1 (PRL-Independent)	
Treatment	[3H]-TdR Incorp. (cpm/well)[a]	Cell Number (cells/ml)[b]	[3H]-TdR Incorp. (cpm/well)[a]	Cell Number (cells/ml)[b]
Cells only	0.06 ± 0.008	1.1 ± 0.04	4.8 ± 0.5	6.5 ± 0.2
Vehicle Control	0.06 ± 0.005	1.2 ± 0.1	4.8 ± 0.1	6.4 ± 0.009
PRL Only	3.9 ± 0.2	3.1 ± 0.2	---	---
PRL + Vehicle	4.2 ± 0.2	3.5 ± 0.2	4.9 ± 0.2	6.9 ± 0.1
PRL+Gen (1 μg/ml)	3.7 ± 0.03	2.8 ± 0.09	4.6 ± 0.3	6.6 ± 0.2
PRL+Gen (2.5 μg/ml)	2.6 ± 0.09	2.4 ± 0.1	4.5 ± 0.09	6.0 ± 0.1
PRL+Gen (5 μg/ml)	1.6 ± 0.008	2.5 ± 0.2	3.1 ± 0.06	4.2 ± 0.1
PRL+Gen (10 μg/ml)	0.6 ± 0.08	1.5 ± 0.1	1.1 ± 0.02	2.0 ± 0.1

PRL: 20 ng/ml; Vehicle: 0.01% ETOH; a: x 10^4 at 48 h; b: x 10^5 at 48 h

These data support the hypothesis that the mitogenic action of PRL is coupled to rapid activation of TK activity in PRL-dependent Nb2 cells. The effect of genistein to inhibit cell proliferation within minutes after PRL addition is consistent with a role for TK as a signal transduction mechanism in these cells. Moreover, genistein also blocked growth in PRL-independent Nb2-SFJCD1 cells. This suggests that uncoupling of a growth requirement for PRL in these cells may reflect alternate hormone-independent pathways for stimulation of TK activity. Supported in part by NIH grant DK44439.

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CONGENIC SYRIAN HAMSTER MONOMORPHIC N-ACETYLTRANSFERASE (NAT1): CLONING, SEQUENCING, AND EXPRESSION IN E. COLI

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N-acetyltransferases (E.C. 2.3.1.5), cytosolic enzymes found in a variety of species and tissues, catalyze the N-acetylation of various therapeutic arylamine and hydrazine drugs and environmental carcinogens. Genetic variability in N-acetylation capacity with respect to the pharmacology and toxicology of these compounds is of significant clinical importance. N-acetylation phenotype may predispose toward a variety of drug and xenobiotic-induced toxicities and carcinogenesis (1). Both humans and Syrian hamsters express two N-acetyltransferase isozymes; one varies with acetylator genotype (polymorphic, NAT2) and the other is acetylator genotype-independent (monomorphic, NAT1). Abu-Zeid *et al.* (2) isolated and sequenced a hamster liver NAT cDNA encoding NAT1. Primers for the polymerase chain reaction (PCR) were synthesized corresponding to areas which flank the *NAT1* coding region (Figure 1). A 910 bp PCR product, containing the complete 870 bp coding region, was amplified via genomic DNA templates from various homozygous rapid and slow acetylator congenic and inbred Syrian hamster lines (Table 1). These *NAT1* PCR products were cloned into pUC18 and sequenced by the dideoxy chain-termination method (3). Two *NAT1* alleles were found; one from the homozygous rapid acetylators (*NAT1*^{r}) and the other from the homozygous slow acetylators (*NAT1*^{s}). The *NAT1* alleles differed in one nucleotide, resulting in no change in deduced amino acid sequence. To characterize the expressed enzymes of the *NAT1* alleles, we developed a prokaryotic-expression system. The *NAT1*^{r} and *NAT1*^{s} alleles were amplified by expression-cassette PCR (4) and subcloned into the *tac* promoter-based plasmid vector pKK223-3 for over-production of recombinant NAT1 in E. coli strain JM105. Induced cultures from selected *NAT1*-inserted transformants yielded high levels of soluble protein capable of N-acetylation, O-acetylation, and N,O-acetylation (Table 2, 3). The recombinant NAT1 proteins exhibited similar substrate-specificity, specific activity, and Michaelis-Menten kinetic properties. Also, the over-expressed NAT1 proteins displayed similar substrate-specificity characteristic of *NAT1* isolated from hamster and colon cytosols. These results confirm that these PCR clones contain hamster *NAT1*. Partially supported by USPHS grant CA34627.

Figure 1. PCR Primers for Hamster *NAT1*.

H3	5'-AGG*GCATGC*GATCATGGACATCGAAGCCTATTTC-3'
H4	5'-TAA*GAATTC*GCAGTTTTGCGCTTTACCCTAAATAC-3'
START	5'-GCGC*GAATTC*AGGAGGAATTTAAAATGGACATCGAAG
	CCTATTTCGAAAGGA-3'
STOP	5'-GCGC*AAGCTT*GCAGTTTTGCGCTTTACCCTAAATACTA-3'

Table 1. Congenic and Inbred Syrian Hamster Strains.

Hamster Strain	Homozygous Acetylator-Genotype	
Bio. 82.73/H-*Pat*^{r}	Rapid	Congenic for the *NAT2* locus
Bio. 82.73/H-*Pat*^{s}	Slow	
Bio. 1.5/H-*NAT2*^{r}	Rapid	Congenic for the *NAT2* locus
Bio. 1.5/H-*NAT2*^{s}	Slow	
Bio. 82.73/H	Slow	Inbred line
Bio. 1.5	Slow	Inbred line
MHA/SsLak	Rapid	Inbred line

Table 2. Recombinant NAT1 Michaelis-Menten constants for N-acetylation.

Substrate	Enzyme	Apparent Km (μm)	Apparent Vmax (nmoles/min/mg)
p-aminobenzoic acid	NAT1^r	4289 ± 1379	547 ± 94
	NAT1^s	4417 ± 1184	676 ± 99
4-aminobiphenyl	NAT1^r	92.9 ± 15	168 ± 9
	NAT1^s	141 ± 30	193 ± 15
2-aminofluorene	NAT1^r	90.1 ± 16.2	177 ± 10
	NAT1^s	129 ± 30	189 ± 16
isoniazid	NAT1^r	509 ± 148	389 ± 40
	NAT1^s	428 ± 126	361 ± 34
3,2'-dimethyl-4-aminobiphenyl	NAT1^r	100 ± 9	129 ± 4
	NAT1^s	127 ± 20	156 ± 9

Results based on sample sizes of 4-6.

Table 3. Recombinant NAT1 O-acetylation and N,O-acetylation specific activities.

Substrate	Enzyme	Reaction	Specific activity (pmoles/min/mg DNA/mg) +10μM paraoxon	without paraoxon
N-hydroxy-2-aminofluorene	NAT1^r	O-acetylation	3358 ± 242	2439 ± 105
	NAT1^s		4232 ± 174	4281 ± 107
N-hydroxy-2-acetyl-aminofluorene	NAT1^r	N,O-acetylation	8825	8278
	NAT1^s		9267	9378

Paraoxon is an inhibitor of microsomal acetyltransferases.
O-acetylation specific activities are based on 3 determinations.
N,O-acetylation results are based on 1 assay.

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METABOLIC ACTIVATION AND DEACTIVATION OF ARYLAMINE CARCINOGENS BY RECOMBINANT HUMAN NAT1 AND POLYMORPHIC NAT2 ACETYLTRANSFERASES

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A genetic polymorphism at the *NAT2* gene locus, encoding for polymorphic N-acetyltransferase (NAT2), segregates individuals into rapid, intermediate, or slow acetylator phenotypes. Both rapid and slow acetylator phenotypes have been associated with increased incidence of cancer in certain target organs related to arylamine exposures, suggesting a role for acetylation in both the activation and deactivation of arylamine carcinogens. A second gene (*NAT1*) encodes for a different acetyltransferase isozyme (NAT1) that is not subject to the classical acetylation polymorphism. In order to assess the relative ability of NAT1 and NAT2 to activate and deactivate arylamine carcinogens, we tested the capacity of recombinant human NAT1 and NAT2, expressed in *Escherichia coli* XA90 strains DMG100 and DMG200, respectively, to catalyze the N-acetylation (deactivation) and O-acetylation (activation) of a diversity of carbocyclic and heterocyclic arylamine carcinogens. Both NAT1 and NAT2 catalyzed the N-acetylation of each of the 17 arylamines tested. Rates of N-acetylation by NAT1 and NAT2 were considerably lower for heterocyclic arylamines such as the dietary carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), particularly those (e.g., IQ) with steric hindrance to the exocyclic amino group. As shown in Table 1, the apparent affinity was significantly ($p<0.05$) higher for NAT2 than NAT1 for carbocyclic arylamines such as 4-aminobiphenyl and β-napthylamine that are present in cigarette smoke. NAT1/NAT2 activity ratios and clearance calculations suggest a significant role for the polymorphic NAT2 in the N-acetylation of carbocyclic arylamine carcinogens (Table 1).

Table 1. Michaelis-Menten constants for N-acetylation of arylamines by human NAT1 and NAT2

Arylamine Substrate	Apparent Km (μM)		Apparent Vmax (nmoles/min/U)		Intrinsic Clearance (Vmax/Km)		Clearance Ratio NAT1/NAT2
	NAT1	NAT2	NAT1	NAT2	NAT1	NAT2	
4-aminobiphenyl	108 ± 14	25.8 ± 3.1[a]	230 ± 17	2.41 ± 0.16	2.13	0.09	24
β-naphthylamine	84.7 ± 9.0	25.5 ± 3.3[a]	224 ± 15	2.92 ± 0.25	2.64	0.11	24
3,4-dichloroaniline	74.9 ± 9.2	17.3 ± 1.5[a]	231 ± 13	2.65 ± 0.04	3.08	0.15	21
3,2'-dimethyl-4-aminobiphenyl	491 ± 39	48.9 ± 0.4[a]	152 ± 12	2.58 ± 0.07	0.31	0.05	6
p-toluidine	77.8 ± 8.9	37.1 ± 2.9[a]	203 ± 8	2.84 ± 0.13	2.61	0.08	33
p-phenetidine	58.8 ± 4.7	16.2 ± 1.2[a]	205 ± 6	2.78 ± 0.10	3.49	0.17	21
Glu-P-2	347 ± 17	194 ± 8[a]	48 ± 5	2.12 ± 0.03	0.14	0.01	14

Mean ± S.E.M. for three to five individual determinations.
[a] NAT2 Km significantly less than corresponding NAT1 Km ($p<0.01$).

Both NAT1 and NAT2 also catalyzed the metabolic activation of carcinogenic arylamines by paraoxon-resistant, O-acetylation of N-hydroxy-2-aminofluorene and N-hydroxy-4-aminobiphenyl to yield DNA adducts. NAT1 catalyzed paraoxon-resistant, intramolecular N,O-acetyltransferase-mediated activation of N-hydroxy-2-acetylaminofluorene and N-hydroxy-4-acetylaminobiphenyl at low rates; catalysis by NAT2 was not readily detectable in the presence of paraoxon. In summary, these studies strongly suggest that the human acetylation polymorphism influences both the metabolic activation (O-acetylation) and deactivation (N-acetylation) of arylamine carcinogens via polymorphic expression of NAT2. These findings lend mechanistic support for human epidemiological studies suggesting associations between both rapid and slow acetylator phenotype and cancers related to arylamine exposures.
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RELATIONSHIP OF HUMAN ACETYLATOR GENOTYPE TO INCIDENCE OF COLORECTAL CANCER

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Colorectal cancer is the second leading cause of death in the United States, killing more than 60,000 each year. Colorectal cancer etiology appears to involve a series of steps in which environmental or endogenous carcinogens such as arylamines induce or promote neoplasia through the accumulation of specific genetic mutations (1).

N-acetyltransferases catalyze the acetyl-CoA dependent acetylation of arylamines encountered from environmental, occupational and dietary exposure (2). Humans exhibit a genetic polymorphism in hepatic N-acetyltransferase (NAT2) expression resulting in rapid, intermediate and slow acetylator phenotype (3). Previous epidemiological studies suggest a genetic predisposition of rapid acetylators (2) to colorectal cancer but a definitive relationship is difficult to establish because of uncontrolled variability. In the present study we have used Restriction Fragment Length Polymorphism (RFLP) to assess the relationship of *NAT2* allelic frequency and genotype with colorectal cancer incidence. Human colon samples from colorectal cancer and non-cancer subjects were obtained from surgical samples through the National Disease Research Interchange or the Cooperative Human Tissue Network. Genomic DNA was isolated from colon tissue and primers selective for the *NAT2* gene were synthesized. *NAT2* was amplified using the Polymerase Chain Reaction (PCR). Following enzymatic digestion, DNA fragments were separated by electrophoresis and *NAT2* acetylator genotypes were determined from the pattern of restriction fragments observed after gel staining.

Our subject population consisted of 72 individuals. Within the group were 44 that had been diagnosed with colorectal cancer while 28 were diagnosed non-cancerous. In our sample population of human colons we identified the *NAT2* wild type (WT) allele and each of the three previously known mutant alleles (M1, M2, M3). The *NAT2* allelic frequency ($X^2 = 0.0177$; $p = 0.991$) and *NAT2* genotype (Table 1) did not differ significantly between the colorectal cancer and non-cancerous group.

Table 1. Comparison of *NAT2* genotypes in non-cancer and colorectal cancer subjects.

	NAT2 Genotype Frequencies[1]	
NAT2 Genotype	Non-Cancer (28)	Colorectal Cancer (44)
M1M1	0.214 (6)	0.250 (11)
M1M2	0.250 (7)	0.250 (11)
M1M3	0.036 (1)	0 (0)
M2M2	0.036 (1)	0.045 (2)
M1WT	0.179 (5)	0.182 (8)
M2WT	0.179 (5)	0.159 (7)
WTWT	0.107 (3)	0.114 (5)

[1]Number in parenthesis indicate number within group. *NAT2* genotype frequencies (except M1M3 omitted from analysis) do not differ significantly between non-cancer and colorectal cancer subjects ($X^2 = 0.157$; $p = 0.995$).

The lack of a relationship between *NAT2* genotype and colorectal cancer incidence in this study strongly suggests that the occurrence of colorectal cancer is not solely dependent upon the expression of *NAT2*. However, these results clearly show that the use of PCR followed by RFLP analysis to determine acetylator genotype from DNA derived from human tissue should facilitate further studies to assess the role of acetylator genotype on the incidence of different cancers. Partially supported by USPHS grants CA-34627, GM-08248, and USEPA 818813.

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3,2'-DIMETHYL-4-AMINOBIPHENYL N-ACETYLATION AND DNA ADDUCT FORMATION IN A CONGENIC HAMSTER MODEL OF URINARY BLADDER CARCINOGENESIS

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The excessive occurrence of urinary bladder cancer in people exposed to arylamine chemicals from the workplace (e.g., rubber and dye industries) and environment (e.g., cigarette smoke and automobile exhaust) has been recognized for many years. Arylamine chemical exposures do not inevitably lead to urinary bladder cancer, which suggests genetic predisposition to tumors in certain subsets of the population.

Human and hamster capacity to N-acetylate arylamines is subject to a genetic polymorphism regulated at a single polymorphic gene locus (*NAT2*). Individuals homozygous for mutant alleles at this locus have significantly lower N-acetylation capacity, and are identified as the slow acetylator phenotype. Slow acetylators constitute over 50% of the American population. Several human epidemiological studies suggest genetic predisposition to urinary bladder cancer in slow acetylators, but a definitive relationship is difficult to establish because of uncontrolled variability in all other genetic and environmental factors that contribute to the etiology of urinary bladder cancer.

To eliminate uncontrolled variation in other genetic factors, our laboratory has constructed congenic acetylator hamster lines that differ only at the *NAT2* gene locus or other closely-linked loci. We have utilized these congenic hamsters to investigate the N-acetylation and DNA adduct formation of 3,2'-dimethyl-4-aminobiphenyl (DMABP), a documented hamster urinary bladder carcinogen. As shown in Figure 1, the N-acetylation of DMABP exhibited *NAT2*-dependent expression in the congenic hamsters, with highest expression in homozygous rapid acetylators (rr), intermediate expression in heterozygous acetylators (rs), and lowest expression in homozygous slow acetylators (ss). This *NAT2*-dependent expression was exhibited not only in liver, but also in tumor target organs such as the urinary bladder. DMABP forms covalent adducts with DNA that serves as one molecular biomarker for carcinogenic lesions. Single intraperitoneal injections of DMABP (33 mg/kg) to male rapid and slow acetylator congenic hamsters (4.5 to 6 months old) yielded two major DMABP-DNA adducts in liver and urinary bladder tissues. These adducts were identified as N-(deoxyguanosin-8-yl)-DMABP (Fig. 2) and 5-(deoxyguanosin-N^2-yl)-DMABP (Fig. 3) and were quantified following ^{32}P-postlabeling analysis. Levels of both DMABP-DNA adducts increased with time up to 36 hrs post-administration and were higher in urinary bladder (tumor target) than in the liver (non-target) DNA.

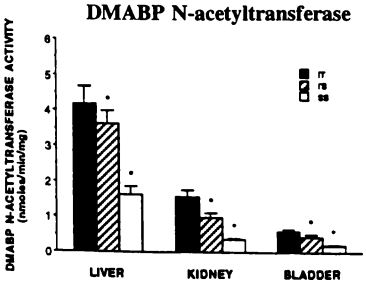

Figure 1

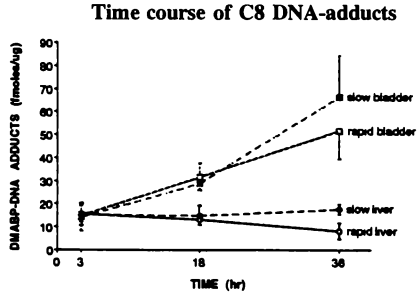

Figure 2

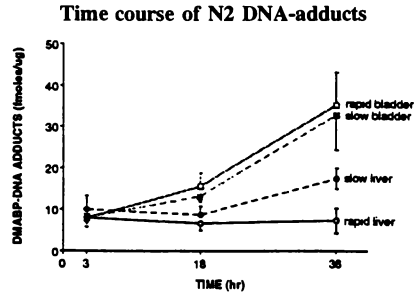

Figure 3

Although *NAT2* genotype significantly affects DMABP N-acetylation in each tissue, the influence of *NAT2* genotype was not significant in preliminary DNA adduct studies. These results suggest that the effect of *NAT2* genotype needs to be assessed further in more comprehensive studies at other dosage levels and time points in conjunction with DMABP tumorigenesis bioassays. These preliminary findings suggest that the congenic hamster model will serve as a unique tool to assess the role of *NAT2* genotype in urinary bladder cancer from arylamine chemicals. Partially supported by USPHS grant CA-34627.

PROFESSIONAL COMMUNICATIONS

MIXED TOPICS

Friday, 30 April

1:30 pm PC Software for Microwave Enginering Design
Frederick E Stevens and David A Rogers*, NDSU, Fargo 58105

1:50 pm Charactersitics of Gunnison's Prairie Dog Burrows in Colorado
Donna M Burns Stockrahm, Stacy L Adolf*, Charles W Peterson, Elizabeth K Harper, Heidi A Olson Brokate, Bruce M Brokate, Todd A Mattson, Bobbi Jo Dickerson, Moorhead State University, Moorhead 56563 and Joshua F Peterson, Steven C Carter, Adam G Stern, Sydney Halperin, School for Field Studies, Beverly 01915

2:10 pm Introduction of Female Cotton-top Tamarin to a Resident Juvenile Male, (Sanguinus oedipus)
Melissa J Brotton, Terry Lincoln, and Louis Rigley*, The Dakota Zoological Society, Bismarck 58501

PC SOFTWARE FOR MICROWAVE ENGINEERING DESIGN

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Although there are several commercial microwave circuit analysis software packages available, the classical MCAP (microwave circuit analysis) program (1) has the advantage of giving the user the opportunity to modify the source code (2). This is especially useful as new developments lead to revisions of MCAP to deal with new devices and higher-frequency applications. A small-signal GaAs MESFET model and a diode model have been developed for MCAP. These enhancements to MCAP give results that are similar to those obtained from a commercial software package. The GaAs MESFET is the active device of choice for microwave amplifiers since it has lower noise, higher gain, and higher output power than a microwave bipolar transistor (3). The diode element can be either shunt connected or series connected and will model mixer, varactor, and pin diodes. Also available in the revised program are complex shunt admittances and series impedances. Certain subroutines have been modified so that they accept real and imaginary values. A subroutine, LCR, has been added so that new values of reactance will be calculated for each frequency being considered.

Variations of MCAP probably have been developed for the special needs of individual researchers, although the authors are not aware of any published in the open literature or available as shareware. Commercial microwave software (4) typically includes many of the algorithms originally developed for MCAP.

The Y-parameters for an older MESFET model (1) were modified for the MESFET model now included in this improved version of MCAP. The lead inductances which are normally placed in series with the drain, gate, and source resistors have been omitted because these reactances may be added by using the standard series impedance element. The Y-parameters of the new model were deduced directly from the circuit and then transformed to the S-parameters (3). The new diode and MESFET elements use typical or actual parameter values. The MESFET element accepts user-determined values of undepleted channel resistance, output conductance, gate-to-source capacitance, drain-to-gate feedback capacitance, transconductance, contact and bulk semiconductor resistances, and drain-to-source capacitance.

The diode element requires typical or actual values of junction capacitance, junction resistance, series resistance, series inductance, and package capacitance. The validity of this new MCAP element was easily demonstrated by comparing MCAP results for the reflection coefficient to those produced by a commercial software package.

The MESFET element was tested by design of a narrowband amplifier with a center frequency of 5 GHz. Initially the MESFET itself was analyzed to obtain its S-parameters. From these parameters the normalized input and output admittance were calculated. This led to the design of an input matching network using an open-circuited stub. The output was matched using a series inductance and a quarter-wavelength line. For stability, a 200-Ω resistor was placed in parallel with the MESFET drain (and, thus, added to the output admittance of the MESFET). The imaginary part of the output admittance was cancelled using the inductor, and the quarter-wavelength line transformed the real part to match the MESFET to the load impedance.

The modified software returned results for the MESFET amplifier that are comparable to those obtained with a commercial software package. The MCAP design yielded a gain of 9 dB with a bandwidth of 175 MHz while a commercial package gave a gain of 11 dB with a bandwidth of 170 MHz. Using a commercial circuit optimization procedure improved the gain to 13 dB while reducing the bandwidth to about 168 MHz. The satisfactory experience obtained with the MESFET and diode elements demonstrates the continued usefulness of the MCAP software.

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CHARACTERISTICS OF GUNNISON'S PRAIRIE DOG BURROWS IN COLORADO

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Prairie dogs of all species have a conspicuous impact on the landscape due to their burrowing activities. Once constructed, their burrow systems might remain intact for many years. Burrows provide a place in which to raise young, shelter from the elements, and protection from some predators. The burrow systems of black-tailed prairie dogs (Cynomys ludovicianus) are often elaborate and have associated dome- and crater-shaped mounds at the entrances (1, 2). Relatively little is known about the burrow systems of Gunnison's prairie dogs (C. gunnisoni), but their mounds often appear to be little more than piles of dirt (3). The purpose of our study was to document characteristics of Gunnison's prairie dog burrows, especially of their entrances.

We examined burrows at 2 Gunnison's prairie dog colonies in Archuleta County, Colorado. Site 1 was located at the junction of the Everett and Catchpole Ranches along Highway 84, southeast of Pagosa Springs (T35N, R1W, S29 and S30). Site 2 was located on the Piedra Valley Ranch, northwest of Pagosa Springs (T36N, R3W, S13).

Sites 1 and 2 were studied during the summers of 1991 and 1992, respectively. On Site 1, 10 plots within an area of 5.85 ha were chosen to represent various microhabitats and slope aspects. For each plot, burrows were measured within a belt transect(s) 2 m wide and encompassing 200 m^2. On Site 2, burrows were measured on 6 transects, each 6 m by 100 m, again representing different microhabitats and slope aspects. For all transects on both sites, aspect and angle of slope were recorded. For each burrow, angle of slope of the opening, direction in which the opening faced, and the presence/absence of a mound were recorded. Vegetative cover around the openings was qualitatively noted. At Site 2, the diameters of the burrow openings and the dirt mounds were also recorded. Angles were measured with either a "Sears Angle Finder" mounted onto a wooden stake or a clinometer.

Burrow densities, based on total burrows counted per total area sampled, were 295/ha for Site 1 and 322/ha for Site 2. Mounds, consisting of piles of dirt, were found on 34% (n = 59) of burrow openings on Site 1 and 69% (n = 116) on Site 2. Sometimes a single mound encompassed more than 1 burrow opening. On Site 1, 7% (n = 59) of the burrows were found on slopes of 10 degrees or greater. On Site 2, 55% (n = 116) of the burrows were on slopes of 11 degrees or greater. Burrows were found on all slope aspects examined. Burrow opening aspects were usually within 45 degrees of the aspect of the slope. On Sites 1 and 2, respectively, 64% (n = 45) and 65% (n = 106) of the burrow openings had a slope between 19 and 36 degrees. The average slope for these openings on Site 2 was 32.3 degrees (S.D. = 14.4, n = 106). Vegetation immediately around the burrow entrance and on the mound was common on both sites.

On Site 2, burrow widths and heights, as measured just inside the outer opening, averaged 15.4 cm (S.D. = 5.5, n = 106) and 14.6 cm (S.D. = 4.2, n = 101), respectively. Mound length averaged 258.4 cm (S.D. = 152.8, n = 71) and mound widths, 180.1 cm (S.D. = 109.9, n = 71). Several burrows that appeared to have been dug out by badgers (Taxidea taxus) were not included in the above measurements.

Overall, Gunnison's prairie dog burrows were found on a wide variety of slope aspects with the openings usually sloping between 19 and 36 degrees. Mounds (dirt piles) did not always exist; however, if present, they were often associated with vegetation.

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Introduction of Female Cotton-top Tamarin to a Resident Juvenile Male, (*Sanguinus oedipus*)

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The Dakota Zoological Society

An adult female cotton-top tamarin was introduced to a resident juvenile male to examine male dominance and space utilization at the Dakota Zoo in the fall of 1992. At the arrival to the zoo, the female was held in isolation for one week to determine her health status. The male was observed for several weeks before the introduction to establish a baseline of normal male behaviors. At the introduction of the female, observations were carried out for three hours and for random one half hour periods for the next three weeks. The observations were either video taped or audio taped for review. The male was housed in a steel wire cage of three meters wide, seven meters long and three meters high. The cage was on display to the public at all times. All observations were made during daylight hours and time sampling was applied.

Before the introduction, the male spent 75% of his time foraging for insects (the cage is in a wooded area of the zoo), scampering about the cage, feeding, sleeping and other normal behaviors. At the introduction of the female, the male switched to a protective territorial role 100% of the time (see fig. 1). He maintained a distance of about one meter from the female at all times, and as visitors approached the cage he would position himself between the female and the visitor. When two visitors approached the cage from different directions, the male scampered back and forth trying to keep himself positioned between each visitor and the female. However, this behavior gradually decreased over the next three weeks.

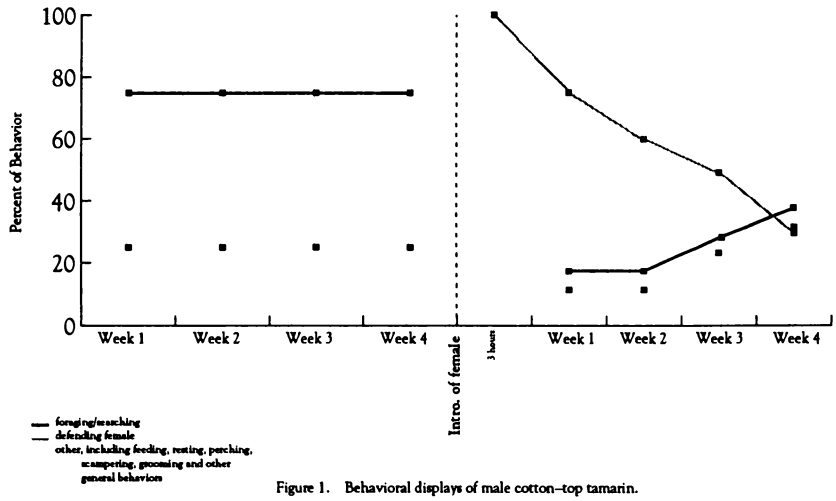

Figure 1. Behavioral displays of male cotton-top tamarin.

According to French and Inglett (1), female tamarins act more aggressively toward intruders while males are more indifferent. Interestingly, this study suggests that male cotton-top tamarins are more aggressive to intruders than females. Price (2) suggests that female tamarins invest in the relationship with the male, but this female remained indifferent to the male for over two weeks. Play behavior is a common interaction between male and female tamarins, but again none was observed (Chalmers and Locke-Haydon, 3). The results suggest that male tamarins do show aggressive behavior when an intruder female is introduced and that time is probably the factor in establishing normal social bonding and normal behaviors.

1. French, Jeffrey A. & Betty J. Inglett 1989. *Female-Female Aggression and Male Indifference to Unfamiliar Intruders in Lion Tamarins*. Anim. Behav., 37, 487-497.
2. Price, Eluned C. 1992. *Sex and Helping: Reproductive Strategies of Breeding of Male and Female Cotton-top Tamarins, (Sanguinus oedipus)*. Anim. Behav., 43, 717-728.
3. Chalmers, N. R. & J. Locke-Haydon 1981. *Temporal Patterns of Play Bouts in Captive Common Marmosets (Callithrix jacchus)*. Anim. Behav., 29, 1229-1238.

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).

2. 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.

3. 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

1. 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.

B Y - L A W S of the NORTH DAKOTA ACADEMY of SCIENCE

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.
4. 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 Corporate 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)

19. The North Dakota Science Research Foundation is established as an operating arm of the 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

OFFICERS and COMMITTEES May 1992 - April 1993

EXECUTIVE COMMITTEE

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Department of Plant Pathology
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North Dakota State University

Robert Seabloom -94
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Clark Markel, Chair -97
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Forrest Nielsen -94
USDA Human Nutrition Research Center

Bonnie Heidell -93
Natural Heritage Program

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State Science Fair

Ron Royer -93
Minot State University
Science Educator Newsletter and
Executive Committee Liaison

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Science Olympiad

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Robinson High School

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AAAS Mini-Grant Coordinator
Junior Academy Liason

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Dorothy Johansen -95
Mayville State University

-95

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Carolyn Brauner, Valley City State University
John Brauner Charles Ault,
Cynthia Ault Jerome Knoblich, Jamestown College
Carl Steffan,

End of Fiscal Year STATEMENT of FINANCIAL STATUS

Fiscal Year	1988	1989	1990	1991	1992	1993
B A L A N C E S H E E T						
ASSETS	31483.43	30902.44	33655.5	34486.88	38027.91	34821.98
Operating Accounts						
Checking	2579.2	611.27	1741.74	3381.24	3205.93	
Savings / Certificates	7634.09	6625.74	6232.93	2000.00		
Trust Accounts						
Scholarship Principal	15361.13	16505.83	17166.26	19536.06	23953.30	23953.30
Research Foundation	5909.01	7159.60	8514.57	9569.58	10868.68	10868.68
LIABILITIES	26106.64	26958.71	28165.83	30865.64	37261.98	34821.98
Advanced Dues Payments	1585.00	1285.00	585.00	760.00	1540.00	
Restricted Purpose Funds						
Scholarship Principal	15361.13	16505.83	17166.26	19536.06	23953.30	23953.30
AAAS Grant	900.00		1900.00	1000.00	900.00	
Research Foundation	5909.01	7159.60	8514.57	9569.58	10868.68	10868.68
Cash	2351.50	2008.28				
ACCUMULATED SURPLUS	5376.79	3943.73	5489.67	3621.24	765.93	
CHANGE in SURPLUS		-1433.06	1545.94	-1868.43	-2855.31	-765.93
O P E R A T I N G C A S H F L O W						
CASH on HAND 1 January	10135.49	10292.90	8415.30	8027.02	5381.24	3205.93
RECEIPTS for Year	14258.92	9748.43	11173.58	9021.40	8144.73	
RESOURCES Available	24394.41	20041.33	19588.88	17048.42	13525.97	3205.93
DISBURSEMENTS	14101.51	11626.03	11561.86	11667.18	10320.04	985.00
CASH BALANCE 31 December	10292.90	8415.30	8027.02	5381.24	3205.93	2220.93
Increase over Year	157.41	-1877.60	-388.28	-2645.78	-2175.31	-985.00
M E M B E R S H I P						
Emeritus	60	58	59	54	56	
Students	43	53	61	104	41	
Professional	283	290	290	312	210	
Deliquent				86	115	
Dropped		58			86	
TOTALS	386	459	410	556	508	

End of Fiscal Year STATEMENT of FINANCIAL STATUS

Fiscal Year	1988	1989	1990	1991	1992	1993
O P E R A T I N G I N C O M E						
DUES	3617.00	2992.00	2680.00	2755.00	3320.00	
Reinstatements	67.00	50.00	90.00	20.00	90.00	
Current year	1965.00	1657.00	2005.00	1975.00	1690.00	
Future years	1585.00	1285.00	585.00	760.00	1540.00	
INSTITUTIONS	1950.00	2200.00	2200.00	1200.00	200.00	
U N D	1000.00	1000.00	1000.00			
N D S U	750.00	1000.00	1000.00	1000.00		
Minot State	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	
Registration Fees	1800.00	2810.00	2191.00	1377.00	1729.00	
Banquet Ticket Sales	1965.00			809.00	423.00	
Assocn ND Geographers		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					
ND Geol Society	50.00	100.00	100.00			
Subsidy	2000.00					
RRV Amer Chem Sco	300.00	300.00	350.00			
NDSU Engineering			772.04			
AWARDS PROGRAMS	1647.50	481.78	2375.20	2355.65	2226.40	
AAAS Sec Schl Research	900.00		1900.00	1000.00	900.00	
Scholarship Dividends	747.50	481.78	475.20	612.15	372.40	
ND Research Foundation				743.50	954.00	
PUBLICATION SALES	167.00	123.00	102.00	52.00	106.00	
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	

End of Fiscal Year STATEMENT of FINANCIAL STATUS

Fiscal Year	1988	1989	1990	1991	1992	1993
O P E R A T I N G E X P E N S E S						
ANNUAL MEETING	6708.68	3564.03	3928.22	3007.59	2915.66	
Speakers Expenses	2651.40	973.83	1122.07	514.00	918.16	
Meals/Refreshments	2734.36	1856.30	1929.39	1903.95	1656.30	
Printing				589.64	320.20	
General Expenses	1322.92	733.90	876.76		21.00	
AWARDS PROGRAMS	1360.00	1725.00	1100.00	1975.00	1750.00	850.00
AAAS Sec Schl Research	710.00	900.00	700.00	1200.00	900.00	
ND Science Olympiad				100.00		
ND Science/Engineer Fair	25.00			50.00	50.00	50.00
Denison Awards	500.00	450.00	400.00	300.00	400.00	400.00
ND Jr Academy Awards				325.00	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	
Proceedings	2103.84	2586.65	3133.37	2633.28	2704.00	
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	
Junior Academy			350.00	132.76		
Exec Committee	475.60	55.80	121.55		255.19	
OFFICE EXPENSES	2171.92	2376.25	1199.49	1857.55	1648.59	49.00
Postage	762.72	403.16	550.56	1194.95	924.08	
Post Office Box Rental	39.00	39.00	39.00	39.00	49.00	49.00
Duplicating	218.68	215.08	208.42	324.95	392.26	
Supplies	414.02	349.01	259.01	228.65	98.25	
Clerical Assistance	137.50	170.00	92.50	70.00	185.00	
Sec Treas Fee	600.00	1200.00	50.00			
MISCELLANEOUS	1031.47	1068.30	979.23	1811.00	1046.60	86.00
Fidelity Bond	26.00	26.00	26.00	26.00	26.00	26.00
AAAS Delegate Expenses	960.57	1000.00	911.73	1000.00		
NAAS Dues	44.90	42.30	41.50	41.50	66.60	60.00
Funds Transfers				743.50	954.00	
TOTAL DISBURSEMENTS	14101.51	11626.03	11561.86	11667.18	10320.04	985.00

End of Fiscal Year STATEMENT of FINANCIAL STATUS

Fiscal Year	1988	1989	1990	1991	1992	1993
				T R U S T	F U N D S	A C
S C I E N C E R E S E A R C H F O U N D A T I O N						
Balance 1 January	4401.44	5909.01	7159.60	8514.57	9569.58	10868.68
Donations from Members	279.00	296.50	270.00	261.50	303.00	
Allocations from Dues	678.00	544.00	438.00	482.00	651.00	
Intrest Accrued	250.57	310.09	396.97	311.51	345.10	
Organization Members	300.00	100.00	250.00			
Balance 31 December	5909.01	7159.60	8514.57	9569.58	10868.68	10868.68
S C H O L A R S H I P F U N D						
CASH INCOME						
S D G E Dividends	257.50	267.50	270.00	205.00	70.00	
I E S Industries	490.00	214.28	205.20	216.00	302.40	
CD Interest					13.59	
AAAS Sec Schl Research	900.00		1900.00	1000.00	900.00	
TOTAL	1647.50	481.78	2375.20	1421.00	1285.99	
CASH EXPENSE						
Denison Awards	500.00	450.00	400.00	300.00	400.00	400.00
Junior Academy Awards				325.00	400.00	400.00
AAAS Mini Grant	710.00	900.00	700.00	1200.00	900.00	
ND Science/Engineer Fair	25.00			50.00	50.00	50.00
Dunbar Award	75.00	175.00				
Henderson Award	50.00					
Abbott Scholarship		200.00				
TOTAL	1360.00	1725.00	1100.00	1875.00	1750.00	850.00
NET CHANGE	287.50	-1243.22	1275.20	-454.00	-464.01	-850.00
ASSETS						
SDGE Shares (1983) 250	277.06	289.48	302.00	315.18	657.40	657.40
Price 18.50	38.25	45.13	43.63	45.00	29.50	29.50
Value 4625.00	10597.55	13064.23	13176.26	14183.10	19393.30	19393.30
IES Industries (1990)			120.00	192.00	192.00	192.00
Price 120 @ 31.63			33.25	27.88	23.75	23.75
Value 3795.60			3990.00	5352.96	4560.00	4560.00
TOTAL Investment Value			17166.26	19536.06	23953.30	23953.30
Change in Total			4102.03	2369.80	4417.24	

Respectfully Submitted

Roy Lamb 11 Jan, 1993

PAST PRESIDENTS
and
Location of the Annual Meeting
of the
NORTH DAKOTA ACADEMY of SCIENCE

1909	M A Brannon	Grand Forks
1910	M A Brannon	Fargo
1911	C B Waldron	Grand Forks
1912	L B McMullen	Fargo
1913	Louis VanEs	Grand Forks
1914	A G Leonard	Fargo
1915	W B Bell	Grand Forks
1916	Lura Perrine	Fargo
1917	A H Taylor	Grand Forks
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1921	L R Waldron	Grand Forks
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1924	O A Stevens	Fargo
1925	David R Jenkins	Grand Forks
1926	E S Reynolds	Fargo
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1929	G A Talbert	Grand Forks
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1931	H E Simpson	Grand Forks
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1949	R B Witmer	Grand Forks
1950	R E Dunbar	Fargo
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1952	Glenn Smith	Fargo
1953	Wilson Laird	Grand Forks
1954	C O Clagett	Fargo
1955	G A Abbott	Grand Forks
1956	H B Hart	Jamestown
1957	W E Cornatzer	Grand Forks
1958	W C Whitman	Fargo
1959	Arthur W Koth	Minot
1960	H J Klosterman	Fargo
1961	Vera Facey	Grand Forks
1962	J F Cassel	Fargo
1963	C A Wardner	Grand Forks
1964	Fred H Sands	Fargo
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1971	Robert L Burgess	Fargo
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1973	John R Reid	Grand Forks
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1975	Arthur W DaFoe	Valley City
1976	Donald R Scoby	Fargo
1977	Om P Madhok	Minot
1978	James A Stewart	Grand Forks
1979	Jerome M Knoblich	Aberdeen, SD
1980	Duane O Erickson	Fargo
1981	Robert G Todd	Dickinson
1982	Eric N Clausen	Bismark
1983	Virgil I Stenberg	Grand Forks
1984	Gary Clambey	Fargo
1985	Michael Thompson	Minot
1986	Elliot Shubert	Grand Forks
1987	William Barker	Fargo
1988	Bonnie Heidel	Bismark
1989	Forrest Nielsen	Grand Forks
1990	David Davis	Fargo
1991	Clark Markell	Minot
1992	John Brauner(elect)	Grand Forks
1993	John Brauner	Jamestown

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