Proceedings of the NORTH DAKOTA Academy of Science

84th Annual Meeting

April 1992 Volume 46

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The PROCEEDINGS contains communications (from four 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 and $5.00 for student membership. Copies of the PROCEEDINGS may be purchased separately for $5.00 per copy, prepaid. 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
o f t h e
N O R T H D A K O T A
A C A D E M Y
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S C I E N C E

Volume 4 6 April 1 9 9 2

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

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84th ANNUAL MEETING

30 April - 1 May, 1992

Grand Forks, 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 XXXIII, the PROCEEDINGS changed to the present format, and 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 46 of the PROCEEDINGS contains 24 presentations in the four symposia offered at the 84th Annual Meeting of the Academy, 30 April - 1 May, 1992. 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 12 collegiate communications, representing all those papers presented in the A. Roger 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 6 papers are from the undergraduate competition (placed in alphabetical order by the last name of the author presenting the paper) and the second group of 6 papers are from the graduate competition (arranged in similar alphabetical order).

The third section of this volume contains the 35 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. An edited version of the PROGRAM for the Annual Meeting has been included as an Appendix.

This issue of the PROCEEDINGS also includes the Constitution and Bylaws of the ACADEMY, a list of officers, 1991-92 Committee Membership, a list of all Academy members as of 1 March, 1992, a copy of the most recent (1991) financial statement of the Academy, and a revision of the paper by Shuler and Nielsen published last year, 45, 27.

Roy Garvey
Editor

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

Department of Chemistry, North Dakota State University, Fargo, ND 58105
Energy and Environmental Research Center, University of North Dakota, Grand Forks, ND 58282
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.

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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 for Cancer Research, Heidelberg, 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 10 minutes with an allowance of 5 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 of the details of methods and procedures.

2. ACADEMY members represent a a variety of scientific disciplines; therefore, speakers should avoid "jargon" and briefly explain or define much specialized terminology as may be judged to be indispensable to the presentation.

3. Projectors for 2" x 2" 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.

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 Roger 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 Secretary, North Dakota Academy of Science. The original must not be folded; a cardboard backing 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 by 11" 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 than "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 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. In particular 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.

O U T L I N E o f M E E T I N G

Thursday, 30 April

8:00- 6:00 pm	REGISTRATION "Link", Center for Aerospace Study
8:30-12:00 am	Paleontology in North Dakota (SYMPOSIUM)
8:20-10:00 pm	Focus on Body (Contributed Papers)
10:00- 5:00 pm	Breath Monitoring and Environmental Toxicology (SYMPOSIUM)
1:20- 3:20 pm	They are not Plants (Contributed Papers)
1:20- 2:00 pm	Computer Applications (Contributed Papers)
1:20- 5:00 pm	Regional Concerns (Contributed Papers) (in conjunction with Assocn of ND Geographers)
7:30- pm	lecture featuring unpublished slides of Earth. Charles Wood, Director of Space Studies
8:30- 8:55 pm	"His just deserts" reception for Chuck Woods
9:00- 9:45 pm	Program at the Atmospherium.

Friday, 1 May

7:30- 3:00 pm	REGISTRATION Second Floor lobby, Union
8:00- 3:00 m	Junior Academy of Science (as required)
8:00-11:40 am	Survival Analysis and its Applications (SYMPOSIUM)
8:00-11:40 am	Denison UnderGraduate Competition Papers
8:00-11:40 am	Dietary Elements (Contributed Papers)
11:40- 1:20 pm	Business luncheon. Denison Competitors and Junior Academy Participants invited to be guests of the Academy at luncheon.
1:20- 5:00 pm	Molecular Mechanisms of Chemical Toxicity (SYMPOSIUM)
1:40- 5:00 pm	Denison Graduate Competition Papers
1:20- 3:00 pm	GeoScience (Contributed Papers)
3:20- 5:00 pm	Solutions looking for Problems (Contributed Papers)
5:00- 6:00 pm	No Host Social Hour Memorial Union ? ? ? Academy Members and Guests
6:00- 7:20 pm	Academy Banquet Memorial Union Ballroom President Brauner Conducting
7:20- 7:40 pm	Academy Awards Ceremony
7:50- pm	Academy lecture

A Symposium on the

PALEONTOLOGY IN NORTH DAKOTA: FOSSILS AS A RESOURCE IN RESEARCH, EDUCATION, AND ECONOMICS

North Dakota Academy of Science 1992 Annual Meeting
University of North Dakota

Symposium Coordinators and Editors
Joseph H. Hartman and Allen J. Kihm

Symposium Agenda

April 30, 1992

Introduction (8:30-8:45 a.m.)

1) *Fossils as a Resource in Research, Education, and Economics in North Dakota*:
Joseph H. Hartman, Energy and Environmental Research Center, University of North Dakota, Box 8213, University Station, Grand Forks, ND 58202 (701) 777-2551

Fossils as a Resource

2) *Fossil Vertebrates as a Resource in Research, Education, and Economics in North Dakota*:
Allen J. Kihm (8:45-9:00 a.m.), Department of Earth Sciences, Minot State University, Minot, ND 58701 (701) 857-3864
3) *Fossil Invertebrates as a Resource in Research, Education, and Economics in North Dakota*:
Joseph H. Hartman (9:00-9:15 a.m.), Energy and Environmental Research Center, Box 8213, University Station, Grand Forks, ND 58202 (701) 777-2551
4) *Microfossils and Fossil Plants as Resources in Research, Education, and Economics in North Dakota*:
Timothy J. Kroeger (9:15-9:30 a.m.), Department of Geology and Geological Engineering, University of North Dakota, Grand Forks, ND 58202 (701) 777-2821
5) *Quaternary Fossils as a Resource in Research, Education, and Economics in North Dakota*:
Allan C. Ashworth (9:30-9:45 a.m.), Department of Geosciences, North Dakota State University, Fargo, ND 58105 (701) 237-7919

Coffee/Discussion Break (9:45-10:00 a.m.)

Management and Utilization of Fossils as a Resource

6) *The North Dakota Geological Survey Fossil Resource Management Program for the State of North Dakota*:
John W. Hoganson (10:00-10:15 a.m.), North Dakota Geological Survey, 600 East Boulevard Avenue, Bismarck, ND 58505 (701) 224-4109
7) *The Role of the Federal Government in the Management of Fossil Resources*:
Dale Hanson (10:15-10:30 a.m.), Bureau of Land Management, P.O. Box 940, Miles City, MT 69337 (406) 232-4331
8) *The Perspective of the Hobbyist in the Utilization of the Fossil Resources of North Dakota*:
Earle H. Campbell (10:30-10:45 a.m.), P.O. Box 1921, Bismarck, ND 58502 (701) 255-3658
9) *Access and Utilization of the Fossil Resources of North Dakota in Primary Education*:
Mike Barnhart (10:45-11:00 a.m.), Center High School, Center, ND 58530 (2704 10th Avenue NW, Mandan, ND 58554) (701) 663-4980

Summary and Discussion (11:00-11:30 a.m.)

10) *Summary and Diagnosis of Paleontology in North Dakota*:
Allen J. Kihm (11:00-11:10 a.m.), Department of Earth Sciences, Minot State University, Minot, ND 58701 (701) 857-3864

The symposium coordinators wish to acknowledge and specifically thank the Energy and Environmental Research Center for providing support for clerical and sundry expenses for this Academy meeting.

FOSSILS AS A RESOURCE IN RESEARCH, EDUCATION, AND ECONOMICS IN NORTH DAKOTA

Joseph H. Hartman*
Energy and Environmental Research Center, University of North Dakota, Grand Forks ND 58202

In many ways, the people of North Dakota have never been more interested in fossils. Most of this interest is associated with the rather phenomenal popularity of dinosaurs. In addition, over the last decade of environmental activism, there has been renewed discussion on the disposition of fossils as a natural resource. How (or if) to regulate the use of fossils as a resource (primarily in regard to their collection) has been the subject of panel discussions, workshops, memoranda of understanding, policy statements, legislation, and a considerable amount of argument among the various vested interests. The purpose of this symposium is to openly present and discuss issues about the varied use of fossils in North Dakota. Towards this end, well-qualified people, with a deep concern about the appropriate use of fossils in North Dakota, will present basic information and their views about fossils as a resource to exploit for the purposes of academic and applied research (including museum exhibits), the public's recreational and hobbyist activities, primary and secondary education, and commercial-scale collecting.

Interest in fossils in North Dakota, particularly in dinosaurs, has been enhanced by press coverage and stimulated by a few vocal individuals, which together have promoted an attitude that North Dakota fossils should remain in North Dakota. Paleontologists from outside the state, who have undertaken research in North Dakota, have been portrayed as looters of North Dakota's fossil treasure trove. The negative publicity that "outsiders" have received for "stealing" North Dakota fossils seems inappropriate in that they have been removed for academic study that will benefit North Dakota by the knowledge gained (at others' expense). The public has been generally poorly informed about the process of paleontological research and the nature of North Dakota's fossil record. In terms of the collection of fossils by North Dakotans for North Dakotans, the state has only very recently directly supported any program that provides the means to maintain collections or establish for itself an active role in understanding the extent of its fossil resources. What we do know about North Dakota fossils has resulted largely from the blending of graduate student and faculty research from both within and outside the state as a result of indirect use of limited state funding for higher education and from a few research-specific federal grants.

Although treated by some only as a commodity to be utilized for tourist dollars or other commercial enterprises, North Dakota's primary interest in fossils should be educational, so as to promote and develop a more thorough understanding of the geological history of the state that will most appropriately serve the needs of all concerned. Education and economic considerations without attendant research by North Dakotans clearly limits how and what knowledge can be passed on to state agencies, institutions of higher learning, and the commercial sector. Only from purposeful state-supported endeavors, such as collections development, can a system be put into place that provides for the transmission of useful information to all concerned. The exhibit of fossils for tourism can be promoted, but this activity must be consistent with maintaining the educational and research value of fossils in existing programs.

Institutions, agencies, and interested citizenry of North Dakota should work together towards the scientific and public use of fossils. Existing research institutions, such as the North Dakota Geological Survey (NDGS) and the University of North Dakota Department of Geology and Geological Engineering (UND), have largely been responsible for the development and maintenance of paleontological collections and resulting knowledge about the state's fossil resources. Paleontologists at the NDGS, UND, North Dakota State University (NDSU), and Minot State University (MSU) should play a fundamental role in providing expertise and information for the purposes of fossil utilization, whether for transmission of knowledge for public education, resource management, or for display. The NDGS is mandated to maintain fossil collections for the purpose of information dissemination to the public on the basis of representative examples of North Dakota fossils. As a state agency, the NDGS can serve as a liaison for public and government agency interaction with the greater paleontological community. UND has been the paleontological research facility for the state for forty years, with its substantial fossil collections and existing undergraduate and graduate programs in geology. UND's responsibilities to the state should naturally continue to include the curation of fossil collections for the purposes of scientific study, undergraduate and graduate education, academic and applied research, and as a paleontological information resource to educators and other institutions and agencies in the state. Information from research collections maintained by other institutions, such as NDSU, MSU, and Dickinson State University, which have resulted from the research activities of their respective scholars, can be incorporated into a continuously augmented data base on the fossil collections of North Dakota. Paleontologists are fundamentally enthusiastic about fossils and, given appropriate opportunities, such as the request for help from the folks of the Pioneer Trail Museum in Bowman, are willing to be supportive and cooperative.

* *The author is an invertebrate research paleontologist with the Energy and Environmental Research Center and an associate professor in the UND Department of Geology and Geological Engineering.*

FOSSIL VERTEBRATES AS A RESOURCE IN RESEARCH, EDUCATION, AND ECONOMICS IN NORTH DAKOTA

Allen J. Kihm*
Department of Earth Sciences, Minot State University, Minot, ND 58701

Upper Cretaceous and Paleogene (lower Tertiary) strata are well represented in North Dakota, while Neogene (upper Tertiary) strata are poorly represented, with extensive Pleistocene glacial and periglacial sediments blanketing much of the state. Of this 90-million-year record, only a few rock units in North Dakota produce many fossil vertebrates. These rock units include the uppermost Cretaceous Hell Creek Formation, the middle to upper(?) Oligocene Brule Formation, and, to a lesser degree, the upper Paleocene Bullion Creek and Sentinel Butte Formations. The vertebrate fauna of the Hell Creek Formation, specifically the dinosaurs, can be used as a paradigm of the vertebrate record and its significance to North Dakota.

The Hell Creek Formation contains a diverse vertebrate fauna. Most species are small animals, including fish, amphibians, lizards, turtles, crocodilians, champsosaurs, birds, and mammals; but most famous are the dinosaurs. Dinosaurs are so popular that they have been suggested as a possible cure to North Dakota's economic troubles. A dinosaur museum, tours, and excavations have all been proposed to lure tourists (and their dollars) into the state.

Programs at the Tyrrell Museum of Palaeontology in Drumheller, Alberta, Canada, the Museum of the Rockies in Bozeman, Montana, and the South Dakota School of Mines and Technology's Museum of Geology in Rapid City, South Dakota, are cited as examples of what could be done in North Dakota. How does the North Dakota dinosaur record compare to the resources on which these successful programs were largely built? The Tyrrell Museum dinosaur collections are based upon specimens from extensive badland exposures along the Red Deer River. These strata have produced numerous complete and nearly complete dinosaur skeletons for more than 100 years (1, 2). Some specimens even preserve skin impressions (3). The Museum of the Rockies' collections feature specimens from Upper Cretaceous beds of central and eastern Montana. These beds contain a variety of dinosaur remains including the first known dinosaur nesting ground in North America (4) and a single bone bed estimated to contain more than 10,000 individuals (5). The Museum of Geology is not as focused on paleontology as the other two museums, with approximately one-third of the display space dedicated to minerals. Fossil exhibits, and the museum collections, include dinosaur material, but the museum's core exhibits are Upper Cretaceous marine reptiles from the Pierre Shale and Oligocene mammals from the White River Group. All three museums have built their programs on a strong research component and on fossil vertebrate resources from their state or province.

How does the North Dakota dinosaur record compare to the material available to these museums? Dinosaurs existed for over 150 million years. At best, the North Dakota record preserves only the last 7 or 8 million years of that history. A much longer record is preserved in the rocks of South Dakota, Montana, and Alberta. Although dinosaur material is relatively common in the Hell Creek Formation of North Dakota, well-preserved specimens are rare, and significant portions of skeletons are exceedingly rare. The most detailed study of dinosaurs of the Hell Creek Formation discovered 316 localities in one field season producing a total of 2083 *in situ* specimens. Of these, 1151 were dinosaurs, but only 19% of the dinosaur specimens were well enough preserved to warrant collection for further study. Only one partial skeleton was found (6). What does North Dakota have to offer? Although fossil vertebrates from the Hell Creek Formation have been known for several decades, they are not well documented. No comprehensive scientific study has been conducted to describe the diversity of the fauna or the stratigraphic distribution of the species. The rocks of the Hell Creek and Ludlow Formations preserve the transition from the Age of Dinosaurs to the Age of Mammals, but no study has made a comparison of the two faunas. Research into the changes associated with that transition could contribute significantly to our understanding of the worldwide extinction of the dinosaurs, as well as related topics. We do not yet know what secrets the North Dakota fossil vertebrate record may hold, because so little work has been done. The North Dakota record may be limited, but it is by no means insignificant. Its significance may, however, be greater for scientific research than it is to economic development.

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1) Cope, E.D. (1892) Proc Amer Philos Soc 30, pp 240-245. 2) Colbert, E.H. (1968) Men and Dinosaurs. E.P. Dutton and Co., New York. 3) Lambe, L.M. (1914) Ottawa Naturalist 27, pp 129-135. 4) Horner, J.R., and Makala, R. (1979) Nature 27 (5736), pp 296-298. 5) Horner, J.R., and Gorman, J. (1988) Digging Dinosaurs. Workman Pub., New York. 6) Gabriel, D.L. (1988) written comm.

* *The author is an associate professor of Earth Science and a research vertebrate paleontologist.*

FOSSIL INVERTEBRATES AS A RESOURCE IN RESEARCH, EDUCATION, AND ECONOMICS IN NORTH DAKOTA

Joseph H. Hartman*
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The macroinvertebrate record constitutes the majority of the fossil localities (containing identifiable taxa) known in North Dakota. Invertebrates from Upper Cretaceous and Paleogene bedrock strata of western North Dakota are represented by a large number of observations and collections of marine, brackish, freshwater, and terrestrial fossil assemblages of mollusks, as well as rarer finds such as crabs, lobsters, echinoids, and corals. The Quaternary glacial and postglacial record also includes a large number of mollusk occurrences.

The first North Dakota fossils discovered and used for scientific study were nonmarine mollusks collected by F.V. Hayden in the 1850s. His collections and geological observations were made along the Missouri River across the state. Hayden, along with his paleontological collaborator, F.B. Meek, used, in part, North Dakota invertebrate fossils to delineate the stratigraphic and temporal framework of what they called the Great Lignite Basin, which corresponds to the Williston Basin of today's usage. Meek and Hayden named a large number of freshwater and terrestrial mollusks, which subsequently have served as the basis for nonmarine molluscan studies in western North America. The formations they named in the Dakotas now serve as part of the stratigraphic reference standard for the northern Great Plains. Thus, North Dakota's fossil and scientific heritage includes important discoveries and collections, and significant pioneering studies.

Subsequent to early research, invertebrate fossil localities are now known from all of the named bedrock formations exposed in North Dakota. Marine invertebrates are known from the Upper Cretaceous Niobrara, Vermillion River, Pierre, and Fox Hills Formations, as well as from the brackish facies of the Breien Member of the Hell Creek Formation, and from the Paleocene Cannonball Formation. Of these strata, the greatest number of the localities have been reported from the Fox Hills and Cannonball Formations. Nonmarine invertebrates, represented mostly by mollusks, are known from the Paleocene Ludlow, Slope, Bullion Creek, and Sentinel Butte Formations, from the Paleocene and Eocene Golden Valley Formation, and from the Oligocene White River Group. Of these strata, a large number of mollusk occurrences are known from the Bullion Creek and lower Sentinel Butte Formations (the type section and "classic" fauna of the Fort Union Group of Meek and Hayden). Along with Quaternary shelly faunas, in excess of 1500 invertebrate localities are known in North Dakota from over 150 publications, thesis works, and unpublished informal sources. About 50 Cretaceous fossil occurrences are known as trace fossils, which may be assignable to invertebrate taxa.

A substantial number of the invertebrate localities known in North Dakota were discovered, collected, and first reported by graduate students through the course of thesis studies mostly undertaken at the University of North Dakota. Significantly, almost 70 percent of the North Dakotan record of Cretaceous life was first studied by graduate students and, in many cases, still represents the basis of our working knowledge of these fossils. Master's and Ph.D. thesis studies represent the first (significant) description of the shelly faunas in North Dakota of the Pierre, Fox Hills, and Hell Creek Formations; the first report of brackish fossils from the Hell Creek Formation; significant upgrades to both the bivalve and gastropod faunas, and an understanding of the paleoecology of the last epicontinental sea in North America; the first attempt to use the Paleocene molluscan record to directly correlate North Dakota nonmarine strata with the North American land mammal ages; and the first report of nonmarine mollusks from the Golden Valley Formation.

Invertebrate fossils, as a resource in research, education, and for economic development, are somewhat limited, compared to other western states that preserve a greater span of exposed geologic time. However, the interval of time available for study in North Dakota spans a particularly interesting period of geologic history, including the uplift of the Rocky Mountains; the transition from a marine- to nonmarine-dominated ecosystem; the extinction of many marine invertebrates, along with the dinosaurs near the end of the Cretaceous; and the subsequent radiation of freshwater and terrestrial molluscan taxa during the time of the dramatic adaptive radiation of mammals during the Paleogene. These geologic events, as recorded in the invertebrate record, have been documented in North Dakota by the faculty and staff of North Dakota universities. Specifically, the invertebrate fossil record has been studied primarily as a medium to train paleontologists and geologists, who, for the most part, have attended the University of North Dakota. Thus, fossil-based research in North Dakota has gone hand in hand with graduate education. Invertebrates have been little used for economic gain in North Dakota. Their primary (and important) use is in providing a means to temporally organize North Dakota's rock record and significantly contribute paleoecological data pertinent to reconstruct the ancient environments that once existed in the northern Great Plains.

* *The author is an invertebrate research paleontologist with the Energy and Environmental Research Center and an associate professor in the UND Department of Geology and Geological Engineering.*

MICROFOSSILS AND FOSSIL PLANTS AS RESOURCES IN RESEARCH, EDUCATION, AND ECONOMICS IN NORTH DAKOTA

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Microfossils and plant macrofossils represent a diversity of life that can be used to provide information on many geological problems including bio- and chronostratigraphic correlation of strata, paleogeographic configurations, and paleoenvironmental reconstructions, such as determinations of bathymetry, water chemistry, water temperature, elevation, and climate. Microfossils are broadly defined as small fossils requiring the use of a microscope during all aspects of their study. Microfossils, as a group, include representatives of each of the five kingdoms of life, and are present in some form in nearly all depositional settings. In some cases, the remains of an entire organism constitute a microfossil, while other microfossils consist of microscopic parts of larger organisms. Microfossils commonly present in North Dakota strata include, but are not limited to, foraminifera, various forms of algae, conodonts, ostracodes, fungal spores, and the pollen and spores of land plants. The study of microfossils has several advantages when compared to macrofossil paleontology: 1) the small size of the fossils permits collection from well cores and cuttings, allowing detailed study of strata found only in the subsurface; 2) microfossils are generally so common that samples containing sufficient specimens can be collected quickly from suitable outcrop sites; 3) microfossil diversity and sizes of populations are generally high within samples, permitting statistical analysis of populations; and 4) microfossils are commonly present in both marine and nonmarine rocks, and, thus, in some cases, chronostratigraphic correlation between marine and nonmarine faunas or floras is possible. Plant macrofossils include the leaves, stems, cones, trunks, and other macroscopic remains of plants, although plant root traces can also be included. Plant macrofossils are generally common, but specimens of quality sufficient for accurate identification are relatively rare and typically found only in fine-grained deposits. Plant macrofossils are generally restricted to continental and marginal marine depositional environments.

Most rocks of Phanerozoic age (last 570 million years) in North Dakota contain a variety of microfossils. Foraminifera, calcareous nannoplankton, ostracodes, pollen, and spores have been collected and studied from the Upper Cretaceous and Tertiary strata that are exposed across much of the state. Subsurface studies in North Dakota have focused on conodonts (1), but ostracodes and foraminifera (2) have also received some attention. All microfossil disciplines have considerable potential for continued research in North Dakota as systematic and associated stratigraphic data is incomplete or lacking for many microfossil groups. As a valuable research and educational resource, the Wilson M. Laird Core Library, operated by the North Dakota Geological Survey on the campus of the University of North Dakota, contains most of the cores produced during the drilling of water and oil wells in North Dakota. Most of the core samples are of Paleozoic strata, but Mesozoic and some Cenozoic strata are also contained in the core library. In addition, the core library preserves samples of well cuttings from most oil wells, although only specified intervals are sampled in development wells. These cuttings are available for use in microfossil, as well as other, studies.

Because the studies of microfossils and plant macrofossils are relatively specialized fields of paleontology, most microfossil study and research is begun at the level of the university graduate student. Undergraduate geology courses generally introduce students to microfossils because of their considerable utility in biostratigraphy and their importance as contributors to total sediment accumulation. Plant macrofossils are also commonly included in undergraduate courses in earth science and botany curricula. Both microfossils and plants could also be utilized effectively if introduced in high school and even elementary school classes devoted to earth science. They might be included in demonstration sets to illustrate the diversity of fossil morphologies and types of fossil preservation.

The primary economic value of microfossils involves their use in oil, gas, and coal exploration studies, although concentrated deposits of microfossils, such as chalk from the Niobrara Formation, could be used by industry. Plant macrofossils are of interest to both professionals and hobbyists, and are commonly available in rock shops, ranging from leaf compression fossils to slabs of petrified wood. Because coal represents concentrated deposits of plant macro- and microfossils, the dominant economic impact of these fossils is through the mining and utilization of coal.

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QUATERNARY FOSSILS AS A RESOURCE IN RESEARCH, EDUCATION, AND ECONOMICS IN NORTH DAKOTA

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North Dakota, except for the southwestern part of the state, is covered by Quaternary deposits. With the exception of the lower strata exposed in the cliff sections on Lake Sakakawea, the Quaternary sediments, assigned to the Coleharbor Formation, were deposited during the latter phase of the Pleistocene glaciation and the Holocene Epoch. As glacial retreat did not begin in the midwest until after 14,000 years ago, North Dakota's landscape is young, mostly postdating 12,000 years ago. Fossils of mammals, amphibians, fish, mollusks, insects, ostracodes, plants, and diatoms have been reported from sediments of lakes that formed on the deglaciated surface. The number of studies of those fossils is small (1), and the best word to summarize the resource is underutilized.

Since deglaciation, the climate of North Dakota has undergone significant changes, which is exemplified by the extinction of woolly mammoths (*Mammuthus primigenius*), which lived along the shores of glacial Lake Agassiz (2). These and other Quaternary fossils of North Dakota are an important resource as we attempt to understand the complexities of significant climate change on plant and animal communities, and will become more important as we try to understand the effects of human-induced climatic change on local and global ecosystems. For example, computer simulations, based on increased values of atmospheric carbon dioxide, project North Dakota summers that will be both warmer and dryer than present. Such a prospect poses many questions. How will global warming affect native plant and animal species? Is the extinction of rare species an inevitability in the fragmented populations as they currently exist? Will wildlife refuges continue to protect species for which they were designed, or will those refuges no longer serve their purpose as organisms will no longer be suited to the altered habitat? What changes might occur between established crops and potentially new insect pests?

Policy-makers faced with these questions will need significant and broad-based information from the scientific community. Directly analogous clues as to the behavior of plants and animals during climatic change are available from studies of the postglacial fossil record. Climatic warming, of similar magnitude to the projected change of global warming, occurred as a natural phenomenon in our recent past. During the Prairie Period, from 8500 to 4000 years ago, summers in western Minnesota and Iowa were warmer and dryer than now. Lake levels regionally were several meters lower. The effects of this change in North Dakota are essentially unknown. No well-dated pollen diagrams, illustrating the changes in the abundance of pollen types (and thus vegetation) through time, exist for anywhere in North Dakota. The only fossil beetle assemblages that have been studied are from the time of deglaciation (3). The only detailed information that is available for the Prairie Period in North Dakota is for the effect that climate change had on the salinity of Devils Lake as interpreted from studies on diatoms (4). Although not evident from its lack of study, North Dakota has an excellent Holocene fossil record. In view of the projected global warming, there is more urgency than ever to undertake directed Holocene fossil studies on climate change to understand the evolution of climate to our present regional and global setting.

Some North Dakota Quaternary fossil assemblages are spectacular in the detail of their preservation. At the 9750-year-old Seibold Site (5), on the Missouri Coteau, fish were preserved with every scale intact. In the same assemblage, part of the gut of a whirligig beetle was still present within its abdomen. The excellence of preservation of these fossils is as fine as that reported anywhere in the world. Fossils that are preserved in such detail may well contain degraded DNA that can be reconstructed using polymerase chain reaction (PCR) and other molecular genetic techniques. Extraction of DNA from these fossils will be attempted by scientists, at the University of North Dakota Biology Department and the North Dakota State University Geosciences Department, through a jointly funded Molecular Population Genetics Laboratory. Extraction of DNA from well-dated fossil assemblages holds the capability of answering fundamental questions about the potential for change in the genome as a result of human-induced global warming.

Dinosaurs are wonderful fossils because their enormous size and strange shapes hold our imaginations like no other fossils. However, when interpreting the recent climatic history that bears on our future survival, the bones of these monsters of the dim and distant past do not hold a candle to the data available from microscopic diatoms, pollen grains, and fossil beetle fragments of the Quaternary record.

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* *The author is a professor in the NDSU Department of Geosciences, codirector of the NDSU Quaternary Entomology Laboratory, and director of the ASEND Population Change and Genetic Diversity Focus Group.*

THE NORTH DAKOTA GEOLOGICAL SURVEY FOSSIL RESOURCE MANAGEMENT PROGRAM FOR THE STATE OF NORTH DAKOTA

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The North Dakota Geological Survey (NDGS) Fossil Resource Management Program was inaugurated in 1983 primarily in response to concerns about the historic and ongoing removal of fossils from the state. The program has evolved to include three primary objectives: 1) to promote public understanding and awareness of the importance of North Dakota's fossil resources through educational activities, 2) to conduct research to determine the types of organisms that inhabited North Dakota at various times in the geologic past and the types of climates and environments in which they lived, and 3) to identify and preserve North Dakota's significant fossil sites and specimens. I will focus on the last of these objectives.

There are three categories of lands in North Dakota: 1) lands administered by agencies of the federal government, 2) lands administered by the state of North Dakota or its political subdivisions, and 3) privately owned lands. The NDGS takes an active role in managing paleontological resources on each of these. The NDGS has signed agreements with the U.S. Forest Service (USFS)-Custer National Forest (1986), federal Bureau of Land Management (BLM) (1988), and the U.S. Army Corps of Engineers (Corps) (1991) to cooperatively identify, manage, and protect paleontological resources found on lands in North Dakota under their jurisdiction. These agencies issue permits for collecting vertebrate fossils from their respective lands. Through the agreements, the NDGS reviews permit applications and recommends to the federal agencies whether or not the permits should be issued. These agreements also provide for information exchange between the NDGS and the federal agencies regarding fossil sites located on the federal lands. When potentially significant paleontological sites are discovered on these federal lands, the NDGS assists the federal agencies in assessing the significance of the sites, and, if the sites are threatened, will recommend appropriate mitigation measures. These federal agencies also now require (USFS, Corps) or highly recommend (BLM) that permit holders not affiliated with a North Dakota institution, after appropriate study, deposit a representative sample of specimens collected from the federal lands in North Dakota with the NDGS (although the fossils remain the property of the federal government).

North Dakota Century Code 54-17.3 (as of 1989) gives the North Dakota Industrial Commission, acting through the State Geologist, the responsibility to manage and protect significant paleontological resources located on land owned by the state of North Dakota or its political subdivisions. The NDGS was given the responsibility to formulate rules that would assure that the scientific and casual (hobby) collecting of fossils from these state lands would be conducted under conditions that adequately protect and preserve the public lands and fossil resources so that information gained about the fossil resources can be incorporated into North Dakota's overall resource management plans. For example, the NDGS evaluates state-owned oil and gas lease tracts for possible impact on paleontological sites.

Under Administrative Code 43-04, a permit is required to collect significant paleontological resources from state lands. Generally, only vertebrate fossils and vertebrate-bearing localities are considered significant paleontological resources. Invertebrate, plant, and trace fossils and the localities where they are found are not considered significant paleontological resources unless they are determined to be significant by the State Geologist. Different types of permits are issued by the NDGS depending on the type of fossil collecting to be done (surface collecting or excavation) and the credentials of the collector (professional or hobbyist). The rules prohibit commercial collecting of fossils from state lands. Also, all significant paleontological resources collected from these lands remain the property of the state, and permit holders not affiliated with a North Dakota institution are required to deposit with the NDGS a representative sample of fossils collected, after an appropriate period of study.

The state of North Dakota has no jurisdiction over paleontological resources found on privately owned land. Collecting fossils from private land is, therefore, at the discretion of the landowner. The NDGS does inform landowners of significant fossil resources on their land, and encourages them to protect those sites. One form of protection for private sites is through the state's Natural Areas Registry Program. Currently, three significant fossil sites are on North Dakota's Natural Area Registry (e.g., Little Badlands Natural Area).

An important part of the NDGS's Fossil Resource Management Program is the preservation and display of North Dakota fossils. North Dakota Century Code 54-17.4 directs the NDGS to operate and maintain a public repository for North Dakota fossils. In July 1991, the State Historical Society and NDGS signed an agreement to cooperate in developing that State Fossil Collection and exhibits of North Dakota fossils at the North Dakota Heritage Center.

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THE ROLE OF THE FEDERAL GOVERNMENT IN THE MANAGEMENT OF FOSSIL RESOURCES

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Most federal land managing agencies have not given a high priority to managing fossil resources. Fossils do not add money to the federal coffers, do not often conflict with other activities that do, and have little specific legal protection. The lack of federal laws directly addressing paleontologic resources is one of the basic reasons for the low level of management interest. There are existing laws that give general guidance to each agency, such as the Federal Land Policy and Management Act of 1976 (FLPMA) for the Bureau of Land Management (BLM) and the National Forest Management Act of 1976 (NFMA) for the U.S. Forest Service, but these rarely address the management of individual resources. Agency regulations are based on laws; but without specific laws, paleontologic regulations have been slow in coming. There have been efforts to create regulations for paleontologic management, prompted by public interest, but none of the proposed regulations have been finalized.

Federal agencies often have very different goals and missions. These range from recreation, protection, and interpretation for the public, typified by the National Park Service, to full multiple use, such as the BLM and the Forest Service, to site-specific project management, such as the Army Corps of Engineers. Because of these widely divergent goals, the paleontologic resources receive widely divergent management and protection by these agencies. Budgets, manpower, and local interest can also affect the level of management of paleontologic resources, even within an agency.

Allocation of fossil resources presents difficult choices for federal agencies, especially those mandated for multiple-use management. Options may include on-site display and interpretation, collection by scientific institutions for study and museum display, commercial operations, and hobby interests. Trying to balance these often opposing uses is rarely an easy job. In some cases, these choices are addressed by existing laws and regulations or agency policy. For example, commercial operations are illegal on federal lands unless specifically authorized by law or regulation.

Collecting of "significant" fossils is allowed only by permit issued to a qualified institution. Significant fossils are defined by policy as all vertebrate fossils and unique invertebrate and plant fossils. Other invertebrate and plant fossils can be collected by anyone without a permit. The permit system is designed to assure that important fossil material will be excavated, prepared, and stored using proper scientific methods. The permit also assures that the fossils remain in public ownership, thereby guaranteeing the material will be available for study and the public benefit.

Commercial collecting of fossils on federal lands is presently not allowed. There is, however, increasing pressure from the commercial sector to allow collecting on federal lands. Specific regulations have to be written to authorize commercial collecting. For commercial operations in general, the federal government collects a fee or royalty based on the value of the resources being removed. A fee system would be very difficult to develop and enforce because accurate assessment of the value of a fossil cannot be determined prior to discovery and until the material is fully prepared and sold. A royalty assessed after a fossil is sold demands detailed record-keeping and inspection, a time-consuming and expensive procedure for both the collector and the government.

Fossils on federal land are public property. The determination that the public would not incur significant losses by allowing fossils to pass into private ownership through commercial sale or hobby collecting is not easily made. The significance of a fossil is determined not only by what the fossil is, but often by where it is found, the rock it is found in, and associated material. A relatively common fossil in one area may be very significant in another, or in an atypical association or setting. Therefore, it is not always accurate to predetermine that a fossil or a group of fossils are not significant. Allowing commercial or hobby collectors to collect only nonsignificant fossils would require a case-by-case, on-site determination of their significance, representing what is probably an unworkable system. The trade-off for allowing hobby and commercial collecting would be the loss of important scientific information. Many commercial collectors are able to assess and record scientific data in the field and workshop, but once a fossil is sold, that fossil may never be available for future research.

Management of fossil resources by federal agencies to date has been limited. The lack of federal laws, money, and interest has lessened the incentive for development of management policies from within agencies. Until specific laws are passed or the public demands increased management, there will be little change in the federal government's handling of the fossil resource. If collecting of fossils by all the various public interests is to be allowed under an increased level of management, then resource trade-offs must occur. Paleontology is a developing science. There are no established guidelines or simple solutions to the problems of managing fossils as a resource.

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THE PERSPECTIVE OF THE HOBBYIST IN THE UTILIZATION OF THE FOSSIL RESOURCES OF NORTH DAKOTA

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North Dakota has an important resource of paleontological material that should be collected, researched, preserved, and displayed. The question is, who should do the work? Should these jobs be left solely to the few professional paleontologists available in North Dakota? Could the professional paleontologist use some help? There are over fifty thousand hobbyists who belong to organizations dedicated to rockhounding in the United States. There are at least twice that number who do not belong to any organized group dedicated to fossil and mineral collecting. Over 250 of these people live in North Dakota, and they could be a great resource to the professional paleontologist. Hobbyists can be the eyes, ears, and legs for the discovery and collection of localities. Hobbyists range in age from five to ninety-five, and their sphere of knowledge of paleontology ranges from knowing very little to the level of expert. These people should not be sold short. They are willing and eager to learn proper techniques for collecting, preparation, study, and display of their finds. Granted, there are those few hobbyists who feel that federal and state laws were meant for others. Those few have given the group as a whole, to some extent, a bad image, which has resulted in the heartburn and anguish (and disfavor) of professional paleontologists.

N. Gary Lane (1) has stated: "The interplay between scientific paleontology and amateur fossil collecting is complex and can be frustrating for both sides. We all know of splendid examples of fruitful cooperation between amateurs and professionals." Of course there are stories concerning the interaction between hobbyists and paleontologists, and vice versa, that could be classed in the horror category. If we learn from these experiences and continue to communicate, we both will be much better for the experience. John Pojeta, Jr. (2) stated that "paleontology ends up speaking with many voices. The net result is that none of them are heard." To rectify this problem he added that the Paleontological Society needs to "accept a leadership role among the widest possible community of persons interested in paleontology, including amateurs and professional collectors."

There are a great many people who collect fossils who do not make their living as professional paleontologists. In fact, the American Federation of Mineralogical Societies (AFMS) alone has over 50,000 members, of which as many as 15,000 are interested in collecting fossils. Until recently, this organization has had very little communication with the professional community that numbers only a few thousand. As an example of their commitment to scholarship in earth science, AFMS has provided 12 awards (of $2,000 per year for two years) to graduate students in earth science programs. In making these scholarships, AFMS honors as well the educators, who are selected through a nomination and review process, to choose the students who will receive the award. Many other scholarships are granted by affiliated AFMS societies interested in the promotion of earth science studies.

The hobbyist is greatly concerned about the limited access to public land to search for and collect fossils. This problem is not limited to federal land, as many states have also considered or passed restrictions on access to much public land. These limitations will eventually take a toll on the entire paleontological community, hobbyist and professional alike. If allowed to continue, these actions could stifle the hobbyist's contribution to the research of the professional paleontologist. Hobbyists feel they should be allowed to surface collect, using hand tools, on state and federal public lands, without obtaining a permit. The question arises, because of what is considered to be a significant fossil, should the hobbyist be allowed to collect vertebrate fossils? Will their collection be the exclusive domain of the professional paleontologist?

The solution to most of the problems facing the hobbyist in paleontology is to have better communication with professional paleontologists and the regulators of state- and federally administered property. Mel Albright (3) wrote: "Far from being a danger to science, amateurs are the wellhead from which progress comes—including the science of paleontology. We simply cannot afford to leave science in the hands and minds of the experts alone. The influence and enthusiasm of amateurs is far too important."

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* *The author is a North Dakota rock and fossil hobbyist, and past president of the Rocky Mountain Federation of Mineralogical Societies, Inc.*

ACCESS AND UTILIZATION OF THE FOSSIL RESOURCES OF NORTH DAKOTA IN PRIMARY EDUCATION

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Nothing fires the imagination of a child like dinosaurs. Dinosaurs, and children's love of them, have been exploited in virtually every way conceivable, as evidenced by the profusion of dinosaur paraphernalia, television programs, snack foods, etc. This is not necessarily a bad thing. Many present-day paleontologists have stated that their fascination with dinosaurs as children led them into paleontology. Just what lies at the root of this fascination is unclear. Maybe it's the size of dinosaurs, or their violence, or maybe it's a kind of thankfulness that we do not have to contend with such monsters in our lives. Maybe it's not important what the appeal is, just that it's there.

There is probably no easier lesson for a teacher of elementary children to prepare than to study dinosaurs. The interest is built in, they will do anything to know more about them. The problem is that often dinosaurs are treated as a separate entity, without the context on how they lived, or on ecosystems in general. Teachers must take the initiative to find out more about the world in which the dinosaurs existed. For, while there is abundant information specific to dinosaurs, and about paleontology in general, there is little else to be found concerning the paleontology in North Dakota.

This is not to say that paleontological research is not being done in North Dakota. One only needs to read the newspaper, or attend sessions of the North Dakota Paleontological Society, North Dakota Academy of Science, or other meetings, to know that there is considerable paleontological research work being conducted in this state. A problem, from the point of view of a teacher, is that this information is not reaching the schools at the primary or secondary level. Aside from the well-publicized dinosaur finds (and, of course, petrified wood), most North Dakotans would be unable to say what kinds of fossils can be found in this state. The fossil resources in this state must be identified and then presented to teachers and others in a form they can understand. To this end, museums or curated displays are essential to the education of North Dakotans about their state's fossil history. To be sure, a museum could be a tourist attraction, and that should be a consideration in its management, but a museum's primary purpose must be to educate. Accordingly, if fossil specimens are not properly studied and prepared, this purpose will be lost.

Many students are surprised to see the condition of fossils before preparation. They can easily be shown that the geologic context in which the fossil is found is as important as the fossil itself. For this reason, in addition to the benefits of museum displays, earth science teachers need access to areas where fossils are known to be present to examine and study both the fossils *in situ*, the methods to prepare them for removal, and the paleoenvironmental context in which they are found. An example of such an area can be found south of Mandan in strata of the Hell Creek Formation. John Stumpf has expressed an interest in allowing student groups to study fossils found on his land. At his request, this area is now under the supervision of the North Dakota Paleontological Society. To provide supervised development, as well as public access, planning is presently underway to make this area a registered Natural Area under the North Dakota Parks and Tourism Department and Nature Conservancy. As stated earlier, a museum display is an important element in the education of students (and adults alike), but access to field areas, where students can study (not simply collect) the fossils in place, provides many additional educational benefits. For example, I teach in an area where there are some of the largest coal fields in North Dakota. I try to use the presence of these coal deposits to relate the geologic past to students in a meaningful way. Coal mining is not some textbook abstraction to these students. Access to these coal fields has made the job of teaching about the prehistory of North Dakota much easier; the student is already interested. In the same way, access to fossiliferous areas in North Dakota will enhance the ability of teachers to teach and students to learn about the methods of science and the fossils and geologic history of North Dakota.

In summary, the education of primary- and secondary-level students in North Dakota would be significantly enhanced by access to 1) information about what fossils are found in North Dakota, 2) areas of fossiliferous strata for student study, and 3) museum exhibits of educational value. Adults need to see the value of the interest in fossils as a means to developing good study habits and general interest in science; students need to know there is more to be gained through study than solely how much money can be generated through the exploitation of dinosaurs.

* *The author is a high school science teacher.*

Presiding: Gwen M Schelkoph, USDA/ARS/HNRC

Physical

10:00 am Demonstration of the Usefulness of Gas Velocity Measurement Devices for the Determination of Industrial Boiler Performance and Long-Term Predicitions of Stack Gas Emissions.
Grant I Schelkoph*, Energy and Environment Research Center, Grand Forks 58202

10:35 am Air Pollution and Air Quality in North Dakota.
Gary D Helbling*, Environmental Engineer, North Dakota State Department of Health and Consolidated Laboratories, Bismarck, 58502

11:10 am Real-Time Measurement of Fine-Particle Emissions from Particulate Control Devices.
Stanley J Miller* and Dennis L Laudal, Energy and Environmental Research Center, Grand Forks, 58202

Biological

1:00 pm Introduction.
Gwen M Schelkoph*, Human Nutrition Research Center, Grand Forks, 58202

1:15 pm Non-Invasive Approach for Detection of Lung Cancer.
Hugh J O'Neill*, Sydney M Gordon, Jan P Szidon, and Robert D Gibbons, IIT Research Institute, Chemical Science Section, Chicago, IL 60616

2:00 pm Analysis of Exhaled Volatiles from Premature Infants with Respiratory Distress: Non-Invasive Health Monitoring.
William T Potter*, Diana Quintero, Paula Morris, Department of Chemistry, University of Tulsa, Tulsa, OK 74104 and George P Giacoia, Division of Neonatology, University of Oklahoma, Eastern Oklahoma Perinatal Center, Saint Francis Hospital, Tulsa, OK 74136

2:40 pm Refreshment / Discussion BREAK

3:00 pm High Resolution Tunable Diode Laser Spectroscopy for Isotope Analysis -- Biomedical Applications.
Peter S Lee*, Biomedical Science Department, GM Research Laboratories, Warren, MI 48090

3:35 pm The Use of Breath Pentane as a Marker of Clinical Illness.
Edwin J Zarling*, Loyola University, Department of Medicine, Maywood, IL 60153

4:10 pm The Role of Breath Monitoring in Exposure Analysis.
James H Raymer*, Edo D Pellizzari, and Richard W Slauter, Research Triangle Institute, Research Triangle Park, NC 27709

THE DEMONSTRATION OF THE USEFULNESS OF GAS VELOCITY MEASUREMENT DEVICES FOR THE DETERMINATION OF INDUSTRIAL BOILER PERFORMANCE AND LONG-TERM PREDICTIONS OF STACK GAS EMISSIONS

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Abstract

In order to better understand the environmental impact of the steam boilers on the campus of the University of North Dakota (UND), UND Plant Services requested the Energy and Environmental Research Center (EERC) to conduct a boiler performance study. This study included the demonstration of the use of permanent gas velocity measurement devices in conjunction with flue gas O_2 measurements for determining individual boiler performance on a continuous basis. From the information collected by this method and particulate sampling data from the stack, long-term predictions for stack emissions from an industrial boiler may be possible.

AIR POLLUTION AND AIR QUALITY IN NORTH DAKOTA

Presented to
1992 North Dakota Academy of Science
Grand Forks, North Dakota

Presented By
Gary D. Helbling
Environmental Engineer
North Dakota State Department of Health
and Consolidated Laboratories

OUTLINE

The outline of this presentation will contain the following topics:

1. Discussion of different types of air pollution.
2. Sources of air pollution.
3. Overview of specific air pollution sources in North Dakota.
4. Different types of air pollution control devices.
5. Effects of air pollution
6. North Dakota Air Pollution Control Rules and Standards.
7. Question and answer period.

REAL-TIME MEASUREMENT OF FINE-PARTICLE EMISSIONS FROM PARTICULATE CONTROL DEVICES

Stanley J. Miller* and Dennis L. Laudal
Energy and Environmental Research Center, University of North Dakota, Grand Forks, ND 58202

Emissions of fine particles from coal combustion systems are of concern because these particles are likely to be deposited in the lower respiratory system through normal breathing, and hazardous trace elements such as selenium and arsenic are known to be concentrated on such fine particles. In addition to causing adverse health effects, fine-particle emissions have an impact on atmospheric visibility. Particles which are the most efficient at scattering light are in the 0.2- to 2-μm range, and, when present in sufficient concentrations, these fine particles will cause serious visibility impairment. Therefore, the emission of fine particles is an issue both because of potential adverse health effects and visibility impairment in the atmosphere.

The Energy and Environmental Research Center (EERC) is conducting research to improve the fine-particle collection efficiency of fabric filters and electrostatic precipitators (ESPs). This required development of superior methods to quantify fine-particle emissions. The standard method for measurement of particulate emissions is EPA Method 5 or 17, which determines the average total particulate emissions over a long time period, such as two hours, by collecting the sample on a heated filter. Because the filter must be cooled and desiccated to a constant weight, results are frequently not available until the following day. Since particulate emissions may be dominated by short-term transient events such as bag cleaning in a fabric filter or plate rapping in an ESP, real-time emissions data were needed to facilitate development of methods to improve collection of fine particles in particulate control devices.

The EERC chose two instruments that are commercially available from TSI, Inc., and adapted them for monitoring particulate concentrations in hot, moist flue gas. Near real-time measurements in the size range from 0.5 to 30 μm are conducted with an aerodynamic particle sizer (APS). The primary advantages of the APS are high resolution and a short sampling time of about 20 seconds. The APS system also includes a dedicated computer for instrument control, on-line data monitoring, and long-term data storage. The second instrument is a differential mobility particle sizer (DMPS), which measures the size and concentration of submicron particles (0.01 to 1.0 μm). The DMPS includes an electrostatic classifier, which removes a predictable fraction of the particles within a narrow size range and passes these on to a condensation nucleus counter (CNC) where the particles are counted. To obtain a complete distribution in this size range requires a sampling time of from 10 to 25 minutes. However, the CNC can be used independently of the electrostatic classifier as a continuous real-time monitor of the total submicron particle count. Since the APS and DMPS were designed for ambient conditions, a sampling and dilution system had to be designed to allow continuous sampling of flue gas while preventing moisture condensation in the instruments. A second purpose of the system was to dilute the sample in cases where particle concentrations were sufficiently high to cause instrument error. After several design iterations, a dilution system was chosen which included a recirculation pump, condenser/dryer, and final filter, which provided dry, particle-free, dilution gas in the ratio of about 10 parts recirculation gas to 1 part flue gas. The system has been successfully employed at EERC to monitor particulate emissions for a number of research projects, and has played a key role in the development of superior fine-particle control technologies.

NON-INVASIVE APPROACH FOR DETECTION OF LUNG CANCER

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Breath analyses were performed to determine whether lung cancers (adeno- and squamous cell carcinomas) could be detected noninvasively by the presence of specific biochemical markers in the expired alveolar air[1]. Subjects were screened in four categories; lung cancers, smoking controls, lung diseases other than cancer, and cancers other than lung. The lung cancer patients were propensity score matched to the smoking control group on the basis of sex, race, pack years smoked, number of years quit, airways distribution, occupational exposures, and socioeconomic status. Staging data were based either on the pretreatment clinical diagnosis or postsurgical treatment, pathological stage.

Sample collection was performed by allowing each subject to inhale purified (charcoal/dry ice trap) zero-grade air through a Rudolph breathing valve and exhaling into a 50 L Teflon bag. Two sequential 20 L aliquots were then removed from the bag by pumping through a Tenax GC sorbent cartridge collector. Sampling was performed both in the AM and PM of the same day, thus generating two "replicate" AM and PM sample subsets. The Tenax GC sorbent cartridges were then thermally desorbed, and the breath components delivered to a high-resolution capillary gas chromatographic column and screened by mass spectrometry using data enhancement algorithms for spectral clean up. Each breath sample contained between 300 and 400 components.

Statistical evaluation of the data was performed using stepwise discriminant function analysis on two different sets of data files. The first screening compared differences between thirteen pre- and postsurgical patients, and the resulting data tested against 25 unmatched lung cancer patients and 25 smoking controls, while the second evaluation intercompared all four subject categories. The components used for screening the pre- and postsurgical patients data files were selected on the basis of; (1) components which occurred sparsely in preoperative patients but absent in postoperative patients, (2) compounds which showed marginal differences between the two groups, and (3) compounds suspected of arising from the mevalonate pathway. These three sources of potential diagnostic markers yielded 22 compounds for screening. The set of six sparsely occurring compounds played a central role in discriminating the two test groups (unmatched lung cancer patients and smoking controls). None of the trials in which they were absent resulted in a small number of false positives in the smoking control group. Four components -- tolualdehyde, oxepanone, and two unidentified components (Nos. 672 and 1424) -- were not present in all lung cancer patients, but 68% of the patients had at least one of these components present. When they are present, they appear to be good markers. When the two additional sets of markers are added to the testing, the ability to pick out positives from the lung cancer group, without appreciably adding to the false positives of the smoking control group, was enhanced. Out of the 22 compounds screened, 11 appeared to be the most useful in discriminating between the two groups.

Discriminant functions analysis of the total subject population included 30 matched presurgical lung cancer patients, 30 matched smoking controls, 14 unmatched presurgical lung cancer patients, 10 subjects with lung diseases other than cancer, and 10 subjects with cancers other than lung. Of the 418 components employed for preliminary statistical screening, 73 compounds were observed, for which the assumption of homogeneous proportions could be rejected at the $\alpha < 0.05$ level of significance. A series of stepwise discriminant analyses were then performed on all 73 compounds and their ability to correctly classify the four subject categories determined.

The application of this technique toward characterizing liver disease, diabetes mellitus will also be discussed, along with the possible role it may play in screening novel intermediates associated with the mevalonate pathway.

[1] Non-invasive Approach for Detection of Lung Cancer, Grant No. CA37056, National Institutes of Health, Bethesda, MD., October, 1990.

ANALYSIS OF EXHALED VOLATILES FROM PREMATURE INFANTS WITH RESPIRATORY DISTRESS: NON-INVASIVE HEALTH MONITORING

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Premature infants can exhibit several clinical conditions which result in poor levels of tissue oxygenation. The two most common conditions are fetal respiratory distress syndrome (FRDS) and persistent fetal circulation. Both of these conditions require oxygen-supplemented artificial ventilation. Relatively high levels of oxygen supplementation (two-fold or higher) may be required for efficient peripheral tissue oxygenation. The prolonged exposure to high oxygen tensions can induce oxygen-mediated free radical injury to several organ systems such as the lung and brain which receive the highest oxygen exposure. Here we present our efforts to determine whether non-invasive air-sampling of exhaled volatiles from newborns can be used to monitor the balance between hypoxic stress induced by the impaired tissue oxygenation and oxidative stress induced from excessive oxygen supplementation.

Exhaled volatiles are collected from newborns with endotracheal intubation. The patient population includes infants on respirators with elevated oxygen tensions for FRDS and infants on respirators for CNS complications or apnea without elevated oxygen tensions. Individual sampling consists of collection and comparison of both the inlet air (filtered through activated charcoal) and the exhalation air. The air samples are adsorbed through calcium sulfate water traps onto mixed-bed activated graphitic carbon/ carbon molecular sieve traps. The samples are analyzed using ballistic thermal desorption gas chromatography with either mass spectrometry or flame ionization detection (TD GC-MS, GC-FID).

The overall chromatographic fingerprints are compared to the daily clinical diagnostic evaluation for each patient and interpatient population variation. Compounds of interest have been initially identified by reference to a computer-based reference mass spectral library and previously published retention indices.

At present, we are still expanding our patient and compound data base, but the studies suggest that several compounds may be correlated to clinical prognosis. Our preliminary results indicate that the concentration ratios of some aldehydes (such as 2-hexenal) to alcohol byproducts may be increased for patients under oxidative stress conditions at high oxygen tensions. Presumably this reflects the greater extent of membrane lipid peroxidation which occurs at the elevated tensions.

There also appears to be a significant variability in alkane production related to improved peripheral tissue oxygenation. It appears that the optimal level of oxygen supplementation for infants with respiratory distress may possibly be determined by monitoring the relative amounts of both hydrocarbon and aldehyde exhalation products.

HIGH RESOLUTION TUNABLE DIODE LASER SPECTROSCOPY FOR ISOTOPE ANALYSIS - BIOMEDICAL APPLICATIONS

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A high resolution tunable diode laser spectroscopy system for isotope analysis is described (1,2). The system combines the spectral specificity of isotopic molecules with the high spectral resolution and power density of infrared diode lasers. The tunable laser is a buried layer design with a 4 micron PbTe active layer (Laser Photonics, Bedford, MA). The infrared laser radiation is collimated, passed through the sample cell and the reference cell, and focused onto the detectors. The sample beam is folded multiple times to provide a detectable optical absorbance for low concentration measurements. A software package controls the system and processes the signal with a PC.

The principle and operation of the system were demonstrated by the noninvasive measurement of physiological levels of isotopic CO naturally present in exhaled human breath with essentially no sample preparation required. Physiological implications of the sample application as well as the capabilities/limitations of the system are presented. Some potential biomedical applications are explored.

The system is suited for the detection and measurement of minute amounts of infrared active compounds present in a huge background of air with minimal or no sample preparation required. This is because of the following:

1) the high spectral resolution of the system allows the measurement of molecular absorption without the interference of other infrared active molecules such as water vapor and carbon dioxide;

2) the transparency of infrared radiation to the main constituents of air (nitrogen, oxygen, argon) allows the detection of minute amounts of compounds in breath with minimal sample preparation.

The simplicity and speed in obtaining such data suggests that fundamental physiological information may be derived from non-invasive measurements. This makes it useful for many biomedical applications.

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THE USE OF BREATH PENTANE AS A MARKER OF CLINICAL ILLNESS

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Pentane is a five carbon hydrocarbon which is released when an omega-6 unsaturated fat undergoes peroxidation by a free radical of oxygen. The lipid is destroyed, and the cellular membrane in which it is located may loose its integrity. Excessive peroxidation results in cell death. Lipid peroxidation is ongoing, and represents a balance between the oxidative forces and the antioxidant activity of both endogenous enzymes (superoxide dismutase, catalase) and exogenous chemicals (retinol, tocopherol, beta carotene, and ascorbate). Studies regarding peroxidation initially focused on food spoilage and later on in vitro biological systems. Over the past decade, pentane has been sought in humans. Initial attempts at measurement were complicated by concentrations lower than the detection limit. This difficulty was handled by the use of rebreathing apparatus or extensive breath collections followed by pentane concentration. In 1987 a modified method for pentane detection described the collection of only the alveolar portion of a single breath sample, which was then subjected to an on line concentration and analysis by gas chromatography (1). Normal values of excreted alkanes were defined, however the tissue source of this basal alkane production was unknown. Two possible sources were non-tissue intraintestinal food degradation and physiologic tissue attrition. To test the first option, normal subjects were fed 25 g linoleic acid (corn oil) and pentane release was monitored for 8 hours. No differences in pentane excretion were found for the fed, fasted, or intestinally purged subjects. This showed that dietary corn oil has no contribution to pulmonary pentane excretion. Next, other healthy subjects were fed a commercial liquid diet which contained high levels of unsaturated fats. Again, no pentane was released after this meal. Collectively, these data show that food in the normal human gastrointestinal tract does not contribute to pulmonary pentane excretion. Physiologic pentane production can be altered by the use of oral antioxidants (vitamin E, beta carotene), exercise, and age. The pentane release associated with aging is correlated with tissue lipofuscin accumulation, but appears to be independent of various antioxidant concentrations, including tocopherol, retinol, beta carotene, lycopene, and zinc.

In clinical situations, lipid peroxidation occurs in three settings. Ischemia followed by reperfusion causes the generation of oxygen free radicals which initiate peroxidation. The effects of ischemic injury in humans has been investigated by the noninvasive technique of breath pentane. Twenty consecutive patients with recent onset of chest pain had breath pentane measured. Standard clinical testing showed that ten of these subjects had suffered a myocardial infarction (MI), and the other ten had not. The pentane in the MI group was significantly ($p < 0.0001$) higher than in the non MI group, indicating the presence of acute cellular ischemia or death (2). Chronic ischemia occurs in congestive heart failure (CHF). Patients with this condition also excrete significantly more ($p < 0.005$) pentane than age matched controls. This pentane release can be decreased in a dose dependent manner by the use of a radical scavenging medication. Further studies are needed to see whether the severity of MI or CHF can be altered by antioxidant medication.

A second setting associated with lipid peroxidation is inflammation. In this case, peroxidation is produced through the release of peroxides and radicals from activated leukocytes. The severity of rheumatoid arthritis, an inflammatory condition, is closely correlated with the magnitude of pentane excretion. The pentane excretion rises and falls with the natural history of this illness, and the excretion may be suppressed by drugs with antioxidant activity. Similarly, the magnitude of inflamed tissue in ulcerative colitis can be predicted by the amount of pentane excretion, however the association is not strong enough in the pilot study to predict a clinical remission or exacerbation. If this trend persists, the insensitivity of pentane levels may be due to hepatic metabolism of pentane which was produced within the splanchnic circulation. Excessive pentane excretion also occurs in patients with a cardiac transplant, and in this case pentane appears sensitive enough to detect the inflammation associated with mild episodes of cardiac rejection. Thus, pentane measurements are helpful in assessing the severity of inflammation in some clinical settings. Perhaps this may obviate the need for more invasive testing, and also aid in assessing the efficacy of specific therapies.

The third category of diseases associated with pentane production does not clearly fit either ischemia or inflammation. Patients with multiple sclerosis (MS) undergo spontaneous focal degeneration in the brain, with resultant neurological loss. Patients with acute MS symptoms have increased pentane excretion compared to inactive patients or controls. Increased pentane excretion has also been identified in smokers (3), ethanol-induced cirrhosis, and in patient exposed to high concentrations of inspired oxygen. It is interesting to speculate whether these examples of tissue destruction are partially related to an antioxidant deficiency. Also, there is some evidence that deficiency of the antioxidant beta carotene is responsible for the initiation of some malignancies. The role of lipid peroxidation and pentane production in this area has yet to be defined.

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THE ROLE OF BREATH MONITORING IN EXPOSURE ANALYSIS

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A significant amount of research has gone into the characterization of volatile organic compounds (VOCs) in the atmospheric environment with the goal of assessing the burden such organic compounds present to the body. Although knowledge about the ambient concentrations of the organic compounds provides valuable information regarding exposure and potential dose, it fails to provide realistic estimations of the internal dose (body burden). It is the internal dose, of course, that is relevant to the toxicological ramifications of the exposure. The measurement of VOCs in exhaled breath allows a direct estimation of the body burden resulting from exposure to an organic compound. Breath is an attractive sample because it is non-invasive, presents a minimal burden to study participants and reflects total dose absorbed from inhalation, ingestion, and dermal exposure routes; we have focused on inhalation exposure. The ability to relate the concentration of a VOC in breath to the VOC concentration in the exposure environment requires knowledge of the uptake and elimination kinetics. Prediction of concentrations of VOCs in individual tissues or tissue groups requires the use of physiologically-based pharmacokinetic (PBPK) models. Our research group has taken two different approaches to the monitoring of breath for the analysis of exposure. One approach has involved humans and the other has utilized an animal model system.

For our human studies, we developed a simple and portable breath collection device (1) and used it to characterize the uptake and elimination of VOCs in human breath after inhalation exposure. Exposures occurred in either common microenvironments, such as a hardware store, or in atmospheres where target VOCs were introduced in a controlled manner. Breath and air samples were collected into evacuated canisters and aliquots of the sample were analyzed by gas chromatography (GC) in conjunction with selected ion monitoring mass spectrometry. The post-exposure VOC concentrations in breath were measured as a function of time and the elimination half-lives for parent target compounds were calculated for a number of people using a classical pharmacokinetic description. The goal has been to improve our understanding of the elimination process and the differences that exist among individuals. Progress in this work will be summarized.

The work with the animal model system has taken a different approach. In this case the focus has been to characterize the normal occurrence of endogenous VOCs produced by Fisher 344 rats and to determine if changes in the types of compounds produced and/or the relative amounts of these compounds are indicative of the biochemical status of the animal. That is, can compounds measured in breath be used as biomarkers of metabolic processes altered as a result of exposure to toxic compounds? A non-rebreathing breath collection system was built based upon the adsorption of VOCs onto Tenax-GC from a large volume (40-90 L) of exhaled breath. Analysis was accomplished by thermal desorption GC with flame ionization detection. The collection and analysis system will be described and an overview of results to date will be presented.

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SURVIVAL ANALYSIS and its APPLICATIONS

Presiding: Ari Wijetunga, MSU, Moorhead

8:00 am NonParametric Estimation in Survival Analysis.
M Bhaskara Rao*, Department of Statistics, North Dakota State University, Fargo 58105

8:30 am Modeling Animal Survival in Nutrition Experiments.
LuAnn Johnson*, USDA/ARS Human Nutrition Research Center, Grand Forks 58202

9:00 am Aspects of Survival Analysis in Animal Ecology.
Douglas H Johnson*, U S Fish and Wildlife Service, Jamestown 58401

9:30 am Refreshment / Discussion BREAK

10:00 am On the Mechanics of Censoring in Survival Analysis.
Rupa Mitra*, Department of Multidisciplinary Studies, Moorhead State University, Moorhead 56563

10:30 am Random and Deterministic Inspection Policies in Survival Analysis.
Kathy M Kraft*, U S Fish and Wildlife Service, Jamestown 58401 and Bhaskara Rao, NDSU, Fargo.

11:00 am The Performance of M-Estimators versus Trimmed Means and the Kaplan-Meier Median in the Presence of Arbitrarily Right Censored Data.
R C Khan-Malek* and Rhonda Magel, Department of Statistics, North Dakota State University, Fargo 58105-5075

NONPARAMETRIC ESTIMATION IN SURVIVAL ANALYSIS

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One of the problems in Survival Analysis is the estimation of the lifetime distribution of members of a certain target population in the presence of censoring. Suppose a machine runs on two components: Component I and Component II. The machine will break down if any one of the components fails. Let T be the lifetime on Component I and C that of Component II. In this context, we can only observe $Y=\min\{T,C\}$ and an indicator variable Δ which indicates clearly whether the breakdown of the machine is due to the failure of Component I or due to Component II. Using the data on (Y,Δ), the main problem is to estimate the distribution of T. The data on (Y,Δ) is called censored data. If the distributions of T and C belong to a well defined parametric families, the estimation problem has been discussed extensively in the literature. See Miller (1981). The problem of estimation of the distribution of T when the distributions of T and C do not belong to any particular family of parametric distributions is the main focus of the current presentation. The talk will present a panoramic view of research in this area.

Estimation under proportional hazards. Mitra (1991) has considered the problem of estimating the distribution of T when T and C have proportional hazards. The estimator derived using the generalized maximum likelihood methods is a refinement of the usual Kaplan-Meier estimator and is best suited for use under the environment of proportional hazards.

Estimation when censored data is interval censored. In many situations, it may not be possible to observe Y exactly. It will only be known that the value of Y is in some interval. Kraft (1992) has studied this problem extensively in a particular parametric environment. A nonparametric solution is the next logical step to pursue.

Estimation under dependent competing risks. A person in the population could die due to any one of the two known causes or leave the study. The two known causes of death are the competing risks on the life of the individual and they could be dependent. Babu, Radhakrishna Rao and Bhaskara Rao (1992) have looked at this problem from a nonparametric point of view and derived estimators of the lifetime distributions of the two causes of death and studied their properties.

We will present some open problems in this area of research.

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MODELLING ANIMAL SURVIVAL IN NUTRITION EXPERIMENTS

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In nutrition studies, animals are commonly fed well-defined diets for a specified length of time. At the end of this time period, all surviving animals are anesthetized and organs or tissues are removed for analysis. If premature death occurs, then the number of animals surviving at a given day or the age at death is traditionally analyzed to determine if there is a differential diet or treatment effect on survival. While in some cases, analysis of these variables may give sufficient information, situations can occur in which the distributions of the survival times differ considerably between treatment groups, yet the median or mean survival time or total numbers of survivors are similar. Important treatment effects can be missed if attention is restricted to a simple analysis of the mean or median survival time.

The Kaplan-Meier product-limit (PL) estimator (1) and the Cox proportional hazards model (2) are two statistical methods used in the analysis of survival times. The PL method is used to estimate the distribution of the survivor function, which is the probability that a subject will survive to a given time point or longer. The PL estimator is calculated by using the number of deaths at a distinct time point given the number of animals at risk at that time. The PL method requires the assumption that the survival times are independent for all animals. Note that the variable of interest need not be death; it could be a specific cause of death, such as ventricular aneurysm, or recurrence of a given condition or disease.

The Cox proportional hazards model is used to study the possible relationships between explanatory variables (covariates) and the observed death rates. The Cox proportional hazards model assumes that death rates may be modelled as log-linear functions of one or more covariates, which are usually coded as categorical variables having the value 0 or 1 to simplify calculations. With the Cox model, the effect of the treatment and the covariates is to multiply the hazard function, which is the instantaneous rate of failure or death at time t given the subject has survived to at least time t, by a constant factor.

A strength of both the Kaplan-Meier estimator and the Cox proportional hazards model is the ability to accomodate right-censoring. Right-censoring occurs when the time of death is not known for an animal; instead, it is only known that the animal survived beyond a certain date. In clinical trials involving human subjects, right-censoring occurs when a person withdraws from the study or can not be located to obtain follow-up data. Right-censoring may also occur if death results from a cause unrelated to the treatment, such as an accident, or if the study is terminated before a subject dies. In animal studies, right-censoring occurs for these latter two reasons and when animals are sacrified during a study, possibly for organ analysis or histological examination.

Data from two longevity experiments (3) will be used to demonstrate the above techniques. In both experiments, male weanling rats were fed a diet containing less than 1 μg copper/g diet. Control animals (C) received demineralized water ad libitum, while the treated animals (T) were given beer ad libitum as their only source of fluids. There were 15 animals in each dietary group in each of the two experiments. Experiment 1 was terminated on day 439 with 2 treated animals remaining. The median survival time for C was 62 days, while the median survival time for B was 204 days. Experiment 2 was terminated on day 502, the day the last C animal died; three T animals were still alive. The median survival time for C was 45 days and for T was 299 days. The survival distributions were significantly different between T and C in both experiments. Right-censoring occurred only at the end of each experiment when the surviving animals were sacrificed.

The data from the two experiments were pooled to achieve a larger sample size to investigate the effects of four possible covariates besides treatment: initial body weight, relative weight gain at 4 weeks, hematocrit, and cholesterol. Categorical variables were created for each potential covariate by coding them as either 1 or 0 depending on whether the actual value of the parameter fell above or below a specified value, usually the median of the combined data. Because the median hematocrits differed between C and T, two categorical variables were used to define hematocrit.

Treatment, relative weight change, and hematocrit < 25.0 significantly affected survival. T animals with hematocrits > 25 at 4 weeks had the greatest probability of survival, while C animals with hematocrits < 25 had the lowest probability of survival. In these experiments, a simple analysis of median survival times indicated a significant treatment effect, yet additional information was gained by using the Cox proportional hazards model.

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ASPECTS OF SURVIVAL ANALYSIS IN ANIMAL ECOLOGY

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Survival rate has major influence on the size of animal populations. Estimation of survival rate is therefore of critical importance in understanding the dynamics of populations. Many methodologies are available, including life tables, the comparison of population sizes at different times, and mark-recapture (e.g., 1). This paper focuses on the intermittent observation of animals, for which methods based on lifetimes can be employed (e.g., 2). Many techniques of reliability theory and medical survival analysis appear to be applicable, but unique features of wild populations pose complications. This paper addresses some of those difficulties and suggests remedies in certain situations.

The intrinsic secretiveness of most wild animals leads to many problems; except in unusual circumstances, they cannot simply be watched. More frequently they must be captured and equipped with a marker, such as a radio transmitter, that allows investigators to find them again and ascertain whether or not they remain alive. Several potential problems immediately arise. First, animals captured are unlikely to be a random sample from the population of interest and in fact may be seriously nonrepresentative. Two possible ways of reducing this problem are using a variety of capture techniques, to avoid biases associated with a particular technique, and carefully comparing marked animals with those not marked. A second problem is that the radio or other marker can influence the animal's behavior and possibly survival prospects. Again, comparison with unmarked animals may offer some insight. A third difficulty is that subsequent observations by the investigators can influence behavior and survival. This influence can be reduced by observing the animals only occasionally and by using great care when doing so. As a result of infrequent observations, either intentionally or because animals can rarely be monitored continuously, deaths are likely to occur between observations, leading to data sets with considerable interval censoring. Analysis of interval-censored data is straightforward with some parametric methods but not so with nonparametric methods.

A second class of problems arises from uncertainties associated with loss of markers. Suppose, for example, that signals from an animal's radio transmitter suddenly cease. If the animal is dead, the observation should be treated as a mortality. If it is alive, it should be treated as right-censored. But without an active transmitter, it is unlikely that the status of the animal can be determined. Unfortunately, such situations are common. Careful searching and the use of ancillary information might reduce, but not eliminate, these uncertainties. One remedy is to analyze the data first treating uncertain terminations as deaths and secondly treating them as right-censored observations. Presumably, resulting estimates of survival should bound the true value, but bounds will be very wide if uncertain terminations are common. An experimental solution would be to double-mark the animals, under the assumption that simultaneous failures would be unlikely unless death had occurred. This step would also permit the estimation of marker loss rates.

Another feature distinguishing studies of survival in wild populations from usual survival applications is the time line of reference. In medical studies and many reliability applications, age is a natural time line. For animals in the natural environment, mortality rates may vary with date as well as age, which may not be known. Stratification may be a recourse if the needed information is available.

A further issue is that survival rates are often highly variable. For young animals, for example, the probability of dying during any particular time interval usually decreases dramatically as the animals age. Even among older animals, survival rates can fluctuate markedly in response to environmental conditions. Survival distributions such as the Weibull can accommodate nonconstant survival, but they invoke a single time line.

A final problem is that lifetimes of individuals in a population of animals may not be independent. Predators are likely to take more than one young from a litter or brood, for example. The statistical treatment of non-independent data is poorly developed, but some recent advances are promising (3, 4).

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ON THE MECHANICS OF CENSORING IN SURVIVAL ANALYSIS

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Let T denote the lifetime of an individual selected at random from a target population and C a censoring variable censoring T. This means that for any individual we observe Y=min{T,C} and an indicator variable Δ which is unity if Y = T or zero if Y = C. The data on (Y, Δ) are called censored data. The celebrated Kaplan-Meier estimate is a nonparametric estimate of the distribution function of T. See Kaplan and Meier (1958). But the Kaplan-Meier estimate is not always a distribution function. More precisely, suppose (y_1,δ_1), (y_2,δ_2), $\cdots$, (y_n, δ_n) are n independent realizations of (Y,Δ). Let $Y_{(1)} \leq Y_{(2)} \leq \cdots \leq Y_{(n)}$ be the order statistics of $y_1, y_2, \cdots, y_n$, and $\delta_{(1)} \leq \delta_{(2)} \leq \cdots \leq \delta_{(n)}$ the corresponding δ_i's. If one looks at the Kaplan-Meier estimate carefully, the estimate is a distribution function if and only if $\delta_{(n)} = 1$. If death occurs at the last observed time $Y_{(n)}$, then the estimate is truly a distribution function. Otherwise it is not.

The current note is concerned about evaluating the probability that the Kaplan-Meier estimator is a distribution function when a random sample of size n is drawn from the target population. We give a precise mathematical expression for the probability and study its asymptotics. Contrary to what was surmized in a meeting on Survival Analysis, the asymptotic probability could take any value between 0 and 1. For further details, see Mitra (1991).

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RANDOM AND DETERMINISTIC INSPECTION POLICIES IN SURVIVAL ANALYSIS

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Many procedures can be used to observe the life-span of a subject. Ideally, we would observe the subject continuously and note the exact life-span, but often this is not practical. In many studies a subject is checked periodically under various regimes which we call inspection policies. Much research has been done on deterministic inspection policies in which all individuals are inspected at predetermined times. No work has been done to determine the effect of random inspection times (i.e., the inspections are driven by some underlying random process) on the statistical properties of estimators even though there are many situations in which inspection times are random. The purpose of our research is to compare statistical properties of estimators for deterministic and random inspection policies under a competing risks model.

We use a simple model with two variables, T and C, which we choose to call lifetime and censoring time, respectively. This model can be viewed as a competing risks model with two risks or a random censorship model. The difference in the two models is only in the outlook of the investigator. If we are interested in both times, T and C, then the term competing risks is usually applied. If we are only interested in one variable, say T, then this can be called a random censorship model. The results we will discuss apply for either model. We assume T and C are independent and both are exponential random variables with parameters α and β, respectively. The observation $Y=\min(T,C)$ is not known exactly but the interval in which the event occurred is known; therefore, we call these data interval censored. In our research, there are two types of censoring. There is the censoring of the lifetime (T) by the random censoring time (C) (or vice versa) and there is the censoring of the observation $Y=\min(T,C)$ by an inspection interval.

We assume the inspection policies are under the investigator's control. We consider a deterministic inspection policy in which all subjects are observed at equally spaced inspection times until all have been observed to die or be censored; i.e., there are an infinite number of disjoint censoring intervals. We denote the inspection times as $0,s,2s,3s,\ldots$, so s is the length of the inspection interval. The random policy is a Poisson process with known parameter λ so that $1/\lambda$ is the average length of the inspection interval. Under the random policy, each subject is visited at different times (these times being determined by the same Poisson process) until the variable $Y=\min(T,C)$ is observed for all subjects. We compare deterministic versus random inspection policies by setting the deterministic length, s, equal to $1/\lambda$. The type of inspection policy used does not affect the value of the estimator as long as the inspection policy is independent of lifetime and censoring time, but the inspection policy does affect the statistical properties of estimators and so can be an important consideration when planning a study. For various values of α, β and $s(=1/\lambda)$ we will discuss the effects of the two inspection policies on statistical properties of the estimators and examine the importance of these effects when planning a study. We will also discuss how the properties of these estimators compare with the properties of estimators derived from exact data; i.e., data obtained by observing each subject continuously.

THE PERFORMANCE OF M-ESTIMATORS VERSUS TRIMMED MEANS AND THE KAPLAN-MEIER MEDIAN IN THE PRESENCE OF ARBITRARILY RIGHT CENSORED DATA

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Let $X_1, X_2, \ldots, X_n$ denote a random sample of size n from an unknown symmetric population with mean θ. The primary objective is to estimate θ when each of the observations is subject to arbitrary right-censorship. This type of data arise often in the areas of medical research or product reliability. What we observe is n pairs of measurements of the form: $(Y_1,\delta_1),(Y_2,\delta_2),\ldots,(Y_n,\delta_n)$ where $Y = \min(X,C)$ and $\delta_i=1$ if $X_i \le C_i$ and $\delta_i=0$ if $X_i > C_i$. X_i is the actual value of the observation and C_i is the censoring time. The random vectors (Y_i,δ_i); i = 1,...,n, are independent and identically distributed. Since the underlying distribution of X_i is unknown, a robust estimator will be used to estimate the mean. Various robust estimators that could be used with censored data were examined. These included various M-estimators (Huber's and Tukey's biweight), several L-estimators (trimmed means), and the Kaplan-Meier median (see 1 and 2). Ten sampling distributions, two uniform censoring distributions, and three sample sizes were examined.

The comparison of the estimators was based on the estimated mean square error (MSE) which is defined by $MSE = E(\hat{\theta} - \theta)^2$ where $\hat{\theta}$ is the value of the estimate and θ represents the mean. The estimated MSE's were based on 5000 iterations.

The sample sizes considered were n=10, 20, and 40. The sampling distributions considered (before the value of θ was added) were: 1. N(0, 1); 2. Cauchy; 3. 90% N(0, 1) and 10% N(0, 9); 4. 50% N(0, 1) and 50% N(0, 9); 5. 25% N(0, 1) and 75% N(0, 9); 6. 90% N(0, 1) and 10% N(0, 100); 7. 75% N(0, 1) and 25% N(0, 100); 8. 90% N(0, 1) and 10% N(0, 1)/U(0, 1); 9. 75% N(0, 1) and 25% N(0, 1)/U(0, 1); 10. 90% N(0, 1) and 10% N(0, 1)/U(0, 1/3). Results are given in Table 1 for samples of size twenty and censoring distribution U(0, 40).

TABLE I -- ESTIMATED MSE'S
U(0,40) censoring, n=20

Estimator / Population	Kaplan-Meier	20% Trimmed	30% Trimmed	40% Trimmed	Huber (K=2.0)	Huber (K=1.5)	Tukey
1	.1056	.0750	.0828	.0926	.0669	.0683	.0698
2	.2051	.2562	.1973	.1866	.4511	.3716	.1801
3	.1228	.0903	.0974	.1086	.0922	.0888	.0864
4	.2492	.2172	.2106	.2197	.2739	.2536	.2459
5	.4641	.4071	.3985	.4137	.4441	.4362	.6500
6	.1299	.1038	.1057	.1148	.1311	.1138	.0853
7	.1840	.2341	.1659	.1646	.4701	.3765	.1167
8	.1178	.0868	.0936	.1030	.0857	.0833	.0803
9	.1386	.1075	.1133	.1224	.1205	.1108	.1005
10	.1229	.0945	.0990	.1087	.1102	.0995	.0831

The use of Huber's M-estimator with censored data is not recommended if one is looking for a robust estimator. It did give good results for the normal distributions, but did not do well for heavier tailed distributions. Tukey's biweight did very well in many cases. It outperformed the trimmed means in seven of the distributions studied. It did well when the underlying distribution was normal as well as for heavier tailed distributions. It did, however, perform poorly when the distribution was 25%N(0,1) and 75%N(0,9).

The 20% and 30% trimmed means did fairly well overall, but the Tukey's estimator did better in more situations. Overall, this study recommends the use of Tukey's biweight estimator. The 20% trimmed mean would be the next choice.

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MOLECULAR MECHANISMS of CHEMICAL TOXICITY

Presiding: David W Hein, UND, Grand Forks

1:30 pm Genetic Predisposition to Cancer from Environmental Chemicals.
David W Hein*, Department of Pharmacology and Toxicology, University of North Dakota School of Medicine, Grand Forks 58203

2:00 pm Glutathione Metabolism and Cadmium Toxicity in Mammalian Cells.
Y James Kang*, Department of Pharmacology and Toxicology, University of North Dakota School of Medicine, Grand Forks 58203

2:30 pm Chlamydomonas Reinhardtii, A Model System for Studying Plant Response to Heavy Metal Toxicity.
Jonathan G Spanier*, Department of Microbiology and Immunology, University of North Dakota School of Medicine, Grand Forks 58203

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

3:30 pm 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Inhibition of Growth: Effects on Hormone Action.
Arthur R Buckley*, Department of Pharmacology and Toxicology, University of North Dakota School of Medicine, Grand Forks 58203

4:00 pm Age-Related Decline in Expression of Cellular Stress Proteins Induced by Heat or Other Environmental Stressors.
Michael J Blake*, Department of Pharmacology and Toxicology, University of North Dakota School of Medicine, Grand Forks 58203

4:30 pm Alterations in the Dopamine Receptor System Induced by Cocaine.
James K Wamsley*, MaryAnne E. Hunt, Neelam Narang and Mario Alburges, Neuropsychiatric Research Institute, Fargo 58103 and Departments of Pharmacology and Neuroscience, University of North Dakota School of Medicine, Grand Forks 58203

GENETIC PREDISPOSITION TO CANCER FROM ENVIRONMENTAL CHEMICALS

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Human exposures to arylamine chemicals result from occupational, environmental, and dietary sources. Environmental sources derive from cigarette smoke, certain herbicides, and coal-derived synthetic fuels. In addition, arylamine chemicals derive from metabolic reduction of polycyclic nitroaromatic hydrocarbons released in diesel and motor vehicle exhaust. The agricultural and energy-related environmental sources of arylamine chemicals suggest that these exposures may be significant in North Dakota.

Arylamine chemicals inflict a number of toxicities ranging from methemoglobinemia to the development of cancer in target organs. It is well established that the arylamine chemicals require metabolic activation (i.e., oxidation) by the enzymes of the host in order to elicit the toxic actions. One very important reaction step in the metabolic cascade of arylamine chemicals is acetylation. N-acetylation forms the amide derivative which is often nontoxic. However, O-acetylation of the N-hydroxyarylamine (following oxidation) yields an acetoxy arylamine derivative which breaks down spontaneously to a highly reactive and toxic arylnitrenium ion, the ultimate metabolite responsible for mutagenic and carcinogenic lesions.

Human capacity to acetylate arylamine chemicals is subject to a genetic polymorphism. Individuals segregate into rapid, intermediate, or slow acetylator phenotypes by Mendelian inheritance regulated by a single gene (*NAT2*) encoding for an acetyltransferase isozyme. Individuals homozygous for mutant alleles lack the acetyltransferase and are slow acetylators, whereas individuals homozygous for the wild-type allele are rapid acetylators. Intermediate acetylators are heterozygous at the *NAT2* gene locus. The percentage of slow acetylators varies with ethnic background, but over 60% of Scandinavian populations are slow acetylators.

The important role of acetylation in the metabolic activation of arylamine carcinogens suggests a possible role for acetylator phenotype in genetic predisposition to the incidence and/or severity of tumors derived from arylamine chemicals. Several human epidemiological studies suggest an association between slow acetylator phenotype and urinary bladder cancer. In contrast, a few studies suggest a relationship between rapid acetylator phenotype and colorectal cancer. The basis for this paradox may relate to the relative importance of N- versus O-acetylation in the etiology of these cancers. However, the conclusions drawn from human data is compromised by a variety of environmental and other genetic factors. To eliminate this variability, our laboratory recently completed construction of homozygous rapid, heterozygous intermediate, and homozygous slow acetylator congenic Syrian hamsters. These hamster lines are isogenic except for a small segment of chromosome from a donor hamster strain that provided the source of the wild-type alleles. The rapid and slow acetylator congenic lines are homologous in greater than 99.975% of their genomes, in order to eliminate genetic variability in virtually all aspects of arylamine carcinogenesis except at the acetylator gene locus. Ongoing studies in these congenic hamster lines should provide unequivocal information regarding the role of genetic acetylator phenotype in susceptibility to arylamine-related cancers. The studies in this laboratory are partially supported by United States Public Health Service Grant CA-34627.

GLUTATHIONE METABOLISM AND CADMIUM TOXICITY IN MAMMALIAN CELLS

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Cadmium is a nonessential, toxic trace element in the environment. Its biological significance lies on the facts that the element is accumulated in almost all of the organs of human and animals, and that environmental exposure of cadmium occurs via food, occupational industries, terrestrial and aquatic ecosystem [1]. At molecular levels, cadmium interacts with macromolecules such as protein and DNA and has a high affinity with free sulfhydryl group [2]. Glutathione (GSH) is a tripeptide non-protein thiol and presents in mammalian cells in concentrations that range from about 0.1 to 10 mM. GSH has been shown to participate in a number of important cellular processes including protection of cells against the toxic effects of oxygen, radiation, and other compounds [3].

Human lung carcinoma A549 cells are highly cadmium resistant and concomitantly have high GSH levels. Therefore, these cells were employed to study the relationship between GSH metabolism and cadmium cytotoxicity. The cells were routinely grown in McCoy's 5A medium supplemented with 10% fetal calf serum. The cells were exposed to different concentrations of $CdCl_2$ and a cadmium dose dependent toxic effect was measured by a long-term cell survival assay. Cellular GSH levels were decreased with buthionine sulfoximine (BSO), a GSH synthesis inhibitor, or with diethylmaleate (DEM) which conjugates with GSH. Tietze's enzymatic assay [4] was used to measure intracellular GSH levels under different treatments. $^{109}CdCl_2$ was used to measure cadmium accumulation by the cells. A further study with normal rat kidney fibroblasts (NRK-49F) was done to evaluate the effect of exogenous added GSH on cadmium toxicity.

Treatment of A549 cells with BSO or DEM depleted cellular GSH and sensitized the cells to cadmium [5]. It was further determined that GSH is importantly involved in the early phase of cadmium cytoprotective response [6]. Studies also revealed that GSH and metallothionein (MT), a cadmium-induced sulfhydryl protein, have a different role in cadmium cytoprotection and that their additive effects contribute to enhanced cellular defense against cadmium [7]. Studies with subpopulations of A549 cells suggested that not only cellular levels of GSH but also other aspects of GSH metabolic status are importantly involved in cadmium cytoprotective response [8].

The effect of exogenous added GSH on cadmium toxicity was investigated with NRK-49F cells. Exogenous GSH of 1 mM, added to culture medium, was found to block cadmium cytotoxic effect on NRK-49F cells. Exogenous GSH, however, did not increase intracellular levels of GSH, but decreased cellular cadmium uptake by the cells. Further studies indicated that GSH may form complex with cadmium outside of the cells and decrease availability of free cadmium in the medium, and therefore decrease cadmium toxicity. This observation suggested a practical potential of GSH as a cadmium chelator.

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CHLAMYDOMONAS REINHARDTII, A MODEL SYSTEM FOR STUDYING PLANT RESPONSE TO HEAVY METAL TOXICITY

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Our objective is to learn how *Chlamydomonas reinhardtii*, an alga, detoxifies and removes potentially toxic heavy metals from its immediate environment. The algal detoxification pathway is similar (if not identical) to that used by higher plants, and distinct from that used by animals. Animals sequester heavy metals with small proteins known as metallothioneins (MTs) while plants utilize a family of small peptides called phytochelatins (PCs) for that purpose.

Metallothioneins are small proteins that are synthesized (transcribed and translated directly from the DNA and RNA, respectively) in response to heavy metal (and other) stimulation. Phytochelatin molecules, though smaller than MTs, are synthesized by a complex series of at least 3 enzymatic steps. We wish to study the steps involved in this detoxification pathway.

Our approach is molecular, genetic and biochemical. We hope to isolate mutants that are either unable to detoxify heavy metals or overproduce the detoxifying compounds, and subject them to a genetic analysis in order to determine the number of steps involved in the detoxification pathway. We will also biochemically characterize the enzymatic activities in the mutants, and clone some of the appropriate genes into *E. coli* where they may be more easily studied.

One of our long range goals is to use the information acquired from these studies to help develop food crops better able to survive environmental challenges (of which toxic heavy metals are only one). We also consider these studies to be our entry into the area of ozone research. Since some of the metabolic intermediates in both heavy metal and ozone detoxification appear to be common, we hope that at least some of the mutants isolated using the selectable heavy metal resistance and sensitivity phenotypes will prove useful in helping us define what cellular processes are most sensitive to ozone and how cells render this chemical harmless.

2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) INHIBITION OF GROWTH: EFFECTS ON HORMONE ACTION

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TCDD is among the most toxic synthetic organic chemicals known. It also serves as the prototype of a large family of aromatic halogenated hydrocarbons that are potent teratogens and carcinogens. There is wide species variation in sensitivity to TCDD toxicity. However, in all species studied, TCDD produces a characteristic wasting syndrome. Loss of weight may reach 50%, and death usually ensues within 2-8 weeks after a single exposure. The ubiquity of this syndrome suggests that TCDD may cause alterations in fundamental hormone mechanisms requisite for growth regulation.

Previous studies (1) have shown that TCDD administration (50 ug/kg, i.p.) in male Sprague-Dawley rats rapidly alters the circadian patterns of prolactin, corticosterone, and thyroid hormone secretion. Compared to pair-fed controls, the earliest endocrinological alteration observed after TCDD administration is a significant reduction in serum prolactin within 4 hr, followed by reduced T_4 by 6 hr. Peak serum corticosterone appeared shifted 2 hr later at 8 hr. By 7 days post TCDD, serum prolactin is significantly elevated and the circadian rhythm was abolished.

This dichotomy of TCDD effects on serum prolactin concentration suggested that a consequence of TCDD toxicity might include interdiction in central mechanisms governing prolactin release by the anterior pituitary. Regulation of prolactin secretion is accomplished by tonic inhibition mediated by hypothalamic dopamine released into the hypophyseal portal system of the median eminence. Therefore, experiments were performed to evaluate the coupling of TCDD to alterations in hypothalamic production of dopamine (2). Administration of TCDD again significantly reduced the serum concentration of prolactin at 4 hr. This effect of TCDD was reversed by pimozide, a dopamine receptor antagonist. These data suggested that TCDD decreased prolactin release either by a direct effect on the pituitary or by altering the dopamine concentration in the median eminence. In in vitro experiments, TCDD had no direct effect on prolactin secretion from pituitary glands maintained in culture. However, in other experiments, median eminence dopamine concentrations in TCDD-treated animals were significantly elevated compared to control levels. These data provide evidence for a hypothalamic site of action for TCDD which may underlie the observed hormonal effects.

The biochemical consequences of TCDD-produced alteration in hormone levels were assessed by determining hormone/growth factor provoked induction of ornithine decarboxylase (ODC) and activation of nuclear protein kinase C (3, nPKC), as indices of receptor-coupled intermediates in a trophic response. Two days after TCDD treatment the induction of ODC activity stimulated by prolactin was markedly diminished in thymus, adrenal, spleen, heart, kidney, and liver from toxin-treated animals (1). In other experiments, PKC responsiveness to growth factor stimulation was determined in nuclei isolated from splenocytes. The stimulatory effect of prolactin, IGF-I, and the direct acting phorbol ester, TPA on nPKC was attenuated in nuclei prepared from TCDD-treated animals.

These data demonstrate that TCDD rapidly alters circulating hormone concentrations which, in the case of prolactin, appears to reflect a direct action of the toxin within the hypothalamus. Moreover, receptor-mediated responsiveness to trophic stimuli is severely blunted by TCDD. Together, these effects may participate in the genesis of the wasting syndrome produced by this environmentally important toxin.

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AGE-RELATED DECLINE IN EXPRESSION OF CELLULAR STRESS PROTEINS INDUCED BY HEAT OR OTHER ENVIRONMENTAL STRESSORS

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In cell culture systems, heat and cellular metabolic stressors such as amino acid analogs, heavy metals, and dinitrophenone are known to induce a rapid and massive expression of a set of highly conserved proteins termed heat shock proteins (HSPs). In mammalian organisms HSPs are induced by direct exposure to elevated environmental temperatures or by heat produced by the administration of endotoxins or physical exertion. In addition, other stressors such as surgical infarction, trauma, neurotoxin lesions and ischemic insult have been shown to induce HSPs in mammalian tissues. While much is known about the regulation of HSP synthesis, relatively little is known about their function, especially in complex mammalian systems. Based on studies in cell culture, it is believed that the induction of HSPs provides protection or adaptation to the presence of a stressor. Whether HSPs have a similar function in mammalian tissues, and their relationship to physiologic stress response mechanism(s) remains to be elucidated.

A reduced capacity of the elderly to respond adequately to stress is well documented. With advancing age, there is a decreased tolerance to a variety of physiologic and environmental stressors including temperature extremes, exercise, surgery and infection. Since HSP induction appears to represent a generalized cellular response to stress we have investigated whether this response is also affected by age using several different stressors. Heat was used as an initial model of stress by comparing the level of HSP expression in tissues of adult and aged rats exposed to elevated ambient temperatures. Results of these studies indicated a significant decline in HSP expression in brain, lung and skin of aged rats. However, the increase in body temperature resulting from the heat exposure was also less in the aged animals. Thus, it appears that the difference in HSP expression due to heat stress could be attributed to altered thermoregulatory mechanisms of the older animals rather than an intrinsic deficit in the molecular mechanisms supporting HSP expression.

Since hypothermia is as persistent a problem in aged individuals as hyperthermia, we also compared the effects of cold exposure on the expression of HSPs between adult and aged animals. With cold stress, HSPs were induced exclusively in brown adipose tissue. As with heat stress, this expression was significantly reduced in aged animals. During cold exposure, aged rats also showed a significantly greater decline in body temperature. Brown adipose tissue is responsible for much of the metabolic heat production in rodents in response to hypothermic temperatures. Thus, these results suggest that the decline in HSP expression is related to a reduced heat generating capacity of this tissue with age.

Restraint was used as a third model of stress to assess differences in HSP expression with age. We have recently reported that placement of rats in rodent restraints results in a selective induction of HSP expression in the adrenal gland and vasculature tissue. Restraint stress also invokes neuroendocrine and autonomic stress responses characterized by the activation of the sympathetic nervous system and the release of several stress hormones. With age, we observed that restraint-induced HSP expression declines dramatically in adrenals and the aorta. We have previously demonstrated that restraint-induced HSP expression in the adrenal gland is dependent on the presence of adrenocorticotropic hormone while expression in the vasculature may be induced by the activation of the sympathetic nervous system. Thus, an age-related deficit in HSP expression in these tissues may contribute to the altered endocrine function and autonomic nervous system activity known to occur with age.

Collectively, these experiments demonstrate that several form of stress can induce a tissue selective induction of HSP expression in mammalian organisms. In all cases the magnitude of the induction declines with age and appears to reflect alterations in several homeostatic control mechanisms. Apparently, this highly conserved cellular response to stress is intimately involved in physiologic stress response systems. A deficit of this cellular stress response in old animals may render them more susceptible to the adverse effects of the stress resulting in a general deterioration of the organism with age.

ALTERATIONS IN THE DOPAMINE RECEPTOR SYSTEM INDUCED BY COCAINE

by
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One of the most important factors contributing to cocaine dependence is an alteration in the concentration of the neurotransmitter dopamine in the central nervous system (CNS). Relatively few neurons in the brain actually contain dopamine, but its significance is reflected by the role dopamine plays in the pathophysiology of neuropsychiatric conditions such as schizophrenia, Parkinson's disease, Tourette Syndrome, attentional disabilities and other related conditions. Changes induced in the dopamine system by the recreational drug cocaine could have ramifications on behavior.

In order to understand some of the neurochemical consequences of cocaine administration, groups of rats were injected with cocaine (15 mg/kg body weight, intraperitoneal, twice a day) for 1,3,7,14 or 21 days. Binding of [^{3}H]cocaine, [^{3}H]SCH23390, [^{3}H]raclopride, and [^{3}H]BTCP was performed in striatal and cortical tissue from the treated animals and compared to vehicle treated controls. [^{3}H]Cocaine binding was increased by the drug in the striatum and cortex at day 14 and 21, respectively. The binding of [^{3}H]SCH23390 to D_1 dopamine receptors was significantly increased at day 3 of exposure. In striatal membranes, [^{3}H]BTCP binding to dopamine uptake sites was significantly increased after day 7 whereas binding in cortical membranes was increased from day 1. [^{3}H]raclopride binding to D_2 (D_3, D_4) dopamine receptors was significantly higher only at day 7 and only in cortical tissues.

These results indicate that repeated exposure to cocaine produces an upregulation (possible supersensitivity) in cortical D_1, cocaine, and dopamine uptake receptor sites which occurs in a time-dependent manner. These alterations occur coupled with an increase in striatal D_1, cocaine and dopamine uptake receptor binding without simultaneous changes in striatal D_2 receptor sites. Thus, the effects of cocaine do not occur in a uniform fashion throughout the brain, but rather are focused within areas of the dopamine system. A consequence of these effects could be reflected as a sensitization to cocaine and the cortex appears most susceptible to this phenomenon. The very rapid response of the dopamine uptake sites and the D_1 receptors indicate that these two alterations could be involved in the post-cocaine craving that accompanies use of the drug.

(Undergraduate) Presiding: Doug Munski, UND

8:20 am Microprocessor Controlled Robotic Checkers. Jason Beck*, Bradley Thorvilson, and Arnold F Johnson, UND, Grand Forks.

8:40 am Iron Mediated [4+2] Cycloaddition of 1,3-Butadiene with Ethyne and Propyne. Jared J Drader*, R Bakhtiar, D B Jacobson, NDSU, Fargo.

9:00 am Hydrologic Changes in the Minot Municipal Well Field (Sundre Aquifer). James Heckman*, MSU, Minot.

9:20 am Effects of Glyphosphate on Small Mammal Populations using Cattail Marshes in North Dakota. Laura A Mendoza*, George M Linz, David L Bergman, and William J Bleier, NDSU, Fargo.

9:40 am * * * * Refreshment BREAK * * * *

10:00 am Design of the Electronics of the Hand Optometer for Measuring Dark Focal Points. Vicki S Link, Aleksandra M Nowok*, and Arnold Johnson, UND, Grand Forks.

10:20 am A Latest Cretaceous (Maestrictian) Lower Vertebrate Faunule from the Hell Creek Formation of North Dakota. Rene Quammen*, MSU, Minot.

(Graduate) Presiding: Robert Crackel, MinSU

1:40 pm Porcine Cycle Modification and Follicular Development D Bauer*, R M Weigl, and J E Tilton, NDSU, Fargo.

2:00 pm Levels of Alpha-Tocopherol in Liver Mitochondria in Diabetic and Normal Rats and Their Relationship to Oxidative Status. Joan Berntson* and Katherine Sukalski, UND, Grand Forks.

2:20 pm Distinguishing Two Species of Melampsora Causing Populus Leaf Rust using Teliospore Wall Thickness as a Criterion. Philip A Mason* and Robert W. Stack, NDSU, Fargo.

2:40 pm Kinetics and Spectroscopy of Acetonedicarboxylic Acid and its Iron Salt. A B Rezvani*, J G Brushmiller, H B Abrahamson, and R J Baltisberger, UND, Grand Forks.

3:00 pm Refreshment BREAK

3:20 pm The Electrochemical Reduction of 1-Bromo-2-(3'-butenyl)naphthalene: Radical vs Anionic Intermediacy. Yizhong Sun* and Duane E Bartak, UND, Grand Forks.

3:40 pm Altering Binding Properties of Tubulin by Proteolytic Modification. K Warren Volker* and Harvey R Knull, UND, Grand Forks.

MICROPROCESSOR CONTROLLED ROBOTIC CHECKERS

Jason Beck*, Bradley Thorvilson, and Arnold F. Johnson
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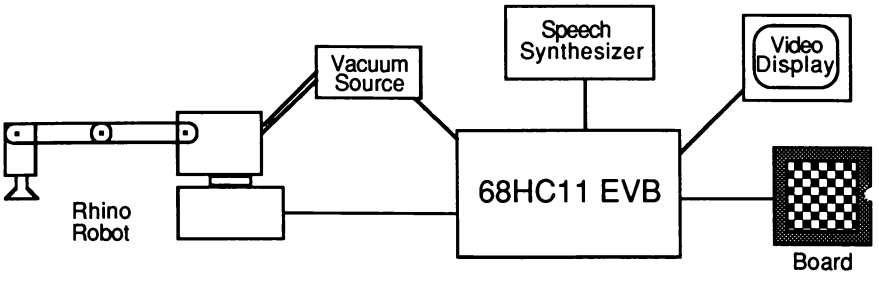
Grand Forks, ND 58202

The School of Electrical Engineering at the University of North Dakota is interested in developing demonstration projects illustrating the integration of several diverse technologies to prospective students and faculty. Several areas of study are incorporated into the design, including microprocessors, robotics, electronics, control systems, computer programming, power electronics, and artificial intelligence. A robotic checkers demonstration is an ideal means of integrating these technologies, as well as to provide a stimulating and entertaining marketing tool for the department.

Depicted in the figure below is a block diagram of the system. A Motorola MC68HC11 Evaluation Board (EVB) is at the heart of the system. With 40 parallel Input/Output (I/O) lines, two RS-232 serial ports, and 8 Analog to Digital (A/D) channels, the EVB is well suited for interfacing to a wide variety of peripherals. MC6811 Assembly Language was employed for software development, and this software resides in an Erasable Programmable Read Only Memory (EPROM) device installed on the EVB. An algorithm, based on Artificial Intelligence (AI) principles, calculates the computer's strategy, using a "most gain-least loss" method. Configuration information, such as the robot kinematic coordinates, are stored in a non-volatile, battery backed Static Random Access Memory (SRAM), allowing the system to adapt to various equipment setups. System status may be monitored and updated via a Video Display Terminal (VDT) connected to an EVB serial port.



For manipulation of the checker pieces, a modified Rhino XR-1 robot was utilized. The EVB communicates with the XR-1 via an RS-232 serial data link. The standard gripper end effector on the XR-1 is not well suited to transporting checkers, thus requiring the development of a vacuum end effector. A microprocessor controlled vacuum source, coupled to a suction cup end effector mounted to the XR-1, proved effective.

Phototransistor sensors were mounted inside of a custom built checkerboard to detect the actions of the human opponent. Signals from these sensors are amplified and buffered by digital Transistor-Transistor Logic (TTL) devices, and transmitted to the EVB. A multiplexed row and column addressing scheme simplifies interfacing, and allows access to 32 sensors with only 7 EVB parallel data lines.

A speech synthesizer was implemented to add personality and novelty to the project, while providing a means of informing the human opponent of various conditions. The design is centered around the SPO256A and CTS256A integrated circuits. These devices convert parallel data signals from the EVB into audio signals which emulate human speech. Presently, the circuit provides a line-level output which must be connected to an external audio amplifier.

This project demonstrates the feasibility of microprocessor based artificial intelligence systems, the physical realization of artificial intelligence via robotics, and several means of effectively interacting with human operators. Given the inevitability of continued advancements in these and related areas, the principles demonstrated here will soon become an integral part of our technological future.

IRON-MEDIATED [4 + 2] CYCLOADDITION OF 1,3-BUTADIENE WITH ETHYNE AND PROPYNE

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The Diels-Alder reaction is a versatile and convenient route to stereospecific synthesis of six membered rings. The low reactivity of unactivated alkynes and alkenes as dienophilic reagents, however, is a major limitation of this [4 + 2] cycloaddition process.[1] Recently, transition metal complexes have been found to facilitate intermolecular and intramolecular dienyne cycloadditions, presumably via η^3-complex formation and reductive elimination.[2] Stereoelectronic perturbations of the ligands around a transition metal center generally have a dramatic effect on both the rate and selectivity associated with catalysis. We have found that [4 + 2] cycloaddition of 1,3-butadiene with ethyne and propyne yielding six membered rings is a facile process for atomic iron cations in the gas phase.

Experiments were performed by using a modified Nicolet FTMS-1000 Fourier transform mass spectrometer.[3] $Fe(1,3\text{-butadiene})^+$ was formed by dehydrogenation of 1-butene, process 1. Fe(1,3-butadiene) reacts readily with both

$$Fe^+ + \text{1-butene} \dashrightarrow FeC_4H_6^+ + H_2 \quad (1)$$

ethyne and propyne exclusively by dehydrogenation, processes 2 and 3. The

$$FeC_4H_6^+ + C_2H_2 \dashrightarrow FeC_6H_6^+ + H_2 \quad (2)$$

$$FeC_4H_6^+ + HCCCH_3 \dashrightarrow FeC_7H_8^+ + H_2 \quad (3)$$

structure of the products of reactions 2 and 3 was probed by both collision activated dissociation (CAD) and specific ion/molecule reactions. The CAD results clearly indicate C-C bond formation between diene and dienophile yielding a single ligand bound to Fe^+. Furthermore, the structural studies indicate formation of $Fe(benzene)^+$ in reaction 2 and $Fe(toluene)^+$ in reaction 3. The proposed mechanism for the above cycloaddition reactions involves initial C-C bond formation yielding an η^3-complex followed by reductive elimination resulting in a 1,4-cyclohexadiene-Fe^+ complex. This complex readily undergoes dehydrogenation yielding the products in reactions 2 and 3. Labeling studies with C_2D_2 are consistent with the above description.

Reactions of $Fe(1,3\text{-butadiene})^+$ with a number of dienophilic agents (ethene, propene, CH_2CF_2, 1,2-propadiene, HCN, CH_3CN) were also studied. All of these agents were unreactive except for 1,2-propadiene which reacts exclusively by dehydrogenation. It is particularly surprising that nitriles (HCN, CH_3CN) due not participate in [4 + 2] cycloaddition. Currently, a number of ligated Fe^+ complexes are under investigation in order to probe stereoelectronic effects in the cycloaddition process.

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HYDROLOGIC CHANGES IN THE MINOT MUNICIPAL WELL FIELD (SUNDRE AQUIFER)

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Contamination prevention and subsequent protection of municipal water systems has become a main concern of governing bodies throughout the U.S. The federal Safe Drinking Water Act amendments of 1986 created the Wellhead Protection (WHP) program and delegated authority to implement the program to the states, under the guidance of the Environmental Protection Agency. This type of program provides an opportunity for a municipality to accumulate baseline data on existing wells, and involve the public in protection of groundwater resources.

The WHP program focuses on the resource requiring protection, rather than controlling a limited set of contamination sources. There are six essential elements which, when used in conjunction with each other, will provide protection for the designated area. 1) Delineation, the limits of the WHP area are determined by the state regulatory agency, in North Dakota, the State Department of Health and Consolidated Laboratories, Division of Water Quality (DWQ). This is accomplished using one or more of four methods: arbitrary fixed radius, calculated fixed radius, zone of contribution, and hydrogeologic mapping. 2) Source Inventory is the identification of potential threats to water quality within the WHP area. Every business, farm, and residence within the delineated area must be contacted to provide necessary information. Personal contact provides site specific information that is used to manage the WHP area and to help develop contingency plans. 3) Management of the WHP area includes a yearly inventory update, as well as regulations relating to new construction, rezoning, & evaluation of existing contaminant sources. 4) Contingency Plans are developed to provide a strategy for contamination emergencies. These should include site specific information about the inventoried area, as well as short and long term solutions to the temporary or permanent loss of all or a portion of the municipal water source. 5) New Wells for municipalities are sited to prevent contamination of the water supply by existing sources of pollution. Plans and specifications must be submitted to DWQ for approval prior to construction. 6) Public Participation is promoted by seminars and symposiums throughout the state. Education of the general public about the potential for contamination is very important if the water supply is to be kept safe.

The City of Minot contracted with the Division of Science at Minot State University to provide chemical analyses of all wells within the WHP area, and produce the source inventory of all properties. Minot's water supply comes from four sources, the Souris River, Souris Valley Aquifer, Minot Aquifer, and Sundre Aquifer. Of these, the Sundre Aquifer is the most important, in 1989 it supplied 42% of Minot's water, in 1991 use increased to 75% of the total (1). The WHP area was delineated by DWQ (2)using a combination of prior hydrogeologic mapping of the Sundre Aquifer System (3,4) which identified the north and south margins of the aquifer. The east and west limits of the WHP area were derived by calculating the estimated distance of travel (X) during a 15 year period (t in days), using the formula $X = (Kit)/n$. The average screened interval of the city wells (64 ft) and the estimated transmissivity of the Sundre Aquifer (36,000 ft^2/day) were used to calculate a hydraulic conductivity (K) of 563 ft per day. The known hydraulic gradient (i) varies from 0.0009 to 0.0008. An aquifer porosity (n) of 0.25 was used, based on the general mixture of fine to coarse sands, with varying amounts of gravel (2).

The City of Minot requested chemical analyses of all the operating wells within the WHP area. Initial tests were conducted for the following: pH, hardness, alkalinity, chloride, nitrites, nitrates, chromium, iron, flouride, and phosphorous. Tests for metals including arsenic, barium, cadmium, lead, chromium and sodium, were run on 10% of the wells that showed high amounts of iron in initial tests. Triazines, Carbofurans, and 2, 4-D tests were conducted on 5% of the wells that showed high amounts of nitrates and phosphates.

Survey forms were completed for each well including infomation on well specifics, sewage disposal, and possible chemical use and/or disposal. Specific forms were used for different types of properties; farms, business, non-farm, and city/urban/non-community. Lithologic information was requested for every well.

In response to several years of extremely low precipitation and river flow, the City of Minot has increased its dependance on the Sundre Aquifer system. This has increased the incidence of private well failures within the WHP area as the elevation of the potentiometric surface decreased. A question of water rights has been raised by citizens forced to redrill wells or convert to the more expensive Rural Water System as their private wells failed.

According to potentiometric surface data from 27 state monitored piezometers within the study area (5), the highest levels occur in the months of March and April. Using April data for comparison, the levels have dropped an average of 20.74 feet from 1987 to 1991. The greatest measured drop was 25.21 feet, the least was a decrease of 14.29 feet.

Increased public awareness of contamination potential is the hidden goal of the WHP program. In educating the public about WHP, the opportunity exists to provide information concerning groundwater conservation. Participation in a WHP program is currently voluntary, and likely will increase as municipalities strive to leave a legacy of clean drinking water.

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EFFECTS OF GLYPHOSATE ON SMALL MAMMAL POPULATIONS USING CATTAIL MARSHES IN NORTH DAKOTA

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From 1989 to 1991, wetlands in Nelson County, North Dakota, were aerially sprayed with glyphosate herbicide to control cattails (*Typha* spp.) used by fall-roosting blackbirds. Fragmenting dense cattail stands may disperse blackbirds responsible for damaging sunflowers. Killing vegetation in and around marshes may affect small mammal populations.

Six marshes were randomly chosen for the study. Four of those marshes were treated for a 70% cattail kill. Two marshes were sprayed in 1990 and two others in 1991. In addition, two control marshes, one from 1990 and one from 1991, were randomly selected from a pool of eight controls. A single side from each marsh was randomly chosen for sampling, and parallel trap lines were established on that side. The length of each trap line was determined and 20 trap stations were placed equidistant from one another. At each station, 2 Museum Special snap traps were set and baited with peanut butter. At alternate stations, pitfall traps were placed into the ground and were half-filled with water to euthanize the animals. Traps were checked at sunrise; all trapped animals were collected, and marsh, station, trap type, and species were recorded.

Ten species and 296 individuals were collected. Species and number of individuals included the following: Microtus pennsylvanicus (n=120), Peromyscus maniculatus (n=101), Sorex cinereus (n=30), Zapus hudsonius (n=24), Clethrionomys gapperi (n=7), Spermophilus tridecemlineatus (n=6), Microtus ochrogaster (n=3), Blarina brevicauda (n=2), Peromyscus leucopus (n=2), and Onychomys leucogaster (n=1). Four of the ten species accounted for 271 or 93% of the total animals collected. One-way ANOVA was used to compare the numbers of mammals trapped in marshes treated in July of 1990 (n=2) to the control marshes (n=2) and those marshes designated for treatment in 1991 (n=2). No significant difference was found among the treatments ($\bar{x}$ = 24.7 ± 14.6 (SD), 1 df, P = 0.4561). In addition, we compared the numbers of mammals trapped in August in those marshes treated in 1990 (n=2), 1991 (n=2), and the controls (n=2). Number of mammals trapped did not differ among treatments ($\bar{x}$ = 24.7 ± 11.4 (SD), 2 df, P = 0.2756).

This preliminary study indicates that alteration of cattail marshes with glyphosate had no effect on small mammal populations.

DESIGN OF THE ELECTRONICS OF THE HAND OPTOMETER FOR MEASURING DARK FOCAL POINTS

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A contemporary topic in optometry is relating the changes of the dark focal point to the influence of fatigue, drug and alcohol abuse, and/or injury. The dark focus is the resting state of an eye in total darkness. In the dark or blank field, no stimulus is provided to the eye for fixation. At that time the eye reverts to the individual's refractive state. The physical and psychological state of an individual changes the resting state of the dark focal point.

A local optometrist, Dr. David Biberdorf, is interested in studying changes in the dark focal point of individuals. He approached the UND Electrical Engineering Department to design a portable device that would measure these changes. Dr. Biberdorf is interested in correlating fatigue, dark focal points and performance of athletes.

To use the hand optometer the subject would simply hold the instrument up to his/her eye (Figure 1). The telescopic tube is adjusted until the blinking light seems to be in focus. The dark focal distance is read from calibrations marked on the side of the optometer.

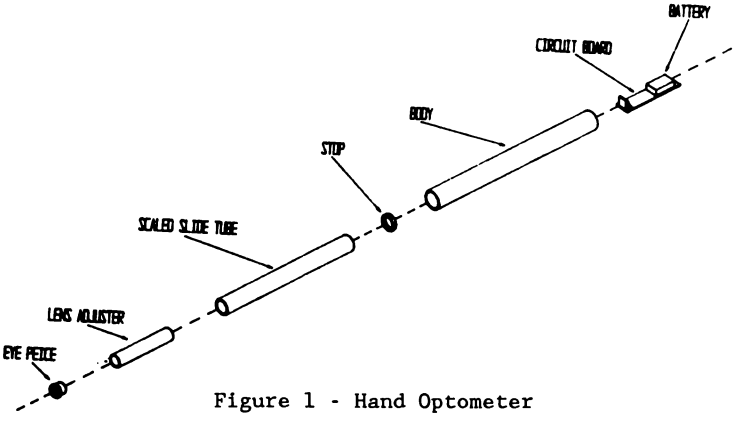

Figure 1 - Hand Optometer

The timing of the blinking light is an important part of the instrument. Previous research has shown that a 200 millisecond impulse of light with 3 second intervals works well for this application. The 200 millisecond light impulse is long enough to see but blinks so quickly that the eye is unable to focus on it. With a timing interval of less than 3 seconds the brain would anticipate the next blink and trigger the focal response of the eye. This would allow the eye to adjust, thus losing the dark focal point.

The electronics of the hand optometer are located near the end of the optometer (see Figure 1). The electronics control the blinking light, Light Emitting Diode (LED). The circuit consists of two Integrated Circuits (555 timers) along with various resistors, capacitors, and diodes. One 555 timer, configured as an astable multivibrator, oscillates with a period of 3.02 seconds. The second 555 timer, configured as a monostable multivibrator, establishes an impulse width of 200 milliseconds. This impulse turns on the LED.

The circuit requires a DC (Direct Current) power source which is supplied by a rechargeable nine (9) Volt battery. The operational characteristics of the 555 timers require a minimum voltage of 5 Volts. Testing has shown that the 9 Volt battery is capable of supplying up to 4 hours of sufficient voltage and current.

The circuit used in the hand optometer is simple, small, and inexpensive to construct. One circuit board and its components cost under $10.

A Latest Cretaceous (Maestrictian) Lower Vertebrate Faunule from the Hell Creek Formation of North Dakota

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The latest Cretaceous (Maestrictian) Hell Creek Formation is well known for its abundance of dinosaurs in North Dakota (1) and elsewhere, but relatively little has been written about the smaller taxa until recently. Work in Garfield and McCone Counties, Montana has resulted in over 700 vertebrate fossil localities with as many as 10,000 specimens and 74 taxa of non-dinosaurian lower vertebrates (2,3,4). In contrast the record from North Dakota is as yet, largely unstudied, or unpublished. This study reports the fauna from a single locality near Pretty Butte, in Slope County, North Dakota.

The locality is in a 1.2 meter thick, gray to medium brown, well-sorted, well rounded, cross-bedded, fine to medium grained, quartz sandstone, with thin interbedded stringers of bentonitic clay. The fossiliferous horizon is 5.7 meters above a 0.2 meter thick coal vein which is traceable for more than 1.2 kilometers. The contact between the Hell Creek and Tullock Formations, as interpreted in this study is 9.1 meters above this coal, and 2.1 meters above the Pretty Butte Locality, identified at the color change from the dominantly gray beds below to the more yellow dominated beds above. This is consistent with the contact as defined previously (1). This places the Pretty Butte faunule in the uppermost Hell Creek Formation, 3.3 to 2.1 meters below the contact with the Tullock Formation.

A total of 34 species are represented in the Pretty Butte faunule, including 28 species of sharks, rays, bony fish, salamanders, turtles, crocodilians and champsosaurs (Table 1). The age of the faunule is Maestrichtian based the presence of five of the species, *Myledaphus bipartitus* (a ray), *Melvius thomasi* (an amiid fish), and *Thescelus insiliens*, *Basilemys sinuosa*, and *Helopanoplia distincta* (turtles), which are restricted to the latest Cretaceous (4). The fauna is most comparable in composition to that described from the upper Hell Creek Formation of Montana which contains 63 species of non-dinosaurian lower vertebrates from localities in the upper 35 meters of the formation (4). Although not as diverse as the Maestrichtian fauna from the Hell Creek Formation of Montana, the Pretty Butte faunule represents only one locality (compared to more than 100 in Montana) and one 2.1 meter thick interval (compared to 35 meters of section in Montana). All of the species in the Pretty Butte fauna, except for one undetermined species of ray, are also represented in the Hell Creek Formation fauna of Montana.

The environment represented by the Pretty Butte faunule is a river system as indicated by the sediments. Aquatic forms in the fauna such as the sharks, rays, and bony fish are consistent with the interpretation. Amphibious forms such as the salamanders, some of the turtles, crocodilians, and champsosaurs represent the river bank community. The presence of *Thescelus insiliens*, a rare land tortoise, and a few mammal and dinosaur specimens from the locality represent the more peripheral floodplain environment.

Table 1. Non-dinosaurian lower vertebrates from the Pretty Butte Faunule

Class Elasmobranchii	Class Reptilia	Class Reptilia (cont')
Lissodus selachos	*Champsosaurus* sp indet.	*Clemmys backmani*
Myledaphus bipartitus	*Contogenys sloani*	*Adocus* sp.
Class Osteichthyes	*Exostinus lancensis*	*Basilemys sinuosa*
Melvius thomasi	*Leidyosuchus sternbergi*	Kinosternid, indeterminate
Kindleia fragosa	*Brachychampsa montana*	*Trionyx* (*Trionyx*) sp.
Lepisosteus occidentalis	*Plesiobaena antiqua*	*Trionyx* (*Aspideretes*) sp.
Class Amphibia	*Neurankylus* cf. *N. eximius*	*Plastomenines* sp. A
Opisthotriton kayi	*Thescelus insiliens*	*Plastomenines* sp. B
Scapherpeton tectum	*Emarginochelys cretacea*	*Helopanoplia distincta*
Lisserpeton bairdi	Chelydrid, indeterminate	*Comsemys* victa

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Porcine Cycle Modification and Follicular Development

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Maximum swine reproductive efficiency theoretically can be achieved through controlled cyclic behavior, early breeding of females, high fertilization rates, and increased litter sizes. Several reports (1) have alluded to the success if using PGF2a to control estrous behavior. Previous studies have shown that gonadotropins can be used to shorten the generation interval and achieve superovulatory responses and estrous synchronization in pigs (2). The objectives of our study were to (1) demonstrate cycle modification in post-puberal gilts using prostaglandin F2a (PGF2a); (2) evaluate follicular development in post-puberal gilts after PF2a; (3) evaluate follicular development in post-puberal gilts treated with follicle stimulating hormone (FSH), pregnant mares serum gonadotropin (PMSG), or saline to determine steroidogenic activity during induced folliculogenesis.

Twenty-four sexually mature gilts were randomly assigned to treatment at the time of onset of estrus (day 0)> All gilts received a 15mg PGF2a intramuscular injection (i.m.) twice (0800,1600h) on day 12. Group A (N=8) was inject i.m. with 25 mg FSH on day 2 and 3 and received 300 I.U. PMSG i.m. on days 13 and 14. Group B (N=8) was treated with 3ml saline on day 13 and 14. Group C (N=8) was given 300 I.U. PMSG i.m. on day 13 and 14. Indwelling cephalic cannulas were surgically implanted in all gilts under halothane anesthesia on day 10 of the cycle. Blood samples were collected twice daily (0800,1600h) on day 11 and 12 and five times daily (0800,1200,1600,2000,2400h) on day 13 and 14. All samples were centrifuged (2500 rpm, 4 C, 10 min.) and the plasma decanted and stored (-20 C) until analyzed for progesterone and estrogen by radioimmunoassay (RIA) procedures. All gilts were slaughtered or ovariectomized on day 15. Ovaries were recovered for morphological analysis and follicular fluid was removed and frozen (-20 C). Analysis of steroidal follicular fluid concentrations were performed using RIA.

PF2a treatment induced regression of the corpora lutea, as evidenced by the decreased ($P<.05$) progesterone concentrations in plasma. A significant change in plasma estradiol concentrations was observed following treatment of PMSG (Fig. 1). In addition, a slight increase in number of follicles greater than 5 mm was observed after treatment with PMSG. Treatment with PMSG had no effect on concentration of estradiol in follicular fluid. Pretreatment with FSH at day 2 and 3 had no observed effect on follicular development. These results indicate prostaglandin F2a will cause regression of the corpora lutea, resulting in cycle modification, but gonadotropin treatment failed to modify follicular steroidogenesis.

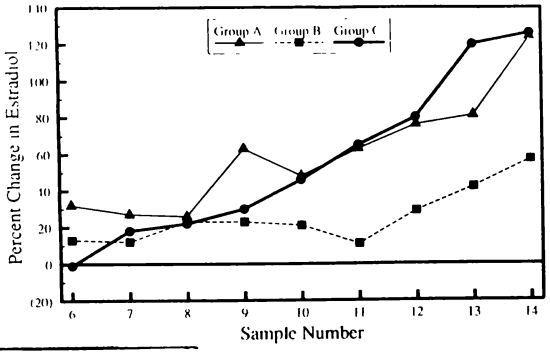

Figure 1. Percent change in Estradiol after Prostaglandin in Gonadotropin Treated Gilts.

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LEVELS OF α-TOCOPHEROL IN LIVER MITOCHONDRIA FROM DIABETIC AND NORMAL RATS AND THEIR RELATIONSHIP TO OXIDATIVE STATUS

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Liver mitochondria from diabetic rats have been shown to be less susceptible than mitochondria from normal rats to oxidative damage upon *in vitro* treatment with iron and a hydrogen peroxide generating system (1). Levels of mitochondrial antioxidant enzymes do not explain this difference (1). As α-tocopherol is an important membrane antioxidant, we investigated potential alterations in α-tocopherol content of the mitochondria from diabetic rats on the same chow diet used in our earlier studies. We then attempted to modify the α-tocopherol content of mitochondria by manipulating the diet of normal and diabetic rats and correlate changes in α-tocopherol levels with alterations in susceptibility to *in vitro* oxidative damage.

Rats were injected with streptozotocin (75 mg/kg body weight) and maintained for four weeks on either Purine Lab Chow or purified diet from TechLad. Littermates of the injected animals were used as normal controls. α-Tocopherol was extracted from mitochondria by a modified method from Lang et al. and quantitated by HPLC using electrochemical detection (3). Mitochondria were incubated with iron (Fe^{3+}) in the presence of a hydrogen peroxide generating system (xanthine and xanthine oxidase) and the resulting sulfhydryl loss (in nmol/mg protein) was determined using a modified method of Fliss (4).

It was first determined that diabetic rats fed a chow diet had liver mitochondria α-tocopherol levels that were seven times higher than controls (1.18 ± 0.24 and 0.17 ± 0.02 nmol/mg protein, diabetic and normal rats respectively). Rats with uncontrolled diabetes consume approximately twice as much food as normal rats (2). A study was designed to determined if increased α-tocopherol intake associated with polyphagia was responsible for the greater α-tocopherol content of the mitochondria from diabetic rats. A 50 ppm α-tocopherol diet was fed to both control and diabetic rats, as this is the approximate level of α-tocopherol in the chow diet. A 25 ppm diet was given to a second group of diabetic rats to approximate the α-tocopherol intake of the control rats on the 50 ppm diet. A second group of control rats was given a 100 ppm diet to approximate the α-tocopherol intake of the diabetic rats on the 50 ppm diet.

Table 1 α-Tocopherol Levels and Sulfhydryl Loss

Mito Source	nmol α-tocopherol/mg prot.	Sulf. Loss	Natural log of α-Tocopherol
Diabetic 25 ppm	1.71 ± 0.51	22.7 ± 3.1	.482 ± .144*
Diabetic 50 ppm	2.49 ± 1.28**	19.9 ± 2.8**	.783 ± .184**
Control 50 ppm	0.85 ± 0.32	32.9 ± 3.5	-.207 ± .124
Control 100 ppm	0.93 ± 0.41	24.4 ± 3.0	-.141 ± .148

Sample size ranged between 7 and 10 for all groups.
*p<.06 for control 50 ppm compared to diabetic 25 ppm.
**p<.01 for control 50 ppm and compared to diabetic 50 ppm.

α-Tocopherol levels were greater in the mitochondria from diabetic compared to the normal rats on the 50 ppm diet and the mitochondria from the diabetic rats were less susceptible to sulfhydryl loss. Matching the diabetic 25 ppm with the normal 50 ppm and the diabetic 50 ppm with the normal 100 ppm, to account for differences in consumption, did not bring about an equalization of α-tocopherol levels. Diabetic 25 ppm and normal 50 ppm were statistically different at the p<0.06 level, when a natural log transformation of the data was done to equalize the variability within the four groups. These data lead us to believe that though increased food consumption undoubtedly plays some role in the increased levels of α-tocopherol seen in liver mitochondria from diabetic rats, α-tocopherol metabolism is probably altered in the diabetic state. Higher α-tocopherol levels in the diabetic animals were consistent with lower susceptibility to oxidative damage.

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DISTINGUISHING TWO SPECIES OF MELAMPSORA CAUSING POPULUS LEAF RUST USING TELIOSPORE WALL THICKNESS AS A CRITERION

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Trees in the genus Populus occur throughout North America. Among those most common in North Dakota and neighboring states are the trembling aspen (Populus tremuliodes Michx.) and the cottonwood (P. deltoides Bartr. ex Marsh.). Many Populus species and interspecific hybrids are planted for wind protection, fiber production or amenity. Populus species are infected by parasitic fungi of the genus Melampsora, causing the disease known as leaf rust. Premature defoliation from rust reduces vigor, making trees more susceptible to environmental injury. It may also predispose trees to other diseases or pests. In trees grown for harvest, defoliation reduces fiber production. Control of leaf rust is best accomplished by genetic resistance (1). Resistant lines are usually identified in test plantings or disease nurseries. In order to usefully interpret results from such trials it is necessary to distinguish which rust species were present at any particular site.

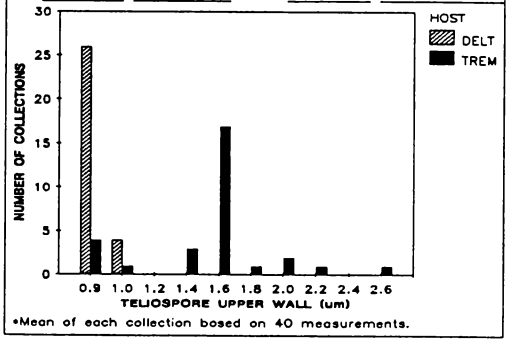
Four species of Melampsora are known on Populus in the region: M. abietis-canadensis (Farl.)Ludw. [MABC], M. occidentalis Jacks. [MOCC], M. medusae Thum. [MMED], and M. albertensis Arth. [MALB]. MABC and MOCC are readily distinguished by spore sizes and both host and geographic ranges (2). Distinguishing between MMED and MALB has been more difficult because these two species have many similarities. Some workers have suggested that they should be considered a single species (3), while others (4) have argued that MMED and MALB were distinct and should be kept separate.

One of the properties of the rust fungi (Uredinales) is existence of several morphologically distinct spore stages. Two spore stages of Melampsora occur on Populus, the summer spore stage (uredospores) and the resting stage (teliospore). Only the former were considered by Stack and Ostry (4). The purpose of this study was to examine teliospores of MMED and MALB to see if taxonomically useful differences existed between the two species.

Populus leaves infected with Melampsora were collected from nine states and two Canadian provinces. Whenever possible, collections included both uredial and telial stages of the rust. There were 60 collections, 30 from P. deltoides and 30 from P. tremuloides.

Small portions of the infected leaves were sectioned on the freezing microtome at 7um. Sections were mounted in a glycerol medium on glass slides, and were viewed with light microscopy. Measurements of the upper-wall of the teliospore were emphasized. Four separate telial sections per slide were measured, 10 spores per section. A mean of the 40 measurements was calculated and served as the value for that collection.

TABLE 1
TELIOSPORE UPPER WALL MEASUREMENTS ON POPULUS DELTOIDES AND POPULUS TREMULOIDES



*Mean of each collection based on 40 measurements.

Upper wall thickness of teliospores collected from Populus deltoides and P. tremuloides were significantly different when compared by analysis of variance ($F(1,58)= 87.9$, $P<0.001$). The upper walls of teliospores from the former were thinner and very uniform from sample to sample (mean=0.98 um ±0.02sd) while those from the latter host were thicker and more variable (mean=1.63 um ±0.38sd). Based on this character, the collections from P. deltoides were identified as M. medusae and those from P. tremuloides as M. albertensis. These measurements fit the two species as originally defined (2). Our results also agree with the host specificity of these two species as originally stated by Arthur (2) and with uredospore identifications presented by Stack and Ostry (4) and support the view that these two Melampsora species are distinct.

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KINETICS AND SPECTROSCOPY OF ACETONEDICARBOXYLIC ACID AND ITS IRON SALT

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Acetonedicarboxylic acid (ADA) is produced (1 and references therein) by the reaction of any of the following with citric acid: a) fuming sulfuric acid, b) Fenton's reagent, c) Vanadium(V), d) *B.Pycyaneus*, and e) singlet oxygen. Datta et. al. (2) reported that ADA is probably an intermediate product in the metal-catalyzed decomposition of citric acid in highly basic pH. The photochemical decomposition of the citrate complexes of iron(III) yields ADA (3).

In this study, the products of the photochemical decomposition of citric acid in the present of iron(III) were examined by HPLC. The kinetics of the decomposition of ADA in the presence and absence of iron(II) or iron(III) were measured using various spectroscopic methods and are discussed.

ADA (from Aldrich) was recrystalized three times from anhydrous ethyl acetate and vacuum-freezed-dried, melting point 129-130 °C. Aqueous solutions of ADA are not stable, two moles of carbon dioxide are released from each mole of the acid with acetone as the final product (4).

UV-VIS: The kinetics of the decarboxylation of aqueous solution of ADA were studied by measuring the decrease in absorbance at 245 nm as a function of time. Solutions of ADA (0.002 M) at various pHs were prepared and the absorbance at 245 nm measured as the decarboxylation reaction proceeded. Decarboxylation of ADA in the pH range corresponding to its first pKa (2.7) was most rapid. At pH 2.0, the decarboxylation of ADA is slower in the presence of iron(II), $k = 0.020\ hr^{-1}$ than in its absence, $k = 0.064\ hr^{-1}$. This is interpreted as meaning that the diprotonated ADA decarboxylate faster than the iron(II) complexed ADA. Solutions containing iron(III) and ADA are red-violet, hence monitoring the changes of absorbance at 245 nm was almost impossible. A fresh mixture of iron(III) ion and ADA at acidic pH (~2) is reddish violet, with an absorption band maxima near 500 nm, this maxima gradually shifts to the UV region.

NMR: Decomposition of ADA was also carried out in D_2O (sodium 2,2-dimethyl-2-silapentane-5-sulfonate, DSS was used as an internal reference) by monitoring the disappearance of the methylene group of ADA (3.6±.1 ppm in ^1H NMR and 53±2 ppm in ^{13}C NMR) and the appearance of the methyl group of acetone (2.2±.1 ppm in ^1H NMR and 32±.5 ppm in ^{13}C NMR). Table 1 summarizes the length of time in hours that takes to reach an equivalent peak height of reactant to product studied by NMR.

Table 1. ^{13}C NMR results on decomposition of ADA and its iron salt to acetone.

pH	t, ADA	t, Fe(III)-ADA	t, Fe(II)-ADA
1-2	30	20	40
4-5	16	3	2
7-8	>>100	100	50

t denotes the length of time in hours that requires to reach an equivalent peak height of reactant to product.

IR: Iron(III)-ADA was prepared similar to the method reported for iron(III)-citrate (5). The broadening and shifting of the acid OH stretching bands in the spectra of iron(III)-ADA salt is indicative of the acid ionization and the participation of the anion OH (of COOH) in the iron(III)-ADA binding. The IR data suggest that the salt also has the enolic chelate character as observed in aqueous solution. The solid phase sample of iron(III)-ADA (pH=3.8) is photoactive with respect to decarboxylation reaction. The appearance of a new band at 2338 cm^{-1} in the IR spectra of KBr pellet of the iron(III)-ADA is indicative of formation of $CO_2(g)$ in the crystal lattice of the complex ion. Despite the appearance of $CO_2(g)$ band, no shifting of bands occurred as observed in iron(III)-alpha-hydroxypolycarboxylate complexes. The intensity of the absorption bands in the irradiated iron(III)-ADA decreases as the intensity of the $CO_2(g)$ stretching frequency vibration increases.

The data on decomposition of ADA indicate that the contribution to decomposition is from the protonated and monoanionic ADA. The rate of decomposition of the ADA to acetone in presence of iron is the sum of first order terms for the free acid and the complex acid.

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THE ELECTROCHEMICAL REDUCTION OF 1-BROMO-2-(3'-BUTENYL)NAPHTHALENE: RADICAL VS. ANIONIC INTERMEDIACY

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The cleavage of the carbon-bromide bond of 1-bromo-2-(3'-butenyl)naphthalene **3** was studied by the methods of the direct and indirect electrochemical reduction. 2-(3'-Butenyl)-naphthyl anions were electrochemically generated upon direct reduction of **3** at a platinum electrode. 2-(3'-Butenyl)naphthyl radicals were produced by the homogeneous reduction of **3** using electron-transfer mediators. Both naphthyl anions and radicals resulted in the formation of 1-methylbenz[e]indan **1** via an intramolecular cyclization reaction.

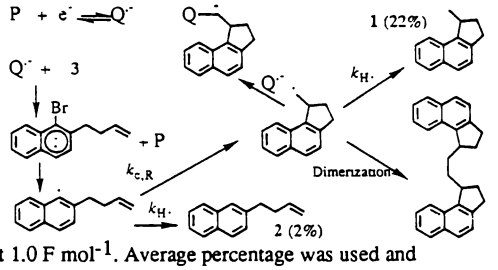
Product analysis data in the presence and in the absence of D_2O can be used to differentiate between anionic and radical precursors with regard to product formation in an overall reaction scheme. In order to determine the above, it was assumed that hydrogen atom abstraction by naphthyl radicals is not competitive with either further reduction of the radical to the anion or a rapid intramolecular radical cyclization reaction. Deuteration experiments (Table 1, entry 2) show that only 17% of the cyclized product, **1**, which was produced, was the result of an anionic precursor. Since the yield of **1** was 25% under these conditions, it can be seen from the deuterium incorporation data that of the 25% produced, 5% was formed from an anion precursor and approximately 20% was the result of a radical precursor. Entry 1 in Table 1 shows that in the absence of D_2O, the yield of **1** is 36%. Since the data in entry 2 show that 20% of **1** is formed by a radical precursor, which should not be effected by the presence of D_2O, it can be concluded that of 36% of 1 formed (entry 1), 20% is the result of a radical intramolecular cyclization reaction whereas the remaining 16% is the result of an anionic intramolecular cyclization reaction. Since **2** can only be produced via an anionic precursor, the 64% yield of **2** in the absence of D_2O (entry 1) in addition to the 16% yield of **1** indicate a total of 80% of products were formed from an anionic precursor.

These results demonstrate the predominant pathway of anion formation (approx. 80%) or ECE mechanism in the direct electrochemical reduction process. However, it should be noted that the 20% formation of **1** from the radical cyclization reaction indicates that the process of intramolecular radical cyclization is competitive with the electrochemical reduction of the radical to the anion.[1] Furthermore, the degree of anion cyclization to produce **1** is substantial (i.e., 16%) and thus this reaction is competitive with the relative rapid protonation reactions that occur to produce **2**. This would indicate that the rate of intramolecular cyclization of the anion under these conditions is considerably faster that observed for a similar aryllithium derivative.[2]

Table 1 The Reduction of 3

Method of Reduction	$E_{app.}$ (vs. SCE)	$[D_2O]$ mM	Product[a] 1	Product[a] 2	Ratio of cyclized/ uncyclized
Direct Electrochemical	-2.2V	none	36%	64%	0.6
Direct Electrochemical	-2.2V	90	25%(17%[b])	75%(85%)	0.3
Homogeneous Redox	-1.9V	none	22%	2%	11
Homogeneous Redox	-1.9V	90	21%(0%[b])	2%(0%[b])	11
Chemical[c]	NA	none	75%	2%	38

REACTION PATHWAY(INDIRECT REDUCTION)



[a]Concentration of **3** was 4.5 mM in all runs. All electrolysis were terminated at 1.0 F mol^{-1}. Average percentage was used and normalized according to the concentration of **3** reacted. [b]Values in the parentheses represented the extent of average monodeuterium incorporation.n [c]4.5 mM of **3** with 12 mM SmI_2 and 70 mM DMPU in 25 ml DMF for 10 minutes at room temperature.

Homogeneous reduction of **3** by the mediators resulted in appreciable intramolecular cyclization of a radical intermediate to yield a product ratio of 11 : 1 for **1** : **2**. These results are consistent with the previously reported rate of radical intramolecular cyclization.[1] Moreover, these results support the assumption which was used in the direct electrochemical case, where hydrogen atom abstraction by the naphthyl radicals was assumed to be insignificant in comparison with the other two radical reactions. Homogeneous reduction of **3** in the presence of D_2O did not result in any deuterium incorporation in either **1** or **2**, which conformed the radical intermediacy in the indirect electrode reduction process. At least 70% of reacted **3** resulted in products other than **1** or **2**. Systematic studies using HPLC in addition to GC/MS were carried to determine other products in the reaction mixtures. Two side reactions appeared to caused the yields of **1** and **2** to decrease: (a) alkylation via the cyclized alkyl radical with the mediator radical anion[3] and (b) radical dimerization. The dimerization of cyclized alkyl radicals was confirmed by an experiment with SmI_2. The proposed mechanism for the indirect electrochemical reduction of **3** is shown above.

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ALTERING BINDING PROPERTIES OF TUBULIN BY PROTEOLYTIC MODIFICATION

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INTRODUCTION. Eucaryotic cells have distinct shapes and a high degree of internal organization. The properties of shape, internal organization, and internal movement depend on complex networks of protein filaments in the cytoplasm that serve as the **"bone and muscle"** and are referred to as **the cell's cytoskeleton.** One of these proteins is tubulin, a heterodimer that polymerizes to form microtubules which are found in high levels in all eucaryotic cells. In addition to cell shape and cytoplasmic organization roles, microtubules are important in orienting chromosomes for cell division at metaphase and they provide physical support that allows for their subsequent separation during late anaphase. Microtubules are responsible for organizing secretory processes as well as providing the anchor for the motor proteins that are involved in transporting various subcellular components in all cells such as axons of neurons for retrograde and anterograde transport.

TUBULIN INTERACTS WITH CYTOPLASMIC PROTEINS. The protein concentration of cells is 20-26% and too high to permit complete independent solvation of all the protein present in the cytoplasm (1). There is evidence for interaction of cytoplasmic proteins with the cytoskeletal proteins (2). Glycolytic enzymes to bind to tubulin with K_d's in the range of 1μM to.1μM *in vitro* and relative to these values the concentration of tubulin in cells is greater than 100μM and the enzyme levels are in the 1μM to 10μM range suggesting the interactions occur *in vivo.* The objective of this study was to elucidate the role of the carboxy terminals of tubulin in enzyme binding and. The working hypothesis is that the acidic C-terminal of the α-subunit of tubulin is responsible for binding proteins from the cytoplasm because it has sequence homology with the human erythrocyte protein **band 3** which also binds glycolytic enzymes (3).

TUBULIN MODIFICATION-MATERIALS AND METHODS. In testing the aforementioned hypothesis the C-termini of α and ß-tubulin were cleaved with **subtilisin.** The three modified derivatives of tubulin (α'ß, αß' and α'ß') were produced by manipulating temperature and the physical state of tubulin (i.e., polymerized or unpolymerized) *see Table 1.* Tubulin was purified from bovine brain (4). To obtain the αß' and α'ß' derivatives, tubulin was polymerized in TRSB(tubulin resuspension buffer), 12M glycerol buffer and 1mM GTP. The addition of 0.025mM Taxol stabilized microtubules which is necessary for subtilisin treatments at temperatures below 15°C. After the polymerization, the microtubule sample was split into two aliquots. Subtilisin was added at 1%(w/w). One aliquot was incubated with the protease at 23°C for 40 minutes to produce α'ß' and the other aliquot was incubated at 6°C for 60 minutes to produce αß'. The α'ß derivative was produced by treating unpolymerized tubulin with the same amount of subtilisin as before and incubating at 6°c for 45 minutes. Each, reaction was terminated with phenylmethylsulfonylfluoride. Polyacrylamide gel electrophoresis was used to identify the derivatives by comparing their migration with unmodified tubulin.

Table I TUBULIN DERIVATIVES

DERIVATIVE	MODIFICATION	TEMP/TIME	STATE
αß	unmodified	---	---
α'ß	cleaved α C-terminal	6°C/45min	unpolymerized
αß'	cleaved ß C-terminal	6°C/60min	polymerized
α'ß'	cleaved α & ß C-terminals	23°C/40min	polymerized

BINDING PROPERTIES OF TUBULIN DERIVATIVES. The derivatives were individually tested for their ability to interact with aldolase and glyceraldehyde-3-phosphate dehydrogenase, two of the glycolytic enzymes which have been shown to bind to native αß-tubulin *in vitro* (5). Two methods were used to test the ability of the derivatives to interact with enzymes. First, both enzymes were assayed in the presence and absence of each of the tubulin derivatives as well as native αß-tubulin to analyze if inhibition existed. Second, each of the tubulin derivatives was covalently linked to CNBr-activated Sepharose to prepare affinity columns. The enzymes were placed on each column to determine if they would bind to the column or immediately elute. An ionic gradient eluted any of the enzyme that bound to each of the columns.

RESULTS. The three derivatives showed different binding properties as compared to the unmodified native αß-tubulin. The α'ß' derivative showed a large decrease in interaction with the two glycolytic enzymes tested. The αß' derivative showed a decrease in interaction very similar to the α'ß' derivative. The αß' derivative was most comparable to native αß-tubulin. Thus, the α C-terminal is suggested to have the highest degree of affinity for these enzymes.

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8:20 am Validity of Body Mass Index Corrected for Sex and Age to Predict Human Body Composition in Adults. H C Lukaski*, W A Siders and C B Hall, USDA/ARS/HNRC, Grand Forks.

8:40 am Body Composition and Collegiate Volleyball Performance. W A Siders* and H C Lukaski, USDA/ARS/HNRC, Grand Forks.

9:00 am Caloric Restriction and Increased Exercise in Mildly Obese Women. I. Effects on Menstrual Symptomatology and Plasma Monamine Oxidase Activity. James G Penland* and Leslie M Klevay, USDA/ARS/HNRC, Grand Forks.

9:20 am Caloric Restriction and Increased Exercise in Mildly Obese Women. II. Effects on Sleep Behavior and Serotonin. Gloria J Krank*, James G Penland, and Leslie M Klevay, USDA/ARS/HNRC, Grand Forks.

9:40 am Measurement of Zinc and Copper Absorption and Retention During Weight Loss in Women. G I Lykken* and L M Klevay, USDA/ARS/HNRC, Grand Forks.

VALIDITY OF BODY MASS INDEX CORRECTED FOR SEX AND AGE TO PREDICT HUMAN BODY COMPOSITION IN ADULTS

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Numerous methods are available to assess human body composition; each method has its own advantages and disadvantages (1). Only a few methods are suitable in epidemiological studies because of requirements of low cost equipment, technical simplicity, and minimal errors in measurement. Whereas recently available methods, such as bioelectrical impedance and near-infrared interactance, offer promise for current and future uses, measurements of body weight and height abound in previous international and national health and nutrition surveys. The use of weight and height indices, specifically the body mass index (BMI; weight/height^2), to infer differences in human body composition is common, but the validity of these interpretations remains unknown. Also, because of differences in body composition between men and women and the age-related increase in body fat mass and the decrease in fat-free mass (2), factors such as sex and age may be useful in improving the specificity of BMI to predict body fatness or percent body fat (%BF).

A sample of 443 men (n=208) and women (n=235) aged 18 to 74 years was studied. Body weight and height were determined, with each volunteer wearing minimal clothing, by using a calibrated scale and stadiometer, respectively. BMI ranged from 15.3 to 43.7 kg/m^2. Body density was determined by underwater weighing and used to calculate %BF (3). Body fatness ranged from 5.1 to 54.0%.

The sample was randomly divided into two groups. Stepwise multiple regression analysis was used to develop a model to predict %BF in each group. These models then were cross-validated in the other group.

In all subjects, %BF was correlated ($p<0.0001$) with height ($r=-0.359$), weight ($r=0.315$), BMI ($r=0.592$), age ($r=0.419$), and sex ($r=-0.552$). As a single predictor of %BF, BMI had a standard error of the estimate (SEE) of 7.61. Inclusion of sex and age in the prediction model (Table 1) reduced the SEE by more than 35%.

Table 1. Regression of Percent Body Fat as Dependent Variable and Body Mass Index (BMI), Sex and Age as Independent Variables

Group	n	BMI	Sex*	Age	Intercept	R^2	SEE
A	200	1.19 ± 0.07[a]	−10.32 ± 0.67	0.18 ± 0.03	−4.72 ± 1.89	0.763	4.73
B	243	1.19 ± 0.07	−10.95 ± 0.63	0.16 ± 0.03	−4.18 ± 1.82	0.730	4.84
Total	443	1.19 ± 0.05	−10.62 ± 0.46	0.17 ± 0.02	−4.44 ± 1.31	0.740	4.78

*Sex: male=1, female=0; [a]Values are mean ± SE

Results of the cross-validation analyses showed no differences ($p>0.05$) between observed and predicted %BF values in Group A (25.7 ± 0.7 vs 25.0 ± 0.7%) and Group B (25.2 ± 0.6 vs 25.8 ± 0.5%). Furthermore, the relationships between observed and predicted values were similar to the line of identity; slopes not different ($p>0.05$) than 1 and intercepts not different ($p>0.05$) than 0.

These findings indicate that the precision of BMI to estimate %BF is improved with the use of sex and age as variables to the prediction model. The precision of the BMI-model is greater (4.78 vs 3.0 %BF) than that of the reference method, densitometry, but less than that (5 %BF) for anthropometry and skinfold thickness measurements (1). Assessment of %BF from BMI, sex and age provides accurate estimates of body composition and will facilitate determination of body composition in large samples of adults participating in community-based health and nutritional surveys.

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BODY COMPOSITION AND COLLEGIATE VOLLEYBALL PERFORMANCE

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The general structural adaptation of a collegiate athlete to a season of sport competition is a decrease in fat weight and an increase in fat-free weight with minimal change in body weight (1). This adaptation is consistent with hypotheses that enhanced physical performance, in which the body is moved horizontally, is related to fat-free weight (2) and that physical performance, involving running and jumping, is inversely related to fat weight (3). However, Johnson, et al. (4) reported that collegiate volleyball players increased fat weight and decreased fat-free weight during a competitive season. The investigators did not relate the athlete's body composition with volleyball performance. The reported changes in body composition seem inconsistent with the physical performance requirements of competitive volleyball. The purpose of this study was to determine the body composition correlates of volleyball performance in collegiate female volleyball players during a competitive season.

Nine women (aged 18-21 years) from the 1991 University of North Dakota varsity volleyball team underwent determinations of body composition by dual energy x-ray absorptiometry (DXA) and somatotype by anthropometry before and at the end of their competitive season. Volleyball performance measures were the individual statistics compiled at each match and accumulated during the season.

A new method of assessing body composition, DXA utilizes a stationery anode x-ray source that alternately pulses x-rays at 70 and 140 kVp to determine whole body and regional bone, fat and lean (fat-free, bone-free) tissue masses. It is a safe method that provides reliable assessment of bone status, and fat and muscle masses in humans.

Preseason subject characteristics of height (172.0 cm ± 5.6) (mean±standard deviation), weight (66.9 kg ± 1.7), fat weight (16.8 kg ± 1.3), percent body fat (25.2% ± 2.3), endomorphy (3.6 ± 0.3), mesomorphy (2.9 ± 0.9) and ectomorphy (2.4 ± 0.8) did not significantly change during the season. Whole body bone mineral and lean weight, and some regional measurements of body composition, increased significantly (Table 1). The significant increase, during a competitive volleyball season, of fat-free weight (bone mineral plus lean weight), especially in the legs, seems intuitively consistent with the running and jumping demands of the sport.

Table 1. Regional and Whole Body Composition Measurements

	Bone Mineral (gm)		Lean Weight (kg)	
	Preseason	End Season	Preseason	End Season
Arms	325± 55+	334± 54*	4.4±0.6	4.6±0.6*
Ribs	162± 14	164± 18		
Upper spine	140± 20	137± 21		
Lower spine	73± 16	80± 16**		
Pelvis	365± 49	386± 58**		
Trunk	741± 99	766±114*	24.8±1.0	25.3±1.3
Legs	1100±114	1144±120**	14.9±1.1	15.8±1.1**
Total	2602±286	2694±310**	47.4±2.4	49.2±3.0*

+ Values are mean, standard deviation
* Mean statistically different from preseason mean, p<.05
** Mean statistically different from preseason mean, p<.01

There were two apparent groups of body composition correlates with volleyball performance (Table 2). Body and leg fat and leg weight were negatively correlated with games played and number of digs. Height and bone mineral density (gm/cm^2) were positively, while trunk fat and percent fat were negatively, related to kills and blocks. These findings suggest that reduced fat in legs, trunk and whole body and increased leg bone mineral density are associated with increased performance among female volleyball players.

Table 2. Body Composition Correlates (r) With Performance

	fat weight	leg weight	leg fat		
Games played	-0.670*	-0.700*	-0.739*		
Digs	-0.704*	-0.672*	-0.818**		
			trunk	leg	
	height	trunk fat	% fat	BMD	
Kills	0.760*	-0.855**	-0.887**	0.707*	
Blocks, solo	0.682*	-0.671*	-0.688*		
Block, assists	0.742*		-0.709*	0.741*	

* p<.05
** p<.01

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CALORIC RESTRICTION AND INCREASED EXERCISE IN MILDLY OBESE WOMEN. I. EFFECTS ON MENSTRUAL SYMPTOMATOLOGY AND PLASMA MONOAMINE OXIDASE ACTIVITY

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Previous research has assessed the effects of obesity, weight loss and exercise on menstrual cycle length and on the volume and duration of menstrual flow (1, 2), but has largely ignored psychological and behavioral symptomatology. Therefore, the present study was designed to assess these latter symptoms in mildly obese women undergoing moderate caloric restriction and increased exercise. Because monoamine oxidase activity (MAO) has been suggested as a factor in premenstrual tension (3), MAO was also assessed in relation to caloric restriction and increased exercise.

Fourteen mildly obese women (age 21 to 38 years) with a body mass index of 28 to 41 kg/m^2 participated in a 5½-month weight loss study while residing on a metabolic unit. Following 4 weeks on a maintenance diet (M; no weight loss), energy intake was reduced to 75% of maintenance levels (75M) for 4 weeks, and then to 50% (50M) for the remaining 14 weeks of the study. Diets consisted of conventional foods and were nutritionally adequate; reduced energy diets were achieved by removing fat and carbohydrates and never contained less than 1200 kcal/day. To facilitate weight loss, a controlled aerobic exercise program of gradually increasing intensity was implemented at the beginning of reduced intake (75M). The Menstrual Distress Questionnaire (MDQ) was administered individually to each woman at the end of each phase of each cycle throughout the study. The MDQ is a retrospective self-report instrument that assesses the presence and severity of 47 symptoms (grouped into 8 scales) which commonly occur during the menstrual, intermenstrual (follicular and early luteal) and premenstrual (late-luteal) phases of the menstrual cycle (4). In addition, venous blood was drawn every 4 weeks and plasma MAO and MAO/mg protein were determined by the colorimetric method described by McEwen (5).

The combination of caloric restriction and increased exercise was highly successful in achieving a significant weight loss in these mildly obese women during the 5½-month study; losses ranged from 12 to 22 kg. Analysis of MDQ scale scores by repeated-measures analysis of variance on ranks showed a significant ($p<0.05$) effect of dieting and exercise on several scores for the menstrual phase of the cycle (Table 1). However, dieting and exercise were not significantly related to any scores for the intermenstrual phase. For the premenstrual phase, only one group of symptoms was affected by dieting and exercise; arousal was significantly higher during M ($\bar{x} \pm$ SD; 2.64 ± 0.39) than 50M (1.55 ± 0.76), but not 75M (1.82 ± 0.68). Analysis of variance also showed that plasma MAO was significantly reduced by caloric restriction and increased exercise (M=13.7 ± 4.9 versus 75M=9.4 ± 3.4 and 50M = 9.4 ± 3.8 clinical units). MAO/mg protein was marginally ($p=0.075$) lower with reduced intakes (M=0.33 ± 0.12 versus 75M=0.24 ± 0.08 and 50M=0.24 ± 0.09).

Table 1. Effects of Caloric Restriction and Increased Exercise on MDQ Scale Scores† for the Menstrual Phase

	Arousal	Autonomic Reactions	Behavior Change	Concentration	Control	Negative Affect	Pain	Water Retention
M	2.05	2.36	2.55[a]	2.64[a]	2.14	2.59[a]	2.45	2.00[ab]
	(0.25)	(0.15)	(0.14)	(0.15)	(0.17)	(0.18)	(0.24)	(0.19)
75M	2.05	1.68	1.95[ab]	1.86[b]	1.77	1.77[ab]	1.77	2.59[a]
	(0.24)	(0.16)	(0.27)	(0.15)	(0.18)	(0.22)	(0.22)	(0.19)
50M	1.91	1.95	1.50[b]	1.50[b]	2.09	1.64[b]	1.77	1.41[b]
	(0.19)	(0.23)	(0.20)	(0.14)	(0.22)	(0.23)	(0.23)	(0.20)
Diet	NS	NS	0.03	0.0008	NS	0.03	NS	0.008
MSE	0.84	0.55	0.72	0.35	0.61	0.73	0.85	0.62

†Values shown are mean ranks (range, 1-3) with standard errors of the mean in parentheses.
[a,b]Means in the same column with different superscripts are statistically different (Bonferroni; $p < 0.05$).

The findings indicate that moderate reductions in caloric intake and increased exercise during weight loss may relieve problematic symptoms associated with behavior, concentration, mood and water retention experienced during the menstrual phase of the cycle in mildly obese women. The reduced MAO activity which paralleled the reduction in symptoms following caloric restriction and increased exercise is consistent with previous findings relating premenstrual tension to MAO, and suggests that the symptom relief observed in the present study may be related to the alterations in oxygen metabolism which occur during weight loss and increased physical activity.

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CALORIC RESTRICTION AND INCREASED EXERCISE IN MILDLY OBESE WOMEN. II. EFFECTS ON SLEEP BEHAVIOR AND SEROTONIN

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Obesity has been associated with several sleep disorders, including obstructive sleep apnea and hypersomnia (1). However, a recent study by Ferini-Strambi and colleagues (2) found no differences in the sleep architecture of mildly obese men and women before and after consuming a very low calorie diet (< 600 kcal/day) for 4-6 weeks. The present study was designed to investigate whether sleep behavior would be affected by a more moderate restriction of caloric intake consumed over a longer period of time and accompanied by increasing exercise of the type commonly found in many weight loss programs. The neurotransmitter serotonin was also assessed because of its involvement in sleep onset and maintenance.

Fourteen mildly obese women (age 21 to 38 years) with a body mass index of 28 to 41 kg/m^2 participated in a 5½-month weight loss study while residing on a metabolic unit. Following 4 weeks on a maintenance diet (M; no weight loss), energy intake was reduced to 75% of maintenance levels (75M) for 4 weeks, and then to 50% of maintenance levels (50M) for the remaining 14 weeks of the study. Diets consisted of conventional foods and were nutritionally adequate; reduced energy diets were achieved by removing fat and carbohydrates and never contained less than 1200 kcal/day. To facilitate weight loss, a controlled aerobic exercise program of gradually increasing intensity was implemented at the beginning of reduced intake (75M). Upon awakening, each woman completed the Sleep Behavior Inventory (SBI) 3 consecutive weekday mornings each week throughout the study, on which bedtime, latency to sleep, nighttime awakenings, dream occurrences, risetime, sleep quality, and feeling upon awakening were recorded. Daytime naps could also be reported and a measure of total sleep time was calculated from responses to these items. In addition, 24-hour urine samples from 3 consecutive days were collected once during M and 75M and three times during 50M. Concentration of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), was determined for each 3-day composite by the high-performance-liquid-chromatography method described by Riggin and Kissinger (3).

The combination of caloric restriction and increased exercise was highly successful in achieving a significant weight loss in these mildly obese women during the 5½-month study; losses ranged from 12 to 22 kg. Repeated-measures analysis of variance of SBI responses and total sleep showed a significant effect ($p < 0.05$) of dieting and exercise on several sleep behaviors (Table 1). Analysis of variance also showed a significantly decreased concentration of urinary 5-HIAA when 50M ($\bar{x} \pm SD$; 1.63 ± 0.50 μg/ml) was contrasted with 75M (2.28 ± 0.56) or M (2.21 ± 0.49); the latter two did not differ.

Table 1. Effects of Caloric Reduction and Increased Exercise on SBI Responses and Total Sleep Times†

	Bedtime[1]	Latency to Sleep[1]	Awakenings[2]	Dreams[2]	Risetime[1]	Total Sleep[2]	AM Feeling[3]	Sleep Quality[3]	Naps[2]	Nap Time[1]
M	2324	14.5[a]	0.67[a]	0.91	568[a]	361	52	64	0.43[a]	34
	(58)	(11.7)	(0.56)	(0.77)	(22)	(50)	(22)	(21)	(0.48)	(44)
75M	2347	8.9[b]	0.29[b]	0.83	558[a]	360	58	71	0.75[b]	38
	(68)	(7.9)	(0.32)	(0.52)	(29)	(39)	(19)	(20)	(0.69)	(33)
50M	2343	13.5[a]	0.31[b]	1.13	597[b]	372	48	69	0.41[a]	25
	(83)	(9.7)	(0.38)	(0.72)	(41)	(53)	(18)	(19)	(0.46)	(31)
Diet	NS	0.022	0.049	NS	0.0003	NS	NS	NS	0.022	NS
MSE	2903	28	0.19	0.21	508	1641	157	114	0.12	692

†Values shown are mean time[1], number[2] or rating[3] with standard errors of the mean in parentheses.
[a,b]Means in the same column with different superscripts are statistically different (Bonferroni; $p < 0.05$).

Because the study was conducted from August through December, the later risetime during the last dietary period may reflect a seasonal rhythm. However, number of nighttime awakenings during 75M was less than half the number during maintenance and remained lower throughout 50M. Interestingly, latency to sleep decreased and number of naps increased initially, but both returned to maintenance levels. A similar transient effect of weight loss has been observed in mood states (4). Collectively, the findings indicate that long-term moderate reductions in caloric intake and increased exercise may reduce the number of nighttime awakenings. Sleep maintenance is generally enhanced by increased seratonergic activity; therefore, the finding of lower serotonin with dieting and exercise would not help to explain the changes in sleep behavior.

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MEASUREMENT OF ZINC AND COPPER ABSORPTION AND RETENTION DURING WEIGHT LOSS IN WOMEN

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The object of this study was to test the hypothesis that obese people have abnormal trace element status, and that weight loss causes alterations in trace element metabolism.

Fourteen women aged 18 to 40 years, between the 80th and 98th percentile of weight (approximate body mass index, wt/ht^2, of 28 to 35 kg/m^2) were selected to reside on a metabolic ward for approximately six months while they lost weight according to a specific diet and exercise program. Following an initial period of 35 days during which energy requirements were determined, a gradually increasing exercise program was begun. This program included aerobic exercise training such that 70 to 85% of peak work capacity was achieved with light muscle activity. A nutritious diet of conventional foods but with 25% less energy than that which maintained weight in the initial period was begun and continued for 35 days. No increments in exercise were permitted during this period. The energy intake was then reduced to 50% of that required to maintain initial weight and held constant for 90 days.

Volunteers were fed a labeled meal containing 0.2 μCi zinc-65 (Zn-65) and 2.5 μCi copper-67 (Cu-67) at the beginning of the maintenance period and four more Cu-67 labeled meals at approximately 28-day intervals. Absorption and retention of these radiotracers were monitored by twice weekly whole body counting of gamma emissions from the subjects. Body self-absorption of internally emitted gamma rays was estimated by weekly measurements of subject absorption of gamma rays emitted from a planar sodium-22 source (UNIS) placed beneath the subject in the whole body counter. These UNIS data were used to correct for changes in counting geometry because of weight loss.

Mean subject weight loss was 16.5 kg with a range from 7.6 to 25.4 kg. Total body Zn-65 gamma activity corrected for gamma ray self-absorption was fit to a two-term exponential retention function for each subject. Percent zinc absorption ranged from 31.4 to 69.6 (mean ± SD, 52.7 ± 11.8); absorption did not differ significantly from reported Zn absorption by normal subjects, although retention function slopes at days 36 and 156 did. However, the zinc associated with the rapidly excreted component, compared to the expected component, apparently was smaller in magnitude (6.5 vs 25%) and had a smaller biological half-life (2.7 vs 11.3 days). The UNIS data were used to correct Cu-67 data, with allowance for greater self-absorption of the less energetic Cu-67 gamma rays. A single-term exponential retention function was used to fit these data over the periods 5 to 19 days following the consumption of labeled meals.

Effect of Weight Loss on Copper Metabolism

Meal	Abs (%)	SD	Bio $T_{1/2}$ (d)	SD
Maintenance	65**	12	38***	24
75%	70**	7	16**	6
50%	65**	13	17**	4
50%	68**	11	22**	9
50%	69**	13	18**	7
mean of means	68	2	22	9

** 14 observations *** 12 observations

These values fell within the limits of reported copper absorption and retention values for normal humans. No significant abnormalities of copper and zinc metabolism were found in women undergoing weight loss except for apparent differences in size and turnover rate of zinc normally associated with the liver. Further investigation of these apparent differences may be of interest to accurately determine short-term zinc metabolism in obese humans.

1:00 pm Cranial Morphometric Variation in Black-Tailed Prairie Dogs from Four North Dakota Dog Towns. Robert E Sorensen, Jerald J Dosch, Bobbi Jo Dickerson*, and Donna M Bruns Stockrahm, MSU, Moorhead.

1:20 pm Observations on Population Structure and Space Utilization by Gunnison's Prairie Dogs in Colorado Donna M Bruns Stockrahm, Elizabeth K Harper*, Todd A Mattson, Sahoko Kawai, Eric B Seplowitz, and Stacy L Adolf, MSU, Moorhead.

1:40 pm Distribution of Rare Small Mammals in Grasslands of Two Western Minnesota Counties. Mat D Goertel*, Craig D Cameron, Theresa E Olson, Elizabeth K Harper, Todd A Mattson, and Donna M Bruns Stockrahm, MSU, Moorhead.

2:00 pm Pitfall Trapped Spiders from the Badlands of Southwestern North Dakota. Daniel J Mott*, DSU, Dickinson.

2:20 pm Status of Eight Rare Butterfly Species in North Dakota. Ronald Alan Royer*, MSU, Minot.

2:40 pm Earthworms (Lumbricidae) of Eastern North Dakota. Rodney A Utter*, Edward J Deibert, and Donald P Schwert, NDSU, Fargo.

CRANIAL MORPHOMETRIC VARIATION IN BLACK-TAILED PRAIRIE DOGS FROM FOUR NORTH DAKOTA DOG TOWNS

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Cranial measurements have commonly been used as a wildlife management tool to assess age structure of a population as well as to measure sexual dimorphism. In this study, greatest skull length, greatest skull width, mastoid breadth, and least interorbital width were evaluated as a means of detecting age differences and sexual dimorphism in adult black-tailed prairie dogs (Cynomys ludovicianus Ord).

As part of a larger population study (1, 2, 3, 4), animals were collected from four dog towns in Billings County, North Dakota. Dog Town 1 (T140N, R102W, S30 center) and Town 2 (T140N, R102W, S29, 30, 31, 32 intersection) were quite small (approximately 3.6 ha and 5.7 ha, respectively), had limited room for town expansion, and had been heavily hunted in years prior to collection. In contrast, Town 3 (T140N, R100W, NW 1/4 of S5 and NE 1/4 of S6) and Town 4 (T140N, R101W, SE 1/4 of S1) were larger (approximately 13.7 ha and 24.3 ha, respectively), rapidly expanding in size, and had not been heavily hunted for approximately 10 years preceding collection.

Prairie dogs were collected from the towns with Conibear traps or by shooting. To increase the likelihood of accurate aging, the left humeri and left eyeballs were collected in addition to the skulls. Animals were placed into age groupings of pups, yearlings, and adults (2 years and older) based on tooth eruption (3), dry eye lens weight (2), epiphyseal closure of the humerus, tooth wear and cementum annuli, and ossification of skull sutures (4). Once these age classes were established, the efficacy of the four cranial measurements as aging techniques and detectors of sexual dimorphism could be assessed. Only adult animals were used for this study because the pup skulls often fell apart during the cleaning process due to their unossified sutures. Skulls were measured with a "Mitutoyo Digimatic Caliper" (Code No. 500-351) to the nearest 0.01 mm.

A total of 228 skulls (102 males, 126 females) were measured, but a few measurements on some skulls could not be taken due to skull damage. Data from Towns 1 and 2 (hereafter referred to as Group A) were pooled because the towns were similar in history and physical characteristics. Likewise, data from Towns 3 and 4 (Group B) were pooled. The largest average cranial measurements were in Group B adult males for skull length ($\bar{X}$ = 64.82, S.D. = 1.12, n = 16), skull width ($\bar{X}$ = 46.85, S.D. = 1.04, n = 17), and mastoid breadth ($\bar{X}$ = 30.97, S.D. = 0.71, n = 16), and in Group A adult males for least interorbital width ($\bar{X}$ = 14.41, S.D. = 0.81, n = 7). The smallest averages were in Group A yearling females for skull length ($\bar{X}$ = 60.78, S.D. = 1.22, n = 17), skull width ($\bar{X}$ = 43.96, S.D. = 1.08, n = 20), and mastoid breadth ($\bar{X}$ = 28.59, S.D. = 0.80, n = 20), and in Group B yearling females for least interorbital width ($\bar{X}$ = 13.03, S.D. = 0.81, n = 45).

Sexual dimorphism (larger males) was evident within all age classes and for all four cranial measurements in both Group A and Group B. Comparisons of the means using t-tests indicated a significant difference ($P < 0.05$ to $P < 0.001$) in every case except for least interorbital width in Group A adults ($t = 0.715$, d.f. = 22, $P > 0.50$).

Further comparisons using t-tests were done between yearling and adult age classes for each sex to determine the efficacy of using cranial measurements as an aging technique. Although adult means for all measurements were greater than those for yearlings, differences were often quite small ($P > 0.05$), especially in males. Differences were not found in any of the four measurements in Group A males or in skull length and mastoid breadth in Group B males. In contrast, means for female measurements were nearly always different ($P < 0.05$) between the age classes with the exception of least interorbital width in Group B.

Overall, the four cranial measurements seemed fairly good detectors of sexual dimorphism in both yearling and adult age classes. However, these same measurements seemed less able to detect age differences between yearlings and adults.

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OBSERVATIONS ON POPULATION STRUCTURE AND SPACE UTILIZATION BY GUNNISON'S PRAIRIE DOGS IN COLORADO

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Gunnison's prairie dog (Cynomys gunnisoni) distributions are limited to the southern Rocky Mountains, primarily the "Four Corners" area, in valleys and plateaus ranging from approximately 1830 m to 3660 m in elevation. Changing land use, poisoning campaigns, and plague (Yersinia pestis) have all contributed to the dwindling numbers of this species (1). During the summer of 1991, a long-term study was begun to study the population dynamics (especially mortality rates) and use of space by this species. This paper describes our preliminary findings on population structure and the use of live-trapping and radiotelemetry techniques to monitor prairie dog movements.

A total of 71 Gunnison's prairie dogs were live-trapped, ear-tagged, and released (69 released, 2 died) on an approximately 2-ha portion of a larger (approximately 9-ha) dog town on the Piedra Valley Ranch, northwest of Pagosa Springs, Colorado (Archuleta County, T36N, R3W, S13). Animals were also observed with binoculars and spotting scopes, and five adult animals were fitted with radiotelemetry collars to supplement trapping data. Transmitters ranged in frequency from 151.005 to 151.800 MHz. Receivers (Model TRX-2000S), 3-element (hand-held) Yagi antennas, and transmitter/battery packs were obtained from Wildlife Materials, Inc.

Trapped pups greatly outnumbered adults (pups = 83.1%, adults = 16.9%, n = 71). The ratio of adult females to pups was 1:8.4 (7 adult females, 59 pups). Visual examination of the trapping area indicated that nearly all adults in the area had been trapped; therefore, at least some adult females had left the area or died because an average litter size is approximately four (1). Pup sex ratios were slightly skewed in favor of males (male pups = 61.0%, female pups = 39.0%, n = 59), while adult ratios were skewed slightly toward females (adult males = 41.7%, adult females = 58.3%, n = 12). However, the sex ratios were not significantly different from 1:1 for either the pups (X^2 = 2.864, d.f. = 1, n = 59, P > 0.05) or the adults (X^2 = 0.333, d.f. = 1, n = 12, P > 0.05). Male pups were the most trappable and adult males the least trappable as indicated by recapture rates. Nearly 80% of the male pups were captured more than once as compared to only 20% of the adult males. Trapping data indicated that both male and female pups moved around considerably, but male pups visited more burrows than other age/sex groups.

Radiotelemetry methods were experimental during the 1991 season to determine the optimal methods for use during the 1992 field season. Five adult dogs were collared, with each transmitter/battery pack being affixed to its leather collar via heat-shrink tubing. The ends of the collars were bonded together with rubber cement. Two animals lost their collars in their burrow systems which were later recovered by holding the antenna at the soil surface and locating the strongest signal. Excavation indicated that the collar location could be pinpointed from the surface to within 35 cm when the collar was at a depth of approximately 1.5 m. One collar appeared to have fallen off in the burrow system due to improper bonding of the glue; the other collar was from an adult female dog which had possibly been predated upon by a badger (Taxidea taxus).

Signals from collared dogs which were below ground were readily discernible from those which were above ground. In fact, signals could usually be located more accurately for those collared prairie dogs below ground than for those above ground unless the animals had gone too deeply into the burrow system. Radiotelemetry as well as trapping data indicated that the use of space by adult females overlapped that of other adult females and males as well as that of both sexes of pups, while adult males seldom overlapped the ranges of other adult males.

Radiotelemetry techniques hold promise as a tool to study prairie dog movements and space utilization. Provided that the ends of the collars are firmly affixed, this technique might be especially useful as a means to study underground movements.

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DISTRIBUTION OF RARE SMALL MAMMALS IN THE GRASSLANDS OF TWO WESTERN MINNESOTA COUNTIES

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Grassland habitats in western Minnesota have rapidly decreased since the turn of the century, primarily due to changing land use including agriculture. Rodent communities are often a forgotten component of these habitats, yet they play an integral part in the dynamics of the food web in such areas. The purpose of this study was to live-trap specified grassland habitats in Clay and Lac Qui Parle Counties with the objective of locating populations of four rodent species which are rare in Minnesota: prairie voles (Microtus ochrogaster), northern grasshopper mice (Onychomys leucogaster), plains pocket mice (Perognathus flavescens), and western harvest mice (Reithrodontomys megalotis).

During the fall of 1990, two study sites (Stockrahm Farm - T138N, R44W, S26 and Aakre Farm - T138N, R44W, S23 and S26) in Clay County were live-trapped using large (8x9x23cm) and small (5x6x16.5cm) Sherman traps baited with a mixture of peanut butter and rolled oats. Traps were set along transects at 10-m intervals. Various trapping arrangements (e.g., one versus two traps per station, efficacy of large versus small traps) and timing of trap checks were tested to determine the best trapping methods for the 1991 field season. During July and August 1991, 9 sites in Clay County and 6 sites in Lac Qui Parle County were trapped. Traps were placed at 10-m intervals along a 500-m transect with one trap per station (i.e., 50 traps per study site), alternating large and small traps. The configuration of a few study sites required using several transects totalling 500 m. Traps were prebaited for one day then checked during the following four days.

During the 1990 field season, none of the target rodent species were captured. However, several relatively uncommon small mammals were trapped including the arctic shrew (Sorex articus), the least weasel (Mustela nivalis), and the boreal redback vole (Clethrionomys gapperi). The most commonly trapped species was the meadow vole (Microtus pennsylvanicus (n = 151). When both a large and a small trap were used at the same station, rarely were small mammals captured in both traps simultaneously (Stockrahm Farm - 0%, n = 770; Aakre Farm - 5.1%, n = 138). With the exception of meadow voles on the Stockrahm Farm, none of the species were captured more readily in one size of trap over the other. When adult meadow voles from the Stockrahm Farm were tested separately from the immature voles, the ratio of captures in large to small traps in either group was not different from 50:50 ($P > 0.05$). However, when the adult and immature meadow vole data were combined, more animals were caught in the small traps ($X^2 = 5.452$, d.f. = 1, n = 31, $P < 0.05$).

During the 1991 season when 15 study sites were trapped, 15 prairie voles, 13 northern grasshopper mice, and various nontarget species were captured. Of these, 11 prairie voles were captured from 3 of the 9 study sites in Clay County (Bicentennial Prairie - T141N, R45W, S5, and 2 separate sites in the Bluestem Prairie - T139N, R46W, S22 and S23), and 4 were captured at 1 of the 6 sites in Lac Qui Parle County (Yellow Bank Hills - T118N, R46W, S4). The 7 grasshopper mice captured in Clay County were from 3 study sites (Bluestem Prairie - T139N, R46W, S23 and 2 separate sites in Buffalo River State Park - T139N, R46W, S 1/2, NW 1/4, S14 and E 1/2 NE 1/4, S14), and the 6 from Lac Qui Parle County were captured at a single site (Yellow Bank Hills - same as above). Prairie voles were always captured in relatively dry prairie, and grasshopper mice were always associated with gravelly/sandy soils. The pocket and western harvest mice appeared to be very rare. All four target species should possibly be considered as species of "Special Concern" in Minnesota, a status currently held only by the prairie vole.

PITFALL TRAPPED SPIDERS FROM THE BADLANDS OF SOUTHWESTERN NORTH DAKOTA

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Little work has been done on the arachnids of the upper Midwest. North Dakota is no exception with a single recent publication by Sauer (1) on the spider family Thomisidae. The North Dakota fauna is of particular interest because the state represents the southern boundary of many northern species and a transitional area between the tall grass and short grass prairie species. The purpose of this study was to produce a list of the spiders of southwestern North Dakota.

Pitfall traps were used to collect spiders at the Dickinson State University Research Station (R102W, T140N, Sec. 25) in Billings County near Medora, North Dakota. Twenty traps consisting of 30 inch sections of 4 inch guttering buried flush with the ground were placed on the 14 hectare study site. Wandering spiders were captured and preserved in a mixture of 80% antifreeze diluted with 50% ethanol. The traps were placed on 11 May 1991 and emptied weekly until their removal on 9 October 1991. Specimens were placed in 70% ethanol for storage.

The 22 week collecting season yielded 3856 mature spiders in 15 families. Males represented 69% of the adult specimens taken. Eighty-four percent of the specimens were in the families Gnaphosidae (1582), Lycosidae (904) or Thomisidae (758). The other typical ground inhabiting spiders in the families Oxyopidae, Hahniidae, Amaurobiidae, Agelenidae, Salticidae and Clubionidae represented only 11% of the total. Families taken in the pitfall traps which are not normally ground inhabiting included the Mimetidae, Pholcidae, Theridiidae, Linyphiidae and Dictynidae. Neither the Oxyopidae nor Mimetidae have been reported from North Dakota. Three adult males and 9 females of *Mimetus epeiroides* Emerton, normally considered an eastern species, were taken from five of the collecting sites. A total of eighty-eight species in forty-six genera were taken. When examined on a per trip basis, June was the most productive month with a mean of 330.8 specimens per collecting trip followed by May (249.3/trip), July (214.5/trip), October (172/trip), August (52.2/trip) and September (44.3/trip).

A review of the literature on the fifteen families collected shows that North Dakota lies in the range of distribution of a possible 262 species in 92 genera. With a single collecting season yielding one half this number of genera and the fact that North Dakota is sure to have an eastern and a western group of families, these numbers will undoubtedly grow with each collecting season.

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STATUS OF EIGHT RARE BUTTERFLY SPECIES IN NORTH DAKOTA

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In company with Gary M. Marrone, in 1991 the author completed field surveys on eight rare butterfly species for the U. S. Fish and Wildlife Service in North and South Dakota. Presented here is basic information on their North Dakota distribution and status.

1) The Powesheik skipper (*Oarisma powesheik*) may be facing extirpation from North Dakota. It is a denizen of undisturbed fresh meadows where its larvae are believed to feed on spikerushes (*Eleocharis*). It is presently known from only two sites in Wisconsin (where it is listed as state-endangered), one site in Iowa, one site in Michigan, and one small area in southeastern Manitoba (1, 2, 3). Minnesota lists it as a "special concern" taxon. Only one Richland County example was observed during fieldwork in 1991.

2) Knowledge of the range of the Dakota skipper (*Hesperia dacotae*), a federal candidate for listing as threatened, was extended by 10 sites to include a total of 30 known sites in 16 counties. The species is believed to have been extirpated by cropland conversion at one previously reported site (4) in Bottineau County. One newly discovered occurrence at the Lostwood National Wildlife Refuge, just 35 air miles from Saskatchewan, constitutes one of the most northwestern records in the range of this species.

3) The Arogos skipper (*Atrytone arogos*), not seen in the Sheyenne National Grassland since the 1970s, was found again north of McLeod in Ransom County. Only two other precise occurrences are documented for the species in North Dakota, both in Ward County. It is associated with undisturbed bluestem prairies, and it may also be facing extirpation from North Dakota.

4) North Dakota range for the Mulberry Wing (*Poanes massasoit*) was confirmed to be limited to a few undisturbed sedge marshes in the Sheyenne Valley east of Lisbon. This species is extremely sensitive to changes in water level. Its larvae feed on *Carex stricta*. In North Dakota it appears to be most common in shrubby (*Alnus, Cornus, Salix*) seeps on the exterior perimeters of ancient oxbows of the Sheyenne River in Ransom and Richland Counties. Most of the ten presently known North Dakota occurrences are in vulnerable private wetlands of low acreage.

5) The Broad-winged skipper (*Poanes viator*) was also confirmed at several new sites in the sedge marshes of the Sheyenne Valley east of Lisbon, North Dakota. Its larvae feed on *Carex lacustris*. It is typically abundant, but appears to be highly local and restricted to dense, open, sunlit sedge mats in the same Sheyenne Valley oxbows as *P. massasoit*. Again, nearly all occurrences are on private land.

6) Before 1991 the Dion skipper (*Euphyes dion*) was known in North Dakota from only two females, both from the now flooded Mirror Pool oxbow system in Ransom County. Two males and a third female were collected at two separate sites west of Mirror Pool Wildlife Management Area on 13 July 1991. This species has never been recorded in South Dakota, and since both new sites are in private wetlands, its long-term survival in North Dakota is uncertain.

7) The Tawny crescent (*Phyciodes batesii*) is another federal candidate species. Its occurrence in South Dakota is limited to the Black Hills, and in North Dakota it appears limited to the drainages of the Little Missouri and Souris Rivers, the Turtle Mountains, and the woodlands south of Devils Lake. It has now disappeared from much of the eastern U. S. The larval foodplant in Manitoba and the Dakotas is believed to be Panicled aster (*Aster simplex*). Its environmental needs remain poorly understood, and the reason for recent disappearances in the eastern U. S. is not known. It must be classed as a rare endemic throughout its North Dakota range.

8) The Regal fritillary (*Speyeria idalia*), the state's third federal candidate for listing, remains secure throughout much of the area south of I-94 from Fargo to the Missouri River. This species requires undisturbed wet to wet-mesic prairies with abundant violets and continuous access to nectar sources (*Asclepias, Cirsium, Monarda, Liatris*). It has disappeared from much of New England and the Ohio Valley, so that the upper Great Plains may come to constitute its final stronghold.

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EARTHWORMS (LUMBRICIDAE) OF EASTERN NORTH DAKOTA

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Reports on the species and abundance of earthworms for North Dakota are limited. Reynolds (1) reported five earthworm species in the state, *Microsolex phosphoreus* and *Eisenia fetida* were found in a greenhouse soil and an earthworm farm, respectively. Included in Reynolds' report was a survey of soil invertebrates from southwestern North Dakota identifying three more earthworm species: *Aporrectodea (Ap.) rosea* from one site in the Heart River floodplain, *Ap. tuberculata* from six sites that included shelterbelts, wooded draws, and a cultivated sugarbeet field under irrigation and *Dendrobaena octaedra* from a green ash-birch *(Fraxinus-Betula)* forest in the Killdeer Mountains. Deibert et. al. (2) identified one species *(Ap. tuberculata)* in a study of earthworm populations affected by long-term tillage systems at the North Central Research Center near Minot.

To establish additional information on the species and abundance of earthworms available to populate agricultural fields, a survey was initiated in eastern North Dakota during the summer of 1991. Areas selected for sampling included State Agricultural Experimental Research Centers, North Dakota Game and Fish Department public lands, and other available private land. Locations of positive earthworm sample sites were recorded for future reference. Random cores were extracted with a spade at each site and the soil hand sorted for mature earthworms. Collected earthworms were placed with site soil into a plastic bag, transported in a cooler, and later stored in a refrigerator. Individual earthworms were killed with 70% ethanol, fixed in 10% formalin and stored in a screwtop test tube with 5% formalin solution. Species identification was performed by noting the external morphology of sexually mature specimens and using Schwert's (3) key to Lumbricidae.

Table 1. Earthworms of Eastern North Dakota

Genus species	Habitats	Number[1] of sites
Aporrectodea tuberculata (Eisen, 1874)	cultivated fields, shelterbelts, prairie grasses and pothole edges	40
Aporrectodea trapezoides[2] (Dugés, 1828)	cultivated fields, shelterbelts, prairie grasses and pothole edges	28
Lumbricus terrestris[2] (Linnaeus, 1758)	private garden and lawn	1
Octolasion tyrtaeum[2] (Savigny, 1826)	moist soil high in organic matter near pothole edge	1

[1] 50 total sites identified as containing Lumbricidae.
[2] Confirmed new species identified for North Dakota.

Lumbricus terrestris has not been identified from North Dakota before this study, but its presence was suspected by Reynolds (1). Ours is the first North Dakota record of *Ap. trapezoides* and *Octolasion tyrtaeum*. This survey recorded 19 sites that contained both *Ap. tuberculata* and *Ap. trapezoides* present at the same site. These latter two species appear to be the most abundant and adaptable to the region.

ACKNOWLEDGEMENT: The authors thank the North Dakota Game and Fish Department for their cooperation in this study.

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COMPUTER APPLICATIONS (Contributed papers)

1:20 pm A Prototype Design of a Computer-Controlled Spreader System for Prescription Farming Technology.
Tak-Lap Tsui and Donald A Smith*, NDSU, Fargo.

1:40 pm Design and Validation Procedures for Professional Antenna Software.
Kory K Evanson and David A Rogers*, NDSU, Fargo.

REGIONAL CONCERNS (Contributed Papers)

1:20 pm Two Multivariate Models of Housing Values for North Dakota.
Mohammad Hemmasi* and Devon Hansen, UND, Grand Forks.

1:40 pm Unemployment in North Dakota.
Fathollah M Bagheri*, UND, Grand Forks.

ASSOCIATION of NORTH DAKOTA GEOGRAPHERS

Program presented in parallel with sessions of NDAS.
Booklet of Communications available at Registration Desk.

A PROTOTYPE DESIGN OF A COMPUTER-CONTROLLED SPREADER SYSTEM FOR PRESCRIPTION FARMING TECHNOLOGY

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Prescription Farming Technology

A modern farming technology, called Prescription Farming or Precision Agriculture, is attracting more and more farmers due to its lower production cost and kindness for our environment. Traditionally, farmers manage large fields as one unit. They apply 'averaged' rates of fertilizers or chemicals on these fields, though most fields contain several soil types and have different crop production potentials and nutrient levels. Research conducted by agricultural scientists has shown that applying uniform production practices across large fields may cost farmers more than they imagine. The costs are both economical and environmental. Applying 'averaged' rates of chemical fertilizer on these fields means an under-application to some of those soils, over-application to others, and decreasing profitability from all of them. In addition, the over applied chemicals may cause serious pollution to our environment.

The first step of developing a Prescription Farming System is to make a soil variability map of the field. The map is used to guide the fertilizer applicator in the field. Some farmers modify equipment to vary rates as they cross into different areas of the map. Others are reshaping fields according to the soil maps. A more technically advanced method is to digitize the maps into a computer memory and use a computer-controlled applicator.

Functionally, this computer-controlled system is composed of four parts: a data base system, a knowledge based system, a micro-controller and a location sensing system. The data base system is an integrated collection of automated data files related to one another in order to support a common purpose, describing the characteristics of each block of the field. They are digitized information about soil types, nutrient levels, yield history, organic levels, PH value, past herbicide use and so on. The knowledge based system consists of recommendation of farming strategy from agricultural scientists. They can be formulas to calculate the needed nutrient amount of each type based on soil nutrient level and user's yield goal, some guides of how to choose a most profitable yield goal according to previous yield history and soil types, or a method of analysis of the soil data to determine the soil types, etc. The location sensing system signals the microcontroller about the location of the fertilizer applicator in the field. Since all block data are based on the block location coordinate, the system has to know where it is in the field so that it can apply variable rates of fertilizer or chemicals accordingly. The microcontroller is filled with control parameters for different areas of the field. As the system carrier moves among the field, the microcontroller reads signals from the location sensing system and calculates the coordinates of the present location. Then it can pick up corresponding control data and send the control signals accordingly to the linear actuators which vary the application rate.

Application Software

BIGWIN is the application software package developed for IBM PC compatible computers under MS-DOS environment. It is a decision making process based upon the data in the database and the rules in the knowledge-based system. It is used to decide the control parameters for the microcontroller. BIGWIN has a database system which can store and retrieve information data from the database. BIGWIN also contains a knowledge-based system. The recommendations of farming strategy are converted to mathematical expressions and formulas which are used in the decision making process. BIGWIN is a user friendly application program. It is designed for the users who do not have strong background with computer applications. BIGWIN is menu driven, so that the user can select what to do by just pointing to the desired function. Whenever the program requires the user's response, there is a hint window which explains what it is about and how to respond. If the answer can be selected from some choices, the user will see a scroll bar selection menu which relieves them from typing in answers. Pleasurable graphic displays have been developed to communicate with the users more efficiently. Moreover, if there is any error that makes the program unable to continue, the user is prompted by some error message instead of getting locked up.

Before establishing the database used for the decision making process, BIGWIN has to be able to obtain some source information from the user. The user is guided, step by step, to input the data about some specifications, soil test results and so on. During the decision making process, BIGWIN consults with the user to make the farming decisions. Intermediate results are shown to the user so that he can correct or agree with what BIGWIN has decided. However, the user may not be familiar with computer applications and hence may get confused if the program assumes too much for them. So BIGWIN has been designed to run in a very user-friendly way. The user sees a map of his field displayed on a monitor. He can see the display of the distribution of the nitrogen, phosphorous, and potassium nutrient levels consecutively. A general nutrition report on each nutrient's needed amount is also displayed.

DESIGN AND VALIDATION PROCEDURES FOR PROFESSIONAL ANTENNA SOFTWARE

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Comprehensive computer software packages for antenna studies now exist that provide powerful analysis tools. At one time found only on mainframe computers, they are now available for the personal computer (PC). This work reports on a rigorous evaluation of the Numerical Electromagnetics Code (NEC), a component of the Numerical Electromagnetic Engineering Design System (NEEDS).

Originally produced for the U.S. Navy, NEC is distributed as shareware by the Applied Computational Electromagnetics Society (ACES). The study reported here had as its objective to establish procedures to allow the engineer to use the package sensibly. NEC provides results that depend heavily on the skill of the user. Little documentation is available to guide the novice. This work is a first attempt at establishing user guidelines. Extensive numerical studies have been done for several configurations. Validity of the calculations is established using studies of convergence and comparison to classical results. In parallel we offer a more efficient technique for writing and editing antenna-model files for use with NEC.

In NEC, excitations are voltage and current sources, and outputs are induced currents, radiated fields, and antenna impedances. Structures that are large compared to several wavelengths will require computer time and storage that exceed the usual capabilities of a PC. The user models antennas with NEC by breaking them down into segments.

The studies done in this project show that the main engineering consideration is that the wire segment length must be much smaller than a wavelength. Table 1 gives numerical results supporting some of the claims made in this study. It can be seen from Table 1(a) that an exaggerated segmentation leads to an erroneous calculation of antenna current. Table 1(b), on the other hand, shows that distributing the dipole source leads to a more reliable impedance calculation even when the segmentation is exaggerated.

Table 1. Half-wave dipole models

a. Antenna current undulation anomalies vs. segmentation ("z" measured from midpoint)

Segments	Position	Variation	Frequency
21	z = 0	4%	1
51	z = 0	8	1
101	|z| = 0, L/4	19	5

b. Impedance variation due to number of sources for tenfold segmentation increase

Type	Number	Resistance	Reactance
Odd	1	39%	-80%
Even	2	21	-18
Odd	3	16	-17

Somewhat longer segments may be acceptable for long wires with no abrupt changes or for wires whose contribution to some overall effect may be minimal. An example is a parasitic radiator several wavelengths away from an active radiator. Shorter segments may be needed to model the more critical regions of an antenna. However, extremely short segments of less than 0.001 times the wavelength will produce numerically unreliable results. In addition, antenna thickness is constrained to be less than appropriate fractions of both the length-to-segmentation ratio and the wavelength.

Convergence testing allows one to obtain the best results for a new model. Its parameters can be varied while the NEC output quantities are studied. NEC will be successful in those cases where the output parameters are stable even though certain model parameters are changed. NEC yields good results for field patterns and currents on linear antennas, provided the antenna is not large compared to several wavelengths and the antenna is modeled properly.

TWO MULTIVARIATE MODELS OF HOUSING VALUES FOR NORTH DAKOTA

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A thriving real estate market and high demand for housing are often considered signs of national and regional economic vitality. Housing values are influenced by housing characteristics such as: age of the house, lot size, and floor space; housing improvements, including presence or absence of a garage, air conditioning, a basement, plus the number of bathrooms. Furthermore, price may vary because of the neighborhood characteristics, such as family income, migration, ethnicity, and demography. Finally, the relative location of the neighborhood plays an important role in overall property values, especially housing prices. During the 1980s, the median value of owner-occupied houses rose by more than 67 percent nationally. In North Dakota, the same housing price index increased only by 15.7 percent. If we take into account the 59 percent increase in the national Consumer Price Index, North Dakota indicates a 27 percent decline, while the US as a whole, registered over 5 percent increase. Likewise, the 1990 census results revealed that the housing values vary significantly across different spatial scales. For example, the median housing values range from $12,600 in Slope County to $67,900 in Cass County. At the Grand Forks's city block level, the price index varies from $15,000 to $187,000, a twelvefold increase. This paper presents two multivariate spatial housing value models for North Dakota, utilizing the 1990 preliminary census results.

The multiple regression models were tested at the county level and city block level. Because of our limited data, several proxy variables were utilized. The dependent variable is the 1990 median value of owner-occupied houses reported by the residents (HOUSVAL). For the county level, we chose five predictor variables: percent population in the 35-55 age group (POPAGE), vacancy rate (VACANCY), net migration rate (MIGRATE), single housing units (SINGLEH), and percent married families (MARRIED). These five variables predict over 80 percent of the variations in median housing values across 53 counties (Table 1). The predictor variables selected for the city block model include: percent married families, percent families headed by a female (FEMALHH), single housing units, rooms per housing units (HSIZE), and two dummy variables. The dummy variables are designed to capture the block's age of houses (NEWHOME) and relative location in respect to the river front (SCENIC). These six variables collectively account for 73 percent of spatial variations in the median house prices over 512 city blocks (Table 1).

The regression coefficients indicate negative relationships between HOUSVAL and VACANCY, FEMALHH, and SINGLEH. These coefficients imply that housing prices tend to be lower in those areas (i.e., counties and blocks) characterized by high residential vacancy rates, high percent of female householders with no husband present, and percent single housing units. One possible explanation for the negative regression coefficients of the SINGLEH variable is the state's Single Family Mortgage (first-time homebuyer) program. This program helps low and moderate householders to purchase their first home. In 1990 alone, 7,565 North Dakota residents received loans through the program. Furthermore, the coefficients for the POPAGE, MARRIED, MIGRATE, and HSIZE variables are, as expected, positive and significant. They reflect the fact that housing prices tend to rise with the increasing presence of residents sharing these socio-demographic traits, and with the existence of large houses in the block. The positive regression coefficients of NEWHOME and SCENIC variables show, respectively, the recent creation of affluent neighborhoods, and the expensive homes located near the river, along south Belmont Road.

Both exploratory models are quite successful, accounting for 80 and 73 percent of the variations in housing values at two extreme spatial scales. Further refinements and wider applications of the model, however, must wait for release of more detailed data by the Census Bureau.

TABLE 1. MULTIPLE REGRESSION MODELS EXPLAINING HOUSING VALUES (HOUSVAL)

Predictor Variable	Regression Coefficient (b)	Beta Coefficient	Probability (t-test)	Coefficient of Determination
A. County Model: N = 53				
POPAGE	2402.4	0.189	0.02	R**2
VACANCY	-298.5	-0.167	0.05	0.804
SINGLEH	-835.0	-0.629	0.0001	
MIGRATE	49.8	0.218	0.01	
MARRIED	457.5	0.202	0.01	
B. City Block Model: N = 512				
MARRIED	187.2	0.192	0.0001	R**2
FEMALHH	-159.5	-0.054	0.03	0.729
SINGLEH	-89.3	-0.116	0.0002	
HSIZE	8041.2	0.376	0.0001	
NEWHOME	16358.0	0.321	0.0001	
SCENIC	36466.0	0.272	0.0001	

UNEMPLOYMENT IN NORTH DAKOTA

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The literature shows that both macroeconomists and policy makers are more concerned with the issue of aggregate unemployment as a measure of labor market performance. However, there are major disparities in unemployment rates across the local labor market areas which have not been addressed in the previous work and are of great importance for area-based policy purposes.

The twofold objective of this paper is first, to identify the spacial dimension of unemployment inequality, as measured by the duration and intensity of the spells of unemployment, for the 53 counties in the state of North Dakota; second, to explore the major determinants of the variations in the state's aggregate level of unemployment.

The method is to classify the counties on the basis of their unemployment records, to look into factors causing the differences in areas of unemployment, and to estimate a multivariate model of the state's unemployment, using data for the period 1975-1988.

The counties were ranked annually based on their level of unemployment for the period 1980-1990. Since regional unemployment is a relative concept, the entire spacial distribution of unemployment was classified into quantiles, each of which represents a specific group in terms of the duration and the intensity of unemployment spells. Thus there are five unemployment areas as follows: persistent high unemployment (H), relatively high unemployment (RH), moderate unemployment (M), relatively low unemployment (RL), and persistent low unemployment (L) areas.

Table 1 identifies these areas by counties and by quantiles. A county may fall into a given quantile for the entire sample period if the relative frequency (probability) of the occurrence of such phenomenon is at least greater than 1/2. A preliminary result shows that differences in the population and employment growth, labor force participation, net migration, and number of firms can explain part of the areas' unemployment differentials.

TABLE 1. DISTRIBUTION OF UNEMPLOYMENT BY QUANTILES

H (1st Quantile)	RH (2nd Quantile)	M (3rd Quantile)	RL (4th Quantile)	L (5th Quantile)
BENSON	BOTTINEAU	BURKE	BARNES	ADAMS
CAVALIER	EMMONS	BURLEIGH	GOLDEN V.	BILLINGS
DUNN	MORTON	FOSTER	GRAND FORKS	BOWMAN
EDDY	OLIVER	GRANT	GRIGGS	CASS
KIDDER	SHERIDAN	HETTINGER	PIERCE	DICKEY
MC HENRY		NELSON	RANSOM	DIVIDE
MC LEAN		RAMSEY	RENVILLE	LA MOURE
MERCER		STARK	RICHLAND	LOGAN
MOUNTRAIL		WALSH	STEELE	MC INTOSH
PEMBINA		WARD	STUTSMAN	MC KENZIE
ROLETTE		WELLS	TRAILL	SARGENT
SIOUX		WILLIAMS		TOWNER
SLOPE				

The estimation results of the state's aggregate unemployment model is as follows:

$$UNND = 1.56 + 0.51\ UNUS - 0.84\ UNMN + 1.05\ UNNE \quad R^2 = 0.82$$
$$(2.35)\ (2.36) \qquad (3.1) \qquad (6.04) \qquad T = 1975, \ldots.1988$$

The predictive variables are the unemployment rates of the nation, Minnesota and Nebraska respectively. All the coefficients are statistically significant at the standard level. The coefficient of the national unemployment measures the extent of the cyclical unemployment. The negative sign of Minnesota's coefficient implies inter-state migration and commutation due to the geographical location of many cities along the border. The positive sign of the Nebraska coefficient also implies the similarity of the economic structure of the two states. The three variables can explain 82% of the total variation in North Dakota's unemployment.

8:00 am The Effect on Arsenic Deprivation in Rats of Diethyl Maleate, an in vivo Chemical Depletor of Glutathione. Eric O Uthus*, USDA/ARS/HNRC, Grand Forks.

8:20 am Dietary Boron Modifies the Effects of Thiamine Nutriture in the Male Rat. Jo Herbel* and Curtis D Hunt, USDA/ARS/HNRC, Grand Forks.

8:40 am Effects of Boron, Training Exercise, and their Interaction in Male Rats. Curtiss D Hunt* and Joseph Idso, USDA/ARS/HNRC, Grand Forks.

9:00 am High Dietary Fructose Affects Plasma Cholesterol Concentrations and Signs of Short-Term Copper Deprivation in Men. Forrest H Nielsen* and David B Milne, USDA/ARS/HNRC, Grand Forks

9:20 am The Effects of Defficient or Marginal Iron Nutriture on Spontaneous Physical Activity of Rats. Carol A Zito*, Janet R Hunt, John Erjavec and LuAnn K Johnson, USDA/ARS/HNRC & UND, Grand Forks.

9:40 am * * * * * Refreshment BREAK * * * * * *

10:00 am The Effect of Vanadium Deprivation in Thyroxine Replete Thyroidecotomized Rats. Rhonda A Poellot*, Carol D Seaborn, Eric O Uthus, USDA/ARS/HNRC, Grand Forks.

10:20 am The Effect of High Zinc on the Uptake of Copper in Caco-2 Cells in Culture. Mary Briske-Anderson* and Philip G Reeves, USDA/ARS/HNRC, Grand Forks.

10:40 am The Effect of Zinc Deficiency on Angiotensin Converting Enzyme Concentrations in Rat Testes: Measurement by Enzyme-Linked Immunosorbent Assay. Kerry L Rossow* and Philip G Reeves, USDA/ARS/HNRC, Grand Forks.

11:00 am Inihibition of Glycolytic Enzymes by Tubulin. Tim J Keith* and Harvey R Knull, UND, Grand Forks.

11:20 am Rat Liver after High-Dose L-Tryptophan Treatment. Donald L Matthies* and Francis A Jacobs, UND, Grand Forks.

THE EFFECT ON ARSENIC DEPRIVATION IN RATS OF DIETHYL MALEATE, AN *IN VIVO* CHEMICAL DEPLETOR OF GLUTATHIONE

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Recent studies have indicated that arsenic has a physiologic role affecting methionine metabolism (1). Methionine metabolism can be categorized into two main aspects, methionine cycling and transsulfuration. Methionine cycling involves the donation of a methyl group via S-adenosylmethionine, and the reactions that regenerate methionine by utilizing methyl sources such as 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 site at which arsenic affects methionine metabolism is unknown. Thus, an experiment was designed to determine the effect of arsenic deprivation in rats when the normal flux of methionine through the transsulfuration pathway was altered by the dietary addition of diethyl maleate (DEM). DEM enzymatically conjugates with glutathione, a cysteine containing tripeptide, which causes a decrease in tissue concentrations of glutathione and an increased rate of glutathione synthesis in the liver; this results in decreased tissue cysteine concentrations and an increased flux of methionine through the transsulfuration pathway (2).

Male weanling Sprague-Dawley rats were assigned to groups of six in a 2 x 2 factorially arranged experiment. Supplemented to the basal diet, based on acid-washed ground corn and casein, were arsenic (as $Na_2HAsO_4 \cdot 7H_2O$) at 0 or 0.5 µg/g and DEM at 0 or 0.25%. The rats were fed *ad libitum* their respective diets for 81 days, fasted overnight, weighed and decapitated subsequent to ether anesthesia and cardiac exsanguination.

Table 1. Effects of Arsenic, Diethyl Maleate (DEM) and Their Interaction on Body Wt., Liver Glutathione and Glutathione S-Transferase (GST), Plasma Cystine, Taurine and Glucose, and Blood Arsenic

Treatment		Body	Liver		Plasma			Blood
As	DEM	Wt.	Glutathione	GST[a]	Cystine	Taurine	Glucose	As
µg/g	%	g	µmol/g	units[b]	nmol/ml		mg/100 ml	µg/ml
0	0	389	5.17	1.15	26.3	159	137	0.08
0.5	0	406	5.38	1.22	29.1	181	152	17.1
0	0.25	376	4.63	1.32	24.5	155	164	0.07
0.5	0.25	358	4.68	1.36	25.2	142	144	17.1
			Analysis of Variance - P Values					
As		NS[c]	NS	NS	NS	NS	NS	0.0001
DEM		0.07	0.08	0.1	NS	0.02	0.06	NS
As x DEM		NS	NS	NS	NS	0.06	0.001	NS
EMS[d]		1609	0.67	0.05	35.9	466	120	2.86

[a]Substrate: 1-chloro-2,4-dinitrobenzene, 1 mM; [b]µmol/min/mg protein; [c]NS=not significant, p>0.05;
[d]Error Mean Square

DEM supplementation to the diet at 0.25% tended to reduce growth and the concentration of glutathione in the liver while only causing a small, but non-significant, increase in the activity of liver GST. Liver glutathione reportedly is a reservoir for cyst(e)ine. Plasma cystine was unaffected by dietary treatment but a cysteine metabolite, taurine, was decreased by DEM supplementation. DEM supplementation increased plasma glucose in the arsenic-deprived rats and decreased plasma glucose in the arsenic-supplemented rats. Blood arsenic was increased by arsenic supplementation and unaffected by DEM treatment.

Altering methionine metabolism changes the severity and nature of signs of arsenic deprivation. DEM supplementation did not affect plasma methionine (data not shown) and had little effect on liver glutathione and plasma cystine. Thus, apparently at this dietary concentration, DEM only slightly stressed glutathione metabolism and, therefore, the flux of methionine through the transsulfuration pathway. However, an interaction between arsenic and DEM affected plasma taurine and glucose in addition to several tissue trace elements (data not shown) which indicates that the DEM stress on methionine metabolism, although apparently small, still affected the physiological site of action of arsenic. The findings support the hypothesis that arsenic has a physiologic role affecting methionine metabolism, but whether the site of action is in methionine recycling or in transsulfuration is still open to conjecture.

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DIETARY BORON MODIFIES THE EFFECTS OF THIAMINE NUTRITURE IN THE MALE RAT

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The water-soluble vitamin, thiamine, is required for the production of energy. Recent findings indicate that dietary boron and thiamine interact to affect energy metabolism. For example, in marginally-deficient thiamine rats, 2 mg of supplemental dietary boron (SDB) in a diet containing 0.1 mg B/kg increased weight (172 vs 190 g) and serum triglyceride concentrations (32 vs 49 mg/dl) (1). Thus, a 2 x 2 factorially arranged experiment was designed to more fully determine the nature of the interaction between dietary boron and thiamine in regards to energy metabolism and body composition.

Sprague-Dawley rats (weanling males, 10 per group) were fed a ground corn-casein (vitamin-free)-corn oil based diet (containing 0.17 mg B/Kg) supplemented with 0 or 2 mg B (as orthoboric acid)/Kg and thiamine at 50% or 100% of the requirement (1 or 6 mg thiamine [as thiamine hydrochloric]/Kg of diet). At age 56 days, the animals were sedated while body composition was determined by bioelectric impedance (2). At age 60 days, the rats were fasted for 16 hours, weighed and decapitated subsequent to Ketamine-Rompun anesthesia and cardiac exsanguination. Biochemical measures were determined by established methods (3).

Table 1. Effects of Boron and Thiamine on Selected Indices of Energy Metabolism and Body Composition

Treatment		Serum					Erythrocyte	
Boron mg/Kg	Thiamine Requirement %	Triglycerides mg/dl	Ceruloplasmin mg/dl	Albumin g/dl	Glucose mg/dl	Body H_2O %	Transketolase U/g Hgb	Body Wt g
0	50	53.0	40.8	2.95	247	74.5	0.0001	301
0	100	58.3	39.3	3.11	165	77.2	0.0009	308
2	50	56.1	43.6	3.11	208	81.3	0.0001	274
2	100	82.2	33.3	2.89	222	74.9	0.0010	336
			Analysis of Variance - P values					
Boron		0.035	NS	NS	NS	NS	NS	NS
Thiamine		0.015	0.001	NS	0.040	NS	0.0001	0.024
Boron x Thiamine		NS	0.011	0.016	0.005	0.004	NS	NS
				Contrasts				
50% Thiamine, 0 vs 2			NS	NS	NS	0.002		
100% Thiamine, 0 vs 2			0.02	0.04	.01	NS		
Mean Square Error		357	21.4	0.048	2340	21.1	3.0×10^{-8}	2010

Thiamine deficiency, verified by decreased transketolase activity, decreased body weight and serum triglyceride concentrations (Table 1). SDB increased serum concentrations of triglycerides. In thiamine-adequate rats, SDB increased serum concentrations of glucose. In these same animals, SDB decreased serum concentrations of ceruloplasmin and albumin. In the thiamine-deficient rats only, SDB increased body water. These findings suggest that physiological amounts of dietary boron modify the affects of thiamine nutriture on energy metabolism. Also, boron nutriture seems to change the thiamine requirement. Therefore, it seems reasonable that dietary concentrations of boron should be controlled in rat experimental models.

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EFFECTS OF BORON, TRAINING EXERCISE AND THEIR INTERACTION IN MALE RATS

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Recent findings indicate that dietary boron influences energy metabolism. In male rats, 2.4 mg of supplemental dietary boron (SDB) in a diet containing 0.06 mg B/kg substantially depressed plasma insulin, pyruvate and aspartate transaminase concentrations, and increased plasma thyroxine (T_4) and triglyceride concentrations (1). In chicks, SDB significantly decreased the abnormally high concentrations of plasma glucose induced by vitamin D_3 deficiency (2). Also, dietary boron modulates hepatic glycolysis in chicks, particularly when dietary vitamin D_3 intake is inadequate(3). Because physiological stressors of energy metabolism seem to enhance the effects of SDB, it was decided to ascertain whether increased energy expenditure modifies the effects of SDB. Thus, a 2 x 2 factorially arranged experiment was designed to determine the effects of dietary boron on indices of energy metabolism perturbed by exercise training.

Weanling male rats (9 per group) were fed a ground corn-casein-corn oil based diet (vitamin- and mineral-adequate; ~0.19 mg B/kg) supplemented with 0 or 2.0 mg boron (as orthoboric acid)/kg. They remained sedentary or, during the last 35 d of the experiment, were placed on a powered running wheel and given incremental (26 d) then routine (454 m in 26 min) training 5 d/week. At age 61 days, the rats were fasted for 16 hours, weighed and decapitated subsequent to ketamine/rompun anesthesia and cardiac exsanguination. Biochemical measures were determined by established methods (1).

Table 1. Effects of Boron, Training Exercise and Their Interaction on Selected Indices Associated with Energy Metabolism in Male Rats

Treatment		Serum					
B mg/kg	Exercise training	Lactate Dehydrogenase U/L	Insulin μU/ml	Cholesterol mg/dL	Aspartate Transaminase U	Body Weight g	Liver wt/ Body wt
0	−	486	5.93	40.4	92.7	316	0.57
2	−	253	6.99	44.9	89.1	301	0.50
0	+	275	5.59	33.0	77.8	307	0.60
2	+	325	6.57	45.9	93.3	311	0.93
			Analysis of Variance - P Values				
B		NS	0.05	0.04	NS	NS	NS
Exercise Training		NS	NS	NS	NS	NS	0.003
B x Exercise Training		0.01	NS	NS	0.04	NS	0.01
Contrasts							
Exercise −: B, 0 vs 2		0.003	NS	NS	NS	NS	NS
Exercise +: B, 0 vs 2		NS	NS	0.03	0.01	NS	0.003
(Mean Square Error)$^{1/2}$		151	1.42	12.3	12.2	41.2	0.21

SDB increased serum concentrations of insulin in both the sedentary and exercised groups of rats (Table 1) but did not affect serum concentrations of glucose (not shown). The different response of insulin to SDB between this experiment and a previous one (1) probably reflects a change in protocol, i.e., the use of serum instead of plasma. Because hemolysis (sometimes present in plasma) affects the insulin assay, the present insulin findings are most likely correct. SDB also increased the serum concentrations of cholesterol. Body weight was not affected by either SDB nor exercise. With some indices, the exercised rats responded to SDB more than the sedentary animals. For example, in the exercised, but not sedentary, rats, SDB increased the liver weight:body weight ratio. Likewise, boron deprivation affected the serum concentrations of aspartate transaminase in the exercised animals only. On the other hand, SDB maintained the serum concentrations of lactate dehydrogenase in the sedentary rats only. These findings suggest that physiological concentrations of dietary boron modulate general energy metabolism, particularly under conditions of exercise training. Also, SDB may affect leucine metabolism, an amino acid which stimulates insulin production and serves as a substrate in cholesterol synthesis.

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HIGH DIETARY FRUCTOSE AFFECTS PLASMA CHOLESTEROL CONCENTRATIONS AND SIGNS OF SHORT-TERM COPPER DEPRIVATION IN MEN

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Attempts to produce signs of copper deprivation in adult humans have not yielded consistent changes in indices of copper status. Because copper is involved mainly in oxidative metabolism, any pathological change induced by copper deficiency probably would reflect changes in free radical metabolism or oxidant damage. However, it seems likely that the extent of oxidant damage or changes in indices related to free radical metabolism in response to copper deprivation would vary depending on the need for oxidative metabolism, the production of free radicals and the activity of alternate anti-oxidant defense mechanisms. Thus, various factors, including the intake of other nutrients which affect the need for anti-oxidant action, may have affected the response to short-term copper deprivation in previous studies. Therefore, an experiment was performed to ascertain whether high dietary fructose would affect the response to copper deprivation. Fructose was chosen because an intermediate in its metabolism, glyceraldehyde, undergoes auto-oxidation and generates free radicals (1), and because it has been shown to enhance the signs of copper deficiency in rats (2). Five men aged 27 to 35 years were housed in a metabolic unit and fed a mixed Western diet that supplied about 0.6 mg Cu/2500 kcal and low, but not deficient, amounts of nutrients involved in antioxidant activity. After an equilibration period of 21 days in which the carbohydrate variable was cornstarch and 0.7 mg of copper was supplemented per day, all men participated in four dietary periods of 48 days. In these periods, supplements of copper (0.0 or 2.0 mg/day), and fructose or cornstarch as 20% of dietary energy, were varied in a 2 x 2 factorial design. Variables indicated in Table 1 were determined by our usual methods (3) during the last week in each dietary period. A P value of 0.05 was considered significant.

Table 1. Effect in Men of Dietary Copper Intake, Carbohydrate Source, and Their Interaction on Selected Serum Variables, Whole Blood Glutathione and Erythrocyte Superoxide Dismutase. Values are means ± SD.

Dietary Period	Cholesterol mg/dl	LDL Cholesterol mg/dl	HDL Cholesterol mg/dl	Glucose mg/dl	RID Ceruloplasmin mg/dl	Glutathione mg/dl	Superoxide Dismutase U/g Hb
LoCu-Fructose	253 ± 54	188 ± 63	37 ± 2	80 ± 6	24.1 ± 2.9	37.4 ± 3.3	2767 ± 433
HiCu-Fructose	250 ± 59	186 ± 61	40 ± 7	85 ± 6	29.5 ± 5.8	32.6 ± 2.6	2983 ± 368
LoCu-Starch	225 ± 66	161 ± 71	40 ± 3	78 ± 7	26.1 ± 3.6	35.8 ± 4.0	3090 ± 676
HiCu-Starch	227 ± 59	164 ± 62	43 ± 7	80 ± 5	23.0 ± 3.3	34.6 ± 3.4	2804 ± 393
Analysis of Variance							
Copper	1.00	0.96	0.15	0.03	0.39	0.06	0.82
Carbohydrate	0.004	0.004	0.12	0.04	0.11	0.89	0.64
Cu x Carbohydrate	0.73	0.68	0.88	0.30	0.006	0.23	0.13

High dietary fructose significantly increased serum cholesterol concentrations; most of the increase was in the LDL-cholesterol fraction. Fructose did not significantly affect HDL-cholesterol concentrations. High dietary fructose also increased serum glucose concentrations but did not independently affect serum RID (immunoreactive) ceruloplasmin, whole blood glutathione, or erythrocyte superoxide dismutase concentrations. In animals, and sometimes in humans, copper deprivation increases serum cholesterol (2, 3). In this study, copper deprivation had no effect on serum cholesterol and high dietary fructose gave no indication of affecting this finding. On the other hand, some variables associated with oxidative metabolism responded or tended to respond to copper deprivation with the response affected by fructose. For example, RID ceruloplasmin was decreased by copper deprivation when fructose was fed, but was increased by copper deprivation when starch was fed. Erythrocyte superoxide dismutase tended to act similarly. An increase in whole blood glutathione during copper deprivation approached significance; the apparent increase was highest when dietary fructose was high. The findings show that dietary fructose can induce changes in serum cholesterol that are often considered detrimental, and possibly affects the response to short-term copper deprivation by increasing oxidative metabolism in men.

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THE EFFECTS OF DEFICIENT OR MARGINAL IRON NUTRITURE ON SPONTANEOUS PHYSICAL ACTIVITY OF RATS

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The effect of iron nutriture on physical activity was investigated in rats. Male Sprague-Dawley rats (97 ± 10 g), 8 rats per group, were fed *ad libitum* for 8 weeks. Diets were modified casein-based AIN-76a diets supplemented with ferric citrate and confirmed by inductively coupled argon plasma atomic emission spectrometer analysis to contain one of three iron concentrations: deficient (5 ppm), marginal (20 ppm), or adequate (108 ppm). The rats were maintained individually in plexiglass cages under a 12-hour light-dark cycle. Hematocrit and hemoglobin were determined weekly on venous blood (.5 ml) drawn from the tail; liver non-heme iron (1) was determined at the conclusion of the study. Marginal rats had normal hematocrit and hemoglobin but reduced iron stores as indicated by liver non-heme iron (Table 1).

Table 1. Iron Status of Rats

(Means ± SD)	Dietary Iron (ppm)		
	5	20	108
Hematocrit (fractional)	0.20 ±0.02	0.45 ±0.01	0.46 ±0.01
Hemoglobin (g/L)	52 ±6	152 ±4	159 ±6
Liver Non-heme Fe (μmol/g dw)	0.79 ±0.24	1.36 ±0.39	4.58 ±0.62

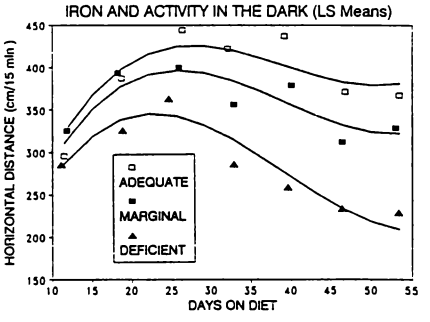

Figure 1

The 23-hour activity of each rat was monitored weekly by placing each plexiglass cage in a Digiscan Animal Activity Monitor by Omnitech.† Horizontal, stereotypic, and circling activities were recorded from infrared beam interference in the X/Y plane and vertical activities from infrared beam interference in the Z plane during 15-minute intervals. Within each phase of the light cycle, individual activities were statistically modeled across time by regression analysis with blocking for individual rats. The regression model contained linear, quadratic, and cubic components. Each component also contained the following contrasts: (a) deficient vs. marginal and adequate and (b) marginal vs. adequate. During the dark cycle, the adequate and marginal animals exhibited more horizontal and vertical movements and spent more time vertical than the deficient animals. In the dark cycle, the adequate animals were more active than the marginal animals, and the deficient animals were substantially less active than the other two groups in activities such as time spent ambulating, total distance traveled (Figure 1), average distance traveled per movement, and number of circling movements. In the dark, (but only during weeks 2 to 4) deficient animals had greater average movement speed than marginal and adequate animals. During the light cycle, deficient animals were least active, but (during weeks 3 to 6) marginal animals were more active than adequate animals in time spent ambulating, total distance traveled, number of horizontal and vertical movements, and time spent vertical.

These results confirm that spontaneous activity was affected by iron nutriture, including marginal nutriture with normal blood hemoglobin values but low iron stores. Animal activity was greater during the dark cycle for all groups; diurnal activity was not reversed by inadequate iron status as found by other methods (2,3). However, greater activity during the light cycle by marginal animals as compared to the other groups indicated moderate circadian effects. The increased speed of deficient animals (dark cycle) early in the experiment and greater activities (light cycle) of marginal animals in the middle of the experiment suggest that early or marginal iron deficiencies produce different effects than severe deficiency. These results may have practical significance for human nutrition. Iron deficiency is the most common nutrient deficiency in the world and 20% of 18 to 44 year-old females in the U.S. have low iron stores.

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THE EFFECT OF VANADIUM DEPRIVATION IN THYROXINE REPLETE-THYROIDECTOMIZED RATS

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In a previous study, vanadium deprivation and an interaction between vanadium and iodine affected several variables associated with thyroid metabolism in rats (1). Vanadium deprivation increased thyroid weight and thyroid weight/body weight ratio. Also, as dietary iodine was increased, thyroid peroxidase activity decreased with the decrease more marked in the vanadium-supplemented than the vanadium-deprived rats. The findings suggested that vanadium may have a physiologic role affecting iodine metabolism and thyroid function. Whether the effect of vanadium on iodine metabolism and thyroid function is direct or indirect is unknown. One possible indirect mechanism through which vanadium could have an effect is by altering the activity of extrathyroidal deiodinase enzymes. To test this hypothesis we determined the effect of vanadium deprivation in thyroidectomized (parathyroid transplant) rats receiving l-thyroxine (T_4). Four-week-old thyroidectomized Wistar-Kyoto rats were used. Seven rats were fed a basal casein-corn based diet which contained 2 ng V/g. Controls (n=6) were fed the basal diet supplemented with 0.5 μg V/g as NH_4VO_3. All rats were implanted with a time-release pellet containing T_4 (0.1 mg T_4 released over 21 days). Each rat received three pellet implants; the first, 2 days after the rats were received and thereafter at 21-day intervals. After 54 days, core temperatures were measured, the rats were then fasted overnight, weighed, and decapitated following ether anesthesia.

Table 1. Effects in T_4 Replete-Thyroidectomized Rats of Vanadium Deprivation on Core Temperature, Body Weight, Plasma T_3 and T_4, Glucose 6-Phosphate Dehydrogenase (G6PDH) and Mucosal (Cecum) Carbonic Anhydrase.

Treatment V μg/g	Body Weight g	Core Temp. °C	Plasma T_3 ng/100 ml	Plasma T_4 μg/100 ml	G6PDH U/10^{12} RBC	Carbonic Anhydrase units[b]
0	367±9.8[a]	38.1±0.2	64.4±3.5	6.9±0.4	239±4	0.065±0.003
0.5	365±4.5	38.3±0.2	60.2±5.7	7.2±0.6	250±3	0.088±0.007
P - Value	NS[c]	NS	NS	NS	0.05	0.035

[a]Mean ± SEM; [b]units = μmol nitrophenyl formed/min/mg protein; [c]NS = not significant, p>0.05

As reflected in the final weights, growth was not affected by dietary treatment. There also were no significant changes in liver, heart, spleen, or kidney weight/body weight ratios (data not shown). Core temperature, an indicator of basal metabolic rate, was not affected by dietary vanadium. Plasma T_4 was within the normal range for a rat; thus, the pellet delivery system functioned adequately. T_3 was also within the normal range with no difference because of dietary treatment. T_3 is produced in the thyroid or by extrathyroidal deiodination of T_4. Because of this, the data suggest that dietary vanadium does not significantly affect the plasma concentration of T_3 formed by extrathyroidal deiodination of T_4. Red blood cell G6PDH, a key enzyme in the pentose phosphate cycle, was decreased by vanadium deprivation. There was no effect of dietary treatment on RBC number (data not shown). One of the controlling mechanisms for G6PDH is through the thyroid hormones; a thyroid hormone stimulus can increase the activity of G6PDH. Thyroid hormones can also affect the activity of carbonic anhydrase; the activity of this enzyme in the cecum decreased with vanadium deprivation. Data from G6PDH and CA still support the hypothesis that vanadium may have a physiologic role affecting iodine metabolism and/or thyroid hormone function. It is also possible that vanadium, through the thyroid hormones, could affect carbonic anhydrase, an enzyme that can affect the metabolism of numerous biosubstances including glucose, fatty acids, and calcium. This could explain some of the myriad effects of vanadium.

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THE EFFECT OF HIGH ZINC ON THE UPTAKE OF COPPER IN CACO-2 CELLS IN CULTURE

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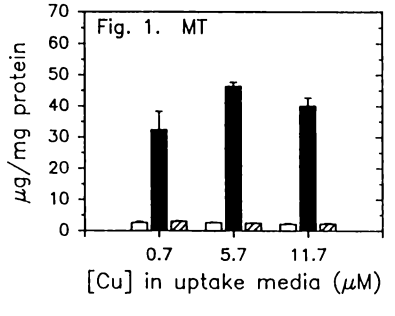
It is currently accepted that the absorption of Cu by the intestinal mucosa is reduced during times of high dietary Zn intake (1). Because metallothionein (MT) has higher affinity for Cu than for Zn, the consensus is that MT induced by the high Zn binds Cu and makes it inaccessible for absorption into the portal circulation (1, 2). Based on this theory, one would expect to see increased Cu concentrations correlating to the increased MT concentrations in the intestinal epithelium. However, a recent report by Reeves and Nelson (3) described a study whereby high parenteral Zn administered to adult male rats increased the MT concentrations of intestinal epithelial cells, but did not increase the Cu concentrations. In order to clarify this discrepancy we evaluated the uptake of Cu into *in vitro* intestinal cells grown in the presence of high Zn. An adenocarcinoma cell line derived from human colon, Caco-2, was used as a model for the intestine.

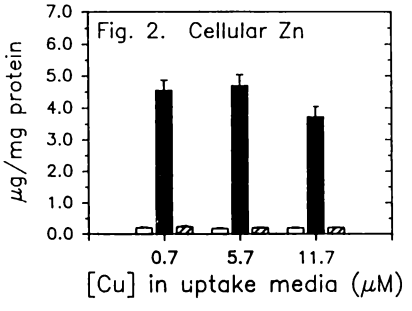
Caco-2 cells were seeded onto transwell inserts (36 per group). Two groups were maintained in normal complete medium (NZn) consisting of 90% Dulbecco's Modified Eagles Medium, 10% fetal bovine serum, 1% nonessential amino acids, 2 mM L-glutamine, and 50 µg/ml gentamicin sulfate. This media contained 5 µM Zn. The third group was maintained in NZn supplemented with 200 µM Zn sulfate (HZn). The growth environment was 5% CO_2 and 95% relative humidity at 36.5°C. At 21 days after seeding, the growth media was replaced with treatment media supplemented with 0.7, 5.7, or 11.7 µM Cu sulfate. The treatment media for cells grown in NZn growth media contained either NZn or HZn in addition to the indicated Cu sulfate concentrations. The cells grown in HZn media were maintained in HZn treatment media (3 treatments/growth group; Table 1). All treatment groups were incubated for 1 hr at 36.5°C in 5% CO_2. The monolayers were washed with a HEPES balanced salt solution, scraped from the inserts, and stored at −20°C until analysis. MT was analyzed by a cadmium binding assay (6 monolayers/treatment). Cellular Cu concentrations were analyzed by graphite furnace and cellular Zn concentrations by atomic absorption (6 monolayers/treatment).

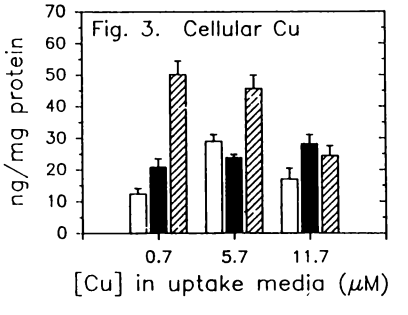
As expected, cells grown in 200 µM Zn (■) for 21 days had higher concentrations of MT than those grown in 5 µM Zn (□). Cells exposed to high Zn for only one hr (▨) did not have higher MT concentrations than those not exposed to high Zn (□) (Fig. 1). High Zn concentrations were seen in those cells with high MT (Fig. 2). However, cellular copper concentrations did not increase in parallel with MT (Fig. 3). These data support the report by Reeves and Nelson (1991) that Cu is not sequestered into MT *in vivo* when Zn is present in high amounts. The increase in copper uptake by cells exposed to high Zn for only 1 hr (▨) is not clear, but experiments are in progress to examine this more closely.

Table 1. [Zn] of Media, µM

[Zn] of Media, µM	
Growth	Treatment
5	5 □
200	200 ■
5	200 ▨







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THE EFFECT OF ZINC DEFICIENCY ON ANGIOTENSIN CONVERTING ENZYME CONCENTRATIONS IN RAT TESTES: MEASURMENT BY ENZYME-LINKED IMMUNOSORBENT ASSAY

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A loss of angiotensin converting enzyme (ACE) activity has been observed in the testes of rats fed a zinc-deficient diet before puberty (1). Whether this is caused by a reduction in ACE protein concentration or by an altered enzyme property is unknown. To solve the first part of this question, an enzyme-linked immunosorbent assay (ELISA) was developed to quantify the ACE protein concentration that is associated with ACE activity. ACE was purified by affinity chromatography using the selective ACE inhibitor lisinopril covalently bound to Sepharose CL-4B gel (2, 3). For the development of antibodies (Ab) to rat ACE, purified ACE was mixed with Freund's adjuvant and injected subcutaneously into a rabbit. Rabbit Ab were isolated from serum by using the Pierce ImmunoPure (A/G) IgG Purification kit. A bio-dot immunosorbent analysis verified the development of high titer rabbit Ab to rat ACE. Ab-coated polystyrene tubes were prepared with 1 μg/ml rabbit Ab in 0.1 M $NaHCO_3$. ACE was conjugated to alkaline phosphatase. Alkaline phosphatase suspension containing 0.3 mg protein was centrifuged to obtain pelleted protein. The protein was resuspended with 0.1 ml of virgin ACE (120 μg). This suspension was dialyzed overnight against phosphate buffered saline (PBS). Glutaraldehyde was added at 0.2% and mixed for 2 hours. The solution was diluted to 1.0 ml with PBS and dialyzed overnight against PBS. The final ACE conjugated alkaline phosphatase (ACEAPC) was used to develop the ELISA (4). The amount of ACEAPC that bound 50% of the Ab sites in the Ab-coated tubes was determined by overnight incubation of serial dilutions of ACEAPC (Fig. 1). P-nitrophenylphosphate, alkaline phosphatase substrate (1 mg/ml), in 50 mM $NaCO_3$ pH 9.8, was added and incubated for 30 min at room temperature, the reaction was stopped with 500 mM NaOH and absorbance read at 400 nm. It was found that approximately 50% of the Ab binding sites were occupied by a 1:50 dilution of the ACEAPC. Standard curve for the ELISA was determined by using virgin ACE purified by the affinity column. ACE was resuspended with 0.1% BSA to a concentration of 10,000 ng ACE/ml and used as the ACE standard. Blanks with no ACEAPC and total bound with only ACEAPC were also assayed. The alkaline phosphatase color that was present after incubation is inversely proportional to the amount of ACE protein present across a given range.

Twenty-one male Sprague-Dawley rats, 40-50 gms, were divided into 3 groups of 7 rats each. Group 1 (+Zn) was fed +Zn diet (55 ppm Zn) ad libitum, group 2 (-Zn) was fed -Zn diet (<1 ppm Zn). Because zinc deficiency depresses food intake, group 3 (+ZnPF) was pair-fed +Zn diet in daily amounts equal to that eaten by the -Zn group. After 4 weeks, the animals were anesthetized with sodium pentobarbital and blood and tissues were removed for analysis. ACE activity (Fig. 2) and ACE protein (Fig. 3) was determined by enzyme analysis and ELISA, respectively. Zinc deficiency caused a decrease in both ACE activity and ACE protein; this decrease was also observed in the pair-fed animals. The latter finding was unexpected. In previous works (1) +ZnPF rats had the same ACE activity as the +Zn rats. However, rats in previous experiments were begun at an older age than in this experiment. This finding suggests that ACE protein expression and activity is affected both by Zn deficiency and inanition. Nonetheless, this experiment has conclusively shown that the depression of ACE activity in -Zn rat testes is caused by a reduction in ACE protein.

Fig. 1

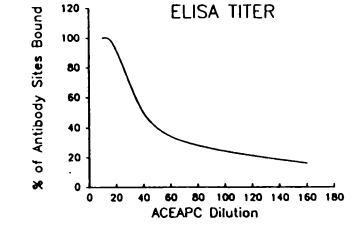


Fig. 2

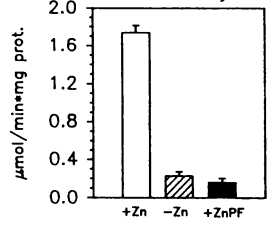
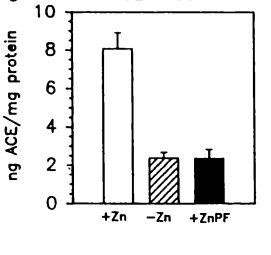


Fig. 3



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INHIBITION OF GLYCOLYTIC ENZYMES BY TUBULIN

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Interactions between the glycolytic enzymes and the structural protein tubulin have recently been reported (1, 2). Several potential functional implications have been suggested for this interaction: among these are channeling and metabolic regulation. In order to determine if the interaction between tubulin and the glycolytic enzymes played a functional role, enzyme activities were determined in the presence and absence of tubulin.

Tubulin was isolated from bovine brain and endogenous glycolytic enzymes were dissociated either by addition of 150 mM KCl to polymerized microtubules followed by centrifugation to pellet the microtubules (2)or by subjecting tubulin to DEAE Sephadex chromatography (3). Enzyme assays were carried out under low ionic conditions in the presence and absence of tubulin. Polyethylene glycol (PEG) was included in the assays to simulate a volume exclusion (2) effect (macromolecular crowding) which occurs in the cytoplasm because of the high protein concentration. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity was determined spectrophotometrically at 10° C in 9.25 mM TEA (pH 7.6), 4 % PEG, 0.2 mM 3-PGA, 0.08 mM ATP, 0.3 mM EDTA, 0.5 mM $MgSO_4$ with 2 U/ml of 3-phosphoglyceric phosphokinase (PGK)to generate 1,3 PGA to initiate the reaction. GAPDH was used at 3 µg/ml in all assays, tubulin concentrations were 0, 0.18, 0.55 or 1.83 µM and NADH concentrations varied from 0.3 - 2.6 µM. Lactate dehydrogenase (LDH_M) activity was assayed fluorometrically at room temperature (approximately 22° C) in 10 mM Pipes (pH 6.8), 4 % PEG, 1 mM DTT, 0.5 mM EDTA and 1 mM pyruvate. LDH_M was used at 0.08 µg/ml in all assays, tubulin concentrations were 0 and 10 µM, and NADH concentrations varied from 1 - 50 µM.

The effect of the interaction between tubulin and GAPDH was an inhibition in the GAPDH activity. This inhibition was seen throughout the range of NADH used in the experiment and was increased with increasing concentration of tubulin. The overall result of the inclusion of the tubulin was a lowered apparent V_{max}. The apparent V_{max} values for the 0.18, 0.55 and 1.83 µM tubulin treatments were 78 %, 32 % and 17 % of the control value respectively. The effect of the tubulin interaction on LDH_M activity was also inhibitory. Under these assay conditions there was an allosteric effect in the range of 1 - 10 µM NADH. At higher concentrations of NADH there were no differences in activity due to the tubulin interaction and indeed the V_{max} was similar in the presence and absence of tubulin. Rates of the inhibited enzyme varied from 17 % to 56 % of the control values in the allosteric range.

In these experiments assay conditions were chosen to allow a high degree of interaction between the enzyme and tubulin molecules in order to determine potential effects. Since the cellular concentration of tubulin, 70 - 270 µM (4) based on 20 % protein, and average protein concentration, 17-26 % (5), is higher than those utilized in these experiments therefore enhancing molecular crowding effects are predicted to occur *in vivo*. As a result of these experiments we feel that tubulin does have a role in the regulation of metabolism in the glycolytic pathway.

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RAT LIVER AFTER HIGH-DOSE L-TRYPTOPHAN TREATMENT

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L-tryptophan (TRP) has been withdrawn from non-prescription access as a result of several reports of adverse effects of this essential amino acid when used medicinally for reduction of sleep latency in insomnia, depression, etc. The most recent adverse effect report is the development in humans of an eosinophilic myalgia syndrome (EMS) which prompted its withdrawal. This effect had been traced to a single supplier and even specific batches of the chemical which had been produced by recombinent DNA technology (1). An earlier reported adverse effect was fatty liver in rats detected in histological sections from animals treated with 250mg/kg TRP by gastric gavage (2). TRP had been used widely by humans for years without problems and certain features of the techniques employed in this paper struck us as less than ideal so we repeated the work with U.S.P. Grade TRP, chemically synthesized.

Using the same investigative methods, viz., frozen histological sections of liver stained for lipid by the oil Red-O, Hematoxylin technique after TRP treatment by gastric gavage we found no liver lipid deposition in our 400gm Sprague Dawley rats. We had delivered the TRP carefully to the stomach as a slurry in a volume of 3.0ml 0.9% saline. The dose volume used by the authors reporting liver damage was 7.5ml. Such a large volume demonstrably stretches the stomach of an adult rat, a condition which would be aggravated by the presence of food as could be expected under ad lib. feeding conditions. We found in separate experiments that this high volume frequently caused a reflux of the gavage vehicle plus TRP up the esophagus with a high incidence of aspiration into the respiratory tract. In rats where this had occurred we did find evidence of liver lipid even when saline alone was administered. We postulated that the fat deposition was caused by impaired feeding occasioned by discomfort due to the large gavage volume and reflux aspiration and that this fasting was the cause of the liver lipid. Liver lipid had been demonstrated earlier in fasting hamsters (3).

By simply fasting our rats (but providing water ad lib.) we found that as little as 24 hours without food was sufficient to induce liver lipid accumulation. We have demonstrated this abnormal condition by several histochemical techniques and although the histological picture is striking, the lipid would not be of such a quantity as to qualify as a fatty liver, which is a stage in liver degeneration. Four papers were cited by the authors of the paper reporting fatty liver in response to TRP as finding similar results. Three of these papers used fasting animals and did not report liver lipid increase while the fourth paper did not mention liver lipid.

We believe that the work reported here demonstrates that when carefully administered TRP does not induce fatty liver in rats at doses many times that used by humans. We suggest that the one paper which reported this result had encountered artifact induced by faulty delivery methods.

Experimental Protocol: Liver Lipid Response to TRP Treatment and Fasting

Group	No. Rats	No. Treatments and Duration	Autopsy	Liver Lipid
1	12	TRP 250mg/kg X 7 over 14 days	Day 17	Negative
2	6	Saline 3.0ml 7 days	Day 17	Negative
3	15	Sham treatment (1)	Day 17	Negative
4	6	Anesthesia only (2)	Day 17	Negative
5	16	No Treatment--Control		Negative
6	9	Fasted 24 hours	End Day 1	Positive
7	6	Fasted 48 hours	End Day 2	Positive
8	9	Fasted 72 hours	End Day 3	Positive

(1) Rats anesthetized and treated with empty gavage tube
(2) Rats lightly anesthetized - no intubation

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1:20 am Topographic Map Analysis of Landforms East of Black Hills, South Dakota.
Eric N Clausen*, MinSU, Minot.

1:40 am Historic Sedimentation at Kindschi Lake, Sheridan County, North Dakota.
Richard Faflak*, VCSU, Valley City.

2:00 am Microfaunal Comparisons Between the Lower and Upper Portions of the Hell Creek Formation (late Maastrichtian) in Southwestern North Dakota.
Dean A Pearson*, Pioneer Trails Museum, Bowman.

2:20 am Blob Geology and Exotic Terranes: A Counter-Example from the Saskatchewan Canadian Shield.
Dexter Perkins*, UND, Grand Forks.

2:40 am Oxygen and Hydrogen Isotope Geochemistry of Groundwaters from a Discharge Wetland Area, Red River Valley, North Dakota, and Evidence for Pleistocene Recharge of the Inyankara Formation.
Ronald K Matheney*, UND, Grand Forks.

Topographic Map Analysis of Landforms East of Black Hills, South Dakota

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Introduction: Clausen (1) described a progressive sequence of asymmetric drainage divides separating drainage basins tributary to the Missouri River. This pattern suggests drainage basins were systematically formed in a single or small number of related geologic events. Immediately east of the Black Hills, drainage divides between the Bad River and the White and Cheyenne Rivers and between several tributaries of the Cheyenne River, while asymmetric, are reversed. The purpose of this study is to develop an explanation for these reversals.

Method and Observations: Analysis of regional landforms observed on detailed topographic maps was done using mosaics of photographically-reduced 7.5 minute series topographic maps. Key observations included:

Asymmetric drainage divides between Cheyenne River tributaries: Divides, separating drainage basins of east-flowing Cheyenne River tributaries, suggest the following sequence: first, South Fork of French Creek, French Creek, Battle Creek, Spring Creek, Rapid Creek, Boxelder Creek, Elk Creek, and finally the Belle Fourche River; all formed by headward erosion associated with a southeast-oriented drainage (not northeast as at present).

Wall-like escarpment between Cheyenne River and Bad River drainage basins: Near Wall, SD, a wall-like, north-south oriented escarpment separates the Cheyenne and Bad River basins, truncates southeast-trending valleys and ridges, and suggests scarp retreat as a southeast-flowing sheet of water stripped the Bad River drainage basin.

Wall-like escarpment between Bad River and White River drainage basins: A wall-like escarpment separating the Bad and White River drainage basins truncates broad, shallow, southeast-trending valleys and ridges. Escarpment shape and orientation suggest formation by uneven scarp retreat, as a sheet of southeast-flowing water stripped the White River drainage basin surface.

Amphitheater-shaped basins along Cheyenne and White River drainage divide: Large amphitheater-shaped basins, opening to the southeast, are present just northwest of the divide separating the Cheyenne and White River drainages. These include the Scenic and Sage Creek Basins. The shape and orientation suggests the basins were formed as large headcuts by a large sheet of southeast-flowing water.

Streamlined erosional hills southeast of Scenic Basin: Small, streamlined erosional hills are present on upland surfaces southeast of the Scenic Basin. Erosional hills such as these have been associated with catastrophic flood events (2) and suggest formation by an extensive sheet of southeast-flowing water.

Trench-like valleys connecting amphitheater-shaped basins with Cheyenne River: Spring Creek and Bear Creek flow northwest from the Scenic Basin to the Cheyenne River in trench-like through valleys similar to flood-produced inner channels described by Kehew and Lord (2). The valleys suggest a large sheet of water, filling the White River drainage basin, broke through the drainage divide to flow northwest into the Cheyenne River.

Southeast-trending valleys truncated by Pine Ridge Escarpment: Valleys of southeast-trending Niobrara River tributaries, notably the Keya Paha, are truncated by the Pine Ridge Escarpment, while White River tributaries, including the Little White River and Bear-in-the-Lodge Creek, begin in truncated southeast-trending valleys, turn abruptly north, and then flow down the escarpment. These truncated, broad, shallow, southeast-trending valleys suggest a large sheet of southeast-flowing water flowed across the region prior to escarpment formation.

Geomorphic History: First, a large sheet of southeast-flowing water, possibly from northwest of the Black Hills area, extended from the Black Hills area to the present-day Niobrara River drainage basin. Second, the Pine Ridge Escarpment was cut as the large sheet of southeast-flowing water was channeled eastward and scoured easily-eroded sedimentary units and lowered the present White River drainage basin surface. Third, flow was progressively (from east to west) diverted into the Bad River drainage basin, leaving a wall-like escarpment as a stark reminder of what had been a retreating scarp, 200 km. or more in width. Fourth, water continued to spill southeastward through what is now the Badlands National Park forming amphitheater-like headcuts, small streamlined hills, and in a progressive sequence the South Fork of French Creek, French Creek, Battle Creek, Spring Creek, Rapid Creek, Boxelder Creek, Elk Creek, and the Belle Fourche River valleys. Fifth, flow into the Bad River drainage basin was diverted into the Cheyenne drainage basin and rapid headward erosion of the Cheyenne River valley, perhaps aided by a large influx of water moving around the south end of the Black Hills, cut off southeast flow through the Badlands National Monument region. Sixth, the large sheet of water, still occupying the White River drainage basin, partially reversed direction to drain northwestward by breaching divides between southeast-oriented basins and the newly formed Cheyenne River valley. Seventh, the flood event abruptly ceased leaving landforms very much as they are today. All events are consistent with a single (or relatively small number of) catastrophic flood event(s) and are consistent with the hypothesis of progressive headward development of the Missouri drainage system.

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HISTORIC SEDIMENTATION AT KINDSCHI LAKE, SHERIDAN COUNTY, NORTH DAKOTA

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Kindschi Lake is located in the NW¼, S34, T148N, R76W of Sheridan County, North Dakota. The non-integrated lake is the remnant of one of the numerous kettles on the Missouri Coteau. The uppermost sediments are all post-glacial deposits of the clay facies of the Oahe formation which have been derived largely from hillslopes, according to Bluemle (1). During the summer of 1990, the lake was dry. Even dunes, approximately 1½m high, comprised of lake sediments had formed along the eastern shore of the lake. The only other time the lake was dry was during the droughts of the mid-thirties, according to the local residents. The dry lake bed thus provided an opportunity to closely examine the lake bed sediments. Of particular interest was the determination of the amount of historic sedimentation from the time of earliest agriculture in Sheridan County, and whether the mode of sediment transport was dominantly aeolian or fluvial.

Field testing consisted of excavating two 1x1 m pits to a depth of one m. A one inch core was also driven to a depth of 2.5 m. The sediments were examined in the wall profile and samples taken for seive analysis. The profiles exhibited a dark, gray-black humic layer (muck) which extended from the surface to a depth of 38 cm. Within this zone, at a depth of 19 cm was found a charred, undecomposed goldenrod gall. Another interesting feature of the profile was the presence of a thin, 5 mm thick layer of sand grains interspersed with pebbles at a depth of 26 cm. This was interpreted as a lag concentrate from a previous dry spell. At the base of the dark, humic layer was another lag of pebbles and cobbles, below which was homogeneous, light colored, sandy silt. Extending below the surface of the lowest lag in one of the pits was a 20 cm deep zone of bioturbation, presumably the hoofprint of a large animal. This level was interpreted to represent the former lake bed surface.

The laboratory procedure consisted of drying, weighing, and wet seiving 70-100 g samples at -1.0Ø, 0.0Ø, +1.0Ø, +2.0Ø, +3.0Ø, and +4.0Ø intervals. The humic samples were dried, weighed, and ignited to determine organic content. The upper 26.7 cm averaged 12.3% organic content, while the lowest-most 11.4 cm averaged 5.2% organic content by weight. The seive fractions from each sample were dried, weighed, and plotted as a cumulative percentage on probability paper to determine the mean grain size (M_z). The 16th, 50th, and 84th percentile values were read directly from the constructed curve and inserted into the formula $M_z = Ø16 + Ø50 + Ø84/3$ to derive mean phi (Ø).

Historic records indicate that Sheridan County was initially settled by cattle and sheep ranchers in the last decades of the 19th century. It wasn't until the railroad reached Denhoff in 1901 that large scale grain agriculture could begin (2). It is thus inferred that the type and rate of sedimentation at Kindschi Lake changed significantly after 1901.

Results of the investigation indicate that the combined observations lead to the conclusion that nearly 38 cm of sedimentation at Kindschi Lake is of very recent origin probably linked to agriculture. Evidence for this hypothesis comes from the observation that 1) there is a sharp boundary between the upper silty humic layer and the underlying non-organic sands; 2) there is a marked lag concentrate at the top of the sand surface; 3) there is a lag concentrate at the right depth to represent a mid-thirties drought; 4) bioturbation near the base of the humic layer may indicate the practice of ranching; 5) the undecomposed goldenrod gall at 19.1 cm suggests recent deposition.

Aeolian origin of the uppermost sediments is suggested by the mean grain size of the sediment. An M_z of 5.7Ø is smaller than the average grain size of loess (4.5Ø), which means that a higher wind velocity and/or disturbance was needed to initially suspend the particles (3). The latter condition was certainly encountered in agricultural practices. The presence of dunes also indicated the susceptibility of the material to movement. A yearly sedimentation rate of .45-.50 cm/yr (including organics) is suggested as a modern rate. The rate from 1901 to the mid-thirties was at least .33 cm/yr, but may have been greater, as an unknown amount of sediment was eroded.

SAMPLE DEPTH (in.)	(cm.)	M_z (Ø)	WENTWORTH SIZE SCALE	REMARKS
0.0	0.0	5.7	medium silt	Surface dunes; 16.5% organics
4.5	11.4	5.7	medium silt	Humic silt; 12.3% organics; snail tests
7.5	19.1	--	----	Charred goldenrod gall
10.5	26.7	--	----	5 mm thick lag sand
13.0	33.0	4.4	coarse silt	Humic silt; 5.2% organics; snail tests
15.0	38.1	--	----	Lag concentrate with bioturbation
18.0	45.7	1.8	medium sand	No organics; yellow-buff sands
22.5	57.2	5.9	medium silt	Yellow-buff silts
27.0	68.6	6.3	fine silt	Yellow-buff silts
34.0	86.4	3.0	very fine sand	
41.0	104	2.6	fine sand	Clayey sands with iron oxide
42.0	106	2.1	fine sand	stains and occasional
55.0	140	3.3	very fine sand	carbonate nodules
66.0	168	3.6	very fine sand	
78.0	200	3.3	very fine sand	
90.0	230	3.4	very fine sand	
98.0	250	4.1	coarse silt	

Table 1. Composite data from pit and core samples

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MICROFAUNAL COMPARISONS BETWEEN THE LOWER AND UPPER PORTIONS OF THE HELL CREEK FORMATION (LATE MAASTRICHTIAN) IN SOUTHWESTERN NORTH DAKOTA

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Analysis of 1152 tooth specimens from four stratigraphically distinct horizons in the Hell Creek Formation of southwestern North Dakota seem to show a vertebrate fauna change commensurate with that of a know floral change (1).

Tooth specimens were chosen for this study because dinosaurs, lower vertebrates, and fish taxa shed teeth throughout their life and the relative abundance of unreworked shed teeth should give an indication of relative abundance of a particular taxon. Since most taxa are not sessile, the teeth shed should be spread throughout the animals feeding range giving an indication of relative abundance. A total of 98 sites were collected yielding 3146 specimens. Of these, four sites producing the 1152 teeth were selected for the comparative analysis. Three of these sites are located in the upper quarter of the formation at 9, 15, and 24 meters below the Cretaceous-Tertiary (K-T) boundary. The fourth site is located at 20 meters above the base or 90 meters below the (K-T) boundary. Using this information trends were plotted for the formation. Specimens were grouped into three taxa assemblages for comparative analysis: Fish, Lower Vertebrates, and Dinosaurs. Johnson (1, 2) reports that only 25% of the floral taxa present in his HCIIb floral subzone survive until the next floral zone HCIII. This zone boundary is located from 15-17 meters below the (K-T) boundary which is also equivalent to my site at 15 meters below the (K-T) boundary. The vertebrate faunas from this site tend to show a decrease in relative abundance of teeth when compared to sites above and below it in the formation. The Fish Taxa Group exhibit a 6.1% decrease along with the Dinosaur Taxa Groups 13.3% decrease for the same interval. The Lower Vertebrate Taxa Group shows an increase of 19.4% during the same period. Conversely, the Fish Taxa Group presents a 21.6% increase along with the Dinosaur Taxa Groups 10.6% during the ensuing six meters of deposition to my site at 9 meters below the (K-T) boundary. The Lower Vertebrate Taxa Group shows a 32.2% decrease for the same distance.

The possibilities of climatic changes preceding the terminal cretaceous event at the (K-T) boundary which include temperature changes (1, 3) and an increasingly wetter climate (1, 4) may account for the changes in aquatic environment taxons. Changes in dinosaurian and land-based taxons may be a result of these climatic changes, floral changes, or both. Further investigations into correlations between floristic and faunal changes tied together stratigraphically will undoubtedly yield new insight into paleoenvironmental coexistences.

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BLOB GEOLOGY AND EXOTIC TERRANES: A COUNTER-EXAMPLE FROM THE SASKATCHEWAN CANADIAN SHIELD

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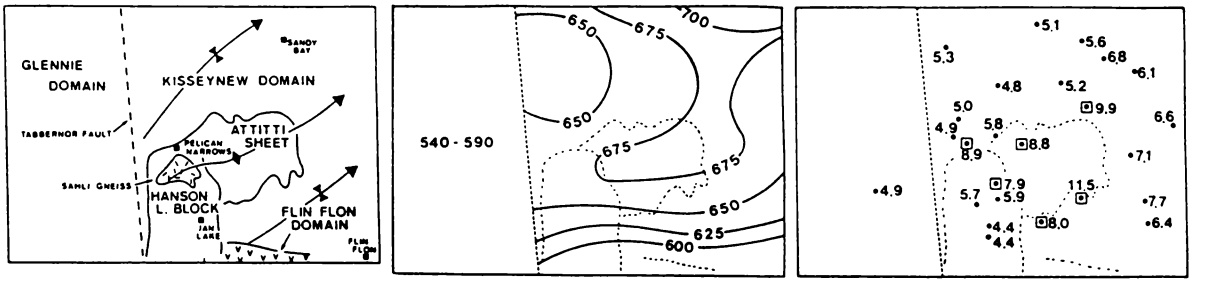
Grand Forks, North Dakota 58102

Maps of the Canadian Shield typically show a number of distinct Precambrian terranes separated by sharp boundaries. The interpretation has been that tectonic juxtaposition has brought together a number of terranes to create the present day craton. Upon closer examination, many of the putative terrane boundaries are hard to find. The boundary between the Grenville and the Superior Provinces, for example, can be traversed in areas with lots of outcrop without noticing a substantial break.

This enigma is especially marked in northern Saskatchewan. The Archean Sahli gneiss of the Hanson Lake Block and Proterozoic micro-terranes, including the Attitti, Glennie, Kisseynew, and Flin Flon are all mapped as distinct units on geological maps. Yet, in the field there is no apparent break across terrane boundaries (1).

The boundary between the Flin Flon volcanic belt and the Kisseynew gneiss belt has traditionally been thought of as equivalent to boundaries between Archean greenstone belts and adjacent sedimentary-plutonic terranes. If so, it results from the accretion of the Kisseynew sediments on an already existing Proterozoic continent (2). Field examination of rocks from both terranes reveals marked differences, but most of the differences can be explained by variation in metamorphic grade, not lithology. Recent detailed mapping suggests that the Amisk and Missi groups in the Kisseynew belt may just be the high-grade equivalents of Flin Flon sediments (3).

Detailed thermobarometric studies suggest the same thing (4). As shown in the figures below (terrane, temperature and pressure maps), there is a smooth regional gradient in both pressure and temperature recorded by the rocks in the region of the domainal boundary. This suggests that the Kisseynew rocks may simply represent an area of greater uplift/exhumation, and may in reality be the roots of the Flin Flon volcanic belt. There is also a strong indication that the Sahli gneiss represents a window into underlying older (Archean) rocks. If so, traditional models for the evolution of the Saskatchewan Precambrian Shield, that involve exotic and other allocthonous terranes, are in error. All the little blobs that appear on geologic maps may in fact simply represent different portions of the same one.



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OXYGEN- AND HYDROGEN-ISOTOPE GEOCHEMISTRY OF GROUNDWATERS FROM A DISCHARGE WETLAND AREA, RED RIVER VALLEY, N.D., AND EVIDENCE FOR PLEISTOCENE RECHARGE OF THE INYAN KARA FORMATION

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An on-going investigation is exploring ground- and soil-water movement in the discharge wetland area at Lunby and Stewart sloughs, Grand Forks County, N.D. The area is immediately underlain by ≈ 30 meters of Pleistocene clayey lacustrine sediment and till, which truncate a westward-dipping artesian sandstone aquifer, the Cretaceous Inyan Kara Formation ("Dakota aquifer")[1]. Surface water and high soil moisture are maintained by meteoric precipitation and upward hydraulic gradients. This report presents stable-isotope ratio measurements of ground- and soil-waters taken from the aquifer and overlying glacial drift, and discusses the isotopic systematics in terms of δD and $\delta^{18}O$ of local meteoric waters, the possible origins of groundwater, and the crude water budget of the wetland.

Despite large variations in salinity (specific conductance), all waters from wells completed in the Inyan Kara Formation show remarkably uniform δD and $\delta^{18}O$ values of approximately -140‰ and -18‰, respectively. The isotopic systematics of these waters do not resemble those of normal saline formation waters[2]; rather, they are compatible with an unmodified meteoric origin. However, these samples are more depleted in D and ^{18}O than average meteoric waters collected at Grand Forks, indicating that the recharge is not simply a time-integrated sample of modern regional precipitation. Two possible explanations are: (a) recharge is modern but preferentially incorporates D- and ^{18}O-depleted winter precipitation; or (b) recharge originated in a colder climate or at a significantly higher latitude than present-day North Dakota. The simplest interpretation is probably that the Dakota aquifer contains Pleistocene-Early Holocene recharge originating from glacial meltwater. Similar recharge mechanisms have been proposed for other north-central U.S. aquifers[3].

Ground- and soil-waters from glacial drift occupy three distinct domains on a δD *versus* $\delta^{18}O$ diagram (Fig. 1). Soil waters and shallow groundwaters are more enriched in D and ^{18}O than waters from deeper wells. Water-table waters are slightly more enriched in ^{18}O than the soil waters, suggesting that the water-table waters may be derived from soil waters by a small degree of evaporation. The $\delta^{18}O$ and δD of drift groundwaters decrease with increasing depth, as illustrated by the shallow (S) and deep (D) sample couplets from nested wells (tie lines in Fig. 1). The field of deeper drift groundwaters is bounded by the field of shallow drift groundwaters and by the field of the Dakota aquifer, suggesting that deeper drift groundwater is a mixture of water from the Dakota (or another source of isotopically depleted Pleistocene/Early Holocene recharge?) and partially evaporated modern meteoric water. Under this interpretation, the component of supposed Pleistocene/Early Holocene recharge increases with depth, but a modern meteoric component has penetrated to at least 12 meters. Thus, groundwater flow occurs throughout the section. Similarly, shallower groundwaters in the drift may contain a component of ancient recharge, since their isotopic composition can be interpreted as a mixture of supposed Pleistocene/Early Holocene water and modified soil water. If so, then either: (a) the drift is currently being flushed of its original glacial-age water; or, more likely, (b) glacial-age recharge from the Dakota aquifer is rising into the drift and becoming entrained in the shallow flow paths.[4]

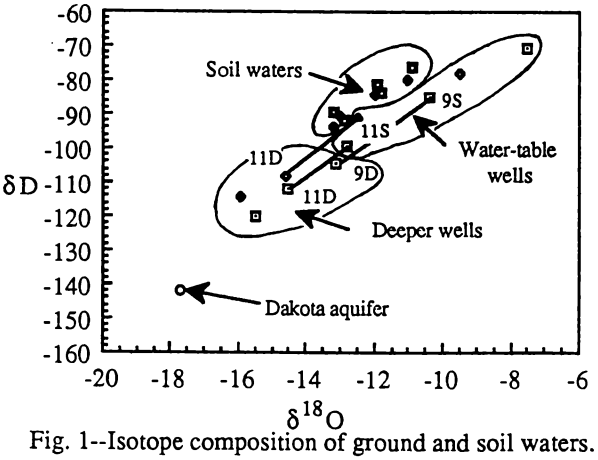

Fig. 1--Isotope composition of ground and soil waters.

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[4]Supported by North Dakota Water Resources Research Institute grant #ND91-06 and by the UND Faculty Research Committee.

3:20 am Levels of Guanine-Nucleotide-Utilizing Enzymes in Mitocondria from Various Sources.
David O Lambeth* and Wallace W Muhonen,
UND, Grand Forks.

3:40 am Leukotriene B4 Induced Airway Hyperresponsiveness is non-Specific in Allergic and non-Allergic Sheep: Blockade by Low Dose Atropine Pretreatment.
William M Long*, UND/SM, Grand Forks.

4:00 am A Quick Method for Studying Binding of Glycolytic Enzymes by Tubulin.
Stephen L Lowe*, MinSU, Minot and Harvey Knull,
UND, Grand Forks.

4:20 am Development and Application of HPLC Assays for Mitochondrial Kinases.
Wallace W Muhonen* and David O Lambeth,
UND, Grand Forks.

4:40 am Procedure for the Isolation of Several Cytosolic Enzymes from Rabbit Liver.
Joseph J Provost*, Paul D Ray, and Dave O Lambeth,
UND, Grand Forks.

LEVELS OF GUANINE-NUCLEOTIDE-UTILIZING ENZYMES IN MITOCHONDRIA FROM VARIOUS SOURCES

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Most endergonic metabolic reactions that require an input of free energy use ATP. From a thermodynamic viewpoint, however, the hydrolysis of any nucleoside triphosphate provides the same energy under a given set of conditions. Two enzymes in major metabolic pathways in mammals, succinate thiokinase (STK) and phosphoenolpyruvate carboxykinase (PEPCK), are highly specific for guanosine triphosphate (GTP). These enzymes are often coupled to ATP/ADP in lower species.

We are interested in the synthesis and use of GTP within the matrix space of mitochondria, and how phosphoryl groups may be transferred between the adenine and guanine nucleotide pools. Although ATP made within mitochondria is exported via ADP-ATP translocase located in the inner mitochondrial membrane, a transport system for GTP/GDP is not known. Therefore, GTP made within mitochondria is presumably used there. GTP is synthesized in the STK-catalyzed reaction. GTP may either be synthesized or used by PEPCK depending on the physiological direction of the reaction (1). The γ-phosphate of GTP can be transferred into the adenine pool either by nucleoside diphosphate kinase (NDPK) or GTP-AMP phosphotransferase (GAP). The uses of GTP within mitochondrial matrix is likely to be highly variable with species and tissue because PEPCK is absent in heart and variably present in liver and kidney mitochondria (2). There is conflicting evidence in the literature regarding the presence of NDPK and GAP within the matrix compartment (e.g., see Reference 3).

We have measured the activities of several GTP-coupled enzymes in isolated mitochondrial fractions by using HPLC to quantitate the nucleotides formed in timed assays. Representative results for GAP and NDPK are shown in Table I. GAP was assayed in the direction of GTP and AMP formation from GDP and ADP while NDPK activity was estimated by measuring the formation of uridine triphosphate (UTP) in the transfer of phosphate from ATP to Ux. These data show that there is great variation in the amount of NDPK, which is very low in rabbit heart and very high in pigeon liver. The activity of GAP in pigeon liver mitochondria is uncertain as explained below.

Table I. Activities of Phosphotransferases in Mitochondria

Tissue	Species	NDPK[1]	GAP[1]
Liver	Rat	190	105
	Rabbit	285	285
	Pigeon	2100	?
Heart	Rat	17	60
	Rabbit	170	615
	Pigeon	50	7
Br. Muscle	Pigeon	33	17

[1]Activities in mU/mg protein

The activities shown in Table I were determined for mitochondrial fractions that had been solubilized using CHAPS, a zwitterionic detergent. Although GAP is widely accepted to be located in the matrix of mitochondria, the location of NDPK has been controversial (3). To determine the location of the activities shown in Table I, we developed a micro procedure for treating mitochondria with digitonin so that the outer membrane could be removed before rupture of the inner membrane occurred. The release of adenylate kinase and citrate synthase were followed as indicators of the release of enzymes from the intermembrane space and matrix compartment, respectively. The location of NDPK was also studied by determining the activities of NDPK in intact mitochondria and mitoplasts as compared to the activities found when these preparations were lysed by CHAPS. The activities in the intact preparations are expected to be low whenever the enzyme is located in the matrix and the inner membrane serves as a barrier to one or more of the substrates. The results of these studies suggest that with the exception of mitochondria from rabbit heart and pigeon liver, NDPK is very low and perhaps absent in the matrix space. We were unable to reliably assay GAP in either direction of catalysis in pigeon liver mitochondria because of the high activities of GTPase, GDPase and NDPK. This may explain why GAP has previously been reported to be absent in pigeon liver mitochondria (3).

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LEUKOTRIENE B_4 INDUCED AIRWAY HYPERRESPONSIVENESS IS NON-SPECIFIC IN ALLERGIC AND NON-ALLERGIC SHEEP: BLOCKADE BY LOW DOSE ATROPINE PRETREATMENT.

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The present study characterizes the effects of aerosol leukotriene B_4 (LTB_4) on bronchomotor tone in sheep previously documented as displaying either acute or dual phase bronchoconstriction or having no airway response to aerosols of a specific antigen, Ascaris suum. The aim of the study is to determine whether LTB_4, a potent neutrophil chemotactic agent, causes specific or differential alterations in airway responsiveness to carbachol in allergic and non-allergic animals. Sheep were restrained in a cushioned cart. An esophageal balloon and a nasotracheal tube were inserted using a fiberoptic bronchoscope through each nostril after pretreatment with topical lidocaine. Mean lung airflow resistance (R_L) was measured by the esophageal balloon technique; specific lung resistance (SR_L = mean R_L · thoracic gas volume) was determined after body plethysmography. Allergic airway responses were characterized after aerosol delivery of 5 cc of Ascaris suum (20:1 dilution, 193,000 protein nitrogen units), and airway mechanics measurements were repeated hourly for 8 hours. Sheep having no airway responses to A. suum were classified as being non-responders (n = 5); allergic sheep displaying only an acute bronchoconstriction occurring immediately after antigen aerosols were classified as being acute responders (n = 7); and allergic sheep showing dual phase bronchial obstruction (an immediate bronchoconstriction resolving within 2 hours with a secondary increase in SR_L 5-6 hours later) after antigen aerosols were classified as being dual responders (n = 5). Two weeks later, the animals received 25 μg of LTB_4 (3 ml phosphate buffered saline in 16% ethanol) by aerosol and mean airway responses were measured for 8 hours. On the following days after LTB_4 aerosols, airway responses to aerosols of carbachol (10 breaths, w/v 0.0, 0.25, 0.5, 1, and 2%) were evoked and the provocative dose (PD_4) causing an increase in SR_L to 4 cm H_2O sec^{-1} was determined. Leukotriene B_4 caused an immediate bronchoconstriction in all three groups of sheep which returned to baseline values within one hour. LTB_4 did not cause a change in thoracic gas volume in any group. Leukotriene B_4 induced a significant ($P < 0.05$) increase in the PD_4 to carbachol in all three groups on the day following LTB_4. Airway responses to carbachol declined on day 2. On day 3, carbachol responses were not significantly different from baseline. There were no differences in the PD_4 to carbachol among the three groups. Both the acute bronchoconstriction to LTB_4 and the development of airway hyperresponsiveness were blocked by low doses of intravenous atropine (20 μg/kg). Atropine treatment had no effect on the carbachol PD_4 in animals given placebo aerosols. These results indicate that LTB_4 causes a bronchoconstriction in both allergic and non-allergic animals concomitant with the development of increased airway responsiveness to carbachol. The potential of an animal to develop late phase responses (dual responders) is not a pre-condition for the development of hyperresponsiveness. The mechanism of atropine blockade of LTB_4 effects is independent of possible antagonism of carbachol responses, and is suggestive of interaction of aerosol LTB_4 with autonomic nervous control of the airways. Thus, LTB_4 generation may play a role in the development of non-specific airway hyperresponsiveness that follows airway inflammation.

A QUICK METHOD FOR STUDYING BINDING OF GLYCOLYTIC ENZYMES BY TUBULIN

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Several studies show that glycolytic enzymes are compartmented in the brain; the cytoskeleton is hypothesized to anchor cytosolic proteins to provide stability in the cytoplasm and organization to metabolic pathways. Much of the work in this area has focused on interactions of tubulin with enzymes of the glycolytic pathway. Evidence of these interactions has been shown by co-pelleting of enzymes with tubulin during centrifugation and binding of enzymes by Sepharose-4B bound tubulin in columns.

Each of these two methods of studying binding (co-pelleting and column studies) has disadvantages: co-pelleting is quick but incomplete and the tubulin cannot be reused; column studies using Sepharose-4B bound tubulin allow reuse of the tubulin but are slow. To overcome the disadvantages of these methods a third method was examined. In this alternative method small aliquots of Sepharose bound tubulin and buffer solution were placed in 1.5 mL centrifuge tubes; enzyme samples added; the Sepharose beads were resuspended using a vortex mixer; the samples were centrifuged briefly and the supernatant was eluted. After the initial elution, buffer containing sodium chloride was added to each tube and the steps repeated. The eluents were examined for the presence of enzymes using the Bradford method of protein determination, appropriate enzyme assays and/or electrophoresis. The Sepharose bound tubulin samples were prepared for reuse by mixing with buffer containing 0.6 M NaCl to remove any remaining enzyme followed by three cycles of resuspension/centrifugation with buffer to wash out NaCl. That virtually all NaCl had been removed was confirmed by conductivity measurements.

The procedure described above was used to examine binding of both muscle lactate dehydrogenase (LDH-M) and aldolase by Sepharose-4B bound tubulin. In these experiments 1/5 strength tubulin resuspension buffer was used for the binding step; this buffer with NaCl added used used for the elution step (concentrations shown in the table below); Enzyme assays were performed on the supernatants and the percents bound by tubulin calculated:

	Relative Activity in Supernatants: (ΔA/min)		
Enzyme	**Buffer only:**	**Buffer+NaCl**	**% Bound** (Without NaCl)
Aldolase	0.010 ±.008	0.290 ±.102	97.1 ± 1.4 %
LDH-M	0.015 ±.005	0.061 ±.014	80.4 ± 2.5 %

For both experiments n = 3 and the ± figures are for 1 standard deviation.
LDH and aldolase assays according to Bergmeyer (1).

Release of bound proteins when ionic strength is increased is consistent with the hypothesis that the binding to tubulin is electrostatic. It has also previously been noted that the carboxyl terminus of tubulin is very rich in acidic residues. If the binding of enzymes to tubulin indeed is the result of elctrostatic attractions between acidic residues on tubulin and basic groups on the enzymes, modification of lysyl residues on the enzyme by pyridoxal phosphate should decrease binding. Modification of aldolase was carried out using the procedure of Forcina et al. (2) with two different concentrations of pyridoxal phosphate. That different amounts of pyridoxal phosphate reacted with the samples was confirmed spectrophotometrically. The percents bound to the different samples were as follows:

Sample:	**% Bound**
Unmodified Aldolase (Control)	89.1 ± 12.6%
1:3 Modified Aldolase	24.0 ± 3.0 %
3:1 Modified Aldolase	2.5 ± 4.3 %

The ratios shown are for moles of pyridoxal phosphate to moles of aldoase lysyl residues in the reaction mixture. %'s based on protein concentrations measured using the Bradford method.
For this experiment n = 3 and the ± figures are for 1 standard deviation.

The results obtained in this study demonstrate the utility of this quick method of studying protein binding. Further experiments should be performed to identify the protein domains involved in these interactions.

The support for this work provided by ASEND is greatly appreciated.

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DEVELOPMENT AND APPLICATION OF HPLC ASSAYS FOR MITOCHONDRIAL KINASES

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The activities of nearly all enzymes that catalyze reactions involving nucleotides have been determined by using coupling enzymes to consume a reactant or form a product that can be followed spectrophotometrically. Many of these reactions have been coupled to NAD^+/NADH or $NADP^+$/NADPH.

Reactions involving nucleotides can be assayed by separating and quantitating the reactants and products by high pressure liquid chromatography (HPLC) (1, 2). We have used a 4.6 x 250 mm reversed phase (C-18) column and an ion-pairing cation, tetrabutylammonium ion (TBA), to separate the nucleotides involved in the reactions catalyzed by nucleoside diphosphate kinase, GTP-AMP phosphotransferase, adenylate kinase, phosphoenolpyruvate carboxykinase (2), and succinate thiokinase (STK). The reactants and products are detected by their UV absorbance at 254 nm. All of the HPLC components, including the autosampler, are software controlled by a microcomputer that also performs electronic integration of the chromatographic data.

We found that all nucleotides of interest could be separated by an elution solvent composed of 100 mM KH_2PO_4, 5 mM TBA and 12-45% acetonitrile (v/v), depending on the assay (2). Except for analyses involving succinyl-CoA, the solvent pH was adjusted to 5.0. A pH of 4.0 was found to stabilize succinyl-CoA in the assay of STK, and 0.05% thiodiglycol (v/v) was added to prevent the oxidation of Coenzyme A (CoA) during the separation. The run time was generally kept under 10 minutes by adjusting the concentration of acetonitrile.

A disadvantage of timed assays is the deviation from linearity of product formation with time caused by a number of factors. These include the kinetic properties of the enzyme, the chemical stability of the product, and competing reactions. In the assay of mitochondrial enzymes, a factor causing nonlinearity is the presence of enzymatic activities, for example triphosphatase, that metabolize the reactants or products of the enzyme being assayed. Some of these competing activities can by inhibited by specific inhibitors such as oligomycin.

An advantage of HPLC-based assays is their applicability to particulate systems. For example, we have used these types of assays to study the latency of enzymatic activities in mitochondria and mitoplasts where light scattering interferes with spectrophotometric assays.

The traditional spectrophotometric assay for STK follows acyl-CoA bond formation at 235 nm where most of the absorbance is due to other reaction components (3). Data obtained by HPLC assays of the STK reaction are shown in Figures 1 and 2.

Figure 1. HPLC Profiles of the STK Reaction After 0, 300 and 600 Seconds

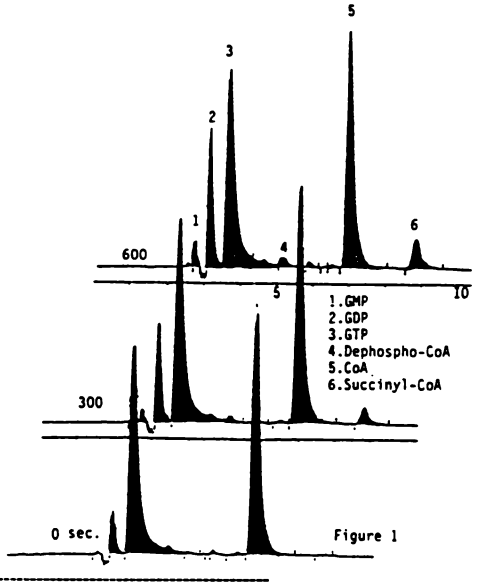
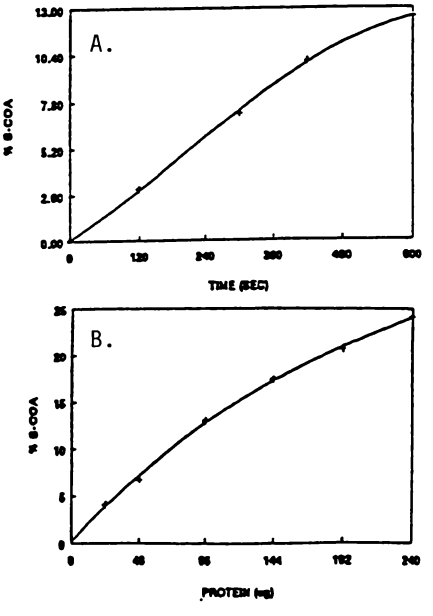


Figure 2. Linearity of Succinyl-CoA Formation With Time (A) and Protein (B)



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PROCEDURE FOR THE ISOLATION OF SEVERAL CYTOSOLIC ENZYMES FROM RABBIT LIVER

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Phosphoenolpyruvate carboxykinase (PEPCK) is a key gluconeogenic enzyme that shares substrates with several other enzymes in cytosol. Phosphoenolpyruvate is also used by enolase and pyruvate kinase, the nucleotides are utilized by nuclceoside diphosphate kinase (NDPK), and oxalacetate is used by malate dehydrogenase (MDH) and aspartate transaminase (AST). Purified preparations of the liver isoenzymes are required to investigate possible physical and kinetic interactions. We have already purified PEPCK from rabbit. Three of the hepatic enzymes are not commercially available from liver while NDPK from bovine liver is available only at a high cost. We report here a procedure to isolate NDPK, AST, MDH and enolase from the same rabbit liver (see scheme I).

Several types of chromatographies are utilized in this procedure. Three different immobilized reactive dye columns are used: Cibacron Blue 3GA (CB), Reactive Green-19 (G-19) and Reactive Red-120 (R-120). Each of these dyes is covalently linked to crosslinked 4% agarose. Reactive dye columns offer several positive features, "nonspecific" affinity, good flow characteristics, high binding capacities and ease in scale up of the purification. Macro-Prep 50 Q and Macro-Prep 50 CM are ion exchangers with the same ion exchange properties as the standard resins, but with a rigid acrylic matrix that is resistant to shrinkage and has a superior flow rate. HA-Ultrogel is microcrystalline hydroxyapatite that is covailently bound to cross linked 4% agarose. This media also has good flow rates allowing it to be easily scaled up. All chromatographies were carried out in running buffer (10 mM Tris-Cl (pH 8.1), 0.1 mM EDTA) unless noted otherwise. The purifications achieved are indicated in Table 1.

Fifteen g of liver from a fasted rabbit is homogenized and the cytosol is isolated by differential centrifugation. The cytosol is brought to 30% saturation with ammonium sulfate and centrifuged at 10,000g for 15 minutes and the supernate is raised to 70% saturation and recentrifuged. The resulting pellet is resuspended in running buffer and 5 mM $MgSO_4$. This preparation is desalted and loaded onto a bed of CB. The CB column is washed with 50 mM NaCl in running buffer to elute enolase and AST. The column is then washed with 1.5 mM NADH and 50 mM NaCl in running buffer to elute MDH. Increasing the NaCl to 350 mM in running buffer elutes unwanted proteins. After baseline absorbance is reached, 3 mM ATP and 5 $MgSO_4$ in 350 mM NaCl is used to elute NDPK.

AST/Enolase: AST and enolase are separated on DEAE Sephacel. The CB 50 mM fraction is precipitated with $(NH_4)_2SO_4$, desalted in running buffer on Sephadex G-25 and loaded onto the ion exchanger DEAE Sephacel. Enolase does not bind to the column, and AST is eluted with a 0 to 200 mM NaCl gradient.

Enolase is further purified by three negative binding chromatography steps: rechromatography on CB, Macro-Prep 50 Q, and MacrPrep 50 CM (at pH 7.0). After the last chromatography the enzyme is applied to a HA-Ultrogel column and eluted with a 0 to 200 mM phosphate buffer. The active fraction is pooled, concentrated in Aquaside III (flaked polyethylene glycol) and chromatographed in Sephacryl S-200.

The AST pooled fractions from DEAE Sephacel is loaded onto a column of G-19. The column is with 100 mM NaCl eluting the enzyme. This sample is loaded on Macro-Prep 50 CM. The activity is found in the flow through eluate.

MDH: The MDH pool from CB is precipitated with $(NH_4)_2SO_4$, desalted and loaded onto a DEAE Sephacel column. The column is developed with a 0 to 100 mM NaCl gradient and the active fractions are diluted with running buffer and loaded onto G-19. G-19 is washed with 100 and 350 mM NaCl. MDH is washed off in the higher salt fraction and is diluted to 50 mM NaCl and loaded onto R-120. MDH is eluted with a gradient of 0 to 3 mM NADH in the 50 mM NaCl running buffer.

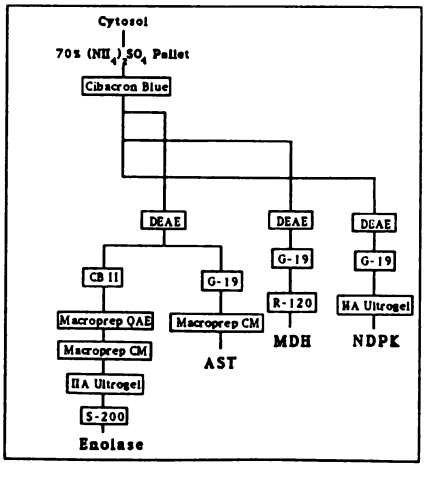
NDPK: As before, the NDPK fraction from CB is precipitated with $(NH_4)_2SO_4$, desalted in running buffer and loaded onto DEAE Sephacel. NDPK is eluted with a 0 to 100 mM NaCl gradient and the active fractions pooled and directly applied to G-19 . The reactive dye column is washed with 100 mM NaCl. After baseline absorbance is reached, the enzyme is eluted with a 0 to 2 mM ATP gradient. The resulting active fractions are directly applied to a HA-Ultrogel and developed with a 0 to 400 mM phosphate buffer gradient (pH 7.4).

Table 1 Purification results

Enzyme	Units	SA	X purification	% yield
Enolase	38.6	27.33	48.5	12.0
AST	61.8	93.73	173.0	20.0
MDH	257.1	707.94	737.6	47.1
NDPK	194.0	396.74	236.26	20.3

(Supported by NIH Grant No DK41631 to PDR)

SCHEME I
Flow diagram of the purification from rabbit liver



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.

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 North Dakota Academy of Science 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 Academy 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 Academy.

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 Academy of Science 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 Academy, 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 academy 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, Academy 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 Academy 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 Academy's 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 December 31 following the nonpayment of dues during the membership year commencing the previous January 1. 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 Academy 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 Academy, 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 Academy.

   b. Editorial Committee.
   Membership: Three members. Three-year terms.

   Duties: The Editorial Committee shall develop and recommend the Academy 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 of whom 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 Academy. 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 Academy. 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 Academy.

b. Student members -- shall be graduate or undergraduate College students in some field of science. Student members may participate in all activities of the Academy, with the exception of holding office.

c. Sustaining members -- are persons or organizations interested in the activities of the Academy. Sustaining members may participate in all activities of the Academy, 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 the support of science and technology in North Dakota.
2. Preference in mounting commercial displays at the annual meetings of the Academy.
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 Academy 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 Academy. (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 North Dakota Academy of Science is instructed to establish a J Donald Henderson Memorial Fund and that the Committee administer this fund so that the proceeds from this fund 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 January 1 to December 31. (1973 Revision)

18. The Academy establishes the North Dakota Academy of Science Achievement Award to be awarded periodically to an Academy member in recognition of excellence in one or more of the following:
    a. Nationally recognized scientific research.
    b. Science education.
    c. Service to the Academy 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 Academy. The purposes of the Foundation are to (1) 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 Academy and shall be separately accounted for annually.

    The Foundation Board of Directors shall be comprised of five members of the Academy, representing different disciplines. Members shall be appointed by the President for staggered five year terms, and 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 Academy at each annual meeting, and the Secretary-Treasurer shall present an accompanying financial report.

Revised May 1989

NORTH DAKOTA ACADEMY of SCIENCE
Officers and Committees for 1991-92

E X E C U T I V E C O M M I T T E E

Clark Markel, Past President -92
College of Arts and Science
Minot State University
Minot, ND 58701 857-3160

John Brauner, President Elect -94
Department of Biology
Jamestown College
Jamestown, ND 58401 252-3467-2482

Roy Garvey, Secretary-Treasurer -93
Department of Chemistry
North Dakota State University
Fargo, ND 58105 237-8697
NUO25304@NDSUVM1

Ronald Royer -94
Division of Science
Minot State University
Minot, ND 58701 857-3209
MNO28909@NDSUVM1

James Waller, Member at Large -92
Department of Microbiology
University of North Dakota
Grand Forks, ND 58202 777-2615

Glen Statler, Member at Large -93
Department of Plant Pathology
North Dakota State University
Fargo, ND 58105 237-7058

EDITORIAL COMMITTEE

James Tilton, Chair -92
North Dakota State University

Bob Stack -93
North Dakota State University

Bob Seabloom -94
University of North Dakota

RESOLUTIONS COMMITTEE

Allen Kihm -92
Minot State University

Dennis Disrud -93
Minot State University

-94

N O M I N A T I N G C O M M I T T E E

David Davis -95
USDA Biosciences Research Lab

Bonnie Heidel -93
ND Parks & Recreation Department

Clark Markel, Chair -96
Minot State University

William Barker -92
North Dakota State University

Forest Nielsen -94
USDA Human Nutrition Research Center

E D U C A T I O N C O M M I T T E T

James Waller
University of North Dakota
Executive Committee Liaison

Allen Kihm -92
Minot State University
National Science Week

Mike Burton -94
Agassiz Jr High School, Fargo
Science Olympiad

Jerome Knoblick -95
Jamestown College
AAAS Mini-Grant Coordinator
Junior Academy Liason

Om Madhok -92
Minot State University
Science Fair Liaison

Ron Royer -93
Minot State University
Science Educator Newsletter

Marcia Steinwand -94
Robinson High School

N E C R O L O G Y C O M M I T T E E

Michael Thompson -93
Minot State University

William Wrenn, Chairman -92
University of North Dakota

D E N I S O N A W A R D S C O M M I T T E E

Robert Crackel, Chairman -92
Minot State University

Doug Munski -93
University of North Dakota

Daniel Mott -93
Dickinson State University

NORTH DAKOTA SCIENCE RESEARCH FOUNDATION BOARD of DIRECTORS

Om Madhok -95
Minot State University

Virgil Stenberg, Chairman -92
University of North Dakota

John Reid -93
University of North Dakota

Larry Campbell -94
North Dakota State University

M E M B E R S H I P C O M M I T T E E

Gary Clambey
North Dakota State University

Vernon Feil
USDA- Bioscience Research Laboratory

Myron Freeman
Dickinson State University

Carolyn Godfread
Bismark

Janet Hunt
Human Nutrition Research Center

Charles Turner
University of North Dakota

Charles Lura, Chair
NDSU - Bottineau

Michael Thompson
Minot State University

L O C A L A R R A N G E M E N T S C O M M I T T E E -- Grand Forks

James Waller, Chair
Microbiology

Elliot Shubert
Department of Biology

John Reid
Department of Geology

Eric Uthus
USDA Human Nutrition Laboratory

John A Williams
Department of Anthropology

THE NORTH DAKOTA ACADEMY OF SCIENCE

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

FINANCIAL and MEMBERSHIP STATEMENT . 1 January - 31 December 1991

A. BALANCE SHEET

	1991		1990	
	Item	Total	Item	Total
I. ASSETS				
Operating Accounts				
Checking	3381.24		1741.74	
Savings			2232.93	
Savings Certificates	2000.00	5381.24	4000.00	7974.67
Trust Accounts				
Scholarship Principal	19534.88		17166.26	
Research Foundation	9569.58	29104.46	8514.57	25680.83
TOTAL ASSETS		34485.70		33655.50
II. LIABILITIES				
Advanced Dues	760.00	760.00	585.00	585.00
Restricted Purpose Funds				
Scholarship Principal	19534.88		17166.26	
AAAS Grant	1000.00		1900.00	
Research Foundation	9569.58	30104.46	8514.57	27580.83
TOTAL LIABILITIES		30864.46		28165.83
III. ACCUMULATED SURPLUS		3621.24		5489.67
IV. CHANGE in SURPLUS		-1868.43		1667.00

B. OPERATING CASH FLOW

	1991	1990
CASH on HAND 1 January	8775.19	7237.01
CASH RECEIPTS for Year	11021.38	21346.83
TOTAL RESOURCES Available	19796.57	28583.84
CASH DISBURSEMENTS	11667.18	19808.65
CASH BALANCE 31 December	8129.39	8775.19
Increase over Year	- 645.80	1538.18

C. MEMBERSHIP STATEMENT

	Emeritus	Student	Professional	Total
1 January, 1991	59	61	290	401
31 December, 1990	54	104	312	470
Net Change	-5	+43	+22	+69
Dues paid 1-Jan-92	54	71	223	348

D. OPERATING RECEIPTS

	1991 Item	1991 Total	1990 Item	1990 Total
DUES				
Reinstatements	20.00		90.00	
Current Year	1975.00		2005.00	
Next Year	760.00	2755.00	585.00	2680.00
SUBSIDIES				
NDSU	1000.00		1000.00	
UND			1000.00	
Minot State	200.00	1200.00	200.00	2200.00
ANNUAL MEETING				
Registration Fees	1377.00		2191.00	
Banquet Ticket Sales	808.50			
Am Chem Soc, Red River VS			350.00	
Assocn ND Geographers				
N D Geological Society			100.00	
Sigma Xi -- UND	50.00		50.00	
Sigma Xi -- NDSU			150.00	
Sigma Xi -- Minot	50.00			
NDSU Engineering			772.04	
Basin Electric Cooperative	100.00			
R R V Sugar Beet Growers	100.00			3613.04
Minot State University	986.00	2663.00		
AWARDS PROGRAM				
AAAS Sec Schl Research Grant	1000.00		1900.00	
Scholarship Dividends	481.78	1481.78	475.20	2375.20
PUBLICATION SALES	52.00	52.00	102.00	102.00
INTEREST on SAVINGS	82.35	82.35	203.34	
SMITS GRANT			17896.92	17896.92
T O T A L I N C O M E		9042.63		28867.16

E. OPERATING DISBURSEMENTS

	1991 Item	1991 Total	1990 Item	1990 Total
ANNUAL MEETING				
Speakers	1500.00		1122.07	
Meals	1903.95		1929.39	
General Expenses	659.64	4063.59	876.76	3928.22
AWARDS PROGRAM				
AAAS Sec Schl Research Grants	1200.00		700.00	
ND Science Olympiad	100.00			
Science/Engineering Fair	50.00			
Denison Awards	300.00		400.00	
Junior Academy Awards	325.00	1975.00		1100.00

PUBLICATIONS				
Editor Fees			750.00	
Proceedings	2631.28	2631.28	3133.37	3883.37
MISCELLANEOUS				
Fidelity Bond	26.00		26.00	
AAAS Delagate	1000.00		911.73	
NAAS Dues	41.50	1067.50	41.50	979.23
PROGRAM OPERATIONS				
Junior Academy	132.76		350.00	
The Dakota Science Teacher	250.00			
Committee Travel		382.76	121.55	471.55
OFFICE EXPENSES				
Postage	604.96		550.56	
Post Office Box Rent	39.00		39.00	
Duplicating	458.06		208.42	
Supplies	165.70		259.01	
Clerical Staff	70.00		92.50	
Sec Treasurer Fee		1337.72	50.00	1199.49
SMITS PROGRAM			16798.24	16798.24
T O T A L D I S B U R S E M E N T S		11457.93		28360.10

F. SCIENCE RESEARCH FOUNDATION

	1991	1990	CHANGE
Balance 1 January	8514.57	7159.60	1354.97
Donations from Members	261.50	270.00	- 8.50
Allocations from Dues	482.00	438.00	44.00
Organization Memberships		250.00	-250.00
Interest Accrued	311.51	396.97	-85.46
Balance 31 December	9569.58	8514.57	1055.01

G. SCHOLARSHIP FUND

	1991		1990	
CASH INCOME				
SDGE Dividends	205.00		270.00	
Iowa Southern Inc (now IES Industries)	216.00		205.20	
T O T A L		421.00		475.20
CASH EXPENSE				
Denison Awards	300.00		400.00	
Junior Academy Awards	325.00		350.00	
Stock Purchase			265.22	
T O T A L		625.00		1015.22
N E T I N C O M E		-204.00		-540.02

FINANCIAL and MEMBERSHIP STATEMENT 1 January - 31 December 1991

ASSETS				
SDGE Shares	(1983)	250.00	315.18	302.00
Price		18.50	45.00	43.63
Value		4625.00	14182.88	13176.26
Iowa Southern, Inc	(1990)	120	192	120.00
Price		31.63	27.88	33.25
Value		3795.00	5352.00	3990.00
Investment Value TOTAL			19534.88	17166.26
CHANGE in INVESTMENT ASSETS			2368.62	-1347.85

Verified by Audit Committee:

Peter W. Aschbacher

David G. Davis

Kenneth Dickerson

Date: Feb. 12, 1992

Respectfully Submitted

Roy Garvey
Secretary-Treasurer

Date: 11-Jan-92

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
1918	R C Doneghue	Fargo
1919	H E French	Grand Forks
1920	J W Ince	Fargo
1921	L R Waldron	Grand Forks
1922	Daniel Freeman	Fargo
1923	Norma Preifer	Grand Forks
1924	O A Stevens	Fargo
1925	David R Jenkins	Grand Forks
1926	E S Reynolds	Fargo
1927	Karl H Fussler	Grand Forks
1928	H L Walster	Fargo
1929	G A Talbert	Grand Forks
1930	R M Dolve	Fargo
1931	H E Simpson	Grand Forks
1932	A D Wheedon	Fargo
1933	G C Wheeler	Grand Forks
1934	C I Nelson	Fargo
1935	E A Baird	Grand Forks
1936	L R Waldron	Fargo
1937	J L Hundley	Grand Forks
1938	P J Olson	Fargo
1939	E D Coon	Grand Forks
1940	J R Dice	Fargo
1941	F C Foley	Grand Forks
1942	F W Christensen	Fargo
1943	Neal Weber	Grand Forks
1944	E A Helgeson	Fargo
1945	W H Moran	Grand Forks
1946	J A Longwell	Fargo
1947	A M Cooley	Grand Forks
1948	R H Harris	Fargo
1949	R B Witmer	Grand Forks
1950	R E Dunbar	Fargo
1951	A K Saiki	Grand Forks
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
1965	P B Kannowski	Grand Forks
1966	Paul C Sandal	Fargo
1967	F D Holland, Jr	Grand Forks
1968	W E Dinusson	Fargo
1969	Paul D Leiby	Minot
1970	Roland G Severson	Grand Forks
1971	Robert L Burgess	Fargo
1972	John C Thompson	Dickinson
1973	John C Reid	Grand Forks
1974	Richard L Kiesling	Fargo
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

ALESSI, Joseph	1210 Eleventh Street South	FARGO	ND	58103
ANDERSON, Edwin M	1151 Twelveth Avenue West	DICKINSON	ND	58601
AUYONG, Theodore	3614 Eleventh Avenue North	GRAND FORKS	ND	58201
BARNEY, William G	1525 Cottonwood	GRAND FORKS	ND	58201
BELINSKEY, Carol R	Minot State University	MINOT	ND	58702
BLISS, Harald N	Post Office Box 522	MAYVILLE	ND	58257
BOLIN, F M	1505 Sixth Street South	FARGO	ND	58102
BROPHY, John A	702 South Drive	FARGO	ND	58103
BROWN, Ralph C	Box 89	STONEHAM	ME	4331
BRUMLEV, Stanley	218 Forty Nineth Avenue South	GRAND FORKS	ND	58201
CALLENBACH, John A	North Dakota State University	HULTZ HALL	ND	58105
CARLSON, Kenneth T	221 Eighth Avenue S E #14	MAYVILLE	ND	58257
CARMICHAEL, Virgil W	1013 North Anderson Street	BISMARCK	ND	58501
CARTER, Jack F	1345 Eleventh Street North	FARGO	ND	58102
CASSEL, J Frank	83 West Boulder Street	COLORADO SPRINGS	CO	80903
CORNATZER, William E	2033 North Washington Street	BISMARCK	ND	58501
DAFOE, Arthur W	515 Third Street North East	VALLEY CITY	ND	58072
DEBOER, Benjamin	312 Alpha Avenue	GRAND FORKS	ND	58203
DINGA, Gustav P	Concordia College	MOORHEAD	MN	56560
EDGERLY, Charles G M	1317 Eighth Avenue South	FARGO	ND	58103
FISK, Allen L	1122 Avenue B West	BISMARCK	ND	58501
FOSSUM, Guilford O	1828 Cottonwood Street	GRAND FORKS	ND	58201
FRANK, Richard E	1010 Boyd Drive	GRAND FORKS	ND	58203
HOEPPNER, Jerome J	2518 Nineth Avenue North	GRAND FORKS	ND	58203
HOFFMAN, Charles A	Minot State University	MINOT	ND	58702
HOLLAND, F D Jr	University of North Dakota	GRAND FORKS	ND	58202
HOLLAND, Jean H	4686 Belmont Road	GRAND FORKS	ND	58201
JACOBS, Francis A	1525 Robertson Court	GRAND FORKS	ND	58201
KIESLING, Richard	Post Office Box 204	FARGO	ND	58107
KLOSTERMAN, Harold J	North Dakota State University	DUNBAR HALL	ND	58105
KOENKER, William E	Whippoorwill Lane	CHAPEL HILL	NC	27514
KRUSCHWITZ, Earl H	431 Sixth Street South West	VALLEY CITY	ND	58072
LAIRD, Wilson M	101 Spanish Oak Lane	KERRVILLE	TX	78028
LOW, Frank N	2511 Saint Charles Avenue	NEW ORLEANS	LA	70130
MARWIN, Richard M	1519 Chestnut Street	GRAND FORKS	ND	58201
MELDRUM, Alan	512 Columbia Road	GRAND FORKS	ND	58203
MCMAHON, Kenneth J	North Dakota State University	VAN ES HALL	ND	58105
NELSON, C N	North Dakota State University	BOTTINEAU	ND	58318
OWEN, John B	1118 Reeves Drive	GRAND FORKS	ND	58201
ROGLER, George A	Box 459	MANDAN	ND	58554
RUDESILL, James T	North Dakota State University	LADD HALL	ND	58105
SCOBY, Donald R	North Dakota State University	STEVENS HALL	ND	58105
SEVERSON, Roland	2682 Catalina Drive	GRAND JUNCTION	CO	81506
SLEEPER, Bayard P	Post Office Box 2236	PAULSBO	WA	98370
SMITH, Glenn S	3140 North Tenth Street	FARGO	ND	58102
SNOOK, Theodore	343 Sheridan Road	RACINE	WI	53403
SOUBY, Armand M	103 Nichols	SAN MARCOS	TX	78666
STARCHER, George W	700 John Ringling Boulvd # 908	SARASOTA	FL	34236
STEWART, James A	Pembroke	ONTARIO	CANADA	K8A 1X2
SUGIHARA, James M	North Dakota State University	LADD HALL	ND	58105
SUMMERS, Lawrence	University of North Dakota	GRAND FORKS	ND	58202
WALSH, Robert G	Rural Route 6 Box 124 CC Acres	MINOT	ND	58701
WEISSER, Wilber O	55 Parkview Circle	GRAND FORKS	ND	58201
WHITMAN, Warren C	North Dakota State University	STEVENS HALL	ND	58105

ALAM, Md Shah	North Dakota State University	SOUTH ENGINEERING	ND	58105
BAHR, James A	Apartment 306	FARGO	ND	58103
BATES, Mark B	Apt 301	FARGO	ND	58103
BERNTSON, Joan	University of North Dakota	GRAND FORKS	ND	58202
BESEMANN, Leonard E	Mayville State University	MAYVILLE	ND	58257
BLAIR, Joseph B	University Village	FARGO	ND	58102
BROOKS, Margaret Ann		MINOT	ND	58701
CAPOCY, Nicole	112 - 2 Siesta Drive	MINOT AFB	ND	58704
CARPENTER, Sharla J	1315 Eighth Street North West #1	MINOT	ND	58701
CASPERS, Lisa		GRAND FORKS	ND	58203
CURTISS, Gene C, Jr	1121 Fifth Avenue South West	MINOT	ND	58701
CURTISS, Randy	Apartment 4	GRAND FORKS	ND	58201
DELBORGO ADAMS, Ann M	3625 East Burdick Expressway #2	MINOT	ND	58701
DEPLAZES, Nick J	Route 5 Box 271	MINOT	ND	58701
DUGAN, Corey	Apartment 301	GRAND FORKS	ND	58201
DWIGHT, Wesley J	Apartment 15	FARGO	ND	58102
EICHHORST, Jean	570 Carleton Court # 102	GRAND FORKS	ND	58203
ELLINGSON, Mark A	1345 North University Drive	FARGO	ND	58102
EASTHOUSE, Douglas L	Apartment 14	FARGO	ND	58102
FALLIS, Mary A	1200 Robert Street	MINOT	ND	58701
FEIST, Susan A	Post Office Box 381	MINOT	ND	58702
FIELDS, Eva L	856 26th Street West	DICKINSON	ND	58601
FOX, Dean	1014 Monte Carlo Drive	FARGO	ND	58102
GIESER, John P	Minot State University	MINOT	ND	58701
GJOVIK, Ann M	2441 Second Avenue Wouth West #2	MINOT	ND	58701
HAIN, Marla	P O Box 552	GRAND FORKS	ND	58206
HANSEN, Jana M	Walster Hall	FARGO	ND	58105
HANSEN, Devon	University of North Dakota	GRAND FORKS	ND	58202
HAUG, William O, Jr	235 Northridge Hills Court	GRAND FORKS	ND	58201
HERMAN, Gregory S	General Delivery	KRAMER	ND	58748
HILL, Lynn	836 Fifth Avenue West	DICKINSON	ND	58601
HJELMELAND, Duane	P O Box 987	STANLEY	ND	58784
JOHNSON, Lori K	13 Bison Court	FARGO	ND	58102
JOHNSON, Paul M	Jamestown College	JAMESTOWN	ND	58401
KANE, Ronald M	1017 Eleventh Avenue N W	MINOT	ND	58701
KOTTSICK, Kori V	Minot State University	MINOT	ND	58701
KUSTERS, Hans C	Apartment 204	GRAND FORKS	ND	58203
LARSON, Jeffrey P	205 Twenty Fifth Avenue North	FARGO	ND	58102
MAC ARTNEY, Lisa L	902 Beacon Street # 1	MINOT	ND	58701
MARDON, Austin A	Edmonton	ALBERTA	CANADA	T5J 2J1
MASON, Philip A	North Dakota State University	WALSTER HALL	ND	58105
MEINKE, Troy	North Dakota State University	HULTZ HALL	ND	58105
MENDOZA, Laura A	1017 North Fourteenth Street	FARGO	ND	58102
MORLEY, James A	Apartment # 6	GRAND FORKS	ND	58203
MUNSKI, Laura	University of North Dakota	GRAND FORKS	ND	58202
NICHOLAS, Joseph W	Mary Washington College	FREDERICKSBURG	VA	22401
OLTHOFF, Kristi K	1309 Fourteenth Avenue N W	MINOT	ND	58701
OMILUSIK, Kevin	808 Holiday Village	MINOT	ND	58701
PEDERSON, JeNell B	Minot State University	MINOT	ND	58701
PEITZ, David J	Apartment # 11	GRAND FORKS	ND	58203
PERRYMAN, Wendy C	1118 28th Avenue South #11	GRAND FORKS	ND	58201
PETRICK, Tim B	2201 Eleventh Avenue South	FARGO	ND	58103
PHILION, Rick A	Pioneer Hall Apartment 1	MINOT	ND	58701
PHILIUM, Richard A	Minot State University	MINOT	ND	58701
PLAINE, Kelly J	3022 South Seventeenth Street	MOORHEAD	MN	56560

QUAMMEN, Rene	Box 785	MINOT	ND	58702
RADI, Curt D	1017 Eleventh Avenue N W	MINOT	ND	58701
REZVANI, Ahmad	Box 8073	GRAND FORKS	ND	58202
RIENIETS, Lisa M	426 Third Avenue East	WEST FARGO	ND	58078
ROCHOCKI, Judith	1211 Eleven 1/2 Street North #6	FARGO	ND	58102
SCHELDRUP, September A	800 Normal Street	MINOT	ND	58701
SCHIFF, Stacey	820 Tenth Avenue West	DICKINSON	ND	58601
SVIHOVEC, Todd A	517 Northwestern Drive	GRAND FORKS	ND	58203
THOMPSON, Susan Joan	6602 Fortieth Avenue South	FARGO	ND	58104
TIBBS, Neile	1086 Natchury Drive #1	DICKINSON	ND	58601
TORKELSON, David R	120 North Twentieth Street	GRAND FORKS	ND	58203
VOLKER, K Warren	University of North Dakota	GRAND FORKS	ND	58202
VOSEN, Wendy M	Apartment 20	EAST GRAND FORKS	MN	56721
WALSH, Naney J	Post Office Box 430	DEVILS LAKE	ND	58301
WASHBURN, Jill R	120 1/2 Nineth Avenue S E	MAYVILLE	ND	58257
WASHEK, Sandra K	Rural Route 1, Box 66	SAWYER	ND	58781
WILLIAMSON, Annette	1000 Fifth Street South West	MINOT	ND	58701
WRETLING, Diane	Rural Route 1 Box 14	GARRISON	ND	58540
YEARGAN, Jeff C	106 First Street	HATTON	ND	58240
YOON, Jazwan	North Dakota State University	FARGO	ND	58105
ZIELINSKI, Richard E	2872 Twenty Second Avenue South	GRAND FORKS	ND	58201

ALTENBURG, Lois Ivers	1146 Fifth Street North	FARGO	ND	58102
ANDERSON, Ordean S	Rural Route 1 Box 269	NEW PRAGUE	MN	56071
ANGEL, Kathleen	7500 University Drive	BISMARCK	ND	58504
ASCHBACHER, Peter	Biosciences Research Laboratory	FARGO	ND	58105
ASHWORTH, Allan C	North Dakota State University	STEVENS HALL	ND	58105
AULT, Charles R	Jamestown College	JAMESTOWN	ND	58401
BAGHERI, Fathollah M	University of North Dakota	GRAND FORKS	ND	58202
BALSBAUGH, Edward	North Dakota State University	HULTZ HALL	ND	58105
BALTISBERGER, Richard	University of North Dakota	GRAND FORKS	ND	58202
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July 5, 1991

Dear Colleague:

In the 1991 issue of the North Dakota Academy of Science Proceedings, we presented some erroneous data on the effect of boron, potassium and amino acids on femur mineral composition. The data in question was so unusual and surprising, we decided to reexamine the procedures that resulted in them. Much to our dismay, we found that a dilution error of two had been made with some of the samples analyzed. In the enclosed communication, the erroneous data has been corrected, and the last paragraph reflects these corrections. Please note that the enclosed communication has been prepared in a form that can be used to staple, tape, paste or insert over the faulty presentation on page 27 of Volume 45; we request that you do so.

We are truly sorry about any inconvenience the erroneous data may have caused.

Sincerely,

TERRENCE R. SHULER
Analytical Chemist

FORREST H. NIELSEN, PH.D.
Center Director

Enclosure

Agricultural Research Service

THE MODIFICATION OF RAT BONE MINERAL COMPOSITION BY CHANGES IN DIETARY ARGININE, METHIONINE, BORON AND POTASSIUM

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Several studies have indicated that dietary boron can affect the composition and structure of bone (1). Moreover, the response of animals to low dietary boron varies markedly as the diet varies in its content of several other nutrients including potassium, arginine and methionine (1). Thus, we performed an experiment for which one objective was to ascertain whether bone mineral composition is influenced by dietary changes in these nutrients because a change in composition would suggest that they are important nutritional factors for normal bone formation or metabolism.

Male weanling Sprague-Dawley rats were assigned to groups of six in a fully crossed, three-way 2x4x2 experimental design. The basal diet, which contained a marginal amount of methionine, was the same as that described previously (2) except potassium chloride was omitted from the diet. Environmental conditions also have been described (2). The experimental variables were: per g fresh diet, boron supplements of 0 and 3 μg; potassium supplements of 1.0, 1.4, 1.8 and 3.6 mg; and either an arginine supplement of 10 mg or a methionine supplement of 2.5 mg. The rats were fed their respective diets for seven weeks, fasted overnight, weighed, then anesthetized with ether for cardiac exsanguination and decapitation. One femur was removed and frozen for later analysis. The femurs were prepared in our usual manner for mineral analysis by inductively coupled argon plasma atomic emission spectrometry (2,3).

Table 1. Effect of boron, potassium and amino acids on femur mineral composition

Dietary Treatment		Femur (dry)							
B, μg/g	K, mg/g	B, μg/g	Ca, mg/g	P, mg/g	Mg, mg/g	K, mg/g	Cu, μg/g	Fe, μg/g	Zn, μg/g
Supplemental amino acid - 10 g arginine/kg diet									
0	1.0	1.61	213	100	2.99	3.29	2.49	68	131
0	1.4	1.59	212	99	2.73	3.19	1.99	56	138
0	1.8	1.70	197	92	2.56	3.17	1.89	55	129
0	3.6	1.49	207	98	2.31	3.14	1.92	53	105
3	1.0	1.92	222	105	3.21	3.24	2.40	58	144
3	1.4	1.85	202	94	2.75	3.22	1.98	55	123
3	1.8	2.26	206	101	2.36	3.12	1.96	58	121
3	3.6	1.73	210	99	2.49	3.11	2.03	48	96
Supplemental amino acid - 2.5 g methionine/kg diet									
0	1.0	1.13	201	94	3.14	2.81	1.85	67	157
0	1.4	0.92	197	93	2.70	2.90	2.21	51	138
0	1.8	0.98	204	95	2.46	2.81	1.97	53	127
0	3.6	0.82	201	94	2.30	2.79	2.05	59	128
3	1.0	1.95	197	94	3.31	2.81	2.05	68	154
3	1.4	1.64	201	94	2.67	2.90	2.19	55	137
3	1.8	1.29	202	94	2.44	2.90	1.92	51	130
3	3.6	1.20	207	96	2.12	2.72	1.85	50	110
Analysis of variance - p values									
Boron		0.0001	NS	0.02	NS	NS	NS	(0.07)	0.008
Potassium		0.02	0.005	0.0004	0.0001	NS	0.0001	0.0001	0.0001
Amino acid		0.0001	0.0001	0.0001	NS	0.0001	0.01	NS	0.0001
B x K		NS	NS	0.004	0.02	NS	NS	NS	0.003
B x AA		NS	NS	NS	NS	NS	NS	NS	NS
K x AA		0.04	0.0001	0.003	0.02	NS	0.0001	(0.07)	0.004
B x K x AA		NS	0.001	0.0001	0.05	NS	0.004	NS	0.01

Femur mineral composition was affected by the dietary variables, especially by the different amino acid supplements which were expected to either enhance (arginine supplementation) or alleviate (methionine supplementation) the effects of a marginal intake of methionine. With all the elements except potassium, the amino acid induced changes were modified by dietary boron and/or potassium. Overall, the compositional changes in bone related to potassium deficiency, boron deprivation and methionine supplementation can be construed as evidence of non-beneficial changes occurring in bone. Thus, excessive dietary methionine or low dietary arginine, boron or potassium may cause changes in bone structure or physical characteristics detrimental to optimal function.

1. Nielsen, F. H. (1990) Magnesium Trace Elem. 9, 61-69.
2. Nielsen, F. H., Shuler, T. R., Zimmerman, T. J. and Uthus, E. O. (1988) Biol. Trace Elem. Res. 17, 91-107.
3. Hunt, C. D. and Shuler, T. R. (1989) J. Micronutr. Anal. 6, 161-174.

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* Identifies the author presenting the communication

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LOCATIONS of SCIENCE PROFESSIONALS

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MINOT STATE UNIVERSITY 857-3000

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VALLEY CITY STATE UNIVERSITY 845-7101

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