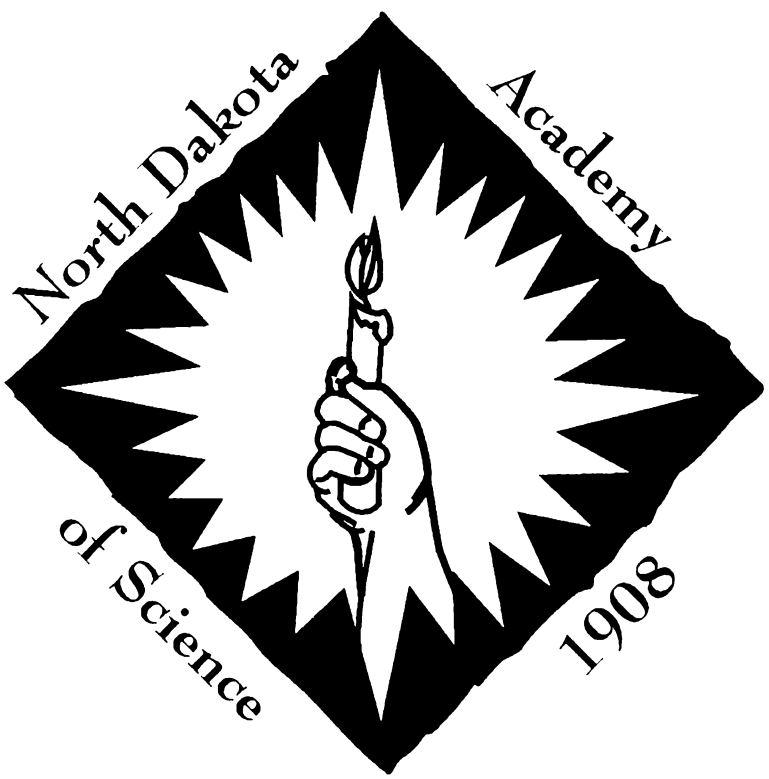
North Dakota Academy of Science

Proceedings
of the
92nd Annual Meeting

April 2000
Volume 54



Proceedings of the North Dakota Academy of Science (ISBN 0096-9214)

Correspondence concerning subscriptions (standing orders), back issues, instructions for authors and other related matters should be directed to:

Secretary-Treasurer
North Dakota Academy of Science
P.O. Box 7081
Grand Forks, North Dakota 58202-7081
USA

Typesetting: Terrifying Typesetting Service, Merrifield, ND

Printed by the University of North Dakota Printing Center

PROCEEDINGS
OF THE
NORTH DAKOTA ACADEMY OF SCIENCE

Volume 54 April 2000

NORTH DAKOTA ACADEMY OF SCIENCE
(Official State Academy; Founded December 1908)

1999-2000

OFFICERS AND MEMBERS OF THE EXECUTIVE COMMITTEE

President ..... Mark A. Sheridan, North Dakota State University
President Elect ..... Ronald K. Jyring, Bismarck State College
Past President ..... Joseph H. Hartman, EERC, University of North Dakota
Secretary-Treasurer ..... Jon A. Jackson, University of North Dakota School of Medicine
Councilors ..... Carl Fox, University of North Dakota

EDITORS

Jon A. Jackson ..... University of North Dakota School of Medicine
Mark A. Sheridan ..... North Dakota State University
Joseph H. Hartman ..... EERC, University of North Dakota

92nd Annual Meeting

April 28–29, 2000

Moorhead, Minnesota

HISTORY

The Proceedings of the North Dakota Academy of Science (NDAS) was first published in 1948, with Volume I reporting the business and scientific papers presented for the 40th annual meeting, May 2–3, 1947. Through Volume XXI, the single yearly issue of the Proceedings included both abstracts and full papers. Commencing with Volume XXII, the Proceedings was published in two parts. Part A, published prior to the annual meeting, contained an abstract of each paper to be presented at the meeting. Part B, published later, contained full papers by some of the presenters.

In 1979 (Volume 33) the Proceedings changed to the present 8 ½ x 11-inch format. It was produced from camera-ready copy submitted by the authors and issued in a single part to be distributed initially at the annual meeting. Commencing with Volume 51 all submissions were on computer disk; the entire Proceedings was then assembled by desktop publishing software. This approach allowed the Editor control over all formatting; many of the papers were reformatted, giving the Proceedings a more consistent look. Also, incorporating all of the submissions on computer allowed production of an electronic copy of the Proceedings for the first time.

VOLUME 54 ORGANIZATION

The communications of this volume of the Proceedings are presented in three sections. The first section lists the titles of papers presented at the NDAS symposium at the 92nd annual meeting. The second section contains the collegiate communications presented in the A. Rodger Denison Student Research Paper Competition. The third section of this volume contains the communications presented in the professional sections of the annual meeting. Readers may locate communications by looking within the major sections of these Proceedings (see table of contents) or by referring to the author index.

Collegiate and Professional Communications

Each Collegiate and Professional 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 results and conclusions, and permit data presentation. The communication conveys much more to the reader than did the abstract, but still provides the advantage of timeliness and ease of production. Commencing with Volume 50, presenters of Symposia at the 88th annual meeting were given the opportunity to contribute an expanded or full-length article consisting of a multiple-page contribution, thus providing a presentation of much greater depth and scope than possible in a singe-page communication.

Constitution and Bylaws

This issue of the Proceedings also contains the Constitution and Bylaws of the Academy, a list of officers and committee members, a list of all Academy members as of March 2000, a copy of unapproved minutes from the 1999 annual meeting, a listing of past presidents of the Academy, and a copy of the 1998 and 1999 financial statements.

IN APPRECIATION

The Academy wishes to specially acknowledge current and emeritus members of the Academy who have supported the mission of the North Dakota Academy of Science Research Foundation through their special gifts. A listing of these supporters is found on page 52 of these Proceedings. The Academy also wishes to express its thanks to the convenors of the symposia for organizing, reviewing, and editing their respective communications. The President of the Academy also wishes to sincerely thank our special guests Dr. Joseph Keenan and Sheila Tobias for speaking at this year's joint gathering.

Jon A. Jackson Mark A. Sheridan Joseph H. Hartman

Symposium Agenda

Organismal Biology of the Northern Plains

Location – Memorial Union

Friday 12:30 - 3:30 p.m. (Room 101)

Organismal Biology of the Northern Plains

A symposium focusing on the behavior, ecology, and evolution of animals on the Northern Plains.

Moderator: *Craig Stockwell,* North Dakota State University

12:30 "Impacts of fathead minnow colonization and removal on a wetland ecosystem." Kyle D. Zimmer, M. A. Hanson[1], and M. G. Butler, North Dakota State University and [1]Minnesota Department of Natural Resources

12:45 "The costs of parasitism in a threatened species." Micheal L. Collyer and Craig A. Stockwell, North Dakota State University

1:00 "Habitat use by migrating blackbirds in east-central South Dakota." Richard S. Sawin, George M. Linz[1], and William J. Bleier, North Dakota State University and [1]USDA/APHIS/WS, National Wildlife Research Center, Great Plains Field Station

1:15 "Individual identification of male barred owl vocalizations using spectrogram analysis and auditory cues." Pam Freeman, North Dakota State University

1:30 "Movement dynamics of an expanding elk herd at Theodore Roosevelt National Park, North Dakota." Ron Richardson, University of North Dakota

1:45 "The ground beetles (Coleoptera: Carabidae) of tallgrass prairie remnants in Minnesota." Paul P. Tinerella and D.A. Rider, North Dakota State University

2:00 "Population genetics of expanding wild pig populations in California." Rod Loggins and Rick Sweitzer, University of North Dakota

2:15 "The distribution of North Dakota stream fishes (an application of GIS technology)." Jeremy Alm, Steven W. Kelsch, and Jeramie Tesky, University of North Dakota

2:30 "Do seniors, mothers or fathers control food distribution among nestling Great Egrets?" Bonnie J. Ploger, Matthew J. Medeiros, and Emily E. Emond, Hamline University

2:45 "Larval life history of the black-belied salamander, *Desmognathus quadramaculatus*: effects of temperature and thyroid hormone on metamorphic timing." Christopher K. Beachy* and Cari-Ann Hein, Minot State University

3:00 "Effects of food and temperature on metamorphic timing of the black-bellied salamander, *Desmognathus quadramaculatus* (Caudata, Plethodontidae)." Cari-Ann Hein*, Evan Barker, and Christopher K. Beachy, Minot State University

3:15 "Bison (*Bison bison*) habitat use and behavior during the breeding season in Theodore Roosevelt National Park in the North Dakota Badlands." Deanna Thompson, Adam Walz, Heather Taylor, Daniel McEwen, Brean Stolz, and Donna Bruns Stockrahm, Moorhead State University.

COMMUNICATIONS

UNDERGRADUATE

A. Rodger Denison Competition – Undergraduate Division
Judges - Ron Jyring, Bismarck State University
Anne Gerber, University of North Dakota

8:20 Welcome and Overview

8:30 "Glucose regulates pancreatic preprosomatostatin I expression." Gregory T. Melroe*, Melissa M. Ehrman, Jeffrey D. Kittilson, and Mark A. Sheridan, North Dakota State University.

8:50 "Low alpha lead and microtome sliced lead for assessment of alpha particle emissions for computer chip manufacturing." Cody Nitschke*, Ben Ziegler, Aaron Kempenich Glenn I. Lykken, and Berislav Momcilovic, University of North Dakota and Institute for Medical Research and Occupational Health, Croatia.

9:10 "Inhibition of scleral gelatinase A activity by captopril." Francine Johnson*, Virginia R. Achen, and Jody Rada, University of North Dakota.

9:30 "Localization of somatostatin mRNA in the brain and pancreas of rainbow trout (*Oncorhynchus mykiss*)." Leslie Alexander*, Darlene Knutson, Jeffrey D. Kittilson, and Mark A. Sheridan, North Dakota State University.

9:50 BREAK

10:10 "Expression of retinoic acid receptors by human sclera." Chrisanne K. Timpe*, Cheryll Perry, and Jody A. Rada - University of North Dakota.

10:30 "Auxin-induced cell wall loosening and the control of tobacco leaf cell expansion." Traci L. Tranby* and Christopher P. Keller, Minot State University.

10:50 "Proliferation of vascular endothelial cells and pericytes in ovine corpora lutea (CL) throughout the estrous cycle." B.J. Bortnem*, D.R. Arnold, K.C. Kraft, K. Fischer, A. Jablonka-Shariff, L.P. Reynolds, and D.A. Redmer, North Dakota State University.

11:15 Judges discussion

LOCALIZATION OF SOMATOSTATIN mRNAs IN THE BRAIN AND PANCREAS OF RAINBOW TROUT (*Oncorhynchus mykiss*)[1]

Leslie Alexander*, Darlene Knutson, Jeffrey D. Kittilson, and Mark A. Sheridan
Department of Zoology , North Dakota State University, Fargo, ND 58105

Somatostatins (SS) are a structurally diverse family of peptide hormones that coordinate the growth, development, and metabolism of animals. Recent work has revealed that the structural heterogeneity stems from the differential processing of a larger hormone precursor (preproSS, PPSS) as well as from the existence of several genes. In rainbow trout, for example, we have isolated three distinct mRNAs from the endocrine pancreas, each encoding a separate precursor: PPSS I, which contains a 14-amino acid sequence at its C-terminus (SS-14) that is highly conserved among vertebrates, as well as two others, PPSS II' and PPSS II", both containing [Tyr^7, Gly^{10}]-SS-14 at their C-terminus (2). We also have shown that the PPSS mRNAs of trout are differentially expressed (2). In this study, we determined the cellular localization of PPSS mRNAs in the brain and endocrine pancreas (Brockmann bodies) of rainbow trout.

For these experiments, Brockmann bodies and whole brains were removed from anesthetized fish. Tissues were fixed (4% paraformaldehyde), dehydrated, and embedded in paraffin. Six micrometer sections were then mounted on poly-L-lysinated slides and the slides stored at 4°C for later *in situ* hybridization. Both antisense and sense (used for controls) gene-specific oligonucleotides were synthesized, labeled with digoxigenin (DIG)-dUTP, and used as probes. Following rehydration, tissue sections were prehybridized with proteinase K, rinsed, then hybridized with DIG-labeled probes. Probes were visualized by bright field microscopy following formation of an oligoDIG-antiDIG-alkaline phosphatase conjugate treated with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium.

In the Brockmann body, the predominate pattern observed was that some islet cells expressed only PPSS I mRNA while other cells expressed only PPSS II mRNAs. There were, however, several instances of co-expression in which a single cell expressed both PPSS I and PPSS II mRNAs. With regard to the pattern of PPSS II expression, many cells were observed to express both PPSS II'and PPSS II" mRNA. However, some cells expressed only PPSS II' mRNA while other cells expressed only PPSS II" mRNA. In the brain, PPSS I mRNA was expressed in the optic tectum (OT), particularly surrounding the ventricle, in nuclei within the pre-optic area [*e.g.*, nucleus rotundus (NR), nucleus anterioris hypothalami (NAH), nucleus preglomerulosus pars lateralis (NPGL), nucleus anterior tuberis (NAT), nucleus lateral tuberis (NLT)] as well as in the pituitary. PPSS II" mRNA was present in the same regions as PPSS I mRNA; however, PPSS II' mRNA was present primarily in OT, NAT, NLT and pituitary.

These results indicate that PPSS mRNAs are expressed differently by different cells. These findings suggest that cell-specific mechanisms are involved with the control of PPSS expression and that particular biological responses may be associated with a specific SS isoform.

---

1. This research was supported by NSF grant IBN 9723058 to M.A.S.

2. Moore C.A, Kittilson J.D., Ehrman M.M., and Sheridan M.A. (1999) Rainbow trout (*Oncorhynchus mykiss*) possess two somatostatin mRNAs that are differentially expressed. *Am. J. Physiol.* 277: R1553-R1561.

PROLIFERATION OF VASCULAR ENDOTHELIAL CELLS AND PERICYTES IN OVINE CORPORA LUTEA (CL) THROUGHOUT THE ESTROUS CYCLE

B.J. Bortnem*, D.R. Arnold, K.C. Kraft, K. Fischer, A. Jablonka-Shariff, L.P. Reynolds, and D.A. Redmer.
Department of Animal and Range Science, North Dakota State University, Fargo, North Dakota 58105

INTRODUCTION

Luteal tissue undergoes rapid growth and vascularization throughout the early and mid stages of the estrous cycle. Cellular proliferation relies on the availability of nutrients to the tissues. The extensive vascular system of the early and mid-cycle CL serves to supply these nutrients as well as providing a means of removing waste products, which could otherwise be toxic. Delivery of hormones to and from this tissue is essential to its role in reproduction. Thus, the female reproductive system, specifically the CL, provides an excellent model for studying the growth and regression of the vasculature.

METHODS

To quantify the rate of vascular cell proliferation, CL were obtained from ewes that were injected with bromodeoxyuridine (BrdU; a thymidine analog) 1h before slaughter on days 4, 8, 12, and 15 of their respective estrous cycle. The CL were removed, fixed in Carnoy's solution, embedded in paraffin, sectioned (6 mm), and fixed onto glass slides. Tissue sections were stained for BrdU, lectin BS-1 (an endothelial marker), and/or smooth muscle cell α-actin (SMCA; a pericyte marker), using immuno-histochemical (ABC) techniques. Cell proliferation was quantified for total (BrdU-labeled) cells, endothelial (lectin + BrdU-labeled) cells, and pericytes (SMCA + BrdU-labeled) by morphometry. The total number of luteal endothelial cells and pericytes also was determined morphometrically.

RESULTS

Total proliferating (BrdU-labeled) cells (per 2.9×10^{4} mm^{3}) decreased ($p<0.01$) throughout the estrous cycle and were 21.9, 7.2, 3.9, and 2.2 on days 4, 8, 12, and 15 respectively. The percentage of total cell proliferation represented by endothelial cells or pericytes did not differ across days of the estrous cycle and was 21.5, 36.8, 16.8, and 33.1% for endothelial cells, and 8.3, 14.8, 12.7, and 13.3% for pericytes, on days 4, 8, 12, and 15 respectively. The total number of luteal endothelial cells increased ($p<0.01$) from day 4 to day 8 (6.4 vs. 28.3×10^{6} cells) then remained constant on days 12 and 15 (19.2 and 22.5×10^{6}). The total number of luteal pericytes increased ($p<0.01$) from day 4 to day 8 (8.3 vs. 25.2×10^{6}) then decreased ($p<0.01$) by day 12 (18.8×10^{6}) and remained constant through day 15 (16.4×10^{6}).

DISCUSSION

These data indicate that the rate of luteal endothelial cell and pericyte proliferation was greatest early in the estrous cycle then decreased throughout the estrous cycle in a pattern similar to that of overall luteal cellular proliferation. The high rate of proliferation of endothelial cells and pericytes on day 4 appears to lead to a large increase in the numbers of these vascular cells by day 8, consistent with the high degree of vascularity of the mature CL.

Supported by NRICGP 98-35203-6222.

INHIBITION OF SCLERAL GELATINASE A ACTIVITY BY CAPTOPRIL

Francine Johnson*, Virginia R. Achen, and Jody A. Rada
Department of Anatomy & Cell Biology; University of North Dakota, Grand Forks, ND 58202.

PURPOSE

Previous reports indicate that the development of myopia in a variety of animal models is accompanied by increased levels of gelatinase A (72 kD gelatinase, MMP-2) activity in the posterior sclera (1,2). In an effort to identify agents potentially useful for the treatment of myopia, we compared the effects of several reported gelatinase A inhibitors on gelatinase A activity in the chick sclera.

METHODS

Punches (3 mm) were obtained from the posterior sclera of chick eyes and immediately placed in multiwell plates containing Dulbecco's Modified Eagle's Medium (DMEM) alone (controls) or DMEM containing Tissue Inhibitor of Metalloproteinase (TIMP) - 2, a natural inhibitor of gelatinase A, Nobiletin, a citrus flavonoid, or Captopril, an inhibitor of angiotensin converting enzyme, which also blocks activity of Zn^{2+}-dependent metalloproteinases. Scleral punches were incubated in organ culture at 37°C for 24 hrs. Medium was harvested and analyzed by gel zymography on 11% gelatin substrate gels. Zymograms were digitized using a flatbed scanner and relative amounts of active and latent gelatinase A were measured on zymograms using NIH Image 1.61.

RESULTS

When placed in organ culture, scleral punches from chick eyes released clearly detectable levels of both the latent and active forms of gelatinase A into the medium. Of the three compounds tested, only Captopril (30 mM) had a significant inhibitory effect on gelatinase A released into the culture medium. Both the latent and active forms of gelatinase A were significantly inhibited by the addition of Captopril at a final concentration of 30 mM ($p < 0.05$).

CONCLUSIONS

The significant inhibitory effects of Captopril on scleral gelatinase A activity in vitro suggest that this agent may prove useful in therapies designed to slow or prevent myopia progression.

---

1. Rada, J.A. and Brenza, H.L. (1995) *Invest. Ophthalmol. Vis. Sci.*, 36:1555-1565.
2. Guggenheim, J.A. and McBrien, N.A. (1996) *Invest. Ophthalmol. Vis. Sci.*, 37:1380-1395.

This research was supported by a Howard Hughes Undergraduate Research Apprenticeship (FJ), National Institutes of Health (NEI RO19391; JAR) and the Macula Vision Research Foundation (JAR).

GLUCOSE REGULATES PANCREATIC PREPROSOMATOSTATIN I EXPRESSION[1]

Gregory T. Melroe*, Melissa M. Ehrman, Jeffrey D. Kittilson, and Mark A. Sheridan
Department of Zoology, North Dakota State University, Fargo, ND 58105

The somatostatin (SS) family of peptide hormones has been shown to regulate various aspects of growth, development, and metabolism in vertebrates. Relatively little is known about the control of SS biosynthesis and secretion. Teleost fishes have proved to be particularly good models for the study of SS because they possess a large principal islet (Brockmann body) that is easily separated from exocrine tissue and because this islet possesses relatively high amounts of SS compared to mammalian islets (2). In this study, rainbow trout were used as a model system to evaluate the control of SS gene expression. We examined the effects and mechanisms of glucose action on the expression of preprosomatostatin I (PPSS I) mRNA. This will contribute to the overall understanding of how the production and secretion of SS is controlled.

For these experiments, juvenile rainbow trout (*Oncorhynchus mykiss*) were anesthesthetized and their Brockmann bodies removed and prepared for culture. After cleaning and bisecting, the hemi-islets were preincubated for 2 hours in 1 ml of basal medium (ca. 2-3 hemi-islets per well). Following preincubation, the medium was removed, the islets were washed gently with 1 ml of fresh basal medium, and 1 ml of test medium was added to each well. Incubation proceeded under the same conditions as preincubation for up to 24 h, after which the medium was removed and the islets were immediately frozen on dry ice. Total RNA was extracted from Brockmann bodies using the TRI Reagent® protocol (Molecular Research Center, Inc., Cincinnati, OH). PPSS I mRNA was measured using a quantitative slot-blot technique in which *in vitro* synthesized cRNA standards were blotted onto a nylon membrane along with sample RNA. The membranes were first hybridized with a gene-specific ^{32}P-labeled oligonucleotide probe and quantified. Sample blots were then stripped and rehybridized with a full-length ^{32}P-labeled α-actin probe and requantified. Following correction for background and normalization for α-actin, mRNA levels were expressed as molecules of mRNA x 10^8 per μg total RNA.

Glucose stimulated PPSS mRNA expression in a dose-dependent manner; the maximal response was at 10mM. Other hexoses (*e.g.*, mannose, galactose, fructose) also enhanced the expression of PPSS I mRNA; the effects of fructose and galactose were particularly pronounced. The mechanisms by which glucose stimulates the expression of PPSS I mRNA were studied in several experiments. Sucrose, a disaccharide that doesn't enter cells readily, had no effect on expression of PPSS I mRNA. In addition, neither 3-*o*-methyl glucose, a compound that enters cells but doesn't get phosphoryated, nor 2-deoxyglucose, a compound which enters cells and gets phosphorylated but not metabolized further, altered the steady-state levels of PPSS I mRNA. On the other hand, metabolites such as glycerol, dihydroxyacetone, pyruvate, and lactate significantly stimulated the levels of PPSS I mRNA. An inhibitor of glycolysis, iodoacetate, significantly reduced the levels of PPSS I mRNA. Dichloroacetate, which increases flux into the Krebs cycle, as well as intermediates of the Krebs cycle, such as acetate and citrate, also enhanced the levels of PPSS I mRNA.

The results of this study indicate that glucose modulates the expression of the PPSS I mRNA. This requires cellular entry and the subsequent metabolism of the sugar. This conclusion is supported by several observations. First, sucrose had no effect on PPSS I expression; this lack of an effect also excludes the possibility of an osmotic action. Second, a variety of hexoses, including glucose, galactose, and fructose, which enter cells and undergo phosphorylation, isomerization, and metabolism to varying degrees, all stimulated PPSS I mRNA expression. Third, that hexose-stimulated PPSS I mRNA expression requires phosphorylation and further metabolism was supported by the failure of the glucose analogs 3-*o*-methyl glucose and 2-deoxyglucose to alter steady-state levels of PPSS I mRNA expression. Lastly, the requirement for the subsequent metabolism of glucose through glycolysis and Krebs cycle was indicated by the ability of metabolites such as dihydroxyacetone, glycerol, lactate, pyruvate, acetate, and citrate to stimulate levels of PPSS I mRNA. Moreover, increased flux through the Krebs cycle, via dichloroacetate action, stimulated the levels of PPSS I mRNA. Together, the present findings suggest that the proximate mediator of glucose-stimulted PPSS I expression is generated after the aldolase step of glycolysis. These findings extend our knowledge of the effects of glucose on SS production.

---

1. This research was support by NSF grant IBN9723058 to M.A.S.

2. Plisetskaya, E.M., Pollock, H.G., Rouse, J.B., Hamilton, J.W., Kimmel, J.R., Andrews, P.C. and Gorbman, A. (1986) *Gen. Comp. Endocrinol.*, 63, 242-263.

LOW ALPHA LEAD AND MICROTOME SLICED LEAD FOR ASSESSMENT OF ALPHA PARTICLE EMISSIONS FOR COMPUTER CHIP MANUFACTURING

Cody Nitschke*, Ben Ziegler, Aaron Kempenich, Glenn I. Lykken, and Berislav Momcilovic
Department of Physics, University of North Dakota, Grand Forks, and
Institute of Medical Research and Occupational Health, Zagreb, Croatia

Soft errors in computer memory chips arise when ^{210}Pb, a radon daughter, generates ^{210}Po which emits high energy alpha particles (5.3 MeV) in lead solder bumps. These emissions change the charge state of individual transistors. Evidently, the smaller the distance between transistors, the greater the possibility for the computer chip soft errors and therefore the greater the need for low alpha lead (LAL), (i.e., lead with low ^{210}Po emissions). Lead ore, galena, free of ^{210}Pb contamination, when processed under normal smelting methods (NSM), contains ^{210}Pb. The source of ^{210}Pb contamination in processed lead is unresolved.

We observed that coke used in NSM was found to emit alpha particles over a range of energies, 4-8 MeV. Activated coke absorbed approximately 5% as much radon as activated charcoal from a dry sump well. Bench-top processes (patented by Heraeus) were used to investigate possible sources of ^{210}Pb contamination. A glove box was used to prepare a baseline sample in a "clean" alumina crucible that was heated in an oven with a flowing nitrogen atmosphere. Atmospheric radon gas and radon progeny (contained in laboratory air) were introduced during the process by replacing the flowing nitrogen with flowing laboratory air supplied by an aquarium air-pump. This introduced radioactive isotopes into the flux during processing so that the resulting lead contained polonium isotopes as determined by alpha particle counting. Because of its long half-life and low energy gamma emissions certification of the ^{210}Pb concentration in LAL is difficult, time consuming, and expensive at the levels required by industry.

We found that the microtome (American Optical Co., Buffalo, NY), a thin slice-cutting device used in biological and mineralogical sciences, could be used to produce thin lead slices of reasonably large surface area, approximately 10 cm^2. Indeed, when the microtome was set to cut 1 micron thick slices the lead slices were approximately 5 microns thick while a setting of 2 microns resulted in samples approximately 8 microns thick. Settings above 2 microns produced slices thicker than ten microns. Attenuation of ^{210}Po alpha particles from a plated source (0.1 μCi, Spectrum Industries, Oak Ridge, TN), as measured in an alpha particle spectrometer (Alpha-King Spectrometer, EG&G Ortec, Oak Ridge, TN), was used to measure peak smearing and energy shift dependence upon lead thickness in both slices and vacuum-deposited thin films of lead. The slices were compressed approximately 67% along the cutting direction and an alpha particle peak showed considerable broadening, approximately three times that of deposited thin lead film and 20 times that of the plated ^{210}Po standard. Energy peak shifts ranged from 1.2 to 2.3 MeV. Thus the compression produced ripples in the slices thereby increasing the effective thickness of lead slices. Interestingly, the LAL cuts better then ordinary lead with increased ^{210}Po. However, after annealing the "contaminated lead" in vacuum for a few hours near its melting point, effectively renders the lead easier to cut. Evidently, high ^{210}Po-associated impurities produces defects which result in difficulties in cutting.

Current assay methods of LAL utilize large area (1000 cm^2) flowing-gas detectors (FGD) which detect gross alpha emissions from samples. It appears that using current FGD to analyze lead slices produced with a microtome should provide the optimum assay method for LAL. This is because the integrity of the lead composition is not affected by the sampling procedure with the microtome slicing method. Furthermore, large areas (10 cm^2) can be produced from reasonably small lead ingots, *i.e.*, 5 cm. It is hypothesized that coke and normal smelter air contain sufficient radon and its progeny to "contaminate" lead during processing.

Supported in part by DEPSCoR under Research Proposal No. 40072-EL-DPS

BETA AMYLOID: THE PEPTIDE THAT KILLS FROM BOTH THE OUTSIDE AND INSIDE TO PRODUCE ALZHEIMER'S DISEASE

Jason Spah*, Katherine Splichal, Kali Wilson and Garl K. Rieke
Department of Anatomy and Cell Biology, University of North Dakota, Grand Forks, ND 58202

The 42 amino acid β-amyloid peptide fragment (Aβ42) is the killer of nerve cells in Alzheimer's disease (AD). We demonstrate here some of the mechanisms underlying Aβ neurotoxicity based upon both light microscopic immunochemistry and electron microscopic immunogold with antibodies specific for epitopes of the Aβ42 fragment. Extracellular challenge of hippocampal neurons with Aβ1-42 (0.22 nmoles/μl for seven days) initiated a cascade of events leading to apoptosis. The Aβ was delivered through an Alzet miniosmotic pump (0.25μl/hr) coupled to a stainless steel cannula, implanted into the dorsal rostral hippocampus. Tissues were fixed in 4% paraformaldehyde and 0.5% glutaraldehyde, dehydrated and embedded in paraffin. Paraffin sections 5 μ thick were cut serially in the coronal plain through the hippocampus and mounted on glass slides. The tissues were immunostained using antibodies against specific antigens (peptides) thought to be involved in the Aβ42-induced signaling pathway leading to apoptosis. DAB, enhanced with nickel, was the chromagen used to label the antibodies. The signaling pathway induced by extracellular challenge with Aβ1-42 involved activation of p53, BAX, cytochrome c, and caspase 3; as demonstrated by immunopositive pyramidal neurons stained by primary antibodies for each of the antigens. Aβ42 is known to induce lipid peroxidation in the plasma membrane and the generation of 4-hydroxynonenal (aldehydic lipid peroxidation product which may activate p53, a tumor suppressor factor. p53 activates BAX, an apoptosis promoter associated with the membranes of mitochondria. BAX reacts with bcl-2 (an apoptosis inhibitor factor), resulting in the loss of the protective effect of bcl-2 on the mitochondria. The membrane potential of the mitochondria is reduced, which leads to the release of cytochrome c (electron transport complex IV) from the BAX effected mitochondria into the cytosol. Cytochrome c in the cytosol promotes the activation of caspase 3 (effector caspase). Caspase 3 migrates to the nucleus and disrupts the enzyme poly-(ADP-ribose) polymerase, thus blocking the ability of the nucleus to repair DNA fragmentation leading to apoptosis. Aβ42-challenged hippocampal neurons demonstrated positive immuoreactivity when stained by the ApopTag *in situ* Apoptosis Detection Kit (Oncor, Gaithersburg, MD). Extracellular Aβ42 induces oxidative stress and perturbations in calcium (Ca^{+2}) homeostasis.

We have demonstrated that the rodent Aβ42 fragment is located within cells, as in AD. Challenge of normal hippocampal neurons with the alkalizing agent chloroquine (60μM, pH=7), results in the intracellular accumulation of Aβ42, thus modeling AD. The Aβ fragments are normal catabolites of the Alzheimer's precursor protein (APP). This large peptide is synthesized in the rough endoplasmic reticulum and degraded by two enzymes (β and γ secretases) located in the membrane of the RER, thus forming the Aβ42 fragment. In our study, the Aβ42 fragments were labeled with two antibodies - one specific for the C-terminal (QCB antibody) and a second that targets the middle region of Aβ42 (4G8 antibody; BM-antibody). The primary antibodies were reacted with specific secondary antibody tags conjugated to 15 nm or 30 nm gold (double-bridge technique of Sternberger). Double-labeled Aβ42 fragments appeared in the rough endoplasmic reticulum (RER), the nucleus (N) and in association with mitochondrial membranes. The presence of double-labeled Aβ42 in the RER indicates that the catabolic proteolytic β and γ secretases are in the membrane of the RER and breakdown of the precursor protein APP occurs before the Golgi and secretory vesicles. Intracellular Aβ42 may interact with the lipids in the RER to induce lipid peroxidation (toxic aldehydic products like 4-hydroxynonenal) as it does with the plasma membrane. The last 15 amino acids of the C-terminal of the Aβ42 fragment are part of the transmembrane domain of APP and may provide lipid solubility to the Aβ42 fragment which permits the fragment to move into the cisternae of the RER or into the cytosol. Aβ42 in the cisternae of the RER can move into the nucleus and may act on the chromatin altering gene functions. Cytosolic Aβ42 may react with the 113 binding domain of bcl-2 (an anti-apoptotic protein in the mitochondrial membrane). By blocking bcl-2 dimerization (bcl-2/bcl-2) and producing a bcl-2/Aβ42 complex, Aβ42 removes the protective effect of bcl-2, the mitochondria depolarizes and releases cytochrome c; a cytosolic trigger for apoptosis. Cytochrome c activates caspase 3 (effector caspase). There may also be an initiator caspase (caspase 8) involved; however, there is no antibody for caspase 8 that works on paraffin embedded tissue. Caspase 3 attacks poly-(ADP-ribose) polymerase and DNA fragmentation is not repaired, thus favoring apoptosis. Lastly, since Aβ42 is lipid soluble, it may form ion channels in the membrane of the RER as it does when added to a lipid bilayer. These Aβ42-induced channels may liberate Ca^{+2} from intracellular calcium stores in the RER. In conclusion, Aβ42 can induce apoptosis by multiple signaling pathways, both of extracellular and intracellular origins. Design of a therapeutic treatment for Alzheimer's Disease must consider all of the signaling pathways.

EXPRESSION OF RETINOIC ACID RECEPTORS BY HUMAN SCLERA

Chrisanne K. Timpe*, Cheryll A. Perry and Jody A. Rada
Department of Anatomy & Cell Biology; University of North Dakota, Grand Forks, ND 58202

Much evidence exists for the presence of a vision-dependent mechanism which regulates the coordinated growth of the ocular tissues of the eye so as to minimize refractive errors. Previous results indicate that this mechanism controls the rate of axial elongation by regulating scleral extracellular matrix synthesis and turnover at the posterior pole of the eye[2]. Of much interest is the identification of the chemical signals which regulate scleral extracellular matrix turnover. Retinoic acid has been suggested as a possible signal which regulates scleral extracellular matrix remodelling[3]. The transcriptional effects of the retinoids are regulated by a family of nuclear receptors, the retinoid acid receptors (RARs) and the retinoid X receptors (RXRs), whose natural ligands are *all-trans* retinoic acid and 9-*cis* retinoic acid, respectively. In order to characterize the possible role of retinoids in mediating scleral extracellular matrix metabolism, we quantified the relative expression of the retinoic acid receptor genes, RARα, RARβ, RARγ, RXRα, RXRβ, and RXRγ by the human sclera.

Human sclerae were obtained within 8 hrs post mortem and immediately snap frozen in liquid nitrogen. The tissue was pulverized under liquid nitrogen with a cryogenic mill and total RNA was extracted from the sclera using Trizol. cDNA, representative of the mRNA pool, was generated from mRNA using random hexamers. PCR primers were selected based on the published sequences using the MacVector (v. 4.1) program and were used to amplify regions of each of the six retinoid receptors and PCR products were visualized on 1.5% agarose gels by ethidium bromide staining. The number of thermocycles was determined for amplification within the linear range for each of the PCR products and the relative steady state levels of RARα, RARβ, RARγ, RXRα, RXRβ, and RXRγ were quantified following PCR amplification in the linear range by measuring the intensity of the ethidium bromide stained bands using NIH Image 1.61.

Human scleral fibroblasts express the retinoic acid receptors: RARα, RARβ, RARγ, RXRα, RXRβ, and RXRγ. Steady state levels of RARs are significantly higher than RXRs with RARβ being the most abundant message expressed by scleral fibroblasts.

Based on the relative abundance of mRNA for RARs, as compared with RXRs, it is likely that *all-trans* retinoic acid, rather than 9-*cis* retinoic acid, plays a role in regulating scleral fibroblast function.

---

1. Goss, D.A. and Criswell, M.H. (1981) *Am. J. Optom. Physiol. Opt.*, 10, 859-869.
2. Rada, J.A., McFarland, A.L., Cornuet, P.K. and Hassell, J.R. (1992) *Curr. Eye Res.*, 11, 767-782.
3. Mertz, J.R., Nickla, D.L., Marzani, D. and Wallman, J. (1998) *Invest. Ophthalmol. Vis. Sci.*, 39, S868.

Research supported by Howard Hughes Undergraduate Research Apprenticeship (CT), National Institutes of Health (NEI R019391; JR) and the Macula Vision Research Foundation (JR).

AUXIN-INDUCED CELL WALL LOOSENING AND THE CONTROL OF TOBACCO LEAF CELL EXPANSION

Traci L. Tranby* and Christopher P. Keller
Department of Biology, Minot State University, Minot, North Dakota 58707

INTRODUCTION

Previous work has established that excised tobacco leaf strips have a substantial growth response following auxin treatment (1). The growth is epinastic, unrelated to auxin-induced ethylene production, and strongly developmentally regulated with responsiveness correlating with the cell expansion phase of growth. Evidence suggests the putative auxin receptor ABP1 is involved in the growth response by tobacco leaf tissue (2).

In other systems (growing stems, hypocotyls, and grass coleoptiles) auxin treatment induces cells to acidify their cell walls, apparently through activation of a plasma membrane H+-ATPase. Increased growth results from increased cell wall extensibility (cell wall loosening) mediated by extracellular loosening enzymes which are only active at low *p*H. Further investigation of auxin-induced growth of tobacco leaf strips has shown, however, the response to be unusual (3). Growth initiates only after a long lag (one hour) and, does not involve the Acid-Growth Mechanism described above. Cell wall acidification does not occur, but auxin-induced growth is apparently still mediated by an increase in cell wall loosening or extensibility. We are interested in determining the mechanism of auxin-induced wall loosening in tobacco leaves. The current project tests the hypothesis that auxin-induced growth of tobacco leaf tissues results either from increased production of wall loosening enzymes and/or from increased susceptibility of the cell wall to be loosened without a lowering of *p*H.

METHODS

Tobacco plants were growth for 6 weeks under greenhouse conditions as previously described (1, 3). Growing leaves 8-10 cm in length were harvested and 1.5 mm by 10 mm strips cut from interveinal regions. The strips were then incubated 5 hours in solutions containing 10 mM sucrose, 10 mM KCl, +/- 0.01 mM naphthlene acetic acid (auxin). Following incubation the strips were killed by freeze/thawing. Cell wall extensibility was then determined by the constant stress method (4) with strips incubated first for 1 hour in 10 mM MES/BTP *p*H 6.5 and then for 2 hours at *p*H 5.0 under tension provided by 4.1 gram weights. Extension was detected by linear displacement transducers and recorded on chart recorders.

RESULTS AND DISCUSSION

In initial trials 10 control and 8 auxin pre-treated strips were tested. Under tension at *p*H 6.5 no distinct difference in extension was evident between the control and auxin pre-treated strips. The mean extension of auxin treated strips under acid conditions was 5.1 +/- 0.3% of initial length over 2 hours while mean extension of the controls was 4.0 +/- 0.5% of initial length. The differences between the treatments was not significant and the trend is sufficiently small as to suggest that auxin-induced growth does not result from an increased sensitivity of the wall to acid conditions.

---

1. Keller C. P. and Van Volkenburgh, E (1997) *Plant Physiol.*, 113, 603.
2. Jones, A.M., *et al.*, (1998) *Science*, 282, 1114.
3. Keller C. P. and Van Volkenburgh, E (1998) *Plant Physiol.*, 118, 557.
4. Cleland, R.E. (1981) In E.A. Loewus and W. Tanner, eds, Encl. Plant. Physiol. New Series. Plant Carbohydrates II. Extracellular. Vol. 13B, pp. 254-273.

COMMUNICATIONS

GRADUATE

A. Rodger Denison Competition – Graduate Division

Judges: Kay Keehr, Grand Forks Human Nutrition Research Center
Mark Wolverton, Minot State University

8:00 Welcome and Overview

8:10 "Immunolocalization of endothelial nitric oxide synthase (eNOS) in ovine corpora lutea (CL) throughout the estrous cycle." Daniel R Arnold*, Vinayak Doraiswamy, Kim C Kraft, Dale A Redmer, and Lawrence P Reynolds, North Dakota State University.

8:30 "Molecular mapping of Fusarium head blight resistance in tetraploid wheat." C.D. Otto*, S.F. Kianian, E.M. Elias, R.W. Stack, and L.R. Joppa, North Dakota State University and USDA-ARS, Fargo.

8:50 "Somatostatin modulates the growth of salmonid fish." Nicole Howe*, Darlene Knutson, Jeff Kittilson, and Mark Sheridan, North Dakota State University.

9:10 "Localization of gap junction protein connexin (Cx)43 durng maturation of the cumulus oocyte complex (COC) of ewes." Tande K. Stenbak*, Lawrence P. Reynolds, Jerzy J. Bilski, Dale A. Redmer, and Anna T. Grazul-Bilska, North Dakota State University.

9:30 "Novel photoaffinity label indicates spatial relationships within the dopamine transporter." Jon D. Gaffaney*, Aloke Dutta^, and Roxanne A. Vaughan, University of North Dakota and ^Wayne State University.

9:50 BREAK

10:10 "Phosphorylation of the type I regulatory subunit of cAMP-dependent protein kinase in rat tissues." Karen M. Boeshans* and John B. Shabb, University of North Dakota

10:30 "Localizing site of protein kinase C stimulated phosphorylation of dopamine transporter." Benchaporn Pananusorn* and Roxanne Vaughan, University of North Dakota

10:50 "Fine structure mapping of the species cytoplasmic specific gene." K.J. Simons*, S.F. Kianian, and S.S. Maan, North Dakota State University.

11:10 "Leptin attenuates cardiac contraction in rat ventricular myocytes: role of nitric oxide." Loren E. Wold*, Peter B. Colligan and Jun Ren, University of North Dakota.

11:30 "An assessment of impact of the Devils Lake emergency outlet on the periphyton communities of the Sheyenne River." Megan Jaskowiak*, Karen Phillips, and Marvin Fawley, North Dakota State University.

11:50 Judges discussion

IMMUNOLOCALIZATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) IN OVINE CORPORA LUTEA (CL) THROUGHOUT THE ESTROUS CYCLE

Daniel R. Arnold*, Vinayak Doraiswamy, Kim C. Kraft, Dale A. Redmer, and Lawrence P. Reynolds
Department of Animal and Range Sciences, North Dakota State University, Fargo, ND 58105

INTRODUCTION

The corpus luteum is a transient endocrine gland which forms from the ovulated ovarian follicle and is critical for normal reproduction because it is the primary source of the progestational hormones such as progesterone. After ovulation, the follicular wall grows and vascularizes extremely rapidly, doubling in size every 3 days, until the mature CL is formed by about day 10 after estrus. Nitric oxide (NO), a potent vasodilator, has been shown to stimulate angiogenesis and production of vascular endothelial growth factor (VEGF), which is an important regulator of luteal vascular development (1, 2, 3, 4). In addition, VEGF stimulates eNOS expression, thus leading to NO production (5). This experiment was conducted to evaluate: 1) eNOS immunolocalization; and 2) co-localization of eNOS and VEGF in ovine CL throughout the estrous cycle.

METHODS

Beginning on the morning of days 13 of the estrous cycle (day 0=estrus), ewes received twice daily (morning and evening) intramuscular injections of follicle stimulating hormone (FSH; to induce superovulation) as follows: Day 13, 5 units/injection; Day 14, 4 units/injection; and Day 15, 3 units/injection. Following detection of estrus, CL were obtained on days 2, 4, 10, and 15 (n=5/group) of the estrous cycle, fixed in Carnoy's solution, and embedded in paraffin. For immunolocalization of eNOS in luteal tissue sections (6 μm), we used a specific monoclonal antibody (Transduction Laboratories) and the ABC method. Positive staining (% of tissue area stained) was quantified by full color computerized image analysis. VEGF immunolocalization utilized a specific affinity-purified polyclonal anti-peptide antibody (6).

RESULTS

The endothelium of the luteal arterioles stained positive for eNOS regardless of day of estrous cycle, whereas thecal-derived connective tissue tracts were devoid of staining. Capillary endothelium stained positive for eNOS in early cycle CL (days 2 and 4), but this staining was reduced dramatically later in the cycle (days 10 and 15). On day 2, eNOS staining was prominent (5.5%; pooled SEM= 0.8) in capillary and arteriolar endothelial cells within the parenchymal lobules; this immunostaining was increased ($P<0.01$) on day 4 (8.2%), but decreased ($P<0.01$) on days 10 and 15 (2.4 and 1.3%, respectively). On days 10 and 15, eNOS immunostaining was localized primarily to endothelial cells of the arterioles. Luteal endothelial eNOS immunostaining was closely associated with peri-endothelial (capillary pericytes and arteriolar smooth muscle) VEGF immunostaining.

CONCLUSIONS

We believe these results are the first to demonstrate eNOS localization in luteal capillary endothelial cells and, in addition, demonstrate elevated concentrations early in the estrous cycle coinciding with the period of rapid CL growth. Proteins for eNOS then decline as angiogenesis in the CL plateaus and the CL matures. In addition, these data provide evidence for a paracrine mechanism that maximizes luteal vasodilation and angiogensis via endothelial eNOS and peri-endothelial VEGF expression.

Supported by NRICGP/USDA grant 98-35203-6222.

1. Chin, K, Y Kurashima, T Ogura, H Tajiri, S Yoshida, and H Esumi. 1997. Induction of vascular endothelial growth factor by nitric oxide in human glioblastoma and hepatocellular carcinoma cells. *Oncogene,* 15:437-442.
2. Ferrara, N, H Chen, T Davis-Smyth, HP Gerber, TN Nguyen, D Peers, V Chisolm, KJ Hillan, and RH Schwall. 1998. Vascular endothelial growth factor is essential for corpus luteum angiogenesis. *Nature Med.,* 4:336-340.
3. Dickson SE and HM Fraser. 1999. Manipulation of angiogenesis on the primate corpus luteum. *Biol. Reprod.* 60(Suppl. 1):279-280.
4. Frank S, B Stallmeyer, H Kampfer, C Schaffner, and J Pfeilschifter. 1999. Differential regulation of vascular endothelial growth factor and its receptor fms-like-tyrosine kinase is mediated by nitric oxide in rat mesangial cells. *Biochem. J.,* 338:367-374.
5. Hood, JD, CJ Meininger, M Ziche, and HJ Granger. 1998. VEGF upregulates eNOS message, protein, and NO production in human endothelial cells. *Am. J. Physiol.,* 274:H1054-1058.
6. Redmer, DA, Y Dai, J Li, DS Charnick-Jones, SK Smith, LP Reynolds, and RM Moor. 1996. Characterization and expression of vascular endothelial growth factor (VEGF) in the ovine corpus luteum. *J. Reprod. Fertil.,* 108:157-165.

PHOSPHORYLATION OF THE TYPE I REGULATORY SUBUNIT OF cAMP-DEPENDENT PROTEIN KINASE IN RAT TISSUES

Karen M. Boeshans* and John B. Shabb
Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND 58202

INTRODUCTION

cAMP-dependent protein kinase (PKA) is a multifunctional enzyme regulating processes such as signaling, gene expression, and cellular proliferation. A single phosphorylation site was identified *in vivo* at Ser-81 of the type I regulatory subunit (RIα) of PKA by electrospray ionization mass spectrometry (1). The role of phosphorylation is unknown. However, modulation of the phosphorylation state of RIα in response to cAMP modulating agents suggests that the phosphorylation may serve some physiological purpose (2,3,4). Hearts of spontaneously hypertensive rats (SHR) have been shown to have reduced levels of PKAI relative to normotensive rats (WKY) (5). It was hypothesized that if phosphorylation modulated the half-life of RIα, then there may be a difference in the phosphorylation state of RIα from the hearts.

METHODS

Rabbit polyclonal phospho-RIα antibody was generated by established procedures (6). 1 mg of peptide (CGGREDEIS(P)PPPPNPVVK) conjugated to keyhole limpet hemacyanin was injected into a New Zealand white rabbit. The serum IgG was purified by ammonium sulfate precipitation followed by protein A affinity column purification. The anti-RI antibodies recognizing all but the phosphorylation site were depleted by passing the IgG fraction over a thiopropyl Sepharose affinity column conjugated to dephosphorylated peptide (CGGREDEISPPPPNPVVK). Rats were anesthetized with pentobarbital and tissues were immediately excised and frozen in liquid nitrogen. The tissue was powdered and homogenized in 3 ml KPE buffer (10 mM potassium phosphate, *p*I 6.8, containing 1 mM EDTA) containing a protease inhibitor cocktail, 50 mM benzamidine, and 17 nM of the phosphatase PP1 and PP2A inhibitor microcystin. The homogenate was centrifuged at 15,000g_{max} for 15 min and supernatant protein concentration was determined by Bradford protein assay. 120 µg of supernatant proteins were separated by SDS-PAGE and transferred to nitrocellulose membrane. Western blot analysis using antibodies against RIα, and antibodies directed specifically against phospho-Ser-81 were used to determine extent of phosphorylation of RIα in rat heart and nine other tissues.

RESULTS

Western blot analysis showed no difference in phosphorylation state of RIα in extracts from the hearts of SHR and WKY rats. A survey of nine other tissues showed ubiquitous RIα phosphorylation with no change in phosphorylation state between animal models. However, the extent of intact RIα phosphorylation was greatly reduced in the brains of WKY and SHR rats relative to other tissues. A 26 kDa fragment unique to the brain was detected by antibodies against the Ser-81 phosphorylation site. A fragment of similar relative mobility was detected by anti-RIα antibodies in all tissues. This indicates a potential major difference in phosphorylation state of this peptide in the brain.

DISCUSSION

Determining the extent of RIα phosphorylation may be useful in assessing the physiological role of RIα phosphorylation. No difference in steady state phosphorylation was detected between the SHR and WKY rats. This suggests that phosphorylation is not involved in modulation of half-life of RIα in the hypertensive rat. However, steady state phosphorylation levels of intact RIα were lower in rat brain than in any of the other tissues tested. A phosphorylated 26 kDa fragment was also detected. The 26 kDa fragment in brain, which does not bind cAMP, may be related to the N-terminus of RIα. This indicates that phosphorylated RIα may be preferentially subjected to proteolytic cleavage in this tissue. Studies are underway to identify the true identity of the 26 kDa fragment and determine if it is an artifact of purification or a physiologically relevant peptide.

---

1. Boeshans, K.M., Resing, K.A., Hunt, J.B., Ahn, N.G., and Shabb, J.B. (1999) *Protein Science,* 8, 1515-1522.
2. Steinberg, R.A., van Daalen Wetters, T., and Coffino, P. (1978) *Cell,* 15, 1351-1361.
3. Steinberg, R.A., and Agard, D.A. (1981) *J. Biol. Chem.*, 256, 11356-11364.
4. Russell, J.L., and Steinberg, R.A. (1987) *J. Cell. Physiol.*, 130, 207-2113.
5. Prashad, N. (1984) *J. Biol. Chem.*, 260, 10125-10131.
6. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

NOVEL PHOTOAFFINITY LABEL INDICATES SPATIAL RELATIONSHIPS WITHIN THE DOPAMINE TRANSPORTER

Jon D. Gaffaney*[1], Aloke Dutta[2], and Roxanne A. Vaughan[1]
[1]Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND, and
[2]Department of Pharmaceutical Sciences, Wayne State University, Detroit, MI

INTRODUCTION The dopamine transporter (DAT) is a Na^+/Cl^- dependent monoamine transporter found on the presynaptic neuron and is responsible for the uptake of the neurotransmitter, dopamine, after nerve cell stimulation. Psychostimulant drugs such as cocaine, amphetamines, and GBR compounds inhibit the uptake of dopamine out of the synaptic cleft, causing continued stimulation. The use of radioactive analogs to these antagonists has allowed for the mapping of potential binding sites (1). Hydrophobic analysis of the DAT amino acid sequence predicted the presence of twelve transmembrane domains (TMDs), positioning the N- and C-termini intracellularly. [^{125}I]-RTI82, a cocaine analog, has been shown to incorporate within the region around TMDs 4-7. Another compound [^{125}I]-DEEP, a GBR analog, incorporates into the region surrounding the first two TMDs (2). A novel radioactive compound, 4-[2-(diphenylmethoxy) ethyl]-1-benzylpiperdine ([^{125}I]-AD96), has recently shown to be specific for DAT and has been used to map the transporter (3). This photoaffinity label has provided new information pertaining to the binding sites and their spatial relationship.

METHODS Membranes were prepared from male Sprague Dawley rats. The striatum was removed and homogenized in ice cold 0.32M sucrose phosphate buffer (SP). Membranes were centrifuged and resuspended at 30mg/ml original wet weight in SP. The membranes were then incubated with the photoaffinity label [^{125}I]-AD96 for one hour on ice, followed by exposure to UV light for 45 minutes. The labeled membranes were then subjected to trypsin digestion. Antibodies were raised against synthesized peptides corresponding to regions within the DAT protein. DAT and tryptic fragments were isolate by epitope specific immunoprecipitation and separated by SDS-PAGE analysis on a 9-16% gradient gel. Serum 16 (S16) recognizes an epitope on the N-terminal tail and Serum 5 (S5) is specific for a region present on the extracellular side of TMD 4 (2). Pharmacological experiments used inhibitors: (-) cocaine, mazindol, GBR 12909, nomifensin and controls: (+) cocaine, desipramine, and imipramine to test specificity for DAT.

RESULTS Protein specificity was confirmed using pharmacological methods. [^{125}I]-AD96 was unable to label DAT in the presence of the antagonists, (-)cocaine, mazindol, GBR 12909, nomifensine and was able to label in the presence of (+)cocaine, desipramine, and imipramine. The inability to label the protein in the presence of these inhibitors shows the [^{125}I]-AD96 is specific, not only for the protein, but for the site involved in controlling DAT inhibition. Immunoprecipitation of the digested [^{125}I]-AD96 membranes with S16 resulted in the co-migration of 45kD and 14kD fragments representative of the fragments that migrate with digested [^{125}I]-DEEP membranes. Immunoprecipitation of [^{125}I]-AD96 membranes with S5 displayed co-migration of 32kD and 16kD fragments characteristic of [^{125}I]-RTI82 digests. This information suggests that the binding region for the two photoaffinity labels, [^{125}I]-DEEP and [^{125}I]-RTI82, are in close structural proximity to one another. The attached photoaffinity label affected trypsin digest efficiency. Resistance to trypsin followed a trend [^{125}I]-RTI82 > [^{125}I]-AD96 > [^{125}I]-DEEP, suggesting that binding to the ligand within the protein causes a reorganization of the structure, hiding or exposing a potential trypsin cleavage site.

DISCUSSION Sequence homology between Na^+/Cl^- dependent monoamine transporters suggested that the transporters share similar mechanisms of transport and structural motifs. Site-specific mutagenisis has provided answers relevant to residue importance, but structural organization of the transporters is not well defined (4,5). In this study we have given evidence that the binding of a novel DAT photoaffinity label is specific and that it is localized within the antagonist binding site. Tryptic digestion and immunoprecipitation of [^{125}I]-AD96 displayed characteristics of two previously studied labels indicating that the binding sites of these two labels are in close proximity to one another. [^{125}I]-AD96, unlike the other photoaffinity labels, has the ability to bind at either site with equal affinity. This model would place the first two TMDs in close structural proximity to TMDs 4-7 forming a binding pocket within the protein. The binding of different photoaffinity labels caused a change in digest efficiency as described previously. The change in digestion pattern is strong evidence that a conformational change is taking place. Whether the binding of a ligand in the first two TMDs causes an increased susceptibility to trypsin or binding within TMDs 4-7 renders the tryptic site inaccessible is unknown. Either scenario suggests exciting new insights into the function of the transporter and how structure is affected by ligand binding.

1. Vaughan, Roxanne A. 1995. *Molecular Pharmacology.*, 47:956-964.
2. Vaughan, Roxanne A and Michael J. Kuhar. 1996. *Journal of Biological Chemistry*, Vol. 271, 35:21672-21680.
3. Aloke K. Dutta, *et al.* 2000. *Life Science*, (In Review).
4. Nanaan Chen, *et al.* 2000. *Journal of Biological Chemistry*, Vol. 275, 3:1608-1614.
5. Sitting In, *et al.* 1999. *Molecular Pharmacology*, 56:434-447.

SOMATOSTATIN MODULATES THE GROWTH OF SALMONID FISH[1]

Nicole Howe*, Darlene Knutson, Jeff Kittilson, and Mark Sheridan
Department of Zoology, North Dakota State University, Fargo, ND 58105

Somatostatins (SSs) are a family of peptide hormones that have been shown to inhibit pituitary growth hormone (GH) release. Extra-pituitary actions of SSs were suggested by previous studies that showed that growth retardation following premature transfer of salmon to seawater was accompanied by elevated plasma SS levels in association with depressed plasma insulin (INS) and insulin-like growth factor-I (IGF-I) levels (2). In addition, growth retardation of rainbow trout resulting from food deprivation was accompanied by altered plasma SS levels and altered expression of pancreatic mRNAs encoding SSs (3). In this study, we used rainbow trout to study modulation of the GH-IGF-1 axis by SSs.

The extra-pituitary role of SSs on growth was evaluated in two experiments. In the first experiment, rainbow trout were anesthetized and given a single injection (10µl/g body weight) of 0.75 % saline or SS-14 (2×10^{-12} mol/g or 10×10^{-12} mol/g). After 6 hours, the fish were anesthetized, weighed, measured, and blood and selected tissues (*e.g.*, liver, and gill) collected for later analyses. In the second experiment, fish were implanted with osmotic mini-pumps containing either saline or SS-14 (1.4×10^{-9} mol/day). After 15 days, fish were anesthesized, weighed, measured, and sampled as before. Plasma concentrations of SS-14, GH, INS, and IGF-I were determined by radioimmunoassay. Hepatic GH binding was determined by radioreceptor assay and hepatic IGF-I mRNA levels were determined by quantitative slot-blot analysis. A bioassay based on $^{35}SO_4$-sulfate incorporation into gill cartilage also was performed.

Short-term (6h) *in vivo* administration of SS-14 reduced plasma levels of GH, INS, and IGF-I as well as reduced ^{35}S-sulfate incorporation into gill cartilage. Implantation of SS-14 for 15 days resulted in significant growth retardation. For example, relative growth based upon length was 6.31 ± 0.34 % in saline-treated animals and 3.43 ± 0.57 % in SS-14-treated animals. SS-14 implantation reduced hepatic GH binding capacity from 331.5 ± 34.2 fmol/µg protein in the saline-treated animals to 58.4 ± 6.4 fmol/µg protein; however, SS-14 had no significant effect on hepatic GH binding affinity. SS-14 implantation also reduced hepatic IGF-I mRNA expression from 3.2 ± 0.5 x 10^9 molecules IGF-I mRNA/µg total RNA in saline-treated animals to 2.2 ± 0.2 x 10^9 molecules IGF-I mRNA/µg total RNA.

These findings indicate that SS-14 plays a extra-pituitary role in the modulation of growth. SS-14 reduced hepatic sensitivity to GH as well as reduced hepatic production of IGF-1, the proximate mediator of growth in vertebrates. These results provide new insight into growth regulation and suggest a mechanism for nutritional modulation of the GH-IGF-1 axis via SS.

---

1. This research was supported by USDA grant 980-3652 to MAS.
2. Sheridan M.A., Eilertson C.D, and Kerstetter T.H. (1998) Changes in plasma somatostatin associated with seawater adaptation and stunting of coho salmon, *Oncorhynchus kisutch*. *Aquaculture*, 168: 195-203.
3. Ehrman M.M., Moore C. A., Kittilson J.D., Boser J.E., and Sheridan M.A. (1997) Nutritional state affects somatostatin gene expression in rainbow trout. The Endocrine Society 79th Meeting, p 568.

AN ASSESSMENT OF IMPACT OF THE DEVILS LAKE EMERGENCY OUTLET ON THE PERIPHYTON COMMUNITIES OF THE SHEYENNE RIVER

M.A. Jaskowiak*, K.A. Phillips, and M.W. Fawley.
Department of Botany/Biology, North Dakota State University, Fargo, ND 58105

The Sheyenne River is a medium-sized river which flows through central and eastern North Dakota. Devils Lake has been flooding for the past several years. The Army Corps of Engineers has proposed to divert water to the Sheyenne River through the Devils Lake Emergency Outlet.

The objectives of this study were to relate the periphytic communities to physical and chemical parameters using appropriate statistical methods and determine the potential impact of the outlet on the periphyton communities of the Sheyenne River. For three years, periphytic algae and water chemistry samples were collected in the Sheyenne River between Highway 30 near Maddock (upstream from the proposed outlet) to Harwood (near the confluence with the Red River of the North).

Three hundred six periphyton taxa (including 161 diatoms) were identified. These data were analyzed using canonical correspondence analysis (CCA). Environmental parameters which explained the most variance in the periphyton communities were *p*H, orthophosphate, hardness, arsenic, sulfate, and nitrate+nitrite. Previous studies have indicated that levels of sulfate and hardness will increase in the Sheyenne River with the influx of Devils Lake water. Based on this analysis study, these influx of Devils Lake water changes would likely impact the periphyton and phytoplankton communities of the Sheyenne River. For example, the diatoms *Cymbella muellerii* and a *Nitzschia sp.* were primarily found at Warwick during periods of low sulfate concentration. Three diatom species (*Epithemia sorex*, *E. adnata* and *Denticula kuetziganii* were only found at upstream sites (Maddock and Warwick) with low hardness. In contrast, the diatoms, *Cyclotella comensis* and *Nitzschia vermicularis* were positively correlated with high hardness at two downstream sites, Lisbon and Harwood. Based on this analysis, the influx of Devils Lake water would likely impact the periphyton and phytoplankton communities of the Sheyenne River.

MOLECULAR MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN TETRAPLOID WHEAT

C.D. Otto*, S.F. Kianian, E.M. Elias, R.W. Stack, and L.R. Joppa
Department of Plant Sciences and Plant Pathology,
North Dakota State University and USDA-ARS, Fargo, North Dakota

Fusarium head blight, more commonly known as scab, is a fungal disease of small-grain crops that causes yield loss and poor grain quality. The extensive damage caused by *Fusarium* head blight (FHB) has made it necessary to develop resistant lines of durum (AABB; 2n=4x=28) and hexaploid (AABBDD; 2n=6x=42) wheat. Unlike hexaploid wheat, there are no usable sources of resistance in domesticated tetraploid cultivars. One species that shows promise as a source for FHB resistance is an accession of *Triticum turgidum* L. var. *dicoccoides* (AABB, 2n=4x=28). Langdon-dicoccoides disomic substitution lines [LDN(Dic)] were analyzed for Type II resistance. The Langdon durum line with a pair of chromosomes from an accession of *Triticum turgidum* L. var. *dicoccoides* [LDN(Dic-3A)] was shown to have reduced infection from FHB relative to the parents. A Recombinant Inbred Chromosome Line (RICL) population of 83 individuals derived from LDN (Dic-3A) has been analyzed over multiple seasons. Phenotypic screening data, molecular marker mapping data (RFLP, AFLP, RGA, and microsatellite), and QTL analysis results have delineated the location of FHB resistance loci on the 3A chromosome. At the present time, 14 markers have been placed on the molecular map of chromosome 3A covering 133.9 cM. Quantitative trait analysis suggests 31% of the phenotypic variance is explained by the locus *Xgwm2* (V_g/V_p=31%). Characterization of the FHB resistance QTL on chromosome 3A will benefit durum, as well as hexaploid wheat breeding, since none of the recently documented major QTL loci are on this chromosome.

LOCALIZING SITE OF PROTEIN KINASE C STIMULATED PHOSPHORYLATION ON DOPAMINE TRANSPORTER

Benchaporn Pananusorn* and Roxanne Vaughan
Department of Biochemistry & Molecular Biology, University of North Dakota, Grand Forks, ND 58203

INTRODUCTION

The dopamine transporter (DAT) is predominantly found in striatum and is responsible for dopamine clearance in the synapse. The presence of multiple potential phosphorylation sites on DAT sequence suggests that its uptake regulation might associate with phosphorylation. Previous studies showed that PMA, a PKC activator, stimulated phosphorylation and reduced dopamine uptake by 35%. Plus, the phosphoamino analysis indicated that serine was the primary type of residues being phosphorylated. Knowing that there are 14 serine residues scattered throughout the dopamine transporter sequence, the localization of phosphorylation site is possible through proteolysis of $^{32}PO_4$-labeled DAT and immunoprecipitation with epitope-specific antibodies. Since DAT is known to contribute to addictive behavior of cocaine users where cocaine binding decreases dopamine reuptake, the attempt to locate phosphorylation site is essential for drug abuse study. Hopefully, this will give insights to its function and regulation and eventually lead to treatments.

METHODS

The striata were obtained from Male Sprague Dawley rats and sliced into 350-μm slices. The slices were incubated in Krebs bicarbonate buffer (1.5 mM $MgSO_4$, 15 μM $CaCl_2$, 4 mM KCl, 25mM Na_2HCO_3, 124 mM NaCl, 10 mM glucose, *p*H 7.3) saturated with 95% O_2/5% CO_2 containing $^{32}PO_4$ (1 mCi/ml) for 2½ hours at 30°C. PMA (10 μM) and/or OA (10 μM), a phosphatase inhibitor, were added and incubated for another 30 min at 30°C. The $^{32}PO_4$-labeled sample was then subjected to proteolytic digestion with endoproteinase Asp-N, which cleaves at aspartic acid residues, or aminopeptidase I, which nonspecifically removes amino acid residues inwards from N-terminus, for 1 hour at room temperature and followed by immunoprecipitation. The precipitation of digested fragments was done with epitope-specific antibodies, 15 (amino acids 6-30) and 16 (amino acids 42-59), directing against N-terminal domain of DAT. SDS-PAGE was used to recover the immunoprecipitated fragments for the purpose of autoradiograph generation.

RESULTS

The treatment of striatal slices with PMA and OA increased $^{32}PO_4$-labeled DAT up to 4 fold when compared to untreated striatal slices. Next, the endoproteinase Asp-N treatment of $^{32}PO_4$-labeled sample generated ~21-kDa fragments that precipitated with both antibody 15 and 16. The fragments were then proven to be DAT with the peptide block, where peptide 16 effectively blocked antibody 16 from precipitating ~21-kDa fragments. When the $^{32}PO_4$-labeled sample was treated with aminopeptidase I, $^{32}PO_4$ was released from the protein and appeared at the gel dye front. The result demonstrated that the serines towards the end of the N terminus were being phosphorylated. And, the immunoprecipitation of aminopeptidase I treated $^{32}PO_4$-labeled sample helped to specify that the serines on the extreme end of the N-terminus were primarily being phosphorylated. This was due to noticeably little or none of $^{32}PO_4$-labeled DAT was being recovered with antibody 15 and some of $^{32}PO_4$-labeled DAT were being recovered with antibody 16.

DISCUSSION

Because antibody 15 and 16 are specifically directed against N-terminal domain, the immunoprecipitation of ~21 kDa fragments from endoproteinase Asp-N treated $^{32}PO_4$-labeled sample indicates that the major site for basal and stimulated phosphorylation is on the N-terminal domain. Peptide block then verifies that ~21-kDa fragments are DAT, seeing that it successfully prevents precipitation with antibody 16. Next, amino-peptidase I was used to further pinpoint which of the 8 serines on N-terminal domain is the one being phosphorylated. As the concentration of aminopeptidase I increases, the antibody 15 shows less ability to recover phosphorylated fragments, indicating that phosphorylation site is localized exclusively on N-terminal domain and near the extreme end of the N-terminus.

---

1. Huff, R.A., Vaughan, R.A., Kuhar, M.J., and Uhl, G.R. (1997) *Journal of Neurochemistry*, 68, 225.
2. Vaughan, R.A., Huff, R.A., Uhl, G.R., and Kuhar, M.J. (1997) *Journal of Biological Chemistry*, 272, 15541.

FINE STRUCTURE MAPPING OF THE SPECIES CYTOPLASMIC SPECIFIC GENE

K.J. Simons*, S.F. Kianian, S.S. Maan
Department of Plant Sciences, North Dakota State University, Fargo

Nuclear-cytoplasmic interactions play a key role in growth and fertility of new and novel wheat cultivars and commercial hybrids. Typically, cytoplasmic male sterility in hybrids has been corrected using *Rf* (restoration of fertility) genes. Not all systems respond to *Rf* genes. Another system consisting of two genes, *scs* (species cytoplasmic specific) and *Vi* (vigor and vitality), works in situations where *Rf* genes do not. The action of these two genes can be observed when a durum [*Triticum turgidum* ($2n = 4x = 28$; AABB)] nucleus is placed in *T. longissima* ($1n = 2x = 14$; s^ls^l) cytoplasm. This alloplasmic combination, (lo) durum, results in non-viable progeny. The *scs* gene alone in (lo) durum produces plump seeds with *scs* which will result in male sterile plants. The *Vi* alone in (lo) durum produces plump seeds with *Vi* which will result in weak but fertile plants. The two genes together in (lo) durum produce a normal plant. It is believed that these genes, when present in euplasmic durum, may increase fertility beyond normal control. A fine structure map of the region surrounding the *scs* gene will be created using various molecular markers. The mapping population is the F_2 generation of a cross between 'Langdon' durum intervarietal chromosome substitution line [*T. dicoccoides* (AABB) chromosome 1A substitution] and a durum line homozygous for *scs*. The *scs* genotype is determined by test crossing. Fine structure mapping is needed for positional cloning of the *scs* gene leading to the detailed characterization of nuclear-cytoplasmic interaction in wheat.

LOCALIZATION OF GAP JUNCTIONAL PROTEIN CONNEXIN (Cx) 43 DURING MATURATION OF THE CUMULUS OOCYTE COMPLEX (COC) OF EWES

Tande K. Stenbak*, Lawrence P. Reynolds, Jerzy J. Bilski, Dale A. Redmer, and Anna T. Grazul-Bilska
Department of Animal and Range Sciences, North Dakota State University, Fargo, North Dakota 58105

INTRODUCTION

Gap junctions are specialized membrane channels that allow cell-to-cell communication between neighboring cells. Gap junctions are formed by one or more types of connexin proteins (2). Connexin proteins, including Cx43, are present in the ovaries of all mammalian species examined to-date and play an important role in coordinating cellular function during growth and development in all tissues (2). Gap junctions also play an important role in oocyte growth and maturation, and Cx43 seems to be a major gap junctional protein in the COC (1).

METHODS

COC were collected from small, growing (1-3 mm; n=12) and large, preovulatory (>5 mm; n=17) ovarian follicles of non-pregnant ewes (n=10). The COC were embedded in agarose gel and fixed in Carnoy's solution immediately after collection, or were incubated in maturation medium (TCM-199 containing 10% FBS, 5μg/ml FSH, 5μg/ml LH, and 1μg/ml estradiol) for 24 h before embedding and fixing. Cx43 was immunolocalized in histological sections (6 μm) of each COC, followed by nuclear fast red to stain cumulus cell nuclei. The area of positive staining for Cx43, the total nuclear area, and the average nuclear area was determined for each COC using computerized image analysis. Cx43 data are expressed as the area of positive staining (μm^2) per cumulus cell.

RESULTS

After incubation in maturation medium, the cumulus cells were expanded in all COC collected from small follicles. For large follicles, the cumulus cells were expanded only in a portion (22%) of the COC. There were no differences between small and large follicles in the area of positive staining for Cx43. For incubated COC in which the cumulus did not expand (*i.e.* non-matured COC, which were observed for large follicles only), the area of positive Cx43 staining per cumulus cell was greater ($0.4 \pm 0.2\ \mu m^2$; $P < 0.05$) than in non-incubated COC or in incubated COC with expanded cumulus ($0.08 \pm 0.03\ \mu m^2$ and $0.11 \pm 0.05\ \mu m^2$; from small and large follicles, which were similar). Size of individual nuclei was greatest ($P < 0.05$) in incubated COC with non-expanded cumulus ($35.5 \pm 1.8\ \mu m^2$), compared with incubated COC with expanded cumulus ($31 \pm 0.8\ \mu m^2$), and least ($P < 0.05$) in non-incubated COC ($27 \pm 0.8\ \mu m^2$).

CONCLUSION

The present data demonstrate that the expression of Cx43 is lower in expanded COC, which suggests that gap junction-mediated communication between cumulus cells and oocytes is diminishing as oocytes reach maturation stage. It has been shown that in the process of oocyte maturation, meiotic resumption is associated with the loss of gap junctions between the oocyte and the granulosa cells (3). However, data concerning the presence or function of gap junctions during oocyte growth and maturation in the COC are very limited at present. In summary, these data indicate that gap junctions are indeed present in the expanded matured COC, and may be necessary for processes, including fertilization, that occur in the oocyte once it has been transported to the oviduct.

Supported in part by NIH grant R29 HD30348 to ATGB and NRI CGP/USDA grant 98-35203-6222 to DAR & LPR.

---

1. Eppig, J.J., Chesnal, F., Hirao, Y., O'Brien, M.J., Pendola, F.L., Watanabe, S., and Wigglesworth, K. 1997. Oocyte control of granulosa cell development: how and why. *Hum. Reprod.*, 12:127-132.
2. Grazul-Bilska, A.T., Reynolds, L.P., and D.A. Redmer. 1997. Gap junctions in the ovaries. *Biol. Reprod.*, 57: 947-957.
3. Senger, P.L. 1999. Pathways to Pregnancy and Parturition. The Mack Printing Group-Science Press, Ephrata, PA.

LEPTIN ATTENUATES CARDIAC CONTRACTION IN RAT VENTRICULAR MYOCYTES

Loren E. Wold*, Peter B. Colligan and Jun Ren
Department of Pharmacology, Physiology, and Therapeutics,
University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND 58202

Obesity is associated with an increased incidence of cardiovascular diseases such as hypertension, stroke, and congestive heart failure[1]. Although it appears that cardiac function may be normal or enhanced in the early stage of obesity[2], cardiac hypertrophy and compromised ventricular function develop as a result of the increased cardiac pressure and/or volume overload. There has been indication that leptin, the product of the human *ob* gene[3], may play a role in obesity-related metabolic and cardiovascular dysfunctions. The purpose of this study was to determine if leptin exerts any direct cardiac contractile action that may contribute to altered myocardial function.

Ventricular myocytes were isolated from adult male Sprague-Dawley rats and contractile properties were analyzed using video-based edge-detection. Contractile properties analyzed in cells electrically stimulated at 0.5 Hz included: peak shortening (PS), time-to-90% PS (TPS), time-to-90% relengthening (TR_{90}) and fluorescence intensity change (ΔFFI).

Leptin exhibited a dose-dependent inhibition in myocyte shortening and intracellular Ca^{2+} change, with maximal inhibitions of 22.4 and 24.2%, respectively. Leptin also stimulated nitric oxide (NO) synthase activity, as reflected in the dose-related increase in NO accumulation in these cells. Addition of an NO donor (S-nitroso-N-penicillamine) to the medium mimicked the effects of leptin administration.

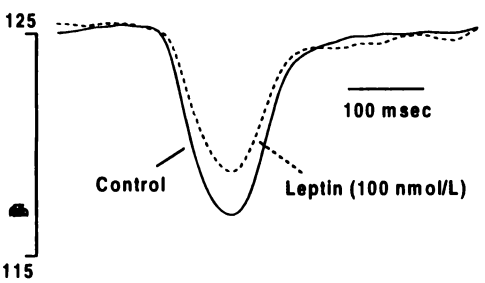


Figure 1. Effect of leptin (100 nM) on cardiomyocyte contractile performance.

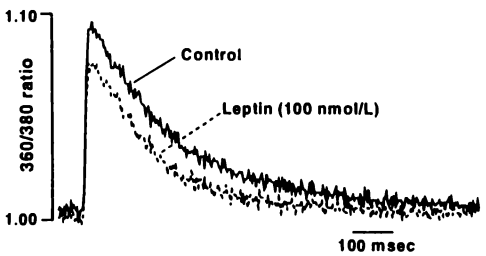


Figure 2. Effect of leptin (100 nM) on intracellular Ca2+ transients using fura-2AM.

In summary, this study demonstrated a direct action of leptin on cardiomyocyte contraction, possibly through increased NO production. These data suggest that leptin may play a role in obesity-related cardiac contractile dysfunction.

---

1. Laitinen J. Obesity is a health problem. 1998 *Int. J. Circumpolar Health,* 57:104-108.
2. Paulson DJ, Tahiliani AG. Cardiovascular abnormalities associated with human and rodent obesity. 1992 *Life Sci.*, 51:1557-1569.
3. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. 1994 *Nature*, 372:425-432.

This work was supported by grants from the AHA-Northland, ND EPSCoR and the University of North Dakota School of Medicine Research Committee.

COMMUNICATIONS

PROFESSIONAL

NDAS SENIOR ACADEMY PROGRAM Room 216

Moderator: Jon Jackson, University of North Dakota.

8:00 "The effect of caffeic acid on root cell membrane potentials in leafy spurge (*Euphorbia esula* L.)." Richard R. Barkosky*, Christopher P. Keller, Minot State University.

8:20 "The role of apically procuded auxin in leaf development in bean (*Phaseolus vulgaris*) plants." Deanna Brodeur and Christopher P. Keller*, Minot State University.

8:40 "Characterization of photoaffinity labeled proleolytic fragments from cloned human dopamine transporter." Margaret Jean Lowe* and Roxanne A. Vaughan, University of North Dakota.

9:00 "Predicted circular dichroic spectra of cyclo(Pro-Gly)3 by the dipole interaction model using revised parameters." Stephen L. Lowe*, Nathan Kadrmas, Tamara Baumgartner, and Kathyrn A. Thomasson, University of North Dakota.

9:20 "A probability problem in drug delivery." M.B. Rao, North Dakota State University.

THE EFFECT OF CAFFEIC ACID ON ROOT CELL MEMBRANE POTENTIALS IN LEAFY SPURGE (*EUPHORBIA ESULA L.*)

Richard R. Barkosky* and Christopher P. Keller
Department of Biology, Minot State University, Minot, North Dakota, 58707

INTRODUCTION

Allelopathy (allelon for 'of each other' and pathos for 'to suffer') is a term that describes the process by which certain compounds (allelochemicals) are released into the environment by one plant species to either positively or negatively impact another plant species. Mechanisms of allelopathic responses in plants can be considered in terms of a sequence of events that lead to disruption of plant growth and development (1). One hypothesis is that exposure to certain allelochemicals depolarize some plant root cell membranes (2). This in turn could induce changes in plant water relationships which ultimately lead to a negative impact on plant growth (3). In this scheme, various interactions between two or more primary and/or secondary physiological events are steps within a signal transduction pathway. It is important to note that this model is limited to just two physiological interactions and the exact nature of physiological integration of the constituents along the pathway is likely more complex. Leafy spurge (*Euphorbia esula L.*), is a noxious weed introduced from Eurasia around the turn of the century. A member of the *Euphorbiaceae*, leafy spurge disrupts both natural and agroecosystems across much of the Northern Great Plains. It has been demonstrated in field and pot studies that the native plant small everlasting (*Antennaria microphylla Rydb.*) is allelopathic to leafy spurge (4). Caffeic acid (CA) is one of three compounds isolated from small everlasting that have been shown experimentally to inhibit leafy spurge seed germination and root elongation (5). Barkosky and Einhellig (6) have shown that 0.25 mM CA significantly ($p < 0.05$) reduced chlorophyll fluorescence and transpiration rates in leafy spurge after chronic long-term exposure. This current experiment was designed as a preliminary test of the hypothesis that CA inhibits the growth of leafy spurge by interfering with normal root membrane function.

METHODS AND MATERIALS

For these experiments, leafy spurge cuttings were propagated in 0.5 Hoagland's nutrient solution and treated with either 0.1 mM or 0.25 mM CA for seven days. Membrane potential was measured using root segments separated from treated and control plants using a conventional microelectrode.

RESULTS

Preliminary results of our current research into the effect of these concentrations of CA on membrane potential, indicate a significant ($p < 0.05$) reduction in leafy spurge root cell membrane potential. While the mean membrane potential of control root cells was -161.3 +/- 6.0 SE mV, 0.25 mM CA treated roots had a mean membrane potential of -105.7 +/- 2.3 SE mV.

DISCUSSION

This preliminary experiment clearly demonstrates that CA can induce changes in the membrane potential of leafy spurge root cell membranes. More specific hypotheses will be tested in order to establish the nature of changes in ion flux and to determine whether normal root activities such as mineral ion uptake are affected by CA.

---

1. Einhellig, F.A. (1995) ACS Symposium Series 582; American Chemical Society Washington, DC, pp 171-188.
2. Glass, A.D.M. (1973) *Plant Physiology*, 51,1037.
3. Barkosky, R.R., Butler, J.L., and Einhellig, F.A. (1999) *J. Chem. Ecol.*, 25, 1611.
4. Selleck, G.W. (1972) *Weed Sci.*, 20, 189.
5. Manners, G.D. and Galitz, D.S. (1985) *Weed Sci.*, 34, 8.
6. Barkosky, R.R. and Einhellig, F.A. (in review), *J. Chem. Ecol.*

LARVAL LIFE HISTORY OF THE BLACK-BELLIED SALAMANDER, *DESMOGNATHUS QUADRAMACULATUS*: EFFECTS OF TEMPERATURE AND THYROID HORMONE ON METAMORPHIC TIMING

Christopher K. Beachy* and Cari-Ann Hein
Department of Biology, Minot State University, Minot, ND 58707

For amphibian species that have a larval stage, metamorphosis is a critical event that can affect the time and size of the animal at first reproduction, *e.g.*, an animal that is large at metamorphosis can reach maturity faster and at a larger size than conspecifics that metamorphose at smaller size. Thus, metamorphosis is a developmental event that is subject to direct natural selection. In most species of amphibian, metamorphosis can be affected by environmental variation, *e.g.*, food, predator threat and temperature. However, the stream-dwelling salamanders of the family Plethodontidae use larval habitats that are less variable in both food and temperature regimes than the extremely ephemeral and high productivity pools and ponds where the typical larval amphibian may be found. These species are found in eastern North America in association with the upland streams of Appalachia. Larval periods are extremely long (8-60 months), and seemingly invariant to resource variation (1, 2). These species also exhibit population differences in duration of the larval period, and these differences can become manifest in population differences in adult body size (3).

Because the plethodontids exhibit geographic variation in metamorphic timing, it is of interest to elucidate the determinants of this variation. Two possible sources of population differences in metamorphic timing are stream temperatures (an environmental effect) and timing of thyroid activity (an endocrine effect). We manipulated temperature and thyroxine in 2x2 factorial experiment, thereby testing the hypotheses that geographic variation in metamorphic timing is due to geographic variation in (a) stream temperatures and (b) timing of thyroxine production, and/or an interaction between these two effects.

Larval *Desmognathus quadramaculatus* were collected from Shelton Laurel Creek in the Bald Mountains, North Carolina in October 1998 and transported to Minot, North Dakota. On 13 November, 32 first-year larvae were weighed to the nearest mg, and placed individually in plastic boxes (8x3x4 cm LWH) with 110 g of sand, 100 g of gravel and 250 ml distilled water. One-half of the animals were placed in one environmental chamber at 7°C, and the rest were placed in another environmental chamber at 11°C. Animals were fed brine shrimp nauplii and *Tubifex* worms *ad libitum*. On 9 April 1999, one-half of the animals were immersed in a 1.2×10^{-9} M thyroxine solution. This thyroxine treatment was administered weekly until the termination of the experiment. In addition, the concentration of the thyroxine solution was doubled every second week, thus bringing the final thyroxine supplements to a 4.8×10^{-9} M solution.

We weighed the animals every 30 days, and also upon termination of the experiment on 10 June 1999, when each animal was also scored for metamorphic progress: a '1' was given for animals that had not initiated metamorphosis, a '4' for animals that had completed metamorphosis, and '2' and '3' were given for intermediate stages (2). Data were analyzed using a two-way multivariate analysis of variance (MANOVA) with two levels of each factor (high vs. low temperature, thryoxine supplement vs. control supplement). Variables analyzed were mass and metamorphic score at termination of the experiment. Initial mass of each animal was included as a covariate in the MANOVA.

MANOVA indicated a significant effect of temperature on the mass/score response vector (Wilks' $\lambda = 0.21$, df = 2, 17, $P < 0.001$); other than initial mass, no other source in the analysis provided significant effects on metamorphic mass or score. Temperature had a significant effect on both metamorphic mass ($F = 41.40$, df = 1, 18, $P < 0.001$) and metamorphic score ($F = 35.11$, df = 1, 18, $P < 0.001$); animals at low temperatures grew more slowly and few animals in low temperature treatments showed any metamorphic progress.

The most remarkable result was that there was no effect of thyroxine on metamorphic score. In every study previous to this one, application of thyroxine to a larval species caused abrupt metamorphosis (4). However, the action of thyroxine on neotenic salamander species (*i.e.*, those that do not undergo metamorphosis and reproduce as gilled animals) has varied impacts, ranging from no effect to complete metamorphosis. In general, the variable effects of thyroxine on these neotenic salamanders result from a lack of cell-surface receptors for thyroxine. We hypothesize that larval *D. quadramaculatus* that are only in first or second year of larval life lack these receptors and that geographic variation in metamorphic timing is due to population differences in receptor synthesis.

1. Beachy, C. K. 1995. *Journal of Herpetology*, 29:375-382.
2. Beachy, C. K. 1997. *Copeia*, 1997:131-137.
3. Bruce, R. C. 1988. *Herpetologica*, 44:144-155.
4. Rosenkilde, P., and A. P. Ussing. 1996. *International Journal of Developmental Biology*, 40:665-673.

THE ROLE OF APICALLY PRODUCED AUXIN IN LEAF DEVELOPMENT IN BEAN (*PHASEOLUS VULGARIS*) PLANTS.

Deanna M. Brodeur and Christopher P. Keller*
Department of Biology, Minot State University, Minot, North Dakota 58707

INTRODUCTION

The presence of a growing shoot apex in many plants exerts an inhibitory control over the proximal lateral buds of the plant. Thus, removing the apex (*i.e.*, "pinching" or decapitating) of a tomato plant stimulates lateral bud growth and results in a bushier plant. Decapitation and hormone manipulation experiments have shown growth control of the lower buds by the shoot apex is due to the plant hormone auxin (indole acetic acid) which is produced primarily in the youngest leaves of the apical bud (1). Though important in lateral branch development, auxin was long thought to have only a limited role in the development of leaves (2). Recent work, however, has established that excised tobacco leaf strips have a substantial growth response treated with auxin (3), though the growth does not involve the so-called Acid-Growth Mechanism found in the auxin growth response of stem tissues (4). Others have since shown that a putative auxin receptor is involved in this auxin growth response by tobacco leaf tissue (5).

What has not been established is whether auxin produced by the shoot apex controls the development of leaves in the intact plant. Others have shown that decapitation of bean plants (*Phaseolus vulgaris*), besides stimulating lateral bud development, enhances the growth of the primary leaves (6). The objective of the project described here is to test the hypothesis that decapitation-induced primary leaf growth in *P. vulgaris* results from the removal of the apical source of inhibitory auxin.

METHODS

Phaseolus vulgaris var. Pinto were grown in moist vermiculite under greenhouse conditions. The plants were watered every 2-3 days and were treated with full strength Miracle-Gro weekly. On day 10, plants with monofoliate leaf blades measuring between 32 and 55 mm in length and with the apical bud 1-2 cm above the monofoliate leaf node were decapitated just below the apical bud. A lanolin paste (+/- 1500 ppm naphthalene acetic acid [NAA; a well characterized auxin analog]) was then applied to the stump. An initial blade length was measured for both monofoliate leaves of each plant. Blade length was then remeasured each day for 10 more days. Leaf length values were normalized to the initial length and expressed as a percent of initial length.

RESULTS AND DISCUSSION

The primary leaves of plants in which the shoot apex was replaced by a lanolin paste containing NAA grew more slowly and reached a smaller final size than those treated with lanolin paste alone. For plants treated both with and without auxin primary leaf growth was complete 7 days following decapitation. Seven days following decapitation the leaves of the control plants had increased in length 97.4 +/- 5.3% of initial length (n = 16), while the leaves of the NAA treated plants had lengthened 64.8 +/- 4.6% of initial length. This experiment was repeated twice with similar results.

The results support the hypothesis that leaf growth is inhibited in intact plants by apically derived auxin. Further planned experiments will be described.

---

1. Cline, MG. (1991) *Bot. Rev.*, 57, 318
2. Went, FW. (1951) *In* F. Skoog, ed, Plant Growth Substances. Univ. Wisconsin, Madison, pp. 287-298.
3. Keller C. P. and Van Volkenburgh, E (1997) *Plant Physiol.*, 113, 603.
4. Keller C. P. and Van Volkenburgh, E (1998) *Plant Physiol.*, 118, 557.
5. Jones, A.M., *et al.* (1998) *Science*, 282, 1114.
6. Van Staden, J. and Carmi, A. (1982) *Physiol. Plant.*, 55, 39.

EFFECTS OF FOOD AND TEMPERATURE ON METAMORPHIC TIMING OF THE BLACK-BELLIED SALAMANDER, *DESMOGNATHUS QUADRAMACULATUS* (CAUDATA, PLETHODONTIDAE)

Cari-Ann Hein* and Evan Barker
Department of Biology, Minot State University, Minot, ND 58707

INTRODUCTION Most species of amphibians have a life cycle that features metamorphosis, the morphological transition from the aquatic larval stage to a more terrestrial adult. Wilbur and Collins (1) proposed a model that predicts that growth rate can influence metamorphic timing. So long as a minimal size threshold has been crossed, metamorphosis should be initiated when the larva's growth rate drops. This decrease in growth rate is thought to represent a cue of a deteriorating habitat (i.e., desiccation, predator threat). If growth rate remains high, the animal should delay metamorphosis in order to take advantage of the growth opportunity. The Wilbur-Collins model was derived from observations of species that use high productivity, highly ephemeral ponds.

While most species of amphibians use these transient, high growth environments for larval development, the salamanders of the family Plethodontidae live in permanent, cool low productivity streams in the southern Appalachian Mountains of eastern North America. These species have long larval periods (2) and it is unknown whether these larval salamanders vary metamorphic timing in response to variation in growth rate. We tested the hypothesis that variation in growth rate would result in variation in metamorphic timing of larval *Desmognathus quadramaculatus*, a plethodontid with a larval period of two to four years.

MATERIALS AND METHODS We tested the effects of two levels of food and temperature (using a multifactoral design) on larval growth rates, metamorphic size and metamorphic timing. On 13 November 1998 we used a random numbers table to assign each larvae to one of four treatment groups. There were 7 salamanders in the first group and 8 in the remaining 3 groups. Each group was assigned one of four treatments with the first group being high temperature/high food; the second high temperature/low food; the third low temperature/high food; and the fourth low temperature/low food. The treatments included exposure to 7° C or 11° C with feeding of four *Tubifex* worms once each week (the high food groups) or once every other week (the low food groups). Beginning 1 April 1999, temperatures in the environmental chambers were raised to simulate typical rising summer water temperatures. The temperatures were raised to 11° C and 14° C respectively. The temperatures were raised 1° C every two days and were stabilized on 9 April 1999.

Water (distilled) was replaced each week. All animals were weighed at the beginning of the experiment and every 30 days thereafter. The salamanders were also weighed upon completion of metamorphosis. A two-factor multivariate analysis of covariance (MANCOVA) was used to evaluate the effects of temperature and growth on metamorphic date and metamorphic mass. Initial mass was used as a covariate in order to compensate for individual variation at the beginning of the experiment. Subsequent analysis of covariance (ANCOVA) on metamorphic date and mass was performed only when MANCOVA indicated a significant treatment effect. In all analyses the significance criterion was set as $p = 0.05$, and Wilks' lambda was chosen as the test statistic. The MANCOVA was used to compare the treatment effects (food, temperature, and food * temperature) and initial mass on metamorphic date and metamorphic mass. The experiment was terminated on day 283, which was 13 August 1999.

RESULTS AND DISCUSSION MANCOVA indicated a significant effect of two of the three factors and the covariate. The three factors indicating a significant result were initial mass (the covariate), food, and temperature. The food * temperature interaction was not significant. Food treatments were not found to significantly affect the metamorphic date. Salamanders in both food treatment groups, exposed to the same temperatures, metamorphosed at the same time. This is inconsistent with the Wilbur and Collins' hypothesis, which predicts that variation in growth rate should result in variation in metamorphic timing.

The effect of temperature on metamorphic timing was significant. The metamorphic date was delayed in the low temperature groups. Similar results have been seen in previous studies and are consistent with earlier observations of effects of temperature on metamorphic timing on plethodontid larvae (3). Initial mass, the covariate, had a significant impact on the metamorphic date and mass. Salamanders with lower initial body mass had longer larval periods and metamorphosed at lower body masses. The results indicate metamorphic timing in *D. quadramaculatus* is closely tied to stream temperatures, and is not related to food regime.

---

1. Wilbur, H. M., and J. P. Collins. 1973. *Science*, 182:1305-1314.
2. Beachy, C. K., and R. C. Bruce. 1992. *American Naturalist*, 139:839-847.
3. Beachy, C. K. 1995. *Journal of Herpetology*, 29:375-382.

DIETARY BORON AND ERYTHRITOL AFFECT REPRODUCTION AND FETAL DEVELOPMENT IN RATS

Kay A. Keehr* and Curtiss D. Hunt
USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202-9034

Previous research indicates that physiologic amounts of dietary boron (B) affect reproductive function and embryonic development in frogs (1), fish (2), and mice (3). For example, frog and fish embryos, in the early stages of development, exhibited increased necrosis and delayed growth when parents were fed a low boron diet. The objective of this experiment was to characterize the role of dietary boron in reproduction and development in rats.

In a 2 x 2 factorially-arranged experiment, weanling Sprague-Dawley female rats (13/treatment) were fed a boron-low basal diet (~0.1 mg B/kg) supplemented with boron (as orthoboric acid) at 0 (0B) and 2 (2B) mg/kg, and l-erythritol (E), a boron-binding biomolecule, at 0 (0E) or 5 (5E) mg/kg diet for a erythritol:boron molar ratio of 4 in the OB/5E group. Dietary treatment was maintained for eight weeks prior to and during gestation. The females were bred with similarly fed males (5/treatment), for a 48 hour maximum mating time per individual breeding pair. On gestation day 19, fetuses were dissected from the uterus, weighed, eviscerated, and then fixed in formalin; subsequently all fetuses were skinned, cleared with glycerol, and stained with Alcian blue and Alizarin red for visualization of skeletons (4). All fetuses were examined for gross morphological and skeletal defects. The cervical, thoracic, lumbar, and sacral vertebrae were scored for degree of ossification and morphological defects.

The overall pregnancy rate was rather low (45%) but highest in the 2B/0E group (62%) and lowest in the 2B/5E group (31%). Physiologic amounts of boron decreased the number of dams with fetal resorptions (FR) in the absence of erythritol (See Table 1), however, boron increased these numbers in the presence of erythritol ($p < 0.005$). The average number of implantation sites tended to be higher in the boron adequate group (data not shown). Dietary erythritol affected rib development as evidenced by increased incidence of discontinuous rib XI of pups from dams fed supplemental erythritol. Fetal thoracic vertebral morphologic maturation in litters fed physiologic amounts of boron was similar to that observed in commercial chow (~12 mg B/kg) fed rats (in a parallel study). However, vertebral maturation ($p < 0.02$) was accelerated in fetuses from dams fed low amounts of boron as indicated by fewer dumbbell-shaped vertebrae, an earlier stage of vertebral development. Neither boron nor erythritol affected fetal body weight.

Table 1. Effects of Dietary Boron, Erythritol, and Their Interaction on Reproduction and Fetal Skeletal Development

Dietary Treatment (mg/kg Diet)		Number of dams	Fetal Resorptions (% of dams with at least one resorption)	Discontinuous Rib XI (Probability of a Positive Response)	Dumbbell-Shaped Vertebra (Probability of a Positive Response)
B	E	(n)			
0	0	5	60.0 (0.20-0.90)*	0.171 (0.10-0.28)	0.062 (0.02-0.16)
2	0	8	37.5 (0.13-0.72)	0.183 (0.07-0.41)	0.190 (0.09-0.37)
0	5	6	16.7 (0.02-0.63)	0.349 (0.25-0.46)	0.044 (0.02-0.10)
2	5	4	100.0 (1.00-1.00)	0.368 (0.24-0.52)	0.141 (0.08-0.25)
- - - - - - - - - - - - - - - - -Logistic Regression Analysis - P Values -					
Boron			NS	NS	0.020
Erythritol			NS	0.020	NS
Boron x Erythritol			0.005	-	-

*Numbers in parenthesis reflect logistic regression analysis confidence intervals.

Because interrupted ossification of rib XI is typically considered a developmental defect (5), the influence of erythritol on normal skeletal development should be examined further. The findings also suggest a reproductive role for boron that is sometimes influenced by dietary erythritol. Further research is needed to determine whether or not accelerated rates of vertebral ossification during boron deprivation have deleterious consequences on postnatal vertebral structure and function.

1. Fort, D.J., *et al.* (1999) *J. Nutr.*, 129, pp 2055-2060.
2. Rowe, R.I. and C.D. Eckhert (1999) *J. Exp. Biol.*, 202, pp 1649-1654.
3. Lanoue, L., *et al.* (1998) *Biol Trace Elem. Res.*, 66, pp 271-298.
4. Newman, S.M., *et al.* (1983) *Trans. Am. Microsc. Soc.*, 102, pp 300-305.
5. Wise, L.D., *et al.* (1997) *Teratology*, 55, pp 249-292.

CHARACTERIZATION OF PHOTOAFFINITY LABELED PROTEOLYTIC FRAGMENTS FROM CLONED HUMAN DOPAMINE TRANSPORTER

Margaret Jean Lowe* and Roxanne A. Vaughan
Department of Biochemistry and Molecular Biology, University of North Dakota, Grand Forks, ND 58202

INTRODUCTION

The dopamine transporter is a transmembrane protein which removes dopamine from the synapse into the nerve cell after excitation. Inhibition of this transport is considered to be the major physiological action of cocaine. To understand the molecular basis of cocaine inhibition, knowledge of the structure of the dopamine transporter is needed. The amino acid sequence of the transporter is known and the sequence puts it in the family of monoamine transporters, all believed to consist of twelve transmembrane helices. How the helices are arranged and the residues involved in binding and transport are still uncertain. Previous work has implicated transmembrane domains 4-7 as a potential binding site for the cocaine analogue RTI-82 (1). The goal of this research is to locate the attachment site of the photoaffinity label, [^{125}I]-RTI-82, for the dopamine transporter. We are investigating protease digestion of photoaffinity-labeled dopamine transporter to produce labeled small peptides. Subjecting such small peptides to mass spectroscopy should determine the precise point of bonding of the label and thereby provide information on the binding site.

METHODS

HEK293 cells transfected with human dopamine transporter (hDAT) were obtained from Dr. J. Justice of Emory University. Cells were broken with a polytron apparatus and cell membranes collected by centrifugation. The cell membranes were photoaffinity-labeled; after SDS-PAGE electrophoresis and autoradiography we identified hDAT bands which were then excised. The excised gel squares were incubated with trypsin, chymotrypsin, or other protease solutions. Supernatants from the in-gel digestions were further electrophoresed; peptide fragments released from the gels into the solutions were identified by autoradiography. Silver staining was done with the Bio-Rad Silver Stain Plus Kit.

RESULTS AND DISCUSSION

Photoaffinity labeling of hDAT with [^{125}I]-RTI-82 or with the GBR analogue [^{125}I]-AD-96 produced preparations which were immunoprecipitated with antibodies to DAT and upon electrophoresis showed labeled bands comparable in mass to the DAT from rat striatal membrane.

For chymotrypsin digests of hDAT labeled with the cocaine analogue, RTI-82, the most highly labeled fragment in limit digests appears to have a molecular weight of about 2 kDa. There are also larger fragments with molecular weights around 30 kDa and 14 kDa. These larger fragments are more evident at lower levels of chymotrypsin. Digestion with chymotrypsin followed by either trypsin or V8 protease did not reduce the size of the 2 kDa fragment. This autoradiograph fragment is located at the position of a band which shows in silver staining of the same gel. In trypsin digests of hDAT labeled with RTI-82, most of the label appears in a fragment of about 30 kD, but there are also some smaller fragments. Trypsin digestion of hDAT that had been labeled with [^{125}I]-AD-96 produced a labeled fragment of about 14 kDa. Trypsin results with both AD-96 and RTI-82 labels are similar to previous results with rat striatal membrane preparations. (2,3)

The 2 kDa fragment which is implicated by our results would be an appropriate size for examination by mass spectroscopy. Mass spectroscopy results should enable us to localize the RTI-82 binding exactly. Further work is aimed at isolating this small fragment.

---

1. Vaughan, R.A. and Kuhar, M.J. (1996) *J. Biol. Chem.*, **271**, 21672-21680.
2. Vaughan, R.A. (1995) *Mol. Pharm.*, **47**, 956-964.
3. Vaughan, R.A., unpublished results.

PREDICTED CIRCULAR DICHROIC SPECTRA OF CYCLO(PRO-GLY)3 BY THE DIPOLE INTERACTION MODEL USING REVISED PARAMETERS

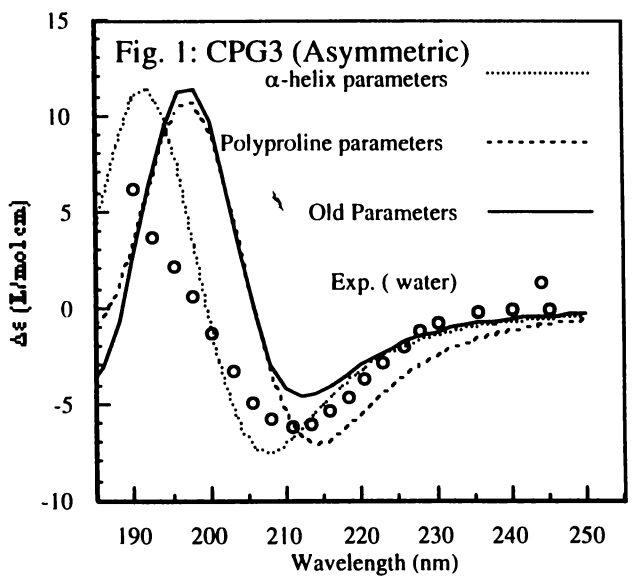
Stephen L. Lowe*, Nathan Kadrmas, Tamara Baumgartner and Kathyrn A. Thomasson
Department of Chemistry, University of North Dakota, Grand Forks, ND 58202

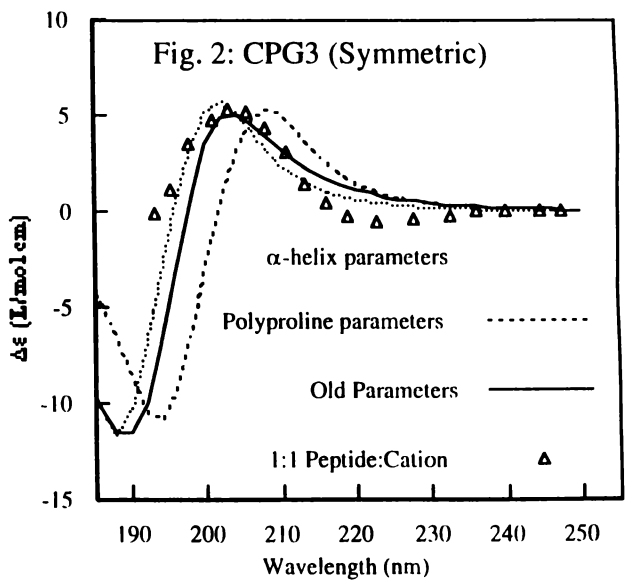
The synthetic peptide cyclo(L-prolyl-glycyl)$_3$ (CPG3) is of interest because it is capable of binding and transporting cations across membranes (1-3). CPG3 has been shown by NMR (1) and by X-ray crystallography (2, 3) to adopt a variety of different conformations depending on solvent and cations bound. In polar solvents, such as water, the isolated peptide adopts an asymmetric conformation; however, when binding cations it assumes various C_3 symmetric forms.

Since circular dichroic (CD) spectra are very sensitive to conformational differences, they are useful in studying solution conformations. The method of choice for predictions of the π-π^* CD spectra of polypeptides is the dipole interaction model. In this method the three atoms (NC'O) of the peptide group chromophore are replaced by a single point; parameters for the anisotropic polarizabilities of the chromophores and isotropic polarizabilities of the other atoms are used with atomic coordinates to predict the CD spectrum. The computer models used in this study to produce files of atomic coordinates were built and energy minimizations performed using the program package INSIGHT II® (MSI, San Diego, CA).

Our previous studies have used the original parameter values of Applequist (4). Recently Bode and Applequist have published revised parameters (5) optimized for simple amides, for α-helices and for proline helices (form II); they have also looked at the effect of slight variations of the position of the point chromophore. We have applied these new parameters to predict CD spectra of different conformations of CPG3. Fig. 1 below shows the experimental values of Madison *et al.* (1) for the asymmetric conformation in water compared to predicted values using old and new parameters. Fig. 2 shows experimental values for the 1:1 peptide:cation complex with magnesium ions compared to the predicted CD for the symmetric form.

Compared to experimental values for the asymmetric form, the old parameters and the polyproline optimized parameters gave somewhat red-shifted spectra; the α-helix parameters gave a better fit. For the symmetric form both the α-helix parameters and the old parameters fit the experimental values fairly well. The parameters optimized for simple amides were completely unsatisfactory for this and other cyclic peptides we have studied; shifting the position of the chromophore tended to produce unsatisfactory wavelength shifts. At this time no one parameter set appears satisfactory for all structures.





Support for this project was provided by: NSF Grant CHE-94009934; NIH R15 GM55929-01; ND EPSCoR

1. Madison V., Atreyi M., Deber C., and Blout E. (1974) *J. Am. Chem. Soc.*, 96, 6725-6734.
2. Kartha G., Varughese K., and Aimoto S. (1982) *Proc. Natl. Acad. Sci. USA*, 79, 4519-4522.
3. Varughese K., Aimoto S., and Kartha G. (1986) *Int. J. Peptide Protein Res.*, 27, 118-122.
4. Applequist J., Carl J.R., and Fung, K.-K. (1972) *J. Am. Chem. Soc.*, 94, 2952.
5. Bode, K.A., and Applequist J. (1996) *J. Phys. Chem.*, 100, 17820-17824 & 17825-17834.

EVALUATION OF REGIONAL BIOELECTRICAL IMPEDANCE MEASUREMENTS TO ASSESS WHOLE BODY COMPOSITION

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

The use of the bioelectrical impedance (BI) method to assess human body composition has progressed from a research method to an at-home self-assessment tool. One commercially available device uses electrodes positioned only on the feet. This approach assumes that the fat-free mass (FFM) in the lower body is proportionally constant relative to the FFM of the whole body and that the measured impedance in the lower body is similar to that of the whole body (1).

We examined the validity of this hypothesis in 15 healthy women aged 33 to 86 years. BI was measured by using adhesive electrodes that were positioned either on the right hand and foot for arm-to-leg or whole body measurements and on the right and left ankles for lower body determinations (1). A radiofrequency current (800 μA, AC, at 50 kHz) was introduced at the distal electrodes and the voltage drop was determined with the proximal electrodes (RJL model 101; Clinton Twp., MI). Fat-free mass was calculated by using the standard bioconductor model (Ht^2/Z_{WB} + constant for whole body and Ht^2/Z_{LB} + constant for lower body determinations; 1) in which Ht is standing height. Body composition was determined by using dual x-ray absorptiometry (DXA; QDR 2000; Hologic Inc., Waltham, MA) with software version 7.3. Lower body composition was defined as the region of interest caudal to a line drawn horizontally across the iliac crests of an individual; it included the hips and legs.

Whole body BI was greater ($p < 0.05$) than lower body BI (583 ± 13 vs 509 ± 13 ohm; mean ± SE). Similarly, FFM of the whole body determined with DXA was greater ($p < 0.001$) than that measured for the lower body (40.72 ± 17.93 vs 20.71 ± 0.91 kg). Impedance predictions of whole body FFM calculated by using lower body BI measurements were greater ($p < 0.01$) than estimates derived from whole body BI measurements (47.06 ± 1.95 vs 43.79 ± 14.1 kg). Comparisons between impedance predictions and reference body composition measurements with DXA indicated that the errors (reference - impedance predicted values) observed for the lower body predictions (-6.34 ± 1.48 kg) were greater ($p < 0.01$) than those found for the whole body BI estimates (-3.07 ± 0.71 kg), and these differences were greater than 0 ($p < 0.05$).

The errors or bias in predicting whole body FFM from lower body BI measurements were not affected by the magnitude of the FFM ($r = -0.11$, $p = 0.68$). Similarly, the bias was not related to the ratio of the lower to whole body FFM ($r = -0.11$, $p = 0.70$). However, the ratio of lower to whole body FFM was highly consistent in this sample (50.8 ± 0.5 %); it ranged from 47.4 to 54.8 %.

This study showed that regional BI measurements are not valid predictors of whole body FFM. This finding suggests that commercial impedance devices that utilize lower body BI measurements may be practical but inaccurate for body composition assessment. Individuals with an over- or under-development of lower body FFM may experience greater errors in prediction of FFM than the errors reported in this study.

---

1. Nunez, C. et al. (1997) *Medicine and Science in Sports and Exercise,* 29:524-531.

LOW DIETARY ZINC AND COPPER NEGATIVELY AFFECT PLASMA AND URINE INDICATORS OF BONE HEALTH

Forrest H. Nielsen*, Cindy D. Davis, and David B. Milne
USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202-9034

Although low dietary calcium receives the most recognition for increasing the risk of becoming osteoporotic, there are a number of other possible nutrients whose lack could increase the susceptibility to osteoporosis; these include zinc and copper. Copper is a cofactor for lysyl oxidase, an enzyme involved in the maturation or cross-linking of collagen to form stable fibrils upon which calcium is deposited to form bone. Copper deficiency decreases the activity of lysyl oxidase and impairs the cross-linking of collagen in bone of experimental animals. Both alkaline phosphatase, which is required for bone calcification, and collagenase, which is required for bone resorption and remodeling, are zinc-requiring enzymes. Zinc deficiency reduces the synthesis and activity of alkaline phosphatase in rats and the activity of collagenase in chicks. Supplementation of both zinc (1) and copper (1,2) has been associated with conserving bone density or preventing spinal bone loss in postmenopausal women. Thus, the effects of low dietary zinc and copper on selected variables used to assess bone health were determined in a controlled feeding study of 21 postmenopausal women housed in a metabolic ward. The experimental protocol was approved by the Institutional Review Boards of the University of North Dakota and the U.S. Department of Agriculture. After a 10 day equilibration period, 10 women were fed a diet containing 1 mg Cu/2000 kcal and 13 women were fed 3 mg Cu/2000 kcal for the remainder of the study. The diet for both groups contained 3 mg Zn/2000 kcal for 90 day after the first equilibration period, and 53 mg Zn/day for the last 90 days of the study after another 10 days of equilibration. Serum and urine variables were determined during the last two weeks of each 90-day dietary period by standard methods using commercially available kits, ion specific electrodes or atomic absorption spectrometry. A p value less than 0.10 was considered significant.

Table 1. Effect of altered zinc and copper intakes on selected bone health indicators.

Group	Urine		Plasma			
	N-telopeptides	Calcium	Osteocalcin	Ionized Ca	Bone API	25-OH-Vit. D_3
		mg/d	ng/mL	mg/dL	IU/L	ng/mL
Low Cu Low Zn	55	168	5.39	5.21	19.0	45
Low Cu High Zn	65	140	3.90	5.14	24.7	48
High Cu Low Zn	50	153	4.29	5.47	20.2	52
High Cu High Zn	58	151	4.23	5.24	21.5	51
	Analysis of Variance - P Values					
Zinc Effect	0.03	0.08	0.03	0.57	0.07	0.60
Copper Effect	0.50	0.99	0.78	0.009	0.76	0.24
Zinc x Copper	0.86	0.12	0.05	0.77	0.23	0.36
Error Mean Square	41	669	1.16	0.02	33	34

As shown in Table 1, a significant interaction between dietary zinc and copper affected plasma osteocalcin concentration; it was increased by the combination of low dietary zinc and copper. Urinary calcium excretion also was highest when both dietary copper and zinc were low. These changes could be considered unfavorable because increased serum concentrations of undercarboxylated osteocalcin and increased urinary calcium excretion have been associated with decreased bone density and increased hip fractures (3). Interestingly, urinary N-telopeptide excretion was higher with high zinc than with low zinc. Increased urinary N-telopeptide usually occurs with increased bone resorption. However, in this case, the decrease with low dietary zinc might indicate a decrease in bone matrix metalloproteinases (collagenases) dependent on zinc for their activity. The reduction in bone alkaline phosphatase supports this suggestion. The findings indicate that low dietary zinc or copper, and especially the combination of low dietary copper and zinc, may be important factors in the incidence of osteoporosis.

1. Strause, L., Saltman, P., Smith, K.T., Bracker, M., and Andon, M.B. (1994) *J. Nutr.*, 124:1060-64.
2. Eaton-Evans, J., McIlrath, E.M., Jackson, W.E., McCartney, H., and Strain, J.J. (1996) *J. Trace Elem. Exp. Med.*, 9:87-94.
3. Vermeer, C., Jie, K.-S.G., and Knapen, M.H.J. (1995) *Annu. Rev. Nutr.*, 15:1-22.

A PROBABILITY PROBLEM IN DRUG DELIVERY

M.B. Rao
Department of Statistics, North Dakota State University, Fargo, ND 58105

INTRODUCTION

The problem that is being presented in this paper came from a pharmaceutical company. The company designed a skin patch, which will release a certain medicine progressively in increasing amounts of dosage. On the first day, it will release one dose of the medicine, second day two doses, third day three doses, fourth day four doses, fifth day five doses, and finally, sixth day six doses. There are six receptors inside the body of the person who has the skin patch attached. At the beginning, all the receptors are inactive. On the first day of the treatment, the single dose released latches onto a receptor randomly and makes it active. On the second day, the two doses released latch onto two receptors randomly and individually, one dose per receptor. If an active receptor receives a dose, then it becomes inactive. If an inactive receptor receives a dose, then it becomes active. On the second day, one could have either three receptors active or one receptor active. On the third day, the three doses released latch onto three receptors randomly and individually, one dose per receptor. This continues until the sixth day at that time all the six doses latch onto six receptors, one dose per receptor. The question is, at the end of the treatment, how many receptors are expected to be active?

A MATHEMATICAL FORMULATION

The problem is synthesized in general mathematical terms. We have n toggle switches each of which is attached to a light bulb. If the bulb is off and you press the switch, then the bulb lights up. If the bulb is already lighted up and you press the switch, then the bulb goes off. At the beginning, all the bulbs are off. There are n individuals serially numbered from 1 to n. On Day One, Individual One selects one switch at random and presses it. The attached bulb lights up. On Day Two, Individual Two selects two switches at random independently of the choice made by Individual One and presses them. On Day Three, Individual Three selects three switches at random independently of the choices made by Individuals One and Two and presses them. This continues until Day n. On Day n, Individual n presses all switches. The question is at the end of Day n, how many bulbs are expected to light up?

On the bulb attached to Switch No.1, we calculated the probability p that the bulb is on at the end of Day n. By the very symmetrical nature of the problem, this probability is the same for each of the other bulbs. Consequently, the expected number of bulbs that will be on at the end of Day n will be np. At a micro level, let p_r denote the probability that the bulb attached to Switch No. 1 is on at the end of Day r, r = 1,2,..., n. The required probability p = p_n. The probabilities p_rs satisfy the recurrence relation,

$$p_{r+1} = p_r\left(1 - \frac{r+1}{n}\right) + (1 - p_r)\left(\frac{r+1}{n}\right),$$

r = 1,2, ... , n,

with the boundary condition $p_1 = 1/n$. We solved the above non-homogeneous difference equation of order one. A key idea in solving the equation is to transform the above difference equation into a homogeneous difference equation of order one, by letting $q_r = p_r - ½$. We obtained the solution that p = ½ if n is even and that p is approximately 1/2 if n is odd. Consequently, the expected number of bulbs that will be on at the end of Day n will be n/2 if n is even and approximately equal to n/2 if n is odd. Suppose n is even. Interestingly, it turns out that $p_{n/2} = ½$. At the end of Day n/2, the probability that the bulb attached to any switch is on is ½. The solution to the mathematical problem translates into a solution to the receptors problem. At the end of Day 6, three receptors are expected to be active.

ADJUSTMENT TO A PEAK PHYSICAL WORK CAPACITY TREADMILL TEST

William A. Siders*, Henry C. Lukaski and Clinton B. Hall
USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202

In a nutrition research setting, the usually recognized function of an exercise stress test is two-fold: a) to determine volunteers' normal response to exercise and their fitness for prescribed exercise; and b) to quantify volunteers' cardiovascular fitness for determinations of dietary related changes (1). Variables such as peak oxygen consumption (commonly regarded as the criterion measure of cardiovascular fitness (2)) and peak heart rate are used in calculations to prescribe exercise. Variables such as peak power, oxygen consumption (VO_2), and carbon dioxide production (VCO_2) are considered together to identify changes in metabolic response to work. Because most people are not accustomed to exercising under the direct observation of laboratory personnel who are trying to quantify their physiological responses, volunteers are usually tested two or three times during the baseline period of the study. Repeated exposure to the testing environment is thought to allow the volunteer to habituate to the anomalies of that environment and thus yield results from the plateau of the learning curve that can be compared with results from future testing. This study examined the reproducibility of physiological responses during graded progressive treadmill tests to determine if two or three were needed to obtain reliable results.

Thirty-two female volunteers aged (mean ± standard deviation) 28.2 ± 4.5 years from three metabolic studies were administered Balke (3) protocol, treadmill physical work capacity tests seven days apart during the baseline period when nutrient intake was adequate. Tests were administered in the mornings prior to the first food consumption of the day. All volunteers (n = 32) performed two Balke treadmill tests and fourteen of those performed a third. During the tests, indirect calorimeters and ECG monitors were utilized to measure ventilation volume (VE), breath rate, the concentrations of O_2 and CO_2 in expired air, and heart rates. The treadmill tests were terminated when a volunteer indicated the desire not to continue.

After the tests, collected data were used to calculate minute by minute power, VO_2 both as L/min and normalized for body weight, VCO_2, and respiratory exchange ratio (RER). The results of the two treadmill tests for n = 32 and of the three tests for n = 14 are summarized in Table 1. The data were statistically analyzed with a repeated measures analysis of variance. The women who

Table 1. Physical Work Capacity Variables (mean ± standard deviation) at Peak Performance by Test

	n = 32		n = 14		
	Test 1	**Test 2**	**Test 1**	**Test 2**	**Test 3**
Body weight, kg	99.5 ± 16.6	99.8 ± 16.6	95.4 ± 14.5	95.2 ± 14.5	95.6 ± 14.3
Peak Time, min	15.8 ± 3.7	16.3 ± 4.0	18.1 ± 3.4	18.1 ± 4.3	18.8 ± 4.2
Power, kg-m/min	8745^{A} ± 1435	8779^{B} ± 1448	8409 ± 1268	8396 ± 1270	8462 ± 1214
VE, L/min	80^{A} ± 14	88^{B} ± 17	83 ± 13	86 ± 13	92 ± 20
Heart Rate, beats/min	182^{A} ± 10	186^{B} ± 10	183 ± 9	184 ± 9	184 ± 12
VO_2, ml/min	2522^{A} ± 432	2684^{B} ± 488	2540 ± 397	2646 ± 389	2666 ± 444
ml/min/kg	25.7^{A} ± 4.2	26.7^{B} ± 4.4	26.9 ± 4.3	27.1 ± 4.5	28.2 ± 4.8
VCO_2, ml/min	2467^{A} ± 425	2670^{B} ± 535	2538^{A} ± 407	$2628^{A,B}$ ± 472	2773^{B} ± 551
RER	0.988 ± 0.06	1.004 ± 0.06	1.020 ± 0.05	1.019 ± 0.06	1.037 ± 0.06

Note: A,B Means with different superscripts are significantly different ($p<0.05$).

performed two exercise tests exercised longer which elicited significant increases in power output, ventilation rate, heart rate, VO_2, and VCO_2. Physiological responses also tended to improve from the first to the third test but only significantly for VCO_2.

These findings suggest a habituation in performance and cardiovascular fitness measures associated with increased test duration over repeated treadmill tests. If the primary goal of exercise stress testing is to generate values for prescribing exercise, then one test may be sufficient. But if the testing is for comparing with values obtained after diet change, then two or three tests may be needed. More than one test is needed to habituate the volunteer to achieve a level of comfort in the test environment that will optimize compliance and reproducibility of functional responses.

1. American Heart Association (1991) Exercise Standards: a statement for health professionals. Dallas, TX: AHA.
2. Astrand, I. (1960) *Acta Physiol. Scand.*, 49 (Suppl., 169): 1-92.
3. Balke, B. and Ware, R. (1959) *U.S. Armed Forces Medical Journal*, 10:6, 675-688.

BISON (*BISON BISON*) HABITAT USE AND BEHAVIOR DURING THE BREEDING SEASON IN THEODORE ROOSEVELT NATIONAL PARK IN THE NORTH DAKOTA BADLANDS

Deanna M. Thompson*, Adam T. Walz, Heather R. Taylor, Daniel C. McEwen, Breann A. Stoltz, and Donna M. Bruns Stockrahm
Department of Biology, Moorhead State University, Moorhead, Minnesota

INTRODUCTION

Herd composition, calf production, habitat use, and reproductive behavior of bison (*Bison bison*) were studied in the South Unit of Theodore Roosevelt National Park (TRNP) in the Badlands of North Dakota during July and August of 1999. We especially wanted to address concerns that bison bulls might be too numerous in the herd for its long-term health. Bull behaviors towards lactating cows versus nonlactating cows were compared. If bulls greatly outnumbered cows, lactating cows might be tended more frequently even though such cows are less likely to ovulate that year than nonlactating cows.

METHODS

Bison were observed with binoculars and spotting scopes from vehicles driven over park roads. Because many roads went directly through or were adjacent to the black-tailed prairie dog (*Cynomys ludovicianus*) towns used by the bison, we could often drive directly into the herd and observe bison from a fairly close distance. Gender and age class (adult, subadult, and calf) of bison were determined when possible. Tending pairs (n = 56) were observed for 5-minute time periods, and behaviors such as lip curling, bellowing, wallowing, aggressive interactions, and mounting were recorded. Locations of other ungulate species were recorded to determine if interaction occurred between these species and the bison.

RESULTS

The largest number of bison that could be accounted for during any one day was approximately 350 animals. Although the herd was often split into various-sized bands and counts varied daily, averages of our best band counts indicated the herd composition was roughly: 18% adult bulls, 33% adult cows, 5% subadult males, 4% subadult females, 4% subadult animals of unknown gender, 21% calves, and 15% animals of unknown age and/or gender. Calf production was fairly high at approximately 0.6 calves/cow.

Some aggression was observed between bulls in the herd at large, but often one bull backed down without an actual fight. In our observed tending pairs, fighting between the tending bull and another (usually challenging) bull had a low incidence, but when it did occur, it always involved a bull that was tending a nonlactating cow. Lip curling was fairly high towards cows with calves (57%) during the height of the rut although the sample size was small (n = 7). No copulations were directly observed by us, but a few mountings were seen, none of which were between bulls and lactating cows. Bulls seldom tended lactating cows until later in the rut, when the percentage of lactating cows being tended increased slightly. Although no calves were seen with the cows being mounted, towards the end of the rut several lactating cows were seen with fresh wounds on their backs, presumably from being mounted. Bellowing was also very common, especially later in the rut. Bison used black-tailed prairie dog towns extensively for their rut activities, and it appeared that the habitat and space use of other ungulates in the Park were independent of those of the bison.

DISCUSSION

Our reproductive behavior data indicated that bulls seldom tended lactating cows until later in the rutting season. Apparently, bulls at TRNP were not spending much of their time and efforts on cows that were not approaching estrus or likely to do so during that season. Most of the longer-term tending (assumed to be associated with cows in estrus) was done by larger, more mature-looking bulls. One of the most frequently observed behaviors was lip curling, evident especially when bulls approached cows. If the female did not have the smell associated with estrus, the male usually left her within a few seconds to a few minutes to approach and test another cow. Generally, the reproductive behaviors and patterns we observed agreed with other published studies. In future studies, we might try to record how many lactating cows are approached, even if they are not tended.

In summary, bull-to-bull aggression levels were sometimes high, but good calf production and normal breeding behaviors suggested that the current number of bulls is not detrimental to the herd. However, we recommend further monitoring as subadult males reach reproductive age.

CONSTITUTION of the NORTH DAKOTA ACADEMY OF SCIENCE

Founded 1908, Official State Academy 1958

ARTICLE I - *Name and Purpose*

Section 1. This association shall be called the NORTH DAKOTA ACADEMY OF SCIENCE.
Section 2. The purpose of this association shall be to promote and conduct scientific research and to diffuse scientific knowledge.

ARTICLE II - *Membership*

Membership in the Academy shall be composed of persons who share the stated purpose of the Academy and who are active or interested in some field of scientific endeavor.

ARTICLE III - *Council*

The officers of the Academy shall be a President, a President-Elect, and a Secretary-Treasurer. The Council, consisting of the officers, the retiring President, and three elected Councilors, shall be responsible for the fulfillment of the scientific and business obligations of the Academy.

ARTICLE V - *Dissolution and Limits of Action*

Section 1. In the event of dissolution of the Academy, any remaining assets shall be distributed to organizations organized and operated exclusively for education and scientific purposes as shall at the time qualify as exempt organizations under Section 501(c) (3) of the Internal Revenue Code of 1954.

Section 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.

Section 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*

Section 1. This Constitution may be amended at any annual Business Meeting of the Academy by a two-thirds vote. Proposed amendments shall be submitted in writing to the Secretary -Treasurer who shall send them to the members at least two weeks before the meeting at which such amendments are to be considered.

Section 2. Bylaws may be adopted or repealed at any regular business meeting by a two-thirds vote.

BYLAWS

BYLAW 1. *Meetings*

Section 1. *Scientific Meetings.* The Academy shall hold at least one annual scientific meeting each year at a time and place determined by the Council. Other scientific meetings, regional, state, or local, may be held at times and places determined by the Council. The Council shall establish regulations governing the presentation of papers at Academy sessions. Such regulations shall be made available to members at least three months before any meeting at which they are to apply.

Section 2. *Business Meetings.* A Business Meeting of the membership shall be scheduled at the regular, annual scientific meeting of the Academy. Ten percent of the active members shall constitute a quorum at the annual business meeting.

Section 3. *Special Meetings.* Special meetings shall be called by the President upon the request of ten percent of the active members and require twenty percent of the active members for a quorum. Notice of the time and place of such meetings shall be sent to all members of the Academy at least four weeks in advance of the meeting. Only matters specified in the call can be transacted at a special meeting.

Section 4. *Procedure.* Parliamentary procedures to be followed in all business meetings shall be those specified in "Standard Code of Parliamentary Procedure" by Alice F. Sturgis.

BYLAW 2. *Financial*

Section 1. *Dues and Assessments.* The annual dues and assessments may be changed from time to time by the Council, subject to approval by a two-thirds vote of the members at an annual Business Meeting. The student member dues shall be one-third (to nearest dollar) of the regular member dues. These dues are payable 1 December of each year.

Section 2. *Supporting Members.* Council shall maintain a program to encourage members to voluntarily contribute funds over and above the regular dues and assessments for the support of activities of the Society.

Section 3. *Sustaining Members.* Any association, corporation, institution, or individual desiring to support the Society with funds or services valued at $50 or greater may be invited by the President or designee to become a Sustaining Associate.

Section 4. *Audit and Reports.* The Nominating Committee shall appoint on a yearly basis one member who is not a member of Council to conduct at least one internal audit per year. The Secretary-Treasurer shall report on the financial affairs of the Society, including the results of an annual audit, as may be requested by the Council.

BYLAW 3. *Membership*

Section 1. *Membership Categories.* Classes of membership shall include the following: (a) Regular, (b) Student, (c) Emeritus, (d) Honorary, (e) Supporting, (f) Sustaining, and (g) Lifetime Members.

Section 2. *Eligibility and Procedure for Membership.* Candidates for membership, except Sustaining Member, may be proposed by any regular or emeritus member of the Academy by submitting the candidate's name to the chairman of the Membership Committee.

(a) *Regular Members.* Any person who is active or interested in some field of scientific endeavor shall be eligible for regular membership. A majority vote of Council shall elect to regular membership.

(b) *Student Members.* Any student who is an undergraduate or graduate student in some field of science shall be eligible for student membership. A majority vote of Council shall elect to regular membership.

(c) *Emeritus Members.* Any member in good standing upon formal retirement is eligible for emeritus membership. A majority vote of Council shall elect to emeritus membership.

(d) *Honorary Members.* 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. A two-thirds vote of members attending the annual business meeting shall elect to honorary membership.

(e) *Supporting Members.* Regular or student members may voluntarily contribute funds over and above the regular dues and assessments for the support of activities of the Society.

(f) *Sustaining Associates.* Any association, corporation, institution, or individual desiring to support the Society with funds or services valued at $50 or greater may be invited by the President or designee to become a Sustaining Associate.

(g) *Lifetime Members.* Any regular member in current good standing for at least one year may become a Lifetime Member by paying an assessment equal to 18 times the current annual dues in one lump sum or in two equal payments over the current and following year.

Section 3. *Privileges of Membership.*

(a) Voting at the annual business meeting is permitted of regular and emeritus members.

(b) Members of all categories may attend business meetings of the Academy.

(c) The Secretary-Treasurer and members of Council must be regular members in good standing.

(d) Regular, student, and emeritus members may submit abstracts or communications for scientific meetings of the Academy.

(e) Emeritus and Honorary Members shall be exempt from payment of dues.

(f) A Sustaining Member is provided a display area at the annual scientific meeting of five linear feet per $50 donation up to a maximum of 20 linear feet.

(g) Every member in good standing shall receive a printed copy or an electronic copy (if available and of equal or lesser cost than the printed copy) of the annual *Proceedings of the North Dakota Academy of Science*, the form to be determined by the member.

(h) Special offices such as Historian may be created by the unanimous vote of the regular members at the annual Business Meeting.

(i) All student research participants shall receive a properly inscribed certificate.

Section 4. *Forfeiture of Membership.*

(a) *Nonpayment of dues.* Members shall be dropped from the active list on 31 November following the nonpayment of

dues during the membership year commencing the previous 1 December. A member may return to the active list by paying the current year dues.

(b) *Expulsion for Cause.* Membership may be terminated for conduct injurious to the Academy or contrary to the best interests of the Academy. The accused member shall be given an opportunity for a hearing before the Council. If a majority of the Council votes to expel the member, the action must be ratified by at least two-thirds of the members present at the next annual business meeting of the Academy. An expelled member shall forfeit all paid dues and assessments.

BYLAW 4. *Duties and Responsibilities of the Council and Council Members*

Section 1. *Council.* The Council shall meet, at the call of the President, at least twice a year. The Council shall:

(a) be the governing board of the Academy, responsible only to the membership.

(b) arrange for programs, approve committee appointments, be responsible for the fiscal affairs of the Academy, an transact such business as necessary and desirable for function and growth of the Academy.

(c) determine the location of the annual meeting three years in advance.

(d) 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.

(e) shall appoint and may compensate a Secretary-Treasurer.

(f) shall appoint and may compensate an Editor of the PROCEEDINGS and other publications.

(g) shall be empowered to charge a publication fee of authors on a per page basis.

(h) shall control all activities of the Academy including grant applications.

Section 2. *President.* The President shall preside at meetings of the Council and over the annual business meeting of the Academy at the close of the regular term of office. The President shall vote only to break a tie. Unless otherwise specified, the President shall, with the approval of the Council, appoint members to serve on Standing Committees and *ad hoc* Committees, designate the chair of each Committee, and appoint representatives to other organizations. The President serves as Coordinator of the Local Arrangements Committee for the annual meeting that occurs at the end of the President's term.

Section 3. *President-Elect.* The President-elect shall be considered a vice president and shall serve as such in the absence of the President.

Section 4. *Past-President.* The retiring President shall serve as Past-President and chair of the Nominating Committee. The Past President shall serve ex officio on those committees designated by the President and shall serve in the absence of the President and President-elect.

Section 5. *Secretary-Treasurer.* The Secretary-Treasurer shall:

(1) Assist Council in carrying on the functions of the Academy including the receipt and disbursement of funds under the direction of Council.

(2) Manage the Academy Offices under Council's general supervision.

(3) Serve as Managing Editor of the *Proceedings of the North Dakota Academy of Science.*

(4) Prepare a summary of the most recent audit and a report of the Academy's current financial status. This information shall be shared with the membership at the annual business meeting and published in the PROCEEDINGS following the business meeting.

(5) Perform all other duties of the Secretary-Treasurer listed in the Bylaws.

(6) Serve as archivist and be responsible for all official records, archives, and historic material which shall be in reposit with the Secretary-Treasurer.

BYLAW 5. *Appointment, Nomination and Election of Members of Council*

Section 1. *Eligibility for Office.* All candidates for election or appointment to the Council must be regular members in good standing. Nominees for President-elect must be members who reside within easy commuting distance of the site of the annual meeting selected by the Council that occurs when the President-elect serves as President.

Section 2. *Nomination Procedures.* The Nominating Committee shall be responsible for all nominations to elective office, shall determine the eligibility of nominees, shall ascertain that nominees are willing to stand for office, and shall be required to advance to the Secretary-Treasurer at least two names for each open position as needed. Academy members shall have been encouraged to suggest nominees to the committee prior to the Committee submitting its report.

Section 3. *Election Procedures.* Election shall be by secret mail ballot. The Secretary-Treasurer shall prepare a printed ballot that bears all names submitted by the Nominating Committee, that contains a brief biography of each candidate, and that

has space for write-in candidates for each office. This ballot is to be mailed to all members no later than 1 November. Each member wishing to vote must return the marked ballot in a sealed signed envelope to the Secretary-Treasurer postmarked not more than thirty days after the ballots were mailed out to members. The President shall appoint tellers who shall count the ballots which have been received by the Secretary-Treasurer and the tellers shall present the results in writing to the President. A plurality of the votes cast shall be necessary to elect and in the case of a tie vote, the President shall cast the deciding vote. The results of the election shall be announced at the annual Business Meeting.

Section 4. *Term of Office.* A President-Elect shall be elected annually by the membership and the following years shall succeed automatically to President and Past President to constitute a three year nonrenewable term. Three Councilors shall be elected by the membership to three-year, non-renewable terms on a rotating basis. All elected Council members shall take office at the end of the next annual Business Meeting following election and shall continue until relieved by their successors. Council is empowered to appoint and compensate a Secretary-Treasurer to successive three-year terms that commence with the beginning of the fiscal year.

Section 5. *Removal from office or position* If for any reason any elected member of Council is unable to fulfill his/her duties, the Council member may be removed from office by two-thirds vote of Council. If for any reason the Secretary-Treasurer is unable to fulfill his/her duties, the Secretary-Treasurer may be relieved of all duties by a majority vote of Council.

Section 6. *Interim vacancies.* Should a vacancy occur in the Presidency, the Council by a majority vote shall appoint a member of the Academy able to coordinate the next annual meeting to fill the unexpired term. A retiring interim President shall succeed automatically to Past President. Should a vacancy occur in the Presidency-elect, the Council shall reassess and change the location of the coinciding annual meeting as necessary and then call for a special election by mail ballot. An interim vacancy in the Past-Presidency shall be filled by the most recently retired Past-President able to fill the duties of the Past-President. Persons appointed to fill the unexpired term of Secretary-Treasurer are expected to remain in the position for a minimum of three years. A vacancy in the office of Councilor shall be filled by a majority vote of Council until the following election at which time the interim Councilor may stand for a full three year nonrenewable term.

BYLAW 6. *Committees*

Section 1. Standing Committees. Standing committees shall include but not be limited to, the following: Editorial, Education, Denison Award, Necrology, Nominating, Resolution, Membership, and Audit Committees. The President shall appoint members of committees other than the Nominating and Audit Committees.

Section 2. *Editorial Committee.* The Editorial Committee shall consist of three regular members appointed to three year terms. The duties are explained in BYLAW 7 (Publications).

Section 3. *Education Committee.* The Education Committee shall consist of five regular members and two high school teachers appointed to five year terms. 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.

Section 4. *Denison Awards Committee.* The Denison Awards Committee shall consist of six regular members appointed to three year terms. 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.

Section 5. *Necrology Committee.* The Necrology Committee shall consist of three regular members appointed to three year terms. The Necrology Committee shall report to the annual meeting on those deceased during the preceding year. Obituaries may be included in the minutes of the annual meeting and/or published in the Proceedings.

Section 6. *Nominating Committee.* The Nominating Committee shall consist of the five most recent past-presidents. The major duties of the Nominating Committee are listed in BYLAW 5 (*Appointment, Nomination and Election of Members of Council*). The Nominating Committee will also administer the selection process, develop a separate funding source for a monetary award, and develop, for Executive Committee approval, the criteria for the North Dakota Academy of Science Achievement Award.

Section 7. *Resolution Committee.* The Resolution Committee shall consist of three regular members appointed to three year terms. The Resolution Committee 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.

Section 8. *Membership Committee.* The Membership Committee shall consist of unlimited numbers of regular members appointed annually.

Section 9. *Audit Committee.* The Nominating Committee shall appoint on a yearly basis one member who is not a member of Council to conduct at least one internal audit per year.

Section 10. *State Science Advisory Committee.* The State Science Advisory Committee (SSAC) shall consist of five regular or emeritus members appointed to four year terms. The SSAC shall serve to direct questions of a scientific nature to the appropriate expert as requested, shall inform regional granting agencies and state and national science policymakers of its expertise and availability and shall counsel those agencies and persons upon their request. The SSAC shall adhere in particular to the guidelines described in Article V, Section 2 of the Constitution.

Section 11. *Ad hoc Committees.* The President may appoint such additional committees as may be needed to carry out the functions of the Academy. Ad hoc committees serve only during the tenure of the president who appointed them. Reports of ad hoc committees shall be presented to Council or to the annual meeting.

BYLAW 7. *Publications*

Section 1. *Editorial Committee.* Three regular members are appointed to the Editorial Committee for renewable three year terms. The Editorial Committee shall develop and recommend the Academy publication program and policies to the Council. It will assist the Editors of each official publication in reviewing manuscripts for those publications that include the *Proceedings* . Chairs of symposia will review manuscripts written for relevant symposia.

Section 2. *Managing Editor.* The Secretary-Treasurer shall serve as the Managing Editor of all Academy publications and as such shall oversee each Editor.

Section 3. *Editor.* Editors shall serve three year terms. The Editors shall edit all official publications of the Academy including the *Proceedings.*

BYLAW 8. *Memorial Fund*

The Council of the Academy shall establish a J. Donald Henderson Memorial Fund and administer this fund so that the proceeds will be used to promote science in North Dakota.

BYLAW 9. *Fiscal Year*

The fiscal year of the North Dakota Academy of Science, for the purpose of financial business, shall be 1 January to 31 December.

BYLAW 10. *Achievement Award*

The Academy establishes the North Dakota Academy of Science Achievement Award to be given 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 Council approval, the criteria for the award.

BYLAW 11. *Research Foundation*

The **North Dakota Science Research Foundation** is established as an operating arm of the Academy. The purposes of the Foundation are:

(1) to receive funds from grants, gifts, bequests, and contributions from organizations and individuals, and

(2) to use the income solely for the making of grants in support of scientific research in the State of North Dakota.

Not less than 50% of the eligible monies received shall be placed in an endowment from which only the accrued interest shall be granted.

The foundation shall be responsible for soliciting the funds for the purposes described. The Foundation funds shall be in the custody of the Secretary-Treasurer of the 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 of staggered five year terms. The chairperson of the Board shall be appointed annually by the President. The Board shall be responsible for developing operating procedures, guidelines for proposals, evaluation criteria, granting policies, monitoring procedures, and reporting requirements, all of which shall be submitted

to the Executive Committee for ratification before implementation.

The Foundation shall present a written and oral report to the membership of the Academy at each annual meeting, and the Secretary-Treasurer shall present an accompanying financial report.

BYLAW 12. *Affiliations*

The Academy may affiliate itself with other organizations which have purposes consistent with the purposes of the Academy. Such affiliations must be approved by the Council and by a majority of those attending a regularly scheduled business meeting of the membership.

BYLAW 13. *Indemnification*

Section 1. Every member of the Council or employee of the North Dakota Academy of Science shall be indemnified by the Academy against all expenses and liabilities, including counsel fees, reasonably incurred or imposed upon him/her in connection with any proceedings to which he or she may be made part, or in which he or she may become involved, by reason of being or having been a member of the Council, or employee at the time such expenses are incurred, except in such cases wherein the member of the Council or employee is adjudged guilty of willful misfeasance or malfeasance in the performance of his or her duties. Provide, however, that in the event of a settlement of the indemnification herein shall apply only when the Council approves such settlement and reimbursement as being for the best interests of the Academy.

The foregoing right of indemnification shall be in addition to and not exclusive of all other rights to which such members of the Council or employee may be entitled.

Minutes (unapproved) of the Annual NDAS Business Meeting - April 16, 1999
South Ballroom, Memorial Union, University of North Dakota
Grand Forks, ND
12:40pm 32 members in attendance

The annual business meeting was called to order by President Hartman. A welcome and thanks was given to organizers and moderators of the symposia and other sessions (Junior Academy, Professional, and Denison division).

Secretary-Treasurer Eric Uthus was called on to give reports.

Kim Michelsen, Director of the Junior Academy, was called upon to present the Junior Academy awards. Award winners were: Junior division - 1st Gregg Schildberger (Hankinson), 2nd Magan Friskop (Hankinson), 3rd (tie) Julianna Fuka and Tracy Jentz (both from Hankinson). Senior division - 1st Landon Bladow and Robert Jentz (tie) (both from Hankinson), 3rd Daniel Krump (Hankinson).

The next order of business was to approve minutes from the April 1998 business meeting. The minutes were approved as presented.

The A. Rodger Dension Award winners were: Undergraduate division – 1st Heather Netzloff (Minot State University), runner up Toni Nardi (University of North Dakota), runner up Sarina Mattson (University of North Dakota), runner up Holly Berginski (North Dakota State University), runner up Amy Erickson (North Dakota State University). Graduate division – 1st Tande Stenbak (North Dakota State University). The awards were presented at the Academy banquet that held the previous night.

Meeting statistics: 169 registered attendees, there were 6 symposia (65 presentations), 16 oral presentations in the Denison competition (15 undergraduate papers, and 1 graduate paper), and 13 professional presentations (oral and posters).

Financial
Academy finances are typical for this time of year after payment of publishing costs for the Proceedings. It was estimated that after the meeting bills were paid, there would be $1000 – 1500 remaining in checking.

There was discussion about Proceedings costs. The cost of printing a volume of the Proceedings similar to this year's is about $5000. The cost of producing an electronic version is minimal and the cost of producing a version on CD-Rom would be about $1000. Although electronic versions are considerably cheaper to produce, the Academy will continue to print paper copies of the Proceedings.

The idea of page charges was brought up. The limited discussion that followed was not supportive of this idea. Secretary-Treasurer Uthus said the Executive Committee had a few ideas of how to help offset cost of Proceedings - these would be sent out in an upcoming mailing. In 1997, the cost of producing the Proceedings was offset by a grant obtained from the Bremer-Bush Foundation. We tried to obtain funding from the Bremer-Bush Foundation this year but were unsuccessful.

This year's Research Foundation Grant ($500) was awarded to Donna Bruns Stockrahm from Moorhead State University. The title of the study to be partially funded by this award is "Bison (*Bison bison*) habitat use and behavior during the breeding season in Theodore Roosevelt National Park in the North Dakota Badlands".

Our next meeting has been scheduled for April 28-29, 2000 (Friday Saturday) at Moorhead State University. It will be a tri-state meeting with North Dakota, South Dakota and Minnesota.

A certificate of appreciation was presented to the outgoing President, Joseph Hartman.

Mark Sheridan, North Dakota State University takes over as President of the Academy.

The meeting was adjourned at 1 pm.

ACADEMY OFFICERS AND COMMITTEES

Executive Committee
Membership:
- Past-President
- President
- President-Elect
- Secretary-Treasurer
- Councilors (three-year terms)

President
Mark A. Sheridan
Department of Zoology
North Dakota State University
Fargo, ND 58205
(701) 231-8110
msherida@plains.nodak.edu

Past-President
Joseph H. Hartman
Energy & Environmental Research Center
PO Box 9018
Grand Forks, ND 58202
(701) 777-2551
jhartman@plains.nodak.edu

President-Elect
Ronald K. Jyring
Bismarck State College
1500 Edwards Avenue
Bismarck, ND 58501
(701) 224-5400
jyring@gwmail.nodak.edu

Secretary-Treasurer
Jon A. Jackson
Department of Anatomy & Cell Biology
University of North Dakota
PO Box 9037
Grand Forks, ND 58202
(701) 777-4911
jackson@medicine.nodak.edu

Councilor
Carl Fox (2000-2002)
Office of Program Development
University of North Dakota
Grand Forks, ND 58202
carl_fox@und.nodak.edu

Committees of the North Dakota Academy of Science

Executive Committee*

Editorial Committee*

Education Committee*

Denison Awards Committee*

Necrology Committee*

Nominating Committee

Resolution Committee*

Membership Committee*

North Dakota Research Foundation Board of Directors*

*** indicates available openings**

PAST PRESIDENTS AND LOCATIONS OF THE ANNUAL MEETING

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 R 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	Bismarck
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	Bismarck
1989	Forrest Nielsen	Grand Forks
1990	David Davis	Fargo
1991	Clark Markell	Minot
1992	John Brauner (elect)	Grand Forks
1993	John Brauner	Jamestown
1994	Glen Statler	Fargo
1995	Carolyn Godfread	Bismarck
1996	Eileen Starr	Valley City
1997	Curtiss Hunt	Grand Forks
1998	Allen Kihm	Minot
1999	Joseph Hartman	Grand Forks
2000	Mark Sheridan	Moorhead, MN

Contributors to the North Dakota Academy of Science Research Foundation (2000)

Carol Belinskey (Minot)
Virgil Carmichael (Bismarck)
Gary K. Clambey (Fargo)
Phyllis Johnson (Bethesda, MD)
W. Thomas Johnson (Grand Forks)
Jennifer Larson (Minot)
James Lindley (Fargo)
Russell J. Lorentz (Bismarck)
Claude H. Schmidt (Fargo)
William Siders (Grand Forks)
Glenn Smith (Fargo)
Armand Souby (San Marcos, TX)
Katherine Sukalski (Grand Forks)

Life Memberships (2000)

George Gillies (Charlottesville, VA)
Eric Uthus (Grand Forks)

Financial Statement, 12/31/98

	1998	1999
ASSETS		
Operating Accounts		
Checking	3771.27	1777.37
Trust Accounts		
Scholarship	27850.12	22357.17
Research Foundation	13797.48	13741.63
Total	$45,418.87	$37,875.17
LIABILITIES		
Advanced Dues Payments		
Restricted Purpose Funds		
Scholarship Principal	27850.12	22357.17
Research Foundation	13797.48	13741.63
Total	$41,647.60	$36,098.80
Accumulated Surplus	3771.27	1777.37
Change in Surplus	**	(1993.90)
DUES		
Reinstatements		
Current year	3077.00	20.00
Future years	00.00	20.00
Sponsor/Patron		
Total	$3,077.00	$40.00
INSTITUTIONAL SUPPORT		
UND	1000.00	0.00
Minot State University	500.00	0.00
Valley City State University	0.00	0.00
NDAS Research Foundation	0.00	0.00
Total	1500.00	0.00
ANNUAL MEETING		
Registration fees	564.00	3292.25
Banquet		
Luncheon		
Sigma Xi - Minot		
Sigma Xi - UND		
Bremer/Bush Grant		
Total	$564.00	$3292.25
AWARDS PROGRAM		
Scholarship Dividends	901.80	866.76
NDAS Research Foundation	0.00	0.00
Total	$901.80	$866.76

	1998	1999
PUBLICATION SALES	154.50	739.00
MISCELLANEOUS INCOME		
Donations	537.00	0.00
Dividend Income	14.32	0.00
Total	$551.32	0.00
TOTAL INCOME	$6,748.62	$4,938.01
MEMBERSHIP		
Emeritus	47	51
Students	36	59
Professional	144	140
Delinquent	35*	10**
Dropped		
Other		7
Total Member Count	227	267
	*1997 or before	**1998 or before
ANNUAL MEETING		
Speakers Expenses	140.00	0.00
Meals/Refreshments	521.32	1600.95
Printing		
General Expenses		
Total	$661.32	$1,600.95
AWARD PROGRAMS		
ND Science/Engineering Fair	50.00	50.00
Denison	100.00	400.00
ND Junior Academy	937.83	450.00
Research Foundation Grant	500.00	500.00
Total	$1,587.83	$1,400.00
PUBLICATION		
Proceedings	1792.46	5286.50
Supplement		
Total	$1,792.46	$5286.50
OFFICE EXPENSES		
Postage	455.03	720.38
Post Office Box Rental	39.00	39.00

	1998	1999
Duplication	303.84	186.75
Supplies	179.33	130.50
Clerical Assistance		
Phone		
Other	131.85	266.03
Bank Fees	9.30	28.30
Total	$1,118.35	$1,370.96
MISCELLANEOUS		
Fidelity Bond	26.00	0.00
NAAS Dues	not paid	51.25
Other		
Research Foundation Loan interest		
Total	$26.00	
Total Disbursements	$5,185.96	$9,709.66
SCIENCE RESEARCH FOUNDATION		
CASH INCOME		
Donations from Members		0.00
Allocations from Dues	0.00	112.00
Interest Accrued	741.91	337.50
Sponsors/Patrons		
Total	$741.91	$449.50
CASH EXPENSE		
Grants	500.00	500.00
Interest Compounding	675.00	337.50
Other Disbursements		
Bank Fees	18.00	13.50
Total	$1,193.00	$851.00
Net Change	($451.09)	($401.50)
ASSETS		
Pass Book Savings, 31 Dec	3797.48	13741.63
T-Note, book value	10000.00	0.00
CD - note value		
Investment Total	$13,797.48	$13,741.63
Change from previous year	$223.91	($55.85)
SCHOLARSHIP FUND		
CASH INCOME		
ENOVA (SDGE)	429.00	429.00
IES Industries	472.80	437.76
Total	$901.80	$866.76

	1998	1999
CASH EXPENSE		
Denison Awards	100.00	400.00
Junior Academy Awards	400.00	450.00
ND Science and Engineering Fair	50.00	50.00
Other Expenses		
TOTAL	$550.00	$900.00
Net Change	$351.80	($33.24)
ASSETS		
ENOVA Shares (1983, 250 shares)	853.52	809.29
Price 18.50	25.38	20.19
Value 4625.00	21658.12	16337.97
IES Industries (1990, 120 shares)	192	218.88
Price 31.63	32.25	27.50
Value 3795.60	6192.00	6019.20
Total Investment Value	$27,850.12	$22,357.17
Change from previous year	$5,891.92	($5,492.95)

A

Harmon B. Abrahamson
Department of Chemistry
University of North Dakota
Grand Forks, ND 58202
701-777-4427
harlan_abrahamson@
und.nodak.edu

Joseph Alessi
PO Box 50643
Idaho Falls, ID 83405

Bonnie J. Alexander
Division of Math, Science and Technology
Valley City State University
Valley City, ND 58072
701-845-7453
bonnie_alexander@
mail.vcsu.nodak.edu

Karl R. Altenburg
709 9th Avenue North
Fargo, ND 58102

Edwin M. Anderson
1151 12th Avenue West
Dickinson, ND 58601

Tom Anderson
623 18th Street NW
Minot, ND 58703
701-852-4383
mot623@yahoo.com

Ordean S. Anderson
Anderson Engineering of New Prague
Rural Route 1, Box 269
New Prague, MN 56071
507-364-8744

Allan Ashworth
Dept of Geosciences
North Dakota State University
Fargo, ND 58105
701-231-7919
ashworth@plains.nodak.edu

B

Evan Barker
Department of Biology
Minot State University
Minot, ND 58707

William G. Barney
1525 Cottonwood Street
Grand Forks, ND 58201

Michael P. Barnhart
2704 10th Avenue NW
Mandan, ND 58554
701-663-4980
barnhart@btigate.com

Christopher Beachy
Department of Biology
Minot State University
Minot, ND 58707
beachych@misu.nodak.edu

Carol R. Belinskey
900 4th Avenue NW
Minot, ND 58703
701-839-2379
cbelin@tv.net

Holly R. Berginski
1350 Broadway, Apt 212
Fargo, ND 58102
701-297-8010

David L. Berryhill
Department of Veterinary & Microbiological Sciences
North Dakota State University
Fargo, ND 58105
701-231-7694
berryhil@badlands.nodak.edu

Brant Bigger
1303 North University Drive
Fargo, ND 58102
701-232-1632

John P. Bluemle
North Dakota Geological Survey
600 East Boulevard Avenue
Bismarck, ND 58505
701-328-8000
bluemle@rival.ndgs.state.nd.us

Karen Boeshans
Department of Biochemistry and Molecular Biology
University of North Dakota
Grand Forks, ND 58202

Lena Bohm
4385 95th St NW
Mohall, ND 58761
701-756-6063

Bryan J. Bortnem
1146 Oak Street
Fargo, ND 58102

John F. Brauner
Jamestown College
6024 College Lane
Jamestown, ND 58405
701-252-3467 x2482
brauner@jc.edu

David W. Brekke
Energy & Environmental Research Center
University of North Dakota
Grand Forks, ND 58202
701-777-5154
dbrekke@eerc.und.nodak.edu

Eric Brevik
Dpt. of Agronomy, Soil Survey
Iowa State University
Ames, IA 50011
515-268-1917
ebrevik@iastate.edu

Ralph C. Brown
PO Box 89
East Stoneham, ME 04231
207-928-2324

C

Candace R. Carlson
312 West 12th Street
Devils Lake, ND 58301
701-662-8256
jccarlson@stellarnet.com

Edward Carlson
Department of Anatomy and Cell Biology
University of North Dakota
Grand Forks, ND 58202
701 777 2101
ecarlson@medicine.nodak.edu

Kenneth T. Carlson
515 13th Street East
Casper, WY 82601

Jeffrey Carmichael
Department of Biology
University of North Dakota
Grand Forks, ND 58202
701-777-4666
carmicha@plains.nodak.edu

Virgil W. Carmichael
1013 Anderson Street North
Bismarck, ND 58501
701-223-7968
virgcarm@btigate.com

Patrick Carr
Dickinson Res. Ext. Ctr.
1089 State Avenue,
Dickinson, ND 58601
701-483-2581
pcarr@ndsuext.nodak.edu

Jack F. Carter
1345 11th Street North
Fargo, ND 58102
701-232-0482

J. Frank Cassel
83 West Boulder Street
Colorado Springs, CO 80903

Gary K. Clambey
Dept. of Biology & Botany
North Dakota State University
Fargo, ND 58105
701-231-8404
clambey@badlands.nodak.edu

Eric N. Clausen
North Dakota Geography Alliance
Minot State University
Minot, ND 58707
701-858-3587
clausen@misu.nodak.edu

Clay Comstock
560 Carlton Court, #11
Grand Forks, ND 58203
701 777 9748
comstock@plains.nodak.edu

William E. Cornatzer
1810 Edgemere Ct SE
Huntsville, AL 35803

Richard Crawford
Department of Biology
University of North Dakota
Grand Forks, ND 58202
701 777 4673
rcrawford@badlands.nodak.edu

Andrea R Culbertson
901 25th Avenue NW
Minot, ND 58703
701-838-1433
cculber@minot.com

D

Gwen M. Dahlen
USDA Grand Forks Human Nutrition Research Center
PO Box 9034
Grand Forks, ND 58202
701-795-8498
gdahlen@badlands.nodak.edu

Dan Daly
Energy & Environmental Research Center
University of North Dakota
Grand Forks, ND 58202
701-777-2822
ddaly@eerc.und.nodak.edu

David G. Davis
USDA Biosciences Research Laboratory
PO Box 5674
Fargo, ND 58105
701-239-1247
davisd@fargo.ars.usda.gov

Andre Delorme
Division of Math, Science & Technology
Valley City State University
Valley City, ND 58072
701-845-7573
Andre_DeLorme@
mail.vcsu.nodak.edu

Stacy Delzer
1111 Park Drive, Apt. B
Grand Forks, ND 58201
701-795-7153
sdelzer@badlands.nodak.edu

Gustav P. Dinga
Department of Chemistry
Concordia College
Moorhead, MN, 56560

Dennis T. Disrud
413 Hillcrest Drive
Minot, ND 58703
701 839 3784

Bruce Dockter
Energy & Environmental
Research Center
University of North Dakota
Grand Forks, ND 58202
701-777-4102
brdockte@badlands.nodak.edu

James R. Dogger
PO Box 208
Gore, VA 22637
540-858-2613

Colleen Dugan
806 3rd St NW
Minot, ND 58703
701-837-6310
warner@minot.ndak.net

Kathy Duttenhefner
North Dakota Parks and
Recreation Department
1835 Bismarck Expressway
Bismarck, ND 58504
701-328-5357
kduttenh@state.nd.us

E

Charles G.M. Edgerly
1317 8th Avenue South
Fargo, ND 58103
701-235-5105

John D. Eide
USDA ARS Plant Physiologist
Northern Crop Science
Laboratory
Fargo, ND 58105
701-239-1354
eidej@ars.usda.gov

Dale G. Elhardt
801 25th Street NW
Minot, ND 58701
701-839-7449

J. Mark Erickson
Department of Geology
Saint Lawrence University
Canton, NY 13617
315-229-5198
meri@music.stlawu.edu

Ned H. Euliss
National Biological Survey
Northern Prairie Wildlife
Research
Jamestown, ND 58401
701-253-5564
ned_euliss@usgs.gov

F

Marvin Fawley
Department of Botany/Biology
North Dakota State University
Fargo, ND 58105

Allen L. Fisk
1122 Avenue B West
Bismarck, ND 58501
701 223 7447

Albert J. Fivizzani
Department of Biology
University of North Dakota
Grand Forks, ND 58202
701-777-2621
fivizzan@badlands.nodak.edu

Guilford O. Fossum
1828 Cottonwood Street
Grand Forks, ND 58201
701-775-7842

Carl Fox
Office of Research and
Program Development
University of North Dakota
Grand Forks, ND 58202
701-777-4280
carl_fox@mail.und.nodak.edu

Ann Fritz
1903 Thompson Street
Bismarck, ND 58501
701-328-5210
afritz@state.nd.us

G

Jon Gaffaney
Department of Biochemistry
University of North Dakota
Grand Forks, ND 58202

Roy Garvey
North Dakota State University
Department of Chemistry
Fargo, ND 58105
701-231-8697
garvey@badlands.nodak.edu

Anne Gerber
Department of Biology
University of North Dakota
Grand Forks, ND 58202
701-777-4667
agerber@prairie.nodak.edu

Phil Gerla
Department of Geology &
Geological Engineering
University of North Dakota
Grand Forks, ND 58202
701-777-3305
phil_gerla@mail.und.nodak.edu

George T. Gillies
Physics Department
University of Virginia
Charlottesville, VA 22901
804-924-3781
gtg@virginia.edu

Hans J. Goettler
Department of Mechanical
Engineering
North Dakota State University
Fargo, ND 58105
701-231-8836
goettler@prairie.nodak.edu

Anna Grazul-Bilska
Department of Animal Science
North Dakota State University
Fargo, ND 58105
701-231-7992
grazul@plains.nodak.edu

Gerald H. Groenewold
Energy & Environmental
Research Center
University of North Dakota
Grand Forks, ND 58202
701-777-5131
ghg@eerc.und.nodak.edu

Larry D. Groth
1801 College Drive North
Devils Lake, ND 58301

Harvey A. Gullicks
Civil Engineering
University of North Dakota
Grand Forks, ND 58202
701-777-3779
harvey_gullicks@
mail.und.nodak.edu

Angela Gunderson
230 Viking Drive
Valley City, ND 58072
701-845-7976
gundie@hotmail.com

H

Katherine Haas
1037 Pinecrest Drive
Annapolis MD 21403
khaas@umd5.umd.edu

Joseph H. Hartman
Energy & Environmental
Research Center
University of North Dakota
Grand Forks, ND 58202
701-777-2551
jhartman@eerc.und.nodak.edu

David J. Hassett
University of North Dakota
Energy & Environmental
Research Center
Grand Forks, ND 58202
701-777-5192
dhassett@eerc.und.nodak.edu

Michael Hastings
Dickinson State University
Department of Biology
Dickinson, ND 58601
701 483-2104
mike_hastings@
eagle.dsu.nodak.edu

Bonnie Heidel
Montana Natural Heritage
Program
1515 East 6th Avenue,
Helena, MT 59620
406-444-0536

Larry J. Heilmann
3535 31st Street SW
Fargo, ND 58104
701-241-9538
lheilmann@worldnet.att.net

Mohammad Hemmasi
Department of Geography
University of North Dakota
Grand Forks, ND 58202
701-777-4592
hemmasi@badlands.nodak.edu

John T. Hobbs
200 15th Avenue
Devils Lake, ND 58301
701-662-1551

Jerome J. Hoeppner
2518 9th Avenue North
Grand Forks, ND 58203

F.D. Holland, Jr.
2303 8th Avenue N.
Grand Forks, ND 58203
701-772-1622
budholland@aol.com

Jean Holland
4686 Belmont Road
Grand Forks, ND 58201
701-775-0995

Peter Hombach
BioDigestor Technologies
PO Box 12015
Grand Forks, ND 58208
701-775-8775

David Hopkins
1128 8th Street N
Fargo, ND 58102
701 231-8948
hopkins@badlands.nodak.edu

Trent D. Hubbard
3719 University Avenue, #210
Grand Forks, ND 58202
701-772-5389

Janis Hulla
Department of Pharmacology Physiology and Therapeutics
University of North Dakota
Grand Forks, ND 58202
701-777-6222
jhulla@medicine.nodak.edu

Curtiss D. Hunt
USDA Grand Forks Human Nutrition Research Center
Grand Forks, ND 58202
701-795-8423
chunt@gfhnrc.ars.usda.gov

Janet R. Hunt
USDA Grand Forks Human Nutrition Research Center
Grand Forks, ND 58202
701-795-8423

Deborah Hunter
PO Box 1165
Minot, ND 58702
701-837-9492

J

Jon A. Jackson
Department of Anatomy and Cell Biology
University of North Dakota
Grand Forks, ND 58202
701 777 4911
jackson@medicine.nodak.edu

Francis A. Jacobs
1525 Robertson Court
Grand Forks, ND 58201
701-772- 2447

Megan Jaskowiak
Department of Biology/Botany
North Dakota State University
Fargo, ND 58105

Douglas H. Johnson
Northern Prairie Science Center
8711 37th Street SE
Jamestown, ND 58401
701-253-5539
Douglas_H_Johnson@usgs.gov

Phyllis E. Johnson
USDA ARS Beltsville
10300 Baltimore Avenue,
Beltsville, MD 20705
301-504-6078
johnsonp@ba.ars.usda.gov

W. Thomas Johnson
USDA Grand Forks Human Nutrition Research Center
Grand Forks, ND 58202
701-795-8411
tjohnson@gfhnrc.ars.usda.gov

Sara Johnson
1801 39th Street SW #206
Fargo, ND 50103
701-282-5779

Michael Jones
Energy & Environmental Research Center
University of North Dakota
Grand Forks, ND 58202
701-777-5152
mjones@eerc.und.nodak.edu

Ron Jyring
Bismarck State College
1500 Edwards Avenue
Bismarck, ND 58501
701-224-5459
jyring@gwmail.nodak.edu

K

Paul Kannowski
Department of Biology
University of North Dakota
Grand Forks, ND 58202
701-777-2199
kannowsk@badlands.nodak.edu

Frank R. Karner
Department of Geology & Geological Engineering
University of North Dakota
Grand Forks, ND 58202
701-772-9501
frank_karner@
mail.und.nodak.edu

Ryan G. Kavlie
1514 5th Ave NE
Jamestown, ND 58401
701-252-8567
kavlie@prairie.nodak.edu

Glenn B. Kays
Department of Geology & Geological Engineering
University of North Dakota
Grand Forks, ND 58202

Christopher Keller
2509 Bel Air Court
Minot, ND 58703
701-852-1978
ckeller@misu.edu

Ross D. Keys
1836 Billings Drive
Bismarck, ND 58504
701-255-4211
ross.keys@mail.house.gov

Allen J. Kihm
Minot State University
500 University Avenue West
Minot, ND 58707
701-858-3864
kihm@misu.nodak.edu

Don Kirby
Animal and Range Science
North Dakota State University
Fargo, ND 58105
701-231-8386
dkirby@ndsuext.nodak.edu

Harold J. Klosterman
1437 12th Street North
Fargo, ND 58102
701-232-1141

Harvey Knull
Dean, Graduate School
University of North Dakota
Grand Forks, ND 58202
701-777-2786
harvey_knull@und.nodak.edu

Ralph H. Kolstoe
2200 29th Street South, #51S
Grand Forks, ND 58201
701-772-3972

Evguenii I. Kozliak
Department of Chemistry
University of North Dakota
Grand Forks, ND 58202
701 777-2145
ekozliak@
mail.chem.und.nodak.edu

Warren D.Kress
Department of Geosciences,
North Dakota State University
Fargo, ND 58105
701-231-7145

Tim Kroeger
Dept. of Biology & Geology
Bemidji State University
Bemidji, MN 56601
218-755-2783
tjkroeger@
vax1.bemidji.msus.edu

Joseph M. Krupinsky
USDA Northern Great Plains Research Laboratory
PO Box 459
Mandan, ND 58554
701-667-3011
krupinsj@mandan.ars.usda.gov

L

David O. Lambeth
Department of Biochemistry & Molecular Biology
University of North Dakota
Grand Forks, ND 58202
701-777-3937
dlambeth@medicine.nodak.edu

Jennifer M. Larsen
529 24th Ave NW
Minot, ND 58703
701-838-0959
larsenj@minot.ndak.net

Omer R. Larson
2663 Rango Pl.
Lake Havasu City, AZ 86406

Jean Legge
Litchville-Marion High School
3212 115th Avenue SE
Valley City, ND 58072
701-845-5749
legge@sendit.nodak.edu

Terry Lincoln
Dakota Zoological Society
PO Box 711
Bismarck, ND 58502
701-223-7543
ndzoo@btigate.com

James A. Lindley
Department of Agriculture & Biosystems Engineering
North Dakota State University
Fargo, ND 58105
701-231-7273
lindley@badlands.nodak.edu

Russell J. Lorenz
1924 North Grandview Lane
Bismarck, ND 58501
701-223-3421

Margaret J. Lowe
Department of Biochemistry & Molecular Biology
University of North Dakota
Grand Forks, ND 58202

Stephen L. Lowe
Departmentof Chemistry
University of North Dakota
Grand Forks, ND 58202

H.C. Lukaski
USDA, ARS Human Nutrition
Research Center
PO Box 9034
Grand Forks, ND 58202

M

Ronald F. MacCarthy
Department of Sociology
University of North Dakota
Grand Forks, ND 58202
701-777-3596
maccarth@prairie.nodak.edu

Om Madhok
1304 13th Avenue NW
Minot, ND 58703

Clark Markell
Science Division
Minot State University
Minot, ND 58707
701-858-3069
markell@misu.nodak.edu

Samuel G. Markell
1502 Dakota Drive N, #13
Fargo, ND 58102
701-280-3184

John Martsolf
Department of Pediatrics
University of North Dakota
School of Medicine
Grand Forks, ND 58202
701-777-4277
martsolf@medicine.nodak.edu

Sarena Mattson
3501 University Avenue
Grand Forks, ND 58201
701-777-9224
samattso@prairie.nodak.edu

Gregory McCarthy
Department of Chemistry
North Dakota State University
Fargo, ND 58105
701-231-7193
gmccarth@prairie.nodak.edu

Donald P. McCollor
Energy & Environ. Res. Ctr.
University of North Dakota
Grand Forks, ND 58202
701-777-5121
dmccollor@eerc.und.nodak.edu

Rose McKenney
500 University Avenue West
Minot, ND 58707
mckenney@misu.nodak.edu

Ronald E. McNair
Student Opportunity Office
North Dakota State University
Fargo, ND 58105
701-231-8028

Paul D. Meartz
Mayville State University
330 3rd Street NE
Mayville, ND 58257
701-786-2539
paul_meartz@
mail.masu.nodak.edu

Alan Meldrum
512 Columbia Road North
Grand Forks, ND 58203
701-772-1166

Kim G. Michelsen
USDA Grand Forks Human
Nutrition Research Center
2420 2nd Avenue N
Grand Forks, ND 58202
701-795-8357
kmichels@gfhnrc.ars.usda.gov

Ray Minette
209 4th Street SW
Rugby, ND 58368
701-776-6484

Earl N. Mitchell
220 Glenhill Lane
Chapel Hill, NC 27514

Karen J.R. Mitchell
North Dak. Geological Survey
600 E. Boulevard Avenue,
Bismarck, ND 58505
701-328-8000
karenm@rival.ndgs.state.nd.us

Patricia Moulton
530 Tulane Drive, #103
Grand Forks, ND 58203
701-777-9583
pmoulton@plains.nodak.edu

Douglas Munski
Department of Geography
University of North Dakota
Grand Forks, ND 58202
701-777-4591
munski@badlands.nodak.edu

Laura Munski
Department of Geography
University of North Dakota
Grand Forks, ND 58202
701-772-8207
munski@badlands.nodak.edu

N

C.N. Nelson
North Dakota State University
Bottineau Branch
Bottineau, ND 58318

Robert M. Nelson
Department of Electrical
Engineering
North Dakota State University
Fargo, ND 58105
701-231-7619
r.m.nelson@ieee.org

Forrest H. Nielsen
USDA Grand Forks Human
Nutrition Research Center
Grand Forks, ND 58202
701-795-8456
fnielsen@gfhnrc.ars.usda.gov

Robert Nordlie
Department of Biochemistry
& Molecular Biology
University of North Dakota
Grand Forks, ND 58202
701-777-3937
rnordlie@medicine.nodak.edu

Paul E. Nyren
Central Grasslands Res. Ctr.
4824 48th Avenue SE
Streeter, ND 58483
701-424-3606
grasland@ndsuext.nodak.edu

O

Carla Otto
509 29th Ave N #7
Fargo, ND 58102

P

Paul D. Pansegrau
Dakota Gassification Company
PO Box 1017
Beulah, ND 58523
701-873-6471
paulpans@btigate.com

Benchaporn Parnanusorn
Department of Biochemistry
& Molecular Biology
University of North Dakota
Grand Forks, ND 58202

Douglas Patenaude
1308 5th Ave NW
East Grand Forks, MN 56721
218-773-6942
dpatenau@plains.nodak.edu

Dean A. Pearson
Pioneer Trails Regional Museum
Box 78
Bowman, ND 58623
701-523-3625

Dexter Perkins
Department of Geology &
Geological Engineering
University of North Dakota
Grand Forks, ND 58202
701-777-2811

Philip C. Pfister
North Dakota State University
Dept. of Mechan. Engineering
Fargo, ND 58105
701-232-5407

Debra F. Pflughoeft-Hasset
Energy & Environmental
Research Center
University of North Dakota
Grand Forks, ND 58202
701-777-5261
dphassett@eerc.nodak.edu

Karen A. Phillips
Department of Biology/Botany
North Dakota State University
Fargo, ND 58105

Ken S. Pierce
1465 14th Street West, #1
Dickinson, ND 58601

Rhonda Poellot
USDA Grand Forks Human
Nutrition Research Center
Grand Forks, ND 58202
701-795-8406
rpoellot@gfhnrc.ars.usda.gov

Lyle Prunty
North Dakota State University
Soil Science
Fargo, ND 58105
prunty@plains.nodak.edu

R

Jody Rada
University of North Dakota
Department of Anatomy &
Cell Biology
PO Box 9037
Grand Forks, ND 58202
jarada@medicine.nodak.edu

Kristen Radtke
Department of Biology
Moorhead State University
Moorhead, MN 56549

Marepalli B. Rao
Department of Statistics
North Dakota State University
Fargo, ND 58105
701-231-8178
mrao@plains.nodak.edu

Banmali Rawat
University of Nevada-Reno
Department of Electrical Engineering 260
Reno, NV 89557
775-784-6927
rawat@ee.unr.edu

Paul D. Ray
University of North Dakota
Department of Biochemistry & Molecular Biology
Grand Forks, ND 58202
701-777-3937

John R. Reid
420 25th Avenue South
Grand Forks, ND 58201
701-777-2131
john_reid@mail.und.nodak.edu

Jun Ren
Department of Pharmacology, Physiology & Therapeutics
University of North Dakota
Grand Forks, ND 58202
jren@medicine.nodak.edu

Garl Rieke
Department of Anatomy and Cell Biology
University of North Dakota
Grand Forks, ND 58202
grieke@medicine.nodak.edu

Randolph Rodewald
721 2nd Avenue NW
Minot, ND 58703
701 858 3066
rodewald@misu.nodak.edu

David A. Rogers
101 J EE Building
North Dakota State University
Fargo, ND 58105
701-231-7216
drogers@plains.nodak.edu

George A. Rogler
1701 Monte Drive
Mandan, ND 58554
701-663-5327

Fariba Roughead
USDA Grand Forks Human Nutrition Research Center
Grand Forks, ND 58202
701-795-8463
froughea@gfhnrc.ars.usda.gov

Ron Royer
Minot State University
500 University Avenue West
Minot, ND 58707
701-858-3209
royer@misu.nodak.edu

James T. Rudesill
1318 12th Street North
Fargo, ND 58102
701-235-4629

Kenneth Ruit
University of North Dakota
Department of Anatomy and Cell Biology
PO Box 9037
Grand Forks, ND 58202

S

Sara Sabin
6655 College Lane
Jamestown, ND 58405
701-252-3467
ssabin@jc.edu

Maryjane Schalk
106-3 Sherwood Circle
Minot AFB, ND 58704
701-727-4939
ndlioness@aol.com

Claude H. Schmidt
1827 3rd Street North
Fargo, ND 58102
701-293-0365
cschmidt@ndsu.nodak.edu

Shiloh Schnabel
1219 Soo Street
Minot, ND 58701
schnabel@ndak.net

Fred Schneider
Department of Anthropology
University of North Dakota
Grand Forks, ND 58202
701-777-4718
frschnei@prairie.nodak.edu

Julie Schroer
Bismarck State College
PO Box 5587
Bismarck, ND 58506
701-224-5411
schroer@gwmail.nodak.edu

Donald P. Schwert
Department of Geosciences
North Dakota State University
Fargo, ND 58105
701-231-7496
donald_schwert@ndsu.nodak.edu

Donald R. Scoby
3302 2nd Street North
Condo #22
Fargo, ND 58102
701-235-3389

Karla J. Scoville
Moorhead State University
Biology Department
Moorhead, MN 56563

Roland Severson
2682 Catalina Drive
Grand Junction, CO 81506

William A. Siders
USDA Grand Forks Human Nutrition Research Center
Grand Forks, ND 58202
701-746-8921

Kristin Simons
166 Loftsgard Hall
North Dakota State University
Fargo, ND 58105

Rodger L. Sims
USDA Grand Forks Human Nutrition Research Center
Grand Forks, ND 58202
701-795-8425
rsims@gfhnrc.ars.usda.gov

Bayard P. Sleeper
PO Box 2236
Paulsbo, WA 98370

Susan C. Slone
484 5th Ave NW
Valley City, ND 58072
susan_slone@mail.vcsu.nodak.edu

Donald A. Smith
Department of Electrical Electronic Engineering
North Dakota State University
Fargo, ND 58105
701-231-7401
dosmith@badlands.nodak.edu

Glenn S. Smith
3140 10th Street North
Fargo, ND 58102
701-235-6785
glenn_6223@msn.com

Irina P. Smoliakova
Department of Chemistry
University of North Dakota
Grand Forks, ND 58201
701 777-3942
ismoliakova@mail.chem.und.nodak.edu

Theodore Snook
343 Sheridan Road
Racine, WI 53403
414-552-8781

Armand M. Souby
103 Nichols
San Marcos, TX 78666
msouby@centurytel.net

Melanie S. Spoor
Department of Biology
University of North Dakota
Grand Forks, ND 58202
mspoor@badlands.nodak.edu

Eileen M. Starr
Division of Math and Science
Valley City State University
Valley City, ND 58072
701-845-7522
eileen_starr@mail.vcsu.nodak.edu

Jamie Stegeman
1806 29th Street Circle South
Moorhead, MN 56560
218-236-8622
stegeman@mhd1.moorhead.msus.edu

Joseph C. Stickler
Division of Math, Science and Technology
Valley City State University
Valley City, ND 58072
701-845-7334
joe_stickler@mail.vcsu.nodak.edu

Ralph Stoaks
5888 Our Way
Citrus Heights, CA 95610
916-965-4045
rdstoaks@pacbell.net

Donna M. Bruns Stockrahm
Department of Biology
Moorhead State University
Moorhead, MN 56563
218-236-2576
stockram@mhdl.moorhead.msus.edu

Katherine A. Sukalski
Department of Biochemistry & Molecular Biology
University of North Dakota
Grand Forks, ND 58202
701-777-4049
sukalski@medicine.nodak.edu

Lawrence Summers
1019 Porter Avenue, #121
Bismarck, ND 58501

Anita Sundstrom
905 4th Ave NW
Minot, ND 58703
701-852-8071

Richard J. Swanson
Hackberry Point Farm
Box 102A
Richville, MN 56576
218-758-2385
dswanson@eot.com

T

Brian Tangen
649 8th Avenue SW
Valley City, ND 58072
701-845-1092
brian_tangen@
mail.vcsu.nodak.edu

Robert K. Tarquinio
1048 Chelsea Avenue
Santa Monica, CA 90403
310-828-7648
rktarquinio@yahoo.com

Heather Taylor
Biology Department
Moorhead State University
Moorhead, MN 56563

Kathryn A. Thomasson
Department of Chemistry
University of North Dakota
Grand Forks, ND 58202
701-777-3199
kathryn.thomasson@
mail.chem.und.nodak.edu

Michael B. Thompson
2208 Crescent Drive
Minot, ND 58703
701-839-6305

Robert G. Todd
221 7th Avenue West
Dickinson, ND 58601
701-225-5056

Paul Todhunter
Department of Geography
University of North Dakota
Grand Forks, ND 58202
701-777-4593
todhunte@badlands.nodak.edu

Stephen R. Townsend
515 8th Steet NE, #18r
Valley City, ND 58072
701-845-6906

U

Michael G. Ulmer
202 East Divide
Bismarck, ND 58501
701-258-6454
mike.ulmer@nd.usda.gov

Eric O. Uthus
USDA Grand Forks Human
Nutrition Research Center
2420 2nd Avenue N
Grand Forks, ND 58202
701-795-8382
uthus@badlands.nodak.edu

Rodney Utter
Department of Soil Science
North Dakota State University
Fargo, ND 58105
701-231-7561
utter@badlands.nodak.edu

V

James B. Van Alstine
Department of Geology
University of Minnesota-
Morris
Morris, MN 56267
320-589-2535
vanalstj@mrs.umn.edu

Richard C. Vari
Department of Pharmacology,
Physiology, and Therapeutics
University of North Dakota
Grand Forks, ND 58202
701-777-3946
rvari@medicine.nodak.edu

W

Robert G. Walsh
CC Acres
6610 14th Avenue NW
Minot, ND 58703
701-838-7103

John R. Webster
912 West Central Avenue
Minot, ND 58701
701-858-3873

Wilber O. Weisser
55 Parkview Circle
Grand Forks, ND 58201
701-772-4013

Rebecca Wentlent
518 Cambridge Street
Grand Forks, ND 58203
701-746-6798
wentlent@badlands.nodak.edu

Loren Wold
Department of Pharmacology,
Physiology and Therapeutics
University of North Dakota
Grand Forks, ND 58202

Z

Richard Zaruba
530 Tulane Drive #103
Grand Forks, ND 58203
701-777-9583
zaruba@medicine.nodak.edu

Membership as of March, 2000

T

V

W

Z