# Proceedings of the NORTH DAKOTA Academy of Science



82nd Annual Meeting

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Volume 44

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The PROCEEDINGS contains communications (from symposia, from professional contributed paper sessions, and from collegiate competition sessions) representing papers submitted and accepted for oral presentation at the April annual meeting of the Academy. The Proceedings appears in April of each year.

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# PROCEEDINGS OF THE NORTH DAKOTA ACADEMY OF SCIENCE

Volume 44

April 1990

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

# 1989-90

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82nd ANNUAL MEETING

April 19-20, 1990

Fargo, North Dakota

# **EDITOR'S NOTES**

The Proceedings of the North Dakota Academy of Science was first published in 1948, with Volume I reporting the business and scientific papers presented to the fortieth annual meeting, May 2 and 3, 1947. Through Volume XXI the single yearly issue of the Proceedings included both Abstracts and Full Papers. Commencing with Volume XXII the Proceedings were published in two parts. Part I, published before the meeting, contained an Abstract of each paper to be presented at the meeting. Part II, published later, contained full papers by some of the presentors.

Commencing in 1979 with Volume XXXIII, a new format appeared. The Proceedings changed to a  $8.5\,\chi$  11" format, is produced from camera-ready copy submitted by authors, and is issued in a single part prior to the annual meeting to be distributed initially at the meeting in late April. Each presentation at the annual meeting is represented by a full page "Communication," which is more than an abstract, but less than a full paper. The communications contain actual results and conclusions, and permit data presentation. The communication conveys much more to the reader than did an abstract, but still provides the advantage of timeliness and ease of production.

The first section of this volume 44 of the Proceedings contains all 43 presentations in the seven symposia offered at the the 82nd annual meeting of the Academy, April 19-20, 1990. These papers are organized by Symposia and are presented in the same sequence as presented at the meeting.

The second section of this volume contains the 43 communications presented in the professional sections of the meeting. All professional communications were reviewed for conformity with the instructions by the Editorial Committee prior to their acceptance for presentation and publication herein. The professional communications have been grouped together in this volume and are arranged alphabetically, by the first author's last name.

The third section of this volume contains 22 collegiate communications, representing all those papers presented in the A. Rodger Denison Student Research Paper Competition. Undergraduate and graduate students reported on the results of their own research activities, usually carried on under the guidance of a faculty advisor. While the student competitors were required to prepare a communication similar to those prepared by their professional counterparts, these communications were not reviewed prior to publication herein. The Denison Awards Committee judges the oral presentation and the written communication in arriving at their decision for first place and runner-up awards in both the graduate and undergraduate student competitions. In this section the first 13 papers are from the graduate competition (placed in alphabetic order by first author's last name) and the second group of 9 papers are from the undergraduate competition (similarly alphabetically arranged).

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

This issue of the Proceedings also includes the constitution and bylaws of the Academy, a list of officers, 1989-90 committee membership, a list of all Academy members as of March 1, 1990, and, for the first time, a copy of the most recent (1989) audited financial statement of the Academy.

SPECIAL NOTICE: The Editor has been informed by formal communication from the United States Geological Survey in Reston, Virginia that Robert L. Houghton, a former USGS employee, is known to have falsified some data related to his work for USGS. After full USGS review the editor has been informed that "some of the information contained in several of the publications (in the Proceedings of the North Dakota Academy of Science) could not be positively validated." Thus, USGS has formally disclaimed eight Academy publications by Houghton which have appeared in previous issues of the Proceedings as follows: 1982 (volume 36), pages 13, 15, 40; 1983 (volume 37),page 59; 1984 (volume 38), page 59; 1985 (volume 39),page 53; 1986 (volume 40), page 94; 1987 (volume 41), page 64.

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# NORTH DAKOTA ACADEMY OF SCIENCE

I. Rules for Preparation of Proceedings Communication

- 1. Each paper presented at the annual meeting of the Academy must be represented by a communication in the Proceedings, including A. Rodger Denison student research competition papers.
- 2. Only communications intended for presentation at the annual meeting will be considered for publication. They must present original research in as concise a form as possible. Quantitative data should be presented with statistical analysis (i.e., means with standard errors). Papers which merely summarize conclusions or ideas without supporting data are discouraged and will not normally be accepted. The communication should include the purpose of the research, the methodology, results, and conclusions.
- 3. Authors are encouraged to utilize the full space available in order to provide sufficient information to fully describe the research reported.
- 4. Communications must be prepared on the special blue-line form and sent, with three legible xerox copies, by first class mail to the Secretary, North Dakota Academy of Science, Box 8123 University Station, Grand Forks, ND 58202. The form must not be folded; a cardboard backing should be used to avoid damage. The Proceedings will be published by direct photo-offset of the submitted communication. No proofs will be prepared.
- 5. All typing, drawing and secured art or photographic materials must be within the boundaries of the blue-line form. Consult the example on the reverse side of the special form for proper style (i.e., titles, authors, address, tables, figures, references, indentations, headings, and punctuation). Indicate the author to present the communication by an asterisk (\*) after that person's name.
- 6. Tables, diagrams, and photographs are acceptable provided they are secured to the special form and do not occupy a total area of more than 100 square centimeters.
- 7. Only essential references should be cited, and should be indicated in the text by numerals and quoted at the end of the communication. Up to three authors' names may be cited in full; with four or more authors only the first should be cited. The following form of citation should be used:
  - Journals: Neary, D., Thurston, H. and Pohl, J.E.F. (1973) Proc. N.D. Acad. Sci. 40, 83. (Abbreviate titles.)
  - Books: Batsone, G.F., Blair, A.W. and Slater, J.M. (1971) A Handbook of Pre-natal Paediatrics, pp. 83-90. Medical and Technical Publishing, Lancaster.
  - Individual chapters in books: Farah, A.E. and Moe, G.K. (1970) in <u>The Pharmacological Basis of Therapeutics</u>, 4th edition (Goodman, L.S. and Gilman, A., eds.), pp. 677-708. Macmillan, New York.
  - Conferences and symposia: Rajewsky, M.F. (1973) Abstr. 2nd Meeting European Association for Cancer Research, Heidelberg, Oct. 2-5, pp. 164-5.
- 8. Use a typewriter with elite type and with a carbon or good quality black silk ribbon. Single space and begin paragraphs with a 3 space indentation. Special symbols, not on the typewriter, must be hand lettered in black ink. Dot matrix type is not acceptable.
- 9. Abbreviations: Only standard abbreviations should be used, and should be written out the first time used with the abbreviation following in parentheses.
- 10. Titles: It is suggested that authors select a sufficient number of keywords to describe the full content of their paper, and then construct a title using as many as these as practicable. Titles normally should not exceed 140 characters in length. In particular, they should be free from unnecessary phrases such as "a preliminary investigation of" or "some notes on" which add little or nothing to their meaning.
- 11. Session Assignment: In order to assist the program committee in organizing the presentations, please indicate on the reverse side of the blue-line form your 1st, 2nd, and 3rd preferences for the topical classification of your paper.
- 12. The authors' permission for the North Dakota Academy of Science to publish is implied by a submission. The Academy does not restrict the right of authors to include data presented in a communication in full papers submitted at a later date to other publishers.

# II. Rules for Oral Presentation of Paper

- 1. All papers are limited to 15 minutes total time, for presentation and discussion. It is suggested that the presentation be limited to 10 minutes with an allowance of 5 minutes for discussion. It is also suggested that major emphasis be placed on the significance of the results and the general principles involved rather than on the details of methods and procedures.
- 2. Academy members represent a variety of scientific disciplines; therefore, speakers should avoid "jargon" and briefly explain or define such specialized terminology as may be judged to be indispensable to the presentation.
- 3. Projectors for 2" x 2" slides only will be available in all session rooms. Opaque projectors will NOT be provided. Only slides which can be read easily on projection should be used. Authors who desire suggestions for preparation of slides are referred to Smith, Henry W. 1957. "Presenting information with 2 x 2 slides." Agron. J. 49. pp. 109-113.
- 4. Timed rehearsals with slides are highly recommended. There is usually time for a maximum of 6 or 7 slides for a presentation of this kind.

# SYMPOSIUM

#### ON

# CONTROL OF PLANT GROWTH AND DEVELOPMENT: HERBICIDES AND PLANT GROWTH REGULATORS

Presiding: David G. Davis

Biosciences Research Laboratory (USDA-ARS)

Fargo, ND

1. Oxidative Metabolism and Bioregulation of Herbicide Activity in Plants. D.S. Frear\*, BRL/USDA/ARS, Fargo.

- Studies on the Control of Flowering in Field Pennycress(Thlaspi Arvense L.). James D. Metzger\*, BRL/USDA/ARS, Fargo.
- 3. Herbicide Mode of Action, Resistance and Selectivity. Richard H. Shimabukuro\*, BRL/USDA/ARS, Fargo.
- 4. The Physiology of Plant Growth Regulator Action. J.C. Suttle\* and J.F. Hultstrand, BRL/USDA/ARS, Fargo.
- Glutathione Conjugation in Herbicide Resistance and Antidoting. Gerald L. Lamoureux\* and Donald G. Rusness. BRL/USDA/ARS, Fargo.
- 6. Polyamines, Plant Development and Flowering. Hector E. Flores\*, Pennsylvania State University, University Park, PA

The number preceding each communication listed above is that assigned to represent the presentation sequence in the meeting program booklet

### OXIDATIVE METABOLISM AND BIOREGULATION OF HERBICIDE ACTIVITY IN PLANTS

D. S. Frear\*

USDA/ARS Biosciences Research Laboratory State University Station, Fargo, ND 58105

In the decade from 1965-1975, extensive herbicide metabolism studies were concentrated primarily on the isolation and identification of major metabolites and the identification of important metabolic reactions and pathways. Primary oxidative reactions were established as important factors in the metabolic detoxification and selective action of the major classes of herbicides.

Recent studies have emphasized the isolation, characterization and genetic engineering of key oxidative enzyme systems responsible for herbicide metabolism and selectivity.

Examples of primary oxidation reactions in the differential metabolism of selected phenylurea, sulfonylurea and aryloxy-phenoxypropanoate herbicides will be used to illustrate the role and significance of microsomal cytochrome P-450 mono-oxygenases in the bioregulation of herbicide activity.

# STUDIES ON THE CONTROL OF FLOWERING IN FIELD PENNYCRESS (THLASPI ARVENSE L.)

James D. Metzger\*
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Of all the phases in the life cycle of a plant, reproductive development (flowering) is probably the most important in terms of the impact on human endeavors. Indeed much of the human diet is directly dependent on the products of plant reproduction (e.g., seeds and fruits) or indirectly through animal feeds. Yet despite its importance to mankind, virtually nothing is known about the underlying biochemical and molecular mechanisms controlling the initiation of reproductive development. Such information is crucial for the development of useful strategies for manipulating flowering in beneficial ways.

Our studies on the mechanisms underlying the control of flower initiation in higher plants have utilized field pennycress (Thlaspi arvense L.), a common cruciferous weed of cultivated fields and roadsides in the Great Plains of North America, as a model system. This species has a vernalization (cold) requirement for the initiation of reproductive development. Field pennycress plants grown above 15°C remain as vegetative rosettes (extremely short internodes). Shortly after returning plants from a 3-6 week cold treatment (0-10°C) to warmer temperatures (ca. 21°C), a sequence characteristic of reproductive development begins. Microscopic flower primordia appear in about 4 days, followed by a large increase in cell division in the shoot sub-apical meristem (4-12 days). Measurable internode elongation is observed in 7-10 days and open flowers appear in about 3 weeks after the end of the cold period.

Probably the major obstacle in studies on the control of flowering is that the production of structures unique to flowering is the result of many highly coordinated processes over a relatively long period of time. Thus, fundamental to our approach was the selection of a component process of reproductive development amenable for in-depth biochemical and molecular studies. The process in field pennycress reproductive development on which we have focused is the bolting or stem elongation response. Early work showed that cold-induced stem growth was mediated by the class of plant hormones known as gibberellins (GAs). This led to the hypothesis that cold-induced stem growth in field pennycress results from the accumulation of a specific GA following activation of one or more steps in the metabolic pathway leading to its synthesis. The bulk of this talk will be devoted to summarizing evidence for this hypothesis. In addition, results indicating specific biochemical steps in GA biosynthesis will be reported. And finally, the use of such data in the development of models for the control of reproductive development will be discussed briefly.

4 Symposia

# HERBICIDE MODE OF ACTION, RESISTANCE AND SELECTIVITY

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The mode of action of a herbicide is the sequence of events that causes death in plants. The mechanism of action is the primary biochemical or biophysical lesion that initiates the events that result ultimately in plant death. Certain plants, including crops and weeds, have an inherent mechanism(s) to resist injury by herbicides. The interaction between the mechanisms of action and resistance in plants results in herbicide selectivity, an important property of modern herbicides. An understanding of herbicide modes of action and mechanisms of resistance in plants is necessary for the efficient use of herbicides and to deal with the emerging problem of herbicide resistance in formerly susceptible plants. The research on the metabolism, selectivity and mode of action of diclofop-methyl illustrates some of the above factors and their dynamic interactions in plants.

Diclofop-methyl (DM) is a postemergence grass herbicide that is phytotoxic to nearly all grasses except wheat (<u>Triticum aestivum</u>). It is totally inactive against broad leaved species. Diclofopmethyl is used as a selective herbicide for the control of wild oat (<u>Avena fatua</u>) and other grassy weeds in wheat and broad leaved crops such as soybean and pea. The sensitive anatomical sites in susceptible grasses are the shoot, root and intercalary meristems where growth and plant development are inhibited. Chloroplasts are very sensitive to ultrastructural damage, resulting in chlorosis to green tissues, but inhibition of photosynthesis is not the primary mechanism of action.

Two mechanisms of action have been hypothesized for DM: (1) a biophysical mechanism located in cell membranes that causes a pertubation of the transmembrane proton gradient, and (2) a biochemical mechanism located in chloroplasts and other plastids of nongreen tissues that inhibits the biosynthesis of lipids. The R(+) enantiomer of DM affects both mechanisms, but the S(-) enantiomer affects only the biophysical mechanism. The R(+) enantiomer causes chlorosis due to the destruction of chloroplasts, resulting probably from the inhibition of lipid biosynthesis. Both the S(-) and R(+) enantiomers inhibit growth of cells in the meristematic zones probably due to their effects on the proton gradient. The auxinic herbicide, 2,4-D, causes indirectly a reversal of the biophysical mechanism, but it has no effect on the biochemical mechanism. Intact susceptible plants will survive and outgrow injury by DM if a timely application of 2,4-D is made to reverse the effects of the biophysical mechanism.

Grasses appear to be sensitive to both mechanisms of action whereas broad leaved species are insensitive. Therefore, the selective action of DM between wild oat and wheat is due to differential metabolism and detoxification whereas the selectivity between grasses and broad leaved plants is due to site-based resistance in the broad leaved species.

#### THE PHYSIOLOGY OF PLANT GROWTH REGULATOR ACTION

J. C. Suttle\* and J. F. Hultstrand USDA/ARS, Biosciences Research Laboratory State University Station, Fargo, ND 58105

By virtue of their diverse spectrum of biological effects, plant growth regulators (PGRs) have the potential of making a significant contribution to agriculture. In spite of this potential, PGR usage has remained constant and presently constitutes only a small fraction of total agricultural chemicals. Inconsistent field performance is a major factor that hinders PGR acceptance. Attempts to enhance PGR performance are hampered by a limited understanding of their mechanisms-of-action and fate in higher plants. Research conducted in this laboratory is directed towards addressing this deficiency through the systematic study of the physiology of PGR action in plants. Past and current studies with an experimental PGR will be used as a paradigm for this approach.

The N-substituted phthalimides (NSPs) are a class of compounds first synthesized by the American Cyanamid Co. Certain NSPs exhibit a moderate degree of gibberellin-like activity and constitute the first documented synthetic gibberellin-like agonists. Initial studies demonstrated a highly variable pattern of bio-activity that seemed to be species dependent. In this presentation, research describing the uptake, translocation and metabolic persistence of a representative member of the NSPs will be presented. In addition, the interactions between the NSPs and an endogenous proteinaceous binding factor (presumed to be a gibberellin receptor) will be discussed. Finally, the synthesis and properties of a group of NSP derivatives to be used as photo-affinity probes of the receptor protein(s) will be presented.

# GLUTATHIONE CONJUGATION IN HERBICIDE RESISTANCE AND ANTIDOTING

Gerald L. Lamoureux\* and Donald G. Rusness USDA/ARS, Biosciences Research Laboratory State University Station, Fargo, ND 58105

The usefulness of modern herbicides is largely due to their selective nature; i.e., their ability to control undesirable weed species in the presence of desirable crop species. The selectivity of many herbicides has been attributed to differences in the rate at which they are detoxified by metabolism in resistant versus susecptible species. Conjugation with glutathione (or homoglutathione) is one of the more common mechanisms of herbicide detoxification by metabolism. To undergo selective detoxification by this mechanism, the herbicide must contain an electrophilic site that is capable of reacting with glutathione in the presence of a glutathione S-transferase enzyme, or it must undergo metabolic activation to produce such an electrophilic site. The tolerant plant species must contain both the necessary glutathione S-transferase and sufficient glutathione to allow for the complete metabolism of the herbicide. If both the weed and crop species contain the necessary glutathione S-transferase and glutathione, little herbicide selectivity based on differential rates of glutathione conjugation can be expected. Also, if the herbicide reacts with glutathione at a high non-enzymatic rate, loss of selectivity may occur. The chloro-s-triazines, methylsulfide substituted triazines, chloroacetanilides, thiocarbamates, nitrodiphenylethers, and miscellaneous members of other classes of herbicides are commonly detoxified by glutathione conjugation in some plant species. These glutathione conjugates usually undergo rapid and extensive secondary metabolism in plants. Because of safety considerations, the nature of these secondary metabolites have also been studied.

There is considerable evidence that the selectivity of some herbicides can be altered by increasing the rate at which the herbicide is metabolized. The tolerance of corn and sorghum to certain herbicides can be increased approximately 10-fold by the use of commercial herbicide antidotes. These antidotes do not significantly increase the tolerance of weed species to these herbicides. In corn and sorghum, these antidotes cause an increase in glutathione biosynthesis and induce specific glutathione S-transferase isozymes which results in a more rapid rate of herbicide metabolism and detoxification. When a herbicide is not sufficiently toxic to control certain weed species, it is also possible to selectively increase herbicide toxicity by the use of herbicide synergists. The synergist, tridiphane, appears to inhibit herbicide detoxification by glutathione conjugation in weed species without significantly effecting the detoxification rate in corn.

Several laboratories have transferred specific glutathione  $\underline{S}$ -transferase genes for herbicide detoxification from corn to yeast and  $\underline{E}$ .  $\underline{coli}$ . Hence, transgenic plants resistant to specific herbicides may be developed by increasing their ability to catalyze specific glutathione conjugation reactions.

# POLYAMINES, PLANT DEVELOPMENT AND FLOWERING

Hector E. Flores\*

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The diamine putrescine and the polyamines, spermidine and spermine are aliphatic amines present ubiquitously in bacterial, animal and plant cells. Research on plant polyamines dates back to the discovery by Richards and Coleman in the 1950's that putrescine accumulates in potassium-deficient harley. These results were extended to include other ionic stresses (low pH, ammonium nutrition and salt stress (1). The pioneering work of Terence Smith established the basic outline of polyamine biosynthesis and catabolism in higher plants (2), which was largely forgotten until the iate 1970's. Interest in the biology of these compounds was stimulated by findings that in animal and bacterial cells, high titers of spermidine and spermine are always correlated with high rates of cell division. The availability of enzyme-activated, irreversible inhibitors of polyamine biosynthesis has made possible the establishment of causal relationships between polyamines and cell division, but the underlying mechanisms of polyamine action are still poorly understood. Studies in higher plants in the last ten years have confirmed the above results, but have also revealed aspects of polyamine metabolism and action unique to plant cells.

Polyamines and ethylene share a common metabolic precursor, S-adenosyl-methionine. Since ethylene is a well known senescence-promoting hormone, and polyamines appear to delay senescence in detached leaves, it is postulated that this process may be at least partly regulated by precursor flow into either polyamine or ethylene pathways (3).

In addition to their involvement in cell division, polyamines have been implicated in the regulation of cell differentiation. In carrot cell cultures, increases in arginine decarboxylase (ADC) and putrescine precede the appearance of somatic embryos. Treatment with difluoromethylarginine (DFMA), a specific inhibitor of ADC, causes a dramatic decrease in somatic embryos, which can be reversed by exogenous addition of putrescine or spermidine. Inhibitors of spermidine synthesis have similar effects, also reversible by polyamine addition. These general findings were confirmed independently by at least two laboratories, and are suggestive of polyamine involvement in somatic embryogenesis. The underlying mechanisms, however, remain unknown (4). Furthermore, these findings are open to some reservations due to the fact that in this system polyamine inhibitors also inhibit both growth and somatic embryo formation, and the two processes are difficult to separate in carrot cell suspensions.

At physiological pH, polyamines are fully protonated polycations, readily extractable in acid. The polyamine titers reported in the literature usually refer to these "free" forms. However, in many cases, most of the polyamine pool in plant cells is in "bound" forms; the best known being the conjugates formed between di- or polyamines and hydroxycinnamic acids (HCAs). These compounds are widespread in higher plants, found mostly in flowers, and may be a significant fraction of the soluble nitrogen of this organ (5). Furthermore, characteristic HCAs patterns are found in male and female flower parts, and a correlation has been established between cytoplasmic male sterility and the absence of a "male" pattern of HCAs. It is therefore been suggested that HCAs may be involved in normal flower development. These results will be discussed in the light of other recent findings: a) the regeneration of tobacco plants from cell lines resistant to polyamine inhibitors, showing peculiar abnormalities in flower development (4); and b) the stimulation of flower bud formation by spermidine addition to thin cell layer explants from flower stems (5).

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#### SYMPOSIUM

#### ON

# BIODIVERSITY OF THE INVERTEBRATE FAUNA OF NORTH DAKOTA

Presiding: Larry D. Charlet

Northern Crop Science Laboratory (USDA-ARS)

Fargo, ND

Introduction. Larry D. Charlet, BRL/USDA/ARS, Fargo.

- The North Dakota Butterfly Fauna. Ronald A. Royer\*, MSU, Minot.
- Coleoptera of North Dakota. Edward U. Balsbaugh, Jr.\*, NDSU, Fargo.
- 9. Chiggers and Ticks of North Dakota. William J. Wrenn\*, UND, Grand Forks, and Don Hyder\*, NDSU, Fargo.
- 10. Functional Diversity Among Aquatic Invertebrates in Prairie Lakes and Ponds. Malcolm G. Butler\*, NDSU, Fargo.
- 11.Introduced Insect Fauna of North Dakota. David Nelson\*, ND Department of Agriculture, Fargo.
- 12.Crop Fauna: Disturbed and Undisturbed Habitats. Michael J. Weiss\*, NDSU, Fargo.
- 13.Insect Diversity in Native and Cultivated Plants: Sunflowers. Larry D. Charlet\*, NCSL/USDA/ARS, Fargo and Gary J. Brewer\*, NDSU, Fargo.

The number preceding each communication listed above is that assigned to represent the presentation sequence in the meeting program booklet

#### The North Dakota Butterfly Fauna

Ronald Alan Royer\*
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North Dakota's butterfly fauna is at once sparse and diverse. Whereas approximately 17,500 species of butterfly are known worldwide, liberal taxonomists Miller and Brown (1) designate just 763 for North America north of Mexico, of which a mere 142 are on formal record for the state of North Dakota (2). That seems at first glance to be a fair share, but the number pales when one recognizes that more than 6,000 butterfly species are known from the American tropics, and even the largely boreal and subarctic province of Manitoba outshines North Dakota, with 145 species on record at this writing (3; Klassen *in litt.*). Despite its limited extent, however, North Dakota's butterfly fauna represents all ten of the families and a majority of the North American subfamilies recognized by Miller and Brown (1) and Ferris (4).

Several factors contribute to this unique combination of faunal impoverishment and relative diversity. Temperature is one such factor. Cold weather naturally limits the number of overwintering species. (Klots (5) reported only six resident species for Greenland.) Consequently, at least 20 species on record for North Dakota cannot normally survive winter in the state, instead

reappearing sporadically through immigration from the south. Rainfall is yet another factor. McCabe (6) demonstrated the relation of rainfall/evaporation ratios to distribution of the potentially threatened Dakota Skipper (Hesperia dacotae Skinner). This humidity gradient undoubtedly likewise applies to a large percentage of the Great Plains fauna. Submesic and even semidesert representatives make up fully a fifth of the badlands contingent of the fauna, while six species exclusive to the more humid (mesic) Sheyenne Valley are at the most western limits of ranges that are bounded eastward only by the coast. Physiographic features and their irrefutable effect on vegetation constitute a third, major factor shaping the North Dakota butterfly fauna (7). As a consequence of these several factors, it can safely be claimed that North Dakota shares at least one butterfly species with every state and province on the continent.



Temperature, humidity, physiographic features and human activity all have helped in demonstrable ways to determine modern distribution of the state's butterfly fauna. Several workers (2, 8, 9, 10, 11) have over the years referred the Turtle Mountain forest region to the Canadian Life Zone, the badlands to the Upper Sonoran Life Zone and the remaining bulk of the state to the Transition Life Zone. While these 19th Century distinctions are in many ways outmoded and imprecise, they nevertheless characterize and predict a great deal of the above-described distribution remarkably well.

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COLEOPTERA OF NORTH DAKOTA<sup>1</sup>
Edward U. Balsbaugh, Jr.
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On a world basis, the largest order of the animal kingdom is the Coleoptera. The order has as many species as the entire plant kingdom, including the algae and fungi. Therefore it is probably true that the most common animals in North Dakota are beetles. For the world, estimates for the numbers of beetle species range conservatively from 300,000 to over 1 million. For North Dakota, a preliminary inventory for the number of beetles is 1,339 species. This count is based on species currently represented in the North Dakota State Insect Reference Collection (NDSIRC) which is housed in the Entomology Department at North Dakota State University, Fargo.

Besides their sheer numbers, there is almost as much diversity in some of the larger families of beetles as is found in all of the other orders of animals combined. Such diversity is reflected by the ecological preferences of beetles. They live in all terrestrial and fresh water habitats. Their species include ectoparasites of vertebrates; some are endoparasites of other insects as larvae and as adult females. They have evolved to occupy the entire range of terrestrial and fresh water ecological niches.

The NDSİRC is fairly representative of the families of beetles that should be expected to occur in North Dakota, however, many more species could be documented. One hundred thirteen families of beetles occur in North America and 68 (60 %) are known for North Dakota. (This compares favorably with the fauna for South Dakota where 69 families have been recorded.) However, a lesser number of ecological niches in North Dakota supports only 44% of the beetle families of the world.

Approximately 70% of all beetle species belong to the 12 largest families: Carabidae, Staphylinidae, Scarabaeidae, Buprestidae, Elateridae, Cantharidae, Cleridae, Coccinellidae, Tenebrionidae, Cerambycidae, Chrysomelidae, and the Curculionidae. Most of the economically important species are in these families. Of the remaining species, most belong to 14 more families and the remainder are in

87 other families (Arnett, 1985).

Of the 12 "common" families, some are reasonably well represented in the NDSIRC for North Dakota species: the Curculionidae (219 spp.) and near relatives - the Attelabidae, Rhynchitidae, and Apionidae - have been documented by Aarhus and Balsbaugh (in press) (South Dakota: 142 species). The Chrysomelidae (183 species) are still only incompletely know. (South Dakota: 306 species). Two hundred and twenty-three species of Carabidae have been recorded but most of these are the larger species. (South Dakota: 353 species.) The Scarabaeidae are fairly well known via the studies of Helgesen & Post (1967) and Lago, Post & Oseto (1979). The disparity with South Dakota (119 species) perhaps reflects its true greater diversity, rather than incomplete collecting efforts for North Dakota. The Staphylinidae is perhaps the fourth largest family of beetles (30,000 species), but only 7 North Dakota species are in the NDSIRC. To a very great extend this discrepancy reflects a bias of North Dakota collectors for larger specimens. Most species of this family are quite small (<4mm). The Cerambycidae and the Buprestidae both include species whose larvae are wood borers. Homogeneous, treeless prairies easily explain the paucity of their numbers in North Dakota.

A table has been compiled which compares the Coleoptera diversity by family for North Dakota

species with that of the world, the United States and South Dakota.

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1 Approved by the Director of the North Dakota Agricultural Experiment Station as Journal Paper No. 1799.

# CHIGGERS AND TICKS OF NORTH DAKOTA

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Members of the acarine family Trombiculidae are called chiggers. The larval stage is parasitic on amphibians, reptiles, birds, and mammals; the active post-larval stages are free living in the soil and feed on other arthropods or their eggs.

Only two species of chiggers previously have been taken in North Dakota: *Neotrombicula subsignata* from Morton and McKenzie counties and *Eutrombicula subsignata* from Billings County. Opportunistic collection and examination of more than 955 potential vertebrate hosts (almost exclusively small rodents) from various localities in eastern and western North Dakota has revealed an additional eight species. These include *Euschoengastia setosa*, *Leptotrombicula mericana*. *Microtrombicula crossleyi*, *Miyatrombicula cynos*, *Neotrombicula autumnalis*, *N. finleyi*, *N. microti*, and *Walchia americana*.

Information is provided concerning the distribution of each of the 10 species and chigger-host relationships are discussed.

Prior to 1988, little was known regarding the diversity and distribution in North Dakota of tick species of the acarine family Ixodidae Murray. Past records were mainly from random samples. Published records list the following species of North Dakota ticks: Dermacentor variabilis, D. andersoni, D. albipictus, Ixodes kingi, I. sculptus, I. marxi, and Haemaphysalis Ieporispalustris., (1, 2, 3, 4, 5). The primary vector of Lyme disease in the North Central United States, Ixodes dammini, previously has not been found in North Dakota, South Dakota, or Montana. The deer tick, however, occurs in Minnesota. In April, 1988, a study was initiated to better determine the species of ticks and their distribution in North Dakota. Survey records from 1988 and 1989 show that I. dammini occurs approximately 70 miles east of the North Dakota- Minnesota border. Because of the potential of Lyme disease, we hoped to establish whether the deer tick, Ixodes dammini, occurs in North Dakota.

We found that the predominant tick species in North Dakota is *D. variabilis*. Two other species of *Dermacentor* were found, *D. albipictus* and *D. andersoni*. Two species of *Ixodes*, *I. kingi* and *I. marxi*, were found occassionally. Neither of these, however, belong to the *Ixodes ricinus* complex which includes *I. dammini*. *Haemaphysalis leporispalustris* was collected from rabbits and ruffed grouse. Incidental tick species include *Amblyomma americanum* and *Rhipicephalus sanguineus*. *Ixodes dammini* has only been found attached to North Dakotans and their pets that had been hunting or visiting in Wisconsin and Minnesota.

A series of maps have been constructed to demonstrate the distribution of tick species in North Dakota based on past records and our survey. Life history and vector potential of several species will be discussed.

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#### FUNCTIONAL DIVERSITY AMONG AQUATIC INVERTEBRATES IN PRAIRIE LAKES AND PONDS

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Aquatic invertebrates are relatively inconspicuous components of the biota in North Dakota's many lakes, ponds, sloughs, and wetlands, yet they have diverse and important ecological functions. Directly and indirectly, these animals can have significant impacts on the quality and value of their aquatic habitats. I illustrate this with examples from three studies conducted by NDSU zoology students on wetland habitats in North Dakota and Minnesota. These examples focus on invertebrates in three very different taxonomic and ecological categories, including detritusfeeding aquatic insects, herbivorous crustacean zooplankters, and a predatory annelid. Each case illustrates a distinct role for invertebrates in their aquatic habitat and three very different mechanisms by which these animals can affect the nature and value of wetland resources.

Midge larvae in the family Chironomidae are often the most abundant benthic invertebrates in prairie potholes, and serve as a trophic link between primary production and vertebrate consumers. These larvae convert primary production to insect biomass by consuming algae, microbes, and plant detritus. In shallow systems where fish are frequently absent, high levels of chironomid biomass can develop and provide an important food base for waterfowl and other aquatic birds. Nelson (1) investigated the seasonal pattern of chironomid abundance in four North Dakota wetlands on the Missouri Coteau. Two chironomid species comprised most of the biomass in all wetlands, but absolute abundance varied greatly among sites. For mobile consumers like birds, seasonal variance in prey abundance is more important than spatial variance. A consequence of the life cycle phenologies of the largest and most abundant chironomids in these wetlands is that highest larval biomass occurs in early spring and late fall. Wetlands with high invertebrate biomass attract large numbers of waterfowl during spring and fall migration. Females that lose their clutch to predators require a high protein, invertebrate—rich diet for renesting. Thus the magnitude and seasonal pattern of aquatic insect abundance directly contribute to wetland quality from the standpoint of attracting and supporting bird populations.

In the water column, crustacean zooplankton graze on planktonic algae and detritus and are themselves fed upon by predatory vertebrates and invertebrates. Large species like  $\frac{Daphnia}{Daphnia}$  are efficient filter-feeders and a 1.5 mm long individual can clear algae and suspended materials from as much as 5 ml of water per day (2). These large zooplankters cannot coexist with planktivorous fish, so the presence and nature of a fish fauna can indirectly influence algal biomass and water clarity. Restructuring of the fish community in a large, shallow Minnesota lake by rotenone treatment and restocking with predatory species led to a dramatic increase in spring water clarity (3). This permitted establishment of extensive beds of aquatic plants that had previously been eliminated by turbidity. These changes appear to have been mediated by a shift in the zooplankton from a heavily-grazed community of small crustaceans to one dominated by large  $\frac{Daphnia}{Daphnia}$ . This latter community could filter 40-100% of the water column per day for much of May and June, maintaining high water clarity and permitting establishment of rooted plants that are an important food resource for waterfowl.

Direct human harvest of aquatic invertebrates from prairie ponds is another way in which these animals may influence the value placed on their habitat. The ribbon or bait leech occurs in fish-free ponds where it preys on invertebrates, especially midge larvae. This species is commercially harvested in Minnesota, where retail sales have been estimated at \$1.5 million annually (4). There is little evidence of leech harvesting in North Dakota, although Pennuto (5) found 12 of 38 ponds surveyed on the Missouri Coteau to have  $N_0$  obscura populations. North Dakota bait dealers sell leeches worth an estimated \$74,100 annually, with most imported from Minnesota (6).

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#### INTRODUCED INSECT FAUNA OF NORTH DAKOTA

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Immigrant insects contribute only a small percentage to the total number of insect species in North America yet their economic significance far surpasses what their mere numbers might suggest. Some of these species have become established in North Dakota.

This paper discusses the introduced insects of significance to agriculture in North Dakota. An historical chronology of their appearance and their relative importance compared to native species is provided. Agricultural crops in North Dakota include both native and introduced plant species and a comparison of the importance of introduced insect fauna on these hosts is made.

Introduction of insect species often occurs through accidental means. Means of entry into North America and subsequent introduction or spread into North Dakota are discussed. The failure of some species to spread into North Dakota may be due to various forms of environmental resistance as well as the actions of man and some of these factors are discussed.

Intentional introductions of beneficial insects into North Dakota have been made in attempts to establish organisms for biological control purposes. These introductions are listed as well as information on establishment. Beneficial insects that have been successfully introduced into other states and subsequently spread to North Dakota are also listed.

#### CROP FAUNA: DISTRUBED AND UNDISTURBED HABITATS

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In the Northern Great Plains of the United States (Western Minnesota, North Dakota, Northern South Dakota and Eastern and Central Montana), conservation tillage systems (no till and reduced till) are used to manage about 22% of the arable land. The major crop is spring wheat which is produced on about 6 million hectares (1). Because of low precipitation (330-380 mm/year) a common cropping system is an annual crop-fallow rotation (2). Currently, about 22% of the arable land is fallowed annually. Annual cropping systems have increased in this area, primarily to prevent saline seeps. Because annual cropping has increased, the need for conservation tillage also has increased in order to preserve soil moisture (1).

Most species of ground beetles are active predators as adults. Some species, particularly of the Harpalini, are phytophagous, feeding on germinating seeds. Some carabids are liquid-feeding predators, feeding on other insects. Unfortunately, very little data are available on the influence of cropping and

tillage systems on their associated ground beetles in the Northern Great Plains.

Study sites were located at the Williston Research Center, Williston, ND, and the North Central Research and Extension Center, Minot, ND. Experimental units (24 x 45 m) were established in 1985 at both locations and arranged in a completely randomized block design with three replications of each treatments. Treatments consisted of three tillage systems paired with two cropping systems: (1) no-till with continuous and annual cropping, and annual fallow; (2) reduced tillage with continuous and annual cropping, and annual fallow. Two pitfall traps were placed perpendicular to the plant rows in the center of each experimental unit (3). To increase collection efficiency, traps were connected with a 70 cm strip of aluminum lawn edging placed in a 5 cm slit in the soil (4). Traps were placed immediately after planting (mid-April in both years) and replaced about every two weeks until mid-August. The total trapping period included 107 days in 1986 and 124 days in 1987 at Williston, and 84 days in 1986 and 124 days in 1987 at Minot. At Minot in 1986, two trapping periods (28 days) were lost due to excessive precipitation. Identification of carabid species were made by Dr. E. Balsbaugh, Dept. of Entomology, NDSU, Fargo, ND 58105.

A total of 40 species of carabids were collected during the two year study. Only 14 species individually represented ≥ 1% of the total number of carabids collected at least one location and year. At Williston, the total number of carabids collected over both years was 26,883 and 45,957 were collected at Minot. Although more individuals were collected at Minot, more species were collected at Williston. Of the 14 species, differences in abundance between locations were observed for some species. At Williston, Amara obesa Say, Harpalus fallax LeConte, Pasimachus elongatus LeConte, and Pterostichus scitulus LeConte were collected in higher numbers than at Minot. At Minot, Harpalus pennsylvanicus DeGeer, Pterostichus corvus LeConte, and Pterostichus lucublandis Say were collected in higher numbers than at Williston. Abundance per species was not consistently affected across cropping and tillage systems and locations for certain species but was for others. The Sorenson similarity index (5) at Minot was higher, especially for similar cropping systems. At Williston, the index values were lower, reflecting greater heterogeneity between treatments. Generally, lower numbers of individuals of a given species were found in cropping systems associated with conventional tillage. However, the influences of tillage and cropping system depended on the individual carabid species. Cropping system may have altered communities to higher degree than the tillage regime.

Table 1. Major carabid species for which tillage and cropping system had an influence on mean number per day collected at Williston and Minot, North

Dakota 1986-87

	a Mean/day								
		b							
				Tillage ar	<u>id croppi</u>	ng system			
Species	NTCC	NTAC	NTAF	RTCC	RTAC	RTAF	CTCC	CTAC	CTAF
Williston									
A. carinata	0.17 a	0.10 abc	d 0.14 ab	0.12 abc	0.06 cd	0.11 abc	0.07 cd	0.05 cd	0.04 d
C. tomentosus	0.12 abc	0.01 d	0.18 a	0.14 ab	0.02 d	0.15 ab	0.08 cd	0.03 cd	0.01 d
H. fallax	1.51 b	0.27 с	2.39 ab	2.17 ab	0.41 c	1.90 b	1.59 b	0.36 с	2.97 a
H. pennsylvanicus	2.12 ab	0.46 cd	0.23 d	2.19 ab	1.56 bc	0.17 d	2.79 a	1.12 bcd	0.12 d
P. scitulus	1.26 bc	0.62 c	0.96 bc	0.76 с	1.93 ab	1.00 bc	0.51 c	2.45 a	0.74 c
Minot									
A. placidum	0.20 ь	0.29 ь	0.24 в	0.19 ь	0.28 ъ	0.13 ь	0.27 ь	0.70 a	0.04 ь
A. obesa	0.63 a	0.36 Ъ	0.65 a	0.66 a	0.41 b	0.40 ь	0.74 a	0.35 ь	0.35 ь
C. calidum	0.24 ab	0.27 ab	0.10 Ь	0.38 a	0.31 a	0.11 Ь	0.34 a	0.26 ab	0.09 b
H. fallax	0.26 b	0.15 b	0.88 a	0.14 b	0.11 ь	1.05 a	0.29 ь	0.15 b	0.21 b
H. pennsylvanicus	8.93 a	5.41 cd	5.29 cd	8.01 ab	5.42 cd	4.48 cd	9.67 a	6.44 bc	3.64 d
P. lucublandis	2.11 a	1.68 ab	1.26 bc	1.39 bc	0.87 с	1.83 ab	0.79 c	1.31 bc	0.90 с

Means within rows and location followed by the same letter are not significantly different (P≥0.10; Duncan's multiple range test [SAS Institute 1985]).

NTCC = no-till continuous crop, NTAC = no-till annual crop, NTAF = no-till annual fallow, RTCC = reduced-till continuous crop, RTAC = reduced-till annual crop, RTAF = reduced-till annual fallow, CTCC = conventional-till continuous crop, CTAC = conventional-till annual crop, CTAF = conventional-till annual fallow.

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#### INSECT DIVERSITY IN NATIVE AND CULTIVATED PLANTS: SUNFLOWERS

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Sunflowers are a botanic group of the Compositae comprising approximately 50 species in the genus \*Helianthus\* and are found growing from southern Canada to northern Mexico (1). The common sunflower, \*Helianthus\* annuus\* L. has become an important food crop worldwide; the only native plant of the United States to do so. Since cultivated sunflower coexists with its native congeners in North America, insects that coevolved with the native species have had the opportunity to move freely between native and crop plants (2). Insect problems in cultivated sunflower have been caused by native insect species in contrast to those of other major crops which are the result of accidentally introduced species. Knowledge about the insect fauna on native sunflower species and their bionomics could aid in the development of ecologically sound management practices of insects in commercially grown sunflower. In addition, information about the susceptibility or resistance of the different species of native sunflower can be utilized in the development of resistant cultivars. Discovery of new natural enemies, especially parasitoids, not currently attacking sunflower pests in commercial plantings could yield more effective biological control agents.

Six species of Helianthus occur in North Dakota and include: H. annuus, H. petiolaris, H. rigidus, H. tuberosus, H. nuttallii, and H. maximiliani. Helianthus annuus has the greatest distribution and is the most common. Over 150 insect herbivores have been reported from native H. annuus (3). These species occupy all niches of the plant including leaves, heads, seeds, stems, and roots. This report did not include insects which also utilize the plant as a source of nectar and pollen or the natural enemies of these herbivores. Research on the insect fauna of the native sunflower of North Dakota has included studies in only the western and eastern ends of the state. Five species of insects were reared from stems of 4 species of native sunflower in western North Dakota. Helianthus annuus had the greatest diversity of fauna including 2 families of Lepidoptera and 3 of Coleoptera. All insects collected had also been recovered from cultivated sunflower. Insect fauna from heads and stems of 6 species of native sunflower in eastern North Dakota yielded insect species in 2 families of Coleoptera, 4 families of Lepidoptera, and 2 families of Diptera. The species of insects collected had previously been reported from cultivated sunflower in North Dakota.

Beginning in the 1970's, the widespread planting of a cultivated variety of *H. annuus* has presented the insect fauna adapted to native sunflower with an enormous increase in habitat. While most of the species associated with native *Helianthus* have successfully transferred to sunflower, only about 20 species are of concern to economic entomologists. Sunflower roots are consumed by a weevil (Coleoptera) and the stalks harbor a dipteran, 2 species of Lepidoptera, and 4 coleopteran species. Sunflower foliage is consumed by a coleopteran species specific to sunflower. Nonhost-specific foliage feeders include a lepidopteran and several species of aphids (Homoptera) and grasshoppers (Orthoptera). Receptacle tissue is habitat for a coleopteran, 2 dipteran and 2 Lepidoptera species. Seeds are consumed by 2 species each of Coleoptera and Lepidoptera and one Diptera. Of these potential economic species, only 5 have proven themselves to be serious rivals for our use of cultivated sunflower.

The majority of the phytophagous insects on sunflower are kept in check, in large part, by entomophagous insects. There are at least 25 identified predaceous or parasitic insects species found on sunflower and all the sunflower herbivores which have been studied are prey to a least one other insect. The sunflower beetle is preyed on by beetles in the families Coccinellidae and Melyridae, 2 species of Chrysopidae (Neuroptera) and several hemipterans (Nabidae and Pentatomidae). Sunflower beetle parasitoids include a dipteran (Tachinidae) and a hymenopteran (Pteromalidae). Seed weevils are preyed on in the soil by stiletto flies (Diptera: Therevidae). On the plant, seed weevils are parasitized by 2 species of Braconidae and a Pteromalidae (Hymenoptera) as well as mites in the family Acaridae.

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#### SYMPOSIUM

ON

#### CURRENT TOPICS IN PLANT BIOTECHNOLOGY

Presiding: Phillip McLean

College of Agriculture

NDSU, Fargo, ND

14.Progress in the Development and Use of Genetic Markers in Plants. Thomas C. Osborn\*, University of Wisconsin, Madison, WI.

- 15. Transformation of Cucumis sativus via Agrobacterium tumefaciens. P.P. Chee\*, The Upjohn Company, Kalamazoo, MI.
- 16.Molecular Genetic Characterization of Lysine Biosynthesis in Corn. B.G. Gengenbach\*, D.A. Somers, D.A. Frisch and S.B. Dotson, University of Minnesota, St. Paul, MN.
- 17.Field Testing of Cucumber Plants Which Express the CMV Coat Protein Gene: Field Plot Design to Test Natural Infection Pressures. Jerry L. Slightom\* and Paula P. Chee, The Upjohn Company, Kalamazoo, MI., and Dennis Gonsalves, Cornell University, Geneva, NY.
- 18. The "Non-Host" Resistance of Peas May be Transferable to Potatoes. L.A. Hadwiger\*, Chin C. Chiang, Ming Mei Chang and Andrew Pettinger, Washington State University, Pullman, WA.

The number preceding each communication listed above is that assigned to represent the presentation sequence in the meeting program booklet

#### PROGRESS IN THE DEVELOPMENT AND USE OF GENETIC MARKERS IN PLANTS

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The use of genetic markers in plants traces back to Mendel's experiments with the garden pea in which he elucidated the basic principles of heredity. Since then there has been tremendous progress in the development and use of various types of genetic markers in plants. Morphological and protein markers have been used during the past several decades to construct linkage maps in a few plant species, such as corn, tomato, barley, wheat and Arabidopis (1). With the development of techniques in molecular biology, methods have recently become available for utilizing sequences of DNA as genetic markers. These markers, called restriction fragment length polymorphisms (RFLPs), do not have pleiotropic effects, such as those associated with the mutant phenotypes of morphological markers, and they are much more abundant than protein markers. In the last few years, detailed RFLP linkage maps have been constructed for several plant species (2,3,4).

Genetic markers have valuable applications for analyzing genome organization and genetic relationships in plants. RFLP markers have been used in corn (3) and <u>Brassica</u> species (4) to obtain strong evidence for intragenome duplication of chromosome segments. They also have been used to compare linkage maps of homologous DNA sequences between tomato, potato and pepper (2) and between <u>Brassica</u> species. RFLP analysis of genetic diversity in <u>Brassica</u> and related genera has provided new insight into species relationships and evolution (5).

The most exciting application of genetic markers in plants is their use to identify and manipulate genes of interest. Although many monogenic traits are easily identified and manipulated by plant breeders without the use of markers, some traits, such as nematode resistance, are difficult to select in breeding programs. Tight linkage of an acid phosphatase allele to nematode resistance in tomato (6) has been used widely by commercial breeders for selection of this trait. Polygenic traits are much more difficult to identify and manipulate in a breeding program due to the small effects of individual polygenes and the influence of environment on trait expression. RFLP markers have been used to identify genes controlling several quantitative traits, such as soluble solids content in tomato (2,7), yield in corn, and morphological variation in Brassica. These studies have provided information on the location and effects of genes controlling quantitative traits of interest and they demonstrate the potential of using RFLP markers for selection in breeding. RFLP markers also are being used to aid in the isolation of cloned genes, for which gene products are not known, by serving as starting points for chromosome walks to the gene of interest.

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- 2. Tanksley, S. D., et al. (1989) Biotechnology 7:257-264.
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# TRANSFORMATION OF CUCUMIS SATIVUS VIA AGROBACTERIUM TUMEFACIENS

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The transfer of genetic materials into Cucumis sativus L cv. Poinsett 76 was accomplished by using an avirulent strain of Agrobacterium tumefaciens. Cotyledons from three to five day-old cucumber seedlings were used as explants. Explants were cocultivated with the avirulent Agrobacterium strain C58Z707 which contained the binary vector pGA482 and cultured on MS medium supplemented with 2.0 mg/l 2,4-D and 0.5 mg/l Kinetin. After three days, the cultures were transferred to fresh medium of the same formulation supplemented with 100 mg/1 kanamycin and 500 mg/l carbenicillin. After 5 weeks, kanamycin resistant embryogenic callus tissues were transferred to MS medium containing 1.0 mg/l NAA, 0.5 mg/l kinetin, 100 mg/l kanamycin and 500 mg/l carbenicillin. These cultures were incubated for an additional two to three weeks. Differentiated embryos were then transferred to MS medium supplemented with 50 mg/l kanamycin for plantlet development. A total of 100 independent  $R_0$  transformed plants were obtained. The expression of NPTII in callus tissues,  $R_1$  and  $R_2$  transformed plants was demonstrated by kanamycin phosphorylase assay. Genomic DNA isolated from NPTII positive plants indicated that the neomycin phosphotransferase gene is integrated into the cucumber genome. These results show that the Agrobacterium-mediated gene transfer system and regeneration via the embryogenic route can be used for the transfer of genetic material into plant species belonging to the family Curcurbitaceae.

#### MOLECULAR GENETIC CHARACTERIZATION OF LYSINE BIOSYNTHESIS IN CORN

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Monogastric animals, including humans, must have several essential amino acids such as lysine provided in their diet. Consequently, the nutritional value of protein in most edible crops has been determined and concerted efforts have been expended to improve deficiencies in amino acid profiles. Despite this effort, the genes and enzymes regulating amino acid biosynthesis have not been well characterized in any plant or crop species. This report describes our recent studies on the control of lysine biosynthesis in corn and the potential for altering lysine synthesis.

Lysine synthesis is controlled by endproduct feedback inhibition. Two enzymes, aspartate kinase (AK) and dihydrodipicolinate synthase (DHPS), in the biosynthetic pathway are inhibited when cellular lysine exceeds certain concentrations. Fifty percent of AK and DHPS activity is inhibited by 10 and 23  $\mu$ M lysine, respectively, in normal corn genotypes (1,2). Tissue culture selection methods have been used in attempts to obtain mutants with altered feedback inhibition properties for these enzymes (3). Two independent, single gene dominant mutants were identified by selecting corn tissue cultures for the ability to survive the growth inhibition exerted by the combination of two amino acids, lysine + threonine (LT). The two mutant genotypes (designated Ask and Ask2) have altered AK enzyme activity that is significantly less inhibited by lysine (4). For example, 75-fold more lysine is needed to inhibit 50% of the AK activity from homozygous mutant Ask2/Ask2 than from the wildtype line. This high concentration completely inhibits wildtype AK.

The reduced lysine feedback inhibition of AK, which is the first enzyme in the pathway for lysine, threonine, methionine and isoleucine synthesis, seems to lead to significant changes in the free and protein-bound amino acid concentrations ( $\mu$ mol/mg dry weight) of mutant kernels. The free amino acid pool in <u>Ask2/Ask2</u> kernels has 174-, 10-, 13- and 2-fold increases in threonine, lysine, methionine and isoleucine, respectively, compared to wildtype corn. Protein-bound threonine changes little, but protein-bound methionine and lysine increased 74% and 20%, respectively, indicating a change in protein composition.

DHPS is the first enzyme specific to the lysine biosynthesis branch of the pathway and this enzyme in the two selected mutant genotypes does not have altered feedback sensitivity to lysine. This means that lysine synthesis is still subject to endproduct feedback inhibition of DHPS in the Ask and Ask2 mutants. To determine the most appropriate means to obtain DHPS mutants with altered feedback inhibition, we have purified wildtype DHPS and have identified corn cDNA clones for the enzyme (5). The cDNA clones were obtained by direct genetic selection in transformed cells of an Escherichia coli dapA auxotroph which is devoid of DHPS activity. We established that the cDNA clones were for corn DHPS based on agreement of the amino acid sequence of the purified DHPS with the nucleotide sequence of the cloned cDNA. DHPS activity expressed in transformed  $\underline{E}$ . coli cells was similar to corn DHPS in lysine inhibition ad distinct from the lysine inhibition observed for wildtype  $\underline{E}$ . coli DHPS. Site-directed mutagenesis and selection in the transformed  $\underline{E}$ . coli cells could provide a means to obtain corn DHPS with altered lysine feedback inhibition properties.

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FIELD TESTING OF CUCUMBER PLANTS WHICH EXPRESS THE CMV COAT PROTEIN GENE: FIELD PLOT DESIGN TO TEST NATURAL INFECTION PRESSURES

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Using a simple procedure for regenerating and transforming cucumber plants (Cucumis sativus L. cv. Poinsett 76) described by Chee (1990, HortSci., in press, and reported at this meeting) we obtained a series of cucumber plants which were transformed with the selection and reporter genes NPTII and Gus, respectively. These plants also contained the engineered CMV strain C coat protein gene. Analysis of  $R_{
m O}$  and  $R_{
m 1}$  plants showed expression of the CMV coat protein gene as determined by ELISA. Several of these Ro and Ro cucumber plants were challenged with CMV infections, in the greenhouse, and two plant lines (T47 and T60) showed significant delay in the onset of virus symptoms. T47 and T60 R1 plants were used for testing in the field. The field test plot was located in a region where both CMV and aphid vector were endemic. To ensure a high level of CMV infection pressure one half of the field plot was designed so that every fifth plant was a susceptible plant which had been mechanically infected with CMV prior to planting. The other half of the plot contained no intentionally infected cucumber plants and was used for a test involving lower disease pressure. Virus spread was assayed in both regions of the plot. Results from this initial field test showed that the CMV expressing transgenic cucumber plants were significantly less infected by CMV than control plants.

#### THE "NON-HOST" RESISTANCE OF PEAS MAY BE TRANSFERABLE TO POTATOES

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One of the quirks of natural resistance of plants to disease is that only a few of the millions of microorganisms in the environment can infect a given plant species. Further, these few tend to be host species specific. Thus a given plant species has the genetic notential to resist most of the plant pathogenic organisms except for those which specifically infect it. This "non-host resistance" can serve as a genetic source of disease resistance if properly defined and transferred to transformable economic crops. We have cloned and characterized some of the major genes expressed in pea tissue as it resists Fusarium solani f. sp. phaseoli, a pathogen of beans (1,2). The promoter of pea gene 49, constructed in line with the CAT reporter gene, has been transferred to both tobacco and The promoter functions consistently without elicitation when transiently incorporated into tobacco cells by electroporation (3). When tobacco or potato tissue was transformed with the gene 49 promoter via the T-DNA of the Ti plasmid of Agrobacterium tumefaciens, the reporter gene is produced only by elicitation and with much less consistency. Thus our present emphasis is directed towards further understanding how this gene and other disease resistance response genes are induced in peas. The 3' and 5' noncoding regions of gene 49 both contain a consensus binding site for an AP-1 transcription factor and clusters of topoisomerase II consensus sites (3). We propose that a potential exists for gene 49 to exist on a short chromosomal loop anchored in the scaffold attachment region via the topoisomerase clusters. Some factors capable of altering the topography of chromatin within the loop can activate gene 49 and influence topoisomerase activity. Antisera directed towards the 17 Kd product of gene 49 has been used to locate the accumulation of this protein following infection.

The biological function of this major inducible protein of legumes is unknown. Since this gene and other major pea genes such as chitinase and  $\beta$ -glucanase are actively expressed in correlation with the cytological expression of resistance, they may collectively generate a level of disease resistance if expressed quickly after challenge by the pathogen. Therefore, it is possible that the pea gene 49 promoter, which enables gene 49 to be expressed quickly in pea tissue challenged by an unsuccessful potato pathogen, when constructed with the major pea response genes and transferred to potato may also afford potato tissue protection from potato pathogens.

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# SYMPOSIUM

ON

# FRONTIERS IN REGULATORY BIOLOGY

Presiding:

Mark A. Sheridan Zoology Department NDSU, Fargo, ND

- 39. Gonadotropin Releasing Hormone: A Neuroendocrine Peptide for All Seasons. P. Michael Conn\*, University of Iowa, Iowa City, IA
- 40.Structure and Regulation of Secretion of Mouse and Hamster Placental Lactogens. Frank Talamantes\*, University of California, Santa Cruz, CA
- 41.Regulation of Growth and Differentiation of Rat Embryos and Fetuses by Hormones and Growth Factors. Charles S. Nicoll, University of California, Berkeley, CA.
- 42.Glucocorticoid Suppression of Hepatoma Cell Growth. Gary L. Firestone\*, Tokuku Haraguchi, Irma Sanchez and Caroline P. Edwards, University of California, Berkeley, CA.

The number preceding each communication listed above is that assigned to represent the presentation sequence in the meeting program booklet

# GONADOTROPIN RELEASING HORMONE: A NEUROENDOCRINE PEPTIDE FOR ALL SEASONS

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Gonadotropin releasing hormone (GnRH) is a neuroendocrine peptide which has been chemically identified:  $pyro-Glu^1-His^2Trp^3-Ser^4-Tyr^5-Gly^6-Leu^7-Arg^8-Pro^9Gly^10-NH_2$ . It is synthesized and stored in neurosecretory cells in the medial basal hypothalamus (brain). GnRH is released into the hypophyseal portal circulation through which it travels to the anterior pituitary. At that site, it binds to target cell receptors on pituitary gonadotropes; these respond by releasing the gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). The gonadotropins are released into the general circulation and act on the gonads to stimulate steroidogenesis and gamete maturation in both sexes. Gonadotropins also appear to be directly involved in negative feedback regulation on GnRH release from the hypothalamus. Gonadal steroids, produced in response to gonadotropin stimulation, are also involved with feedback regulation on the hypothalamus, as well as on the pituitary.

GnRH can be used therapeutically to treat a variety of human disease states. Pulsatile administration of low doses of GnRH or GnRH agonists results in maintenance or enhancement of gonadotropin and gonadal steroid production. Therefore, GnRH has been used to successfully treat conditions in which increased secretion of LH, FSH, and/or gonadal steroids is needed. Specific examples include hypogonadotropic hypogonadism, cryptorchidism (undescended testicles) and some cases of infertility in both men and women. Conversely, sustained treatment with high levels of GnRH or agonists results in decreased gonadotropin and gonadal steroid production. This effect of GnRH is used to treat conditions in which gonadotropins and gonadal steroid levels are elevated. Examples include prostatic cancer, breast cancer, polycystic ovarian disease, endometriosis, and precocious (early) puberty in boys and girls. Similarly, sustained administration of GnRH and its agonists can be utilized as a contraceptive in both men and women. Cystic ovaries, mammary tumors, perianal tumors, and prostatic cancer have been successfully treated with GnRH or GnRH antagonists. In addition, suppression or stimulation of the estrous cycle can be induced with GnRH administration.

Because of its profound effects on the reproductive system, GnRH analogs have important roles in veterinary medicine. Further, in European and Middle Eastern countries, GnRH analogs are used for establishment of synchronized ovulation and fertilization in the breeding of catfish.

The tremendous clinical and veterinary utility of GnRH and its analogs has been a catalyst for industry, government, and private laboratories to develop research programs associated with the development of new analogs—both agonists and antagonists. To date in excess of 3,500 GnRH analogs have been developed—more than for any other peptide or protein—and these have resulted in a precise understanding of the ligand receptor interaction. Also of significant interest is the mechanism of action of GnRH—the description of the molecular changes within the cell which occur as a result of the interaction with GnRH and lead to: (1) release of LH and FSH release, (2) regulation of cell sensitivity to a subsequent dose of GnRH, (3) regulation of its receptor, and (4) regulation of biosynthesis of the gonadotropins.

It is well-established that the first step in its mechanism of action is the interaction of GnRH with its plasma membrane receptor. The activation of the receptor by binding of an agonist leads to increased intracellular calcium levels (this ion, itself, serves as a second messenger in the release of gonadotropins), lipid turnover, the production of diacylglycerols, and the activation of intracellular molecules including protein kinase C and calmodulin. Advances in our understanding of GnRH action at the cellular and molecular levels have resulted in markedly improved treatment of human disorders and new compounds for veterinary applications.

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Molecular Mechanisms:

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# STRUCTURE AND REGULATION OF SECRETION OF MOUSE AND HAMSTER PLACENTAL LACTOGENS

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Rodent placental lactogens (PL's) are prolactin (PRL) and growth hormone (GH)-like polypeptide hormones produced by the fetal component of the placenta. Mouse placental lactogen-I (mPL-I) is a glycoprotein composed of species that range in molecular weight from about 29K to 42K, as determined by SDS polyacrylamide gel electrophoresis (I). Most of the molecular weight heterogeneity between mPL-I species is due to differences in glycosylation. Sequence analysis of a cDNA for mPL-I suggests that it is synthesized as a 224-amino acid precursor and secreted as a 194-amino acid species (2). The predicted amino acid sequence includes two potential sites for asparagine-linked glycosylation and 5 cysteline (cys) residues. The odd number of cys residues in mPL-I is an unusual feature among PLs. The "extra" cys, cys 167, is thought to form a disulfide bond with cys 169, which in other members of the GH-PRL-PL family participates in the formation of the large disulfide loop structure that is characteristic of this family of hormones. Formation of a disulfide bond between cys 167 and 169 is thought to prevent the formation of the large disulfide loop in mPL-I. The N-linked oligosaccharides of mPL-I are of the complex or hybrid type and contain sialic acid. Including the signal sequence, mPL-I shares 44% amino acid homology with mouse placental lactogen-II (mPL-II), 33% identity with mouse prolactin (mPRL) and 20% identity with mouse growth hormone (mGH).

Mouse PL-II is a single chain polypeptide having a molecular weight of 21, 812 (3). Sequence analysis of the cDNA indicates that the hormone is synthesized as a 222-amino acid precursor which is cleaved to yield a 191 amino acid mature mPL-II molecule. There are four cys residues. Although the positions of the disulfide bonds have not been determined, they are predicted to occur between cys 51 and 166 and cys 183 and 191 by analogy to mPRL and mGH. Mouse PL-II exists as several charge isoforms with isoelectric points ranging from 7.0 to 6.6 (4). The amino acid sequence of mPL-II shows 51% sequence homology with mPRL and 31% sequence homology with mGH.

Sequence analysis of the cDNA for hamster placental lactogen-II (haPL-II) codes for a 221 amino acid precursor protein which is cleaved to yield a 191 amino acid mature haPL-II protein (5). As is the case for mPL-II and rat placental lactogen-II (rPL-II), the mature haPL-II does not contain a consensus sequence for Asn-linked glycosylation (Asn-X-Ser/Thr). The most obvious structural difference between haPL-II and the other rodent PLs is the presence of an additional pair of cysteine (cys) residues. The four cys residues of haPL-II which correspond to those of mPL-II (at positions 51, 166, 183 and 191) are conserved in all known members of the GH-PRL-PL family. In addition, haPL-II contains a pair of cys residues not present in the other rodent PLs. These occur at residue 21 (Asn in mPL-II and Tyr in rPL-II). This unique pair of cys residues may be responsible for the extreme tendency of haPL-II, compared to other members of the GH-PRL-PL, family, to form disulfide-bonded hormone-serum protein complexes (6). Overall, haPI-II has essentially identical sequence homology to both mPL-II (70% identity) and rPL-II (68% identity). This homology is slightly less than between mPL-II and rPL-II (79% identity).

Utilizing a homologous radioimmunoassay (RIA), the concentration of mPL-I has been found to be detected as early as day 6 of pregnancy with peak levels on day 10 of gestation. In contrast, circulating mPL-II is first detected on day 10 of pregnancy, and the hormone level increases until term. The maternal serum mPL-II concentration is regulated by the number of conceptuses, the genotype of the fetoplacental unit, by the pituitary via GH, the maternal nutritional status, ovaries and by the decidua. An increase in the production rate of mPL-II appears to play a role in determining the gestational profile of the hormone but a change in half-life does not. (Supported by NSF grant PCM-8217382 and NIH grants RD 14966 and RR 08131).

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REGULATION OF GROWTH AND DIFFERENTIATION OF RAT EMBROYS AND FETUSES BY HORMONES AND GROWTH FACTORS.

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Studies on the factors that regulate growth and tissue differentiation in mammalian embryos and fetuses  $\underline{\text{in utero}}$  are complicated by their inaccessibility and other factors. To avoid these problems transplantation of whole 10-day embryos or parts of 14-16-day fetuses were used to study these processes in an  $\underline{\text{in vivo}}$  system. When placed under the kidney capsule of intact syngeneic hosts, fetal paws or intestinal segments grow almost as well as they do  $\underline{\text{in situ}}$ , and tissue differentiation occurs normally. By contrast, although transplanted 10-day embryos grow rapidly and their tissues differentiate normally, their growth is severely retarded compared to that which occurs in embryos left  $\underline{\text{in situ}}$ . Thus, while the internal milieu of intact host rats is well suited for supporting essentially normal growth of fetal structures, it appears to lack some factor or factors that are essential to support normal growth of the embryos.

In hypophysectomized (Hx) hosts growth of embryos and fetal tissue transplants was inhibited by 50-65%, and in diabetic hosts their growth was impaired by 25-35%. Tissue differentiation was retarded only in the Hx hosts. Replacement therapy with growth hormone (GH) or insulin in the respective hosts restored transplant growth to normal, and GH treatment corrected the impaired differentiation. The restorative effects of insulin were exerted directly on the transplanted tissues but the effects of GH were indirect, apparently mediated by insulin-like growth factors (IGFs).

The role of several growth factors was studied by infusing either the factors or antiserum to them into the arterial blood supply of kidneys bearing the transplants. Antiserum to fibroblast growth factor (FGF) caused a striking inhibition of the growth of embryo transplants and prevented the differentiation of all mesodermal and many endodermal tissues. Infusion of recombinant FGF significantly stimulated growth of the embryos. By contrast, the antiserum to the growth factor and FGF itself had only slight effects on growth and tissue differentiation in transplanted fetal structures. Studies with the IGFs indicate that embryos are more responsive to IGF-II than to IGF-I, whereas fetal tissues show the opposite responsiveness. Preliminary results indicate that epidermal growth factor inhibits growth of embryo transplants.

Overall our results indicate that the dependence of rat tissues in hormones and growth factors changes during development. Embryos are more dependent upon FGF and IGF-II than are fetal tissues but the opposite is true for IGF-I. However, embryonic and fetal tissues appear to be equally dependent on insulin for tissue growth, but not for tissue differentiation. Growth and development of tissues in the rat does not become dependent on GH or thyroid hormones until after birth.

GLUCOCORTICOID SUPPRESSION OF HEPATOMA CELL GROWTH Gary L. Firestone\*, Tokuko Haraguchi, Irma Sanchez and Caroline P. Edwards Dept. of Molecular and Cell Biology and The Cancer Research Laboratory

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We have documented that glucocorticoid hormones suppress the growth rate and final cell density of in vitro cultures of Fu5 rat hepatoma cells [1] which are derived from the minimal deviation Rueber H35 tumor. The suppression of Fu5 cell growth requires a strict dependence on glucocorticoid receptor occupancy by known glucocorticoids while the powerful synthetic glucocorticoid antagonist, RU38486, efficiently prevents this response. Expression of cloned glucocorticoid receptor genes in a glucocorticoid receptor minus variant restored the ability of these cells to respond to the growth suppression effect of dexamethasone and thereby demonstrating that this regulation is a glucocorticoid receptor-mediated process (1). Glucocorticoids confer a "normal-like" phenotype on these epithelial tumor cells since cellular proliferation is arrested in a controlled and synchronous fashion while the level of serum determines the absolute arrested cell density. Glucocorticoid withdrawal triggers a rapid and synchronous reinitiation of DNA synthesis concomitant with an induction cascade of transcripts for several cell cycle regulated genes which peak prior to and during the onset of DNA synthesis (c-fos at 30 min. cmyc at 2 hours, c-rasKi at 4 hours while c-rasHa and ornithine decarboxylase transcripts rose steadily over the course of 16 hours) without any rapid changes in intracellular Ca++ levels or pH (2).

To genetically dissect the key cellular events responsible for the growth suppression, unique Fu5-derived variants that are either resistant or hypersensitive to the growth inhibitory effects of glucocorticoids have been recovered by different selection procedures (2). One class of glucocorticoid resistant variants, represented by EDR3, fail to express glucocorticoid receptor transcripts, whereas, a second class of variants, represented by EDR1, expressed a defective receptor and is partially responsive to glucocorticoids. A third class of dexamethasone-resistant variants, DRG13, are selectively defective in the glucocorticoid-regulated growth suppression pathway since they were constructed to contain excess copies of the wild type glucocorticoid receptor gene and genetically defines a new class of glucocorticoid-regulated proliferation suppressor genes. The expression of known glucocorticoid-regulated gene products was examined in this panel of glucocorticoid-resistant proliferation variants. Interestingly, the expression of alpha1-acid glycoprotein, a liver-specific acute phase protein, strikingly correlated with the suppression of growth suggesting a potential role for this glycoprotein in the growth suppression response.

Characterization of a a variant (BDS1) hypersensitive to the dexamethasone-mediated growth suppression, has revealed a secreted 30,000 M<sub>r</sub> glucocorticoid suppressible mitogenic activity (denoted GSM) that stimulates DNA synthesis and alters cellular morphology of quiescent, serum starved Balb/c 3T3 cells (3). The regulated expression of the secreted GSM activity represents a new glucocorticoid response. The GSM activity fails to be expressed in certain glucocorticoid-resistant variants while partially purified GSM does not stimulate the DNA synthesis of glucocorticoid suppressed Fu5 hepatoma cells suggesting that hepatoma cell growth does not require the presence of this mitogenic activity or result from its actions. Thus, characterization of the glucocorticoid-resistant and hypersensitive proliferation variants have defined a complex multifactor system composed of intracellular and extracellular gene products which are associated with the glucocorticoid suppression of Fu5 hepatoma cell growth. We are attempting to define potential glucocorticoid-regulated growth suppression genes and to determine the identity, and function of the glucocorticoid suppressible mitogenic activity.

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### SYMPOSIUM

ON

# ENGINEERING DESIGN EDUCATION: RESEARCH, TEACHING AND ECONOMIC DEVELOPMENT

Presiding: David A. Rogers

Department of Electrical and Electronics
Engineering

Engineering

NDSU, Fargo, ND

and

Girish Kumar

Department of Electrical Engineering

UND, Grand Forks, ND

- 43. Design Links Engineering Education with Industry and Community. David A. Rogers\*, NDSU, Fargo, and Girish Kumar, UND, Grand Forks.
- 44. Some Case Studies on the Teaching of Electromechanical Design. Arturo R. Miles\*, UND, Grand Forks.
- 45.Optimization in Engineering Design. Sudhir I. Mehta\*, NDSU, Fargo.
- 46.Robotics Design Projects. Arnold F. Johnson\*, UND, Grand Forks.
- 47. Computers and Design in Teaching Electromagnetics. Douglas B. Miron\*, SDSU, Brookings, SD
- 48. Senior Design with a Purpose. Daniel J. Krause\*, Jenny L. Rawson and John D. Enderle, NDSU, Fargo.
- 49.A Power System Project for Engineering Student Design Experience. Paulo F. Ribeiro\*, Dordt College, Sioux Center, IA.
- 50. Innovative Electronics Design Techniques Leading Towards Research and Economic Development. Girish Kumar\*, UND, Grand Forks.
- 51.Organizing the Advanced Electronics Design Course. David A. Rogers\* and Robert Nelson, NDSU, Fargo.

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#### DESIGN LINKS ENGINEERING EDUCATION WITH INDUSTRY AND COMMUNITY

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Girish Kumar University of North Dakota Grand Forks, North Dakota 58202

As is pointed out in one of the papers in this session, many engineering instructors are called upon to teach design courses which are often required for engineering students in the last year or two of their undergraduate studies. It is not uncommon for either a senior professor with long years of experience in teaching lecture-oriented theory classes or the new assistant professor just out of several years of high-level research in graduate school to be called upon to teach this course.

What resources are available to such instructors (and their students)? We will explore this question among others in this session. For many of us design is a major emphasis in the senior year. It should, of course, be integrated throughout the engineering curriculum. However, it has become especially significant in recent years as industry has heavily recruited the new engineering graduate who shows promise of becoming a productive contributor in a few months or so. Gone apparently are the days of the one- or two-year orientation or apprenticeship that was common in large companies twenty or more years ago.

Papers in this session deal with design in engineering education, and its impact on the curriculum and the community. Electrical engineers with many different specialties teach design. Engineers in other disciplines are also conducting intensive design efforts. A paper in this session by Professor S.I. Mehta, a mechanical engineer, is an example of this. Each presenter in this session teaches a senior design course and/or is involved in a course with strong design components.

Under requirements established by the Accreditation Board for Engineering and Technology (ABET), design experience must exist across the engineering curriculum. This means that there will be design components in almost every engineering course, constituting perhaps twenty-five percent or more of a single course in some cases. In addition, most schools find it useful to have one or two courses that are specifically in engineering design. In electrical engineering, design tends to emphasize electronics since this is a core study area for our students and because of the availability of electronic components and test equipment.

Since design is typically a senior-year course, most students who enroll are well prepared in at least some of the subject areas they studied in the past, but not all are able to perform at a high level immediately in each, and some will resist having to do a design project in certain areas (i.e. their "worst" subjects). Many of these courses have been good theory courses, but practical design experience is lacking. Gaps in student background and experience must be repaired in senior design.

The instructor may choose to focus student interest in one area such as projects for the disabled (see paper by Krause and Rawson), robotics projects (see paper by Johnson) or projects to stimulate economic development (see papers by Kumar and by Rogers and Nelson). This latter area is a concern of all session authors. Can design contribute toward the goal of fostering small- to mediumsize industrial development in their local communities? All session authors would respond, "Yes!" Through the leadership of Professors John D. Enderle and Daniel J. Krause, North Dakota State University has become involved in a program to foster design projects to aid the disabled. (1) These programs are funded by the National Science Foundation through the Bioengineering and Research to Aid the Disabled (BRAD) program. Many of the design projects require strong student interaction both with teachers of the disabled and with their disabled students. An immediate consequence of this is the development of good relationships between engineering students and members of the local community.

In the spring of each year students from the electrical engineering departments of UND and NDSU participate in the Institute of Electrical and Electronics Engineers (IEEE) Red River Valley Section student paper contest held as part of a regular monthly professional meeting of the Section. Most of these presentations are based on senior design projects and have been received with enthusiasm by IEEE members. This is a significant learning experience for the students involved and, at the same time, it serves to challenge and update the practicing engineers in attendance. Faculty members from UND and NDSU have long supported this event.

Design prepares students for professional productivity. Design courses and projects have the potential for a positive impact on students, the university, and the community.

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#### SOME CASE STUDIES ON THE TEACHING OF ELECTROMECHANICAL DESIGN

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Traditionally, electromechanical design was aimed mostly at rotating machines (motors and generators), as they are, in many ways, much harder to analyze than the simpler relays, contactors, etc. Recently, our focus has shifted in the above mentioned course to including a greater variety of devices. An effort is made to always address some motor design but only enough to convey one exposure.

The motivation for this change is due to at least two factors: on the one hand we have had a declining student interest in motors and generators over the years, and couching the design in the context of rare topologies has been thought to be a good catalyst for renewed interest. On the other hand, the students curiosity for such down-to-earth devices like a doorbell, or a speaker, is not addressed in any other parts of the curriculum and so when the opportunity is there it is exciting. It should be noted here that the course is offered once a year in the last senior semester, with about 8 to 14 students signed up. They work either individually or in pairs, and are required to design, build and test their devices, with a final report and formal presentation at the conclusion.

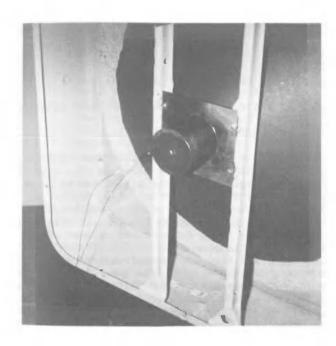


Figure 1 - A Loudspeaker Design

Note the cloth glued to the outer edge of the cone, so as to provide "springiness", and the ease with which the floor fan was adapted.

Figure 1 - A Loudspeaker Design

The projects are built with as much local hardware store material as possible. A typical case is that of a loudspeaker, which was built with a frame from a floor fan, a cone built of construction paper, and a main field electromagnet from hollow pipe and solid steel bar stock. The accompanying figure is a photo of the device. Cases that have been addressed are relays, contactors, telephone ringer, stepper motor, induction motor, special transformer, linear motor and eddy-current brake.

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References are standard in the individual device specialties, and rarely are texts specified for the course, as there would be too many. A video-cassette illustrating some of the projects is available on a loan basis from the author.

# OPTIMIZATION IN ENGINEERING DESIGN

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Optimization methods are finding an increasing number of applications in the area of engineering design. This is due to the availability of the optimization software packages, the availability of relatively low-cost high-power computers and the competition in industry. Some of the recent magazines have reported, "Once the exclusive domain of a few mathematicians, design optimization is finding a home in commercial analysis codes," [1] and "optimization methods are being offered for the ANSYS FE code because ... engineers can no longer afford to make merely adequate products."[2]

An optimization problem is formulated in the following standard form.

Minimize 
$$f(X)$$
  
With respect to  $X$   
Subject to  $g_j(X) \le 0$ ;  $j = 1$  to  $J$ ,  $h_k(X) = 0$ ;  $k = 1$  to  $K$ ,  $x_i^1 \le x_i \le x_i^u$ ;  $i = 1$  to  $n$ 

where f(X) is an objective function, X is an (nx1) design vector,  $\mathbf{g_j}(X)$  are J inequality constraints,  $\mathbf{h_k}(X)$  are K equality constraints, and  $\mathbf{x_i}^1$  and  $\mathbf{x_i}^u$  are the lower and upper bounds of each of the n design variables  $\mathbf{x_i}$ .

In engineering, many design problems can be formulated in the above standard form. However, very often the objective function and the constraints do not have any specific standard form with respect to the design variables. These conditions then require a special class of algorithms to solve the optimization problems. These algorithms are referred to as Non-linear Programming Methods of Optimization. There are a few textbooks available which deal with this class of optimization problems [3,4,5]. At North Dakota State University we have been teaching a course on this subject for the last six years. The course is offered at a graduate level and is taken by most of the graduate students of all the engineering departments. In this course the students learn the mathematical concepts behind the optimization algorithms and they use the software packages [6] to apply to the engineering problem. Some of the projects done by the students are: Optimal Design of a Steam Condenser, Minimization of the Chip Area of an IC, Optimization of Vehicle Transmission, Optimum Design of an Analog to Digital Converter, Design of a Transformer, Optimization of Drum Brake Design, Optimization of a Pressure Vessel, and Maximization of the Torque of an Inductive Motor. Results of some of these projects and the details of the software packages will be presented at the annual meeting.

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#### ROBOTICS DESIGN PROJECTS

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The Electrical Engineering Department at the University of North Dakota has offered a course in Robotics Fundamentals since 1984. The course has traditionally been offered every fall semester. The last two semesters I have had the opportunity to teach this class. There are usually about 20 students in the class. It is made up of mostly seniors, but there are usually several graduate students who take the course also. Most of these students are in Electrical Engineering, but we also get

As a part of this class the students are required to do a design project using the knowledge and experience gained from this class. Even though this course is not specified as a laboratory course, there is an informal laboratory associated with it. Students are required to complete approximately eight short laboratory exercises. These exercises may take from fifteen minutes to as long as two hours each. Thus the students become familiar with our lab robot (Rhino XR-1), the microprocessors in the lab and also with some of the basic equipments used in robots such as stepper motors and optical encoders.

The following is a listing of the design projects that were completed by the students during the last two years.

- \* Interfacing the Rhino with an IBM Compatible PC
- \* Intergrating the Rhino, Photosensors, a Conveyor & the Z-8 Microprocessor
- \* Joystick Control of a Robotic Cart
- \* A Teach Pendant for the Rhino Robot
- \* A Line Following Robot
- \* A Computer Controlled Conveyor Belt
- \* A Model of the Stanford Manipulator
- \* Computer Controlled Velocity Profile
- \* Automatic Control of the Rhino via EPROMs
- \* Stepping Motor Control via a MC6809 Microprocessor
- $\mbox{\tt {\tt \#}}$  Assembly Line Simulation with Phototransistor Pairs
- \* A Pencil Factory Process (Robotic Pencil Sharpener)
- \* Search and Detect (Implementing a Magnetic Detector)
- \* A Material Sensitive Gripper for a Robot

The philosophy behind the class is to introduce the students to the multidisciplined topic of robotics. The students are taught both forward and inverse kinematics as well as trajectory planning. The main premise is that our students will be involved in the design of robots or robotic manipulators so that they need to know some of the underlying concepts of robotics. That is ... they learn how to develop the equations that describe the robot's motion rather than just walking the robot through a specified set of motions using a teach pendant.

The class is also intended to present a design component to the student. Therefore, the student is challenged with a design project at the end of the course. This project is worth 25% of the student's grade. The intention of the design project is to help the student make the transition from the theory learned in class to the development of hardware/software that has practical use in the industrial world. The students like this challenge and for most of them it is the most interesting part of the course.

At the beginning of this last semester the students toured several industrial facilities which used robots in their manufacturing operation. This helped give them a feel for possible robotic projects. Also, this last semester the students were required to give semiformal presentations of their projects which were videotaped. This gave an opportunity for future classes to observe the type of projects taken on in the course. It also gave the students experience in making presentations. Both of these ideas were well received by the students.

This course has been very popular with the students. It has limited enrollment because we only have one Rhino robot in our laboratory. Thus the course usually fills rather early during the pre-registration period. For most students this course ends up being a very enjoyable and satisfying design experience.

## COMPUTERS AND DESIGN IN TEACHING ELECTROMAGNETICS

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Last Fall, I decided to introduce a little design experience into the Electromagnetic Field Theory course. At the beginning of the semester I didn't have a clear idea about what kind of design problem I would eventually give them. We have heard that kids have had personal computers (pcs) available for some years now, and expect some degree of computer competence in our students. I thought I would use this strength to improve their understanding through writing programs to use basic field equations to calculate field values for specific problems. In the first program assignment, I asked them to write a program that would allow the user to specify an arbitrary point-charge distribution in a plane, and a line along which the program would calculate the components and magnitude of E. The program would do these calculations and then plot the results. They were to do this on a pc in any language. Well, it turned out that most of them had done very little programming on a pc. They all had the mainframe course in FORTRAM. Some had done a little programming in BASIC on pcs but claimed to be rusty. None had ever done any programming to do plots. With some coaching, we got over these hurdles and produced a few programs that worked. The next program assignment was similar, in that it concerned the potential from line sources perpendicular to the plane in which potential was to be calculated and plotted. By the time we got through this one, the gap between those who were starting to catch on and those who weren't was widening so that some dropped the class. For the fourth program assignment I asked them to calculate the H-field in the symmetry plane of a finite-length cylindrical current. This was a little more ambitious in that I wanted them to study the effect of step-size choice in the numerical integration, and observation-point distance, by comparing the calculated result to the infinite-line value. While mostly successful programs were written, little understanding of how to study parameter dependence was developed.

In this class, I hand out a chapter on impedance-matching using simple networks, starting with L-C lumped-element design, and then three transmission-line approaches, short-line approximations to low-pass lumped-element designs, resonated load and quarter-wave transformer, and the single-stub system. I decided to give them the impendance function for an electrically-small hemispherical monopole and ask them to do matching-network designs using a lumped-element network and two transmission-line methods. I gave them limits on the value ranges they could use for parts and line parameters, and center frequency, source impedance, and passband requirements to meet. We worked through the resonated-load and quarter-wave transformer method in class, to the point of choosing substrate material and using charts to find the line widths and phase speeds. They had three weeks to do the assignment. It became apparent from the questions I started to get in and out of class that most of them hadn't started by halfway through the second week. In the third week, panic had set in. Most seemed to be trying to use formulas without reading the words in the text or using the figures to even understand the geometry of the system they were trying to design. In the end, I didn't include this assignment in the points base for the course. I used whatever they did accomplish as extra credit for the programming part of their course grade.

From the experience described above, and from my previous experience teaching design in the

Linear Controls course, I offer the following points.

1. The level of programming preparation of most students is inadequate. They have zero experience in plotting, make logic and syntax errors which they cannot find (see below), and they generate a lot of spaghetti. They generally don't know what a flow chart is for, and none write the flow chart first.

2. Field theory is hard enough to learn. To raise their stress level by requiring them to learn proper programming and do some self-study in a short time for a design assignment is more than any in that class could bear.

3. Generally, students do not understand trouble-shooting. This is true whether they are in the lab or working on paper. They seldom do intermediate checks, and if the result is not right, they will start over from the beginning, the equivalent of tearing the circuit apart, and frequently repeat their mistakes. Even when they understand signal tracing in the lab, they do not transfer the concept to mathematical work, paper circuit design, or programming. Even though I stand there in class, check my units as I go, make approximate estimates of what values should turn out to be, and make numerous sketches of aspects of the problem as I go, very little of this seems to be imitated by the students. These deficiencies do not show up strongly in simple problems but they become critical stumbling-blocks in anything approaching real design.

critical stumbling-blocks in anything approaching real design.

Design is a multi-stage iterative process which frequently involves learning as well as the application of one's current knowledge. At the present state of our curriculum, even minor design projects should be limited to one per course. A course in problem-solving might be useful. Its purpose should not be to teach any new physics or math, but to use what the students have already

seen to find the errors in incorrect solutions.

#### SENIOR DESIGN WITH A PURPOSE

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Design in an engineering curriculum has been identified by ABET as very important and is a required part of an accredited curriculum. Yet many design projects remain as academic exercises without a specific purpose and without much connection to real world problems. It is difficult for either faculty or students to find suitable design projects for all engineering students. Cooperative education experiences help in exposing students to the design process, but have the problem of not being directly supervised by faculty.

In 1988 a National Science Foundation grant was received by the electrical engineering department at NDSU to fund design projects for the disabled. This has given students an opportunity to work on projects that will be used by handicapped people in the region. What will be presented here will be how suitable projects are identified and how students are involved with the selection process.

The problem is to find about 120 student projects per year oriented toward the disabled. Students are encouraged but not required to do a disabled design project, but they all are required, as part of senior design, to participate in the identification of possible design projects that could help the disabled. Having the students involved in the selection process should be motivational. During the writing of the proposal to NSF, various organizations in the Fargo-Moorhead area that dealt with the disabled were contacted and asked if they would like to participate. This was at an administrative level with few contacts directly with the disabled, physical therapists, or teachers. Once the proposal was accepted, additional contacts with teachers and therapists were made so that there would be means for the students to identify potential projects.

Historically, design projects at NDSU have been individual projects carried out by students working alone. Now, cooperation and interaction is encouraged by having students assigned to peer groups. Students are randomly assigned to peer groups of six. Each group then elects a group leader and a group teacher is assigned to each group. Next, a site visit to an organization or individual for each group is arranged. Transportation is provided by NDSU.

At the site, students are to find as many projects as possible independent of simplicity, complexity or suitability for a design project. In some cases they observe and other cases they meet with individuals and in still others they are interviewed on what electrical engineers could design. In all cases they record the possible projects in their design notebooks. Each student then fills out at least one site report describing one project. These site reports are collected and shared with all so that individual interests can be matched with needs of the disabled community. A full spectrum of projects covering all areas of electrical engineering taught at NDSU were identified.

In the remainder of the quarter, each student undertakes to complete a paper design of project. The building of the projects is not required except for students in the bioengineering option so that many projects are not yet completed. Some of the more successful are listed(1).

"Emergency Telephone Dialing System" by Megan Everett

"Light Talker" by Andrew Beauchamp

"Pneumatic Sewing Machine Control" by Darin Westly

"An Ultrasonic Ranging System for the Visually Impaired" by Eric Durfee

"Writing Skills Improvement Board" by Charles Breid

Students generally have appreciated the chance to work on a real problem that could provide an immediate benefit. A few do not like the involvement and some are permitted to work on other projects that they have already identified. The projects for the disabled have given a higher visibility to electrical engineering by inviting the local news media to project demonstrations at the end of terms.

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### A POWER SYSTEM PROJECT FOR ENGINEERING STUDENT DESIGN EXPERIENCE

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Cooperative programs between university and industry (particularly in the area of power engineering) exist essentially to support research at the graduate level. Very few of those programs seem to be related to the undergraduate curriculum. Undergraduate research provides not only an economic means of exploring new ideas but also gives a new dimension to the engineering education process. With those ideas in mind, a sophisticated professional load flow program was introduced in the curriculum of a power system analysis course. The project was given to the students at the beginning of the semester in order to create the motivation for learning the concepts necessary to develop the project.

The students used the Distribution System Analysis and Simulation (DSAS) program, IBM-PC version, developed by the Electric Power Research Institute (EPRI). The primary function of the DSAS program is to provide detailed simulation of a distribution feeder utilizing load energy models and three-phase circuit modeling. The DSAS program is capable of predicting energy changes as a function of voltage at the circuit level.

The project's goal was to investigate the possibility of energy conservation on a distribution system by a controlled reduction of the voltage levels along the feeders. Applying this approach to a specific case is not always simple. The benefit of using the DSAS program is the use of complex simulation to predict the quality of the system and promote ideas for energy savings. Many state regulatory agencies are either suggesting or requiring that utilities study or perform voltage reduction experiments to see if it is possible to conserve energy by this means. As this project investigates an actual problem confronted by engineers in the power industry, it is expected that the experience will lead the students to a better understanding, not only of the basic concepts, but also of the complexity of real life problems. Since this kind of project, together with many others being developed in the Engineering Department, also satisfies the ABET design content in the courses, it is expected that this approach might help strengthen the department's effort regarding the accreditation process.

It is also expected that this approach will bring more excitement to the study of power engineering, as undergraduate students realize that they are discovering, opening up, and unfolding an actual technological system, rather than just repeating old experiments. Based on this experience it is expected that future projects for a power system analysis course will be developed by expanding the use of DSAS and other sophisticated computer programs available in the engineering department.

The results of this project were well received by the utility people, although they have a natural reluctance to implement the ideas without further studies. This is perfectly understood considering that the voltage reduction technique for energy conservation is a relatively new concept and may be applied only under well-controlled situations. The success of this project has encouraged the Engineering Department at Dordt College and the Sioux Center Utility to proceed with this cooperative program. From the results obtained in this undergraduate teaching/research experiment, the potential is clear for energy savings by operating the feeders of the electric system of the City of Sioux Center in the lower 5% of the acceptable voltage band. Even in a small utility, the savings could justify the implementation of such procedures. It must be noted, however, that operating at a reduced band may require additional capital expenditure, especially if the feeder length is rather long. These expenditures would have to be offset by the resulting benefits in order to justify installation of additional equipment.

This project has investigated an actual problem confronted by engineers in the power industry. The experience has led the students to a better understanding, not only of the basic concepts, but also of the complexity of real-life problems regarding power engineering. Finally, it is expected that this kind of project, that has integrated a senior project, the work-study program, a power utility problem, and a research effort as part of an undergraduate power system analysis course, may demonstrate both the possibility of the development of meaningful undergraduate research projects, and a model relationship between colleges and communities, regarding power engineering education.

# INNOVATIVE ELECTRONICS DESIGN TECHNIQUES LEADING TOWARDS RESEARCH AND ECONOMIC DEVELOPMENT

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I have been teaching undergraduate electronics courses (Electronics I and II, and Electronic Circuits) for the last three years at UND. Electronic Circuits is one of the four elective courses (other three courses are Robotics, Machine Design, and Power Systems) which satisfy the design requirements of ABET (Accreditation Board for Engineering and Technology). This course (more popularly known as Electronics III) is offered every spring semester and has both formal lecture and design components. The lectures are primarily concentrated on the use and applications of integrated circuit chips. For the design component, a list of design projects is given to the students. They can either select a project from the given list or choose their own project, which is subject to approval. Oral presentations by the groups (typically 2 to 3 students) is a routine part of the class, which helps them in developing their communication skills, and learning about not only their own projects but other's projects as well. A test based on the general aspects of all the projects is given towards the end of the semester. Overall grading of the course is strongly based on the group performance, hardware operation, final report quality, and presentation/demonstration of the project at the end of the semester in front of other faculties and students.

The prerequisites of the above design course are Electronics I and II, which are required courses for all the undergraduate electrical engineering students. The labs associated with these courses have been modified to give students some design flavor. In the Electronics II laboratory course, students spend half of their lab time on the mini-design project. Students are encouraged to choose their own project, and many a times they have come up with great ideas. They are required to submit biweekly progress reports on their projects which are reviewed to monitor their progress and graded. At the end of the semester they submit a final report and give a demonstration of their project. This prepares them well for the Electronic Circuits design course.

Some of the mini-design projects undertaken by the students in Electronics II are: Electric guitar amplifier, digital thermometer, digital appliance timer, grain bin indicator, car alarm system, heart rate monitor, breathalizer, speech synthesis, 12 channel fiber optic data link, infrared deer detector, scanner for WOLF System, ultrasonic distance ranger, electronic alarm clock, mono-stereo synthesizer, etc. On some occasions, students undertook a large design project, worked on its sub-system in Electronics II and then continued working on it in the Electronics Circuits course. This gave them an edge over other students in the latter course as they did not have to spend time on deciding and getting familiar with a new project. Some of them even worked on their project during the Christmas break!

Some of the design projects done in the Electronic Circuits course are: Digital bicycle speedometer/odometer, hail meter, digital anemometer, traffic light model, LED oscilloscope, ASCII generator for speech synthesis, speaker dependent and speaker independent voice recognition system, 7-band graphic equalizer with spectrum analyzer, digital weather sensing module, voice synthesized clock, etc. As can be seen, a wide variety of projects have been undertaken by the students, which gives them a broad spectrum of electronics design. Some of these projects have been done for Aerospace Studies and the Mechanical Engineering Department at UND. Talks are underway to get projects from other departments and local industries.

The electronic circuits course is very popular among the students even though it requires lots of extra time and effort. Besides learning about the various aspects of electronics, this course has provided some additional rewards for the students. Some of their projects have been displayed during Engineer's Week and have won several awards. It is being planned to demonstrate these projects to area high school students to get them interested in science and technology. Students have presented projects in the students' paper contest sponsored by the IEEE Red River Valley Section and won all the three prizes in April 1989. One student presented a paper in the ND Academy of Science and two more papers based on these projects have been communicated. One of the students opened his own company and one of the products is based on his design project.

#### ORGANIZING THE ADVANCED ELECTRONICS DESIGN COURSE

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Many engineering instructors are called upon to teach design courses which are now often required courses for students in their senior year. In the electrical engineering context this is an advanced electronics design course. The instructor must organize a solid course that will help the student move from textbook knowledge to professional performance. In addition to their own experience, instructors can find the tools and materials needed for the class by going to the following sources: the previous experience of other instructors, design-oriented textbooks, industrial databooks and design software, and electronics design and trade journals. The challenge will be to select those items that will be most helpful and to organize a class that integrates the use of these materials appropriately to prepare each student to propose, plan, and design his or her system, device, or process.

In electrical engineering, a student takes this course after taking most of the formal courses in analog and digital electronics including microprocessors. Many of these courses have been good theory courses, but few have brought the student into contact with the problem of device selection and purchase. Thus a significant topic in the course will be an introduction to the product selection guides available from industry, such as those published by Motorola, National/Fairchild, and Texas Instruments. Some instructors and students will prefer textbooks that introduce the process of device selection in a way that is more compatible with their previous experience in lecture-oriented courses. Of many possible candidates we will mention four books. Horowitz and Hill (1) comes out of a physics background, but is a very helpful design textbook. DeMassa (2) is an innovative electronics engineering textbook that is thoroughly design oriented. Franco (3) is a senior/graduate text in advanced electronic design. Meiksin and Thackray (4) is written to serve the general reader and is now a little bit dated, but it contains fine examples of completed designs along with descriptions that can serve as models for student reports. A careful choice of material from the selection guides and textbooks will give students a good foundation for their design work.

The instructor could focus student interest on one area such as projects for the disabled or projects for regional economic development. This can be a significant aid to student motivation. A student begins by writing a short project proposal. A written progress report on the design about midway through the term is expected. This is followed by a final report that details both the theory and the hardware realization of the device.

When teaching electronic circuit design, another topic which is very important is electromagnetic compatability (EMC). Ott states that "some of the most difficult and frustrating problems faced by design engineers concern elimination of noise from their circuits or systems." (5) Assuring the electromagnetic compatability of a design has a two-fold thrust. First, the equipment being designed should be able to function in its intended environment. The design should incorporate measures to ensure that the equipment is not susceptible to external sources. Second, the design should ensure that the equipment is not a source of electrical noise either to itself or to another piece of equipment. Introducing design students to such considerations is essential. Studies of potential EMC problems associated with each student project is a goal that we strongly recommend.

The advanced electronics design course prepares students for professional productivity. There is a wealth of topics and material available in these areas. Diligent preparation and openness to new ideas will lead the instructor to present a solid professional experience to engineers in formation.

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## SYMPOSIUM

ON

# GROUND WATER OF THE NORTHERN PLAINS

Presiding: Philip J. Gerla

Geology and Geological Engineering Department

UND, Grand Forks, ND

- 79.Past and Future Ground Water Research in the Northern Plains. Philip J. Gerla\*, UND, Grand Forks.
- 80.Regional Aquifers of North Dakota and Adjacent Areas of Canada. Joe S. Downey\*, USGS, Lakewood, CO.
- 81.Modelling Flow in the Dakota Aquifer in Norh Dakota. R.D. Butler\*, EERC/UND, Grand Forks.
- 82.Ground Water Recharge Through Till. A. Barari\*, T. Cowman and D. Iles, SDGS, Vermillion, SD
- 83. The Effects of No-Till and Moldboard Plow Tillage on the Movement of Nitrates and Pesticides Through the Vadose Zone. J. Bischoff\*, A. Bender and C. Carlson, SDSU, Brookings, SD.

The number preceding each communication listed above is that assigned to represent the presentation sequence in the meeting program booklet

#### PAST AND FUTURE GROUND WATER RESEARCH IN THE NORTHERN PLAINS

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Our knowledge of the physical and chemical aspects of ground water movement has increased dramatically as a result of several important tools and concepts that originated in research related to Northern Plains hydrological systems. Darton's documentation of flowing wells in the "Dakota" sandstone (Inyan Kara Formation) and the recharge of the same unit at higher elevations in the west is the classic example of a confined regional aquifer system (1). His predictions on water quality and the longevity of artesian head, however, were overly optimistic. Toth (2) pioneered work on the mathematical simulation of ground water flow, beginning with his analytical models of ground water drainage basins in Alberta. The results of his work developed into the current widespread use of numerical models to characterize ground water flow systems at all scales. Simulation of ground water movement in the regional bedrock aquifer complex of the Northern Plains has been recently completed (Downey, this volume).

Applying Toth's work, Maclay and Winter demonstrated the influence of flow path length on the variability of ground water composition in the Red River Valley (3) and the lakes of the Missouri Coteau (4). A later numerical analysis of the interaction of lakes and ground water. based on Winter's field observations in North Dakota, has been recognized as an important contribution to both limnology and ground water hydrology (5). The dynamic interaction between surface water, ground water, and plant communities was first characterized in Saskatchewan prairie pothole wetlands by Meyboom (6); water levels and quality in the widespread and ecologically important wetlands of the Northern Plains are maintained in large part by ground water and are likely to receive continued research interest in the future.

Although Freeze and Banner (7) were able to relate precipitation, infiltration, and recharge at a single highly instrumented site in Alberta, only a qualitative knowledge of regional "depression-focused" recharge (8) exists at the present time. Environmental isotopes have been used to determine the age and movement of ground water and will likely be applied to a greater extent in characterizing recharge. Active areas for future research include quantification of regional recharge through low-permeability materials (Barari et al., this volume) and the impact of long-term climate change on ground water resources.

Contamination of ground water from agricultural chemicals is a current focus of research in the region. This work is greatly improving our analytical techniques and theoretical concepts of subsurface transport (e.g., Bischoff, this volume). In comparison to other parts of the U.S., contamination of Northern Great Plains ground water systems has been minimal. Nevertheless, easily obtained potable water is commonly derived from vulnerable small and shallow Pleistocene aquifers; future studies will include additional evaluation with increasing emphasis on protection from impact.

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# REGIONAL AQUIFERS OF NORTH DAKOTA AND ADJACENT AREAS OF CANADA

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Rocks of Paleozoic and Mesozoic age underlie the entire northern Great Plains. These rocks have been subdivided into at least five major artesian aquifers by Downey (1). These regional aquifers are recharged in high mountain areas of Montana and Wyoming and in the Black Hills of South Dakota. The aquifers extend for more than 600 miles to discharge areas along the Red River Valley in the northeastern part of North Dakota and in the Canadian province of Manitoba (2). From the recharge areas the regional flow is generally to the east and northeast, with most of the discharge from the system occurring in eastern North Dakota and in the province of Manitoba. High-density brine (200,00-300,000 mg/l) is present in the deeper aquifers on the eastern flank of the Williston basin, and freshwater flow appears to divert to the north and south around the brine area or to leak upward into aquifers overlying the Madison. Fracture and lineament systems traverse the entire geologic section and exert a major influence on both vertical and horizontal flow as they provide conduits or act as barriers to ground-water flow.

Prior to Pleistocene glaciation, the flow system probably was similar to that which exists today. During the Pleistocene Epoch, ice sheets covered the northern and eastern parts of the study area, causing extensive changes in recharge and discharge patterns. These changes probably did not cause major shifting in the position of the high-density brines over any long periods of time.

The ground-water flow system was analyzed with the help of a three-dimensional mathematical simulation model using data collected by the Northern Great Plains Regional Aquifer Study (3).

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#### MODELLING FLOW IN THE DAKOTA AOUIFER IN NORTH DAKOTA

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The Dakota Aquifer is a complex of Lower Cretaceous sandstones that are present over most of the Northern Plains region. In North Dakota, the Dakota Aquifer consists of several hundred feet of water-bearing sandstones in the Inyan Kara Group and Newcastle Formation. For the past 100 years, groundwater from these sandstones has been vital in meeting the water needs of farms and small cities in eastern North Dakota. Wells in the Dakota have typically yielded groundwater suitable for most needs (except irrigation) in appreciable amounts, often under artesian pressure.

An effort to characterize the hydrogeology of this valuable resource was inititated about 10 years ago as part of an inventory of groundwater resources in the United States. Stratigraphy, hydrogeology, and water quality of the Dakota Aquifer were studied in detail. A digital model of flow was prepared to examine relationships between hydraulic behavior and water quality. This presentation will focus on results of the digital flow model, with emphasis on eastern North Dakota where regional groundwaters discharge from the system.

eastern North Dakota where regional groundwaters discharge from the system.

Work related to modelling flow in the Dakota Aquifer (3-dimensional finite-difference method) resulted in a series of maps that delineate distinct zones of active and inactive flow in the system (1). The relationships between these zones to variations in thickness and hydraulic conductivity within the complex of sandstones were examined. A key map in this series shows higher quality groundwater occurring in zones extending across the eastern half of the state to the Red River Valley. Expectedly, these zones tend to follow the permeable pathways through the aquifer created by the combination of substantial aquifer thickness, and possibly better hydraulic interconnection with permeable zones in overlying glacial sediments. The flow zones appear to be more restricted and focused along the eastern edge of the aquifer.

At the eastern end of the model, the total lateral flow (underflow) past a pre-determined line extending north-south across the state is calculated to be about 95 cfs. East of this line, some of the flow discharges to wells, but most is physically constrained to exit the system and leak upward through overlying bedrock shale and glacial sediment. Leakance values and vertical head differences at each model node determine the amount of leakage associated with a given nodal area. Model calculations indicate that leakage ranges from 0.01 to 12 cubic feet per second per node area (15 miles square), most of it upward in the Red River Valley. The average is 2 cfs per node, which would be about 9 gallons per day per acre for each of the 144,000 acres in the node. This is only a modelling estimate, and the model does not distinquish how this amount is physically distributed in the field, whether through fracture zones, direct connection with glacial sands, or otherwise.

Some of the more direct indications of Dakota discharge in the valley can be found in the water chemistry from well samples or from saline sloughs. There is also the signature of brackish waters (and brine) that appear to have leaked to the Dakota from underlying Ordovician and Cambrian aquifers, and mixed with the lateral flow. For the most part, field data on subcrop discharge from the Dakota is too limited for quantitative study of leakage.

Studies that include field instrumentation and sampling are needed to identify and describe the zones and pathways that focus the leakage and transmit groundwater from one aquifer to another across so-called confining layers. This type of information has a high value, not only for improving the predictive capability of a model, but also for basic understanding of how regional flow systems work. Future use of groundwater in the Dakota Aquifer may increase in periods of extended drought. Having a predictive model that works well would benefit management of the resource.

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GROUND-WATER RECHARGE THROUGH TILL

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Many prior investigations have been conducted throughout the world regarding recharge through permeable materials such as sand and gravel. However, little research has been conducted to determine the mechanisms and rates of recharge through clayey till.

From 1983 through the present, the South Dakota Geological Survey (SDGS) has been conducting investigations to better understand water movement in till. Results of these investigations show that from a hydrologic perspective, till can be divided into a weathered and an unweathered zone. The weathered zone contains fractures, whereas the unweathered zone is predominately void of fractures. Observation wells completed in weathered till show seasonal water level fluctuations which are indicative of recharge and discharge. Observation wells completed in unweathered till do not exhibit fluctuations indicative of recharge. The hydraulic conductivity of the fractured, weathered till is highly variable and averages approximately 10(-6) centimeters per second (cm/sec), while the hydraulic conductivity of the unweathered till is less variable and averages about 10(-8) cm/sec. Results of radioisotope age dating show that water in the weathered till is of recent origin and water in the unweathered till and underlying outwash is mostly connate water. These data support the concept of very little water movement through the unweathered till zone.

One of the SDGS till research sites is located in southeastern South Dakota near the city of Dolton. A portion of this area is underlain by the Dolton aquifer, which is a buried-confined aquifer of glacial origin that provides water for the Hanson Rural Water System (Hanson RWS), the TM Rural Water System (TM RWS), and many private wells. This outwash aquifer has a known areal extent of 82 square miles, consists of sand and gravel with an average thickness of 21 feet, and is buried under approximately 135 feet of till.

The water level in this aquifer has been declining at least since the Hanson RWS began pumping in 1982. The water-level decline is occurring over the entire known extent of the aquifer. Near the Hanson RWS the water level has declined more than 40 feet between 1982 and 1989.

The amount of recharge to the Dolton aquifer can be semi-quantitatively defined by the following comparison. During the time after the Hanson RWS began pumping and before the TM RWS began pumping from the Dolton aquifer, the amount of recharge over the known extent of the aquifer necessary to compensate for ground-water withdrawal by the Hanson RWS would have been 0.1 inches per year. However, because water levels throughout the entire known extent of the aquifer were declining during this time, it can be concluded that the amount of recharge, through unweathered till or from any other source, to the Dolton aquifer was less than 0.1 inches per year. Age dating of water in the unweathered till and in the Dolton aquifer and calculations of ground-water movement based on hydraulic conductivities and prevailing ground-water gradients also indicate a very small rate of recharge to the Dolton aquifer through the till.

THE EFFECTS OF NO-TILL AND MOLDBOARD PLOW TILLAGE ON THE MOVEMENT OF NITRATES AND PESTICIDES THROUGH THE VADOSE ZONE

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A field experiment was conducted using an innovative approach of collecting percolating water (1) moving through the unsaturated soil (vadose zone) at three depths (0.60m, 1.20m, and 1.80m).  $T_{WO}$  tillage treatments (moldboard plow (MP) and no-till (NT)), and three crops (corn, oats, and alfalfa) were evaluated on their effect on the quantity and quality of water moving in the soil preferential paths (macropores)  $\underline{during}$  a rainfall. These two tillage management extremes were evaluated to determine if groundwater may be protected from agricultural chemicals leaching through soils in macropores.

Plots were rotated with oats and corn each year with alfalfa remaining in the same area. Oats treatments received 112 kg/ha/yr nitrogen fertilizer as N in the form of ammonium nitrate dropped on the surface and corn treatments received 225 kg/ha/yr. The alfalfa treatment received no fertilizer. The fertilizer was spike-tooth harrowed for the MP treatments and remained on the surface for the NT treatments. Pesticides were applied at the same time with equal rates with equal methods of application between tillage systems. The soil was a Poinsett silt loam (fine-silty, mixed Udic Haploboroll) with 0.70-1.50m of silt loam soil overlying weathered clay loam glacial till.

After receiving five substantial rainfall events over the growing season, the NT corn treatments consistently had higher concentrations of nitrates in water samples collected from 1.20m and 1.80m than the MP. At the 0.60m depth the MP corn treatment consistently had significantly higher concentrations of nitrates in water samples collected from 1.20m and 1.80m than the MP. At the 0.60m depth the MP corn treatment consistently had significantly higher concentrations of nitrates. Water table position for all treatments were measured hourly. Although the amount of water reaching the water table was the same after a given time frame for both MP and NT corn treatments, percolating water reached a given depth faster for the NT corn treatment. This can be explained by the following theory: The MP acted more like a sponge in receiving rainfall than the NT. The tilled soil, consequently, required near saturation values before releasing the water to subsurface depths as the macropores were much less numerous. This allowed more contact time and contact area of the water with the fertilizer mixed in the soil. In the NT, a larger percent of the water percolated in the more numerous macropores which had a much higher hydraulic conductivity than the soil matrix. This transport of water picked up surface nutrients and carried them to deeper depths.

The number of pesticide detections from samples collected in the corn treatments were not significantly different between tillage systems for the first few samples analyzed. The most important finding from the preliminary data suggests that some chemicals which were not applied for two to three years prior to sampling were dominant in the analysis. This suggests that residual agricultural chemicals may persist in the soil complex longer than anticipated. The average number of pesticide detections per sample collected decreased with depth.

Cropping history and climate had a significant impact on the potential of leachate to reach the groundwater. Heavy water-use crops mined the subsurface soil water. Rainfall events on those treatments did not increase the water table. It appears that percolating water reaches the water table mainly when the soil profile is near field capacity.

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### SYMPOSIUM

ON

### PHYSICAL ACTIVITY AND NUTRITIONAL STATUS

Presiding: Henry C. Lukaski

Human Nutrition Research Center (USDA-ARS)

Grand Forks, ND

- 84. The Role of Protein and Fat in Physical Activity. Holly A. Dieken\*, NDSU, Fargo.
- 85. Interactions of Carbohydrate and Water. Donna J. Terbizan\*, NDSU, Fargo.
- 86.Physical Training and Trace Element Status. Henry C. Lukaski\*, HNRC/USDA/ARS, Grand Forks.
- 87. Exercises, Diet and Bone. A.D. Martin\* and D.T. Drinkwater, University of Manitoba, Winnipeg.
- 88.Body Composition and Performance. W.W. Bolonchuk\*, UND, Grand Forks.
- 89.Eating Disorders Among Athletes. Reuchele Tweed-Hadrava\*, Dakota Medical Center, Fargo.
- 90.Basic Eating Plan for Active People. S.J. Crockett\*, NDSU, Fargo.

The number preceding each communication listed above is that assigned to represent the presentation sequence in the meeting program booklet

# The Role of Protein and Fat in Physical Activity

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There has been significant debate over the utilization and requirements of the macronutrients in physical activity. Protein especially has been scrutinized related to its role in muscle anabolism during training for strength and power activities. More recently, protein utilization in endurance events has been investigated. While the results of studies are are not unanimous, there is adequate evidence to consider the need for additional protein for both individuals engaged in power and endurance sports.

The 1989 Recommended Dietary Allowances continue to subscribe to the belief that physical activity has little effect on protein requirements citing a 1977 study by Torun et al. [1]. While a 1987 supplement to Medicine and Science in Sports and Exercise presents numerous review papers to validate the need for additional protein in regular physical activity [2, 3,]. The problems related to assessing protein requirements in physical activity are numerous and include type of method used ie., in vivo or in vitro; procedures used to measure protein requirements - labeled isotopes, urinary urea nitrogen, 3-methylhistidine and nitrogen balance; the training status of the individual, and the frequency, type, intensity and duration of exercise as well as the conditions under which they occur [2]. In reviewing amino acid and protein turnover in exercise, Brooks [3] found that numerous studies report enhanced leucine oxidation to as high as three times normal turnover. And that leucine metabolism is linked to alanine in an anaplerotic role during exercise. Other studies using infused, labeled <sup>15</sup>N leucine support the enhanced turnover of leucine in exercise but do not find increased urea production clouding the overall amino acid kinetics situation in exercise [4]. Pivarnik et al. [5] evaluated muscle catabolism in athletes involved with progressive resistance weight training using 3-methylhistidine excretion and strict dietary controls. They found 3-methylhistidine excretion increased beginning the third day of the study and continued over the duration of the study [19 days].

While evaluation of protein requirements of individuals involved in physical activity is difficult due to a variety of characteristics related to the activity, there is good evidence to support increased muscle catabolism and increased nitrogen loses which should increase requirements.

While recent research has focused on the relationship between exercise and serum lipids, little attention has been given to the role of dietary fat in physical activity. While it is recognized that free fatty acids provide fuel to spare muscle glycogen and that lipolytic enzyme activity is enhanced by training, investigators perception of the significance of fat's role in activity is best addressed by the omission of studies from the literature. Of interest has been the proposal that "fat loading" may enhance endurance. However, carbohydrate is usually reduced in the diet to compensate for fat reducing glycogen and therefore limiting performance [6].

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### INTERACTIONS OF CARBOHYDRATE AND WATER

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physical activity requires energy. The body gets this energy from adenosine triphosphate (ATP). ATP is produced through the energy systems through the breakdown of carbohydrate, fat, and protein. The substrate for anaerobic glycolysis and the aerobic system is carbohydrate. Carbohydrate utilization during activities is dependent on a number of factors

Muscle glycogen utilization appears to be affected by the length of exercise. The greatest number of usage occurs during the first few minutes of moderate exercise. The first 20 minutes of exercise utilizes glycogen at a rapid rate, while the body is reaching a steady state of oxygen consumption. This is followed by a slower usage as the levels of muscle glycogen decline, and finally is depleted, with other fuel sources then utilized.

Exercise intensity and physical training capacity also affect muscle glycogen utilization. Slow twitch and fast twitch muscle fibers utilize muscle glycogen relative to the intensity of the exercise. Trained and untrained individuals utilize glycogen at the same rate relative to the same exercise intensity, but additional energy is needed at the same relative work rate. This "glycogen sparing" effect will allow the trained individual to perform progressively longer exercise than the untrained individual.

Initial muscle glycogen levels can be influenced by diet and exercise patterns. Ingestion of high carbohydrate diets provide higher initial muscle glycogen levels, and will provide longer participation times. Changes in pre - event diets or training will greatly affect muscle glycogen levels. Glycogen loading can enhance muscle glycogen concentration, thereby providing more substaate to the energy systems for ATP production. The "pregame" meal eaten 3 - 4 hours before activity will aid in recovery rather thanduring the event, so the meals the day prior to the event become critical. Training should be tapered prior to the event to provide restoration of muscle glycogen levels. After exercise, all effort should be made to replenish muscle glycogen stores depleted during the event.

Water is probably the most essential nutrient for the body, as approximately 60% of the body is composed of it. Water is important for the following reasons: 1) It provides building material for cell protoplasm; 2) It protects the brain and spinal cord; 3) It controls the osmotic pressure in the body; 4) It is the main transportation mechanism of the body; 5) it aids in the proper function of the senses; and 6) It regulates body temperature.

Standard fluid requirements are based on three elements: caloric intake, body surface area, and body weight. These basic requirements change as a person becomes more active, develops as a training program is followed, or exercises in different climates. Water loss, termed hypohydration, can effect the energy systems' ability to produce ATP, decreasing aerobic endurance performance, as well as cardiovascular functions and temperature regulation.

Fluid lost by the body is best replaced by water. Additional electrolytes or glucose may slow gastric emptying, and therefore fluid absorption. The water should be about 15°C, hypotonic to the system, and taken in smaller than 500 ml volumes. Both the American College of Sports Medicine and the American Dietetic Association have developed guidelines in regards to fluid ingestion and replacement.

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#### PHYSICAL TRAINING AND TRACE ELEMENT STATUS

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An increasing emphasis has been placed on the role of trace elements in human health. By definition, the essential trace elements are those ions required in very small amounts for optimal body function. The concentration of these elements in biological tissues is in the range of micrograms to nanograms per gram tissue. The importance of trace elements rests in their roles in biological systems. Some trace elements are components of biological fluids and behave as electrolytes. Some serve as co-factors in enzymatic reactions and also bind, transport, and release oxygen. Thus, trace elements have the potential to be involved in many energy requiring and energy producing processes that may be affected by physical training.

Knowledge about the nutritional status of athletes during training is limited. Studies of athletes participating in intense physical conditioning generally describe changes in energy intake. There is a paucity of data about micronutrient status. The purpose of this review is to summarize information on the influence of training on the status of some essential trace elements (copper, Cu; iron, Fe; zinc, Zn).

Copper is associated with many enzymes involved in oxidation and reduction. Some important enzymes include cytochrome oxidase, the terminal component of the electron transport system, superoxide dismutase, a free radical scavenger, and ceruloplasmin, a Cu-transport protein. Several cross-sectional studies among athletes and sedentary controls have shown no difference in plasma Cu concentration (1-4) between the groups. Ceruloplasmin concentrations also have been found to be similar among athletes and non-athletes. Recently, however, red blood cell superoxide dismutase activity has been shown to increase in swimmers after training (4). This represents a metabolic adaptation in Cu metabolism to aerobic training.

In a 70 kg man, there is about 1.5-2.5 g of Zn in contrast to 50-70 mg of Cu. The major depot of Zn in humans is skeletal muscle which contains 65% of body Zn. Zinc-containing enzymes are found in all the major pathways, including protein, carbohydrate, lipid, and nucleic acid metabolism. With this variety of Zn-dependent enzymes, therefore, cellular deficiency of Zn may lead to disturbances in many metabolic activities. Reduced serum Zn concentrations have been reported among some endurance athletes (3,5,6). Other studies found no differences between trained and untrained men and women (2,4).

The Fe status of an athlete can significantly affect performance. Anemia reduces peak oxygen uptake and cardiovascular function during work by reducing the oxygen carrying capacity of the blood. Iron deficiency without anemia also affects work capacity by reducing muscle Fe and causing a reliance on anaerobic metabolism during submaximal and maximal work. Anemia and Fe deficiency have been reported in competitive and recreational athletes (6-8).

Any assessment of the influence of physical activity on nutritional status should include an estimation of nutrient intake and blood biochemical variables. Most studies (1,3,5,7,8) that have found significant differences in blood nutritional variables have not monitored food intake. In contrast, those studies (2,4,6) that have reported no differences in blood biochemical variables between trained and untrained people have shown that nutrient intakes were within recommended guidelines. Thus, it appears that athletes who consume diets that are adequate in nutrients are at no significant risk of developing nutritional deficiency. Evidence of unique trace element requirements dependent upon physical activity and not diet is not available.

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## EXERCISE, DIET AND BONE

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Thinning of the human skeleton with age is a universal phenomenon, but in western countries bone loss is often excessive, resulting in osteoporosis which manifests as low-trauma fractures, typically of the forearm, hip and vertebrae. These are at epidemic proportions in our elderly; a fifty year old woman has a 15% probability of a future hip fracture, the most serious of the osteoporotic fractures. Fracture incidence is increasing rapidly, due partly to the aging trend in our population and also to an unexplained increase in the age-specific incidence rates in both men and women. No full assessment of cost has been done but the direct medical costs just for women in 1986 were estimated to be \$5.1 billion. The effect of estrogen therapy in stemming postmenopausal bone loss in women has been well established, but no other safe, proven therapy exists thus emphasizing the need for prevention.

Other than hormonal factors both diet and exercise play roles in the maintenance of the skeleton, but evidence is incomplete. Potentially important dietary factors include vitamin D, protein, calcium and phosphorus, but Parfitt has stated that "bone mass normally depends less on nutrition than on age, sex, race and other features of genetic constitution, muscular activity and hormonal state" (1). Calcitriol, the most important metabolite of vitamin D, acts to increase the availability of calcium by increasing gut absorption, inhibiting bone collagen synthesis and increasing bone resorption. The finding that serum calcitriol levels in patients with osteoporosis are similar to those of healthy age-matched controls supports the conclusion that vitamin D should not be used in treating postmenopausal osteoporosis, where it has been shown to have either no effect or to increase the loss of cortical bone. Its effectiveness in treating senile osteoporosis has yet to be assessed. Though extremely low intakes of calcium cause osteoporosis in animals, the optimal level in humans is highly controversial. Epidemiologic evidence does not support the hypothesis that larger intakes of calcium are associated with increased bone density or decreased incidence of fractures. The populations of many third-world countries have low calcium intakes but little osteoporosis, and in Western countries measurements of bone mass and calcium consumption are poorly related. Prospective controlled studies in postmenopausal women have generally failed to demonstrate that calcium supplementation significantly reduces bone loss (2). However, if pooled, these studies may suggest a trend that might be confirmed by trials of longer duration than two or three years. A much-cited Yugoslavian study reported a significantly lower incidence of hip fractures in people taking large amounts of calcium than in those taking small amounts (3). However, age-related bone loss was greater in the first group and the authors concluded "the data suggest that nutrition (in particular the calcium intake) is an important determinant of bone mass in young adults but seems to have little effect on age-related bone loss in either males or females." It may be that increases in calcium intake during growth can increase the peak bone mass of early adulthood, but this important hypothesis has yet to be proved.

The effects of mechanical loading on the skeleton have been known for over a century. Complete unloading of bone induces the most rapid change in bone mass, greater by far than that resulting from hormonal shifts such as those occurring around menopause. Of current interest is the effect of varying degrees of supra-normal loading of bone. Cross-sectional studies show that bone density differs between groups whose activity level is different, e.g. athletes vs. non-athletes and manual vs. sedentary workers. Prospective studies of exercise intervention in animals show a positive effect on bone in a wide variety of species. Similar studies in humans are limited in number and subject to limitations such as lack of randomization. Nonetheless, they suggest that regular weight-bearing exercise can slow bone loss, even in the elderly (4). The intermediate variable between loading and bone formation appears to be the mechanical strain within bone. Animal studies show that loading must be dynamic and that both strain level and strain rate are important. Perhaps the most striking finding is that relatively few loading cycles per day are required for maximum bone gain, if strain rate is high. Thus loading for optimal bone formation may be the same as for maximum muscle hypertrophy, i.e. few repetitions at high loads, but this needs to be confirmed in humans. Also needing further investigation is the possible synergistic effect of dietary calcium and exercise on bone.

While regular exercise is bone-promoting, intensive training can impair reproductive function in women, ranging from a shortened luteal phase to overt amenorrhea. The resulting hypoestrogenic state, as at menopause, causes increased bone loss which, over time, will increase fracture susceptibility. Amenorrheic athletes have lower bone density and higher incidence of stress fractures than normally menstruating athletes training at the same intensity. Similar characteristics are exhibited by those whose amenorrhea is associated with anorexia nervosa. Such young women have the lowest bone mineral densities of any subjects we have measured, especially at the lumbar spine where densities may be 50% below normal. The cause of the amenorrhea is not known, but it is of the hypothalamic type and is associated with leanness, low caloric intake and activities where low body weight is desirable. Though the role of adipose tissue in the etiology of menstrual dysfunction had been challenged, exercise per se cannot be the causative factor since famine victims also become amenorrheic and swimmers, despite high intensity training, rarely become amenorrhic.

In summary, in the normal hormonal milieu, increased mechanical loading is the only proven modality to increase the formation of normal bone. Nutrients such as calcium have not yet been shown to prevent bone loss though their role in growth may be important.

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#### Body Composition and Performance

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From the ponderous bulk of a football lineman to the diminutive physique of a jockey, physical performance is structurally selective by physique and body composition. Although training may alter body composition and thus physique, these changes are small (1). So small that the relationship between body composition and performance may be the result of genetic endowment, not training.

The interest in body composition of athletes was established by the findings of Welham and Behnke (2) in 1942. This study of 25 football players indicated that 17 of the players were overweight according to the height-weight tables, although 11 of the 17 had less than average percent body fat. Subsequent research on the body composition of adult athletic groups has indicated that both male and female athletes have less percent body fat and greater lean weight per unit of height than non-athletes (3). Parizkova et al (4) observed that spontaneously active preschool children were leaner than their inactive peers; this suggests that body composition may influence activity patterns at a very early age.

Fat-free weight is generally associated with powerful physical performance. Shot-put, javelin throw and weight lifting depend on the combination of forceful muscle contraction and the stability provided by large bones. A large, fat-free mass would be an advantage in these performances. However, a large, fat-free mass may be a disadvantage when the performance depends upon moving the body mass through space such as the high jump, sprinting or playing tennis. The large, fat-free mass in these examples may contribute to the work and the metabolic cost of the effort and thus have a negative effect on the performance.

Fat weight or percent fat tends to vary with the sport. Long distance runners and athletes in other activities with a high endurance component generally have less than 10% body fat (1). Professional football players, by contrast, vary from 9-10% body fat for defensive and offensive backs to 18% for defensive linemen (1). Mechanically, excess fatness is detrimental when the body weight must be accelerated either horizontally or vertically because it adds non-force-producing mass to the body (3). These data suggest less body fat among athletes than among non-athletes in an average, college-aged population where mean percent fat has been reported as 15% and 22% for males and females, respectively. However, these facts do not account for a thorough explanation of fat and performance because some athletes tend to have greater relative fat than the average adult; for example, football linemen. Bolonchuk and Lukaski (6) found that in a college-aged sample, average relative fat was 25% for females and 18% for males. Body composition by physique indicated significant differences by somatotype with ectomorphs having the least fat, 12% and 20% for males and females, respectively, and endomorphs have the most fat, 26% and 31% for males and females, respectively. As it has been suggested by other studies, the relationship between body composition and physical performance is likely a combination of changes in fat weight and fatfree weight with training and the genetic endowment of the athlete.

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#### EATING DISORDERS AMONG ATHLETES

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Eating disorders are a form of addicitive behavior. Personality traits include perfectionism, an intense desire for control, and an obsession with body weight. Family history typically includes chemical dependancy, divorce or other dysfunctional situations. In the case of athletes, it has been questioned whether athletics causes the addictive behavior or if the addict turns to athletics as a means of escape.

It is not known exactly what percentage of athletes suffer from eating disorders, but the numbers are high compared to the non-athletic population. Anorexia nervosa is an act of deliberate self-starvation in the pursuit of increasing levels of weight loss. Bulima is an act of excessive eating followed by a method of purging. Characteristics of these disorders include:

#### Anorexia nervosa

- 1. Refusal to maintain normal weight
- 2. An intense fear of gaining weight
- 3. A severe body distortion
- 4. Amenorrhea for 3 months or longer

#### Bulima

- 1. Recurrent episodes of binge eating
- 2. Feeling out of control during binge
- Self-induced vomiting, diuretics, or laxatives
- 4. At least 2 binges/week for 3 months
- 5. Persistant concern with body shape

Early recognition and intervention are keys to the prevention of serious disorders. A treatment program involving a physician, qualified counselor and an experienced dietitian are needed. Medical management, nutrition support and counseling, insight-oriented therapy and in some cases, pharmacotherapy will contribute to long term success. Average length of corrective therapy is two years. Short term treatment programs do not assure continued improvement. Drossman (2) lists the following long-term prognostic factors in anorexia nervosa:

## Good Prognosis

High educational achievement Early age at onset Good educational adjustment Improvement in body image after weight gain

## poor prognosis

Late age of onset P
Overestimation of body size
Premorbid obesity H
Self-induced vomiting D
Laxative abuse P
Low social class
Long duration of illness
Disturbed parental relationship
Male sex
Marriage
Marked depression, obsessional

No effect on prognosis

Premorbid personality type or psychologic disorder Hyperactivity Degree of weight loss Pharmacotherapy

An eating disorder is a disturbance in an individual's eating behavior which will jepordize that person's physical or psychologic health. Eating disorders, if noticed and treated early, can be avoided in athletes. Increased awareness of symptoms by parents, family members, coaches, peers, and medical professionals can aid in the prevention and early treatment of these disorders to ensure long-term success.

behavior, somatic complaints

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50 Symposia

## Basic Eating Plan for Active People

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In the last two years, two major national reports have highlighted the importance of diet in the prevention of chronic disease for Americans: the Surgeon General's Report on Diet and Health (1) and the National Academy of Science Report of Diet and Health: Implications for Reducing Chronic Disease Risk (2). These documents are important not only because they have added new knowledge about the importance of nutrition, but also because they reflect a relatively new consensus among respected nutrition scientists about the pivotal role of diet in the health of Americans.

Eating plans for active people and athletes differ only slightly from diet recommendations for the general public in the United States today. Those national recommendations can be summarized as follows:

- 1) Reduce total fat consumption to 30% or less of calories, saturated fat to less than 10% of calories and cholesterol to less than 300 mg per day.
- 2) Eat five or more servings daily of vegetables and fruits, especially green and yellow vegetables, dried beans/peas and citrus fruits plus six or more servings of starches and other complex carbohydrates daily to bring total carbohydrates to more than 55% of calories.
- 3) Maintain only a moderate intake of protein, meeting the Recommended Dietary Allowance (RDA) for protein every day, but do not exceed 1.6 g/kg of body weight which is twice the RDA.
- 4) Balance food intake and physical activity in order to maintain ideal body weight. Increase energy expenditure through regular and sustained physical activity.
  - 5) Either avoid alcoholic beverages or consume no more than 1 ounce of alcohol per day.
- 6) Limit total daily salt intake to 6 grams or less. Limit the amount of salt added in food preparation and at the table.
- 7) Maintain adequate calcium intake by meeting the RDA. Adolescent girls and women should be especially careful to increase calcium consumption.
  - 8) Avoid taking dietary supplements, especially in excess of the RDA.
  - 9) Drink fluoridated water to prevent tooth decay.
- 10) Children, adolescents and women of child bearing age should be sure to consume foods that are good sources of iron, such as lean meats, fish, certain beans and iron-enriched cereals and whole grain products. This is especially an issue with low income families.

Current guidelines (3) recommend that all athletes should adhere to a diet pattern providing at least 55% of calories from carbohydrates. Athletes who train exhaustively on successive days or who compete in prolonged endurance events should follow a diet which has 65 to 70% of calories from carbohydrates.

Based on our knowledge of food consumption in this country (4-6), adherence to these dietary guidelines for either normal adults or for athletes will require definite changes from the current practices of most Americans. Current dietary practices of Americans will be discussed as well as appropriate strategies for change for active people.

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# DIABROTICA VIRGIFERA VIRGIFERA ESTERASE-m CHARACTERIZATION USING MONOCLONAL ANTIBODIES

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Corn rootworms (<u>Diabrotica</u> spp.) are considered to be major pests of corn in the United States. Current methods of control which include crop rotation and insecticides have helped reduce the economic damage, but there is a need to develop more effective and environmentally safe control procedures. A possible control method this research examines is population regulation by manipulation of reproduction at the molecular level.

A sex-limited esterase ( $\underline{\text{Est-m}}$ ) thought to be involved in the reproduction of corn rootworms has been discovered in the accessory glands of males and is currently being characterized (1,2). Monoclonal antibodies reactive with the esterase have been developed to aid in the characterization of this enzyme.

Monoclonal antibodies were obtained following the injection of BALB/C mice with various preparations of  $\underline{\text{Est-m}}$ . Lymphocytes from these mice were subsequently fused with P3 myeloma cells to form hybridomas. The different immunization procedures were used to obtain antibodies reactive with both native and denatured forms of the enzyme. Hybridomas secreting antibodies reactive to the various  $\underline{\text{Est-m}}$  markers were screened using an ELISA procedure developed in our laboratory. Purified  $\underline{\text{Est-m}}$  is first attached to the wells of 96-well plates by adsorption. These plates are then incubated with 3% BSA to block the remaining attachment sites for protein. Hybridoma supernatant is then added to allow possible binding of antibodies to the  $\underline{\text{Est-m}}$  protein. Specific antibody binding is detected using a second antibody system (goat anti-mouse antibody conjugated to horseradish peroxidase). Cultures containing hybridomas capable of secreting reactive antibody were thus identified and isolated using limit dilution cloning methods.

A panel of monoclonal antibodies have been selected that react against both native and denatured  $\underline{Est-m}$  by western blotting procedures. This involved running semi-pure  $\underline{Est-m}$  on either an 8% non-denaturing polyacrylamide gel or on a 5-10% gradient SDS polyacrylamide gel followed by electrophoretic transfer to nitrocellulose membranes using a Hoefer Transphor apparatus. The nitrocellulose membrane was then cut into strips and incubated with 3% BSA to block any unoccupied protein binding sites. The blocked nitrocellulose strips were then incubated with individual monoclonal antibodies to detect specific binding to  $\underline{Est-m}$  protein components. Bound monoclonal antibodies were detected using goat anti-mouse antibody conjugated to horseradish peroxidase. Several reactive protein components from  $\underline{Est-m}$  have been detected using this procedure. The panel of monoclonal antibodies have also been screened using an enzyme inhibition assay to obtain antibodies possibly reactive with the active site of this enzyme. Subsequent uses for the panel of monoclonal antibodies include: 1) evaluation of  $\underline{Est-m}$  biological activity  $\underline{in\ vivo}$ , 2) affinity purification and largescale recovery of  $\underline{Est-m}$ , 3)  $\underline{Est-m}$  peptide mapping, and 4) as immunological probes for CDNA expression libraries currently being developed.

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## ZINC UPTAKE BY ENDOTHELIAL CELLS

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The mechanism by which zinc enters a cell is poorly understood. Most cells of vertebrates exchange nutrients (including zinc) and waste products with their surrounding extracellular fluid. This interstitial fluid exchanges nutrients with the blood of the vascular system through the capillary walls. Therefore, before zinc can be available for entry into a pericyte it must pass through the endothelial cells of the capillary. Thus, we are investigating the mechanism by which zinc enters and passes through endothelial cells. We are using tissue cultures of bovine pulmonary aorta cells as a model system.

Cells were cultured as a monolayer in T-25 flasks with Eagle's minimum essential medium and 10% fetal bovine serum (6  $\mu$ M Zn) until 4 d post-confluent. The media was then replaced with a similar one containing 50 nCi <sup>65</sup>Zn/ml and incubated for 1 to 300 min. The cells were then harvested and the amount of zinc within the cells was calculated based upon their specific activity. Zinc uptake was biphasic; it was initially rapid and slowed after 60 min. A linear uptake of zinc with time was evident between 1 and 16 min.

Similarly grown cells were incubated for 10 min in a media containing EDTA dialyzed serum, zinc concentrations of 1 to 500  $\mu$ M, and 250 nCi <sup>65</sup>Zn/ml. Zinc uptake was again biphasic; it apparently followed saturation kinetics up to approximately 32  $\mu$ M Zn (Figure 1), which suggests that an active transport system was involved. Then it was linear (Figure 2), indicating that uptake was under the influence of a passive diffusion mechanism. Analyzing the results up to 32  $\mu$ M in an Eadie-Hofstee plot indicated that the saturable component had a maximum rate of Zn uptake (V<sub>MAX</sub>) of 21 pmoles Zn/(mg protein·min), and it was transporting Zn at half the maximal rate (K<sub>m</sub>) when the Zn concentration was 1.4  $\mu$ M.

The results suggest that zinc uptake into endothelial cells is under the control of both passive and active transport systems. The active process predominates at the zinc concentrations encountered physiologically, i.e., from 10 to 20  $\mu$ M. The passive uptake mechanism predominates above 32  $\mu$ M, which is also the point at which the zinc to albumin molar ratio begins to exceed 1:1 in the media. As the principal zinc binding ligand in serum, albumin may have significant influence on its uptake. Future research will investigate the effect of albumin and other factors upon the zinc transport process.

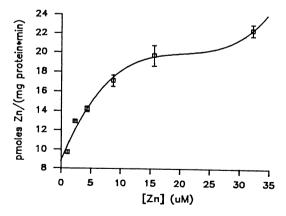


Figure 1. Zn uptake by endothelial cells. Points are the means of 5 flasks. Standard errors for each mean are indicated by bars.

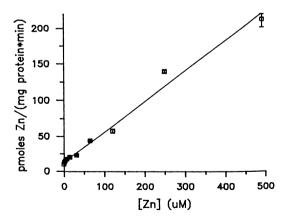


Figure 2. Zn uptake by endothelial cells. Points are the means of 5 flasks. Standard errors for each mean are indicated by bars.

# THE EFFECT ON THE ANALYSIS OF GROUPING DATA BY SOMATOTYPE COMPONENT OR BY SOMATOTYPE DOMINANCE

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Most of the studies which have examined the association between somatotype and performance compared a measure of performance with the rating for each somatotype component (1,2). A few studies analyzed performance measures after the data were grouped by somatotype dominance (3,4). These studies suggest that the assumption of grouping data by somatotype dominance rather than somatotype component will increase the sensitivity of the analysis to detect differences in variable responses related to somatotype. However, none has analyzed data to determine whether or not this assumption is tenable.

A sample of 82 adult males was studied to examine this assumption. Tests of body composition by hydrodensitometry, physical work capacity by a standard work test on the cycle ergometer, and plasma analysis for lipids, metals, and enzymes were administered. Correlation estimates were computed between the somatotype ratings and the measurements which were grouped two ways: by somatotype component (N-82), and then by somatotype dominance (n-19 endomorphs, n-41 mesomorphs, and n-22 ectomorphs).

Correlations between plasma metals and the endomorphic and ectomorphic groups were unaffected by the groupings for analysis. The mesomorphic group showed a small increase in all plasma metal correlations except for plasma Mg which remained unchanged. Correlations for plasma lipids were increased for the endomorphs, mesomorphs, and ectomorphs when the data were grouped by somatotype dominance. Correlations between percent fat and fat weight and somatotype dominance remained unchanged for the endomorphic group and increased (r- .33 to .43 and .45 to .57, respectively, p > .05) for the mesomorphic group; however, the correlation between the endomorphic group and fat-free weight decreased (r- .31 to .04, p > .05) and the correlation between the mesomorphic group and fat-free weight was unchanged. These changes in fat weight and fat-free weight tend to complement one another and, although each change is moderate, the direction of the change contributes to a combined effect of differentiating the body composition of the endomorphic from the mesomorphic group. Correlations for the dominant ectomorphic group decreased for percent fat, fat weight, and fat-free weight (r= -.48 to -.35, r= -.51 to -.35, and r= -.39 to .05, respectively, p > .05). Since these correlation coefficients were negative, a decrease in the value of the coefficient means an increase in the positive relationship between the dominant somatotype and the correlated variables. Analysis of the physical work capacity measurements indicated that correlations for heart rate, oxygen consumption, carbon dioxide production, and maximum power output were greater when the measurements were grouped by somatotype dominance.

Although some correlation estimates remained unchanged, the general effect of grouping by somatotype dominance was to increase the correlation estimate between the performance measures and the somatotype. However, the changes in correlation estimates were not great enough to achieve significance at the .05 level.

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ECOLOGICAL ASSESSMENT OF A WATER CONTROL PROJECT ON A SMALL TRIBUTARY
IN THE SOUTHERN RED RIVER BASIN

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Variable climatic and hydrologic conditions are common to the southern Red River basin, as most recently evidenced by the drought and low flow of 1988 followed by the flood of spring 1989. The problem of flooding at times of high precipitation or rapid snowmelt is exacerbated by the limited topographic relief of the Lake Agassiz plain and relatively fine-textured soils with low permeability. Since late-19th century settlement began, a primary effort in dealing with excess water has been creation of an extensive artificial drainage network. While facilitating water movement from parts of the basin, that has also had the effect of hastening and focusing water movement. A second approach to flooding problems, whichis now being considered, is to temporarily hold back part of the runoff in selected retention sites and then release it in a controlled fashion.

The latter effort has been initiated by the Buffalo-Red River Watershed District of western Minnesota. The Buffalo River, which empties into the Red River 15 miles north of Fargo-Moorhead, has as one of its tributaries Stony Creek. This stream originates in the formerly glaciated uplands near Barnesville, Minnesota and flows through a large natural lowland before continuing across part of the Lake Agassiz plain.

This natural lowland is a suitable site for water impoundment, and in 1985 the Watershed District Board finalized plans for the Stony Creek Flood Detention Project. Included would be an earthen dam, a permanent floodpool of approximately 345 acres and a temporary floodpool of about 800 acres. Because of significant natural areas in the vicinity and part of the floodpool would encroach on a wild-life management area maintained by the Minnesota Department of Natural Resources, an ecological assessment was included as part of the project. The objectives were to (a) establish a pre-construction baseline inventory, (b) analyze historical information pertaining to the site, and (c) predict possible changes induced by the altered water levels.

The flora and vegetation were inventoried during the summer of 1986 by ground reconnaissance, transect sampling, and interpretation of aerial photographs. Within a four-square-mile area encompassing the dam and impoundment, 228 vascular plant species were found, including 9 tree species, 22 shrubs and vines, 35 grasses, 23 monocots (other than grasses) found in wet soils or shallow water, 129 forbs, 10 floating or submergent species. Vegetation within the proposed floodpools was mapped into eleven units: deep marsh (164 acres), emergent wetland vegetation (278), sedge meadow (244), wet meadow (28), reed-willow-dogwood (29), shrub-carr (152), deciduous woodland (7), mesic and lowland prairie (103), non-native grassland (49), cropland (106), beaver pond (3).

Historical information was gathered from the General Land Office Survey of 1870, multiple sets of aerial photographs spanning 1939 to 1986, and interviews with local people. The pattern of wetlands, stream, and hillside springs on nearby slopes, which was documented in 1870, parallels closely the pattern seen 116 years later. Changes include an apparent increase of woody vegetation in and around the lowland, together with wholesale conversion of nearby upland prairies to cropland. In 1910 part of the lowland was ditched, and aerial photographs beginning in 1939 reveal use of portions for pastures, haylands, and some cropland. With changes in ownership, farming practices, and prevailing moisture conditions, many of these areas later reverted to natural vegetation, while a few areas were later brought into farm operations. About 1980, with a several-year increase in regional water balance and damming activity by beaver, a wetland was restored, which corresponded closely to that intended with the permanent floodpool.

That wetland was temporarily drained in July 1986 to facilitate dam construction, and the permanent floodpool began to fill later that year. Lower portions of the temporary floodpool have been filled briefly with runoff early each spring since then. However, below-normal precipitation in the summers of 1987, 1988, and 1989 has meant partial drawdown of the permanent floodpool and no summer inundation of the temporary floodpool. Over longer periods of time, which will undoubtedly include high water episodes, the deep marsh zone will expand, and, around the margins, 30 acres of sedge meadow, 11 acres of low prairie, and 6 acres of non-native grassland are likely to become permanent wetland vegetation. Because water retention at higher elevations will be relatively brief and the vegetation now present is growing in moist to saturated soils, temporary floodwater detention will probably not cause widespread vegetation change. Other significant natural sites, e.g., fens formed on deep peat deposits, woodlands, and upland prairies, are far enough upslope to experience little or no impact. Due to the particular setting for this flood control project and the limited detention time within the temporary floodpool, a degree of flood control has been accomplished with limited ecological impact.

## Northern Great Plains Geomorphic History as determined from Asymmetric Drainage Divides

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The principle of cross cutting relationships can be applied to erosion surfaces and river valleys in regions of homogeneous bedrock. If erosion surface A is cut by erosion surface B, then erosion surface A must predate erosion surface B. Likewise, if river valley C cuts erosion surface B, then erosion surface B must predate river valley C. Relationships observed on United States Geological Survey 1:250,000 topographic maps (names indicated in parentheses) are used to construct the sequential development of Missouri River tributaries.

White River - Niobrara River sequence: An asymmetric divide, the Pine Ridge Escarpment, separates the Niobrara River drainage basin from the White River drainage basin (Alliance, Nebraska). The Niobrara River erosion surface

predates the White River basin.

Cheyenne River - White River sequence: An asymmetric divide separates the White River drainage basin from the Cheyenne River drainage basin (Hot Springs, South Dakota). The White River erosion surface predates the Cheyenne River drainage basin.

Belle Fourche River - Cheyenne River sequence: An asymmetric divide separates the Cheyenne River drainage basin from the Belle Fourche River drainage basin (Rapid City, South Dakota). The Cheyenne River erosion surface predates the Belle Fourche River valley.

Grand River - Moreau River sequence: An asymmetric divide separates the Moreau River drainage basin from the Grand River drainage basin (Lemmon, South Dakota). The Moreau River erosion surface predates the Grand River

drainage basin.

Knife River - Heart River sequence: An asymmetric divide, the Russian Springs Escarpment, separates the Heart River drainage basin from the Knife River drainage basin (Dickinson and Watford City, North Dakota). The Heart River erosion surface predates the Knife River basin.

Little Missouri River - Moreau, Grand, Cannonball, Heart, and Knife River sequence: An asymmetric divide separates the Moreau, Grand, Cannonball, Heart and Knife River drainage basins from the Little Missouri River valley (Dickinson and Watford City, North Dakota, and Lemmon, South Dakota). The Moreau, Grand, Cannonball, Heart, and Knife River erosion surfaces predate the Little Missouri River.

Yellowstone River - Little Missouri River sequence: An asymmetric divide separates the Little Missouri drainage basin from the Yellowstone drainage basin (Miles City, Montana). The Little Missouri River erosion surface predates

the Yellowstone valley.

Powder River - Little Missouri River sequence: An asymmetric divide separates the Little Missouri River drainage basin from the Powder River drainage basin (Ekalaka, Montana). The Little Missouri erosion surface predates the Powder River drainage basin.

Redwater River - Yellowstone River sequence: An asymmetric divide separates the Yellowstone River drainage basin from the Redwater River drainage basin (Glendive, Montana). The Yellowstone River erosion surface predates

the Redwater River drainage basin.

Prairie Dog Creek - Redwater River sequence: An asymmetric divide separates the Redwater River drainage basin from the Prairie Dog Creek drainage basin (Glendive, Montana). The Redwater River erosion surface predates the Prairie Dog Creek drainage basin.

Big Dry Creek - Prairie Dog Creek sequence: An asymmetric divide separates the Prairie Dog Creek drainage basin from the Big Dry Creek drainage basin (Jordan, Montana). The Yellowstone River erosion surface predates the

Redwater River drainage basin.

Musselshell River - Big Dry Creek sequence: An asymmetric divide separates the Big Dry Creek drainage basin from the Musselshell River drainage basin (Jordan, Montana). The Big Dry Creek erosion surface predates the Musselshell River drainage basin.

Big Dry Creek and Musselshell River - Yellowstone River sequence: An asymmetric divide separates the Yellowstone River drainage basin from the Big Dry Creek and Musselshell River drainage basins (Jordan, Montana). The Yellowstone River erosion surface predates the Big Dry Creek and Musselshell River drainage basins.

Armells Creek - Musselshell River sequence: An asymmetric divide separates the Musselshell River drainage basin from the Armells Creek drainage basin (Lewistown, Montana). The Musselshell River erosion surface predates the

Armells Creek drainage basin.

Dog Creek-Armells Creek sequence: An asymmetric divide separates the Armells Creek drainage basin from the Dog Creek drainage basin (Lewistown, Montana). The Armells Creek erosion surface predates the Dog Creek drainage basin.

Judith River - Dog Creek sequence: An asymmetric divide separates the Dog Creek drainage basin from the Judith River drainage basin (Lewistown, Montana). The Dog Creek erosion surface predates the Judith River drainage basin.

Arrow Creek - Judith River sequence: An asymmetric divide separates the Judith River drainage basin from the Arrow Creek drainage basin (Lewistown, Montana). The Judith River erosion surface predates the Arrow Creek valley.

Conclusions: Northern Great Plains drainage basins formed in the following sequence (from oldest to youngest): Niobrara River, White River, Cheyenne River, Belle Fourche River, Moreau River, Grand River, Cannonball River, Heart River, Knife River, Little Missouri River, Powder River-Lower Yellowstone River, Redwater River, Prairie Dog Creek, Big Dry Creek, Musselshell River, Armells Creek, Dog Creek, Judith River, and Arrow Creek. Explanations of northern Great Plains drainage development must account for this sequence.

WISCONSIN FAST PLANTS - A TOOL FOR RESEARCH AND TEACHING OF PLANT BIOLOGY AT ALL LEVELS OF EDUCATION

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Brassica species have been selected over the past 15 years for the characteristics of small size and rapid development. Dr. Paul H. Williams, plant pathologist at the University of Wisconsin, Madison, has conducted numerous workshops over the past few years promoting the use of these plants. The materials used are inexpensive and frequently can be obtained from commonly-used items that may even be free of charge. One example is the use of plastic film cannisters that many people discard because they don't have any use for them. Plastic 2-liter soft drink containers are another example of materials that usually are discarded, but can serve as inexpensive growth chambers for these small plants. Pollination of flowers by the use of bee thoraxes glued onto toothpicks for pollination of flowers is another example of a simple but very effective use of materials that might be available to just about everyone.

Morkshops are being conducted as time permits, usually under the auspices of scientific organizations. The American Society of Plant Physiologists (ASPP) sponsored a one-day workshop in Toronto in July, 1989, with the cooperation of Dr. Ellen Weaver of the ASPP Education Committee. One of us (DGD) had the opportunity to attend that workshop, while DSG attended a one-half day workshop at the University of California, Davis, and a two-day workshop at the University of Wisconsin at Madison.

Manuals and kits for use in classrooms are available in the most recent catalog of Carolina Biological Supply, although as mentioned much of the materials can be accumulated at very little expense if a school district is short on money. A newsletter is distributed with useful information for the aid of educators. Contact with Dr. Williams and his associates is encouraged.

Many of the experiments are useful also for general principles and rapid turnaround for scientists engaged in research as well as teaching. Since the life cycle of <u>B. rapa</u> is about 35 days (shorter than for <u>Arabidopsis</u>) it is possible to do experiments over the entire life of these plants in a much shorter time than for almost all other higher plants. These plants are larger than Arabidopsis, so for research purposes they may be more useful.

Although this sounds like a commercial for the Wisconsin Fast Plants, it really is only a suggestion to anyone interested in teaching plant biology in such a way as to excite young (and old) students or researchers. It seems to us Wisconsin Fast Plants may serve admirably in this capacity.

VIRUSES INVOLVED IN MOSAIC SYMPTOM EXPRESSION IN NORTH DAKOTA CERTIFIED SEED POTATO FIELDS

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The identification of mosaic-infected plants is a major concern of seed potato certification agencies across the country. Several viruses or combinations of viruses are capable of inducing mosaic symptoms in potato plants (1,2). Environmental influence and cultivar reaction may also affect symptom expression. The North Dakota seed certification standards (3) refer to severe and mild mosaics. This is intended as an indication of disease severity, but these two descriptive terms are also accepted as the common names for diseases caused by potato virus Y (PVY) and potato virus A (PVA), respectively. Symptoms may vary enough that the only way to confirm the virus involved is either by inoculating indicator hosts or through serological techniques.

In addition to mosaics caused by PVY and PVA, several other viruses or combinations of viruses may induce mosaic symptoms. Potato virus X (PVX) and potato virus S (PVS) cause latent mosaics, and potato virus M (PVM) causes leafrolling mosaic (1,2). Some of these viruses have not been reported from North Dakota. The objective of this study was to determine the viruses responsible for mosaic diseases in North Dakota in the 1986 growing season.

Samples were collected from symptomatic plants in North Dakota certified seed potato fields by inspectors from the State Seed Department. Collected plants were mailed to the laboratory and tested by ELISA for PVA, PVM, PVX, PVY, PVS, and potato leafroll virus (PLRV). Several samples tested were free from mosaic viruses in their indexing profiles using ELISA. No plants were detected with infection by PVA, PVM, or PLRV. Multiple infections were common, usually involving PVS which was found in 82.5% of the samples tested. Potato Virus X was found in 22.8% of the samples tested, but only once was it found to occur alone. Potato Virus Y occurred in 63.2% of the samples (Table 1). Samples that were tested, but did not show infection by any of the six viruses, can be attributed to nitrogen deficiency, which may be confused with mosaic under certain conditions. The large amount of nutrient leaching is probably also responsible for the samples submitted for testing while infected only with latent mosaic viruses (PVS and PVX).

PVS was prevalent among the samples tested, occurring more than 80% of the time. It has long been suspected that PVS was common in North Dakota, but its role in the mosaic complex of diseases and in actual losses in yield are yet to be established. Samples testing negative for PVS always originated from early generation seed fields in their first or second year from PVS free tissue culture.

The remaining two viruses, PVX and PVY, are the viruses most commonly associated with mosaic. A light mosaic may occur in association with PVX infection, but this is rare in North Dakota and may be strongly influenced by environment. This study does suggest that the primary cause of mosaic in North Dakota in 1986 was PVY. This virus was found in 63.2% of the samples tested and is the only virus capable of inducing severe mosaic symptoms in North Dakota, as shown by this study. Samples which tested negative for all viruses may have been selected in error due to the high number of fields with chlorosis due to nitrogen deficiency in 1986.

Cultivar	Occurrences	PVA	PVM	PVS	PVX	PVY	PLRV	Cultivar	Occurrences	PVA	PVM	PVS	PVX	PVY	PLRV
Chieftain	(1)	-	-	+	+	+		ND 671-4Russ	(1)	-		+	-	+	- '
Chieftain	(1)	-	-	+	+	-	-	NUT9-1068-11R	(4)	-	-	+	-	+	-
Crystal	(1)	-	-	+	-	+	-	Red LaSoda	(1)	-	-	٠	-	+	-
Kennebec	(1)	-	-	+	-	-	-	Red Norland	(1)	-	-	+	-	+	_
Kennebec	(1)	-	-	-	-	-	-	Red Pontiac	(2)	-	-	+	-	-	-
Krantz Russet	(1)	-	-	+	-	+	-	Red Pontiac	(2)	-	-	+	+	+	-
LaChipper	(1)	-	-	-	+	+	-	Red Pontiac	(1)	-	-	+	-	+	-
LaRouge	(2)	-	-	+	-	+	-	Red Pontiac	(1)	-	-	-	-	-	-
Monona	(1)	-	-	-	-	+	-	Redsen	(1)	-	-	+	-	+	-
Norchip	(3)	-	-	+	-	-	-	Russet Burbani	(1)	-	-	+	-	-	-
Norchip	(8)	-	-	+	-	+	-	Russet Burbank	(1)	-	-	+	-	+	-
Norchip	(1)	-	-	-	-	+	-	Russet Burbank	(1)	-	-	-	+	-	-
Norchip	(1)	-	-	-	-	-	-	Shepody	(5)	-	-	+	+	+	-
Norchip	(3)	-	-	+	-	-	-	Shepody	(1)	-	-	+	-	•	
Norgold-Super	(1)	-	-	+	-	+	-	Shepody	(2)	-	-	+	-		-
Norgold-Super	(1)	-	-	-	-	+	-	Shepody	(2)	-	-	-	+	+	-
ND 671-4Russ	(1)	_	_	+	-			Superior	(1)	-	-	+	+	-	-
Total Positive	•									υ	o	47	-i3	36	()
Percent Positi	ive									0	0 8	32.5	22.8	3 63.	2 0

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### ON CHARACTERIZING THE QUALITY OF URBAN RUNOFF IN FARGO

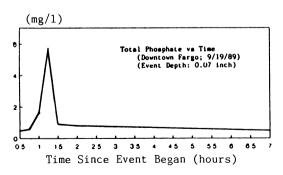
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Urban runoff can contribute a significant pollutant load to receiving waters. In many locations around the country urban runoff is being scrutinized for its pollution potential and methods to treat these waters have been implemented. Considering this, a study was undertaken by the North Dakota Water Resources Institute to investigate the characteristics of runoff from a subbasin which drains downtown Fargo and assess the impact on the Red River.

Three rainfall events, one each in July, August and September of 1989, were investigated. The subbasin drains a predominantly commercial area of approximately 61 acres with 80% imperviousness. Samples were collected at the outfall to the Red River and analyzed for total and volatile suspended solids (TSS & VSS), total and volatile dissolved solids (TDS & VDS), PO<sub>4</sub>, NO<sub>3</sub>, chemical oxygen demand (COD), and pH. Additionally, a one hour composite sample was analyzed for the metals Cu, Zn, Hg, Cr, Ni, As, Se, Cd and Pb. Observations were also made regarding the visual characteristics of the discharge, floating materials, and first flush characteristics. Sampling and analysis were performed in accordance with the procedures described in "Standard Methods" (1). The first flush phenomenon was evident in the concentrations of most constituents as well as the visual characteristics of the discharge. Some pollutographs are shown in figures 1 and 2.

The quality of runoff must be characterized first for assessing its impact on the receiving The Red River, as a Class I stream, is expected to meet certain general conditions and specific water quality standards set forth by the Department of Health. This outfall intermittently exceeds minimum conditions for state waters. Floating debris, oil and scum were observed at the Runoff produced an off-color plume in the river. Hydrocarbon sheen was observed at the outfall and surrounding shoreline. Solid waste in the form of bottles, cans, cigarette butts and styrofoam was flushed into the river. The water quality criteria of the discharge showed a great deal of variability throughout the study and at times the levels of pollutants were in excess of the specific water quality standards; however, it does not necessarily follow that the water quality of the receiving water was impaired beyond standards from this outfall. Peak concentrations above minimum standards are indicative only of intermittently high values of pollutants entering the mixing zone in the river. Upon mixing, the water quality standards are then applied to the river. Such peak value excesses occurred in the concentrations of TSS, Phosphorous, Nitrate, and BOD. relation for biochemical oxygen demand (BOD) of commercial land use runoff was estimated as 1/6 of the COD (2). In the sample analyzed for metal content, no concentration exceeded acute standards. Mercury, lead and cadmium did exceed the chronic standards (3).

The estimated mean concentrations (EMC's) for an entire event determine the mass of pollutants entering the river. The determination of an event EMC requires instantaneous discharge information in the form of an event hydrograph at the sampling site; however, the flow was not measured; therefore, the actual EMC's were not determined. A logical next step contemplated in the ongoing study is to couple simulated hydrographs with the observed runoff quality and thereby determine event EMC's.



Total Suspended Solids vs Time
(Downtown Fargo: 7/19/89)
(Event Depth: .175 inch)

Fixed Solids
Volatile Solids

Time Since Event Began (hours)

Figure 1. Total PO, pollutograph

Figure 2. TSS pollutograph

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### SNOWMELT FLOOD POTENTIAL IN THE SOURIS RIVER DRAINAGE

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 $_{
m floods}$  begin in the form of excess water moving over the land surface. Spring snowmelt may initiate  $_{
m floods}$  when the rate of snowmelt is in excess of surface storage. In regions with snow cover, flood  $_{
m hazards}$  in early spring are augmented by low evaporation losses and warming temperatures.

The entire length of the Souris River in both North Dakota and Canada is prone to flooding. Low-lying areas between one-half to one mile wide are periodically inundated to depths from a few inches to several feet. The principal flood damage within the Souris River valley occurs between Tolley and Upham, which includes 510 acres of urban development in Minot. The towns of Sawyer and Velva are also vulnerable to flood damage, but to a lesser extent. Overbank conditions at Minot result whenever the flow rate exceeds the channel capacity of 1,500 cubic feet per second (1). The purpose of this study was to determine the snowmelt runoff threshold which leads to sporadic spring floods in the Souris River drainage.

A base map of the Souris River drainage was constructed from topographic maps. Twenty subdrainages consisting of contributing tributaries were delineated, each with a gaging station at the mouth of the subdrainage. The size of each subbasin was planimetered for use in calculating runoff. Weather records from 48 stations in North Dakota, Manitoba, and Saskatchewan covering a time span of 40 years were employed in calculation of winter precipitation and potential evapotranspiration (PE). A modified water year budget (November through mid-April) was used to calculate snow-derived precipitation, as most floods on the Souris River occur between April and May (1). The precipitation values were computer interpolated (SYMAP) to produce a density distribution map for calculation of runoff in each subdrainage. The mid-value of each contour interval was multiplied by the corresponding area in each subbasin. PE was also calculated (2) and subtracted from the total to determine runoff. The derived runoff values were compared to published gaging station volumes. An assumption was made that infiltration during spring was minimal.

The results of the investigation revealed that winter precipitation in the Souris River drainage ranged from a low of 2.2 inches to a high of 4.4 inches, with an average of 3.2 inches water content. After PE was subtracted, the runoff averaged 2.7 inches. However there is year-to-year variability of this value and any consecutive year is as likely to be higher or lower than the previous year. By ranking the top eight flood years (Table 1) with greatest runoff, it was found that five out of eight floods occurred when runoff equaled or exceeded the threshold value of 3.48 inches (Fig. 1).

In conclusion, the flood potential of the Souris River drainage for overbank conditions is calculated to occur at 3.48 inches of runoff. However, historic records indicate that this condition may be attenuated by other factors such as slow warming in spring, ground infiltration, lack of rainfall during spring breakup, a dry autumn, and artificial storage. An average spring runoff of 2.7 inches was calculated. However, this value was realized only five times within a 40 year time span.

Table 1
Top Ranked Flood and Non Flood Years

	Flood You		Non-Flood Year Winter					
Year	Precip. (in.)	Runoff (in.)	Year	Precip. (in.)	Runoff (in.)			
1949	4.4	3.6	1967	4.2	4.0			
1974	4.4	4.0	1970	3.9	-3.6			
1948	4.4	4.2						
1969	4.2	3.5						
1943*	3.9	3.1						
1951	3.9	3.6						

\*Second flood peak on April 26

Data:(3)

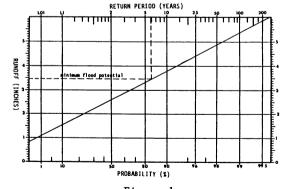


Figure 1

Calculated Probability and Return Periods for Floods on the Souris River Resulting from Snowmelt Runoff

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## A LOW COPPER DIET INFLUENCES THERMOREGULATION OF THE RAT

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The possible role of copper in thermoregulation has not been fully explored. There are indications that copper may influence thyroid hormones regulating body temperature (1). Children with Menkes' kinky hair syndrome reportedly have body temperature instability (2). Also, copperdeficient rats often feel cool to the touch. This study was conducted to determine if temperature homeostasis by thyroidal control is influenced by low copper status.

Sixty-three male Sprague-Dawley rats were matched by weight and divided into two groups. The copper-deficient group (CuD) was fed a purified, low copper diet (0.7~ppm), while the copper-adequate group (CuA) was fed a purified diet containing 10.0~ppm copper, for six weeks. Copper-adequate and -deficient diets were prepared as described by Moore and Klevay (3). Both groups received deionized distilled drinking water. The animals were housed individually in stainless steel cages and kept under controlled environmental conditions. Body temperatures were taken at the same time each morning using a digital thermometer. At week two and continuing for each successive week, six rats were sacrificed from each group. Plasma samples were obtained for determination of copper, cytochrome oxidase, ceruloplasmin, cholesterol, triiodothyronine  $(T_3)$ , and thyroxine  $(T_4)$ . Livers were removed for copper and cytochrome oxidase determination.

Body temperatures at the time of sacrifice are shown in Table 1. Significant differences were found in body temperatures between groups on days 35 and 41.

TABLE 1. MEAN BODY TEMPERATURES (°C)

Day	14	21	28	35	41
CuA	37.9 ± 0.1°	37.6 ± 0.2	$38.1 \pm 0.1$	$38.2 \pm 0.1^{b}$	$38.0 \pm 0.2^{b}$
CuD	37.8 ± 0.1	37.6 ± 0.1	$38.0 \pm 0.2$	$37.4 \pm 0.3$	$37.2 \pm 0.2$

\*Mean ± SEM \*Significantly Different (P<0.05, ANOVA) from CuD.

Liver copper declined significantly (P<0.05) in the CuD over time. Other indicators of copper deficiency were also present in the CuD: increased cholesterol, decreased cytochrome oxidase and ceruloplasmin, anemia, and poor growth.

The  $T_4$  concentrations were lower in CuD than CuA animals (Table 2). Reduced concentrations of  $T_4$  in CuD rats have been reported previously (1). The  $T_3$  concentrations in both groups were similar but showed great variability.

TABLE 2. HORMONE ANALYSIS

Day of I	Experiment	14	21	28	35	41
CuA	T <sub>3</sub> ng/dl	98 ± 10°	87 ± 11	71 ± 11	89 ± 15	75 ± 10
	$T_4 \mu g/dl$	$3.1 \pm 0.7$	$3.6 \pm 0.6$	$3.4 \pm 0.5$	$3.6 \pm 0.4$	$3.3 \pm 0.6$
CuD	T <sub>3</sub> ng/dl	125 ± 11	89 ± 11	60 ± 5	75 ± 9	74 ± 10
	$T_4 \mu g/dl$	$3.4 \pm 0.6$	$3.4 \pm 0.6$	$2.3 \pm 0.6$	2.4 ± 0.5	2.1 ± 0.4

Mean ±SEM

Declining  $T_4$  concentration during copper deficiency suggests some impairment of thyroid function. This may be the result of defects in either synthesis or release of  $T_4$ . Conversion of  $T_4$  to  $T_3$  apparently is unaffected by copper deficiency. Further work is needed to establish where the lesion of  $T_4$  metabolism occurs.

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# THE USE OF PUBLIC DOMAIN AND ANCILLARY SOFTWARE FOR DIGITAL IMAGE PROCESSING INSTRUCTION IN REMOTE SENSING

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Probably the greatest use of digital image processing techniques is found in the field of remote sensing. Given the ever-increasing amount of available digital data from satellites, aircraft, and ground-based sensors, and rapid advances in hardware and software to exploit them, an understanding of the rudiments of this technology by students of contemporary remote sensing is paramount. Given the cost involved, however, some departments and institutions may be sorely pressed to procure the commercial hardware and software specifically developed for this application (1).

public domain software (software developed through the use of government funds and available at no charge to the public) and/or "Do It Yourself" (DIY) programming are two viable options for departments that are "resource-impaired." This paper highlights the development of a remote sensing course focusing on digital image processing using a combination of public domain software and newly-developed computer programs. All of the software operated on standard IBM-PC/XT or compatible micro-computers; the only "specialized" equipment required was an EGA graphics adapter. Developed by Major Scott Loomer at the Department of Geography and Computer Science at West Point Military Academy, the "Landsat" image processing package was obtained. This system enables supervised classification of multispectral data, provides the capability to construct histograms and performs gray-scale and color composites of multispectral channels. Eleven utility and image enhancement programs were developed to supplement the "Landsat" software package. These included: File Read, Spectral Range, Histogram Bin Calculation, 4-Gray Dither, 8-Gray Dither, Gray Ramp, Magnify, Filter, Vertical Edge Detection, Horizontal Edge Detection, and Ratio. This subset was designed according to the precept that the software was to "...educate rather than facilitate the processing of...data" (2). This is a departure from a "data oriented" image processing subsystem which minimizes user interaction. All of the software was written using the QuickBasic programming language.

In the lecture component of the course, topics are presented in a sequence which leads students through a progression of more difficult digital image processing techniques. Using the "Landsat" system, students become proficient in gray-scale image manipulation and display and are then introduced to additional image enhancements using the image processing software developed for this course. The overall structure of lab exercises is varied and includes: 1) display of select imagery using the appropriate software; 2) written evaluation of enhanced digital imagery; 3) short answers; and 4) calculations.

Cost and flexibility constitute the primary advantages of this hardware and software configuration. Cost factors include only the purchase price of an IBM or compatible microcomputer. Flexibility advantages include student acess to equipment and the ability to tailor and/or modify the software to meet specific or changing requirements. This setup can help to take the "squeeze" off of other hardware and software systems or can be utilized for introductory-level instruction, reserving the state-of-the-art, commercial resources for more advanced training or research. Installing the data and software on floppy disks may allow students to utilize other equipment available on campus, thus providing them with increased times. The ability to modify the system to meet changing levels of student expertise or to augment other facilities is a prime advantage.

Computer memory is the most significant developmental consideration. Any manipulation of scene pixels which requires that multiple files or arrays be made memory resident quickly exceeds the available 640 KB of memory. The QuickBasic language provides some help in this regard (e.g., the Dynamic and Chain statements, and the QB/AH command). De-installing memory-resident programs also increases available memory. All methods, however, invariably result in very slow manipulation and display times, key disadvantages.

In summary, the scenario presented here offers a viable focus for instruction in remote sensing and digital image processing. The low cost factor involved in establishing this scenario enhances the ability of even the most budget-constrained department to offer some instruction in this arena.

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# AN IMPROVED DIGESTION PROCEDURE FOR THE ATOMIC ABSORPTION DETERMINATION OF SELENIUM BY HYDRIDE GENERATION

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Hydride generation atomic absorption analysis is a convenient and sensitive technique for the determination of a number of elements that form volatile hydrides. Selenium hydride is formed from selenium in the +4 oxidation state by reduction with sodium borohydride. Selenium in the +6 oxidation state is hardly reduced at all because of kinetic factors. Since samples may contain selenium in several oxidation states, an initial step is required to ensure that selenium is present in the +4 oxidation state.

The digestion procedure selected used an oxidation with acidic permanganate to convert all selenium present to the +6 oxidation state. This has been reported to prevent reoxidation of selenium and thus provides a more stable solution (1). After destruction of excess permanganate with hydroxylamine the sample was mixed with an equal volume of HCl and heated for a specified time period of 10 minutes to reduce the selenium to the +4 oxidation state. The procedure was carried out in Erlenmeyer flasks heated on hot plates. Although adequate, there were several difficulties such as sample loss due to bumping and evaporation and the procedure was time consuming. To ensure a heating period of 10 minutes for the reduction, flasks were heated to boiling over a Fisher burner, and transferred to a hot plate. After 10 minutes the flasks were placed in an ice bath to stop the reaction. Sample volume was then adjusted for evaporative losses by adding water to each flask to compensate for lost mass.

An improved, simplified, procedure was developed as follows: Erlenmeyer flasks were replaced with 50 mL screw-top test tubes. The reactions were carried out in a dry block heater at 95°C. To reduce pressure buildup due to expansion of cold air the tubes were placed in the block heater with the screw caps loose. After a minute the caps were tightened for the remainder of the heating period. Results have indicated that a precisely timed reduction step was not critical in sealed tubes. It had been speculated that analyte loss occurred due to some reduction of Se to the 0 state with heating times longer than 10 minutes (2). It is more likely that selenium was volatilized in the heating step (3). This is prevented by the use of sealed tubes, thus the necessity of a critically controlled timed heating period was eliminated.

Verification of this improved digestion procedure was performed using Environmental Protection Agency Quality Assurance check samples. Results are shown in Table 1.

TABLE 1

Analytical Results on EPA Quality Assurance Check Samples

SAMPLE	FLASK	TUBE	TRUE VALUE	LIMITS
1 1 (DUP)	43.3 43.8	42.0 40.9	48.0	33.0 - 58.5
2 2 (DUP)	12.1 11.8	14.1 14.1	12.0	6.24 - 16.7

The digestion procedure outlined in this communication has simplified sample preparation for the determination of selenium in various sample matrices while maintaining a high degree of accuracy and precision. The revisions are inexpensive and utilize readily available laboratory equipment.

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# EFFECTS OF SHORT-TERM DIETARY ZINC DEFICIENCY ON SEMEN MINERAL CONCENTRATIONS IN HUMANS

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 $_{
m Zinc}$  is required for many of the phases of sperm production and maintenance (1) and is found in significant amounts in both sperm and seminal fluid. Calculations based on reported values for seminal zinc concentrations and volume (2) suggest that one-half of the daily zinc intake of males maintained on 1 mg zinc/d (inadequate) may be lost in a single ejaculate. It therefore seemed appropriate to determine the effects of short-term zinc deficiency on semen mineral concentrations and volume. Eleven young men living on a metabolic unit were fed a diet composed of a mixture of a semisynthetic formula and conventional foods supplemented with ZnSO, to supply a total of 1.2.3.4. or 10 mg Zn/day. After an equilibration period of 28 days (10 mg Zn/day), all treatments were presented for 35 days each, the first four in random order and the fifth last. Semen samples obtained at the end of each treatment period were collected in latex condoms (determined to contribute negligible mineral contamination) under prescribed sanitary conditions, transported to the laboratory in insulated boxes, and transferred to plastic tubes for determination of sample volume. Small aliquots of sample for other analyses were removed immediately using plastic pipettes. After allowing the remaining sample to liquefy at room temperature for 30 minutes, the  $r_{\text{sample}}$  was vortexed gently, and a 1000  $\mu$ L aliquot was transferred to microcentrifuge tubes and kept frozen at -80°C until analyzed. As described in detail elsewhere (3), each sample was thawed at room temperature, vortexed and weighed directly into a Teflon\* tube. The samples were then oxidized (<120°C) by double-distilled 15.9 M HNO3 for 48 hours, followed by 30% H<sub>2</sub>O<sub>2</sub> for 12 hours, in open tubes placed in a 150°C sandbath. All samples and blanks were then diluted 1:3 with double-distilled, deionized water and stored in polypropylene tubes until analyzed. The digestates were analyzed by inductively coupled argon plasma spectroscopy (ICAP) for boron, calcium, copper, iron, magnesium, manganese, molybdenum, phosphorus, vanadium, and zinc.

Table 1. Effects of Short-Term Dietary Zinc Deficiency on Seminal Mineral Concentrations In

Treatment	Semen	Ser	nen Mineral Co	oncentration	(mg/ml)	
	Volume	Ca	Fe	Mg	P	Zn
Zn, mg/day	<u>m1</u>	$\mu_{\rm g/ml}$	$\mu_{\rm g/ml}$	$\mu g/ml$	$\mu_{\rm g/ml}$	$\mu_{\rm g/ml}$
1	2.24	283	3.38	110	1016	119
2	2.75	251	1.40	83	958	103
3	2.98	275	2.19	95	1065	119
4	2.96	281	1.92	96	1029	131
10	3.30	270	1.47	91	905	124
		Analysis of V	<u> ariance - P V</u>	<u>lalues</u>		
	0.03	NS	0.014	NS	0.012	NS
		<u>Cont</u>	rast P Values	<u>i</u>		
1 vs. 10	0.002	NS	0.002	0.054	0.018	NS
2 vs. 10	NS	NS	NS	NS	NS	NS
3 vs. 10	NS	NS	NS	NS	0. <b>0</b> 01	NS
4 vs. 10	NS	NS	NS	NS	0.012	NS

Of the minerals analyzed, phosphorus was found to be the most abundant element in human semen, followed in descending order by calcium, zinc, magnesium, and iron (Table 1). Reliable values for boron, copper, manganese and vanadium could not be established because a combination of small sample volume and necessary dilution to 2 ml lowered the concentration of those elements to concentrations below detection limits. Compared to when they were consuming 10 mg Zn/day, volunteers consuming 1 mg Zn/day had lower semen volumes but elevated concentrations of seminal iron, phosphorus, and magnesium. Total ejaculatory mineral loss also differed with that dietary comparison for some certain elements (not shown). Thus, compared to 10, a 1 mg Zn/day treatment decreased total seminal calcium, magnesium, phosphorus and zinc, although from a clinical viewpoint, impact on overall balance from changes in seminal mineral content seems important only in the case of zinc. On the other hand, absolute calcium, iron and phosphorus seminal concentrations may be important for normal spermatozoa function. Because there were no differences in the number of ejaculations between any two dietary treatments, the data also suggest that body zinc stores are partially conserved during short-term zinc deprivation by a decrease in the amount of seminal secretions.

\*Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the Product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

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# LATE PLEISTOCENE (ILLINOIAN?) MOLLUSCS FROM THE LORAFF FARM SITE NEAR MILBANK. NORTHEASTERN SOUTH DAKOTA

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One of the very few exposures of pre-late Wisconsinan fossiliferous sediments in the upper Midwest occurs at the Loraff Farm site near Milbank in extreme northeastern South Dakota. These sediments contain the remains of molluscs, insects, ostracods, microtine rodents, macroplants, and pollen. We describe here the molluscs and discuss their paleoenvironmental significance. The exposure is an east-facing cutbank of the Whetstone River in the NW1/4SW1/4SW1/4 sec. 13, T. 121. N., R. 47 N., Grant County. Wood from organic-rich "gastropod silts" has produced indefinite radiocarbon ages (>56,000 yr B.P.), and an amino acid racemization age of 140,000+70,000 years was obtained from gastropod (Pupilla muscorum) shells (1). The "gastropod silts" were presumably deposited during late Illinoian time (1). If this age is correct, the "gastropod silts" are older than the fossil-bearing Gervais Formation exposed in northwestern Minnesota, inferred as early Wisconsinan in age (2).

The Loraff Farm site exposure consists of 10 m of Hawk Creek till underlain by the "gastropod silts." In our measured section, 16-20 cm of organic-rich, dark gray clay directly underlies the till. Underlying the clay is 7-30 cm of light brown, fine to medium sand. Gray sandy silt, 100 cm thick, underlies the sand to river level. All sediments exposed beneath the Hawk Creek till are considered as "gastropod silts," but only the upper 50 cm are fossil-bearing. In the subsurface the "gastropod silts" overlie the Whetstone till (1).

We collected samples at 10-cm intervals from the base of the Hawk Creek till to river level. Molluscs were extracted by wet sieving. We also examined several hundred specimens supplied by Jay P. Gilbertson (South Dakota Geological Survey) and R. Sanders Rhodes II (University of Iowa), from approximately the upper 40-50 cm of the "gastropod silts." Collectively, several hundred kilograms of sediment were processed for molluscs.

The "gastropod silts" molluscan assemblage consists of 1 species of pill clam, Pisidium nitidum (R); 5 aquatic snails, Valvata sincera (R), V. tricarinata (R), Fossaria parva? (A), Gyraulus parvus (R), and Physa jennessi (U); and 8 land snails, Vallonia gracilicosta (U), Columella alticola (C), Pupilla muscorum (C), Vertigo modesta (A), Catinella sp. (A), Discus cronkhitei (U), Euconulus fulvus (U) and the presumably extinct Deroceras aenigma (U) (R=Rare, U=Uncommon, C=Common, A=Abundant). Presumed snail eggs were also identified. The present ranges of the extant taxa are broad, and the Loraff Farm site is at the central or south-central part of the ranges of these taxa. Specimens of terrestrial molluscs exceed those of aquatic molluscs. Except for the abundant Fossaria parva? the aquatic molluscs are sparse. Most of the land snail species occur today in moist, wooded habitats. All taxa have been reported previously from Illinoian or older sediments.

Because the extant taxa have broad latitudinal ranges, they are of little value for paleoclimatic inferences. The aquatic species could have existed in either standing or flowing-water bodies of variable size. The relative sparseness of most of the aquatic species, except for F. parva?, frequently an amphibious species, suggests a semi-permanent water body for deposition of the "gastropod silts." Most of the land snails imply that the immediate site area was probably wooded during the time of deposition of the "gastropod silts."

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# EFFECTS OF SHORT-TERM DIETARY ZINC DEFICIENCY ON SPERM MORPHOLOGY AND MOTILITY IN HUMANS

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Zinc is required for the G1 and S phases of spermatogenesis and for sperm nuclear condensation during spermiogenesis and is implicated in the antibacterial activity of prostatic fluid (1). In addition, the integrity and fertility of spermatozoa are dependent upon a physiological concentration of zinc in the semen (2). It therefore seemed appropriate to determine the effects of short-term zinc deficiency on semen morphology and motility. Eleven young men living in a metabolic unit were fed a diet composed of a mixture of a semisynthetic formula and conventional foods supplemented with ZnSO4 to supply a total of 1,2,3,4, or 10 mg Zn/day. After an equilibration period of 28 days (10 mg Zn/day), all treatments were presented for 35 days each, the first four in random order and the fifth last. Under prescribed sanitary conditions semen samples were collected at the end of each treatment period in latex condoms and transported to the laboratory in insulated boxes. The samples were transferred to plastic tubes for the determination of sample  $_{
m volume}$ . A semen smear was then prepared within 5 minutes by transferring 20  $\mu {
m L}$  of sample to a glass slide coated with dried hairspray. The smear was air-dried, stained, and coverslipped. At the same time, 20  $\mu L$  of sample were transferred to a glass slide and sperm motility was assessed by visual analysis with a binocular microscope. After allowing the remaining sample to liquefy at room temperature for 30 minutes, the sample was vortexed gently, and up to 1600 \( \mu \)L were removed for other analyses. Then a second semen smear and motility slide were prepared and 20 µL of sample were removed for sperm density measurements. As described in detail elsewhere (3), the semen smears collected at 30 minutes were analyzed for sperm head and acrosome and postacrosomal region areas, and sperm head shape using a Cambridge 970 Quantimet Image Analysis System. Frequency of ejaculations for each volunteer was recorded daily.

Table 1. Effects of Short-Term Dietary Zinc Deficiency on Human Sperm Morphology and Motility.

Treatment	Sperm	Sperm	Motility at	t 30 min.		Sperm	
	Density	Mobile	Sluggish	Immobile		Head	Acrosome
					Area	Length/Breadth	Area
Zn, mg/day	$10^6/\text{m}1$	<u>x</u>	<u>%</u>	<u>%</u>	um		<u>um</u>
1	31.2		13	36	10.83	1.61	4.45
2	25.1	37	19	46	11.10	1.59	4.39
3	26.7	41	17	38	11.65	1.63	4.39
4	24.0	48	14	40	10.36	1.60	3.98
10	25.9	36	13	51	10.51	1.64	4.29
			Analysis o	of Variance	- P Valu	<u>ies</u>	
	NS		•		NS	NS	NS
Friedman	Test-P values	for Motility	Data				
		NS	NS	NS			

The percentage of mobile, sluggish, or immobile sperm at either 5 (not shown) or 30 minutes did not vary significantly between any two treatments (Table 1). However, compared to when they were consuming 10 mg Zn/day, volunteers consuming 1 mg Zn/day were found to have a tendency for higher sperm densities and percentage of mobile sperm. There were no differences in the number of ejaculations between any two treatments. The treatments did not affect sperm head area or shape or acrosome area; the coefficients of variation for those variables were within acceptable limits (12, 12, and 4% respectively). Findings described elsewhere (4) suggest that body zinc stores were partially conserved during short-term zinc deprivation by a decrease in the rate of seminal secretions. The findings to date suggest that there are two separate pools of zinc related to sperm and seminal fluid production. Thus, it is hypothesized that during short-term zinc deficiency, the zinc pool required for normal spermatozoa development is maintained while the pool required for seminal fluid production and/or normally transferred to the seminal fluid is utilized by the body elsewhere.

\*Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the Product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

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#### EFFECT OF ENERGY INTAKE ON TRACE ELEMENT BALANCE

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The effects of different energy intakes on trace element nutriture have not been extensively evaluated. Atinmo et al. (1) reported that in healthy Nigerian college students consuming diets typical for that culture, a low energy diet was associated with decreased retention of calcium and magnesium and increased retention of zinc, in comparison to a high energy intake. The purpose of the present investigation was to determine the effect of high and low energy Western diets on trace element balance in healthy young men.

Thirteen healthy men, 23 to 42 years old, with body weights within 95 to 125 % of average weights for height (2), consumed low and high energy diets, each for four weeks. Half the participants received the high energy diet first and half the low energy diet first. The low energy diet provided 25 kcal/kg body weight and recommended amounts of nutrients, and was typical of well-balanced weight control diets recommended in the U.S. Using a similar menu, the high energy diet supplied 45 kcal/kg with additional energy provided by fat and refined carbohydrate without substantially altering the daily intake of protein and micronutrients. Elemental balance was determined during the last 12 days of each dietary period.

Participants lost (mean  $\pm$  SD) 4.7  $\pm$  1.2 kg while consuming the low energy diet and gained 0.9  $\pm$  1.0 kg while consuming the high energy diet. Retention of calcium was not affected by dietary energy, and the small differences in magnesium and manganese retention were less than the differences in the dietary magnesium and manganese content (Table 1). In contrast, copper, iron, and zinc balances were substantially lower on the low energy diet.

TABLE 1.	Elemental Programme 1	Balance	as	Affected	bу	High	and	Low	Energy	Diets.

	CALCIU	1, mg/d	MAGNESI	UM, mg/d	ZIN	C, mg/d		
	<u> High kcal</u>	<u>Low kcal</u>	<u>High kcal</u>	Low kcal	<u> High kcal</u>	Low kcal		
Diet	1471 ± 32+	$1379 \pm 32$	404 ± 8	367 ± 8	$21.1 \pm 0.4$	$19.6 \pm 0.4$		
Feces	780 ± 23	685 ± 23**	192 ± 7	189 ± 7	16.6 ± 0.7	$18.7 \pm 0.7$		
Urine	181 ± 9	147 ± 9	$133 \pm 3$	$124 \pm 3$	$0.5 \pm 0.02$	$0.7 \pm 0.02$		
Balance	509 ± 16	547 ± 16	78 ± 5	54 ± 5**	$4.0 \pm 0.4$	$0.2 \pm 0.4**$		
	COPPE	R, mg/d	IRON,	mg/d	MANGANE	SE, mg/d		
	High kcal	Low kcal	High kcal	Low kcal	<u>High kcal</u>	Low kcal		
Diet	$1.58 \pm 0.03$	$1.45 \pm 0.03$	$24.8 \pm 0.5$	$22.3 \pm 0.5$	6.14 ± 0.14	5.62 ± 0.14		
Feces	$1.34 \pm 0.05$	$1.43 \pm 0.05$	17.9 ± 0.7	$18.2 \pm 0.7$	$5.22 \pm 0.18$	$5.01 \pm 0.18$		
Balance	$0.25 \pm 0.03$	0.02 ± 0.03**	$7.0 \pm 0.4$	4.1 ± 0.4**	$0.93 \pm 0.10$	0.62 ± 0.10*		

<sup>+</sup> Mean  $\pm$  SEM, \* p < 0.05, \*\* p < 0.01

The results of this study are in contrast to those in the study by Atinmo et al. (1). However, the lower elemental balances observed with the low energy diet in the present study are consistent with a reduction in lean body mass. Average body surface losses of copper and zinc are 0.04 mg/d (D. Milne, personal communication) and 0.5 mg/d (3), respectively. Thus, although measured zinc and copper balances were positive, it is likely that the measurement of surface losses of copper and zinc would have revealed a negative balance of these nutrients on the low energy diet. The high, positive iron balances observed probably overestimate true iron retention but may indicate a real difference between diets. These diets exceeded current recommended intakes for iron and zinc of 10 and 15 mg, respectively, and provided 1.5 mg copper, which is in the range suggested to be safe and adequate (4). Despite seemingly adequate intakes of these nutrients, the results of this study suggest that nutritional status of copper, zinc, and possibly iron may be negatively affected by weight-loss diets.

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### STATISTICAL COMPARISON OF NEST SUCCESS RATES Douglas H. Johnson\* U.S. Fish and Wildlife Service Jamestown, North Dakota 58402

Mayfield (1) suggested a method of estimating the success rates of bird nests. The estimator commonly used before that was severely biased in many situations. Mayfield proposed that the number of nests destroyed be divided by the exposure, the number of days a nest was under observation and available to be destroyed. This estimator possesses several desirable properties (1,2). Johnson (2) developed a variance estimator for Mayfield's estimated daily mortality rate and indicated how it can be used to compare rates between two groups with a Z test. This note extends the argument to K > 2 groups. Assume there are K (K  $\geq$  2) groups of nests and the true daily mortality rate of nests in group i is  $\rho_i$  (i = 1, ..., K). Suppose a number of nests in each qroup are observed and the total exposure for the ith group is  $e_i$ , which we  $_{\mbox{\scriptsize assume}}$  is fixed in advance. Suppose that  $\mbox{\bf d}_{\mbox{\scriptsize i}}$  of the nests in group i are destroyed, which results in an estimated daily mortality rate of  $\hat{\rho}_i = r_i$  $d_{i}/e_{i}$ . Interest is in testing the hypothesis  $H_{0}$ :  $\rho_{1}=\rho_{2}=\ldots=\rho_{K}=\rho$ , say. Define the sums  $e_t = \sum e_i$  and  $d_t = \sum d_i$ , along with the pooled estimator of  $\rho: \hat{\rho} = r_{+} = d_{+}/e_{+}$ , where summation is j = 1, ..., K throughout. Consider the test statistic

$$T = \sum e_j (r_j - r_t)^2$$
, which we will write in terms of  $z_j$ , where  $z_j = \sqrt{e_j} r_j - \sqrt{e_j} \rho$ .

Because the  $r_{i}$  are independent, so will be the  $z_{i}$ . Then asymptotically each  $z_i$  will have a normal distribution with mean zero and variance  $\rho(1-\rho)$  (3).

Since 
$$r_j = z_j / \sqrt{e_j} + \rho$$
,

we have 
$$r_t = \sum e_j r_j / \sum e_j = \sum \sqrt{e_j} z_j / e_t + \rho$$
  
and  $T = \sum e_j [(z_j / \sqrt{e_j} + \rho) - (\sum \sqrt{e_i} z_i / e_t + \rho)]^2 = \sum z_j^2 - (\sum a_j z_j)^2$ ,  
where  $a_j = \sqrt{e_j} / \sqrt{e_t}$ ,  $j = 1$ , ..., K.

Hence, writing in vector and matrix notation,  $\underline{z}' = (z_1 \ z_2 \ \dots \ z_K)$  and  $\underline{\mathbf{a}}' = (\mathbf{a}_1 \ \mathbf{a}_2 \ \dots \ \mathbf{a}_K)$ , we have  $\underline{\mathbf{T}} = \underline{\mathbf{z}}'\underline{\mathbf{z}} - (\underline{\mathbf{a}}'\underline{\mathbf{z}})^2 = \underline{\mathbf{z}}'(\underline{\mathbf{I}} - \underline{\mathbf{a}}\underline{\mathbf{a}}')\underline{\mathbf{z}}$ . Now

$$(I - \underline{aa}')(I - \underline{aa}') = I - \underline{aa}' - \underline{aa}' + \underline{aa}'\underline{aa}' = I - \underline{aa}',$$

because  $\underline{a}'\underline{a} = \Sigma a_j^2 = \Sigma (\sqrt{e_j} / \sqrt{e_t})^2 = 1$ . So (I -  $\underline{aa}'$ ) is idempotent with rank  $rank (I - \underline{aa}') = tr(I - \underline{aa}') = tr(I) - tr(\underline{aa}')$ =  $K - \Sigma(e_{i}/e_{t}) = K - 1$ .

So from Cochran's theorem (e.g., 3), the quadratic form z'(I - aa')z is distributed as  $Var(z) \times \chi^2$  with K-1 degrees of freedom. Also, Var(z) =ho (1 - ho). Test statistics such as T result from performing an analysis of variance on daily mortality rates  $(r_i)$ , using exposure  $(e_i)$  as a weight. Instead of using the within-group error as the denominator in an  $\underline{F}$  test, the error sum of squares T is divided by  $r_t(1 - r_t)$  and referred to a Chi-square distribution.

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## ABSORPTION AND BIOLOGICAL HALF-LIFE OF MN-54 FROM INTRINSICALLY AND EXTRINSICALLY LABELED FOODS IN HUMANS

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The ability to use an extrinsic  $^{54}$ Mn tracer to study dietary effects on Mn absorption and bioavailability is desirable because production of intrinsically labeled foods is difficult, time. consuming, and expensive. There is only one report on the validity of using an extrinsic Mn tracer; Davidsson et al (1) labeled chicken liver intrinsically with  $^{54}$ Mn and extrinsically with  $^{52}$ Mn and found no difference in the absorption of the two tracers by human subjects.

Adult men and women consumed a series of three test meals consisting of a food labeled intrinsically, the same food labeled extrinsically with  $^{54}$ Mm, and a control "meal" of MnCl $_2$ , served in random order. Foods tested were wheat, spinach, lettuce, and sunflower seeds. Each meal also contained 5 g vegetable oil, 0.15 g NaCl, and 10 g Ritz crackers. Lettuce meals and their controls contained 530  $\mu$ g Mm; other meals contained 1230  $\mu$ g Mm. Each meal was labeled with 1.0  $\mu$ Ci  $^{54}$ Mm. Subjects were counted in a whole-body gamma counter immediately after consuming each labeled meal and weekly thereafter for eight weeks. Absorption of  $^{54}$ Mm was calculated as the y intercept of a semilogarithmic plot of percent  $^{54}$ Mm retention (corrected for decay) vs time after the labeled meal. Biological half-life (BH) of  $^{54}$ Mm was calculated as BH - -ln2/slope, using the slope of the linear portion of the semilogarithmic plot of retention vs time.

Intrinsically labeled hard red spring wheat and confectionery sunflowers were labeled by injection with <sup>54</sup>Mm. Intrinsically labeled lettuce and spinach were labeled by foliar application of the isotope. Identical, unlabeled lettuce and spinach were grown for preparation of extrinsically labeled foods, while wheat and sunflower seeds of the same variety as the intrinsically labeled plants were purchased for preparation of the extrinsically labeled meals. All foods were wet-ashed with a mixture of trace mineral grade nitric acid and 30% hydrogen peroxide. Mn analysis was done by inductively coupled argon plasma emission spectroscopy (ICAP). Whole blood and plasma were digested by a similar method and analyzed for Mn using graphite furnace atomic absorption spectroscopy with Zeeman background correction (2).

For each of the foods tested, absorption of intrinsically and extrinsically added  $^{54}$ Mn was the same. Biological half-life of  $^{54}$ Mn was also independent of the source of the  $^{54}$ Mn label in the test meal. Although each of the test foods was eaten by a different group of subjects,  $^{54}$ Mn absorption from the control meals did not differ significantly among groups of subjects. This lack of difference in absorption occurred despite the fact that the Mn dose in the control meal for the lettuce group was 530  $\mu$ g, compared to 1230  $\mu$ g in the other control meals. Because there were no significant differences in  $^{54}$ Mn absorption or BH from intrinsically and extrinsically labeled foods, and because control  $^{54}$ Mn absorption and BH were the same in all four groups of subjects, we combined the intrinsic and extrinsic data for each food and compared  $^{54}$ Mn absorption and BH among foods (Table 1). The biological half-life of  $^{54}$ Mn did not vary among foods, but  $^{54}$ Mn absorption was significantly greater (p<0.001) from lettuce than from sunflowers or wheat. Because no differences in Mn absorption were found between the Mn doses of 530  $\mu$ g and 1230  $\mu$ g Mn as MnCl<sub>2</sub>, we believe the significantly higher Mn absorption from lettuce is not related to the lower dose of Mn used compared to the other foods, but to some inherent property of the lettuce.

TABLE 1. Co	omparison o	f Mean 54Mn	Absorption and Biologi	cal Half-Life from	n Test Foods
	x	Absorption	Biological Half-I	Life (d) n	
Lettuce		5.20ª	40.4	1	6
Spinach		3.89 <sup>ab</sup>	42.2	1	5
Sunflower Se	eeds	1.51	38.6	1	7
Wheat		2.16 <sup>b</sup>	37.1	1	8

There were no significant correlations between whole blood Mn or plasma Mn concentration and <sup>54</sup>Mn absorption or biological half-life in our subjects.

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# Stratigraphic distribution of <u>Eumys</u> in the Brule Formation of North Dakota and its biochronologic implications

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Analysis of 53 specimens of the cricetid rodent <u>Eumys</u> from two localities in the Brule Formation of North Dakota shows that two and possibly three species are represented. Collections from the lower portion of the Brule Formation exposed at White Butte in Slope County include 27 specimens; all but one are referable to <u>Eumys elegans</u>. This is the common species of the middle Oligocene (Orellan land-mammal biochron) found in South Dakota, Nebraska, Montana and Colorado (1,2), as well as in the late Oligocene (Whitneyan) of Nebraska (1) and Wyoming (3). The remaining specimen may represent <u>E. obliquidens</u>, a species of arguable validity (4, 5, 6) which has been reported from the Orellan of Colorado, South Dakota and Nebraska (1, 2).

The second locality producing a sample of <u>Eumys</u> is from the Schmidt Ranch locality of Stark County. The 26 specimens of <u>Eumys</u> from Schmidt Ranch differ from <u>E. elegans</u> from White Butte in being significantly larger; M1-3 length, and M2 and M3 lengths all differ significantly (P < 0.01 that the samples are the same). The M1 lengths do not differ significantly (0.70 < P < 0.80 that the samples are the same) but this is probably due to a smaller anteroconid on the Schmidt Ranch specimens. The Schmidt Ranch species also differs from <u>E. elegans</u> in having a consistently different M2 morphology. The anterior cingulum of M2 extends buccally to the margin of the tooth, but does not extend lingually beyond the midline of the metaconid. In <u>E. elegans</u> the anterior cingulum always extends lingually beyond the midline of the metaconid. The M2 morphology of the Schmidt Ranch specimens fits that of <u>E. brachvodus</u> (1) and the material can be confidently assigned to that species.

Identification of <u>E. brachyodus</u> has chronologic implications for the Brule Formation in North Dakota. Galbreath (2) noted that in northeastern Colorado <u>E. elegans</u> and <u>E. obliquidens</u> are restricted to the Orellan fauna and that <u>E. brachyodus</u> is restricted to the Whitneyan fauna. Most other reported occurrences of <u>E. brachyodus</u> are from Whitneyan faunas (1, 3) with few, if any, unquestioned occurrences of the species from Orellan localities. This suggests that <u>E. brachyodus</u> may be an index fossil for the Whitneyan, and in turn, that the Schmidt Ranch locality is Whitneyan. Most workers have considered most or all of the Brule Formation in North Dakota to be Orellan (7, 8, 9). The Schmidt Ranch locality is in the lower third of the Brule Formation section, possibly stratigraphically below the Fitterer Ranch bed which has produced the largest concentration of fossils. Some authors have noted that at least some of the fossils from the Brule Formation in North Dakota indicate a younger (Whitneyan) age for some of the section (9, 10, 11). However, if <u>E. brachyodus</u> can be used as an index fossil for the Whitneyan, then the middle-late Oligocene transition occurs lower in the Brule Formation of North Dakota than has previously been suggested and much of the Oligocene fauna reported from the North Dakota is Whitneyan rather than Orellan.

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Chronostratigraphic Implications of the Mammal and Nonmarine Mollusk Record of the Paleocene Fort Union Group in North Dakota

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Although fossils are common in the strata of the Fort Union Group in North Dakota, their present utility does not permit refined chronostratigraphic correlation of most Paleocene formational contacts. Mammal fossils provide the greatest potential for resolving the temporal relations between formations because they have been intensively and extensively studied throughout the western interior of North America resulting in a relatively well defined sequence of Paleocene biochrons. The main limitation in utilizing mammals to provide a detailed chronostratigraphic framework for Paleocene strata in North Dakota is the general scarcity of mammal occurrences, especially of diverse local faunas, and the difficulty of chance discovery of new localities. Of the known localities, only two local faunas, comprising six sites, were discovered by paleontologists, and two of these sites were discovered with forehand knowledge of the lithofacies of nearby producing sites. The remaining thirteen localities were discovered by interested local residents or scientists pursuing other geological or biological studies. Of the nineteen known localities in North Dakota, only ten have received any publication (1-5), and only six have produced more than two taxa. Further limiting the chronostratigraphic utility of the localities is the distribution; five are probably from the Slope Formation and one from the Sentinel Butte Formation, but all six are late Paleocene in age, representing the middle part of the Tiffanian biochron (Ti3-Ti4).

In contrast to the mammal record, nonmarine mollusks are known from more than 300 Fort Union Group localities in the state (6). Unfortunately, the current knowledge of many of the taxa does not permit delineation of well defined clades. Like the mammalian record, most mollusk localities are known from upper Paleocene strata; effectively post-Cannonball Formation deposition. Lower and middle Paleocene mollusk localities are known exclusively from southwestern North Dakota in Bowman and Slope counties. Detailed stratigraphic and sedimentologic observations by a number of geologists in this area have discovered relatively few shell concentrations. The presence of essentially in situ claystone shell impressions at a number of localities indicates at least the existence of mollusks in North Dakota during this time in restricted lacustrine and paludal environments. With the taxonomic studies that have so far been completed, nonmarine mollusks can be used to delimit three biozones approximating lower (Puercan), middle (Torrejonian), and upper (Tiffanian) Paleocene mammal biochrons. More generally, on the basis of historic and current identifications, the Paleocene is divisible into two assemblage biozones that also reflect the abundance and diversity of nonmarine mollusks in North Dakota. The upper biozone includes the Slope (in part), Bullion Creek, and lower Sentinel Butte Formations, and corresponds, with available evidence, to at least the medial and late Tiffanian. This assemblage can be exclusively defined by a number of the "classic Fort Union" taxa described by F.B. Meek and F. V. Hayden between 1856 and 1876. Other Meek and Hayden taxa are more long-ranging with some identified from lower and middle Paleocene strata.

Mammal fossils, which are generally uncommon in the Paleocene, have received considerable study, especially outside of North Dakota, due in part to interest in mammalian evolution at the beginning of their diversification. Mammal local faunas currently under study, including reevaluation of localities already discovered but only vaguely understood, will permit more refined chronostratigraphic correlations in North Dakota. Investigations should also include searches for mammal fossils in strata near formational boundaries to resolve specific correlation problems. Nonmarine mollusks, which are generally abundant in the Paleocene, are known from limited studies dealing primarily with the introduction of new taxa. Familial-level taxonomic synthesis is fundamental to improving the resolving power of nonmarine molluscan chronostratigraphy. In addition, field studies of "non-shell bearing" molluscan localities in the lower portion of the Fort Union section will provide the opportunity to understand the marked change between uppermost Cretaceous and upper Paleocene nonmarine molluscan faunas.

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## EFFECT OF CATTLE GRAZING ON SHARP-TAILED GROUSE NEST-SITE SELECTION AND NESTING SUCCESS

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Information on the effects of livestock grazing on sharp-tailed grouse nesting and nesting success is inconclusive. Light-to-moderate grazing has been suggested by some as being beneficial to sharp-tailed grouse (1). However, grazing has generally not been recommended for intensively managed grouse habitat (2). The objective of this research was to examine the effects of various grazing treatments on sharp-tailed grouse nest-site selection and nesting success.

The study was conducted between 1984 and 1986 on the Central Grasslands Research Center in southcentral North Dakota. The Center is located in the mixed-grass prairie which is dominated by western
wheatgrass (Agropyron smithii), needle-and-thread (Stipa comata), Kentucky bluegrass (Poa pratensis),
blue grama (Bouteloua gracilis), sedges (Carex spp.), and western snowberry (Symphoricarpos occidentalis). Approximately 8% of the grazed areas of the Center are seasonal and permanent wetlands.
Grazing treatments were initiated in 1982 and 1983. The seasonlong (SL) grazed treatment was 130 ha
grazed by 45 cow-calf pairs. The short duration (SD) grazing treatment was implemented on 130 ha as
8-16.25 ha pastures. Each year 65 cow-calf pairs were allocated to this system and rotated between
pastures every 5 days. A replicated twice-over rotation grazing system was located on 260 ha adjacent
to the previous treatments. The 4-32.5 has pastures in each replication were grazed by 60 to 65 cowcalf pairs on a 20 day graze, 60 day rest cycle. Cattle were allocated to all grazed treatments in
late May and removed by November for a 160 day grazing season. Approximately 200 ha of contiguous,
idled rangeland was available on the Center to serve as a control.

All sharp-tailed grouse dancing grounds within 1.6 km of the Center's boundaries were censused each spring. A total of 36 hens were trapped on dancing grounds or on nests found through chain dragging operations, and fitted with radio-transmitters. Eggs were counted, stage of incubation estimated, and nesting hens monitored daily to determine nest fates. Visual obstruction readings (VOR's) (3) of the vegetation were taken at nests and along permanent transects in each grazing treatment. Species and frequency of vegetation occurring around nests were recorded.

Displaying males totaled 123, 135, and 119 between 1984 and 1986 on fifteen dancing grounds. Over 65% of sharp-tailed grouse nests were located in western snowberry communities. VOR's at nests averaged 1.8 dm while average grazed pasture VOR's were 1.0 dm indicating a high selection for nesting habitat. Median date of nest initiation was mid-May each year which is prior to allocation of cattle to treatments. Total nests were 20 and 16 for grazed and idle treatments, respectively (Table 1). Apparent nesting success and nests per 40.5 ha were not different (P>0.05) between grazed and idle treatments. Adjusted nesting success and nests per 40.5 ha were greater (P<0.05) on the grazed treatments yet successful nests per 40.5 were similar. Properly grazed rangelands can produce similar numbers of sharp-tailed grouse when compared to idled rangelands.

TABLE 1. APPARENT AND ADJUSTED SHARP-TAILED GROUSE NESTING SUCCESS, NESTS PER 40.5 HA, AND

SUCC	CESSFUL NESTS	PER 40.5 HA				
		APPARENT			ADJUSTED <sup>1</sup>	
TREATMENT	Total	Nesting	Nests/	Nesting <sup>2</sup>	Nests/2	Successful
	Nests	Success		Success		Nests/
		%	40.5 ha	%	40.5 ha	40.5 ha
GRAZE	20	60	1.6	42	2.3	1.0
IDLE	16	38	3.2	26	5.2	1.4

Mayfield method using 36 exposure days (4).  $^{2}$ Treatments were different (Chi-square, P<0.05).

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#### FORGOTTEN OR IGNORED EXPERIMENTS WITH RADON AND RECENT FINDINGS

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Radon-induced lung cancer can be traced back to 1576 in the Erz mountains of Germany. More recently (circa 1940) uranium miners were observed to have developed lung cancer. As long ago as 1967 Pohl and Pohl-Ruling[1] reported that the equilibrium concentration of radon in the human body is about 30% of that in the air and concentrations in fatty tissues may be 700%. They also found relatively high radon concentrations in the bone marrow, adrenal and thyroid glands of guinea pigs. Furthermore, about one third of the inhaled radon decay products was found to pass from the lungs into the bloodstream[2]. Unfortunately, most researchers ignore these facts and maintain that radon gas flows quickly in and out of the lungs never lingering long enough to cause damage while the radon progeny, attached to particulate matter, lodge in the bronchial tree, where their emissions may produce cancer. All present day estimates of lung cancer risks have been extrapolated from underground miners' data. Scully[3] reported in 1933 that inhaled radon was distributed thoughout the body; in particular, the brain, bone marrow, nervous system and lipoids (lipids). He further reported that the blood-making organs, the lymphatic tissues, the ductless glands, the liver, the kidneys and the brain were most strongly affected. Recent studies on cancer incidence as related to radon in potable water indicate that radon released from the water and breathed into the lungs may be responsible for certain cancers including: brain, breast and skin, digestive system, hormonal system, bone, lung (non-smokers), and uterus[4,5]. We performed a study to determine the dependence of body radon and radon progeny contents on bedroom radon concentration. A mean body content of 330 Bq (9 nCi) was found in 102 free-living subjects whose mean bedroom radon concentration was 480 Bq/m $^3$  (13 pCi/L). This mean body content is in agreement with "scaled" quinea pig data from a radon and radon progeny inhalation study [1].

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#### DEVELOPMENT OF INSECT RESISTANT INTERSPECIFIC SOLANUM HYBRIDS

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Because of increasing concern over environmental safety and decreasing effectiveness of pesticides, host plant resistance to insects offers an attractive supplement to pest control programs. In potato, effective means of resistance are mechanical in the form of glandular trichomes (1), or chemical, in the form of glycoalkaloids (2). The goal of this project was to produce hybrids with high levels of insect resistance, preferably due to both mechanical and chemical barriers to feeding. Although the cultivated potato is susceptible to insect pests, several wild relatives express high levels of resistance.

The insect resistant wild diploid species Solanum berthaultii was crossed to haploid S. tuberosum S. chacoense and haploid S. tuberosum - S. tarijense hybrids. Progeny were screened for the presence of two types of glandular trichomes which confer resistance to small insects. Clones with high trichome densities were crossed to S. berthaultii and S. tarijense. Progeny were then screened for resistance to first instar Colorado potato beetle larvae and green peach aphid nymphs, using direct feeding tests on intact leaves. Attempts to cross hybrids to S. chacoense clones with high levels of the glycoalkaloid leptine were unsuccessful.

In all 16 S. tuberosum haploid x wild species hybrid families, clones were identified with high levels of resistance compared to the S. tuberosum check cultivar. Results from one family are listed in Table 1. Clones with resistance to both the Colorado potato beetle and the green peach aphid were identified in all families. Data from a limited number of highly resistant clones planted in the field indicate that tuberization is poor.

Glandular trichomes provide effective resistance against Colorado potato beetle larvae, but not adults. Consequently, future efforts will concentrate on combining both trichomes and leptines into clones. Studies of the relationship between resistance and tuberization have also been initiated in an effort to increase the horticultural quality of insect resistant clones.

Table 1.	Resistance to Colorado potato beetle (CPB) and green peach aphid (GPA)
	in the interspecific hybrid [(S. tuberosum x S. tarijense) x S.
	berthaultiil x S. berthaultii.

	CPB	GPA		CPB	GPA
Clone	Score#	Score##	Clone	Score#	Score##
Cultivar	6.0 a	0.0 a+	17	3.9 bc	0.4 abc
20	4.3 b	0.5 abc	1	3.9 bc	0.7 abcd
14	4.3 b	1.4 cde	9	3.9 bc	1.5 cdef
3	4.3 b	0.8 abcd	6	3.9 bc	0.2 ab
24	4.2 b	0.7 abcd	7	3.6 c	1.0 abcd
19	4.1 b	0.1 a	5	3.1 d	1.2 bcde
22	4.1 b	1.7 def	4	1.2 e	2.5 f
16	4.1 b	0.8 abcd	13	1.0 e	2.5 f
23	4.0 b	0.5 abc	18	1.0 e	2.5 f
12	4.0 b	0.9 abcd	2	1.0 e	2.5 f
10	4.0 bc	0.6 abc	25	1.0 e	2.5 f
21	3.9 bc	2.1 ef	11	1.0 e	2.5 f
15	3.9 bc	1.0 abcd			

instar + ln(weight+1)

<sup>\*\* {(</sup>no. dead GPA + 0.5)/[no. dead GPA + (no. live GPA-5) + 0.5]} + 1; analyzed and presented as a ln(x) transformation.

<sup>+</sup> Mean separation by Duncan's multiple range test, P = 0.05.

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EFFECTS OF BORON. STREPTOZOTOCIN AND THEIR INTERACTION ON ORGAN MINERAL CONCENTRATIONS IN VITAMIN D3-DEFICIENT RATS

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Previous findings indicate that the effects of dietary boron on calcium metabolism in vitamin D<sub>3</sub>-deficient chicks are modified by magnesium status (1). Thus, in magnesium-inadequate chicks boron supplementation elevated plasma calcium and inhibited initiation of cartilage calcification Adequate amounts of dietary magnesium reversed those effects of boron. Also, dietary boron lowered plasma glucose concentrations independent of magnesium status. In view of other findings that indicate pancreatic insulin release is impaired in vitamin D, deficiency (2), the results suggest that boron deprivation perturbs a vitamin D, dependent system. It therefore seemed appropriate to test the effects of dietary boron on mineral metabolism. Thus, a 2x2 factorially arranged experiment was designed to determine the effects of dietary boron on mineral concentrations in body organs most susceptible to damage induced by a short-term diabetic state.

Male, weanling, Sprague-Dawley rats were assigned to groups of 24 and were fed a ground corncasein-corn oil based diet which was vitamin D<sub>1</sub>-deficient and contained 0.034 mg boron/kg. The diet was supplemented with either 0 or 3 mg boron (as orthoboric acid)/kg. After 49 days, 12 rats from each of the two dietary groups were placed in metabolic cages for urine and fecal collections. At 57 days, one-half of the rats in each dietary group, including those in the metabolic cages, were injected with 75 mg streptozotocin/kg body wt. and fed one-half normal mineral supplements to compensate for increased food consumption. At 60 days, the rats were fasted for 16 hours, weighed and decapitated subsequent to pentobarbital anesthesia and cardiac exsanguination with a heparin-coated needle and syringe. The whole brain, kidney, femur, spleen, and liver were removed and lyophilized prior to analysis. The organs were wet-digested and subsequently analyzed for mineral concentrations by inductively coupled argon plasma spectroscopy as described elsewhere (3).

Table 1. Effects of Boron, Streptozotocin, and Their Interaction on Organ Mineral Concentrations

	in Rats.										
Treatment			Brain			Femur		Kidney		Liver	
В	Streptozotocin	Mn	Ca	В	Mn	Ca	В	Mn	Ca	Mn	Ca
			µg/g		mg/	′g	μg/g	μ	g/g		ug/g
0	-	1.82	850	.400	0.33	164	0.50	3.45	300	7.79	140
3	-	1.78	<b>3</b> 70	.440	0.34	165	0.82	3.33	290	6.58	150
0	+	1.84	840	.580	0.42	156	0.79	3.64	250	7.35	110
3	+	1.87	1050	.570	0.35	159	0.72	3.24	250	7.98	120
					Studen	t T-te	<u>est</u>				
St	rep:0 vs 3 B	NS	0.000	6 NS	NS	NS	0.005	NS	NS	0.03	NS
	rep.+:0 vs 3 B	NS	NS	NS	0.04	NS	NS	0.04	NS	NS	NS_

Of the organs analyzed, the highest concentrations of boron on a dry weight basis were found in the spleen (4.09), followed in descending order by liver (1.62), femur (0.708), brain (0.497), and kidney  $(0.095 \, \mu \text{g/g})$ . The food consumption of the streptozotocin-injected rats did not double as expected; therefore comparisons between injected and non-injected animals could not be made. Boron concentrations were increased in the femurs of non-injected animals fed supplemental boron. Boron supplementation had no effect on femur, kidney, or liver calcium concentrations. In the noninjected animals, boron supplementation greatly reduced the concentration of calcium in the brain. Boron supplementation also affected manganese metabolism in some of the organs analyzed. Thus, in the injected rats, boron supplementation decreased concentrations of manganese in the femur and kidney. The findings suggest that the brain is highly sensitive to boron as evidenced by changes in calcium concentrations in response to dietary boron supplementation. The effects of dietary boron on mental function, as well as manganese metabolism, need to be elucidated.

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### PROFESSIONAL COMMUNICATIONS

## AN ASSESSMENT OF WORLD BASIC PLACE VOCABULARY KNOWLEDGE IN AN INTRODUCTORY CULTURAL GEOGRAPHY COURSE

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Assessing the levels of student knowledge of world basic place names has been a philosophical concern of professional geographers since Williams's (1) seminal work in the 1950's through to Helgren's (2) controversial study in the 1980's. In addition, periodic assessment of an undergraduate's knowledge of world basic place names in general education requirement courses in geography is a useful means to determine how much fundamental map work is needed in such classes. Such testing at the University of North Dakota's Department of Geography relies upon using the International Geographical Union's World Basic Place Vocabulary Test. Since 1987, composite student place name levels at that campus can be compared to the 1986 benchmark results reported by Munski and Jensen (3), and map exercises can be adjusted accordingly. During the fall semester of 1989, students in GEOG 151 (Introduction to Cultural Geography) were tested using this instrument, and the overall results apparently reveal that undergraduates continue to have significant "blindspots" in world basic place name knowledge, requiring increased attention to fundamental map exercises in the university classroom.

Based upon previous studies at the University of North Dakota using the International Geographical Union's World Basic Place Vocabulary Test, it was expected that the pre-test of GEOG 151 students should indicate low levels of place name knowledge of locations in Africa, Asia, and South America with a class average of 38.5 points on a scale of 50 whereas the post-test of those students should show an improvement in identifying Third World locations such that the class average should rise to at least 42.5 points (3).

Fifty-five students took the pre-test in the last week of August, 1989. The class average was 39.42, slightly higher than expected. Using a cut-off of 80 percent correct, the pre-test results for fall of 1989 were consistent with respect of anticipated ability to identify African and Asian locations. However, the pre-test results were inconsistent with respect to expected levels of knowledge of South American place names at the standard of 80 percent correct. South American places falling far below the expected levels of being known were: Columbia (72.72% correct), Peru 58.18% correct), Rio de Janeiro (54.54% correct), and Buenos Aires (41.8% correct).

The post-test, taken in the middle of December, 1989, also involved 55 students -- the majority of whom had taken the pre-test. The class average rose to 43.62, again a result slightly higher than the benchmark level of knowledge. Identification of Third World locations improved to the point where no place was known by fewer then 63% of the undergraduates. However, using the 80 percent correct cut-off, African place names remained the least well-known of the Third World Places; for example, four of the eight African locations were below the cut-off mark: Ethiopia (74.54% correct), Algeria (69.09% correct), Nigeria (63.63% correct), and Zaire (63.63% correct), Asian locations being slightly better know. South American place names, other than the tested cities, barely improved above the standard of 80 percent correct.

As a result of this assessment, consideration was given to having the type of map exercises for GEOG 151 for the spring of 1990 developed to include an even stronger emphasis upon Third World places. Such an action has been pre-testing of the GEOG 151 class in the spring semester of 1990: continued low levels of Third World Place name knowledge at the time of entering the course. If American students are going to be successful in interacting with the people of the Third World in trade, diplomacy, and culture, then it is imperative that those undergraduates begin to eliminate their "blindspots" relative to locations in Africa, Asia, and South America.

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#### MAGNESIUM DEPRIVATION EFFECTS ON PLASMA CHOLESTEROL AND ERYTHROCYTES OF HEALTHY POSTMENOPAUSAL WOMEN

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Efforts to produce symptomatic magnesium deficiency in healthy humans simply by restricting dietary intake have been generally unsuccessful. However, a recent study (1) indicated that low dietary magnesium affected the response to dietary boron; this suggested that magnesium deprivation signs could be induced in healthy adults. Thus, two experiments were each performed with 13 postmenopausal women housed in a metabolic unit. In the first experiment, all women participated in four dietary periods of 42 days in which magnesium supplemented at 0 and 200 mg/day, and boron supplemented at 0 and 3 mg/day, were varied in a Latin-Square design. At an intake of 2000 kcal the 3-day menu rotation diet provided 115 mg magnesium and 0.23 mg boron. In experiment 2. all women were immediately placed on a magnesium-low diet (109 mg/2000 kcal). A magnesium supplement of 200 mg/day was started at day 52 for two women, day 64 for two women, and day 78 for nine women The experiment ended on day 156. Some women began supplementation earlier than others because they showed heart rhythm abnormalities suspected to be caused by the low magnesium intake. variables were determined by our usual methods (2). Values given in Tables 1 and 2 were from the last 21 days and from the last 5 weeks of each dietary period in experiments 1 and 2, respectively

Table 1. Effects of Magnesium Deprivation in Postmenopausal Women - Experiment 1

Dietary	reatment		Plasma chole	Erythrocyte			
Mg, mg/day	B, mg/day	Total	LDL	HDL	VLDL	Volume, $\mu^3$	Hemoglobin, %
115	0.23	248	163	59	26	89.6	33.9
115	3.23	248	165	58	27	90.1	33.8
315	0.23	256	171	59	26	89.5	34.0
315	3.23	255	171	58	26	88.9	33.7
		Ana	lysis of Var	iance - P Va	lues		
Boron effect	t	0.60	0.41	0.53	0.37	0.56	0.18
Magnesium e	ffect	0.02	0.09	0.68	0.47	0.08	0.52
B x Mg		0.32	0.46	0.41	0.94	0.27	0.37
ВхMg		0.32	0.46	0.41	0.94	0.27	0.3

Table 2. Effects of Magnesium Deprivation in Postmenopausal Women - Experiment 2

Dietary Mg,	P1	asma choles	Erythrocyte			
mg/day	Total	LDL	HDL	VLDL	Volume, μ <sup>3</sup> H	emoglobin, %
109	236	155	59	28	93.2	33.2
309	258	168	59	30	91.3	34.0
P Value	0.0003	0.05	0.68	0.16	0.0005	0.0001

In both experiments, the magnesium deprivation depressed total plasma cholesterol; most of the change was in the LDL-fraction. In experiment 1, magnesium deprivation tended to elevate mean corpuscular volume. In experiment 2, the elevation in mean corpuscular volume and depression in mean corpuscular hemoglobin concentration caused by magnesium deprivation was highly significant.

The changes in erythrocyte volume and hemoglobin may be a reflection of changes in the erythrocyte membrane. The membrane is more flexible, and apparently more permeable to cations in magnesium-deficient rats than in magnesium-adequate rats (3). The change in plasma cholesterol may be a reflection of a modified lipid exchange between blood vessel walls and blood. The above findings indicate that significant effects other than on urinary magnesium can be obtained through the dietary restriction of magnesium in otherwise healthy adults.

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THE DEVELOPMENT OF A CAMERA MOUNT FOR COLLECTING LOW ALTITUDE MULTISPECTRAL IMAGES

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The use of geographical information systems (GIS) has increased in recent years in response to the need for more efficient methods of land management classification. The evolution of computer software for analyzing and interpreting multispectral data has played a large part in the increased use of this land management tool. The labor intensive job of dot grid area measurement and hand drawn soil classification on aerial photographs has been replaced with efficient and accurate computer assisted raster-vector overlays and vegetation classification using semi-automatic classification algorithms. These developments have made the use of multispectral analysis available to land managers who do not have extensive training in photo interpretation or access to large mainframe computers with expensive software.

A research project was started in 1989 at the Central Grasslands Research Center to evaluate the use of low altitude aerial photographs to determine vegetation utilization and above-ground biomass. In an attempt to keep the data collection as economical and simple as possible, 35mm cameras are being used to collect the images. Color infrared (CIR) and visible color transparency film is used to obtain four spectral bands. In order to collect simultaneous photographs on two types of film two cameras are being used. To accomplish this a camera mount was built that would hold both cameras in alignment and allow for both shutters to be tripped simultaneously. A periscope was constructed out of 4cm PVC pipe so the camera operator could look through the viewfinder of one of the cameras and align on the intended target.

Once developed, the film is scanned into separate 3-band raster files and analyzed using the Map and Image Processing computer software. The use of color infrared and visible color film produces CIR, red, green, and blue spectral bands for analysis.

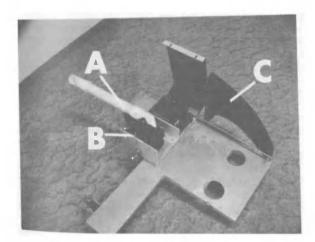


Fig.1. Camera mount with camera pod in film changing position, A) periscope
B) camera pod, C) wind deflector.

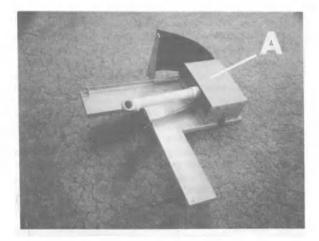


Fig. 2. Camera mount with camera pod (A) in position to take photographs.

#### DIETARY BORON AFFECTS BRAIN FUNCTION IN MATURE LONG-EVANS RATS

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Dietary boron has recently been associated with brain function in healthy adult men and women Electroencephalographic (EEG) measures made under resting conditions showed that, when compared to higher boron intakes (3.25 mg/d), lower boron intakes (0.25 mg/d) resulted in decreased 8-12 Hz activity (dominant alpha) across the brain, and a shift in the proportion of electrical activity in the posterior regions of the brain from higher to lower frequencies. participants in that study were fed low magnesium (115 mg/d) throughout and there were no experimental controls for possible time effects. The animal study reported here was run concurrently in an attempt to further investigate boron effects while controlling for the effects of dietary magnesium and time.

Eighty 100-day old Long-Evans rats (40 male & 40 female) were assigned on the basis of sex and weight to one of four dietary groups created by the factorial combination of boron (0 & 3 ug/g) and magnesium (100 & 400 ug/g). After seven days of equilibration, rats were fed their assigned diets for 60 days, at which time cortical electrodes were implanted over the left and right hemispheres of the brain (2). Following a 2-week recovery period during which all animals continued on their respective diets, a 60-sec electrocorticogram (ECoG) was recorded without anesthesia during the light phase of the light-dark cycle. Rats were sacrificed within 24-hour of the ECoG and femur samples were analyzed to verify the efficacy of the dietary manipulation. Spectral analysis of cortical electrical activity yielded measures of power (amplitude) across the 2-12 Hz frequency spectrum and separately for each frequency in that spectrum for each hemisphere. In addition, a measure of the relative distribution of power among the different frequencies for each hemisphere was determined by calculating the proportion of total power across the frequency spectrum represented in each frequency (3).

Contrasted with higher boron (3  $\mu g/g$ ), lower dietary boron (0  $\mu g/g$ ) was associated with the following significant (p < .05) effects: 1) decreased left hemisphere log-power in the 3, 6, 7 9-12 Hz frequencies; 2) decreased right hemisphere log-power in the 8, 9 and 11 Hz frequencies; 3) increased proportion of total power in the 4 and 5 Hz frequencies in both hemispheres; 4) decreased proportion of total power at 8 Hz in the left hemisphere; 5) decreased total power in the 5-9 and 8-12 Hz bands in both hemispheres; 6) decreased total power across the spectrum in the left hemisphere; and 7) increased proportion of low-to-high frequency activity in the right hemisphere. Dietary magnesium was not significantly related to cortical electrical activity, nor were there any significant interactions between boron and magnesium with respect to cortical activity. There was significantly less boron and less magnesium in the femurs of rats fed low boron and low magnesium, respectively, indicating the appropriate differences in dietary intakes and absorption.

These results indicate that dietary boron systematically influences brain electrical activity assessed by an ECoG in mature rats under conditions which control for the effects of dietary magnesium and time. Additionally, when considered in conjunction with the EEG findings of the human study noted above, these data strongly suggest that the principal effect of dietary boron is on the frequency distribution of the brain's electrical activity. Such an effect may have important implications for the potential role of boron in the maintenance of brain activation in both animals and humans.

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A TECHNIQUE FOR REMOVAL OF INTERFERING CATIONS FOR THE DETERMINATION OF OXYANIONIC SPECIES BY AA AND ICAP SPECTROSCOPY

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One of the challenges in atomic absorption (AA) and inductively coupled argon plasma (ICAP) spect-roscopy is the determination of analytes of interest in the presence of many potential interferences from complex and variable sample matrices. A number of elements, such as As, B, Mo, and Se, generally exist as oxyanions in aqueous solution under conditions found in natural groundwater systems. There are a number of documented interferences for these elements, most of which are common elements that exist as cations in aqueous solution. These include Al, Ca, Fe, and Mg. Thus, a cation exchange resin in the H<sup>+</sup> form should remove the potentially interfering cationic species with no effect on the concentrations of oxyanions of interest.

Research on ion exchange pre-treatment has been performed on samples containing Mo and high concentrations of Mg. A determination of Mo performed by ICAP on samples containing high concentrations of Mg showed an unexpectedly high concentration of Mo. In addition, the apparent analyte peak was slightly offset from the reported Mo wavelength indicating the presence of an interfering spectral signal near the wavelength of interest. The Mo wavelength chosen was 281.6 nm, but the peak observed was offset as illustrated in the example shown in Figure 1. This offset was determined to be due to the presence of a strong Mg line at 280.3 nm and represented the summation of the two emission lines rather than the isolated analyte line. The sample contained over 500 mg/L Mg. Tables of spectral lines listed Al, Cr, Fe, Mn, and Ti as potential interferents in the ICAP determination of Mo at 281.6 nm although these elements were not present at significant concentrations in our sample matrix.

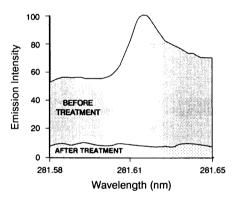


Figure 1. Sample scanned at the Mo 281.6 nm wavelength before and after ion exchange treatment

The sample used to prepare the scan in Figure 1 was subsequently treated with an H<sup>†</sup> ion exchange resin using a commercially available On-Guard H<sup>†</sup> cartridge manufactured by Dionex Corporation. Analysis of the treated sample for Mo and Mg indicated a near quantitative removal of the Mg. The resulting measured Mo concentration was significantly lower than the original analysis had indicated. Spike recoveries were acceptable. These results are shown in Table 1.

Figure 1 also shows a scan of the Mo line after sample treatment by ion exchange. The peak, centered on the analytical wavelength, indicates the absence of spectral overlap and is the true analyte peak.

This sample preparation method has been utilized in the determination of arsenic and selenium that commonly exist as oxyanions in aqueous solution. Although these results have not been formalized, preliminary experiments indicated quantitative recovery of oxyanionic species with near quantitative removal of potentially interfering cations. This inexpensive and convenient method of sample preparation has found numerous uses in our analytical protocols for use in both AA and ICAP determinations.

TABLE 1

Analytical Data for Samples Treated with Ion Exchange Resin

Sample	Mo CONC.	CONC. Spike (ug/mL)	Mo CONC.(ug/mL)	% Spike
Number	(ug/mL)		of Sx + Spike	Recovery
MW-2	0.290	0.500	0.820	106
SWS-5	0.103	0.100	0.204	101

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## DIETARY MARGARIC ACID AFFECTS THE RESPONSE TO NICKEL DEPRIVATION AND THE INTERACTION BETWEEN NICKEL AND VITAMIN B<sub>12</sub> IN THE RAT

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The hypothesis that nickel is an essential element for higher animals continues to gather support. However, conclusive demonstration of a biochemical function for nickel in higher animals has been elusive. Nielsen et al. (1) suggested that nickel has a biological role closely related to vitamin  $B_{12}$  metabolism. A previous report from our laboratory (2) showed that, when vitamin  $B_{12}$  status is low, nickel deprivation depresses the activity of methylmalonyl-CoA mutase (EC 5.4.99.2) (MMM), a vitamin  $B_{12}$ -dependent enzyme. MMM catalyzes the last step in the propionate pathway of branched-chain amino acid and odd-chain fatty acid metabolism. The following experiment was performed to ascertain whether the relationship between nickel and vitamin  $B_{12}$  would be affected by margaric acid, an odd carbon (C-17) fatty acid.

Male weanling Sprague-Dawley rats were assigned to groups of six in a 2x2x2 factorially arranged experiment. Supplemented to the basal diet (about 25 ng Ni/g), based on acid-washed ground corn and skim milk, were nickel (as NiCl<sub>2</sub>) at 0 and 1  $\mu$ g/g, vitamin B<sub>12</sub> at 0 and 50 ng/g and margaric acid at 0 and 0.5%. The rats were fed their respective diets ad libitum for 10 weeks, fasted overnight, weighed and decapitated subsequent to ether anesthesia and cardiac exsanguination with a heparin-coated syringe and needle. The livers were removed and immediately frozen in liquid nitrogen for future analyses. A portion of the liver was lyophilized and prepared for trace element analyses by our usual method (3). Liver MMM activity was determined by the method of Kolhouse and Allen (4).

Table 1. Effects of Nickel, Vitamin B<sub>12</sub>, Margaric Acid and Their Interaction on Body Wt., Liver Trace Elements. and Liver MMM Activity.

Treatment			Body			Liv	er			Methylmalonyl-CoA
Ni	B <sub>12</sub>	Margaric	Wt.	Ca	Cu	K	Mg	Mo	Mn	Mutase
μg/g	ng/g	<u>x</u>	g	μg/g	μg/g	mg/g	μg/g	μg/g I	ug/g	nmoles/min/mg protein
0	0	0	270	112	46	9.9	739	1.49	9.1	0.138
0	0	0.5	243	97	27	9.7	674	1.04	9.0	0.120
0	50	0	269	113	42	10.4	741	1.43	9.8	0.338
0	50	0.5	260	107	61	9.7	701	1.50	8.4	0.342
1	0	0	251	111	49	9.8	699	1.36	8.2	0.164
1	0	0.5	263	111	43	9.9	705	1.48	8.2	0.153
1	50	0	280	109	42	9.7	682	1.28	8.1	0.350
1	50	0.5	288	118	52	10.8	758	1.52	9.4	0.375
				Analysis	of Var	Lance -	P Values			
Nicke:	1		NS	0.08	NS	NS	NS	NS	0.09	NS NS
Vit B	12		0.007	NS	NS	0.03	NS	NS	NS	0.0001
Nix	B <sub>12</sub>		NS	NS	NS	NS	NS	0.08	NS	NS
Marga			NS	NS	NS	NS	NS	NS	NS	NS
Nixl	Margario	2	0.03	0.008	NS	0.001	0.000	2 0.00	4 0.06	NS NS
	Margari		NS	NS	0.05	NS	0.05	.01	NS	NS
Ni x	B <sub>12</sub> x Ma	rgaric	NS	NS	NS	0.02	NS	NS	0.08	NS NS

Margaric acid supplementation increased body weight in nickel-supplemented rats, but decreased body weight in nickel-deprived rats. Margaric acid supplementation decreased liver calcium, potassium, and magnesium in nickel-deprived rats, but increased liver calcium, potassium, and magnesium in nickel-supplemented rats; the effect with potassium was more marked in the vitamin  $\mathtt{B}_{12} ext{-supplemented}$  rats. In both the nickel-deprived and -supplemented rats, vitamin  $\mathtt{B}_{12}$ supplementation increased liver copper in margaric acid-supplemented rats but tended to decrease liver copper in rats fed no supplemental margaric acid. Margaric acid supplementation increased liver molybdenum in nickel-supplemented rats. On the other hand, margaric acid supplementation depressed liver molybdenum in the nickel- and vitamin B12-deprived rats but not in the nickeldeprived, vitamin B12-supplemented rats. Although the dietary manipulations seemed to affect liver manganese in a manner similar to potassium, the effects did not reach significance. Vitamin  $\mathtt{B}_{12}$ deprivation depressed MMM activity. Nickel deprivation tended to depress MMM, especially when vitamin B<sub>12</sub> was low. Margaric acid did not markedly affect MMM activity or its rèsponse to dietary vitamin B<sub>12</sub> and nickel. The findings indicate that margaric acid, a fatty acid whose oxidation is influenced by the vitamin B12-dependent enzyme MMM, affects the response to nickel deprivation. The findings also suggest that animals with an elevated propionic acid metabolism benefit from a diet containing nickel and that a low propionic acid metabolism (vitamin  $B_{12}$  deficiency and no margaric acid) is more detrimental to nickel-supplemented than nickel-deprived animals.

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## THE ROLE OF PHYSICAL GEOGRAPHY IN NORTH DAKOTA'S SEVENTH GRADE SOCIAL STUDIES COURSES

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While studies on geographic education in North Dakota have focused upon the nature of the discipline in junior high schools and high schools during the 1950's through 1970's (1, 2, 3), it was only in the late 1980's that research began emphasizing the role of physical geography as a major component of social studies in grade seven (4). In order to better understand the relationship of physical geography to the teaching of geographic issues at that grade level, research efforts were focused upon conducting a comprehensive, statewide survey of North Dakota's seventh grade teachers in 1989.

This research had six objectives. First, to discover whether or not physical geography concepts were being taught vis-a-vis spatial relationships of geographic phenomena. Second, to determine if teachers were using a problem-solving techniques as part of their teaching of physical geography. Third, to explore whether of not there is a spatial distribution of the content and approach to physical geography instruction. Fourth, to reveal teacher perceptions of the importance of physical geography as a subfield of geography. Fifth, to determine teacher materials. Finally, to assess whether or not sufficient interest existed among teachers for taking inservice education in physical geography as part of the social studies.

Research was undertaken in the spring of 1989 using a mail-back survey consisting of 24 questions. The sample was drawn using a stratification of the state consisting of eight regions (North Dakota's Social Service Unit Areas) and four levels of school sizes. Of the 256 possible respondents, 133 teachers (52% of the population) returned usable questionnaires.

Analysis of the responses of the educators in terms of the six objectives revealed the following results. First, it appeared that spatial distributions of physical geography phenomena are taught in seventh grade social studies but that coverage varies by physical geography topic. Second, only 28.7% of the teachers used problem-solving techniques to present physical geography to their students. Third, there seemed to be only marginal differences in the content and approach to the teaching of physical geography among the eight regions. Fourth, teachers throughout the state held the perception that physical geography was important to the social studies curriculum. Fifth, most teachers perceived that their laboratory materials and computer software were "poor" but that available printed materials were most frequently rated as "adequate." Finally, there was an overwhelmingly positive interest in having inservice education on physical geography, notably in the topics of landforms and climatology.

Follow-up activities to this research have resulted in increased efforts to provide more physical geography inservice to seventh grade teachers. Such activities focus upon the earth system science approach, a version of the science, technology, and society paradigm. The ultimate benefactors of this change in teacher education in geography will be the youth of North Dakota.

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#### EFFECTS OF CHOLESTEROL FEEDING AND COPPER DEFICIENCY ON INFLAMMATION AND THROMBOSIS

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Cardiovascular effects of dietary copper (Cu) deficiency in experimental animals include structural and functional defects of the heart and large blood vessels, anemia and hypercholesterolemia (1).

We have recently reported that small blood vessel phenomena, specifically inflammation and thrombosis, are also affected by Cu deficiency (2). In that study, the <u>in vivo</u> responses of microvessels of the cremaster muscle of Cu-deficient (CuD) and Cu-sufficient (CuS) rats were compared via intravital microscopy. After injection into the circulation of a fluorescent dye conjugated to serum albumin, the dye was photoactivated by blue light (450-490 nm), an event known to induce platelet aggregation and eventual plugging of microvessels of normal rats (see CuS group in Fig.1). Vessels of CuD rats showed no tendency to plug with photoactivation (Fig. 1), an evident impairment of thrombosis. In a second experiment, the influence of compound 48/80 on protein leakage from the microcirculation was monitored by measuring the increase in fluorescence in the extravascular space. Compound 48/80 simulates inflammation by causing mast cell release of histamine and serotonin. CuD rats showed a much greater increase in extravascular fluorescence with 48/80 than did CuS rats (see Fig. 2). This indicates that the potential for inflammation was increased in Cu deficiency.

Because dietary Cu deficiency leads to hypercholesterolemia (1) and, conversely, cholesterol feeding causes a reduction in Cu status (3), it is reasonable to postulate that the cardiovascular effects caused by either treatment will be similar. If the effects were similar, one might then presume a common cause.

The above postulate was tested in rats fed a diet of Chow or Chow supplemented with 1% cholesterol - 0.5% cholic acid for five weeks. Their Cu status was assessed and microvascular responses to photoactivation and compound 48/80 were measured as described above.

Rats fed cholesterol had higher plasma cholesterol (191 $\pm$ 21 vs. 59 $\pm$ 5 mg/dl, p<0.05) and lower liver Cu (7.9 $\pm$ 0.4 vs. 14.8 $\pm$ 0.7  $\mu$ g/g dry wt, p<0.05) than Chow-fed rats. The effects of hypercholesterolemia on photoactivation-induced thrombosis and compound 48/80-induced protein leakage are shown in Figs. 1 and 2, allowing comparison to the Cu deficiency study (2). In contrast to CuD-rats, which showed reduced thrombosis and enhanced inflammatory response (protein leakage), hypercholesterolemic rats showed enhanced thrombosis, (p<0.05, repeated measures ANOVA) and reduced inflammatory response (p<0.03), each relative to their respective controls.

We conclude that, in spite of their apparent interrelationship, copper and cholesterol have separate roles in the regulation of inflammation and thrombosis.

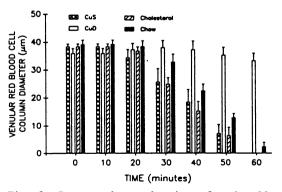


Fig. 1. Progressive reduction of red cell column diameter by photoactivationinduced platelet aggregation.

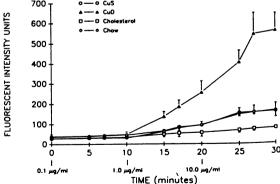


Fig. 2. Increase in extravascular fluorescent intensity (protein leakage) with increasing compound 48/80 concentration.

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#### PROCEDURES FOR A HISTOLOGICAL STUDY OF WESTERN GALL RUST IN PONDEROSA PINE

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Various staining procedures were examined to describe the microscopic characteristics of the fungus causing western gall rust (<u>Peridermium harknessii</u> J.P. Moore) on <u>Pinus ponderosa</u> (Dougl. ex Law. var. <u>scopulorum</u>). The most apparent symptom of this disease is stem gall formation. Stem galls, however, are the end result of a sequence of macroscopic symptoms that generally begin with stem discoloration. Several histological and cytological staining procedures were surveyed 1) to determine their usefulness in describing microscopic aspects of disease development that correspond to the sequential macroscopic symptoms, and 2) to examine the nuclear condition of axenic cultures of <u>P. harknessii</u>.

p. ponderosa seedlings were inoculated with spores of P. harknessii (1). Inoculated epicotyls, collected prior to and after appearance of various symptoms, were fixed by soaking in FAA for 48 hours after an initial 10-minute aspiration. Samples were then dehydrated through a graded six-step series of ethanol/butanol. Host tissues were embedded in paraffin wax and sectioned at a thickness of 10 m. Sections were dewaxed in xylene for five minutes, after which they were dehydrated in a graded ethanol series to various concentrations, dependent upon the type of stain. The following stains (and stain specificities) were utilized: Safranin-Fast Green (general differential), Safranin-Crystal Violet-Orange G (general differential), Crystal Violet-Iodine (nuclear), Acid Fuchsin (protein), FeCl<sub>3</sub> (phenols), Toluidine Blue (polysaccharides/phenols), Silver Nitrate (reducing substances), and Sudan Black B (lipids) (2,3). The differential stains resulted in limited success in differentiating the fungal cells from the pine tissues. Adjusting staining periods, however, has allowed better differentiation of cell type. Staining for tissue-specific compounds in the host tissue has revealed changes in proteins, polysaccharides and phenols in response to infection.

Axenic cultures of P. harknessii representing three colony types were examined to determine the nuclear status of each type. Acetocarmine, Modified Carbol Fuchsin and Feulgen methods had little or no use for determining the number of nuclei per cell under the conditions studied. The fluorescent stains, DAPI and Calcofluor White, provided limited success because of difficulties delimiting cell boundaries. The Giemsa staining method (4) proved best for determining the nuclear status. This may be attributed to lengthened hydrolysis and staining times. Specifically, small fractions of each culture were spread on slides containing Haupt's adhesive and placed into a 30°C oven until dry. Dried smears were fixed for 10 minutes in Singleton's fixative and quickly rehydrated through a three-step graded ethanol series. Hydrolysis was carried out in two stages using 1N HCl: seven minutes at 2°C immediately followed by eight minutes at 60°C. The slides were rinsed in distilled water for five minutes, a phosphate buffer (pH 7.2) for five minutes, stained in Giemsa overnight followed by sequential rinses through distilled water and buffer. Preliminary observations indicate that the nuclear status differs among colony types. The orange mycelial type appears to be dikaryotic and the white mycelial forms are monokaryotic.

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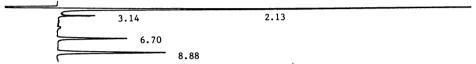
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#### DETECTION OF SULFITE AND SULFATE IN SERUM AND URINE USING ION CHROMATOGRAPHY

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We have developed of an ion chromatography (IC) assay using conductivity detection installed with an PAX-100 analytical column to quantify sulfite (SO3") and sulfate (SO4") simultaneously in The desired resolution was acquired by using a sodium hydroxide (20 mm)/5x methanol eluant and an anion membrane suppressor using 25 mN sulfuric acid. Serum and urine samples were collected and transported to a glove bag under a helium atmosphere. The samples were then deproteinized and centrifuged; the supernant layer was removed and added to a 10% formaldehyde solution (pH 9.3) to prevent oxidation by atmospheric oxygen (1). Formaldehyde forms a complex with the SO," (HCHO-SO), which is resolved in relation to SO," as shown in Figure 1. Standards were made from stock solutions of 1000 ppm SO3" and SO3" containing the formaldehyde stabilizer solution. Recoveries of SO<sub>3</sub> and SO<sub>4</sub> in urine were 90-102% and 98-105%; in serum their recoveries were 95-108% and 93-99%, respectively.



Chromatogram showing resolution of 1 ppm standards for SO<sub>3</sub> (6.70 min.) and SO<sub>4</sub> (8.88 min.) in formaldehyde stabilizer solution.

The described assay was utilized in a pilot study designed to test our ability to assess the activity of sulfite oxidase. This enzyme is responsible for conversion of SO, to SO, the last step in the metabolism of sulfur amino acids. Our objective was to alter sulfur status in rats by feeding them SO<sub>3</sub>, cystine (a sulfur amino acid) and/or tungstate (a sulfite oxidase inhibitor (2)), and then determining whether  $SO_3$  or  $SO_4$  in urine or serum were indicators of those alterations.

Thirty six male Sprague-Dawley rats weighing 130 to 150 grams were divided into 6 groups of six rats each. Group 1 was fed a purified basal diet (control group), group 2 a high SO3 (0.10 mg/g as  $Na_2SO_3$ ) diet, group 3 a high cystine (0.05 M) diet, group 4 a high tungstate (0.7 mg/g as Na, WO, diet, group 5 a high SO, and tungstate diet, and group 6 a high tungstate and high cystine diet. After being fed their respective diets for fourteen days animals were anesthetized and urine and serum samples were taken.

No samples from any of the groups had detectable SO3, although standard curves (and spiking) showed resolution down to 0.5 ug/ml. Tables 1 and 2 illustrate the dietary effects on urine and serum SO4" content. Two x two factorial analysis of variance (ANOVA) indicated no effect of feeding SO<sub>3</sub> or tungstate on either urine or serum SO<sub>4</sub> concentration (Table 1). Cystine feeding caused a significant increase in both urine and serum SO4" (Table 2); tungstate significantly inhibited the increase in urine SO4" and tended to inhibit the increase in serum SO4". We conclude that cystine feeding, but not SO<sub>3</sub> feeding, caused an observable increase in SO<sub>4</sub> production and that this production was inhibited by tungstate.

These experiments provided an opportunity for the development and an IC assay for SO3" and SO4" to biological fluids; the findings suggested that we could assess the activity of sulfite oxidase by the assay.

TABLE 1. Effect of feeding SO," and Tungstate on Urine and Serum content of SO.".

lungstate	on orrne and	serum concent of so,
Diet	Urine SO <sub>4</sub>	Serum SO <sub>4</sub> -
	(mg/ml)	(ug/ml)
Control	2.5±1.6	78±15
SO <sub>3</sub>	3.1±1.3	86±11
WO4"	2.4±3.3	79±18
SO <sub>3</sub> x WO <sub>4</sub> "	3.4±1.7	65±18
Source of	Variation Al	NOVA, p values
SO <sub>3</sub>	0.4	0.7
WO4"	0.9	0.2
SO <sub>3</sub> x WO <sub>4</sub>	0.9	0.2

Tungstate on urine and serum content of SO. Urine SO4" Serum SO4-Diet (ug/m1)(mg/ml) 78±15 Control 2.5±1.6 Cystine 103±13 7.5±2.6 79±18 WO. 2.4±3.3 \ Cystine + WO4 85±19 3.4±1.0 Source of Variation ANOVA p values

0.005

0.04

0.05

0.03

0.3

0.2

Table 2. Effect of feeding cystine and

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The technical assistance of Jackie Keith is gratefully acknowledged.

Cystine WO4"

Cystine x WO4"

### GENERATING FUNCTIONS FOR HUNTER-GUERRIER POLYNOMIALS

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The following problem arises in the study of critical phenomena in statistical mechanics. Suppose p(t) is a function for which N terms of the convergent Taylor series

$$p(t) = \sum_{n=0}^{\infty} p_n t^n \tag{1}$$

are known exactly. Suppose further that p(t) is known to be of the form

$$p(t) = (1 - t/s)^{-v} r(t) + a(t)$$
 (2)

with s real and r(t) and a(t) analytic for  $|t| < \rho$  with  $\rho > |s|$ . The real number v is either non integral so that s is a branch point, or a positive integer so that s is a pole. Physically v may be a critical exponent controlling the singular behavior of some thermal property. The problem is to determine s and v as accurately as possible using the N known coefficients  $\{p_n\}$ .

There are two kinds of methods for solving the current problem.[1] The first kind comprises the Padé-type methods which, though often effective, do not in general rest on rigorous theorems. The other kind is based on Darboux's theorem.[2,3].

Hunter and Guerrier (HG) have constructed a method of the Darboux's-theorem type [4]. Since r(t) has a Taylor expansion

$$r(t) = \sum_{k=0}^{\infty} b_k (t-s)^k \tag{3}$$

convergent for  $|t-s| < \rho - |s|$ . Darboux's theorem gives

$$p_n \sim \sum_{k=0}^{\infty} \frac{(-1)^k b_k s^{k-n} \Gamma(n+v-k)}{n! \Gamma(v-k)}.$$
 (4)

By eliminating the dominant terms in 1/n between different  $p_n$  as expressed by Eq.(4), HG have shown[4] that s and v can be found approximately as simultaneous roots of the polynomials  $X_n^m(s,v)$  and  $X_{n-1}^m(s,v)$  defined by  $X_n^0(s,v)=p_n$  and the recursion

$$X_n^{m+1}(s,v) = sX_n^m(s,v) - (n+v-2m-1)X_{n-1}^m(s,v). \quad (5)$$

- 1. Pearce, C. (1978) Advances in Phys., 27, 89.
- 2. Darboux, G. (1878) J. de Math. 4, 5 and 377.
- 3. Henrici, P. (1977) Applied and Computational Complex Analysis. Vol.II, Wiley, NY.
- 4. Hunter, C. and Guerrier, B. (1980) SIAM J. Appl. Math. 39, 248.
- 5. McBride, E. (1971) Obtaining Generating Functions. Springer, NY.
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For maximum precision, one chooses n = N - 1 and considers a sequence of m values. For m = 1, the equations are linear with unique roots. Thus, at m = 2, in which other (extraneous) roots are introduced, the m = 1 values are used to make the appropriate choice, etc.

We have computed a formal generating function

$$g(s, v, u, y) = \sum_{m,n} \frac{1}{m!} X_n^m(s, v) u^n y^m,$$
 (6)

assuming  $X_n^m(s, v)$  is zero if n or m < 0 or m > n. Since p(t) is known only by finite approximation Eq.(1), the expression we obtain for g(s, v, u, y) is meaningful primarily as formal series manipulation.

Directly from Eqs.(5) and (6) one can see[5] that

$$g(s, v, u, y) = \sum_{m} \frac{1}{m!} B^{m} p(y) = e^{B} p(y)$$
 (7)

where the operator B is

$$B = \frac{1}{\xi^2 \eta} (s - v\xi \eta) - \frac{\partial}{\partial \xi}$$
 (8)

in the variables  $\xi = (uy)^{-1}$  and  $\eta = u^2y$ . Using the integrating factor  $\phi = -s(\xi\eta)^{-1} + v \ln \xi$  such that  $e^B = e^{\phi}e^{-\frac{\partial}{\partial \xi}}e^{-\phi}$  one has

$$g(s, v, u, y) = e^{\phi} e^{-\frac{\phi}{\delta \ell}} (e^{-\phi} p(y(\xi, \eta)))$$
 (9)

which, in view of Taylor's theorem, gives

$$g(s, v, u, y) = (1 - uy)^{v} exp(\frac{sy}{1 - uy}) p(u(1 - uy)). \quad (10)$$

The formal result Eq.(10) gives a method for extracting the HG polynomials mechanically using the algebraic processing languages such as REDUCE, Macsyma, or Mathematica. We have studied percolation transitions using Mathematica by this means. One can often perform analytical operations more easily using the generating functions than by using the HG polynomials themselves.[6]

NESTING SUCCESS OF UPLAND WATERFOWL IN GRAZING SYSTEMS IN SOUTHCENTRAL NORTH DAKOTA

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Range scientists began studying specialized grazing systems at the Central Grasslands Research Center (CGRC) near Streeter, North Dakota in 1981. Grazing treatments researched include the short duration (SD) and seasonlong (SL) since 1982, two replications of the twice-over rotation (TOR) since 1983, the complementary (COMP) since 1985, and two replications of the switchback (SWB) since 1986. The ongoing range studies provided an opportunity to compare the nesting success of waterfowl in relation to these grazing treatments. An idle mixed grass-prairie, ungrazed since 1979, was also compared to the grazing treatments. The idle area was mowed in late-July 1988, eliminating all idle area in 1989. Funding was provided by the N.D. Game and Fish Dept., and NDSU-Agricultural Experiment Station.

The SL has been grazed by one cattle herd at the recommended stocking rate of 1.8 AUM/ha (1) since 1984. Livestock were free to graze any area within the 130 ha SL pasture. The SD consisted of eight 16.2 ha pastures each grazed by one herd at a stocking rate 2.7 AUM/ha since 1984. Each pasture of the SD was grazed for five days during each of four rotations per grazing season, with 35 days rest between rotations. Each replication of the TOR consisted of four 32.4 ha pastures, each grazed by one herd at a stocking rate of 2.7 AUM/ha since 1986. Each pasture of the TOR was grazed for 20 days and then rested for 60 days per each of two rotations. Each replication of the SWB consisted of two 16.2 ha pastures each grazed by one herd at a stocking rate of 2.6 AUM/ha since 1987. Each pasture of the SWB was grazed for 20 day periods and then rested for 20 days per each of four rotations. The COMP consisted of three domestic or tame grass pastures and one native grass pasture. Livestock began grazing the COMP in a 12.1 ha crested wheatgrass (Agropyron desertorum) pasture and were then rotated sequentially to a 32.4 ha native pasture, a 12.1 ha Russian wildrye grass (Elymus junceus) pasture, and a 12.1 ha altai wildrye grass (Elymus angustus) pasture at an average stocking rate of 2.3 AUM/ha since 1985.

The grazing season was 160 days long on the SL, SD, TOR, and SWB with the mean starting date 28 May. The grazing season on the COMP was approximately 190 days long with the mean starting date 24 April. The drought of 1988 shortened the grazing season. Cattle were removed from the grazing treatments by 1 September and stocking rates were decreased 33% on the SL; 40% on the SD, TOR, and SWB; and 50% on the COMP.

Waterfowl nests were found by dragging an 8-mm diameter, 30.5 m long chain between two 200 cc all terrain cycles as illustrated by (2). Four nest searches were conducted between 1 May and 15 July at three week intervals in 1983, 1984, 1987, 1988, and 1989. Three nest searches were conducted at four week intervals in 1985 and 1986. Each site from which a duck flushed was examined and considered as a nest if at least one egg was present. Study area, species identification, number of eggs, stage of embryo development, cover diversity, and visual obstruction reading were recorded. Nests were revisited every 7-10 days and those nests in which at least one hatched were classified as successful (Table 1). In conclusion, cattle grazing has continually had a positive impact on enhancing waterfowl nesting habitat when properly managed. All grazing treatments, excluding the COMP, provided safer nesting cover in all seven years of the study when compared to idle areas (Table 1). We recommended the specialized grazing systems because mean waterfowl and livestock production rates exceed those of SL.

Table 1. Number of nests found for all years combined, average number of nests per 40.5 ha (100 ac), average number of ducklings produced per 40.5 ha, average Mayfield nesting success, and average gain (AG) of calves per ha.

				•	
	Number of	Avg. number of	Avg. number of	Avg. Mayfield	AG
Treatment	nests	nests/40.5 ha	ducklings/40.5 ha	success	Kg/ha
Twice-over rotation	549	13.2	63.3	34.7	55
Seasonlong	216	9.6	48.1	26.6	39
Short duration	294	13.0	58.5	25.6	54
Switchback	92	19.0	64.6	22.7	54
Complementary	48	5.6	10.1	8.8	53
Idle	289	18.7	47.3	11.3	

<sup>1.</sup> U.S. Soil Conservation Service. (1984). Notice ND-35. Bismarck, ND 58501

<sup>2.</sup> Higgins et al. (1977). J. Wildl. Manage. 41:232-242

#### COPPER DEPRIVATION AFFECTS THE PLASMA AMINO ACID PROFILE IN RATS

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Experiments in our laboratory have shown that sulfur amino acid nutriture can markedly affect the nature and severity of the signs of copper deficiency (1). Furthermore, copper deficiency affects plasma urea and glucose (1) two components of the glucose-alanine cycle. Thus, because there apparently is a relationship between copper nutriture and some amino acids, we decided to ascertain whether copper deprivation affects the amino acid profile in plasma of rats.

The plasma used in this study came from female Sprague-Dawley rats from experiments described elsewhere (1). The rats used were those fed supplements of 0 and 6  $\mu$ g Cu/g diet and amino acid supplements (in g/kg diet) of methionine (MET) 2.0, and cystine (CYS), 6.3 plus methionine, 6.3. The basal diet contained 0.2  $\mu$ g Cu/g. Females were chosen because effects on plasma amino acids caused by muscle breakdown as the result of decreased food consumption was expected to be small; unlike males, the females exhibited only small changes in body weight as the result of the copper deprivation. The two amino acid supplements were chosen to represent normal and a high intake of the sulfur amino acids (SAA). The rats were fed their respective diets for six weeks, then fasted overnight, weighed and decapitated subsequent to ether anesthesia and cardiac exsanguation with a heparin-coated needle and syringe. Plasma was obtained and frozen for later analysis. Plasma urea was determined by a urease method. The amino acids were determined on an ion chromatograph. The plasma proteins were precipitated with 20% sulfosalicylic acid. The supernatant were put through a 0.2 micron filter. The eluants used were 100 mM nitric acid, distilled deionized water 20 mM nitric acid and 25 mM potassium oxalate. The amino acids were detected using a fluorescence detector with o-phthalaldehyde as a fluor. The column was regenerated with 100 mM hydrochloric acid. Selected amino acids are presented in the following table.

Table 1. Effects of Copper on the Concentrations of Selected Amino Acids in Plasma of Rats.

Troa	tment	Plasma Urea	Plasma Amino Acid, nmol/ml						
Cu, µg/g		mg/100 ml	Alanine	Arginine	Cystine	Serine	Threonine	Valine	
0	2.0 MET	20.6	1169	181	105	1393	774	381	
0	6.3 MET	20.3	1031	175	112	1184	651	324	
	+								
	6.3 CYS								
6	2.0 MET	12.0	542	263	102	632	509	252	
6	6.3 MET	13.9	578	213	108	727	537	249	
	+								
	6.3 CYS								
			<b>Analysis</b>	of Variance	- P value	<u>s</u>			
Copper ef	fect	0.0001	0.0001	NS	NS	0.002	0.03	0.05	
SAA effec	:t	NS	NS	NS	NS	NS	NS	NS	
<u>Cu x SAA</u>		NS	NS	NS	NSNS	NS	NS	NS	

Contrary to expectations, plasma concentrations of amino acids (arginine and cystine) which were found to affect the signs of copper deficiency (1) were not affected by the dietary manipulations. On the other hand, four of the gluconeogenic amino acids, alanine, serine, threonine and valine were elevated in plasma by copper deficiency. The elevations were especially marked for alanine and serine. In the liver, the rate of glucose synthesis from alanine and serine is far higher than that observed from all other amino acids. Moreover, the liver constantly takes up from plasma large quantities of alanine which apparently is the vehicle for nitrogen transport in plasma. The major pathway of nitrogen excretion is as urea synthesized in the liver, released into the blood, and cleared by the kidney. Perhaps the elevated plasma glucose and urea found in the copper-deficient rats were the result of elevated alanine and serine in plasma and an impaired kidney function (2). The increased alanine and serine in plasma may be the result of copper deficiency causing oxidative damage and tissue breakdown (3), thus increasing the amount of nitrogen to be excreted.

<sup>1.</sup> Nielsen, F.H. (1988) <u>J. Trace Ele. Exp. Med.</u> 1, 153-166.

<sup>2.</sup> Moore, R.J., et al. (1989) <u>J. Lab. Clin. Med</u>. 113, 516-528.

<sup>3.</sup> Saari, J.T. (1989) <u>Proc. Soc. Exp. Biol. Med.</u> 190, 121-124.

#### BODY COMPOSITION AND SWIMMING PERFORMANCE: A TWO-YEAR STUDY

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In 1984, Stager, Cordain and Becker (1) studied 284 female competitive swimmers (aged 12 to 17 years) and reported significant (p<.001) correlations between time in a 100-yard, free style swim and 1) body height (r = -.25) and 2) lean body mass (r = -.26). They concluded that women who were tall and had a high lean body mass had lower times in the 100-yard swim and that body fat was relatively unimportant. Others have reported that competitive swimmers respond to swim training with decreases in body fat (2,3) and increases in fat-free weight (3). The purpose of this study was to determine body composition correlates of swimming performance in competitive collegiate female and male swimmers.

Nine women and six men from the University of North Dakota varsity swim teams were studied over two consecutive competitive seasons. The subjects underwent determinations of body composition by hydrodensitometry with simultaneous determination of lung volume (4) the week prior to the first swimming workout at the beginning of each season and the week prior to the conference meet at the end of each season. The swimming performance measure was the 100-yard time in each subject's primary competitive stroke. Times were recorded at the first meet of each season and at the conference meet at the end of each season.

Subject characteristics at the beginning of the study are presented in Table 1. Partial correlations were calculated (for performance versus each physical dimension to account for the

TABLE 1. Descriptive Statistics of Swimmers at the

<u>Beginning</u>	of Comp	<u>etitive</u>	Seasons
	Women	(n = 9)	Men $(n = 6)$
	Mean	SD	<u>Mean</u> SD
Age (years)	18.8	0.8	20.0 1.8
,Height (cm)	167.2	9.0	179.4 4.3
₩eight (kg)	61.6	6.8	75.3 4.1
Body Fat (%)	22.2	3.8	12.0 2.5
Weight (kg)	13.8	3.6	9.0 2.2
Fat-Free Weight (kg)	47.7	3.8	66.3 2.9
Time (sec)	64.9	6.6	58.7 6.4

effects due to individual differences) for each sex separately to generate correlates of body composition with performance. Partial correlations between swim time and body composition dimensions are presented in Table 2. For both women and men, swim time was negatively (but not significantly) correlated with fatfree weight. For women, height and swim time were positively correlated, whereas for men the correlation was negative and significant. For women, the correlations between percent body fat and fat weight

and swim time were positively related and statistically significant; the same correlations were also positive for men but not statistically significant.

This study did not find the same negative correlation between body height and swim time in

females as did Stager, Cordain and Becker (1). We did find the same large negative correlation between fat-free weight and swim time. We also found positive correlations (significant in females) between fat and swim-time. The results of this study indicate that swimming performance within individuals is probably not unlike other forms of athletic performance: enhanced performance is related to body

TABLE 2. Correlations	Between Boo	dy Composition
<u>Dimensions a</u>	and Swimming	Performance
	Women	<u>Men</u>
Age (years)	043	220
Height (cm)	.102	532*
Weight (kg)	.150	. 251
Body Fat (%)	.359*	. 290
Fat Weight (kg)	. 364*	. 296
Fat-Free Weight (kg)	275	218
* p<.05		

fat-free weight and impaired performance is related to body fat.

- 1. Stager, J.M., et al. (1984) <u>J. Swimming Res.</u> 1, 21-26.
- 2. Wade, C.E. (1976) Res. Quart. 47, 292-295.
- 3. Siders, W.A., et al. (1989) Res. Quart. Submitted.
- 4. Lukaski, H.C. (1987) Am. J. Clin. Nutr. 46, 537-556.

### THE EFFECTS OF DIETARY PROTEIN INTAKE ON BONE COMPOSITION IN THE GROWING RAT

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Growing rats fed diets containing 30% egg white deposited significantly more zinc into bone than rats fed diets with 15% egg white despite adequate dietary zinc concentrations (1). Although these amounts of egg white were chosen to meet or exceed requirements for protein quality and quantity, growth curves of the rats indicated that the 15% protein diet may have initially limited growth, an effect which disappeared within 5-6 weeks. Thus, a follow-up study was conducted to determine whether differences in bone zinc concentrations reflected early inadequate protein nutriture, or effects of protein within a range of adequate dietary protein concentrations.

Twenty-four weanling, male Long-Evans rats were fed, ad libitum, purified diets containing 15, 25, 35, or 45% egg white protein for 25 days. Dietary minerals were adjusted to similar concentrations for all protein concentrations, dietary fat remained constant at 5%, and carbohydrate with a constant ratio of corn starch to sucrose varied inversely with the protein concentrations. After dry ashing and dilution in HCl, tibia, duodenum, kidney, and gastrocnemius muscle were analyzed for Ca, Cu, Fe, Mg, Mn, P and Zn by inductively-coupled argon plasma emission spectroscopy (ICAP). Tibia nitrogen was analyzed using a micro-Kjeldahl procedure. Differences between diets were assessed by analysis of variance with Tukey contrasts.

The rats grew similarly during the first 2 weeks regardless of dietary protein concentration. Growth rates of rats fed 35 or 45% protein were reduced during the last 10 days, a difference which was statistically significant only at 25 days (data not shown). The 15% protein diet did not limit growth at any time during the study.

Dietary protein concentrations did not affect muscle or duodenal mineral concentrations. Kidney concentrations of Fe and Mn were significantly greater when rats were fed 35 or 45% protein than when fed the lower dietary protein concentrations (data not shown). There were no differences in tibia weight, length, width, or ash weight (Table 1). However, concentrations and total amounts of nitrogen, Cu, Fe, Mg, and Zn in tibia were significantly affected by dietary protein (Table 1). Slight reductions in tibia Ca and P concentrations with the diet containing 25% protein probably occurred by chance, as concentrations of Ca and P were greater at both higher and lower dietary protein concentrations.

Table 1: Effects of dietary protein concentration on body weight and tibia composition.

	15% Protein	25% Protein	35% Protein	45% Protein
Final body weight, g	224 ± 11*	221 ± 16	205 <b>±</b> 10	209 ± 9
Tibia weight, mg fresh	269 ± 12	252 <b>±</b> 19	258 ± 15	251 ± 18
Tibia ash, mg	153 ± 6	$142 \pm 12$	141 ± 7	141 ± 10
Tibia N, mg/g dry	50.8 <b>±</b> 2.7 <sup>a</sup>	50.8 ± 2.4 <sup>a</sup>	46.6 ± 3.1 <sup>ab</sup>	45.7 ± 4.1 <sup>b</sup>
Tibia Ca, mg/g dry	225 ± 2 <sup>a</sup>	214 ± 9 <sup>b</sup>	225 ± 9 <sup>a</sup>	222 ± 3 <sup>ab</sup>
Tibia Cu, µg/g dry	4.6 ± 0.4 <sup>a</sup>	4.9 ± 0.6 <sup>a</sup>	$3.3 \pm 0.4^{b}$	3.7 ± 0.5 <sup>b</sup>
Tibia Fe, μg/g dry	67 ± 6 <sup>a</sup>	68 ± 7 <sup>a</sup>	80 ± 5 <sup>b</sup>	89 ± 4 <sup>b</sup>
Tibia Mg, mg/g dry	3.1 ± 0.2 <sup>a</sup>	3.3 ± 0.1 <sup>ab</sup>	3.5 ± 0.2 <sup>b</sup>	3.5 ± 0.2 <sup>b</sup>
Tibia P, mg/g dry	101 ± 1 <sup>ab</sup>	96 ± 4 <sup>a</sup>	103 ± 4 <sup>b</sup>	101 ± 1 <sup>ab</sup>
Tibia Zn, µg/g dry	$243 \pm 14^{a}$	262 ± 26 <sup>a</sup>	301 ± 20 <sup>b</sup>	332 ± 19 <sup>b</sup>

Within a row, means with different letters are significantly different, p <0.05.  $^{\star}$ Mean  $\pm$  SD (n=6).

The slightly reduced growth and greater kidney Fe and Mn concentrations in rats fed 35 or 45% Protein indicate that those diets exceeded desirable protein concentrations for growth and development. However, the stepwise changes in tibia Zn and nitrogen concentrations with increasing dietary protein confirm the results of the previous study (1), and indicate that changes in dietary protein above amounts required for growth affect deposition of nitrogen and zinc into bone. In addition, the wider range of dietary protein concentrations used in this study revealed differences in bone composition of Cu, Fe and Mg. It is not known whether these differences in bone composition may be associated with any functional advantage. If they are, these findings could be of practical importance to human infant nutrition, as infant formulas vary widely in protein concentration.

Hunt, J. R. and Johnson, L.K. (1989) Proc. N. D. Acad. Sci. 43, 54.
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### EFFECTS OF DIETARY METHIONINE ON SIGNS OF ARSENIC DEPRIVATION IN RATS

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Previous studies using 16% casein, 75% ground-corn based diets have shown an interaction between arsenic and methionine in rats (1). In those studies the lowest achievable amount of dietary methionine and/or cystine was dependent upon the basal level of casein and corn. Thus. in a 16% casein, 75% corn based diet, the lowest achievable amount of methionine was about 0.58% and of cystine, about 0.18%. A portion of the requirement for methionine can be met by dietary cysteine/cystine, thus the diets could not be utilized for studies of methionine deficiency because the methionine and cystine content exceeds the requirement of rats for total sulfur amino acids (0.60%). Therefore, in order to study the effects of an altered methionine metabolism on arsenic deficiency we have had to feed guanidoacetic acid (2,3) or high dietary arginine (4). compounds utilize a methyl group in their metabolism, in effect lowering the amount of methionine available for other metabolic processes. Therefore, the purpose of this study was to ascertain the effects of methionine deprivation on arsenic deficiency utilizing an amino acid, ground-corn based diet. In a 2x2 factorially arranged experiment, male weanling Sprague-Dawley rats were assigned to groups of six and fed a 14% amino acid, 77% ground-corn based diet. The basal diet contained 0.24% methionine, 0.44% cystine and less than 10 ng arsenic per gram. supplements were arsenic as  $As_2O_3$ , 0 or 1  $\mu$ g/g and L-methionine, 0 or 4 g/kg. The rats were fed their respective diets for 6 weeks, fasted for 16 hours, weighed and decapitated subsequent to ether anesthesia and cardiac exsanguination with a heparin-coated needle and syringe. methods and environmental conditions have been described (5).

Table 1. Effects of Dietary Arsenic, Methionine and Their Interaction on Body Wt., Hemoglobin, Mean Corpuscular Hemoglobin, Bone Mg and Zn, and Blood As in the Rat.

Tre	eatment	Body			Bon	е	Blood
As	Methionine	Wt.	Hemoglobin	MCH	Mg	Zn	As
µg/g	g/kg	g	g/100 ml	pg	mg/g	μg/g	$\mu_{\rm g/ml}$
0	1	104	14.8	19.0	2.62	165	0.21
1	1	142	15.3	20.1	2.81	170	21.0
0	4	221	14.5	20.3	3.02	185	0.11
1	4	211	15.3	20.4	2.81	170	22.3
			Analysis of Var	iance - P V	<u>alues</u>		
As		NS	0.02	0.005	NS	NS	0.0001
Methion	ine	0.0001	NS	0.0006	0.008	0.04	NS
As x Me	thionine	0.03	NS	0.035	0.006	0.05	NS
Error M	Mean Square 624 0.40 0.21 0.026 135		135	1.41			

Arsenic deprivation decreased body wt., mean corpuscular hemoglobin (MCH), and bone magnesium and zinc in rats fed 1 g methionine/kg but either tended to increase or have no effect on those variables in rats fed 4 g methionine/kg; as a result, the interactions between arsenic and methionine were significant (Table 1). Regardless of dietary methionine, hemoglobin and blood arsenic were decreased by arsenic deprivation. Bone, heart and plasma iron tended to be increased by arsenic deprivation in rats fed 1 g methionine/kg (data not shown).

These results indicate that there is a relationship between methionine and arsenic. Other signs of arsenic deprivation include depressed activities of S-adenosylmethionine decarboxylase (6), ornithine decarboxylase (6) and cystathionase (unpublished observation) in rat liver. Also, rat liver polyamine and rat and hamster plasma taurine concentrations were decreased by arsenicdeprivation (6). The findings from the present and previous experiments support the hypothesis that arsenic has a role that influences methionine metabolism. Because many of the changes observed in the present and previous experiments can be attributed to alterations in vitamin  $B_6$ metabolism, the possibility that arsenic is necessary for proper vitamin B6 utilization, either directly or indirectly, is being investigated. The present study also indicates that using an amino acid based diet is a viable option in studying the effects on arsenic deprivation of altering dietary sulfur amino acids.

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# COMPREHENSIVE LOCAL WATER PLANNING: A MINNESOTA APPROACH TO LOCAL WATER RESOURCE MANAGEMENT

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Comprehensive Local Water Planning (CLWP) began in Minnesota in 1985 when a state-wide citizens planning committee was formed to make recommendations to the legislature about local water management. The legislature, in the spring of 1936 after a year-long effort, passed Comprehensive Local Water planning into law as Chapter 110B. The legislation attempted to forge a partnership between local units of government and state agencies to address water and resource issues. The Legislative Commission on Minnesota Resources (LCMR) made water its number one priority, and created the opportunity for counties to submit proposals to LCMR for money to initiate CLWP. A total of 52 counties in Minnesota ware funded for comprehensive water planning in 1986 and 1987, and the majority of the remaining counties initiated the process in 1989.

The purpose of a Comprehensive Local Water Management Plan is:

- 1. To identify existing and potential problems or opportunities for protection management and development of water and related land resources in a county.
- 2. To develop and implement a plan of action to promote sound hydrologic management of water and related land resources in the county, and
- 3. To work toward effective environmental protection and management in a county.

The essential constituent during development of a plan is the establishment of a continuing process whereby local government officials and the general public can understand the amount, characteristics, and distribution of their water resources. Based on the understanding of existing conditions, county officials can then decide what water resources are necessary and desired for future growth and development, and on a course of action to achieve and maintain the quality of life desired in the county. Counties recognize that whether growing, remaining stable, or declining in population, they must develop their own local plan for managing water resources. Counties also recognize that a well developed comprehensive water plan can integrate local initiatives and existing state or federal water-related programs and funding sources for more efficient management of all these programs for protection of water resources and the general environment.

Comprehensive Local Water Plans focus on water and related land resources. The counties assemble and study available information relating to the physical environment, surface and groundwater resources, and related land use. Surface water and groundwater quality and quantity and related land uses that affect water resources are analyzed, and relationships between these resources and their current potential usage are defined. Problems are analyzed within the context of watershed units and groundwater systems where appropriate, but the plan applies to the entire area within the county. The plan is based on principles of sound hydrologic management and recognizes interrelationships between surface and groundwater as well as the potential cumulative effects of land use on both water quality and quantity. The plan is consistent with other plans that exist at the county, watershed, Soil and Water Conservation District, or groundwater system level.

Lajor water management initiatives addressed in the plan include: education; development of a more complete data base; coordination of activities with state agencies to enforce existing regulations, to develop new programs and ordinances and acquire easements in sensitive or target areas; and funding for capital expenditures.

#### THERMAL IONIZATION MASS SPECTROMETRY OF BORON

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Indications that boron may be an essential trace element suggest the importance of developing human metabolism studies using stable boron isotopes (1). There are three reports of boron isotope ratio studies of biological samples: rye grass (2), olive leaves, orchard leaves, skimmed milk (3), and apples (4). There are no boron isotope absorption studies involving humans or animals. These studies require rapid and reproducible isotope measurements; knowledge about the natural variability of boron isotope ratios in commercial foods; and techniques for the isotopic enrichment of plant products. Establishment of a protocol for boron isotope studies, as a step towards metabolism studies, includes the development and modification of techniques for measuring boron isotope ratios.

Single tantalum filaments were degassed followed by air oxidation for a minimum of one week. Samples were loaded onto oxidized filaments by a 5 uL pipet with acid washed tips and dried with a 700 mA current through the filament. After samples were loaded, hydrated water was removed by heating each sample to 2000 mA for 10 sec and 1700 mA for 30 sec. A standard sample consisted of boric acid (0.06 M), CsOH (0.03 M), and La(NO<sub>3</sub>)<sub>3</sub> (0.003 M). Both La(NO<sub>3</sub>)<sub>3</sub>.6H<sub>2</sub>O (99.999%) and CsCl (99.9999+%) were placed through a boron specific ion exchange column before use and CsCl was converted to CsOH by ion exchange techniques (5).

Isotope ratio measurements were obtained with a magnetic sector thermal ionization mass spectrometer dedicated to isotope ratio measurements. The mass spectrometer had a single Faraday cup detector used in a jumping mode. Filaments were rapidly heated to 1400 mA and held for 8 minutes, to 1450 mA at 100 mA/min for 4.5 minutes, and finally to 1500 mA at 100 mA/min for 4.5 minutes. Generally a 500 mV Cs $^{+}$  signal was obtained and used for spectrometer optimization. To obtain a  $Cs_2BO_2^{+}$  signal, the filament current was raised to 1650 mA at 100 mA/min in three steps of five minutes. The  $Cs_2^{11}BO_2^{+}$  signal is typically 200 mA.

Three studies have been used for development and comparison to this work. An NIST special publication has described the boron isotope ratio determination in NIST SRM-951 (natural abundance standard) (7). The NIST study measured the  $Na_2^{11}BO_2^+/Na_2^{10}BO_2^+$  ratio in SRM-951. However,  $Cs_2BO_2^+$  is the ion presently used to determine boron isotope ratios (5,6).

Measured  $^{11}B/^{10}B$  (Cs<sub>2</sub> $^{11}BO_2^+/Cs_2^{-10}BO_2^+$ ) ratios were normalized to NIST SRM-951 by R(obs)\*K, where  $K = 0.9991_4 \pm 0.0002_4$  and R(obs) =  $4.0471_1 \pm 0.0008_5$  (95% Confidence limit, Table 1, Reference 7). While an RSD(°/°) of 0.53 is within usable bounds (Table 1), improvements in instrument calibration should improve the standard deviation.

Table 1. Boron isotope ratios and 95%

confidence limit on ratios.							
$R(^{11}B/^{10}B)$	95% CL	RSD(°/。)	Source				
4.04362	0.00054	0.049	7				
4.04558	0.00033	0.012	5				
4.04488	0.00090*	0.026	6				
4,04711	0,00085	0.053	This Work				
*Calculated							

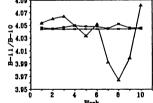


Figure 1. Boron ratios without La³\* (▲) & with La³\* (■). -\*- for SRM-951.

The described isotope ratio procedure is a modification of several other reported techniques. Metal nitrates have been shown to enhance both signal intensity and stability of  $Na_2BO_2^+$  and  $BO_2^-$  ion beams (8). Signal intensities are increased an order of magnitude for  $Na_2BO_2^+$  and three orders of magnitude for  $BO_2^-$  ion beams. Addition of 0.003 M La<sup>3+</sup> had a comparable effect for  $Cs_2BO_2^+$  ion beams with an order of magnitude increase in signal strength, 20 to 200 mV. This increased signal strength and resulted in a steadier signal (Figure 1). In other reports filament oxidation was three days (5) or not discussed (6). However, both oxidation conditions resulted in erratic signals and variable filament currents required to obtained a 200 mV ion beam. A one-week oxidation resulted in more stable signals and reproducible filament currents. While high purity CsCl and  $La(NO_3)_3$ .6H<sub>2</sub>O were used, passing the compounds through a boron specific ion exchange column lowered the observed isotope ratio approximately 0.005.

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#### A PERSONAL COMPUTER SOFTWARE FOR HYDROLOGIC FLOOD ROUTING

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A commonly used technique for flood routing is the Muskingum method. The continuity of flow through the channel reach for this method can be given in linear form as Equation [1] or in nonlinear form as Equation [2] or [3]

$$S_{t} = K[xI_{t} + (1-x)O_{t}]$$
 [1]

$$S_{t} = K[xI_{t}^{n} + (1-x)O_{t}^{n}]$$
 [2]

$$S_t = K[xI_t + (1-x)O_t]^n$$
 [3]

in which  $S_{t}$ ,  $I_{t}$ , and  $O_{t}$  are the simultaneous storage, inflow and outflow during the passage of a flood through the reach, and K and x are the parameters to be determined from past flood hydrographs through the reach (1). K, the storage coefficient, can be physically interpreted as the travel time of a flood wave through the reach while x is a weighting factor of inflow compared to outflow or is an external factor characteristic of the reach in question in relation to the catchment. These two parameters are expected to capture the flood propagating characteristics of the reach in its entirety. The reliability of Muskingum routing technique depends on the accuracy of estimates of paratmeters x and K. Traditionally, determination of these parameters has been based on the linearity of a loop formed by graphical plotting of accumulated storage versus storage of a given reach calculated using Equation [1]. This trial and error graphical procedure, which has been used for decades, is time-consuming, subjective, and may not minimize the error of estimation. Furthermore, the visual judgment may not correctly identify the best among several sets of x and K when all may appear acceptable. In addition, the linear Muskingum model may be inappropriate for representing some reaches. It is possible that the storage versus weighted flow relationship is not always linear as implied in the original Muskingum equation. If the relationship is nonlinear, applying the linear equation may not be justified, and may introduce considerable error. If the nonlinear models given in Equation [2] or [3] are used, n is also a parameter to be estimated from actual hydrographs.

To obviate these difficulties, a menu-driven microcomputer program package was developed. The package is capable of checking linearity of data sets, so that linear or nonlinear model can be used appropriately. The package has twelve application programs: one to check linearity and others to estimate parameters using different methods. Each program as listed below except CHKLNR can also calculate routed outflows. Hydrograph plotting options are available in the program.

CHKLNR preliminary linearity check of data sets TRERR graphical trial and error method LSOREG iterative Least-squares Regression OTHREG iterative Orthogonal Regression LEAST direct Least-squares Optimization FBOPT Graphical Optimization **FLTDAT** Outliers filtering method Linear Programming method 0P Quadratic Programming method CONSEG Conjointed Segmental regression DISSEG Disjointed Segmental regression NONLR nonlinear Optimization method

An example problem from (2) is used to demonstrate the application of the package. Parameter estimates obtained by using the different methods are presented in Table 1. For want of clarity, only the outflow hydrograph routed by using OTHREG parameter estimation on the linear model and the outflow hydrograph routed by using NONLR are shown in Fig. 1 along with the actual outflow hydrograph.

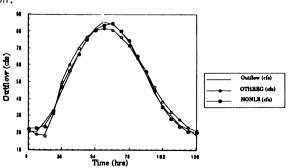


Figure 1. Outflow Hydrographs

Table 1. Parameter Estimates

		К
TRERR	0.200	28 99
LEAST	0.234	17 29
LSQREG	0.248	28.979
OTHREG	0.248	28.977
FBOPT	0.229	29.03
FLTDAT	0.124	27.02
LP	0.499	22.78
QP .	0.009	1.58
CONSEG	0.248	18.00 & 43.30
DISSEG	0.248	18.77 & 42.71
NONLR (Eq. [2])	0.274	0.066 (n=2.36)
NONLR (Eq. [3])	0.215	0.054 (n=3.36)

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#### EFFECT OF SILICON DEFICIENCY ON THE MINERAL COMPOSITION OF BONE

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Most of the signs of silicon deficiency found in experimental animals indicate an aberrant metabolism of connective tissue and bone (1). Chicks and rats fed silicon-deficient diets exhibit skull abnormalities associated with a depressed collagen content. Chicks exhibit long-bone abnormalities characterized by small, poorly formed joints and defective endochondral bone growth. Tibias of silicon-deficient chicks contain depressed contents of articular cartilage, water, hexosamine and collagen. Although silicon deficiency depresses the ash content of bone (1), to our knowledge no marked effect of silicon deficiency on bone mineral composition has been found. The objective of the experiment described here was to ascertain whether such an effect occurs.

Male weanling Sprague-Dawley rats were assigned to groups of six in a two-way  $2x^2$  factorially arranged experiment. The treatments were supplements ( $\mu$ g/g diet) of silicon, 0 and 50, and molybdenum, 0 and 10. The basal diet was based on acid-washed ground corn and casein (2) and was formulated to contain one-half (2.5 g/kg) the reported rat requirement for calcium. The analyzed silicon content was 4.4  $\mu$ g/g. Molybdenum was made a variable because it was found to depress the incorporation of silicon in tissue (1). Calcium was fed at an inadequate amount because the effect of silicon on bone ash was found to be most marked when dietary calcium was low (1). Environmental conditions for the rats have been described (2). The rats were fed their respective diets for six weeks, weighed, fasted overnight, anesthetized, and decapitated. Tibias and skulls were frozen for later analyses. They were cleaned with cheesecloth and dried in a vacuum oven at 85°C for 24 hours. The dried samples were weighed in platinum crucibles and ashed at 490°C in a muffle furnace. The ash was handled as described elsewhere for mineral analyses using a Perkin-Elmer ICP/5000 System\* (2).

Table 1. Effects of Silicon, Molybdenum and Their Interaction on Tibia and Skull Mineral

Tre	atment,	Body		Tibia			Skull			
	<u>g diet</u>	wt., g	Ca,	Мg,	Ρ,	Si,	Ca,	Mg,	Ρ,	Si,
<u>Si</u>	Mo		mg/g	mg/g	mg/g	μg/g	mg/g	mg/g	mg/g	μg/g_
0	0	252	168	3.47	86	18	139	2.34	62	29
0	10	260	183	3.60	91	26	140	2.35	62	27
50	0	264	186	3.74	95	35	157	2.74	71	34
50	10	273	187	3.60	94	17	157	2.48	69	15
		<u>An</u>	alysis of Va	riance	- P Valu	es				
Si	effect	NS	0.0001	0.02	0.0001	0.10	0.009	0.02	0.005	0.09
Мо	effect	NS	0.001	NS	0.04	0.03	NS	NS	NS	0.0001
Si	x Mo	NS	0.009	0.02	0.02	0.001	NS	NS	NS	0.001

Silicon deprivation only tended to depress the growth of the rats. This lack of a marked effect on growth is similar to findings by others (1). Thus, the changes in bone mineral concentrations probably were not influenced by differences in growth or food consumption. Silicon deprivation depressed the tibia concentrations of calcium, magnesium and phosphorus; however, the depression was most marked when the diet was not supplemented with molybdenum. Molybdenum supplementation depressed the silicon concentration in tibias of rats fed supplemental silicon, but apparently elevated the concentration in tibias of rats fed the silicon-deficient diet. Silicon deprivation also depressed the calcium, magnesium and phosphorus concentrations in skulls. Unlike the finding with tibias, dietary molybdenum apparently did not affect the response of these three elements in skulls to silicon deprivation. However, the changes in skull silicon concentration caused by the dietary manipulations were similar to those in the tibia. The findings indicate that, in rats fed a low-calcium diet, silicon enhances the incorporation into bone of elements that are major components of the mineral fraction. The findings also indicate that high dietary molybdenum does not enhance the effect of silicon deprivation, and support the concept that silicon is involved in bone calcification.

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<sup>\*</sup>Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the Product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

### HYDROGEN ABSTRACTION FROM 9-BORABICYCLO[3.3.1]NONANE BY BIS[TRICARBONYL(METHYLCYCLOPENTADIENYL)TUNGSTEN] USING VISIBLE AND ULTRAVIOLET IRRADIATION

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Photochemical reactions of  $Cp_2W_2(CO)_6$  ( $Cp = C_5H_5$ ) have been extensively investigated (1) using both visible and ultraviolet irradiation. Two pathways can be photochemically induced, the homolytic cleavage of the W-W bond leading to a 17-electron radical species, and the loss of a carbonyl ligand. Most trapping reactions have involved species such as disulfides, halocarbons or phosphines (2). Our objective is to understand the reaction of a tungsten-centered radical with compounds containing hydrogen bonded to an electron deficient atom such as boron. The results expected would be hydrogen abstraction by the tungsten radical to form a tungsten hydride and/or formation of complexes containing boron-tungsten bonds. Analogous products are formed in the photoreaction of  $Cp_2W_2(CO)_6$  with anyl thiols (RSH) (3).

The reaction of the tungsten dimer  ${\rm Cp'}_2{\rm W}_2({\rm CO})_6$  ( ${\rm Cp'}={\rm C}_5{\rm H}_4{\rm CH}_3$ ) with 9-borabicyclo[3.3.1]nonane (9-BBN) [structures shown below] is not an efficient process, but is more efficient under ultraviolet (366 nm) than visible irradiation. The use of the methylcyclopentadienyl ligand in place of the usual unsubstituted cyclopentadienyl increases the solubility of the complex (as high as 0.6 mM in hexane), allowing higher concentrations of tungsten dimer in solution, giving better light absorption. The reaction is run in either hexane or benzene and requires an excess of 9-BBN to insure complete reaction of the tungsten dimer. During the reaction there is a noticeable color change from red to murky orange. The reaction is conducted under inert atmosphere conditions (argon or nitrogen atmosphere) due to the reactivity of both 9-BBN and the products of the reaction.

 $Cp'_2W_2(CO)_6$ 

The progress of the photoreaction is followed by infrared spectroscopy in the carbonyl stretching region (1700-2200 cm<sup>-1</sup>) where the original dimer peaks (1948,1901,1889 cm<sup>-1</sup> in benzene) decrease as two new peaks (2017,1924 cm<sup>-1</sup> in benzene) grow as the reaction proceeds. The products have also been characterized by nuclear magnetic resonance (nmr) spectroscopy. Proton nmr shows unreacted tungsten dimer and the tungsten hydride Cp'W(CO)<sub>3</sub>H which gives a <sup>1</sup>H proton shift of -6.98 ppm with a W-H coupling of 36.6 Hz [compare to CpW(CO)<sub>3</sub>H:  $\delta$  -7.21 ppm,  $J_{W-H}$  36.6 Hz (3)]. After completion of the reaction, only one <sup>11</sup>B nmr resonance is seen (57.12 ppm); no starting material (9-BBN) is seen. The boron-containing product is very similar to methoxy-9-BBN (4) giving possible evidence of carbonyl insertion after carbonyl loss from the tungsten dimer. Carbonyl insertion usually occurs in boranes under high pressure situations. The present reaction could involve possible metal-initiated carbonyl insertion.

The tungsten hydride was an expected product due to previous work where hydrogen abstraction has occurred (3). There is no evidence for W-B bonding, unlike other abstraction reactions of photoproduced radicals which often give products which contain tungsten-heteroatom bonds (e.g. Cp(CO)<sub>3</sub>W-SR (5)). Another unusual feature of the current reaction is the excess consumption of borane. Note that no 9-BBN is observed among the final products, even when the B/W ratio is 2:1 and not all of the tungsten dimer is consumed.

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# INTERACTION OF SODIUM, SULFUR, AND SILICA DURING THE COMBUSTION OF LOW RANK COALS

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Western coals, primarily lignite and subbituminous are used to generate electricity by producing steam in large scale pulverized coal-fired boilers. A 450 MegaWatt boiler produces approximately 22.3 tons of ash per hour along with the electricity causing fouling and slagging of the boilers. This corrosion greatly reduces the thermal efficiency of the boiler and requires significant capital investment to remove.

One of the major components of the corrosive ash is sodium, most commonly found as sodium sulfates and sodium silicates. The size distributions of the formed fly ash particles are of great importance due to their ability to impinge upon sticky surfaces and aid in defining the combustion process taking place (i.e. coalescence vs. fragmentation).

To study the interaction of sodium, sulfur and silica (quartz), a synthetic coal, made from a furfuryl alcohol polymer, with the appropriate amount of minerals was created. The coal was burned in a laminar flow reactor at four different temperatures. The resultant ashes were studied with Scanning Electron Microscope (SEM) using an electron dispersive detector with an ultra thin window. The ash was sized with the aid of a Tracor Northern IA8500 image analysis system. The resultant sizes of the ashes are shown in Table 1.

Table 1 Size Data for Ashes

Temp	Size
(°C)	(um)
( 0)	(um)
900	27.0
1100	0 7 7
1100	27.7
1300	17.6
1500	3.5
1300	3.5

The size of the quartz particles bound in the coal were approximately 6 um equivalent spherical diameter. If all of the quartz in each coal particle were to coalesce upon combustion to form one particle, the size of that particle would be approximately 28 um in size.

The above results indicates the formation of the fly ash at temperatures below 1100°C is governed by coalescence thus producing one fly ash particle for every coal particle resulting in a particle size average of 28 um for this study. For temperatures above 1100°C, fragmentation is the method of combustion resulting in multiple fly ash particles for each coal particle with the actual size distribution depending on the temperature.

Lower temperatures produce larger particles which will be more prone to impact and stick on the surface of the heat transfer tubes inside a boiler. The smaller particles will tend to follow gas streams thus forming fewer deposits.

#### ZINC TRANSPLACENTAL TRANSFER IN THE RAT

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The development of the placenta results in the formation of a complex membrane barrier between the maternal compartment and the developing fetus. In most cases this means prevention of the simple diffusion of nutrients, including the important growth factor, zinc. A zinc deficiency during pregnancy has been shown to generate anything from a modest decrease in birthweight to fetal fatality. The purpose of this study was to investigate protein substituents that regulate the flux of zinc from maternal to fetal compartments. Metallothionein, a small molecular weight metalloprotein, has been shown to have a relatively high affinity for the mineral zinc, and it is found in substantial concentration in various tissues including the placenta. Consequently, the role of metallothionein (MT) in adjusting the concentration of zinc in maternal, placental, and fetal tissues on days 19 (D-19) and 21 (D-21) of gestation was examined in Long-Evans rats.

Maternal liver and kidney, placenta, and fetal liver tissues were analyzed for MT based on a competitive binding assay (1). The reaction mixture containing MT was labeled with radioactive cadmium (Cd-109), incubated, and the excess Cd-109 removed by centrifugation. The MT·Cd-109 pellet was counted for radioactive emissions at 228.8 nm in a gamma counter. Maternal liver, kidney, tibia, placenta, fetal liver, and one whole fetus from each dam were dry ashed in a muffle furnace at a final temperature of 450°C and diluted with 0.12N HCl. The absorption at 213.9 nm was measured on a flame atomic absorption spectrophotometer to determine total zinc concentration. Finally, placentas were homogenized, centrifuged at 200,000 x g, and an aliquot of the cytosolic fraction was placed on a gel chromatographic column. The G-75 column was calibrated with the following molecular weight standards: aprotinin (6,500 KD), cytochrome-c (12,400 KD), carbonic anhydrase (29,000 KD), and albumin (66,000 KD). The column was eluted with tris-acetate buffer, and the eluent passed through a U.V. monitor measuring absorption at 280.0 nm. The absorption served as a measure of the separation of proteins. The eluted fractions were analyzed for zinc on a flame atomic absorption spectrophotometer to establish which molecular weight proteins were associated with zinc.

 $\label{thm:metallothionein} \mbox{Metallothionein concentration values for those tissues so analyzed are shown in the table below along with the corresponding changes in zinc concentration.}$ 

Tissue	Variable	D-19*	D-21*	P-value**
Maternal	Zn(ug/g)	95.5±2.2	87.7±1.4	P<0.05
Liver	MT(ug/g)	24.0±1.9	22.7±1.0	NS
Maternal	Zn(ug/g)	93.1±1.5	85.6±0.9	P<0.05
Kidney	MT(ug/g)	61.9±2.4	31.1±2.5	P<0.05
Placenta	Zn(ug/g)	71.9±0.9	65.0±0.5	P<0.05
	MT(ug/g)	16.2±0.7	11.2±0.6	P<0.05
Fetal	Zn(ug/g)	242.8±15.1	290.3±6.1	P<0.05
Liver	MT(ug/g)	397.8±32.1	592.9±36.0	P<0.05

MT and Zn Concentration Changes in Late Gestation

As shown, results suggest that as zinc concentration decreases in the maternal and placental tissues, there is a corresponding decrease in MT concentration. Conversely, the increase in fetal tissue zinc concentration coincides with an increase in fetal tissue MT concentration. In addition, the percent of total cytosolic zinc associated with a 10 kdalton protein in the placenta also decreased significantly (D-19,  $13.8\pm2.4\%$ ; D-21,  $11.0\pm1.7\%$ ).

Thus, it appears that fetal liver plays a major role in regulating zinc transfer across the placenta in late gestation. In addition, placental MT may act as a reservoir supplying zinc to the fetus during this time of rapid fetal growth.

<sup>\*(</sup>Value±SEM)

<sup>\*\*(</sup>Statistical significance determined by Student's t-test)

#### GENETICS OF LEAF RUST RESISTANCE IN BARLEY

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Nine genes have been associated with the various reactions to leaf rust pathogen (<u>Puccinia hordei</u> Otth.) in barley (<u>Hordeum vulgare</u> L.) (1). These genes are designated as <u>Rphl</u> to <u>Rph9</u> (<u>pal to Pa9</u>). Some of the <u>Rph</u> genes have a broad spectrum of resistance to the races of <u>P. hordei</u>, and  $\frac{Rph2}{Rph2}$ ,  $\frac{Rph3}{Rph}$  and  $\frac{Rph7}{Rph}$  have been used in breeding programs in recent years. This study was undertaken to map  $\frac{Rph}{Rph}$  genes on barley chromosomes using morphological traits as genetic markers, to verify the linkage relationships and allelism among  $\frac{Rph}{Rph}$  genes, and to investigate the dominance of and interaction between  $\frac{Rph}{Rph}$  genes. Previous reports suggested that the  $\frac{Rph2}{Rph3}$ ,  $\frac{Rph3}{Rph3}$  and  $\frac{Rph7}{Rph3}$  genes may be located on chromosome 3 which carries a major gene for barley yellow dwarf resistance.

No linkage was found between Rph3 from 'Estate' and genetic markers on chromosomes 1, 2, 3, 5, 6, and 7. No linkage was found between the Rph7 from 'Cebada Capa' and genetic markers located on chromosomes 1 through 7. Based on previous trisomic analysis (2,3) and the negative linkage data, Rph3 may be located on the short arm of chromosome 1 and Rph7 may be near the end of the long arm of chromosome 3. The only morphological marker located near the end of chromosome 3 is cu2 which was not tested because of poor plant vigor. If Rph3 is located near the Rpg1 (T) locus on chromosome 1s, this gene may be difficult to incorporate into North Dakota barley cultivars because the Rpg1 gene is needed to adequately control wheat stem rust, incited by P. graminis f. sp. tritici.

Single dominant resistant genes were detected in 'Quinn' and 'Triumph' using the ND8702 culture of race 8 P. hordei. Segregation ratios in F2 progenies fit an expected F2 ratio of 3 resistant to 1 susceptible plants at 15 C and 25 C. The data plus prior allelism tests indicated that the Triumph gene is not an allele to Rph1 through Rph9. The gene from Triumph was found to be linked with the R and S loci on chromosome 7 with recombination values of  $26.1 \pm 2.3\%$  and  $39.5 \pm 2.9\%$ , respectively. No linkage was found between the Rph2 from Quinn and genetic markers on chromosome 3.

Progeny tests indicated that <u>Rph3</u> and <u>Rph7</u> are not linked, as had been suggested previously (1). Tests for allelism between <u>Rph3</u> from 'Roland' and the resistant gene from Triumph were negative and progeny screening showed that these two resistant genes segregated independently.

The heterozygous condition of each <u>Rph</u> gene studied produced infection type(s) different from the infection type(s) produced by the homozygous condition. Therefore, incomplete dominance was a common trait of these four <u>Rph</u> genes and heterozygotes can be identified in segregating progenies. No epistatic interaction was found between <u>Rph</u> genes because of the high level of resistance displayed by each gene.

Of the genes conferring resistance to races of  $\underline{P}$ .  $\underline{hordei}$  common in North America, only the gene from Triumph was located using morphological markers. The chromosome locations of  $\underline{Rph3}$  and  $\underline{Rph7}$  can be assigned tentatively to 3L and 1S, respectively, based on negative linkage data  $\underline{And}$  F<sub>2</sub> progenies from allelism tests. The  $\underline{Rph2}$  probably is not located on chromosome 3.

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#### MICROPROCESSOR BASED REMOTE WELL DEPTH RECORDING SYSTEM

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Research professors at South Dakota State University are interested in investigating the recharge of local public wells from the spring thaw. They would like to monitor about 10 wells in differing locations and conditions recording water depth and time of measurement. Since these wells are located some distance apart each site requires its own data acquisition system. The cost of commercially available systems to perform these functions ranges in price from about five hundred to several thousand dollars depending upon their features. A more economical solution needed to be found. To meet these needs a microprocessor based data acquisition system was designed and built for less than two hundred dollars per unit for parts.

The system is based on the Motorola 68HCll microcontroller. A pressure transducer is placed in the well following calibration. This sensor will give an analog voltage proportional to the well water depth. This voltage passes through some conditioning circuitry and then into the HCll where the onboard 8-bit Analog-to-Digital converter converts it into a binary number between 0 and 255. This value is stored in random access memory (RAM) for later retrieval.

The unit is small, battery operated and interfaces with any personal computer with an RS-232C serial interface. The user needs only one portable PC unit and visits each well site to retrieve the stored data as an ASCII file on floppy disk for later examination. The user chooses one option from the main menu which includes:

- 1) Begin Logging Data
- 2) Read Out Logged Data
- 3) Clear Memory
- 4) Set the Clock
- 5) Configure System
- 6) Calibrate

The system can be configured to read and record as many as 8 different signals even though for this application only a few channels are needed. The interval between readings is user selected from seconds up to one day. The user can also specify what time information (Month, Day, Year, Hours, Minutes and Seconds) he wishes to have recorded. Each piece of information takes up space in the RAM and only pertinent data should be recorded. The system can be loaded with either 8K or 16K of RAM which can store 8192 or 16,384 pieces of information. The 8K capacity would allow for a retrieval rate in excess of 2 months if the interval between readings is one hour and all available information is stored.

Many new technologies are being implemented in this data acquisition system. The Motorola MC68HCll microcontroller has many notable features. It has a built-in 8 bit Analog-to-Digital converter which eliminates the need for a conventional separate chip to perform the A/D conversion. It also features 512 bytes of Electrically Erasable Programmable Read Only Memory (EEPROM), which can hold data even when power is lost. The design utilizes this feature to permanently store the system configuration. Because this sytem is to be battery operated power consumption must be kept to a minimum. The HCll is constructed of High-density Complementary Metal-Oxide Semiconductor (HCMOS) which are known for their low power consumption; furthermore, the microcontroller has wait and stop states which further reduce power consumption between readings.

The design includes the use of a 68HC68Tl real time clock. This 16 pin chip provides the needed time keeping function as well as the alarm interrupt function. The real time clock communicates to the HCll through a four wire serial communication interface. It also is connected to the Interrupt Request (IRQ) line on the microprocessor. If, for instance, the user has selected fifteen minutes as the interval between readings the microprocessor will read the present time, add fifteen minutes to it and store it back in the alarm register of the real time clock. The system will then go into a low power consumption mode and wait for the IRQ line to be driven to a logic low state by the real time clock when the interval has expired. The system will then wake up, record the requested time information, take the requested channel readings and then reset the real time clock alarm for the next reading.

This system is not limited in application to just the measurement of well depth, rather it can be used to measure up to 8 different signals within the voltage range of the system. For instance, it could be used to measure weather conditions such as temperature, barometric pressure, wind speed and direction, etc. The system is low cost, convenient, flexible and benefits from the effective application of new technologies.

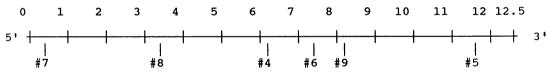
#### DETECTION OF HETEROGENOUS BVD VIRUSES USING SYNTHETIC OLIGONUCLEOTIDES

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Rovine viral diarrhea virus (BVDV) is a ubiquitous pathogen of cattle worldwide. Although RVDV is associated with numerous disease manifestations, the most devastating economic losses are due to reproductive failures and persistently infected animals. The persistently infected cattle provide for easy spread and maintenance of BVDV within a herd due to the difficulties in detecting infected animals. Many of the problems caused by BVDV could be prevented by identification and elimination of persistently infected animals. The current methods of detection are time consuming, moderately successful at best, and not always practical for analysis of large numbers of animals. For these reasons we have begun to investigate the potential use of synthetic oligonucleotides as diagnostic probes for the BVDV genomic RNA. (1-3)

The oligomers were selected by computer analysis of the two known sequences of BVDV-RNA, Osloss and NADL; the BVDV genome is a single stranded RNA 12.5 Kb in length. Six regions of extensive homology were chosen and 20-mers of the genome complement were synthesized. BVDV isolates were obtained from the NDSU Veterinary Diagnostic Laboratory. These viruses were propagated in BVDV-free bovine turbinate (BT) cell cultures including the laboratory strains Singer, NADL, and NY-1 as positive controls. RNA was extracted from lysates of BVDV-infected cells, minifected cells; negative virus controls used were bovine herpesvirus type-I and feline calicivirus. The rapid extraction was done in SDS and Proteinase K at 37 C for 30 min. followed by phenol:chloroform extraction. The sample was then treated with formaldehyde and denatured at 65 C for 10 min. prior to dot blot analysis on nylon membrane. Plasma samples from known BVDV persistently infected animals were treated in the same fashion. Hybridization was performed as previsouly described at 50 C with 32P end-labeled oligomers, subsequently washed and autoradiographed. (4)

Figure 1. Relative Location of NDSU Synthetic Oligonucleotides



The results of hybridization demonstrated a marked genome heterogeneity in field isolates of BVDV. The oligomers provided a variety of hybridization patterns on the dot blots (Table 1). Oligonucleotide #7 detected 10 of 12 NDSU isolates and in comparison Oligo #8 detected none of the field isolates. A cocktail-mixture of all of the oligomers did not detect any more cases than oligo #7. Stringent washing was necessary to remove background and to provide a clear picture of the hybrization. Plasma from persistently infected animals were also positive using a cocktail of oligomers.

Table 1. Number of BVDV-Isolates Detected by Selected Oligonucleotides

NDSU S	ynthetio	oligor Oligor	nucleot	ides -	20-mers
4	5	6	7	8	9
9/21	11/21	12/21	18/21	4/23	11/21

Potential benefits to be derived from the use of nucleic acid probes, and more specifically oligonucleotide probes as diagnostic tools, are a high degree of specificity, less technical time and expertise when compared with current techniques. Oligomers are more stable and have rapid hybridization times compared to longer nucleic acid probes. Disadvantages at this time include less sensitivity; the oligomers we tested did not detect all BVDV isolates. It should be noted that we have analyzed only 1% of the genome at this time.

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# TRIHALOMETHANE PRECURSOR REDUCTION AT THE GRAND FORKS WATER TREATMENT PLANT

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In 1974, trihalomethanes (chloroform, bromodichloromethane, dibromochloromethane and bromoform) were discovered to be formed during the disinfection step of drinking water treatment if free chlorine was the disinfectant (1). The City of Grand Forks has encountered difficulties in meeting the maximum contaminant level (MCL) of 0.1 mg/l when using chlorine as the disinfectant. The goal of this research was to identify cost effective operational modifications that would lead to the reduction of trihalomethane (THM) precursors at the Grand Forks Water Treatment Plant. THM precursors are removed by the coagulation process at the head end of the treatment plant.Improved coagulation practices were investigated in this research using jar test procedures to enhance THM precursor removal.

The concentration of THM precursors was found to vary seasonally at the Water Treatment Plant. Precursor concentrations were found to be lower in snow melts and winter months when compared to that of the summer months. Studies were conducted on both rivers according to the seasonal changes, taking into consideration the facilities available and the capabilities of the Water Treatment Plant.

Experimental design techniques were used to reduce the experimental testing necessary to gather the needed data. The Box-Behmen experimental method for five factors was used in setting up the sequences for the jar testing. The five test variables were pH, alum, potassium permanganate (KMnO4), polyaluminum chloride (A12Cl6), and Nalco 8793 (polymer). The monitored parameters of the experiments were turbidity, total organic carbon (TOC), ultraviolet-visible range spectrophotometry (UV- VIS), and bromoform formation potential (BFP).

The Statistical Analysis System program (SAS) was used to develop polynomial regression expression for the experimental data. The program was conclusive in determining the significant variables for the reduction of turbidity, TOC, UV-VIS, and BFP. Alum dosage and pH were found to be the most effective for the reduction of turbidity, while alum gave the best result for the reduction of TOC and UV-VIS. The Al2Cl6 was effective for the reduction of BFP.

Results of the research indicate that, due to the variation of 40 to 50 percent in TOC content on the Red lake and Red river, the Grand Forks Water Treatment Plant should use the Red river as its main source of water supply if THM precursor removal is the sole decision criteria. Problems associated with using the Red river as a water source is its consistently higher hardness which increase chemical costs and sludge quantities.

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#### PREFORMED ALUMINUM HYDROXIDE AS AN ALTERNATIVE TO ALUM

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Use of aluminum salt coagulants in potable water treatment may lead to the increased levels of aluminum in finished water compared to original raw water. The objective of this research was to conduct a comparison study of the coagulants, alum and pre-formed aluminum hydroxide, for soluble aluminum concentration in treated water.

Jar tests were performed using 1000 mL beakers at room temperature. The samples were stirred rapidly for 1 minute at 80 rpm following addition of coagulant and acid or base for pH adjustment. After rapid mixing, 15 minutes of slow mixing at 20 rpm was provided. The pH values reported are those measured at the end of slow mixing. The samples were filtered through 0.2 micron membrane filters. Five milliliters of the filtrate was withdrawn for the determination of the soluble aluminum residual using nV/VIS spectrophotometer at a wavelength of 535 nm.

The data covers a pH range from 5.5 to 8.0 and a dosages range from 30 mg/l to 70 mg/l for alum and pre-formed aluminum hydroxide coagulants. The results presented in rigures 1 and 2. The test was conducted using various dosage of coagulants, which was considered to be representative of the dosages used in water treatment processes for different raw water conditions. The results show minimum dissolved aluminum contents at a pH of 6 to 7 for both alum and pre-formed aluminum hydroxide. The dissolved aluminum concentration is lower for model water samples using preformed aluminum hydroxide coagulant as compared to the ones using alum coagulant. The pH is the major parameter affecting the concentration of dissolved aluminum in water. soluble aluminum concentration for alum coagulation processes is 89 ppb at a pH 6 when using a alum dosage of 70 mg / 1. Figure 1 shows that the soluble aluminum concentration in water for samples using alum increases as pH increased. At a pH of 8.0, the soluble aluminum concentrationis 974 ppb, which is a significant increase compared to that of pH 6. For Figure 2, it can be noticed that the lowest soluble aluminum concentration in water is 7 ppb at pH 6.5 when using a pre-formed aluminum hydroxide concentration of 50 mg / 1. Using different pre-formed aluminum dosage shows little effect on the soluble aluminum concentration in water. However one can notice the effect of pH is significant as the pH increases.

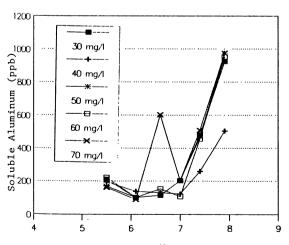


Fig.1 - Soluble Aluminum Concentration for Alum

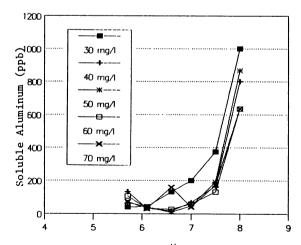


Fig.2 - Soluble Aluminum Concentration
for Preformed Aluminum Hydroxide

#### DISTRIBUTION OF MELAMPSORA LEAF RUST ON POPULUS IN THE NORTHWEST

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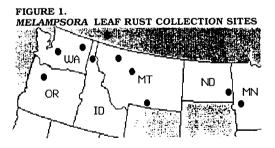
Melampsora leaf rust is an important foliar disease of the <u>Populus</u> species and hybrids used for forestry and shelterbelt plantings. Repeated infections result in premature defoliation, reducing vigor and predisposing trees to other pathogens (2). In North America, five <u>Melampsora</u> species have been reported on <u>Populus</u>: <u>M. medusae</u>, <u>M. albertensis</u>, <u>M. occidentalis</u>, <u>M. abietis-canadensis</u>, and <u>M. aecidioides</u> (1). Resistant hybrids offer the best control for this disease, but little information exists for <u>Melampsora</u> specificity on hybrid poplars. At present, most hybrid poplars are screened only for resistance to <u>M. medusae</u>, the predominant rust species in the central United States. Other <u>Melampsora</u> species may be important, particularly in northern states such as North Dakota. The purpose of this study was to examine the occurrence of <u>Melampsora</u> species on both native and hybrid poplars.

Leaves of Populus infected with Melampsora were collected from the north central and northwestern United States and western Canada. There were 22 collections in 1988 and 72 in 1989 from the following states: MN, ND, MT, ID, WA, OR, as well as British Columbia, Canada (Fig. 1).

Collections were gathered from stands of eastern cottonwood (P. deltoides), black cottonwood (P. trichocarpa), trembling aspen (P. tremuloides), bigtooth aspen (P. grandidentata) and hybrid poplars. To examine host specificity of Melampsora species, leaves were collected from sites where two or more Populus species were growing together. This occurred at 9 locations in 1988 and 18 in 1989. Melampsora species were identified by urediospore characters (width, length, and echinulation) as determined by light microscopy (1,3). The weather was unfavorable for leaf rust development in 1988 and 1989 in central and western North Dakota and eastern Montana, and no rust was found in these locations either year. Collections from these areas had previously yielded mixtures of Melampsora species (3).

The occurrence of the four Melampsora species found are summarized in Table 1. M. medusae was found in collections from MN and ND; M. albertensis was identified from MN, MT, ID, WA, and BC; M. occidentalis from OR, WA, ID, MT; and M. abietis-canadensis from MN. As shown in Table 1, each Melampsora species occurred only on certain Populus species. At 27 of the sites more than one Populus spp. was present; and even though they were in close proximity, different Melampsora species were always associated with different Populus species. For example: at all 1988 sites where two or more Populus species were present, M. albertensis infected only trembling aspen while M. occidentalis only black cottonwood; 1989 sites were similar.

Results of this study support the view of host specificity for the <u>Melampsora</u> species infecting <u>Populus</u> species. No mixed infections were found in these collections, although mixed infections have been reported on hybrid poplars (3). Future screening practices for hybrid poplars to be used in northern states may well need to include other Melampsora species in addition to M. medusae.



OCCURRENCE OF MELAMPSORA SPECIES ON POPULUS Number of Collections 1988-1989 MELAMPSORA SPECIESa ABI POPULUS HOST MED ALB occ P. DELTOIDES 23 P. TREMULOIDES 34 2 31 P. TRICHOCARPA . P. GRANDIDENTATA BMED=M. medusae; ALB=M. albertensis; OCC=M. occidentalis; ABI=M. abietis-canadensis.

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#### PHOTO-REDUCTION OF IRON(III) BY SELECTED CARBOXYLIC ACIDS

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Naturally occurring carboxylic acids such as tartaric, malic, citric and succinic are believed to enhance dietary iron absorption. These acids complex with iron(III) both in the food and through the gastrointestinal tract and provide for an optimum rate of iron exchange with the mucosa/serosa acceptors. These ligands solubilize the iron at pH = 7.5 by chelation making it available for absorption (1). Ascorbic acid enhancement of nonheme iron absorption is well documented (2). It should be noted that in addition to complexing iron(III) the ascorbic acid can reduce iron(III) to iron(II), the bioavailable form of iron.

In our laboratories we observed that the iron(III) complexes of citric, malic and tartaric acid underwent a slow thermal redox reaction at pH's 1.8 and 3.0 producing iron(II) (3). The redox reaction is fast in solutions exposed to room light but slow in the dark. The role of citric acid and other organic acids in promoting photo-reduction of ferric salts have been known for over a century [(4) and references therein]. Photo-reduction of the iron(III) carboxylate complex is accompanied by the oxidative decarboxylation of the organic acid. The reducing nature of iron(III) complexed carboxylic acids has not been thoroughly studied but the reaction could potentially play an important role in controlling iron bemostasis.

In this study, the photo-reduction rate of iron(III) to iron(II) in presence of the biologically and nutritionally important dicarboxylic and tricarboxylic acids (tartaric, malic, citric and succinic) is compared. Factors influencing the rate of formation of iron(II) under variety of experimental conditions were examined and are discussed.

The concentration of soluble iron in an oxidizing aqueous environment is limited by the precipitation of ferric hydroxide (the  $K_{sp}$  is  $10^{-39}$  and the equilibrium concentration of ferric ion at pH=4 is about  $10^{-9}$  M). To prevent the precipitation of iron(III) hydroxide, direct addition of base to the iron containing solutions was avoided in this study. Reaction mixtures to be studied were prepared by adjusting the pH of the ligand solution first, and then while it was being stirred vigorously in an inert atmosphere an exact volume of the iron-sulfate stock solution ( $pH\sim0.5$ ) was pipetted into the vortex of the ligand solution. The reaction of iron with the carboxylate ligand is assumed to be rapid; thus, the reactants reached equilibrium in the time of mixing. The mixtures were protected from light. The solutions were deoxygenated to prevent the reoxidation of iron(II) to iron(III) by dissolved oxygen. Irradiation was carried out using a Hg lamp equipped with a filter transmitting 366 nm light. o-Phenanthroline was added right after irradiation to quantitatively trap the photo-reduced iron(II) and allow the spectrophotometric determination of the ferrous ion formed in the solution.

The rate of photo-reduction of the ferric-carboxylate complex was determined for the conversion of the initial 20% of the iron(III) present (1-3 min). The photo-reduction rate depends on both the pH and the nature of the carboxylate ligand. As the pH increases the rate of reduction of carboxylate complexed iron(III) increases. The relative rate of photo-reduction of the ferric-carboxylate complexes under the same reaction conditions varies with the ligand, the order being: tartarate > malate > citrate > succinate.

The rate of photo-reduction of iron(III) complexed with alpha hydroxylated carboxylic acids is faster than with the structurally analogous non-hydroxylated carboxylic acids, that is, malic acid reduces faster than succinic acid. The hydroxyl group(s) on a polycarboxylic acid may somewhat alter the speciation, reduction potential, solubility and thermodynamic ability of the iron enhancing ligands.

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## CATALYST AND TREATMENT TEMPERATURE EFFECTS ON THE SURFACE AREA OF THREE COALS

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An extended Box-Behnken factorial design was undertaken to determine the most influencial factors towards the inhibition or enhancement the surface area of three coals. The coals in descending rank of high to low were Chinook (CN), a sub-bituminus; Wyodak (WY), a bituminus; and Coteau (CT), a lignite. The three coals were treated at 700, 750, and 800°C with and without the presence of the catalysts, Trona or Limestone (Lmstn.). Surface areas were measured using a dynamic flow adsorption apparatus. Each sample was outgassed and then the volume of CO<sub>2</sub> adsorbed at five different partial pressures of CO<sub>2</sub> in helium was measured and placed into two mathmatical models to determine the surface area of the coal sample. The Dubinin-Polanyi(D-P) (1) and the Brunauer-Emmett-Teller (BET) (2) models were used to determine the surface areas. The values obtained are shown in Table 1. The (3) values indicate the sample points that extend beyond the Box-Behnken factorial design.

Table 1

Coa1	Treat.	Temp. °C	D-P m <sup>2</sup> /g	BET m <sup>2</sup> /g
<b>#</b>				
∦WY	None	800	241.1	202.9
#CT	None	759	194.9	196.2
#WY	None	700	239.1	190.0
WY	None	75 <b>0</b>	221.1	192.5
WY	None	750	196.9	130.4
WY	None	750	208.3	185.4
WY	Lmstn.	300	191.9	166.8
IJY	Lmstn.	700	172.8	150.6
1:JY	Trona	300	190.9	119.6
₩Y	Trona	700	134.1	154.6
CT	Lmstn.	750	210.9	175.6
CN	Lmstn.	750	127.4	117.2
CT	Trona	750	97.4	69.8
CN	Trona	750	133.4	115.6
CT	None	300	226.6	183.8
CN	None	800	175.6	147.3
CT	None	700	197.3	131.2
CN	None	700	165.9	133.6
#CT	Trona	750	77.8	58.4
#CT	Lmstn.	750	180.8	147.4

A statistical analysis of the data indicates that there is an appreciable difference in the surface area of the three coal types and that catalyst treatment affects surface area. However, treatment temperature has no significant effect on the surface area. The D- $^{\rm p}$  model consistantly predicted a 23.7 m<sup>2</sup>/g higher surface area than the BET model. Statistical analysis of the difference between the two models indicates that the difference is not dependent on coal type, catalyst or treatment temperature.

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# REACTION OF CARBONYLCHLOROBIS(TRIPHENYLPHOSPHINE)IRIDIUM(I) WITH TRANS-STILBENE EPISULFOXIDE

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Although the reaction of episulfoxides (thiirane-S-oxides) and square planar metal complexes have been reported (1,2), the mechanism of the thionyl (SO) transfer has not been determined. To generate molecules of sulfur monoxide (SO), episulfoxides are heated (typically at 100°C) to produce an alkene and SO. Sulfur monoxide, a classically unstable small molecule, quickly disproportionates to elemental sulfur and sulfur dioxide over several steps (3). However, when episulfoxides are in the presence of square planar complexes, the episulfoxides undergo the same decomposition but at much lower temperatures (-30°C). The metal apparently acts as a catalyst in this reaction, allowing the episulfoxide to decompose at -30°C at rates normally seen near 100°C (4). The metal acquires SO fragments from the episulfoxide and becomes six-coordinate, requiring a 2:1 ratio of the episulfoxide to metal complex to form M(L4)(S<sub>2</sub>O<sub>2</sub>) (4).

trans-Stilbene episulfoxide was chosen as the episulfoxide for this reaction because of the steric factors provided by the alkene, trans-stilbene, produced in the reaction. The presence of bulky substituents prevents the alkene from reacting with the metal complex to form an adduct. Vaska's complex, [IrCl(CO)(PPh<sub>3</sub>)<sub>2</sub>], is the square-planar metal complex used to catalyze the decomposition, owing to a long history of oxidative addition reactions (5). Typically, the reaction is run at -30°C in chloroform solvent using a ratio of episulfoxide to iridium slightly larger than 2:1. The order of addition is episulfoxide to Vaska's complex. The presumed IrCl(CO)(PPh<sub>3</sub>)<sub>2</sub>(S<sub>2</sub>O<sub>2</sub>) product is soluble in chloroform, but can be precipitated using hexane.

A  $^{31}P$  NMR analysis of the product showed a low intensity impurity peak at -3.30 ppm while the desired product showed a strong peak at -10.28 ppm (see Table 1). It is important to identify and eliminate the formation of the impurity during the reaction for simplification of the analysis to identify the reaction's intermediates. Efforts to identify the complex responsible for the unknown peak include attempts to oxidize the sulfur with *meta*-chloroperoxybenzoic acid (MCPBA) to  $SO_x$  (x=2 or 3) and purging with  $N_2$  while irradiating with UV light to remove the carbonyl ligand. A reaction of Vaska's complex with elemental sulfur will be carried out to determine if possible sulfur contamination of the episulfoxide produces a disulfide complex with the iridium. Additionally, bulk reactions will be completed after further purification of the episulfoxide and removing oxygen contamination from the Vaska's complex. Reactions will be run at lower temperatures (-78°C) in preservative-free methylene chloride. This will prevent side reactions of the Vaska's complex with non-hindered alkenes.

A ring-opening reaction, with the iridium atom inserting itself into the thiirane ring, is being hypothesized as a likely mechanism, because of possible chemiluminescence taking place in this reaction (very bright green intermediate). Tests for chemiluminescence will be performed throughout the reaction, as well as low temperature proton and phosphorus NMR analysis.

Table 1. Major <sup>31</sup>P NMR Resonances Produced in Various Reactions

Conditions <sup>a</sup>	δ <sup>31</sup> P (ppm)	Identity <sup>a,b</sup>	Conditions <sup>a</sup>	δ <sup>31</sup> P (ppm)	Identity <sup>a,b</sup>
VC with	-10.28(s)	VC(S <sub>2</sub> O <sub>2</sub> )	VC(S <sub>2</sub> O <sub>2</sub> )	-31.41(w)	?
episulfoxide	- 3.30(w)	?	4 hrs. UV irradiation	-22.46(w)	?
	- 0.47(vw)	?		-17.48(w)	?
	12.32(trace)	?		-15.96(w)	?
	43.41(trace)	SPPh3		-12.74(w)	?
		•		-3.17(m)	?
VC(S <sub>2</sub> O <sub>2</sub> )	-10.12(s)	VC(S2O2)		-2.14(m)	?
1:1 with MCPBA	-6.02(vw)	7		-0.32(vs)	?
	-3.15 (vw)	'n		5.34(vs)	$VC(O_2)$
	-0.30 (w)	'n		24.15(m)	VC
	0.50 ()	•		29.39(w)	OPPh3
				43.62(s)	SPPh3
				137.41(w)	?

 $<sup>^{</sup>a}$  VC = IrCl(CO)(PPh<sub>3</sub>)<sub>2</sub>

<sup>b</sup>The compounds have been identified through tables compiled by R. Vanderpool from work of various researchers (see reference 4).

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# Insecticide Release Rates from Organophosphate and Organophosphate/Pyrethroid Impregnated Ear Tags

Mark B. Bates\* and H.J. Meyer

Pesticide impregnated ear tags have become a widely used and effective means of controlling pests of cattle in North Dakota. Ear tags are generally targeted for horn fly, Haematobia irritans (L.), control. Effective control of this pest using ear tags has been documented throughout much of the United States.

Two types of ear tags are currently in use; multiple component ear tags, which contain more than one pesticide type and single component ear tags. Many brands of both types are marketed and may contain several different insecticides or combinations of insecticides. The first insecticidal tag used contained the organophosphate insecticide stirofos (Rabon®); however control using single component ear tags was only successful when synthetic pyrethroids were introduced. Control using pyrethroids was extremely effective until horn fly populations developed resistance to these pesticides. Single component organophosphate and multiple component ear tags combining an organophosphate with a pyrethroid insecticide were introduced to control pyrethroid resistant flies.

In this study, we determined the pesticide release rates of several currently registered ear tags of both types. We examined pesticide quantity and rate of release as influenced by time of exposure, temperature fluctuations and tag placement on the animal. Finally, pesticide release rates from tags exposed on and off the cow were compared to determine the influence of the microclimate provided by the cow.

Three single component organophosphate ear tags were used in this study; Terminator, Optimizer (both diazinon impregnated) and Tomahawk (pirimiphos-methyl impregnated). Multiple component ear tags containing chlorpyrifos (Dursban®) in combination with a pyrethroid used in this study were Ear Force Ranger (chlorpyrifos-permethrin), Max-Con (chlorpyrifos-cypermethrin) and an experimental ear tag (Car-Mac Company) (chlorpyrifos-permethrin). Tags were removed from cattle at the NDSU Beef Unit and the Carrington Research Extension Center every 7 and 14 days, respectively. Tags fastened to the north and south faces of boards attached to posts in exposed locations were also removed every seven days for off cow comparisons. All tags were stored in sealable polyethylene bags at -4°C prior to analysis.

Tags were weighed, diced into small pieces (2 X 2 mm) and extracted using methods submitted with registration data by the manufacturers. A

Tags were weighed, diced into small pieces (2 X 2 mm) and extracted using methods submitted with registration data by the manufacturers. A sample of 1.25 grams of single component tag material was placed into 50 ml of chloroform in a 250 ml erlenmeyer flask for extraction. This procedure was repeated twice with the same tag so that a total of 2.50 g of material was extracted. Extractions proceeded for eight hours with agitation using an orbital shaker. The solution was then filtered through a 0.45 μm filter and 1 μl of the solution was injected into a Shimadzu GC-9A gas chromatograph equipped with a 3% OV-101 on Chromsorb W-HP (2m X 6mm dia.) column and a hydrogen flame detector. Pesticide remaining in the tag was quantified using a Shimadzu CR3-A computing integrator.

Multiple component ear tags were extracted using a two step procedure. After dicing, 5 grams of diced tag material was placed into 100 ml of tetrahydrofuran (THF) contained in a 250 ml erlenmeyer flask. The tag material was dissolved in THF for 10 hours with agitation on an orbital shaker. Five ml of the THF solution was then added to 20 ml of methanol placed into a 50 ml glass centrifuge. Precipitate which formed was centrifuged for approximately two minutes until a solid pellet formed on the bottom of the centrifuge tube. The supernatant solution was pipetted from the centrifuge tube and filtered through a 0.45 µm filter. Twenty microliters of the solution was injected into a Shimadzu LC-6A high performance liquid chromatograph equipped with a Zorbax CN® 250mm X 4.6 mm column (60:40 acetonitrile: water). Pesticide remaining was quantified using a Shimadzu CR3-A computing integrator.

Quantification of tag pesticide was used to determine fraction released from and release rate for each pesticide over time. Analysis of single component tags from cows showed that the pirimiphos-methyl tag (Tomahawk) reached half-life by 98 days after initial application. The two diazinon tags (Optimizer and Terminator) reached half-life by 120 days. Multiple component tag analysis showed that the chlorpyrifos release from Max-Con tags is significantly greater than chlorpyrifos release from Ear Force Ranger tags; Max-Con half-life was reached by day 98 while Ear Force Ranger chlorpyrifos half-life was reached in 154 days. A cypermethrin half-life from Max-Con tags of 63 days is significantly greater than the permethrin half-life of 154 days of Ear Force Ranger tags. A release rate analysis of single component ear tags showed that the Tomahawk tag has an overall higher release rate than either of the diazinon tags; however, this difference was not significantly different from the Terminator tag diazinon release while being significantly greater than the Optimizer diazinon tag (figure 1). The release rates for the two diazinon tags were not significantly different. None of the release rates of chlorpyrifos or the pyrethroid from multiple component tags on cows were significantly different (figure 2).

A comparison of tags off or on cows showed that overall pesticide release rates were significantly greater when tags were placed on cattle. Max-Con and Tomahawk tags showed higher release rates on the cow, but these differences were not significantly different. Temperature correlation analyses were inconclusive; no relationship could be demonstrated between temperature fluctuation and release rates between sampling periods. Analysis of tags fixed to the front vs. the back of cows ears showed that even though the release rate on the back of the ear was numerically higher, neither placement was significantly different.

These results support the following tag use strategies. Ear tags should be applied as late as possible, but before fly counts reach 100 per side, to maximize use of the insecticide contained in the tags. Tags should be applied at the rate recommended (usually two tags per cow) to provide an adequate dose of insecticide at the peak fly season. Optimum pesticide release will then coincide with the major portion of the fly season and can minimize chances for development of resistance in the horn fly population. Tags should be removed to eliminate late season control when fly numbers are declining and when pesticide release form the tags is low. This will also aid in delay of appearance of resistant flies.

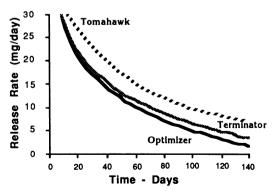


Figure 1. Organophosphate release rate from single component insecticide impregnated ear tags.

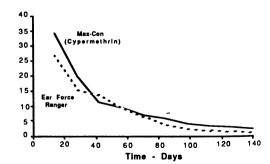


Figure 2. Pyrethroid release rate from multiple component insecticide impregnated ear tags.

## **Deprotonization of Acetic Acid**

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In the gas phase reaction between acetic acid and fluoride or hydroxide anions, the expected result is abstraction of the more acidic carboxyl-group proton. However, Grabowski and Cheng (1) found that anion-induced deprotonization of acetic acid tended to show less selectivity than expected, resulting in formation of significant amounts of both carboxylate and enolate ions. The intention of this research project is to investigate these results by quantum mechanical methods.

Individual reactions are reasonable approximations for gas phase studies at low to moderate pressures. Such approximations, along with more sophisticated and powerful computational devices and programs such as GAUSSIAN86 (2), offer an opportunity for the application of quantum mechanical theory to the study and characterization of reactions by looking at reaction paths and molecular and transitional energies. procedure starts by finding optimal structures for both products and reactants. three-dimensional geometry is decided upon by finding accepted values for bond lengths and angles in as much of the structure as possible (e.g., the general bond length for a carbon-to-oxygen double bond). Unavailable data, left to the best guess of the researcher, are then entered in as the 3n-6 unique internal coordinates (3n-5 for linear molecules). Bond lengths and angles are then manipulated in such a way that energy is decreased and parameters such as the rms force are brought below specified values. Once those criteria have been met, a frequency or hessian run certifies that all energy paths leaving the structure are positive. This corresponds to the potential well expected of a stable structure. After the researcher achieves stable structures in this way, reactants are allowed to react. This time a transition state is sought. energy found for reactants and that for the transition state, the barrier potential can be calculated.

If the transition state is hard to find, the energy map provides an alternative. The process entails setting the distance between the attacking group and the molecule as a constant and optimizing the rest of the structure. This is done for a variety of distances. If the change of energy in relation to the bond length is a steady increase the assumption that a barrier potential does not exist is supported. If there is a point where there is an increase followed by a decrease in energy, there is indication of a barrier potential, and a good idea of where to look for it exists.

The following data are for the hydride addition to the oxygen proton resulting in carboxylate ion formation. These data (energy in hartrees and lengths in angstroms) indicate no barrier energy to this reaction.

<u>H - H-</u>	<b>Energies</b>	<u>H - H-</u>	<b>Energies</b>	<u>H - H-</u>	<b>Energies</b>
0.9	-229.04045449	1.4	-229.01219612	3.0	-228.98332818
1.0	-229.03142860	1.6	-229.00696025	3.5	-228.97932515
1.1	-229.02435001	1.8	-229.00233795	4.0	-228.97661718
1.2	-229.01921232	2.0	-228.99809283		
1.3	-229.01533679	2.5	-228.98933043		

- (1) Grabowski, J. J., and Xucheng Cheng (1989) J. Am. Chem. Soc. 111, 3106-3108
- (2) GAUSSIAN86. Carnegie-Mellon Quantum Chemistry Publishing Unit. Pittsburgh.

# THE QUANTITATIVE RELATIONSHIP OF INDUCED INFLAMMATION WITH CORTICOSTERONE RESPONSE IN THE RAT

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Experiments presented herein were designed to define the quantitative relationship of carrageenan-induced inflammation with plasma corticosterone response. Plasma corticosterone is regulated by the hypothalmo-pituitary adrenal system (1). Time course of an induced corticosterone response has been demonstrated by injecting a 1% carrageenan suspension into a rat hind paw which elicited two distinct responses. First response occurred within 30 minutes and was due to the stress of injection itself. Second response with a maximum at 7 hours was not duplicated by the solvent and can be attributed to carrageenan (2).

The methods and procedures used in these experiments followed those of Stenberg et. al. (2). The modifications are noted herein. To cause inflammation each rat received a 0.1 ml injection of water, 0.9% saline or 60, 180,  $540 \,\mu g/ml$  carrageenan. Blood samples were collected exactly 7 hours after carrageenan injections and timed to be between 11:00 and 12:00 am. In this manner diurnal variation of corticosterone concentration of the afternoon would not complicate the data (3). The plasma of each rat was individually analyzed for corticosterone using a radioimmunoassay.

Anesthesia and blood sampling procedures were designed and performed to minimize effects of stress on plasma corticosterone concentration. A mean basal concentration of plasma corticosterone from control rats was found to be 8 ng/ml which is in agreement with Hilfenhaus's value for basal level of corticosterone of unstressed rat (3). Stenberg et. al. found a second corticosterone pulse response could be induced by injecting 0.05 ml of a 500 µg carrageenan suspension into a hind paw of the rat. Plasma corticosterone concentration reached a maximum value of 292 ng/ml within 7 hours after carrageenan was injected (2).

The results of Table 1 demonstrate a direct quantitative relationship of the carrageenan amount with the corticosterone response. The lower dosage injection of 60-180  $\mu$ g/0.1 ml carrageenan into one paw did not elicit a corticosterone response. Injection of 540  $\mu$ g/0.1 ml into one paw did induce a corticosterone response which was half of that induced by 540  $\mu$ g/0.1 ml injected in each of two paws. Injection of 540  $\mu$ g/0.1 ml into each of three or four paws failed to induce a higher corticosterone response than that of the two-paw injection. This evidence suggests that there is a minimum amount of inflammation required to stimulate a corticosterone response and there is an amount of inflammation beyond which no additional corticosterone response occurs.

These results are of importance for control of inflammation in rheumatoid arthritis. This study shows that hypothalmopituitary-adrenal system is stimulated to a varying degree with varying amount of inflammation present. A base line level of inflammation must be exceeded before the hypothalmo-pituitary-adrenal system is stimulated and there is a maximum level where the increase in the number of points of inflammation did not increase the amount of corticosterone produced.

Table 1
Mean Plasma Corticosterone Concentration 7 Hours After Injection of Carrageenan into One or More Paws of the Rat

Agent Dosage (µg/ml)	Number of Rats	Number of Paws Treated	Plasma Corticosterone Concentration (ng/ml)*
controls	20	0	8+5
water	8	1	9+10
saline	8	1	24+14
carrageenan (60)	8	1	12+13
carrageenan (180)	8	1	8+9
carrageenan (540)	20	1	140+70
carrageenan (540)	11	2	363+44
carrageenan (540)	12	3	396+65
carrageenan (540)	12	4	386+73

<sup>\*</sup>Mean determined at 95% confidence

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# GEOLOGIC FACTORS AFFECTING THREE RURAL STREAM CROSSINGS IN POLK AND NORMAN COUNTIES OF MINNESOTA

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Three rural road crossings on an unnamed tributary to the Sand Hill River in Polk and Norman counties of Minnesota have historically presented a chronic washout problem in times of flood. While the most heavily used of the three crossings (Crossing B) has apparently been stabilized by the installation of a redesigned culvert system, the other two crossings (Crossings A and C) remain of concern. Although the dollar amount of the washout damage is not large, the cumulative impact of repeated repairs has been considerable.

Crossing A, the furthest downstream of the three, has a severely underdesigned culvert system. The flow of the stream, rated at 490 cubic feet per second with a velocity of 8 feet per second at design flood levels(1), requires 61 square feet of outlet culvert or channel. The current situation is two 36" metal culverts which, if entirely unobstructed and flowing at full capacity, provide 14 square feet of outlet. The stream flows through nearly a mile of mature woodland before reaching these culverts, and usually blocks one or both to some degree with branches and debris. This crossing is not used by heavy equipment or farm machinery, so a light-duty bridge or a larger culvert system would suffice. The soils are stable enough to support either option.

Crossing B was originally serviced by three 36" culverts. These were replaced in 1978 by two 102" by 62" reinforced concrete pipes. In addition, the road was regraded to provide a spillway 100 feet wide in case of overflow, thus localizing damage due to unexpectedly high floodwaters. After nearly twelve years in place, the system seems to be working well, with only one overflow (1979), resulting in insignificant damage.

Crossing C, furthest upstream, presents the most challenging problem of the three. Situated on an organic muck soil, the roadway is used quite heavily at times by farm equipment and heavy machinery, so a light-duty bridge will not work in this case. The organic nature of the soil precludes the effective use of culverts, which are soil-supported structures. The present culverts, one 48" and one 36", wash out nearly every year, even without large-scale flooding. Although the culverts themselves are capable of handling the discharge of the stream at this point, the soil will not support culverts. Piping and other forms of erosion begin to attack the roadway embankment immediately upon thawing in the spring and result in undercutting of the road itself. The organic soils are underlain at a depth of 60" by alluvial sands, which would support bridge pilings quite well. The sands in turn, are underlain by the tills of the surrounding ground moraine landscape, which also exhibit excellent engineering properties(2). The obvious resolution to the problem is a medium-duty bridge. While this solution may cost more at the outset, the reduced or eliminated repair costs would offset the investment in a few years.

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#### PHOTOCHEMISTRY OF Cp2W2(CO)6 AND Ph2Se2

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Irradiation of single-bonded metal carbonyl dimers gives organometallic radicals (1). Disulfides have been used as trapping agents for photoproduced CpW(CO)<sub>3</sub>(2); this work was designed to explore if diselenides could play the same role.

A solution of  $Cp_2W_2(CO)_6$  ( $Cp = C_5H_5$ ) and diphenyl diselenide in toluene was irradiated with a white (tungsten) light. To hinder any competing thermal effects, the solution was kept at 0°C. Due to sensitivity to  $O_2$ , the solution was made, reacted and stored under an inert ( $N_2$ ) atmosphere. An infrared (IR) spectrum was taken of the solution, and two peaks were dominant - one at 1953 and another at 1904 cm<sup>-1</sup>. As the reaction progressed, the solution color changed from the original crimson to red-orange, and the prominent IR peaks changed to 2021 and 1935 cm<sup>-1</sup>. The reaction was monitored throughout by IR spectra to determine the finishing point. The reaction is:

An NMR was run and there were two multiplets with peaks at 7.541 and 7.572, and 7.238, 7.215, and 7.189 ppm, respectively (phenyl); and a singlet at 5.6995 ppm (Cp). These are all different from the  $Ph_2Se_2$  and  $Cp_2W_2(CO)_6$  peaks -  $Ph_2Se_2$  has two multiplets with major peaks at 7.677 and 7.6510, and 7.3356, 7.310, and 7.285 ppm. The  $Cp_2W_2(CO)_6$  peak is a singlet occurring at 5.386 ppm. The IR and NMR spectral data are very similar to those for the Mo analog (3). A melting point was taken (under inert atmosphere) and determined to be 99-102°C. The corresponding Mo complex melts from 92-94° (3).

Light and air sensitivity tests were conducted. The CpW(CO)<sub>3</sub>SePh in toluene solution (under an inert atmosphere) was exposed to light for short, then increasingly longer, periods of time and monitored by visual observation and IR spectra. A similar solution was exposed to an O<sub>2</sub> atmosphere in the dark to determine air sensitivity. The compound appears to be stable to both air and ambient lighting, at least for periods of hours.

Ph<sub>2</sub>Se<sub>2</sub> was obtained from Aldrich Chemical Company and used without further purification. Cp<sub>2</sub>W<sub>2</sub>(CO)<sub>6</sub> was prepared using modified literature methods. The first step was to make the tris-nitrile derivative of W(CO)<sub>6</sub>, i.e., (CH<sub>3</sub>CN)<sub>3</sub>W(CO)<sub>3</sub> by refluxing W(CO)<sub>6</sub> in excess acetonitrile for 40 hours (4). (This could have benefited from longer reflux time and monitoring by IR.) The Birdwhistell technique (5) was used to prepare the Na<sup>+</sup>Cp<sup>-</sup> salt, which was then reacted with the (CH<sub>3</sub>CN)<sub>3</sub>W(CO)<sub>3</sub> to form the CpW(CO)<sub>3</sub>- anion (6). The anion was oxidized (5) with aqueous ferric sulfate to yield Cp<sub>2</sub>W<sub>2</sub>(CO)<sub>6</sub>. The dimer was washed with water, methanol and hexane to remove ferric sulfate, ferrocene, and W(CO)<sub>6</sub> respectively.

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#### INFRARED DEER DETECTOR AND TIME RECORDER

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When hunters take to the woods in pursuit of deer, their main concern is the time of day in which the deer are moving about. There are devices on the market today which when placed across a deer trail will record the time a deer passes. These devices, however, use a string across the trail as a detection method and are only able to record one such event. The device we have designed is a multiple event time recorder which could be taken out into the woods, attached by means of a strap or similar device to a fixed object such as a tree, and left for a period to record the passage of creatures. It uses an infrared beam reflected across a nearby trail to detect the passage of deer. It will then record the time that such events occur to Random Access Memory (RAM) chips for later review by the user. This system can record up to 100 such events which is a vast improvement over available devices.

Our device has three modes of operation, record, review, and clear memory. In record mode, the breaking of the infrared beam causes the current time to be loaded into memory. The memory is advanced to the next memory position after a twenty second delay to prevent one animal or a small group of animals from making multiple recordings. In review mode, the times recorded in memory are displayed and the memory is slowly advanced through all its memory positions to allow the user access to the event times. Finally, to clear the memory, all the memory inputs are connected to ground and the memory is sequenced through all its positions. The position counter is then set to the beginning and the detector is again ready to record.

The block diagram of our deer detector is shown in Figure 1. The clock section generates a 12-bit word representing the present time and sends it into a tri-state 12-bit data bus. This data bus connects the clock and display sections with the RAM memory chips. There is an infrared source consisting of an infrared diode and a 40 KHz modular circuit which generates the proper frequency for the infrared detector. The detector is a module which detects the presence or absence of the 40 KHz infrared beam. When the beam is interrupted, a trigger signal is sent to the three RAM chips which make up the memory and the address counter. This causes a time to be recorded in the memory. The address counter is a two stage counter which generates an 8-bit address word for addressing the memory. Finally, for the display we chose seven segment LED displays and decoders as this would allow for reading the stored data in low light conditions common in hunting. Four of these LEDs are used to display the time and two for the memory address.

As mentioned before, our detector can store up to 100 event times which is more than sufficient for a day or two of tracking the movement of deer down a trail. Our device was built from off the shelf integrated circuit chips with a cost of approximately \$70 which would give hunters an accurate record of deer movement with reasonable investment of time and money.

Since this device is basically an event time recorder, with minor modifications it could be used in many other applications where there is a need for tedious observation of the occurance of a specified event such as traffic flow surveys, science projects, wildlife studies, or security control systems. A demonstration of the circuit operation will be given at the time of the presentation.

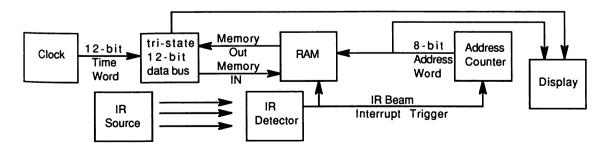


Figure 1 - Block Diagram of Infrared Deer Detector and Time Recorder

Conditional Symmetric Instability in the Atmosphere
(A Case Study)

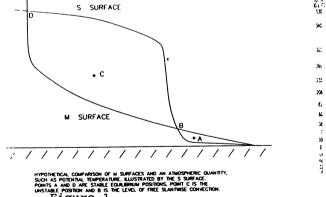
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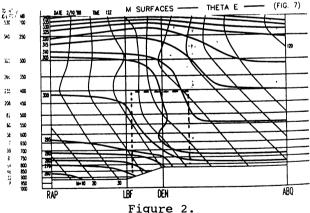
Linear bands of precipitation have been observed in many extratropical cyclones and were often assumed to be associated with frontal boundaries. However, with the advent of Doppler radar, it became evident that this assumption was not always valid. Conditional Symmetric Instability (CSI) is a concept which offers an explanation as to why convective bands may form in some large scale extratropical storms without the presence of a frontal boundary.(2) CSI is based on conditions established in a Boussinesq inviscid rotating stratified fluid, under which, vertical instability is produced by a forced slantwise ascent. (1) This concept is of major concern in forecasting and detecting in-cloud icing for aircraft. The means to determine this phenomena involve computations and display of the pseudo-angular momentum, (M) and equivalent potential temperature surfaces utilizing the following equations.

M=V+fx  $\mathcal{O}_e = T_e * (\frac{1000}{p})^{-X}$ 

For the M surface, V is the meridional velocity, f is the coriolis force and x is the distance between sounding stations. While for  $\mathcal{O}_{e}$ ,  $T_{e}$  is the equivalent temperature, P is the pressure altitude of  $T_{e}$ , and X is the ratio of gas constant to molar heat capacity at constant pressure. To visualize the data, a vertical cross section normal to the vertical shear vector must be constructed and a contour analysis performed. The ideal case (Fig. 1) consists of  $\mathcal{O}_{e}$  surfaces being of steeper slope than the M surfaces.

Field operations were conducted on 10 February 1988 at the UND Doppler radar site in Denver Colorado, for what at first appeared to be an upslope induced icing event. However, a banded appearance to the echoes and higher reflectivity cores suggested that imbedded convection was occurring. To perform a CSI analysis, the synoptic pattern required that the cross section be constructed from Rapid City SD, North Platte NE, Denver CO and Albuquerque NM, and is illustrated below with the finished products in Figure 2.





Comparing the outlined area in figure 2 with the ideal case of figure 1 indicates the atmosphere in the Denver area was experiencing CSI. In this case, the convection was produced by forced lift along the M surfaces oriented from west to east, which in turn produced a convective cloud slanted from south to north. UND aircraft also confirmed the presence of in cloud icing and of imbedded convection as suggested by the radar echoes. This concludes that CSI does play a role in not only forecasting icing, but its detection as well. However, applications of CSI to other types of weather may also apply, and must be researched on a case by case basis.

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  North Dakota Academy of Science Proceedings, Volume 44, 1990

#### 12 CHANNEL FIBER OPTIC DATA LINK

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This is a new design for a fiber optic data link to be used for two-way transmission of serial computer signals (RS-232) with baud rates up to 19.2K baud. The system is designed for multiplexed communication between 12 remote terminals and their respective computer ports. The distance between the terminals and ports can be a maximum of 2Km. The goal of this design is to eliminate damage caused by lightning-induced voltage spikes in outdoor signal carrying lines. One solution to this lightning-induced voltage problem would be to install surge suppressors on each terminal and computer port. A more eloquent solution is to remove the copper cables from the electromagnetic field and use fiber optic cables instead. This application will be used to protect the data gathering equipment in the Radar Equipment and Computer Trailer (REACT). REACT is a mobile research facility owned by the Department of Atmospheric Sciences at the University of North Dakota.

The data to be transmitted from the terminals is multiplexed into a serial data stream and sent through a fiber optic cable to the computer system. A second fiber optic cable carries the data that is received by the terminals; this data is also a multiplexed serial stream. The receiver circuit demultiplexes the serial stream back into the respective RS-232 data channels. For full duplex operation (two-way transmission), two transmitter-receiver pairs are needed: one for the terminal end and one for the computer end. The four main parts to this design are: (1) the transmitter circuit, (2) the receiver circuit, (3) the sync detect circuit, and (4) the power supply. The sync detect circuit is contained in the receiver. Figure 1 shows the block diagram.

The transmitter accepts data from the remote terminals through a terminal block consisting of six telephone jacks (RJ-11 block). The data then goes to the ICL232 chips which allow conversion from RS-232 to TTL (0 and 5V logic levels) using only a single 5V power supply. The parallel data is latched and then scanned by the 16 channel multiplexer. The first four inputs to the multiplexer are hard-wired to create a sync pattern embedded in the data stream. This will be used by the sync detect circuit in the receiver section. From the multiplexer, the serial data stream goes to the optical transmitter module (698-LS-T1) which converts the TTL to light pulses to be sent through the fiber optic cable.

For the receiver section, the light pulses from the second fiber optic cable go to the optical receiver module (698-LS-R1) and are converted back to the TTL serial data stream. The serial data then goes to the clock recovery chip (AT&T T7033) which has two derived outputs: (1) the original clock frequency of the transmitter circuit, and (2) the serial data stream retimed to the clock output. When the sync field is detected (as determined by the sync detector), the serial data stream is demultiplexed back into 12 discrete channels. The TTL channels are then converted back to RS-232 using the same ICL232 I.C.s used in the transmitter circuit.

The sync detector circuit determines if the right terminal is communicating to the right computer port. The data and recovered clock from the T7033 I.C. go to the sync detector. After three consecutive sync patterns (created in the transmitter circuit) have been detected, the circuit provides an output to enable the rest of the receiver circuit and demultipelxing begins. If the data stream is interrupted, the sync circuit also signals the system to stop.

The total parts cost of each transmitter-receiver pair is approximately \$200.00. A similar system on the market is priced at approximately \$900.00.

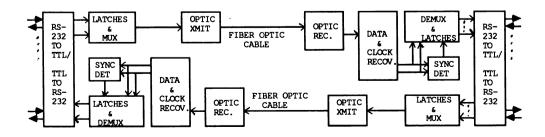


Figure 1 - Block Diagram of the 12 Channel Fiber Optic Data Link

#### Basalts from Tutuila, American Samoa

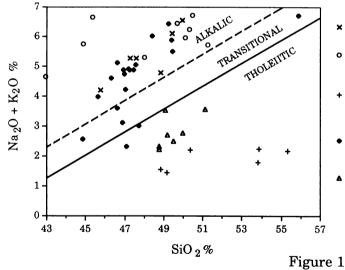
Jody K. Solem Geology Department, North Dakota State University Fargo, N.D. 58105

Seven rock samples were collected from an ancient village site on the island of Tutuila, part of the American Samoan islands, by Dr. J. Clark, Anthropology Dept. NDSU. The samples were studied using petrographic microscope, x-ray powder diffraction, and scanning electron microscopy (by Dr. R.J. Stevenson, UND) in order to characterize their physical properties, determine their mineralogies and compare them to the known petrography of the island. Archaeological interest in the petrographic and geochemical comparison was to determine whether or not the samples, which represented prehistoric stone tool specimens, were made locally or imported from elsewhere.

The Samoan Islands lie geographically near the Figian Islands which have distinctly different types of rocks forming on them. The Samoan Islands represent oceanic basalts and the Figian Islands represent continental basalts. This made it possible to study the stone tools and make valid comparisons of possible sources.

The mineralogies of all the samples included plagioclase feldspar, augite, varying amounts of olivine, and mafics (predominantly ilmenite). A few of the samples contained magnetite and biotite. None of the samples showed any quartz present, which would be characteristic of an andesitic of dacitic (continental) rock type. The normative mineralogies of the samples were undersaturated with normative olivine and minor nepheline which classifies them as alkali olivine basalts (oceanic).

A comparison of the alkali vs.  $SiO_2$  composition (Figure 1) of various samples showed that Clark's samples were geochemically very similar to samples previously collected from the Pago Shield, the remnant of a shield volcano located near the archaeology site where the samples were collected. It seems clear from available petrographic information that the samples were not imported from Tonga but were collected locally.



- ➤ Dr. Clark's Samples..... collected in 1988 near Aoa, Tutuila.
- o Engel Alkali Basalts... collected from the east Pacific rise (Engel, 1965).
- + Bryan Tonga Basalts.... collected from various sea mounts and islands from Tonga (Bryan, 1972).
- Wright Tutuila Basalts. collected from various locations on the island (Wright, 1986).
- ▲ Lau Basin ...... lies between Tonga and Samoa (Hawkins, 1974).

Bryan, W.B., G.D. Stice, and Edward A. Ewart (1972). "Geology, petrology, and geochemistry of the volcanic islands of Tonga." from: <u>The Geology of Continental Margins</u> by C.A. Burk and C.L. Drake (1974); Springer-Verlag, New York-Heildeberg-Berlin.

Engel, A.E.J., Celeste G. Engel, and R.G.Havens (1965). "Chemical Characteristics of oceanic basalts and the upper mantle"; Geological Soc.of Am., V. 76, pt.2, July-Dec., 1965.
 Hawkins Jr., James W. and James H. Natland (1974). "Geology of the Lau Basin, a marginal sea behind the Tonga Arc." from: The Geology of Continental Margins by C.A. Burk and C.L. Drake (1974); Springer-Verlag, New York-Heildeberg-Berlin.

Wright, B.E. (1986). "Petrology and geochemistry of shield building and posterosional lavas of Samoa; implications for mantle heterogeneity and magmagenesis. Ph.D. dissertation, University of California, San Diego.

#### **OBITUARY**

#### Norma E. Pfeiffer

13 January 1889 - 23 August 1989

Norma E. Pfeiffer, President of the North Dakota Academy of Science in 1922-23, died at the age of 100 years in Dallas, Texas. She had joined the University of North Dakota Biology Department faculty in 1912, and received her Ph.D. degree in botany from the University of Chicago in 1913. At that time she was the youngest person ever to have received that degree from that University

While at UND she taught all of the botany courses and carried out research on ferns. After reaching the rank of Associate Professor and serving UND for eleven years, she resigned to join the Boyce Thompson Research Institute for Plant Research in Yonkers, NY and worked on ferns and flowering plants, especially lillies. She developed several types of hybrid lillies in her career.

Although she retired in 1959, she continued to publish until 1976. She lived alone in Dallas until recently. She had joined the Academy in 1912 and served as Vice-President prior to assuming the Presidency in 1922.

118 CONSTITUTION

#### C O N S T I T U T I O N of the NORTH DAKOTA ACADEMY OF SCIENCE

(Founded 1908; Official State Academy 1959)

#### ARTICLE I - Name and Purpose

- 1. This association shall be called the North Dakota Academy of Science.
- 2. The purposes of this association shall be to promote and conduct scientific research and to diffuse scientific knowledge.

#### ARTICLE II - Membership

- 1. Membership in the North Dakota Academy of Science shall be composed of persons active or interested in some field of scientific endeavor. Candidates for membership may be proposed by any active member of the Academy by submitting the candidate's name to the chairman of the Membership Committee for approval. Specific categories of membership shall be defined in the bylaws of the Academy.
- 2. Annual dues for the various categories of membership shall be determined by the members present at the Annual Meeting.

#### ARTICLE III - Officers

- 1. The officers of the Academy of Science shall be a President, President-Elect, and the Secretary-Treasurer who shall perform the duties usually pertaining to these offices. The President-Elect shall be chosen by ballot at the Annual Meeting and will hold the office for one year and then assume the office of President for one year. The Secretary-Treasurer shall be appointed for a three-year term by the Executive Committee.
- 2. The Executive Committee, consisting of the above-named officers, the retiring President, and three members-at-large, shall have charge of the ordinary executive duties. The members-at-large shall be elected for a three-year term on a rotation basis.

#### ARTICLE IV - Meetings

- There shall be an Annual Meeting each year held at such time and place as the Executive Committee may determine.
- 2. Special meetings shall be called by the President upon the request of ten percent of the active members. Only matters specified in the call can be transacted at a special meeting.
- Ten percent of the active members shall constitute a quorum at the Annual Meeting. Special
  meetings require twenty percent of the active members for a quorum.

#### ARTICLE V - Miscellaneous

- 1. In the event of dissolution of the Academy, any remaining assets shall be distributed to organizations organized and operated exclusively for educational and scientific purposes as shall at the time qualify as exempt organizations under Section 501(c) (3) of the Internal Revenue Code of 1954.
- 2. No substantial part of the activities of the Academy shall be the carrying on of propaganda, or otherwise attempting to influence legislation, and the Academy shall not participate in, or intervene in, any political campaign on behalf of any candidate for public office.
- 3. No part of any net earnings shall inure to the benefit of, or be distributable to, Academy members or officers, or other private persons, except that the Academy may authorize the payment of reasonable compensation for services rendered.

#### ARTICLE VI - Amendments

- 1. This Constitution may be amended at any Annual Meeting of the Academy by a two-thirds vote. Proposed amendments shall be submitted in writing to the Secretary who shall send them to the members at least two weeks before the meeting at which such amendments are to be considered.
- Bylaws may be adopted or repealed at any regular meeting by a two-thirds vote.
   North Dakota Academy of Science Proceedings, Volume 44, 1990

BYLAWS 119

#### NORTH DAKOTA ACADEMY OF SCIENCE

#### BY-LAWS

1. The Academy's official guide for parliamentary procedure shall be the "Standard Code of parliamentary Procedure" by Alice F. Sturgis. (1965 Rev.)

- 2. The annual dues shall be determined by a two-thirds vote at an Annual Meeting. These dues are payable January 1 of each year. (1965 Rev.)
- Members shall be dropped from the active list on December 31 following the nonpayment of dues during the membership year commencing the previous January 1. A member may return to the active list by paying the current year dues and a membership renewal charge of \$5.00. (1975 Rev.)
- 4. Every member in good standing shall receive a copy of the annual Proceedings of the North Dakota Academy of Science. (1965 Rev.)
- $_{5.}$  Special offices such as Historian may be created by the unanimous vote of the members at the Annual Meeting. (1965 Rev.)
- 6. The Executive Committee shall annually appoint an Academy representative to the National Association of Academies of Science and to Section X (General) of the American Association for the Advancement of Science. (1979 Rev.)
- 7. The Committee structure of the Academy shall be as follows, the President appointing the members and chairpersons for all except the Executive Committee:

#### a. Executive Committee

Membership: Past-President, President, President-Elect, Secretary-Treasurer, three members-at-large. Three-year terms.

Duties: The Executive Committee shall be the governing board of the Academy, responsible only to the membership. It shall arrange for programs, approve committee appointments, be responsible for the fiscal affairs of the Academy, and transact such business as necessary and desirable for function and growth of the Academy.

#### b. Editorial Committee

Membership: Three members, three-year terms.

Duties: The Editorial Committee shall develop and recommend to the Executive Committee the Academy publication program and policies. It will assist the Editor in reviewing manuscripts for the Proceedings.

#### c. Education Committee

Membership: Seven members, two of whom shall be high school teachers. Five-year terms.

Duties: The Education Committee shall work with high school students and teachers in the state, in visitation programs, Science Talent Search programs, and other programs to stimulate an interest in science by the youth of the state. It shall operate the Junior Academy of Science program and administer the AAAS high school research program.

### d. Denison Awards Committee

Membership: Six members, three-year terms.

Duties: The Denison Awards Committee shall have as its prime duty the judging of student research and paper competitions, both undergraduate and graduate, and any other similar competitions. The committee shall also maintain the criteria to be used in the judging and selection of papers, such criteria to be circulated to prospective competitors. (1985 Rev.)

#### e. Necrology Committee

Membership: Three members, three-year terms.

Duties: The Necrology Committee shall report to the annual meeting on those departed during the preceding year. Obituaries may be included in the minutes of the annual meeting and/or published in the Proceedings.

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#### f. Nomination Committee

Membership: The five most recent past-presidents.

Duties: The Nominating Committee shall propose a slate of at least two nominees for each of the offices as needed. The committee report shall be submitted to the President prior to the annual meeting as well as reported to the

membership at the appropriate time for action.

#### g. Resolution Committee

Membership: Three members, three-year terms.

Duties: The Committee on Resolutions shall prepare such resolutions of recognition and thanks as appropriate for the annual meeting. Further, the Committee shall receive suggested resolutions for the membership and transmit such resolutions and the Committee recommendation to the membership.

### h. Membership Committee

Membership: Unlimited number, appointed annually.

Duties: The Membership Committee shall promote membership in the Academy. It shall conduct an annual canvass of the institutions of higher education, government agencies, and other related organizations for purpose of providing opportunity for prospective members to join the Academy. Further, this Committee shall make recommendations to the Executive Committee of potential candidates for emeritus and honorary memberships.

- 8. The Nominating Committee shall be responsible for all nominations to elective office and shall be required to advance at least two names for each open position. Academy members shall have been encouraged to suggest nominees to the committee prior to the Committee submitting its report. A ballot, incorporating brief biographical information, shall be distributed by the Secretary-Treasurer to all members prior to the annual meeting. Those ballots may be returned by mail, or in person at the annual meeting, until the announced deadlines. The results of the election shall be announced at the annual meeting.
- 9. Categories of membership:
  - a. Active members shall be persons interested or actively participating in some scientific endeavor. Active members may participate in all activities of the Academy.
  - b. Student members shall be graduate or undergraduate college students in some field of science. Student members may participate in all activities of the Academy, with the exception of holding office.
  - c. Sustaining members are persons or organizations interested in the activities of the Academy. Sustaining members may participate in all activities of the Academy, with the exception of voting or holding office. Sustaining members may be of three types: Individual, Corporate, or Institutional. (1965 Rev.) This bylaw is implemented by the following action of the Executive Committee (10-25-85):

There shall be two categories of Corporate Sustaining Membership, Patron members and Sponsor members. The annual membership fee shall be \$100 for Patron members and \$50 for Sponsor members.

Benefits accuring to Corporate Sustaining Members include:

- Positive public relations through the support of science and technology in North Dakota.
- 2. Preference in mounting commercial displays at the annual meeting of the Academy.
- 3. Early access to research results and early awareness of research programs through first hand association with scientists and engineers.
- 4. Improved commercial opportunities through association with members, institutions, and other sustaining members.
- 5. Improved future commercial opportunities through exposure to students contemplating careers in science or technology.

BYLAWS 121

- Until action is taken otherwise, the Corporate Sustaining Membership fees shall be placed in the North Dakota Science Research Foundation for the support of scientific research.
- d. Emeritus membership. Any member in good standing upon formal retirement is eligible for emeritus membership. Nominations may be forwarded to the Membership Committee by any member, and it shall be the responsibility of the Membership Committee to review the membership list for possible candidates. The Executive Committee shall approve nominations. Emeritus members shall retain all rights of active members but will be exempt from payment of dues. (1973 Rev.)
- e. Honorary Membership. The Academy may recognize, by awarding honorary membership, any person (nonmember or member) who has in any way made an outstanding contribution to science. It shall be the responsibility of the Membership Committee to be aware of individuals whom it would be fitting for the Academy to honor in this fashion. Any member may submit nominations along with supporting data to the Membership Committee. Approval of nominations shall be by a two-thirds majority of those attending the annual meeting. (1973 Rev.)
- 10. The President, with the approval of the Executive Committee, shall appoint members to serve on ad hoc committees. Reports of ad hoc committees shall be presented to the Executive Committee or to the annual meeting. Ad hoc committees serve only dring the tenure of the president who appointed them. (1965 Rev.)
- 11. The Executive Committee shall appoint an Editor who shall edit the Proceedings. The Editor shall be appointed for a three-year term. The salary of the Editor shall be set by the Executive Committee. (1975 Rev.)
- 12. The annual dues shall be \$12.00 per year for professional members, with \$2.00 designated for the North Dakota Science Research Foundation, and \$5.00 per year for student members. (1985 Rev.)
- 13. The Executive Committee is empowered to charge a publication fee of authors of up to \$10.00 per page. (1965 Rev.)
- 14. All student research participants shall receive a properly inscribed certificate and be invited to the dinner as the guests of the Academy. (1965 Rev.)
- 15. All activities of the Academy, including grant applications, are to be handled through the Academy offices from now on. (1966 Rev.)
- 16. The Executive Committee of the North Dakota Academy of Science be instructed to establish a J. Donald Henderson Memorial Fund and that the committee administer this fund and that the proceeds from this fund be used to promote science in North Dakota (1967 Rev.)
- 17. The fiscal year of the North Dakota Academy of Science, for the purpose of financial business, shall be January 1 to December 31. (1973 Rev.)
- 18. The Academy establishes the NDAS Achievement Award, to be awarded periodically to an Academy member, in recognition of excellence in one or more of the following:
  - a) Nationally recognized scientific research.
  - b) Science education.
  - c) Service to the Academy in advancing its goals.

The Nominating Committee will administer the selection process, will develop a separate funding source for a monetary award, and will develop, for Executive Committee approval, the criteria for the award. (1988 Rev.)

19. The North Dakota Science Research Foundation is established as an operating arm of the Academy. The purposes of the Foundation are to (1) receive funds from grants, gifts, bequests, and contributions from organizations and individuals, and (2) to use the income solely for the making of grants in support of scientific research in the State of North Dakota. Not less than 50% of the eligible monies received shall be placed in an endowment from which only the accrued interest shall be granted.

The Foundation shall be responsible for soliciting the funds for the purposes described. The Foundation funds shall be in the custody of the Secretary-Treasurer of the Academy and shall be separately accounted for annually.

The Foundation Board of Directors shall be comprised of five members of the Academy, representing different disciplines. Members shall be appointed by the President for staggered five year terms, and the chairperson of the Board shall be appointed annually by the President. The Board shall be responsible for developing operating procedures, guidelines for proposals, evaluation criteria, granting policies, monitoring procedures, and reporting requirements, all of which shall be submitted to the Executive Committee for ratification before implementation.

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

Revised May 1989

#### ACADEMY OFFICERS AND STANDING COMMITTEES 1989-90

## EXECUTIVE COMMITTEE

David Davis, President USDA, BRL, Fargo

Forrest Nielsen, Past-President Human Nutrition Res. Ctr.

Clark Markell, President-Elect Minot State Univesity

A. William Johnson, Sec.-Treas. University of North Dakota (1989-92)

Carolyn Godfread, Member-At-Large

James Waller, Member-At-Large
University of North Dakota (1989-92)

Jimmie Richardson, Member-At-Large North Dakota State Univ. (1989-90)

#### EDITORIAL COMMITTEE

Claude Schmidt (1988-91)
USDA, BRL, Fargo--Chairman

James Tilton (1989-92)
North Dakota State Univ., Fargo

Douglas Johnson (1987-90) NPWRC, Jamestown

#### RESOLUTIONS COMMITTEE

Richard Baltisberger (1988-91) Univ. of North Dakota--Chairman

Allen Khim (1989-92) Minot State University

Lee Manske (1987-90)
North Dakota State University

#### NOMINATING COMMITTEE

Elliot Shubert (1986-91) Univ. of North Dakota

Michael Thompson (1985-90)
Minot State University

Forrest Nielsen (1989-94) Human Nutrition Res. Ctr.--Chairman

William Barker (1987-92) North Dakota State Univ.

Bonnie Heidel (1988-93) ND Parks & Recreation Dept.

#### EDUCATION COMMITTEE

Jimmie Richardson(1989-92) North Dakota State University Executive Liaison--Chairman

Mike Burton (1989-94) Agassiz Jr. High School, Fargo Science Olympiad

Jerome Knoblich (1986-90) Jamestown College AAAS Minigrants Coordinator Junior Academy Director

Om Madhok (1987-92) Minot State University Science Fair Liaison

Allen Khim (1987-92) Minot State University National Science Week

Ron Royer (1988-93) Minot State University Science Educator Newsletter

Marcia Steinwand (1989-94) Robinson High School

#### DENISON AWARDS COMMITTEE

Roy Garvey (1989-1992)
North Dakota State University

Robert Crackel (1989-92) Minot State University

Louis Rigley (1987-90)
Dickinson State University

Curtiss Hunt (1988-91) Human Nutrition Res. Ctr.

Jimmie Richardson (1987-90) North Dakota State University

John Brauner (1988-91)
Jamestown College--Chairman

#### NECROLOGY COMMITTEE

Benjamin DeBoer (1988-91)
Grand Forks--Chairman

Charles Lura (1987-90) NDSU-Bottineau

William Wrenn (1989-92) University of North Dakota

# ND SCIENCE RESEARCH FOUNDATION BOARD OF DIRECTORS

Virgil Carmichael (1987-91) Bismarck--Chairman

Harry Holloway (1986-90) University of North Dakota

Virgil Stenberg (1987-92) University of North Dakota

Larry Campbell (1989-94) North Dakota State University

John Reid (1988-93) University of North Dakota

#### MEMBERSHIP COMMITTEE

Myron Freeman Dickinson State University

Michael Thompson Minot State University

Gary Clambey North Dakota State University

Vernon Feil USDA, BRL, Fargo

Charles Lura
NDSU-Bottineau--Chairman

Charles Turner University of North Dakota

Janet Hunt Human Nutrition Res. Ctr.

Carolyn Godfread Bismarck

#### LOCAL ARRANGEMENTS -- Fargo

Lee Manske Animal Science--Chairman

Claude Schmidt USDA, BRL

William Barker Botany

David Berryhill Bacteriology

Berlin Nelson Plant Pathology

David Davis USDA, BRL

## NORTH DAKOTA ACADEMY OF SCIENCE

# Financial and Membership Statement January 1, 1989-December 31, 1989

#### A. BALANCE SHEET

	1988 Items	1988 TOTAL	1989 Items	1989 TOTAL
I. ASSETS				
Operating Accounts				
Checking Account	\$2,579.20		\$611.27	
Savings Account	\$2,634.09		\$1,625.74	
Savings Certificate	\$5,000.00	\$10,213.29	<b>\$5,000.0</b> 0	\$7,237.01
Trust Accounts				
Scholarship Principal	\$15,361.13		\$16,505.83	
Research Foundation	\$5,909.01	\$21,270.14	\$7,159.60	\$23,665.43
Receivables				
SMITS Grant	\$0.00	\$0.00	\$1,098.68	\$1,098.68
TOTAL ASSETS		\$31,483.43		\$32,001.12
II. LIABILITIES				
December Operations	\$0.00		\$121.06	
Advance Dues	\$1,585.00	\$1,585.00	\$1,285.00	\$1,406.06
Restricted Purpose Funds				
ScholarshipPrincipal	\$15,361.13		\$16,505.83	
Cash	\$2,351.50		\$2,008.28	
AAAS Research Grant	\$900.00		\$0.00	
Research Foundation	\$5,909.01	\$24,521.64	\$7,159.60	\$25,673.71
TOTAL LIABILITIES		\$26,106.64		\$27,079.77
III. ACCUMULATED SURPLUS		\$5,376.79		\$4,921.35
IV. CHANGE IN SURPLUS		(\$566.57)		(\$455.44)

#### B. OPERATING CASH FLOW

	1988	1989
CASH ON HAND JANUARY 1	\$10,055.88	\$10,213.29
Cash Receipts for year	\$14,258.92	\$11,851.51
Total Resources Available	\$24,314.80	\$22,064.80
Cash Disbursements	\$14,101.51	\$14,827.79
CASH BALANCE DECEMBER 31	\$10,213.29	\$7,237.01
Increase over the year	\$157.41	(\$2,976.28)

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### C. MEMBERSHIP STATEMENT

	Actions	Emeritus	Student	Professional	Total
Jan.1,1989		60	43	283	386
Terminations	Resigned Dropped(Dues) Deceased SUBTOTAL	1 0 2 3	8 13 0 21	11 25 0 36	20 38 2 60
Additions	New Reinstated Change Status SUBTOTAL	0 0 1 1	32 0 - 1 31	37 6 0 43	69 6 0 75
Net Changes		- 2	10	7	15
Dec.31,1989		58	53	290	401
Dues Paid 1/1/90		58	19	119	196

### D. OPERATING RECEIPTS

	1988 Items	1988 TOTAL	1989 Items	1989 TOTAL
DUES				
Reinstatements	\$67.00		\$50.00	
Current Year	\$1,965.00		\$1,657.00	
Next Year	\$1,585.00	\$3,617.00	\$1,285.00	\$2,992.00
SUBSIDIES				
Univ. of ND	\$1,000.00		\$1,000.00	
NDSU	\$750.00		\$1,000.00	
Minot State Univ.	\$200.00	\$1,950.00	\$200.00	\$2,200.00
ANNUAL MEETING				
SD Academy	\$233.20		\$0.00	
Registration Fees	\$1,800.00		\$2,810.00	
Meals	\$1,965.00		\$0.00	
Am. Chemical Soc.(RRVS)	\$300.00		\$300.00	
Assoc. ND Geographers	\$0.00		\$50.00	
ND Geol. Soc.	\$50.00		\$100.00	
Sigma XiUND	\$50.00		\$50.00	
NDSU	\$0.00		\$100.00	
Minot	\$0.00		\$50.00 °	•
Program Subsidies	\$2,000.00	\$6,398.20	\$0.00	\$3,460.00
AWARDS PROGRAM				
AAAS Research Grant	\$900.00		\$0.00	
Scholarship Dividends	\$747.50	\$1,647.50	\$481.78	\$481.78
PUBLICATION SALES	\$167.00	\$167.00	\$123.00	\$123.00
INTEREST ON SAVINGS	\$479.22	\$479.22	\$491.65	\$491.65
SMITS GRANT	\$0.00	\$0.00	\$2,103.08	\$2,103.08
TOTAL INCOME		\$14,258.92		\$11,851.51

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## E. OPERATING DISBURSEMENTS

	1988 Items	1988 TOTAL	1989 Items	1989 TOTAL
ANNUAL MEETING				
Speakers	\$2,651.40		\$973.83	
Meals	\$2,734.36		\$1,856.30	
General Expenses	\$1,322.92	\$6,708.68	\$733.90	\$3,564.03
AWARDS PROGRAM				
AAAS Research Grants	\$710.00		\$900.00	
Dunbar Award	\$75.00		\$175.00	
Henderson Award	\$50.00		\$0.00	
Science Fair	\$25.00		\$0.00	
Denison Awards	\$500.00		\$450.00	
Abbott Scholarships	\$0.00	\$1,360.00	\$200.00	\$1,725.00
PUBLICATIONS				
Editor Fee	\$250.00		\$250.00	
Proceedings	\$2,103.84	\$2,353.84	\$2,586.65	\$2,836.65
MISCELLANEOUS				
Fidelity Bond	\$26.00		\$26.00	
AAAS Delegate	\$960.57		\$1,000.00	
NAAS Dues	\$44.90	\$1,031.47	\$42.30	\$1,068.30
PROGRAM OPERATIONS				
Junior Academy	\$0.00		\$0.00	
Committee Travel	\$460.30		\$55.80	
Research Foundation	\$15.30	\$475.60	\$0.00	\$55.80
OFFICE EXPENSES				
Post <b>ag</b> e	\$762.72		\$403.16	
Postal Box Rent	\$39.00		\$39.00	
Telephone	\$0.00		\$6.38	
Duplicating	\$218.68		\$208.70	
Supplies	\$414.02		\$349.01	
Clerical Staff	\$137.50		\$170.00	
Sec. Treas. Fee	\$600.00	\$2,171.92	\$1,200.00	\$2,376.25
SMITS PROGRAM	\$0.00	\$0.00	\$3,201.76	\$3,201.76
TOTAL DISBURSEMENTS		\$14,101.51		\$14,827.79

## F. SCIENCE RESEARCH FOUNDATION

	1988	1989	CHANGE
Balance January 1	\$4,401.44	\$5,909.01	\$1,507.57
Donations from Members	\$279.00	\$296.50	\$17.50
Allocations from Dues	\$678.00	\$544.00	(\$134.00)
Organization Memberships	\$300.00	\$100.00	(\$200.00)
Interest Accrued	\$250.57	\$310.09	\$59.52
Balance December 31	\$5,909.01	\$7,159.60	\$1,250.59

#### G. SCHOLARSHIP FUND

	1983	1988	1989
CASH INCOME			
SDGE Dividends		\$257.50	\$267.50
Pinwest Dividends		\$490.00	\$214.28
TOTAL		\$747.50	\$481.78
CASH EXPENSES			
Denison Awards		\$500.00	\$450.00
Dunbar Award		\$75.00	\$175.00
Henderson Award		\$75.00	\$0.00
Abbott Scholarships		\$0.00	\$200.00
TOTAL		\$650.00	\$825.00
NET INCOME		\$97.50	(\$343.22)
ASSETS			
SDGE shares	250.00	277.06	289.48
Price	\$18.50	\$38.25	\$45.13
Value	\$4,625.00	\$10,597.43	\$13,064.23
Pinwest Shares	275.00	302.46	313.00
Price	\$21.00	\$15.75	\$11.38
Value	\$5,775.00	\$4,763.70	\$0.00 ***
Money Fund	\$0.00	\$0.00	\$3,441.60
Investment Value Subtotal	\$10,400.00	\$15,361.13	\$16,505.83
Operating Account	\$0.00	\$2,351.50	\$2,008.28
TOTAL	\$10,400.00	\$17,712.63	\$18,514.11
CHANGE IN TOTAL ASSETTS		(\$363.64)	\$801.48
INVESTMENT VALUE CHANGE		(\$461.14)	\$1,144.70

\*\*\* PINWEST Stock was sold 10/19/89\*\*\*

Verified by Audit Committee:

1100

Gaula V. Hay

Date: Jan. 23 1990

Respectfully Submitted

A. William Johnson Secretary-Treasurer

Date: Jan .- 12 1990

ABRAHAMSON, HARMON B.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
ACHEN VIRGINIA	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
ATSETH DANIEL	506 11TH STREET N.W. APT. D	MINOT	ND	58701
AL BRECHT, STEVEN	JAMESTOWN COLLEGE	JAMESTOWN	ND	58401
ALESSI, JOSEPH	1210 11TH STREET SOUTH	FARGO	ND	58103
ATTENBURG, LOIS IVERS	1146 FIFTH STREET NORTH	FARGO	ND	58102
ANDERSON, EDWIN M.	1151 12TH AVENUE WEST	DICKINSON	ND	58601
ANDERSON, ORDEAN S.	RURAL ROUTE 1, BOX 269	NEW PRAGUE	MN	56071
ANGEL KATHLEEN	7500 UNIVERSITY DRIVE	BISMARCK	ND	58504-9652
ARMFIELD, LARRY	ROUTE 1, BOX 530	DETROIT LAKES	MN	56501
ASCHBACHER, PETER W.	NORTH DAKOTA STATE UNIVERSITY	`FARGO	ND	58105
ASHWORTH, ALLAN C.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
AUDET, PATRICK	MINOT STATE UNIVERSITY	MINOT	ND	58701
AUYONG, THEODORE	3614 11TH AVENUE NORTH	GRAND FORKS	ND	58201
RALSBAUGH, EDWARD, JR.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
BALTISBERGER, RICHARD	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
BARBER, ROBERTA	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
BARKER, WILLIAM T.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
BARNEY, WILLIAM G.	1525 COTTONWOOD	GRAND FORKS	ND	58201
BARNHART, MICHAEL	2704 10TH AVENUE, NW	MANDAN	ND	58554
BARTAK, DUANE E.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
BASSINGTHWAITE, DAVID	P.O. BOX 640	DEVILS LAKE	ND	58301
BATES, MARK B.	304 31ST AVE. N. APT. 104	FARGO	ND	58102
BAUMBERGER, THOMAS R.	1035 BOYD DRIVE	GRAND FORKS	ND	58203
BEHM, MARLA	516 NORTH 19TH STREET	BISMARCK	ND	58501
BEHRINGER, MARJORIE	1613 CRIPPLE DRIVE	AUSTIN	TX	78758
BELINSKEY, CAROL R.	MINOT STATE UNIVERSITY	MINOT	ND	58702
BERDAHL, JOHN D.	NORTHERN GREAT PLAINS RES. LAB	MANDAN	ND	58554
BERKEY, GORDON B.	MINOT STATE UNIVERSITY	MINOT	ND	58702
BERRY, EUGENE S.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
BERRYHILL, DAVID L.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
BICKLER, SCOTT	14 - 11TH AVENUE N.W.	MINOT	ND	58701
BITZAN, EDWARD F.	2200 UNIVERSITY AVENUE	GRAND FORKS	ND	58203
BLEIER, WILLIAM J.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
BLISS, HARALD N.	P.O. BOX 522	MAYVILLE	ND	58257
BLUEMLE, JOHN P.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
BOBILYA, DENNIS J.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
BODE, ANN M.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
BOLIN, F.M.	1505 SIXTH STREET SOUTH	FARGO	ND	58102
BOLONCHUK, WILLIAM W.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
BRAUER, MICHAEL G.	HC 1-53	BALDWIN	ND	
BRAUNER, JOHN F.		JAMESTOWN	ND	58521
BREKKE, DAVID	JAMESTOWN COLLEGE			58401
	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
BREKKE, GARY	419 - 11TH AVENUE SOUTH #308	FARGO	ND	58103
BRISKE-ANDERSON, MARY	1504 COTTONWOOD	GRAND FORKS	ND	58201
BROPHY, JOHN A.	702 SOUTH DRIVE	FARGO	ND	58103
BROWN, RALPH C.	BOX 89	STONEHAM	ME	04331
BRUMLEVE, STANLEY	218 49TH AVENUE SOUTH	GRAND FORKS	ND	58201
BRUSHMILLER, GEORGE	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58201
BUCK, MICHAEL	607 1ST S.W.	CROSBY	ND	58730
BURT, NANCY	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
BURTON, MICHAEL	34 PRAIRIEWOOD CIRCLE	FARGO	ND	58103
BUTLER, MALCOLM G.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
CALLENBACH, JOHN A.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
CAMPBELL, LARRY G.	USDA, ARS, NORTHERN CROP SC LAB	FARGO	ND	58105-5677
CARLSON, CHRIS R.	RURAL ROUTE 2, BOX 3	BOTTINEAU	ND	58318
CARLSON, EDWARD C.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
CARLSON, KENNETH T.	320 SECOND AVENUE NORTHWEST	MAYVILLE	ND	58257
CARMICHAEL, VIRGIL W.	1013 NORTH ANDERSON STREET	BISMARCK	ND	58501
CARR, DONNA	3904 UNIVERSITY AVENUE #213	GRAND FORKS	ND	58203
CARTER, JACK F.	1345 11TH ST., NORTH	FARGO	ND	58102
CASPERS, LISA	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
CASSEL, J. FRANK	U.S. AIR FORCE ACADEMY	COLORADO SPRI	co	80840
CHALLEY, JOHN R.	1349 SECOND STREET NORTH	FARGO	ND	58102
CHERIAN, K. SEBASTIAN	JAMESTOWN COLLEGE	JAMESTOWN	ND	58401
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CLAUSEN, ERIC N.	MINOT STATE UNIVERSITY	MINOT	ND	58702
COLE, DUANE	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
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CONNELL, MARVIN D.	2606 FIFTH AVENUE NORTH	GRAND FORKS	ND	58203
CONVARDEL LEGISLAM E.	307 PARK AVENUE	GRAND FORKS	ND	58203
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CURTISS, GENE C. JR.	1121 5TH AVENUE SW	MINOT	ND	58701
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DALY, DANIEL	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
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DEBOER, BENJAMIN	312 ALPHA AVENUE	GRAND FORKS	ND	58203
DEES, BONNIE A.	RURAL ROUTE 1, BOX 430	GARRISON	ND	58540
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FISK, ALLEN L.	1122 AVENUE B WEST	BISMARCK	ND	58501
FIVIZZANI, ALBERT J.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58 <b>2</b> 02
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FREEMAN, MYRON L.	DICKINSON STATE UNIVERSITY	DICKINSON	ND	58601
FROELICH, STACIE J.	507 SWANSON HALL	GRAND FORKS	ND	58202
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GABRIELSON, VICKI	NORTH DAKOTA STATE UNIV.	FARGO	ND	58105
GARVEY, ROY	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
GERLA, PHILIP	711 25TH AVENUE SOUTH	GRAND FORKS	ND	58201
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GLENN, JULIE	510 TULANE DRIVE, APT.#11	GRAND FORKS	ND	58203
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GODFREAD, CAROLYN	409 ASPEN AVENUE	BISMARCK	ND	58501
GOETTLER, HANS J.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
GOOS, ROBERT J.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
GOSNOLD, WILLIAM	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
GREELEY, SHARON	405 11TH AVENUE SOUTH	FARGO	ND	58103
GREENWALD, STEPHEN	1729 NORTH 4TH STREET	FARGO	ND	58102
GRIFFIN, JUDITH	2603 7TH AVENUE SOUTH, #38	GRAND FORKS	ND	58201
GROENEWOLD, GERALD	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
HAIN, MARLA	625 9TH STREET, NE	MINOT	ND	58701
•	3633 KIMBERLY COURT	GRAND FORKS	- ND	58201
HALL, CLINT				
HALVORSON, GARY A.	BOX 459	MANDAN	ND	58554
HAMMEN, JOHN L.	1339 SOUTH 18TH STREET	GRAND FORKS	ND	58202
HANSEN, BRYANT W.	NORTH DAKOTA STATE UNIV-BOTTINEAU	BOTTINEAU	ND	58318
HARMONING, ARLEN	1708 NORTH 4TH STREET	BISMARCK	ND	58501
HARRIS, ROBERT	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
HARTMAN, JOSEPH H.	UNIVERSITY OF NORTH DAKOTA	<b>GRAND FORKS</b>	ND	58202
HASSETT, DAVID J.	20 FENTON AVENUE	GRAND FORKS	ND	58203
HASSETT, DEBRA	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
HASTINGS, MICHAEL	DICKINSON STATE UNIVERSITY	DICKINSON	ND	58601
	NORTH DAKOTA STATE UNIVERSITY	FARGO	/ND	58105
HAYES, ROBERT M.				
HEIDEL, BONNIE	402 N. MANDAN STREET	BISMARCK	ND	58501
HEMMASI, MOHAMMAD	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
HENDERSON, WILLIAM	3014 NORTH ELM STREET	FARGO	ND	58102
HENJUM, DAN	2521 VILLA DRIVE #105	FARGO	ND	58103
HERBEL, JOLAYNE	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58203
HERTSGAARD, DORIS	BOX 5194	FARGO	ND	58105

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utntz, DENNIS D.	BOX 235	GLEN ULLIN	ND	58631
HORRS, JOHN T.	BOX 264	FORDVILLE	ND	58231
HOEPPNER, JEROME J.	2518 NINTH AVENUE NORTH	GRAND FORKS	ND	58203
HOFF, DONALD L.	402 EAST FIRST STREET	VELVA	ND	58790
UOFFMAN, CHARLES A.	MINOT STATE UNIVERSITY	MINOT	ND	58702
HOFSTRAND, PHILIP	721 30TH STREET, N.W., APT.6	FARGO	ND	58102
HOGANSON, JOHN W.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
HOGANSON, SHELLY	722 BELMONT ROAD	GRAND FORKS	ND	58201
HOLLAND, F.D., JR.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
HOLLAND, JEAN H.	4686 BELMONT ROAD	GRAND FORKS	ND	58201
HOLLOWAY, HARRY, JR.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58201
HOLLOWAI, HARRI, JR.		MINOT		
HOPKINS, CHARLES	1917 9TH STREET N.W.		ND	58701
HOWELL, FRANCIS L.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
HUNG, YUNG-TSE	CLEVELAND STATE UNIVERSITY	CLEVELAND	OH	44115
HUNT, CURTISS D.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
HUNT, JANET	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
HURLEY-WILSON, BARBARA	815 - 40TH AVENUE S, #155 NORTH	GRAND FORKS	ND	58201
HUSAIN, SYED	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
JACKSON, JON A.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
JACOBS, FRANCIS A.	1525 ROBERTSON COURT	GRAND FORKS	ND	58201
JANSKY, SHELLEY H.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
IOHANSEN, DOROTHY	MAYVILLE STATE UNIVERSITY	MAYVILLE	ND	58257
JOHNSON, A. WILLIAM	629 HIGH PLAINS COURT	GRAND FORKS	ND	58201
JOHNSON, ARNOLD R.	449 EAST BRONDON DRIVE	BISMARCK	ND	58501
JOHNSON, DOUGLAS H.	BOX 2096	JAMESTOWN	ND	58402
JOHNSON, LESTER E.	P.O. BOX 224	BOTTINEAU	ND	58318
JOHNSON, PHYLLIS E.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
JOHNSON, WILLIAM T.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
JORDE, DENNIS	U.S. FISH AND WILDLIFE SERVICE	LAUREL	MD	20708
JUDKINS, WAYNE	10 COUNTRY ACRES TRAILER COURT	MINOT	ND	58701
JUHL, NYLA H.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
KANNOWSKI, PAUL B.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
KANTRUD, HAROLD A.	ROUTE 7	JAMESTOWN	ND	58401
KARNER, FRANK R.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
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KELLEHER, JAMES J.				
KEYS, ROSS D.	1617 EAST CAPITOL AVE. #6	BISMARCK	ND	58501
KHAVANIN, MOHAMMAD	1115 24TH AVENUE SOUTH	GRAND FORKS	ND	58201
KIESLING, RICHARD	P.O. BOX 204	FARGO	ND	58107
KIHM, ALLEN J.	MINOT STATE UNIVERSITY	MINOT	ND	58702
KILLINGBECK, JAMES	P.O. BOX 5520	BISMARCK	ND	58502
KIM, TAHNEE M.	525 PARK STREET #5	MINOT	ND	58701
KIRBY, DON	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
KLOSTERMAN, HAROLD J.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
KNOBLICH, JEROME	233 14TH AVENUE NORTHEAST	JAMESTOWN	ND	58401
KNUDSON, CURTIS L.	711 NORTH 25TH STREET	GRAND FORKS	ND	58203
KNULL, HARVEY	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
KOENKER, WILLIAM E.	WHIPPOOR WILL LANE	CHAPEL HILL	NC	27514
KOLSTOE, RALPH H.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
KONTZ, BRAD	402 N. 23RD STREET, #2	GRAND FORKS	ND	58202
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KOTCH, ALEX	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
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KRESS, WARREN D.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
KROGSTAD, KEVIN D.	163 LANCASTER DRIVE	MOORHEAD	MN	56560
KRUPINSKY, JOSEPH M.	BOX 459, USDA-ARS	MANDAN	ND	58554
KRUSCHWITZ, EARL H.	431 SIXTH STREET SOUTHWEST	VALLEY CITY	ND	58072
KUBE, DIANNE A.	630 BOYD DRIVE	GRAND FORKS	ND	58203
KUIPERS, GILBERT	VALLEY CITY STATE UNIVERSITY	VALLEYCITY	ND	58072
KU <b>MAR, GIRISH</b>	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
LAIRD, WILSON M.	101 SPANISH OAK LANE	KERRVILLE	TX	78028
LAMBETH, DAVID	1909 20TH AVENUE SOUTH	GRAND FORKS	ND	58201
LARSON, LINDA	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
LARSON, OMER R.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
LAURSEN, SUSAN E.	3435 S. 10TH STREET, #8	GRAND FORKS	ND	58201
LEADBETTER, LARRY	717 PRINCETON PARK	GRAND FORKS	ND	58201
I FADDETTED MADY				
LEADBETTER, MARY	717 PRINCETON PARK	GRAND FORKS	ND	58203
LEAGUE, LARRY	DICKINSON STATE UNIVERSITY	DICKINSON	ND	58601
LEHR, EUGENE R.	BOX 724	LINTON	ND	58552
LEWIS, TERRY	317 24TH AVENUE NORTH	FARGO	ND	58102
LINDLEY, JAMES A.	1421 NORTH UNIVERSITY DRIVE	FARGO	ND	58102
LIPP, WILLIAM V.	3024 NORTH 10TH STREET, #19	FARGO	ND	58102
LOBDELL, FREDERICK	UNIVERSITY OF WISCONSIN	OSHKOSH	WI	54901
LOCKWOOD, KARL L.	MAYVILLE STATE UNIVERSITY	MAYVILLE	ND	58257
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MANSKE, LLEWELLYN	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
MARDON, AUSTIN A.	1007 7TH AVENUE SOUTH	LETHBRIDGE, A	CA	
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MARKELL, CLARK	MINOT STATE UNIVERSITY	MINOT	ND	58702
MARTIN, ALAN	UNIVERSITY OF MANITOBA	WINNIPEG	MA	R3T2N2
MARTIN, DEWAYNE C.H.	2104 SEVENTH AVENUE NORTHWEST	MINOT	ND	58701
MARWIN, RICHARD M.	1519 CHESTNUT STREET	GRAND FORKS	ND	58201
MASON, HARRY	P.O. BOX 1116	JAMESTOWN	ND	58401
MATHENEY, RONALD	3425 SOUTH 10TH STREET #5	GRAND FORKS	ND	58201
MATHSEN, DON	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
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MATTHIES, DONALD L.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
MCCOLLOR, DONALD P.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
MCDONALD, CLARENCE E.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
MCGILLIVRAY, GERALD	15 UNIVERSITY AVENUE EAST	MINOT	ND	58701
MCMAHON, KENNETH J.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
MEARTZ, PAUL D.	MAYVILLE STATE UNIVERSITY	MAYVILLE	ND	58257
MELCHIOR, ROBERT C.	615 S. MOVIL LAKE ROAD, NE	BEMIDJI	MN	56601
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MELDRUM, ALAN	512 COLUMBIA ROAD	GRAND FORKS	ND	58203
MESSENGER, THEODORE	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
MESSMER, TERRY A.	2109 SOUTH 10TH STREET	FARGO	ND	58103
MEYER, DWAIN W.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
MILLER, JAMES E.	3807 MICHAEL LANE	GLENVIEW	IL	60025
MITCHELL, E.N.	220 GLENHILL LANE	CHAPEL HILL	NC	27514
MOK, TECK S.	110 STATE STREET, NO. 14	GRAND FORKS	ND	58201
•	823 SIXTH STREET SOUTHWEST		ND	
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MORLEY, JAMES	3712 BERKELEY DRIVE, APT. 6	GRAND FORKS	ND	58203
MUDDERMAN, DENIS	BOX 8274, UNIVERSITY STATION	GRAND FORKS	ND	58202
MUESSIG, KAREN D.	2824 B 22ND AVENUE SOUTH	GRAND FORKS	ND	58201
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NALEWAJA, JOHN D.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
NEEL, JOE K.	2221 CHESTNUT STREET	GRAND FORKS	ND	58201
NELSON, BERLIN D.	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
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NELSON, HARVEY K.	10515 KELL AVENUE SOUTH	BLOOMINGTON	MN	55437
NELSON, KERRY	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
NELSON, ROBERT	NORTH DAKOTA STATE UNIVERSITY	FARGO	ND	58105
NICHOLAS, JOSEPH W.	UNIVERSITY OF GEORGIA	ATHENS	GA	30602
NIELSEN, FORREST H.	UNIVERSITY OF NORTH DAKOTA	GRAND FORKS	ND	58202
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